Impact of ozone on the water relations of ash (*Fraxinus excelsior* L.)

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$AOT40_{24}$	accumulated dose over threshold 40 ppb calculated for 24 hours per day
CF	charcoal-filtered air
$CF+O_3$	charcoal-filtered air to which 150 ppb of ozone were added
d	droughted
E	exposure day
E+1	day following an exposure
E+2	day following two days after an exposure
O	day where at least six days had elapsed since the last O_3 exposure
P	probability
PAR	photosynthetically active radiation
VPD	water vapour pressure deficit
w	well-watered

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Abstract

During the field seasons 1993 and 1994, five-year-old field-grown ash trees as well as potted two-year-old saplings and one-year-old seedlings of ash (*Fraxinus excelsior* L.) were exposed to ozone episodes in open-top chambers. The plants received either charcoal-filtered air (CF) or charcoal-filtered air to which 150 ppb of ozone were added (CF+O_3). Plants in unchambered plots, receiving ambient air (Ambient), were included into the investigation for comparison. Half of the two-year-old saplings of each of the three pollution treatments were subjected to three drought cycles of 7-14 days, while the rest of the plants were well watered as a control.

The two-year-old saplings were the main object of the investigation. On these, the main parameters investigated were: stomatal conductance; the growth parameters - extension growth, radial increment at the stembase and radial increment at the base of the new shoot; leaf area; aboveground biomass production; the microscopic determination of ringwidth; and the structure of the latest annual ring for samples taken from the stembase. Additionally, stomatal conductance was measured in the five-year-old trees and total biomass accumulation, photosynthate allocation and ring parameters of the latest annual ring were investigated in the one-year-old seedlings.

The ozone episodes were shown to influence stomatal conductance in plants of different water status differently. Drought stress led to a significant decrease of stomatal conductances, and the drought-ozone interaction caused a further decrease which was also significant. The ozone episodes also affected stomatal responsiveness of the plants, restricting stomatal aperture of the droughted two-year-old saplings, when the drought cycles were finished and the plants were maintained at high soil moisture again. The drought cycles alone, however, left the functioning of the stomata unimpaired.

The restriction of stomatal aperture which was found for the droughted CF+O_3 treatment caused reductions of growth and biomass accumulation, but a significant decrease was found only for radial growth at the stembase of those plants. Analysis of the annual rings showed that this was caused by a reduction of xylem growth, while phloem development did not seem to be affected. Radial increment at the base of the new shoot was less affected by the pollution treatment. Here only a significant impact of the drought could be found, and the growth reduction was less pronounced.

In the well-watered plants, ozone caused a slight increase in stomatal conductance which led to increased aboveground biomass accumulation, but concomitantly biomass allocation was slightly altered, favouring the crown rather than the lower plant organs. A reduction of photosynthate allocation to the roots became evident from measurements on the one-year-old saplings.

Due to chamber effects, stomatal conductance as well as growth, biomass accumulation and allocation differed from those of plants from the Ambient treatment.

The responses to ozone that were found in this study could lead to increased crown growth during periods of good water availability and to a strong reduction of water uptake during and following drought conditions. Thus crown demand for water may increase in ash trees exposed to elevated ozone concentrations, while the supply of water to the crown may become limiting.

1 Introduction

Ozone (O_3) - translated from greek as "the fragrant" - is becoming increasingly popular and important in many science related discussions and political debates. This is due both to its stratospheric decrease and tropospheric increase during the last few decades and to the negative effects of these two processes on living organisms. While the decreasing thickness of the stratospheric ozone layer leads to an increased transmission of UV-B, the increase in tropospheric ozone concentrations affects organisms directly.

Among the known effects of tropospheric ozone on plant growth and physiology is its influence on stomatal behaviour, leading to decreased stomatal responsiveness in many plant species (Koch & Maier-Maercker, 1992; Pearson & Mansfield, 1993; Reich & Lassoie, 1984). This further implies an impact on the water relations via the water use of the plants.

Growth (Cooley & Manning, 1987; Edwards *et al.*, 1992) and biomass allocation (Edwards *et al.*, 1994; Spence *et al.*, 1994) can also be influenced. According to Cooley & Manning (1987) the storage organs of plants are usually most severely affected by pollutant-induced alterations in the biomass allocation pattern. In woody plants these are mainly the roots and to a lesser degree the stems, plant organs which are important for water absorption, storage and water transfer within the plants and thus partly determine the plant's water relations. Disproportionate biomass allocation can lead to reduced root-shoot ratios (Edwards *et al.*, 1994; Oshima *et al.*, 1979), which may make plants more susceptible to drought stress.

According to Innes (1993) drought stress is a particularly frequent cause of forest dieback. Such dieback, seemingly connected to dry summers, was experienced by ash trees in England in recent years. Since dry summer weather often coincides with increased ozone concentrations, ozone might be considered as an additional factor contributing to the dieback.

In this connexion, the impact of elevated ozone concentrations on the water relations of *Fraxinus excelsior* L. was investigated in this study, also giving consideration to the interactions of ozone and drought stress.

The following parameters - relevant for the plant's water relations - were investigated: firstly, stomatal conductance, which was measured on two-year- old potted saplings and on five/six year old field-grown plants; secondly, the three growth parameters (extension growth, and radial increments at the stembase and at the base of the new shoot growth) were assessed on the two-year-old saplings. These parameters were measured regularly throughout both years of the investigation.

Biomass and the morphology of annual rings were also investigated in one and two year old saplings after the destructive harvest of these plants. Further, the effect of ozone on the assimilate translocation pattern of one-year-old seedlings was investigated, using $^{14}CO_2$ as a tracer in a laboratory experiment.

With the investigation of the impact of ozone - and drought - on these parameters, which are responsible for water uptake, its transport, and final loss to the atmosphere, it was intended to cover the different aspects of the water relations of ash under the influence of these stressors.

2 Literature review

2.1 Ozone - protector and pollutant

Ozone is found in both layers of the lower atmosphere - troposphere and stratosphere - but in different concentrations and with different effects. While stratospheric ozone, making up about 90% of the total atmospheric ozone content, is working as a screen against UV-B, tropospheric ozone is a pollutant harmful to living organisms (Deutscher Bundestag, 1989).

2.1.1 Stratospheric ozone

In the stratosphere, ozone is generated photochemically from oxygen. The molecule is unstable, but under natural and undisturbed conditions its generation and decay are in equilibrium. The main factor that causes the destruction of the ozone layer is the release of chlorofluorocarbons (CFC). These compounds decay in the stratosphere due to photolysis and reactions with hydroxy radicals (OH) or activated oxygen generating ClO_x radicals. The ClO_x radicals then react with the ozone molecule in a catalytic process and decompose it to oxygen.

With this thinning of the ozone layer, the atmospheric sunglass effect decreases and the transmission of the "mutagen" ultraviolet radiation increases. Estimates are that the decrease of the ozone concentration by 1% increases UV radiation by 2% (Council on Environmental Quality and US Foreign Ministry, 1980).

This destruction of the ozone layer is found particularly above the Antarctic, where in the Antarctic spring more than 50% of the total stratospheric ozone, and more than 95% of the ozone at a height of 15-20 kilometres is destroyed (Deutscher Bundestag, 1989).

2.1.2 Occurrence of tropospheric ozone

The mean ozone mixing ratio in the troposphere during the Arctic summer is about one percent of that found at a height of 30 kilometres (Deutscher Bundestag, 1989). A small part of the ozone in the troposphere is transported there from the stratosphere by turbulent exchange, but most of it is generated in a photochemical process from nitrogen oxides (NO_x) and hydrocarbons (C_nH_m). In the latter case, peroxyradicals formed from oxidation of hydrocarbons lead to the oxidation of NO. The pollutants derive from combustion processes in power stations and motor vehicles.

Between O_3, NO_2 and NO a photo-stationary equilibrium

$$NO_2 + O_2 \rightleftharpoons NO + O_3$$

develops, caused by the photochemical generation of ozone by photolysis of NO_2, while simultaneously the NO molecule, which derives from this photolysis, destroys the O_3 molecule.

In urban areas, with a low C_nH_m/NO_x ratio, the ozone concentrations depend mainly on the NO_2/NO ratio and the level of irradiance. Thus the peak concentrations are almost invariably found during the afternoon and the minima occur during the early hours of the morning. In cities the night-time ozone concentrations decrease almost to zero, due to the proximity to the source of the pollution that provides not only the precursors, but also the destroyers of the ozone molecules.

Ozone pollution in rural areas, however, derives not only from transport of the precursors, but also of ozone itself. In addition, a much higher C_nH_m/NO_x ratio provides an additional pathway of NO_2 generation, involving the hydrocarbons. Thus the NO_2/NO ratio increases and the photo-stationary equilibrium rises to a higher level (Baumann & Baumbach, 1992). Due to the relative lack of pollutants in rural areas, the nighttime ozone concentrations do not decline as drastically as in the cities and the diurnal variations are smaller. Thus mean concentrations at rural sites tend to be higher than in urban areas.

Throughout the industrialized countries the annual mean ozone concentrations are rising slowly, but continuously. Since 1970 a mean annual increase of 0.5-1% (in polluted areas: 2%) was found (Deutscher Bundestag, 1988; Loppow & Schnabel, 1993) and an increase of 50% in emissions of the precursors, the nitrogen oxides, has been reported for the period between 1966-1978 (Ashmore *et al.*, 1985). During recent years NO_x concentrations have been broadly constant without a clear trend, but a change in the relative contributions from different source types was shown by Eggleston *et al.* (1992). Accordingly the percentage contribution of transport has increased from 60 to about 80%, while at the same time the contribution of commercial and industrial users declined. Emissions from soils, generated by microbial activity, are about 10% of those from combustion processes (Atkins, 1995).

Due to the significant variation of ozone concentrations in space and time, the general trend of an increase does not necessarily apply to all sites. Thus Bower *et al.* (1994) reported a downward trend in summer O_3 levels for Central London. Analysis suggested that the trend was related to a corresponding increase of NO_x in the area. In the UK (Bower *et al.*, 1994) as in Europe in general (Simpson, 1993) a clear north/south gradient of concentrations is apparent, with a continuous increase from the north of Sweden to the Alps, where ozone concentrations additionally increase with altitude. However, while in 1985 the means of daily maximum ozone concentrations for the months April to September did not exceed 70 ppb anywhere in Europe, in 1989 they did so in the area of the Alps. Values in the range of 60-70 ppb had been the highest mean concentrations in 1985, and they were then locally restricted to the Mediterranean, the Alps and the Carpathians. In 1989 such concentrations were measured in all Central Europe, as well as in parts of Eastern and Western Europe, including southeastern parts of Britain (Simpson, 1993).

2.2 General effects of ozone

Among the range of unwelcome consequences of increasing ozone pollution is its impact on human health. Here ozone mainly affects the respiratory tract, causing irritations and increasing the frequency of asthma attacks. Extreme pollution, as in Southern-California, can lead to a permanent impairment of lung function. Irritation of the eyes and headache are further symptoms frequently observed at ozone concentrations above 100ppb (Dekant & Vamvakas, 1994).

However, ozone pollution not only affects humanity, but also vegetation. Thus the pollutant is known to affect plant growth, physiological processes within plants and the competitiveness of some species, either directly or indirectly, by predisposing the plants to injury from other abiotic or biotic stresses (Chappelka & Freer-Smith, 1995). It has also been shown that ozone plays a role in some forms of forest decline, particularly in the United States, but also in Germany (McLaughlin, 1985; Ashmore *et al.*, 1985; Rehfuess, 1991). Among the most commonly described symptoms of forest decline, attributed to ozone injury, are chlorotic mottle and premature needle abscission. A dieback of branches is often observed. The species involved in these forms of forest decline are in general conifers, but adverse effects on deciduous trees were also described frequently. These include foliar chlorosis in *Betula pendula*, *Fraxinus excelsior*, *Liriodendron tulipifera* and other species (Ashmore *et al.*, 1985). Similarly Davis & Skelly (1992) detected a dark, adaxial stipple as a foliar symptom induced by their O_3 treatments. In their study they found *Prunus serotina*, *Liquidambar styraciflua*, *Liriodendron tulipifera* and *Fraxinus americana* to be sensitive species, while *Quercus rubra* and *Q. alba* did not develop foliar symptoms.

Forest decline, however, is not a continuous process, and while deterioration of forests occurs during hot dry summers, the decline may be checked or reversed in wet years (Chappelka & Freer-Smith, 1995).

2.3 Plant physiological response to ozone

The response of the stomata as the plant's entrance for gaseous substances is of major importance for all subsequent plant reactions. However, results of investigations into the impact of ozone on stomatal behaviour differ substantially and are often contradictory. Differences seem to be dependent on various factors, such as species, exposure regime or the water and nutrient status of the plants. At higher exposure concentrations

(> 200ppb) the majority of species react with stomatal closure (Darrall, 1989). However, for low pollutant concentrations the reported results cover a range of different responses.

These are:

(a) reduced stomatal conductance (Eamus & Murray, 1991 Pearson & Mansfield, 1993; Le Thierc *et al.*, 1994 - *Fagus sylvatica*; Rosen *et al.*, 1978 - *Vitis labrusca*; Reich *et al.*, 1985 - *Glycine max*)
and decreased transpiration (Keller & Häsler, 1984 - *Abies alba*; Skärby *et al.*, 1987 - *Pinus sylvestris*)

(b) increased stomatal conductance (Freer-Smith & Dobson, 1989 - *Picea sitchensis*, *P. abies*; Freer-Smith & Taylor, 1992 - *Populus deltoides* × *nigra*; Eamus *et al.*, 1990 - *Picea abies*)
or intensified transpiration (Keller & Häsler, 1984 - *Picea abies*).

All these results apply to well-watered plants. Under conditions of water stress the same plants often respond differently. Thus increased stomatal conductance under water stress conditions was found for *Fagus sylvatica* by Davidson *et al.* (1992) and by Pearson & Mansfield (1993) as well as by Le Thierc *et al.* (1994), and for *Picea abies* by Maier-Maercker & Koch (1992). Similarly, increased water use by ozone and drought-stressed *Picea abies* was reported by Van den Driessche & Langebartels (1994).

Stomatal response of the ozone-affected plants as compared to unpolluted plants not only changes with increasing soil moisture deficit, but also with differences in air humidity (Jensen & Roberts, 1986; Maier-Maercker & Koch, 1991). This differential response to air humidity and thus water vapour pressure deficit may cause changes in the diurnal variations of stomatal conductance, as found by Skärby *et al.* (1987). In their study on the effects of ozone on the physiology of *Pinus sylvestris* they found a reduction of transpiration during daytime, while the night-time conductance was increased. This could indicate a decrease in the range of conductance and of stomatal responsiveness, as was reported by Reich & Lassoie (1984), Keller & Häsler (1984) and Koch & Maier-Maercker (1992). Such diminished stomatal control is typical for ageing leaves (Reich, 1984).

With regard to longer-term trends caused by the effects of ozone, Olszyk *et al.* (1991) reported small but consistent changes of transpiration rates during the course of the season for "Valencia" orange trees in ambient air as compared to others in filtered air. Similarly for *Fraxinus excelsior*, Wiltshire *et al.* (1994) found a decrease of daily integrated stem flow values for their ozone treatment relative to the clean air treatment.

From studies which considered both stomatal conductance and photosynthesis, it can be shown that the described changes in stomatal behaviour can be accompanied by alterations of the photosynthetic capacity (Scott Clark *et al.*, 1995). However, these changes in photosynthesis are not necessarily concomitant with changes in stomatal behaviour, as can be seen from reports of altered water use efficiency (WUE). Decreases of WUE were reported by Freer-Smith & Dobson (1989), Reich & Lassoie (1984) and Wallin & Skärby (1992). On the other hand, Eamus & Murray (1991) found significantly reduced stomatal conductance in their ozone-treated beech trees, while the photosynthetic response was not influenced by the treatment. Beyers *et al.* (1992) reported no detectable effect of O_3 on stomatal conductance, but increased photosynthetic capacity in current year needles of *Pinus ponderosa*, which led to a photosynthetic compensation of ozone induced needle loss. Further, increased photosynthesis in the autumn and in spring prior to budburst were observed by Barnes & Davison (1988); Eamus *et al.* (1989) and Mikkelsen & Ro-Poulsen (1994). Such increased photosynthesis may lead to a certain degree of photosynthetic compensation, but it may also decrease the frost hardiness of the trees.

Although often no clear effect on photosynthesis is found, dark respiration of the plants may be affected, as was shown for *Pinus* species (Barnes, 1972; Yang *et al.*, 1983; Skärby *et al.*, 1987).

In the case of longer-term exposure, the described effects on stomatal behaviour and photosynthesis often result in alterations of growth and biomass accumulation. Growth response therefore reflects the cumulative and lasting response to the pollutant, which can alter the vitality of the plant. Like the effects on stomatal conductance and photosynthesis, those on growth vary widely and can either apply to whole plant growth or be restricted to certain growth parameters.

Many studies on whole plant growth have shown a reduction of total biomass accumulation (Cooley & Manning, 1987; Edwards *et al.*, 1994; Jensen, 1981; Matyssek *et al.*, 1993; Matyssek *et al.*, 1995; Pearson, 1995; Retzlaff *et al.*, 1992). In *Fagus sylvatica* Braun & Flückiger (1995) measured a 10% biomass reduction within three years and further correlated this change to a cumulative O_3 dose of 7 ppm h > 40 ppb. In contrast, Billen *et al.* (1990) found increases in dry matter production in ambient air, as compared to filtered air.

Where individual growth parameters were investigated, diameter increment of the shoots showed the most uniform tendencies among species, and was shown to decrease in plants which are sensitive to pollution stress (Edwards *et al.*, 1992; Matyssek *et al.*, 1993; McLaughlin *et al.*, 1982; Swank & Vose, 1990/91; Temple & Miller, 1994; Woodbury & Laurence, 1994). An increase in stem diameter as well as in height growth was reported for *Pinus halapensis* by Wellburn & Wellburn (1994). In contrast to diameter increment, extension or height growth response seems to be more variable among species. For this parameter, growth stimulations were found for *Liriodendron tulipifera* (Kress & Skelly, 1982), *Pinus taeda* (Edwards it et al., 1994), and *Picea rubens* (Thornton *et al.*, 1992), while Jensen (1973) reported significant growth reductions for *Platanus occidentalis*, *Acer saccharinum* and *Acer saccharum*. Similar results were obtained by Kress & Skelly (1982) for *Pinus taeda*. For many other species, no effects on extension growth were reported (Jensen, 1973; Kohut *et al.*, 1990; Kelly *et al.*, 1993; Roberts & Cannon, 1992; Taylor *et al.*, 1986).

Few workers have included root growth or below-ground biomass accumulation into their investigations, and due to the difficulties in the assessment of these parameters the information available in general derives from studies on potted seedlings. Reductions of root biomass in response to ozone stress were reported for *Fraxinus pennsylvanica* (Chappelka & Chevone, 1986), *Populus* × *euramericana* (Landolt *et al.*, 1994), *Quercus rubra* (Edwards *et al.*, 1994), and *Pinus sylvestris* (Skeffington & Roberts, 1985). Differential effects on root growth of beech, depending on the water status of the plants, were described by Davison *et al.* (1992). In their study they found root dry weight to decline with increasing ozone concentrations in well-watered plants, while the opposite response of an increase of root dry weight with increasing pollutant concentration was seen in plants that had experienced soil water deficit.

Corresponding to the well known decline of root biomass in ozone stressed plants, Qiu *et al.* (1992) found a decrease of root surface area in an ozone sensitive family of *Pinus taeda*, while in the insensitive family that they investigated no such effect could be detected. The growth reductions, however, do not necessarily affect the differential root components in the same manner. Temple *et al.* (1993), although finding a reduction of coarse roots, reported an increase in fine roots for their well-watered *Pinus ponderosa*, while Braun & Flückiger (1995) found significant reductions in fine root biomass in response to ozone. In addition, the stress can alter root structure; this was shown for *Fagus sylvatica*, where root biomass was reduced in ambient air, but simultaneously root length was found to be increased (Taylor *et al.*, 1989).

According to the review of Lechowicz (1987), the reduction of root biomass is generally more pronounced than the decrease in shoot biomass. Mean biomass reductions during pre-reproductive growth of 34% and 23% were given for roots and shoots respectively. These values indicate changes in assimilate partitioning which, according to Cooley & Manning (1987), affect storage organs most severely. In particular, species with high relative growth rates readily change their allocation pattern in response to stress (Laurence *et al.*, 1994), while the partitioning in slow-growing species appears to be unaffected.

These changes in biomass allocation can be seen as the predominant way in which plants compensate for stress (Pell *et al.*, 1994). In the case of ozone, stress compensation for oxidative damage to the leaves determines the shift in carbon allocation. This damage, and a widespread tendency to early senescence and leaf abscission (Matyssek *et al.* 1993; Qiu *et al.*, 1992; Shertz *et al.*, 1980; Temple & Miller, 1994; Wiltshire *et al.*, 1993), are possibly tied to more rapid initiation of new foliage (Pell *et al.*, 1994). The younger leaves then can show elevated photosynthetic activity (Beyers *et al.*, 1992, Greitner *et al.*,1994; Pell *et al.*, 1994) as a mechanism of photosynthetic compensation. Beyers *et al.* (1992) also measured higher tissue nitrogen concentration in current year foliage as a response to the loss of older needles and assumed increased inorganic phosphate cycling.

According to Laurence *et al.* (1994), increased senescence must be primarily expected in species with high stomatal conductance, although the longer lifespan of evergreen foliage also effects a high cumulative uptake of the pollutant, which often leads to accelerated senescence in older age classes of the foliage. The earlier senescence of leaves can be preceded by visible damage to the green tissue, in the form of chlorotic mottle, or even of necrotic lesions (Polle *et al.*, 1993; Temple & Miller, 1994), although in most studies with low exposure concentrations no such visible damage preceded the premature senescence and abscission (McLaughlin *et al.*, 1982; Matyssek *et al.*, 1993; Swank & Vose, 1990/91).

An adaption of plants to ozone stress has been observed by Walmsley *et al.* (1980). It showed in the slower senescence of late-developing radish leaves, as compared to that of cotyledons or first leaves. Similarly, Günthardt-Georg *et al.* (1993) found an adaption in the leaves of *Betula pendula* that had expanded during low ozone treatment. In their experiments the stomatal density of leaves increased with pretreatment ozone dose, but guard cell size decreased. Furthermore, when exposed to ozone fumigations, these leaves were abscised later as compared to those that had expanded in filtered air. In contrast to birch, poplar showed decreasing stomatal density in response to ozone (Matyssek *et al.*, 1993a) and correspondingly Dean (1972) found much lower stomatal densities in those tobacco varieties which were resistant to ozone induced weather fleck, as compared to susceptible varieties.

However, partitioning of biomass to foliage for repair is not the sole reason for the reduced gain of root biomass in ozone stressed plants. Inhibition of sieve-tube loading, as was postulated for plants under SO_2 stress (Noyes, 1980; Teh & Swanson, 1982), is understood to be a further cause for the altered allocation pattern. Correspondingly Matyssek *et al.* (1993) reported starch accumulations along the leaf veins of ozone fumigated birch plants and McCool & Menge (1983) and Smeulders *et al.* (1995) observed increased retention of ^{14}C in the foliage of the species they investigated. For *Pinus taeda* also, reductions in phloem transport to the roots were reported, although in this case photosynthate accumulated in the shoots (Spence *et al.*, 1990). Highly contrasting effects were reported by Rantanen *et al.* (1994), who found stimulated mycorrhiza development and a decreased shoot-to-root ratio as early effects of low level ozone exposure. Since they also observed increased height growth without a simultaneous increase in stem biomass, they suggested that radial growth of the stems might have been retarded by preferential translocation of carbon to the roots.

For poplar, such reductions in radial width were described by Landolt *et al.* (1994), combined with a lower amount of starch granules in the bark tissue. They also detected an increase in callose formation on the phloem sieve plates of the ozone treated material. Such callose formation narrows or even blocks pores in the sieve plates and thus impedes phloem transport. This could lead to a decreased carbohydrate content of roots and shoots, as was reported for *Picea rubra* (Amundson *et al.*, 1991). Since these tissues are relevant as sites of carbohydrate storage, and contain reserves which will be used for the following season's growth, an impact of ozone on plant growth may occur not only during the period of exposure to high ozone concentrations, but also as a carry-over effect on the following year's performance. Such an effect on the growth of beech saplings was described by Pearson & Mansfield (1994).

Besides these direct effects of ozone on plant growth, ozone and air pollutants in general are discussed as factors predisposing plants to injury from other abiotic and biotic stresses. Chappelka & Freer-Smith (1995) reviewed this aspect, particularly with regard to trees and to the dominant factors limiting tree growth and distribution: soil moisture deficit and low temperature stress. For the interaction of pollutant and drought stress, they concluded that air pollutants cause disruptions to metabolic responses to water deficits and thus the two stressors occurring together can result in serious effects on tree condition.

2.4 Characterization of ash - and its expected response to ozone

Within the group of broadleafed trees, *Fraxinus excelsior* is among those species with the highest stomatal conductances in Europe (Körner *et al.*, 1979).

According to Wieser & Havranek (1993), the response of plants to air pollution is a function of the pollutant that enters the tissue rather than of the external, ambient pollutant concentrations. Pollutant uptake is mainly determined by stomatal conductance (Heath, 1980) and it has been shown that, in general, those species with higher stomatal conductances were likely to be less tolerant to ozone (Darrall, 1989). This is due to their uptake of a higher effective dose. Accordingly, crop species, which usually exhibit a higher stomatal conductance and higher inherent rates of net photosynthesis than broadleafed tree species or even conifers, are affected by the pollutant at a lower unit dose (Reich & Amundson, 1985). Nevertheless, in the longer term, damage to trees cannot be expected to be less than to crops, since trees, due to their perennial nature are exposed to pollutants for a longer time.

For ash, results from an experiment using ambient and filtered air, conducted at a rural site in southeastern England, confirmed the expectation that the plant may be sensitive to ozone stress, since there was a significant increase in foliar chlorosis for the plants that received ambient air (Ashmore *et al.*,1985).

Corresponding to its high transpiration rates, ash is naturally found in rather moist localities, both as regards soil and atmosphere (Hegi, 1975). In general, the distribution of the tree includes the whole of Europe and the Caucasus, and it belongs to the few species that were indigenous to Britain in earliest historical times (Nisbet, 1893). Pure *Fraxinus excelsior* woods however are very rare and mainly occur in Britain, particularly in Derbyshire (Hegi, 1975).

Besides its preference for a high soil moisture content, the species also requires fertile soils and good aeration of the soil to grow well. However, it also can be adapted to dry limestone soils and it has been suggested that there might be differing physiological races (Wardle, 1962). Plants growing on the different soil types also show differences in their stomatal behaviour. This has been described by two French studies on ash trees growing in a floodplain of the Haut-Rhône and a tree in a mesoxerophytic mountain stand of the north-western Alps. The trees growing in the floodplain exhibited high values of stomatal conductance throughout most of the day and variations in conductance were closely related to changes in light intensities (Besnard & Carlier, 1990). For mountain ash, generally lower stomatal conductances were found, even under conditions of good water supply. Further, the stomata partially closed at noon or even earlier (Carlier *et al.*, 1992). However, the survival of the plants during drought is attributed to tolerance, rather than avoidance - the tolerance mechanism being osmotic adjustment (Peltier *et al.*, 1994).

Due to its high stomatal conductance, which, apart from causing the comparatively high transpiration rate, also leads to a similarly high CO_2-uptake, ash belongs to one of the fastest growing tree species and is only occasionally beaten by *Acer pseudoplatanus* or *Acer platanoides* (Braun, 1977). While the mature trees are very light-demanding, the seedlings and juveniles are comparatively shade-tolerant and can survive for many years below a closed canopy, then only producing a minimal height increment. The juveniles respond to the occurrence of tree-fall gaps with a substantial increase of growth, an ability that remains unreduced for several years in slow-growing ash juveniles (Tapper, 1993). However, unlike its ability to tolerate a shading canopy of deciduous trees, the plant cannot persist in competition with a herbaceous field layer.

In recent years, ash trees in England experienced a dieback seemingly associated with dry summers (Hull & Gibbs, 1991). The prevailing symptoms were early loss of leaves and a dieback of branches. Similar diebacks in many habitats in Derbyshire have been reported by Wardle (1962) for the severe drought in the summer of 1959. There were also reports of early abscission of leaves and damage to branches at a site near Teltow (Germany) in the hot summer of 1911, when the water table dropped to a very low level (Hegi, 1975). These reports demonstrate the susceptibility of *Fraxinus excelsior* to drought stress.

However, since dry summer weather often coincides with increased ozone concentrations, an interaction of ozone and drought stress might occur and the pollutant could be an additional factor contributing to the damage. In this context, ozone, with its known effects on stomatal behaviour, might affect the plant's ability to optimize its water relations in the face of drought.

3 Materials and Methods

3.1 Plant material and growth conditions

The *Fraxinus excelsior* L. plants for both field seasons, 1993 and 1994, were obtained from Oakover Nurseries, Ashford, Kent, where they had been grown from seeds derived from a single seed source. Three different age groups were used for the experiments. These were field-grown trees, which were five years old at the start of the first field season, two-year- old potted saplings and one-year-old pot-grown seedlings.

3.1.1 Field grown trees

36 saplings had been transplanted directly into the soil in March 1991, when they were three years old. The soil was a stony, sandy loam, overlying Keuper marl at a depth of about 1m (Wiltshire *et al.*, 1994). These trees were rainfed and did not receive any fertilizer applications. However, competition from herbaceous species was kept low, by manual weeding of the plots.

3.1.2 Two-year-old saplings

In April 1993 and 1994, 96 two-year-old bare-rooted saplings were obtained from the nursery. The plants for the first season were potted on 20 April 1993, and those for the second season on 26 April 1994.

In the first season, 18.5 l pots were used to provide sufficient rooting volume for the plants. The pots were filled with a mixture of sieved field-site soil (mesh size: 10mm) and fine sand (4:1). This substrate had been chosen to facilitate the extraction of roots from the soil, when harvested at the end of the season. However, since the root systems of the bare-rooted saplings were very variable at the date of potting, due to damage when being dug up, root biomass was not assessed. Six of the pots had soil psychrometers inserted, four in each pot, at the following depths: 0.10, 0.15, 0.20 and 0.25 m. The soil water potential was monitored with a Microvoltmeter (Wescor Inc., Utah, USA).

The plants were then randomly distributed to the twelve plots of the field-site (see section 3.2.1 and figure 2). There were eight plants per plot. One pot containing soil psychrometers was placed into two of the plots of each pollution treatment; the plot numbers were 1,2,4,8,9 and 11.

To insulate the soil against overheating and highly varying temperatures, the black pots were wrapped into a loose fit double layer of bubble polythene and a covering layer of black and white polythene with the white side exposed. The pots were placed on inverted gardening saucers, which themselves were situated in other larger saucers. This was both to protect the plants in pots from getting waterlogged, and to shield the purely rainfed field grown trees from receiving excess water from the watering of the pots.

On 2 August 1993, 2 l of commercial fertilizer (NPK = 10:10:27; Phostrogen, Phostrogen Ltd., Corwen, Clwyd, Wales, U.K.) were applied to all plants (fig. 1a).

Until 12 July 1993 all plants received the same watering regime. After that date half of them were subjected to droughting conditions (fig. 1a). These plants - randomly selected - were sheltered with a flexible square plastic cover. The cover with a hole in the middle and slot on one side to fit round the stems was fixed to the pots with a string. At the location where the cover was fitted, the tree stems were wrapped with bubble polythene and black and white polythene to protect them from possible injuries on windy days. To ensure ventilation of the soil, wooden sticks were pushed into the soil at the sides of the pots, and thus the cover was fixed to a height of about 10 mm above the pots. To reduce the light reflection of the white plastic, and to make sure there was no transmittance of light through the plastic sheeting, the covers were painted green. The covered plants were then subjected to two drought cycles of two to three weeks, while the others were watered regularly as a control. This treatment was

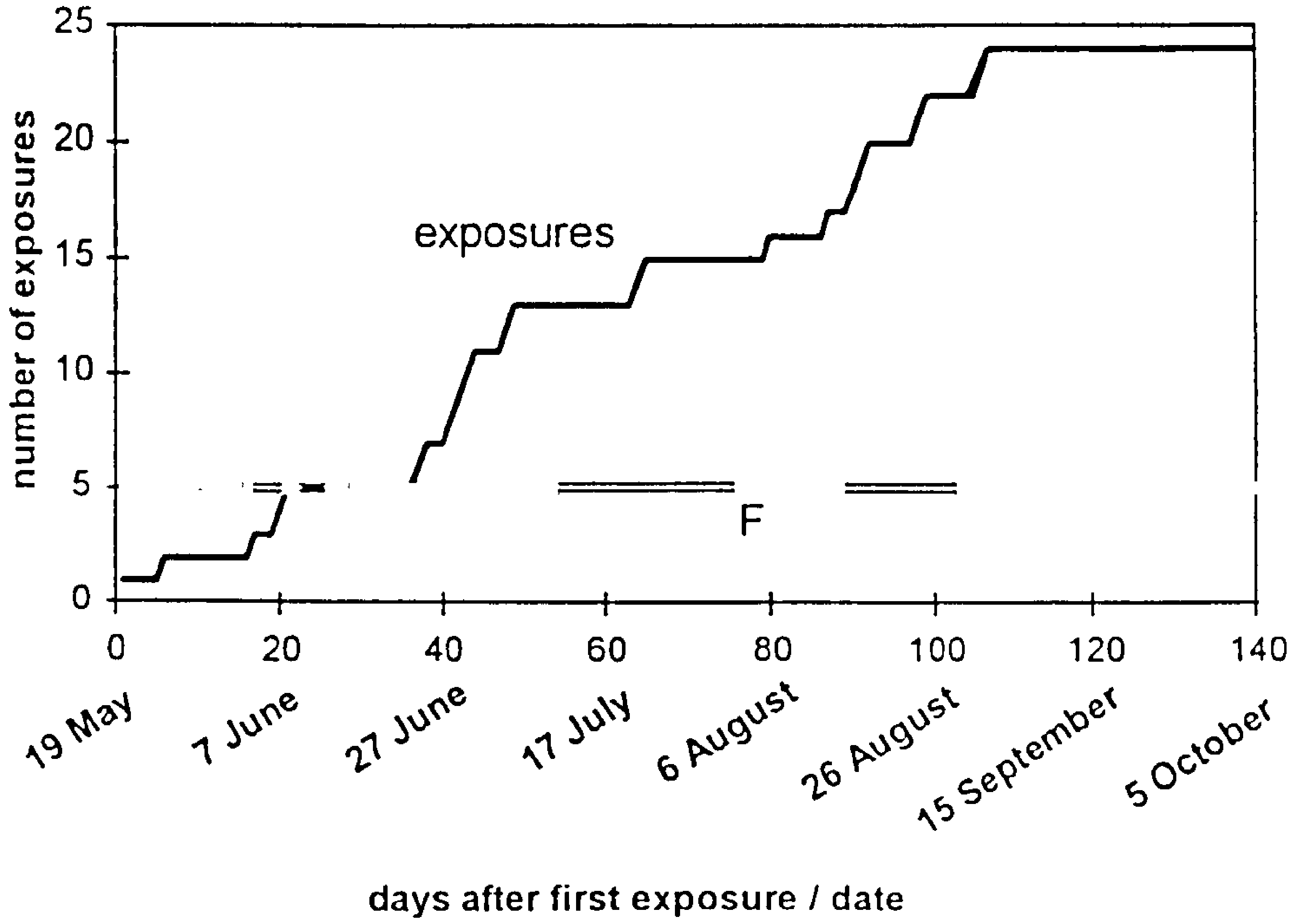

Fig 1a Exposure regime, drought treatment and fertilizer application 1993

—— exposures

watering of all plants

═══ irrigation of well watered treatment only

F: fertilizer application

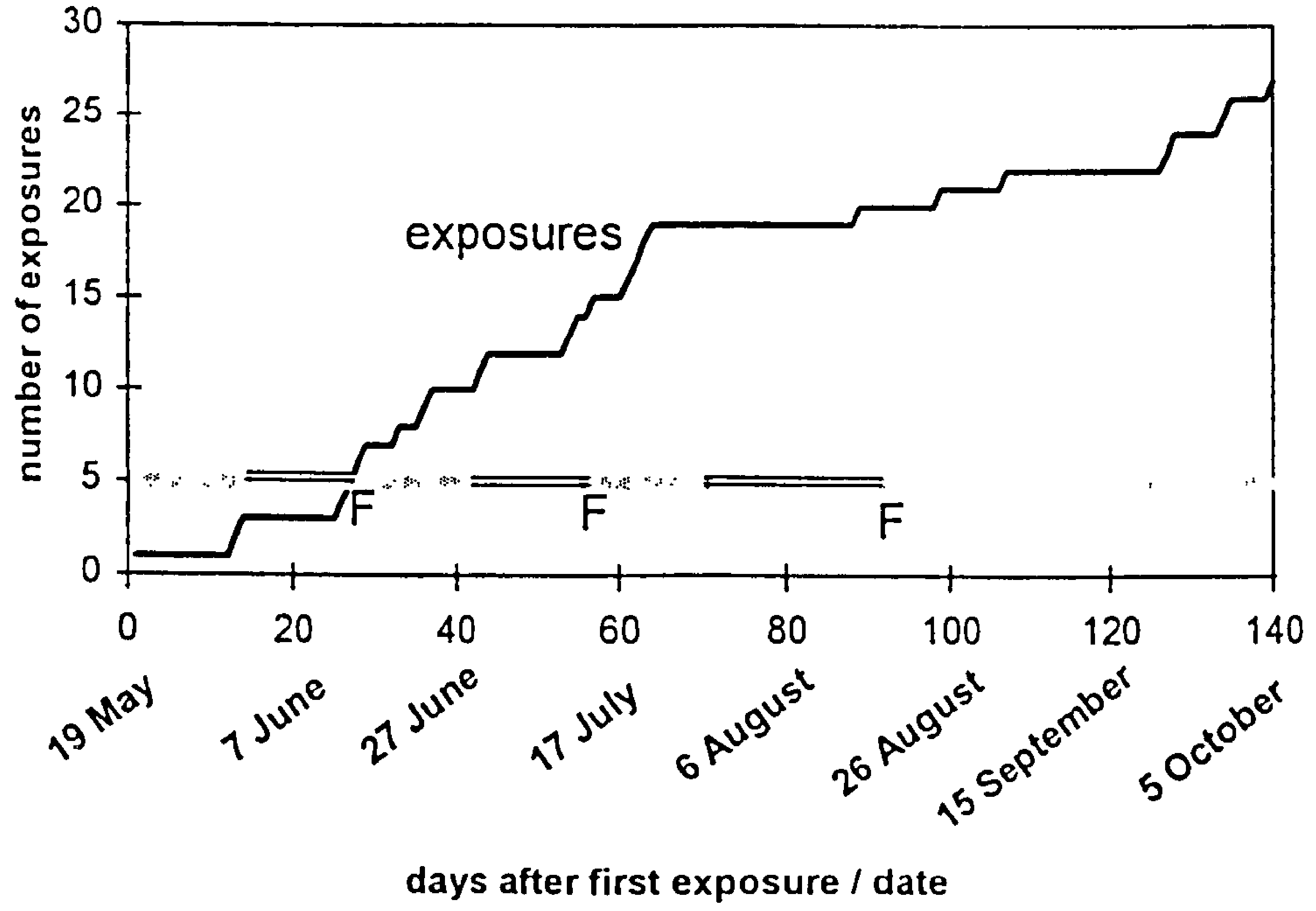

Fig 1b Exposure regime, drought treatment and fertilizer application 1994.

maintained until the plants were harvested on 13 September.

For the 1994 season, 12 l pots were used. This choice was made to facilitate the moving of the pots within the chambers, when other experiments were conducted there. Furthermore, the experience with the plant material in the first season had shown that smaller pots would be sufficient to accomodate the root systems of the plants. Since the bare-rooted saplings used for the 1993 season had proven not to be suitable for the determination of root biomass, the pots were filled with sieved soil only.

As a further modification to the first year's experimental design, the drought cycles were started earlier in the season. Altogether there were three drought cycles of 7-14 days (fig. 1b). During the second field season the pots were not covered by plastic sheeting, and therefore the droughting proceeded more rapidly and more effectively, than it had during the first season.

Due to the smaller pots that were used for the second field season, three fertilizer applications had to be given, to ensure a good nutritional status of the plants (fig. 1b). The respective application dates were 15 June, 14 July and 18 August. On all three dates two litres of fertilizer solution were given to each of the pots.

3.1.3 One-year-old seedlings

On 9 June 1993 more than 600 seedlings - then about five weeks old - were obtained from the nursery. The plants were potted up in Sutton Bonington on the same day. A mixture of soil and sand (4:1, diameter of the pots: 90 mm) was used. The pots were kept in the open. If necessary, they received additional watering. The pots were weeded by hand regularly to prevent competition for nutrients. During the first week of August the plants were transferred to bigger pots (Ø: 130mm) with an identical soil mixture.

(a) In autumn 1993, 100 plants, which were to be used for the assimilate translocation experiment, were pretreated by a short day and low temperature regime to induce early dormancy and to stimulate early budburst for the experiment. To achieve this, the plants were transferred to a growth cabinet by mid-October. The temperature of the growth cabinet was controlled to 3 ^{0}C and the plants received light for nine hours per day. According to Heide (1993), dormancy of woody plants is mainly induced by the decreasing daylength, whereas low temperatures are mainly required for the dormancy release.

On 19 December 1993 - during dormancy - these plants were removed from the pots with their complete root system. They were put into moist plastic bags and transferred to the Universität Freiburg, Germany. There the roots were washed and the plants were transferred to solution culture, based on a modified Hoagland & Arnon (1938) solution (details are given in appendix A). The plants were put into 2.5 l pots filled with nutrient solution. Each of the pots accomodated three plants. They were then grown at 20 ^{0}C (day) and 12 ^{0}C (night) temperature in a greenhouse. The plants received daylight, and additional light from two mercury-vapour lamps. In this way the photoperiod could be extended to twelve hours. The lighting was indirect, since the lamps were arranged laterally at a distance of about 1.5 m. Air humidity was controlled to 60 % R.H.

The concentration of the solution used to cultivate the plants varied throughout the experiment. During the first three to four weeks, until the swelling of the apical bud, a Hoagland solution of $\frac{1}{8}$ strength was used. When the apical buds were swollen the strength was increased to $\frac{1}{6}$. From then until budburst the strength was gradually increased to $\frac{1}{2}$ of Hoagland solution. The plants grew rapidly. According to Jarvis & Jarvis (1964), positive effects of solution culture on growth have been found for all woody species investigated. The time of budburst varied by 18 days, the earliest date being 3 February 1994.

On 14 February 1994, 60 plants that showed at least one pair of fully developed leaves were chosen for the experiment. They were randomly distributed to three plastic boxes (12 l) filled with $\frac{1}{2}$ strength Hoagland solution, each box accommodating 20 plants. At the height of their hypocotyl, they were fitted with a collar of "mosy" (Hartschaumwerk Waldmann, Theddinghausen, Germany). These collars were inserted into the punched holes of the polystyrol sheeting that was used to carry the plants on the surface of the culture solution. During the following fumigation and tracer experiment, the plants received new culture solution every second day to ensure good nutrient availability.

(b) The remaining plants, which were not to be used for the assimilate translocation experiment, were kept outside during the winter. They were transferred to the field site on 12 May 1994. There the plants were randomly distributed to the plots, 24 plants per plot. They were placed on trays, each tray accommodating 8 pots. All plants were watered regularly and fertilizer applications were given on the same dates as for the two-year-old plants (cf. fig. 1b). During the 1994 season, these plants were harvested in three batches, on 29 June - 2 July, 4 - 7 August and 26 - 29 September.

3.2 Experimental facilities for the ozone treatment

3.2.1 Field site

The field experiments were conducted on an experimental site at Froghole Farm, The University of Nottingham, Sutton Bonington Campus, UK (latitude 52^0 50' N). The plants were treated with air of different qualities during the field seasons 1993/94. The plants were subjected to three different treatments, which were replicated four times (a field plan is shown in figure 2). These were: charcoal-filtered air with 150ppb ozone added ($CF+O_3$), ambient air (AA), and charcoal-filtered air (CF). To apply the $CF+O_3$ and CF treatments, open-top chambers were used, while the ambient treatment was "chamberless". The open-top chambers were 3.8 m high and 3.5 m in diameter (cf. Wiltshire *et al.*, 1992). The sides of the chambers could be covered or uncovered rapidly, allowing trees to be uncovered when treatments were not being applied.

3.2.2 Laboratory experiment

The ozone treatment for the plants used in the carbon allocation experiment was conducted at the Gesellschaft für Strahlenforschung (GSF) in Munich, working group

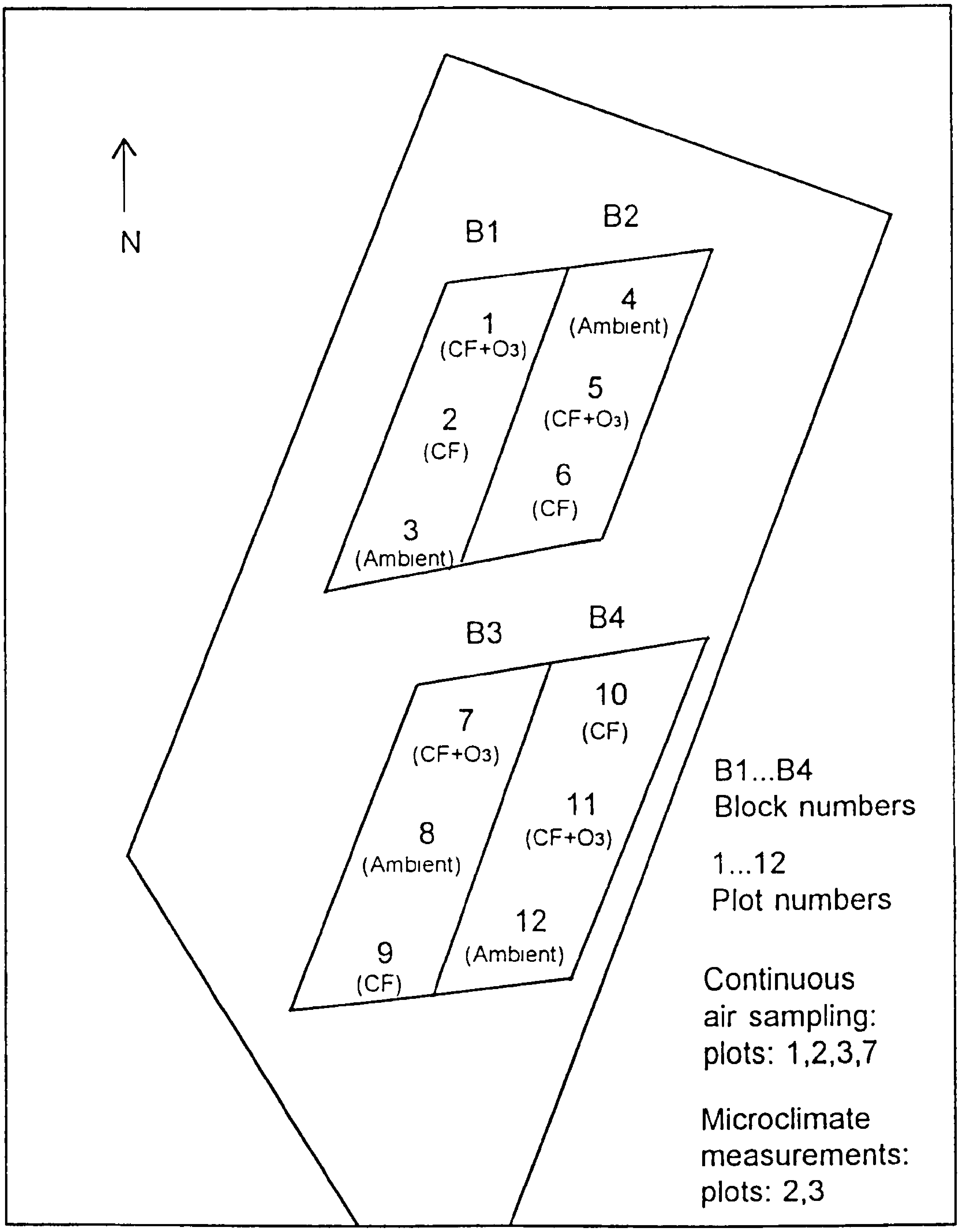

Fig. 2 Field plan, showing the four blocks on the experimental field site. Each block consisted of three plots

Dr. C. Langebartels. The plants were subjected to three different levels of ozone (150ppb, 75ppb and 0ppb) for twelve consecutive days. Three Perspex chambers (1.08 × 0.71 × 0.95m), situated in a walk-in growth cabinet ($10m^2$), were used as exposure chambers. Chamber conditions were similar to those of the pre-cultivation period in the greenhouse with 20/12 ± 0.5 ^{0}C day/night temperatures and 60 ± 5% RH. The photoperiod (light intensity: $100\mu E/m^2 \cdot s$) was twelve hours, with half an hour of "twilight" ($50\mu E/m^2 \cdot s$) preceding and following this period. The subchambers drew conditioned air from the walk-in cabinet and used a vertical, single-pass air circulation (air exchange 2.5 volumes per minute) (cf. Langebartels *et al.*, 1991).

When plants were not subjected to ozone treatment, they received filtered air. The filter system consisted of five units; particle, activated charcoal, Purafil II ($KMnO_4$-coated alumina granules), charcoal and particle. For the pollutant treatments, ozone, generated by electrical discharge in dry oxygen (500 M, Fischer, Köln, Germany), was added to the filtered air. Ozone concentrations were measured at the plant level with a UV O_3 analyzer (Model CSI 3100, Messer-Griesheim, München, Germany) every five minutes.

3.3 Application of ozone and drought treatments

3.3.1 Field site

The open-top chambers, in which plants were exposed to the treatments at the field site, were operated only on exposure days and on days with the risk of high ambient ozone concentrations ($\geq$ 50ppb). This was to protect the plants of the CF treatment from exposure to high ozone. To ensure that all chambered plots were subject to the same microclimate, the operation schedule was always applied to all open-top chambers. Whenever the chambers were not operated, the sides were uncovered and plants in all plots were exposed to ambient air, so that the cumulative thermal effects of the chambers were decreased (Wiltshire *et al.*, 1994), and simultaneously the plants received unattenuated radiation.

Which days were selected for O_3 exposure depended on the ambient conditions. The days chosen were characterized by high irradiation, low windspeeds and above-average temperatures. The plants were subjected to the ozone treatments for 8h/d (10-18h GMT), for 1-4 days in succession. The number of exposure days in 1993 amounted to 24, with 13 exposure days before the start of the drought treatment (fig. 1a). In 1994 there were 27 exposures, but only 22 of the exposure dates were before the final harvest of the one and two year old plants; since the drought cycles were started early in the season, the first drought cycle was preceeded by only 3 exposure days (fig. 1b).

For the drought treatment, four of the eight potted two-year-old plants per plot were selected randomly. The well-watered plants were irrigated regularly throughout the season; water was applied on evenings when the soil surface appeared dry and the pots were watered until water was observed running from the drainage holes.

The droughted plants were subjected to two drought episodes of 14-21 days in 1993 (fig. 1a), while in 1994 there were three drought episodes of 7-14 days (fig. 1b). After each drought episode, the droughted plants were irrigated regularly for two weeks, to allow recovery from the drought stress.

Thus the experimental treatments were:

Exposed to ozone, well-watered: CF+O_3(w)
Exposed to ozone, droughted: CF+O_3(d)
Protected from ozone, well-watered: CF(w)
Protected from ozone, droughted: CF(d)
Ambient, well-watered: Ambient(w)
Ambient, droughted: Ambient(d)

3.3.2 Laboratory experiment

In the laboratory experiment, the pollutant treatment was imposed for eight hours every day, except for the twelfth day, when it was applied for only four hours. At all other times the plants received filtered air.

On the last treatment day after the fumigation was terminated, the plants were left in the chambers until no trace of ozone remained. Then the seedlings were removed from the chambers and transported back to Freiburg, where they received the same greenhouse conditions as before the ozone exposures, until the harvest for the determination of ^{14}C allocation.

3.4 Measurements

3.4.1 Stomatal conductance

Stomatal conductance was measured with a Porometer (AP4, Delta T Devices, Cambridge, UK). During 1993, routine measurements were taken every five to ten days depending on weather conditions. Due to the results of the 1993 season, the measurement schedule for 1994 was changed. Thus in 1994 measurements concentrated on the exposure periods, and on days preceding or following the exposures. Stomatal conductance was investigated both in the two-year-old potted saplings and in the five/six year old field-grown trees. On the young plants, four fully developed leaves were chosen randomly for porometry each time, whereas on the mature trees 15 leaves per plant were investigated. These 15 leaves were located on five different branches, which were chosen at the beginning of the season, labeled and kept as measurement branches throughout the season. The branches had a westerly-northwesterly orientation. On days with no exposure, measurements were mainly taken during the morning between 6.00-10.00h GMT, while on exposure days assessments were made between 11.00-14.00h GMT. The measurements were taken in the order of the plot numbers 1-12.

Furthermore, diurnal changes of stomatal conductance were investigated in the two-year-old plants, on six days in 1993. In these cases, measurements were taken three to five times per day in one selected plot.

3.4.2 Growth parameters

In 1993 the three growth parameters, extension growth, stem diameter increment at the stembase and at the base of the new shoot growth, were assessed for the two-year-old plants every second week between 25 May and 13 September. Growth assessments in 1994 were made at similar two-week intervals between 4 May and 14 September.

For the measurement of extension growth a tape measure was used and assessments of stem diameter were made using calipers. Measurements were always made from the same marked positions on the stems.

3.4.3 Leaf area

Leaves were counted at intervals of one week. After the harvests of the plants on 13 September 1993 and on 4 October 1994, leaf area was determined for all leaves of every individual plant. Measurements were taken with a DeltaT-Areameter. Division of the assessed leaf area by total leaf number at harvest produced a standard leaf area for every individual plant, as leaf size was highly variable between plants. Multiplication of the weekly leaf numbers by the standard leaf areas led to an estimation of the leaf area development over the season.

3.4.4 Dry weight

At the final harvest of the two-year-old saplings, as well as at the three harvests of the one-year-old seedlings, dry weight of leaves, stems and branches was assessed for every plant individually. For stems and branches, the parts grown during the current growing season ("new shoot") were distinguished from the older parts ("old shoot").

The leaves were dried in an oven at 80^{0}C for two days, whereas the woody parts were dried for a whole week at the same temperature. The dried material was weighed after cooling down to room temperature.

3.4.5 Morphological analysis of annual rings

3.4.5.1 Preparation of stem sections for light microscopy

From all harvested plants from the chambered treatments, stem sections of about 3 mm thickness were taken on 14/15 September 1993 and on 5/6 September 1994. On the second and third harvests of the one-year-old seedlings in 1994, samples were also taken from the bases of the stems of these plants. The sections were fixed in glutaraldehyde solution (3%) in 0.02 M sodium-phosphate buffer (pH 7.0) for one day and at 4 ^{0}C. Then they were rinsed with buffer solution 3-4 times for one hour. Afterwards the samples were dehydrated in an alcohol series (25-100%) for two days. Where air had entered the tissue, samples had to be evacuated, since the air would have hindered fixation and particularly the subsequent impregnation.

After dehydration, the sections were impregnated with resin for one week at 4 ^{0}C, and cured at 60 ^{0}C for 24 hours. Sections of 3-5 μm thickness were cut with a microtome (Ultratome II, Upsala, Sweden). For the staining toluidin blue was used.

3.4.5.2 Assessment of ringwidth

Ringwidth was determined at a magnification of 50 with a binocular microscope (Wild, Heerbrugg, Switzerland). The width of the latest annual ring was measured at ten locations for three sections from each plant.

3.4.5.3 Assessment of cell number

The number of cells per ringwidth was determined by counting the cells in the cell-lines orientated with the pith rays. Eight cell-lines were counted per section.

3.4.5.4 Assessment of cell size

At a magnification of 500, two pictures from every section were taken with a Leitz Laborlux S microscope (Ernst Leitz, Wetzlar, Germany). The prints were overlaid with a plastic foil. 10 × 10 cells per photo - randomly selected within the vessels - were encircled on the plastic foil and blackened. The area of these cells was measured with an areameter.

3.4.6 Determination of photosynthate translocation

3.4.6.1 $^{14}C0_2$-labeling

One-year-old seedlings, which had been pretreated with ozone in the exposure chambers at the GSF in February 1994, were used for an experiment to determine ozone influence on carbon partitioning in ash seedlings. Pulse-labeling was conducted at the open-air facility of the Botanical Institute of the Universität Freiburg at 44, 116 and 188 hours after the ozone treatment. For every labeling, six plants were randomly chosen from each of the three pollution treatments (0, 75, 150 ppb).

16 hours before labeling, the plants were transferred individually to 250ml PE-bottles with $\frac{1}{2}$-strength Hoagland solution. The bottles were wrapped in aluminium foil in order to protect the roots from receiving light. Three hours before pulse-labeling, the plants were taken to the open-air laboratory. After acclimatisation, the plants were put into pots in threes. Every pot contained one plant from each of the three treatments. A strip of Terostat sealant (Teroson, Heidelberg, FRG) was taped round the rim of the pots and a translucent, flexible polythene bag (16 l) was fixed to this Terostat strip. Before the bag was closed, a small sealed 'zip-lok' bag was placed between the plants. The 'zip-lok' bag contained 1ml of a ^{14}C-$NaCO_3$ solution (specific activity 1μCi/ml) and 1ml of 10% H_2SO_4, which had been injected with a syringe beforehand. After the polythene bag was closed tightly, the 'zip-lok' bag could be opened through the flexible bag allowing the generated $^{14}CO_2$ to escape. The complete above-ground biomass was exposed to the radioactive pulse.

After one hour of labeling, 10% KOH was injected into the zip-lok bags to absorb excess $^{14}CO_2$. Five minutes later the 'zip-lok' bag was sealed, and the polythene bag was removed from the pots.

3.4.6.2 Assessment of labeled photosynthate

Following a 4-hour chase period the plants were removed from the PE-bottles and divided into leaves, stems and roots. For every single plant the fresh weight of the individual organs was determined; the samples were then immediately frozen and homogenized in liquid nitrogen. An aliquot of 300±5mg was extracted from each sample and transferred to a vial. 4.5 ml of a scintillation cocktail (Rotiscint Eco plus, Fisons, Loughborough, U.K.) were added. The samples were counted on a liquid scintillation counter (model LS 1122, Beckman Inst. Inc., Fullerton, California, USA).

Since the pigmentation of the suspended organs and therefore quenching was highly variable, a quench-correction was required. The corrected counts in each fraction were summed for every plant and a percentage of counts for those fractions was calculated. The relative distribution of ^{14}C in the organs represents the translocation of assimilate.

3.5 Determination of soil water status

For the pots containing the two-year-old plants, which were partly subjected to drought stress, soil water status was determined by gravimetrically measuring the soil water content. Since it was impossible to weigh the pots in the field, small soil cores were withdrawn from the pots with a brass pipe (Ø: 8 mm), long enough to be driven to the bottom of the pots. Thus the soil cores provided a good integration of the soil moisture content throughout the pot.

Individual samples were immediately sealed into a zip-lok plastic bag and the fresh soil was weighed in the laboratory directly after sampling. The soil was dried in an oven at 105 ^0C for three days. When dry, the samples were weighed again and soil water contents were calculated for each sample.

In addition to this gravimetric method, it was intended to use a potentiometric determination of the soil water to assess the soil water status of the pots. For this reason soil psychrometers were inserted into the pots in 1993, as was described in chapter 3.1.2. However, of the psychrometers available, most failed early in the course of the measurements.

3.6 Statistical analysis

Analysis of variance was performed on the obtained data using the statistical package GENSTAT 5. Data were blocked, with the location of the experimental site as the blocking criterion.

Split plot analysis of variance was performed on data sets deriving from measurements on the two-year-old plants, which were subjected to the pollution treatment as well as to drought stress. For all other data, simple analysis of variance was used.

4 Climatic conditions, pollution load and soil water status

4.1 Local climate

The experimental site at Froghole Farm, Sutton Bonington Campus, is in the central region of the East Midlands, and the climate already displays traits of continentality, which are found to show an eastwards increase across the low ground (Dury, 1963). This particularly derives from an eastward decrease in precipitation and an increase in the same direction of the fraction falling in the summer months. The long-term average (1916-1994) of annual precipitation measured at the meteorological site at Sutton Bonington Campus is 606 mm, while values across the East Midlands range from about 50 inches (1279 mm) in the area of Buxton in the Pennines to below 22.5 inches (571.5 mm) at the eastern border of the region (Dury, 1963).

4.2 Microclimatic conditions at the field site during the two growing seasons, 1993/4

Microclimatic and pollution data were measured at the experimental site, within open-top chambers and in ambient plots, throughout the two growing seasons. The data were recorded as hourly means. From these data, monthly means were calculated for the months May - October.

4.2.1 Temperature

In 1993, the course of the mean temperatures was very uniform and moderate throughout the months June to August, ranging between 14 and 16 ^{0}C (fig. 3). Except in June, when they amounted to 0.6 ^{0}C, the differences between the chambered and the unchambered ambient plots were not very pronounced. In 1994, the start of the growing

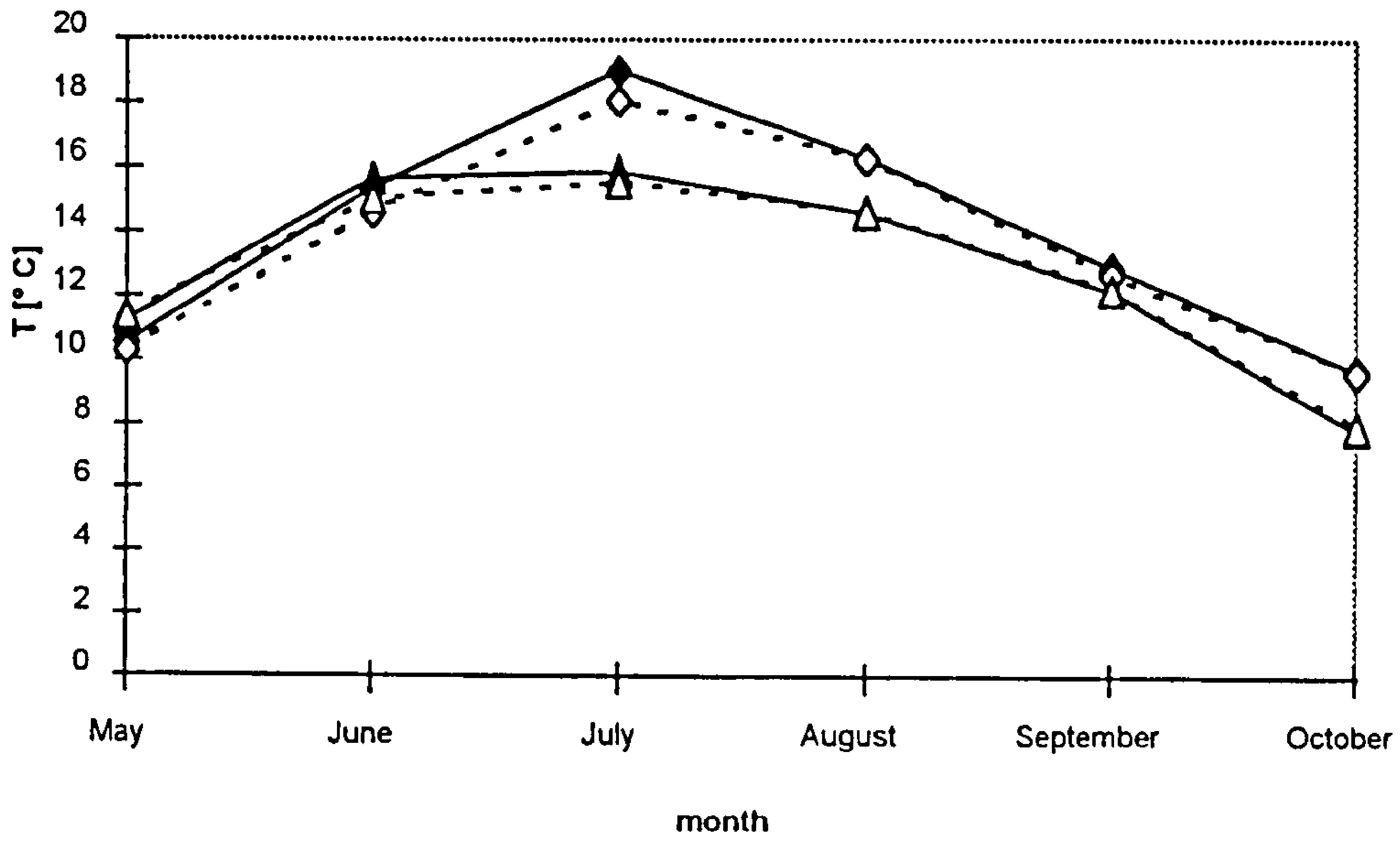

Fig. 3 Monthly 24h mean temperatures for May to October 1993 and 1994. Measurements were taken in a chambered (p2) and in an unchambered ambient (p3) plot.

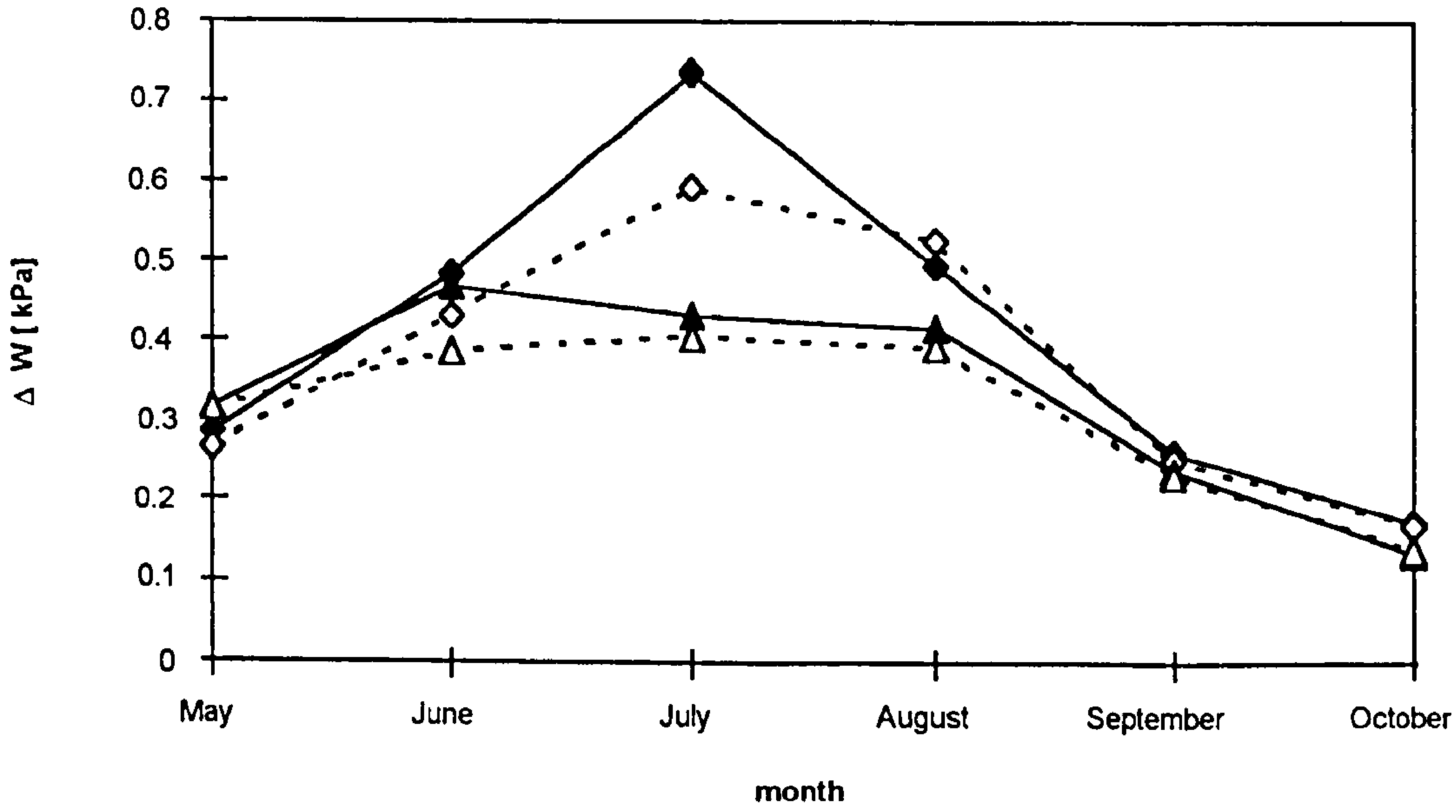

Fig. 4 Monthly 24 h means of VPD (Δ W) for May to October 1993 and 1994.

season was cooler than in 1993, and the mean temperature in May was almost one degree lower than it had been the year before. However, the increase in temperature was steeper in 1994 and showed a pronounced peak in July, with 19.0 ^{0}C for the chambered plots and 18.1 ^{0}C for the unchambered ambient plots. Here the differences between the chambered and the unchambered treatments was also highest. The decline of the temperatures in September/October was less in 1994 and October temperatures were almost two degrees higher in 1994 than they had been in 1993.

4.2.2 Water vapour pressure deficit

The monthly means of the water vapour pressure deficit (VPD), which are given in figure 4, show similar general trends to those described for temperature. However, here the the July values between the two years varied more markedly than those for temperature, and also the difference between chambered and ambient treatments was much more pronounced. The differences between the October values of the two years were less, as compared to the temperature.

4.2.3 Example of a typical exposure day

Hourly mean values of temperature, VPD and pollution concentrations for a typical exposure day (30 June 1993) are given in figures 5a, b. The courses of temperature and VPD (fig. 5a) for chambered and ambient plots showed similar trends, with lower values in the open plots throughout most of the day. Both parameters exhibited the lowest values in the early hours of the morning, and peaked in the late afternoon. The morning increases and evening decreases of the VPD-curves were steeper than those of the temperature-curves. Comparison of VPD of chambered and open plots showed lower values for the open plots throughout most of the day; however, during the morning, between 6.00 and 9.00 GMT, VPD in the open plots increased more rapidly, so that during this period values in chambered and ambient plots were almost identical.

Figure 5b gives the hourly means of the O_3 concentrations in an open-top chamber with ozone fumigation and in an ambient plot, as well as those of the SO_2 concentrations

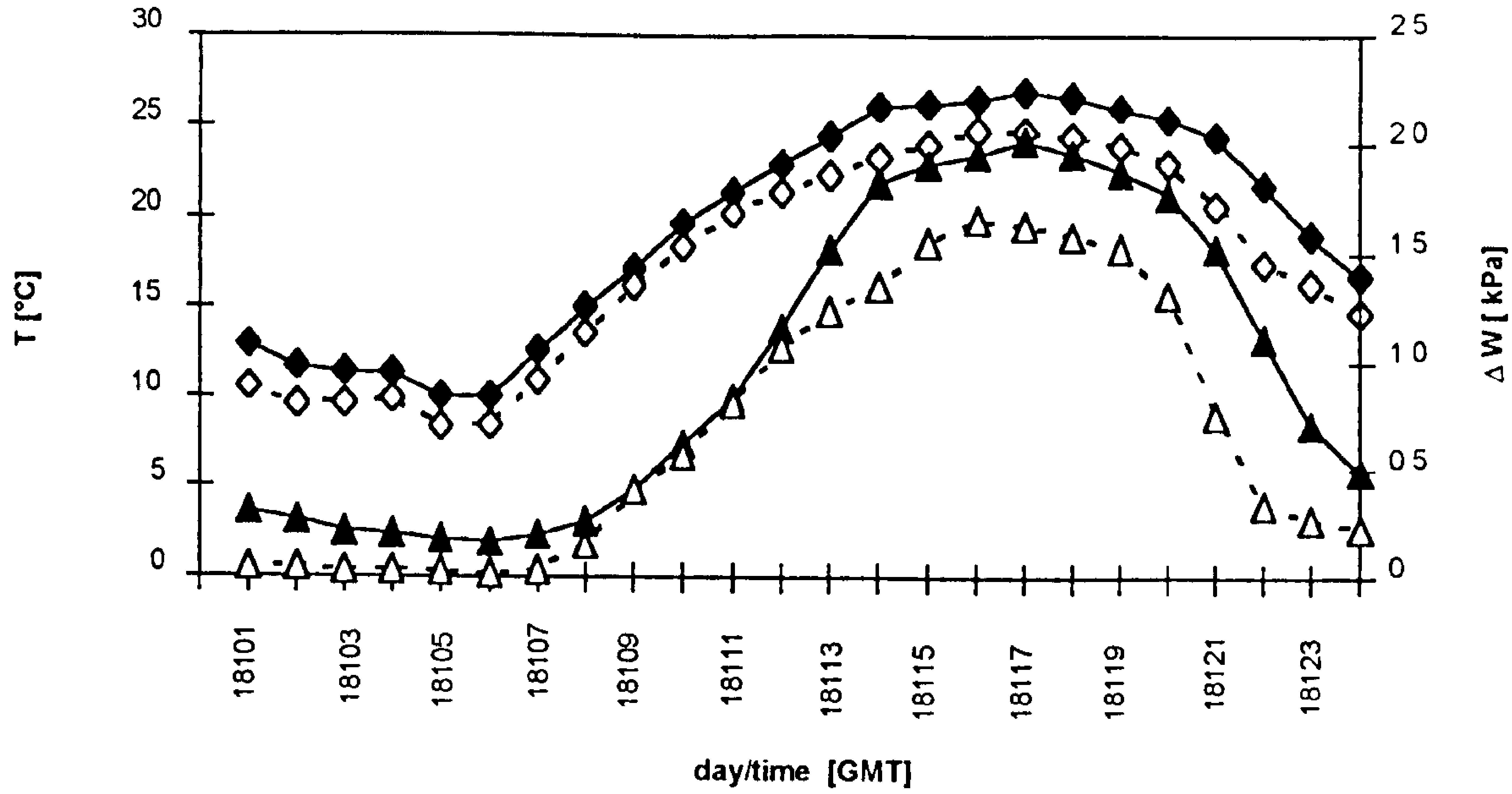

Fig. 5a Diurnal courses of temperature and VPD (Δ W) on a typical exposure day (30 June 1993). Temperature: —◆— (open-top chamber), - - ◇ - - (unchambered plot); VPD: —▲— (open-top chamber), - - △ - - (unchambered plot).

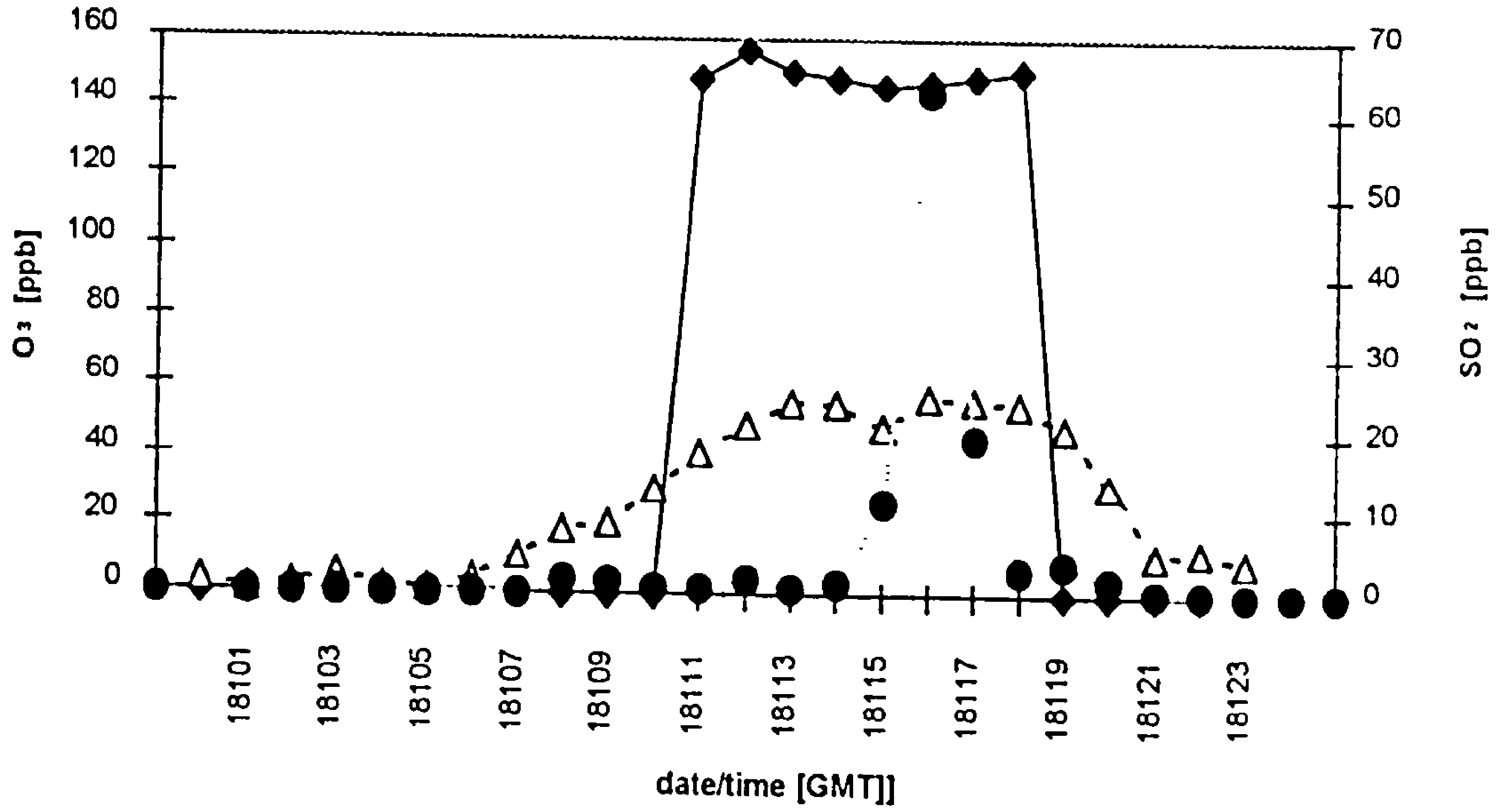

Fig. 5b Diurnal courses of the O_3 concentrations in an open-top chamber with ozone exposure (plot 7): —◆— and in an unchambered plot receiving ambient air (plot 3): - - △ - for 30 June 1993. The ozone concentrations for the CF treatment are represented by the null-line. In addition the diurnal course of the SO_2 concentration on the same day is plotted on the second y-axis: ● .

in the ambient plot.

In the plot with the ozone fumigation, the course of the O_3-concentrations was determined by the exposure regime, consisting of 8 hours ozone exposure to 150 ppb and 16 hours of exposure to filtered air. Due to the constant air flow through the chamber, the increase of the O_3-concentrations in the morning, as well as their decline in the evening, were both very rapid. In the ambient plot, the O_3 concentration showed a similar course to the VPD, with the lowest values in the early hours of the morning and the highest ones in the afternoon. However, the diurnal course of the O_3-concentrations was less steep than that of the VPD. Since the sides of the open-top chambers were covered throughout the day, there was no ozone detectable in those chambers receiving filtered air (CF).

In contrast, the SO_2 concentration curve for ambient air showed a marked peak in the afternoon, while during the rest of the day the values were nearly nil. The course of the SO_2 concentration curve was possibly due to the operation scheme of the nearby power station, Ratcliffe on Soar, and to the wind direction.

4.3 Seasonal ozone load

Monthly 24 hour means (table 1) and monthly totals (table 2) of the ozone concentration were calculated for the three different treatments. Since for all months, between 3 and 56 hourly recordings were missing, the monthly totals were corrected by adding the product of the number of hours missing and the monthly mean to the calculated totals.

In 1994, the ozone concentrations for all three treatments were generally higher than in 1993. This becomes evident from the monthly, and particularly the seasonal, totals of the ozone concentrations. Comparison of the latter shows that in 1994 the seasonal ozone concentrations were up to 84% higher than in 1993 (O_3 - 45%, Ambient - 84 %, CF 51%).

In both years, the highest monthly totals of the ozone concentration were found

1993						
	May	June	July	August	September	October
$CF+O_3$	21	21	15	20	9	3
Ambient	20	14	9	8	6	3
CF	18	8	7	6	6	3
1994						
	May	June	July	August	September	October
$CF+O_3$	24	24	22	18	24	13
Ambient	25	19	23	17	17	13
CF	21	10	8	16	14	12

Table 1 Monthly 24h mean ozone concentrations for May to October 1993 and 1994. All values are given as ppb.

1993							
	May	June	July	August	September	October	sum
$CF+O_3$	15894	15137	11021	13727	6591	2351	64721
Ambient	14751	10380	6817	5983	4492	2351	44773
CF	13613	5503	4941	4846	4180	2351	35433
1994							
	May	June	July	August	September	October	sum
$CF+O_3$	18009	17598	15936	14347	17580	9805	93761
Ambient	18451	13355	17191	12545	11972	8716	82230
CF	15558	1268	5843	11937	10324	8595	53525

Table 2 Monthly cumulative O_3 doses for May to October 1993 and 1994. "Sum" gives the totals for the respective investigation periods for the three different pollution treatments. All values are given in ppb· h.

in either May or June. This was true for all three treatments, and can be attributed mainly to the comparatively high ambient O_3-concentrations at the beginning of the season. Since the numbers of fumigations in May of both years were low, the differences between the mean ozone concentrations of the three treatments were only marginal. While in 1993 a steady decline of the monthly mean ozone concentrations was apparent for the Ambient and the CF plots, the respective concentrations in 1994 did not show a steady trend. In 1994 the October values of all three treatments were about four times higher than those of the year before.

The monthly increase of the ozone dose ($ppm \cdot h$), which is plotted against time in figs. 6a, b, shows more clearly the differences in the ambient background O_3 concentrations between the two years, particularly for the exposure periods. While in 1993 the difference between cumulative dose of the $CF+O_3$ and Ambient treatments was about twice that between the Ambient and CF treatments, the opposite situation was found in 1994. Here only a minor difference could be detected between the $CF+O_3$ and the Ambient treatments during the first four months of the season, with little change during September and October.

Similarly, figure 7, which shows the distribution of hourly means of O_3 concentrations in defined concentration ranges, emphasizes the large differences in background ozone concentrations between the two years of the investigation. It becomes evident that in 1994 the percentage of low-level O_3 concentrations (up to 19 ppb) was decreased for all months, as compared to the year before, and that particularly the percentages of concentrations in the ranges of 20-39 ppb and 40-59 ppb were increased. These alterations affected all three treatments, but due to the experimental conditions they were most pronounced for the Ambient treatment.

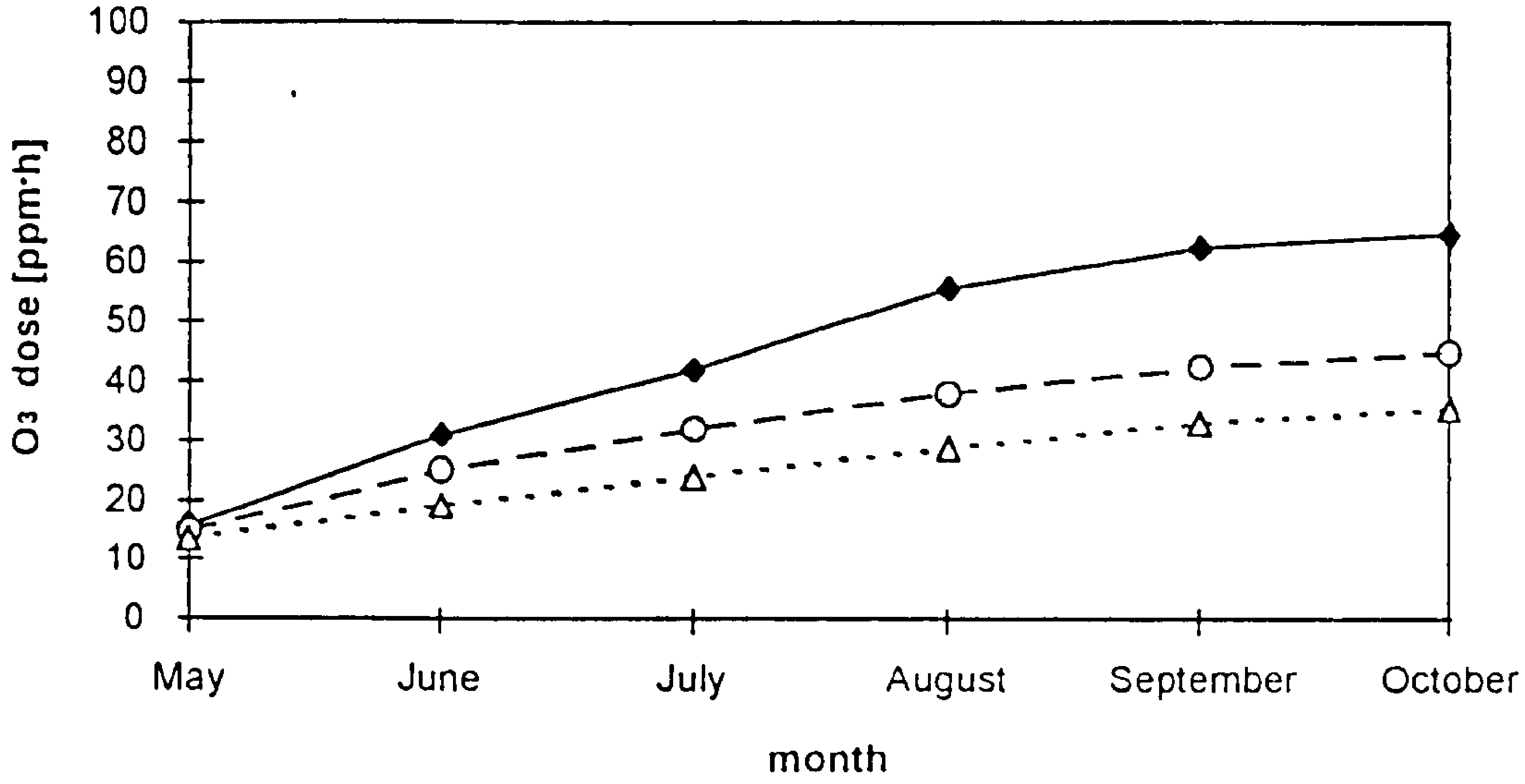

Fig. 6a Cumulative O_3 dose for May to October 1993.

—♦— $CF+O_3$; – ○ – Ambient ; ··△·· CF

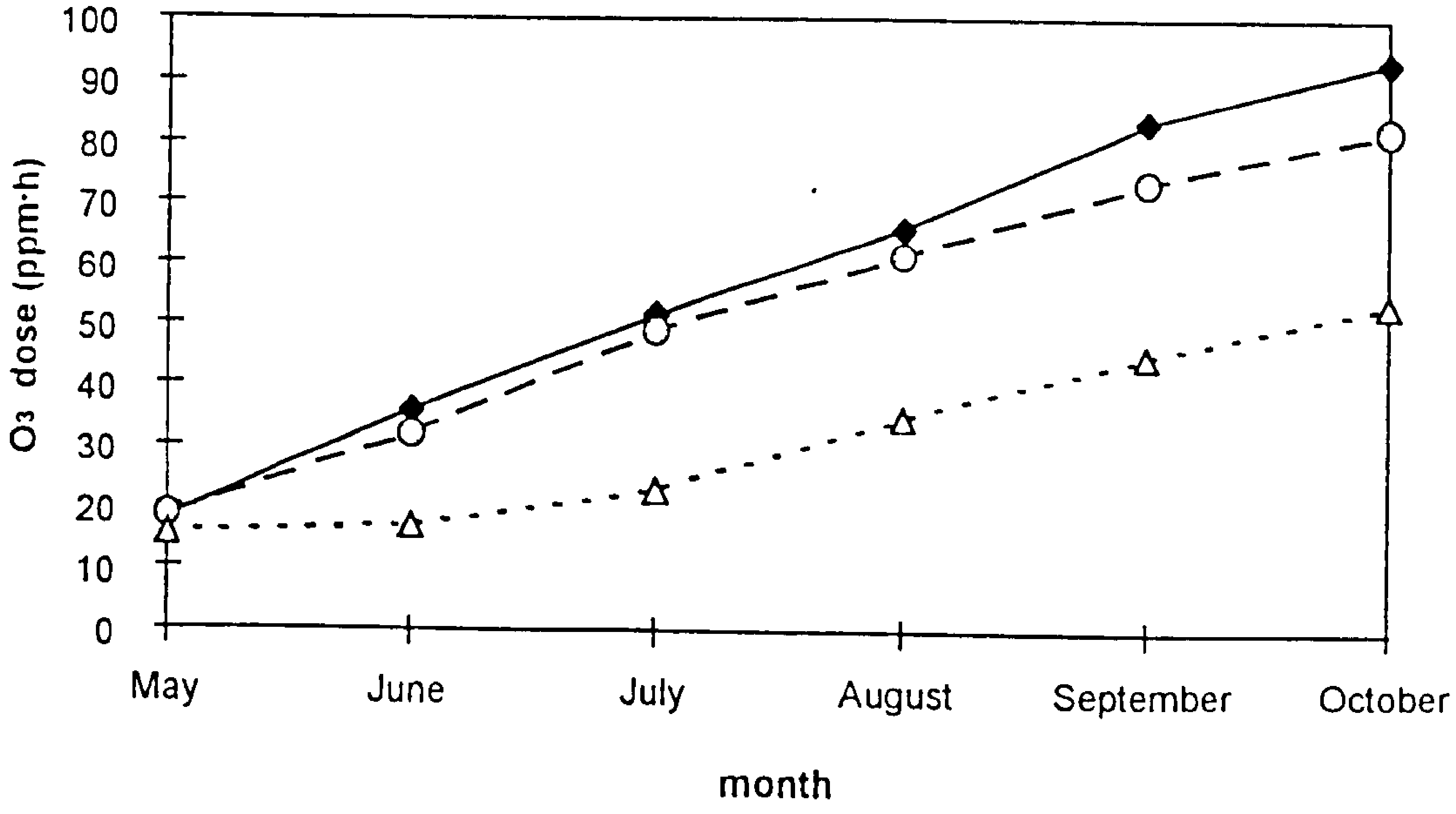

Fig. 6b Cumulative O_3 dose for May to October 1994.

—♦— $CF+O_3$; – ○ – Ambient ; ··△·· CF

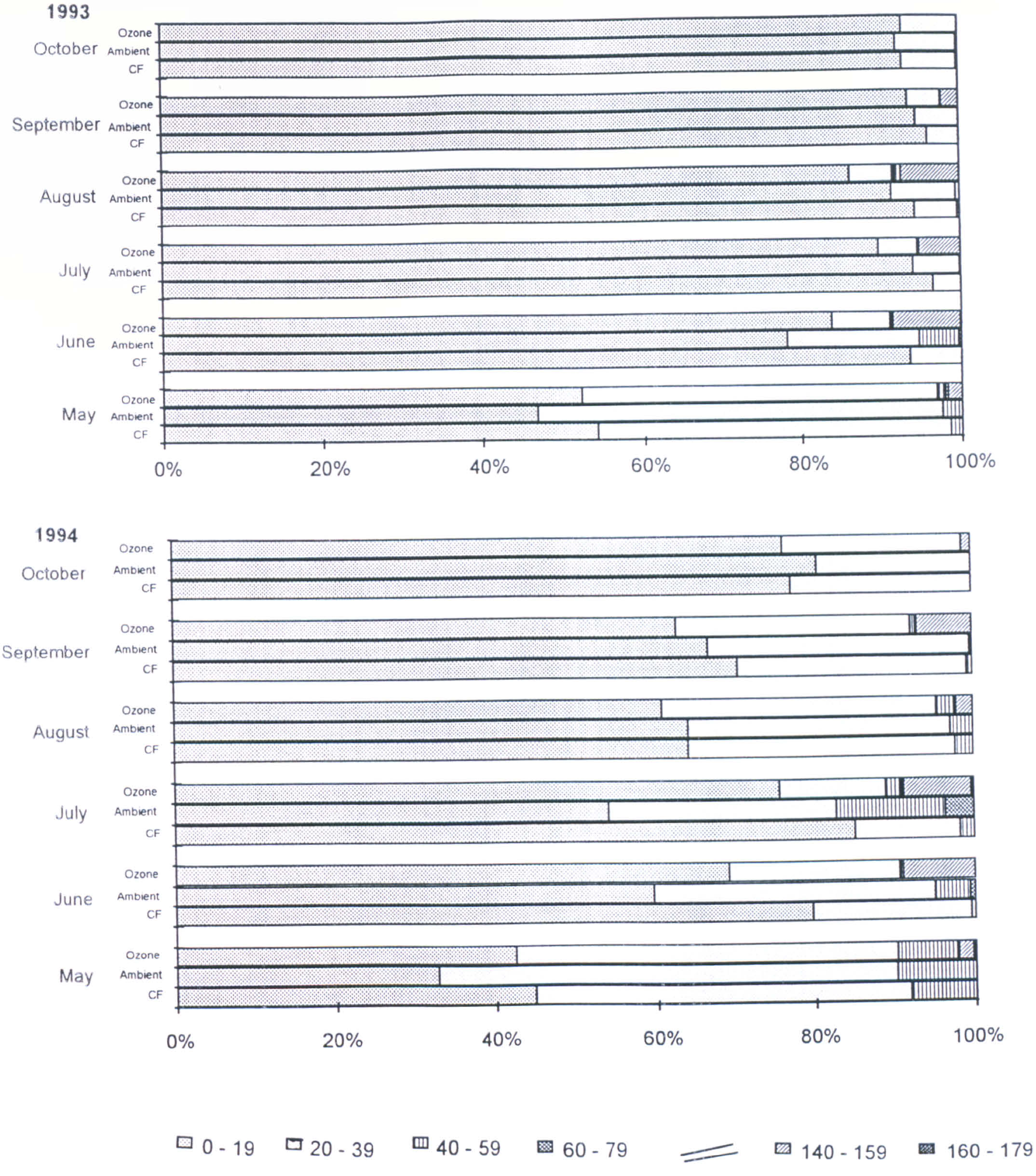

Fig. 7 Percentages for the distribution of hourly means of O_3 concentrations in defined concentration ranges for the three different treatments. The data cover the investigation periods in 1993 and 1994.

4.4 AOT40$_{24}$

It is commonly considered appropriate to characterise the plant's exposure to ozone by calculating a cumulative dose over a threshhold and often this threshold is set at 40 ppb, since few effects are known to occur at lower concentrations (Braun & Flückiger, 1995). Thus the applied dose is also separated from the natural background concentration, which is assumed to be lower than 40 ppb (Volz *et al.*, 1986). This cumulative ozone dose above 40 ppb - AOT40 - was calculated from the 24 hourly means of each day and will be referred to in the text as AOT40$_{24}$. Nighttime ozone dose was included in the calculation, because it has been reported to affect plants similarly to daytime exposure (Matyssek *et al.*, 1995; Skärby *et al.*, 1987).

Unlike the total ozone dose, which in 1994 differed only marginally between the CF+O$_3$ and Ambient treatments, the AOT40$_{24}$ values for both years varied substantially between all three treatments.

1993							
	May	June	July	August	September	October	sum
CF+O$_3$	1551	6992	4467	5939	1709	0	20657
Ambient	72	297	0.5	11	0	0	381
CF	39	0	0	11	0	0	50
1994							
	May	June	July	August	September	October	sum
CF+O$_3$	2090	7138	7104	1870	5396	888	24486
Ambient	456	304	1753	128	4	0	2645
CF	283	16	40	109	4	0	452

Table 3 AOT40$_{24}$ for the months May to October 1993 and 1994 and the total AOT40$_{24}$ for the respective investigation periods. All values are given in ppb · h.

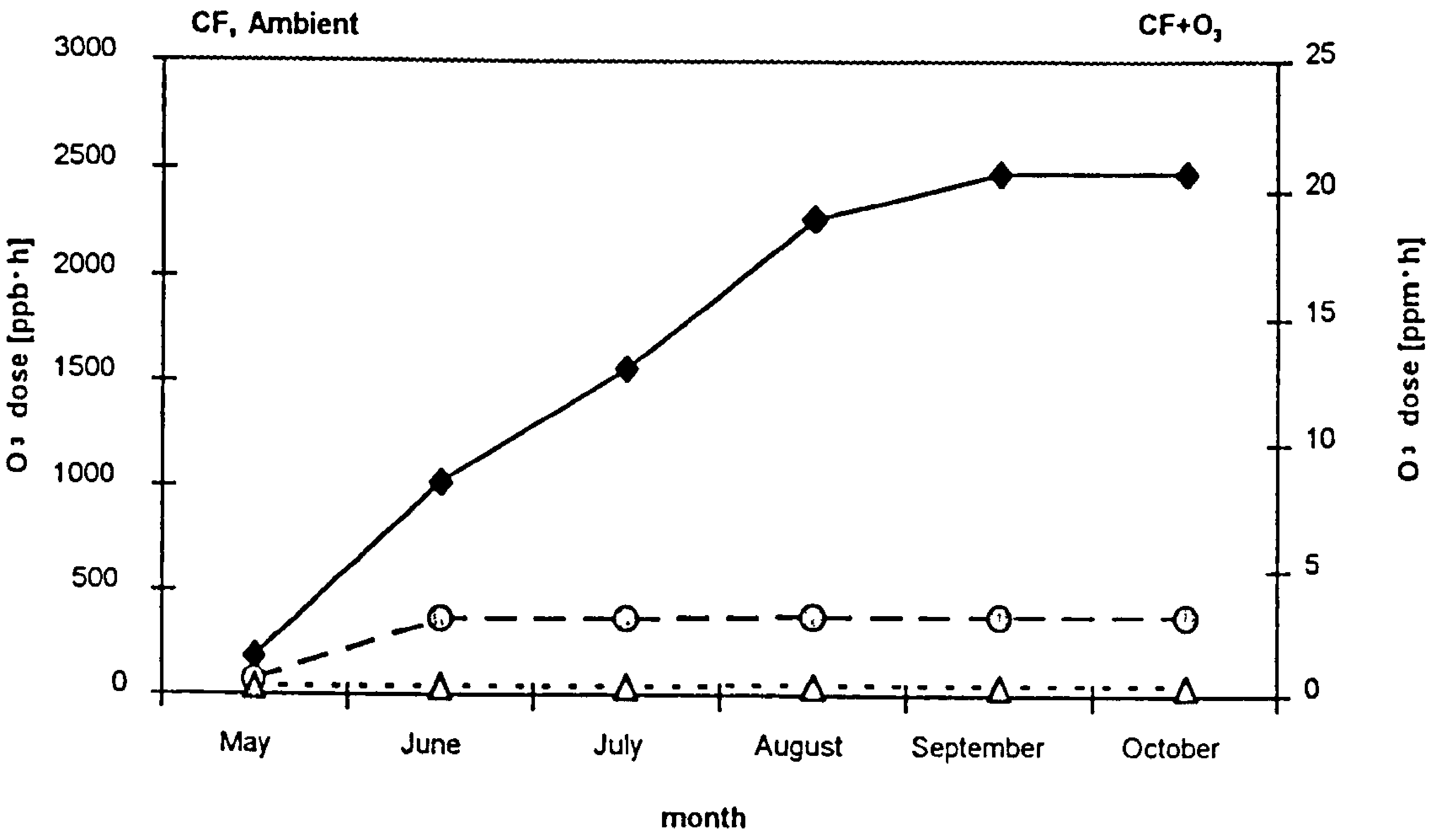

Fig. 8a Cumulative monthly AOT40$_{24}$, 1993.

——◆——CF+O$_3$; - - △ - - CF ; —○— Ambient

Values of CF and Ambient treatments are given on the left y-axis, while values for CF+O$_3$ are shown on the right y-axis.

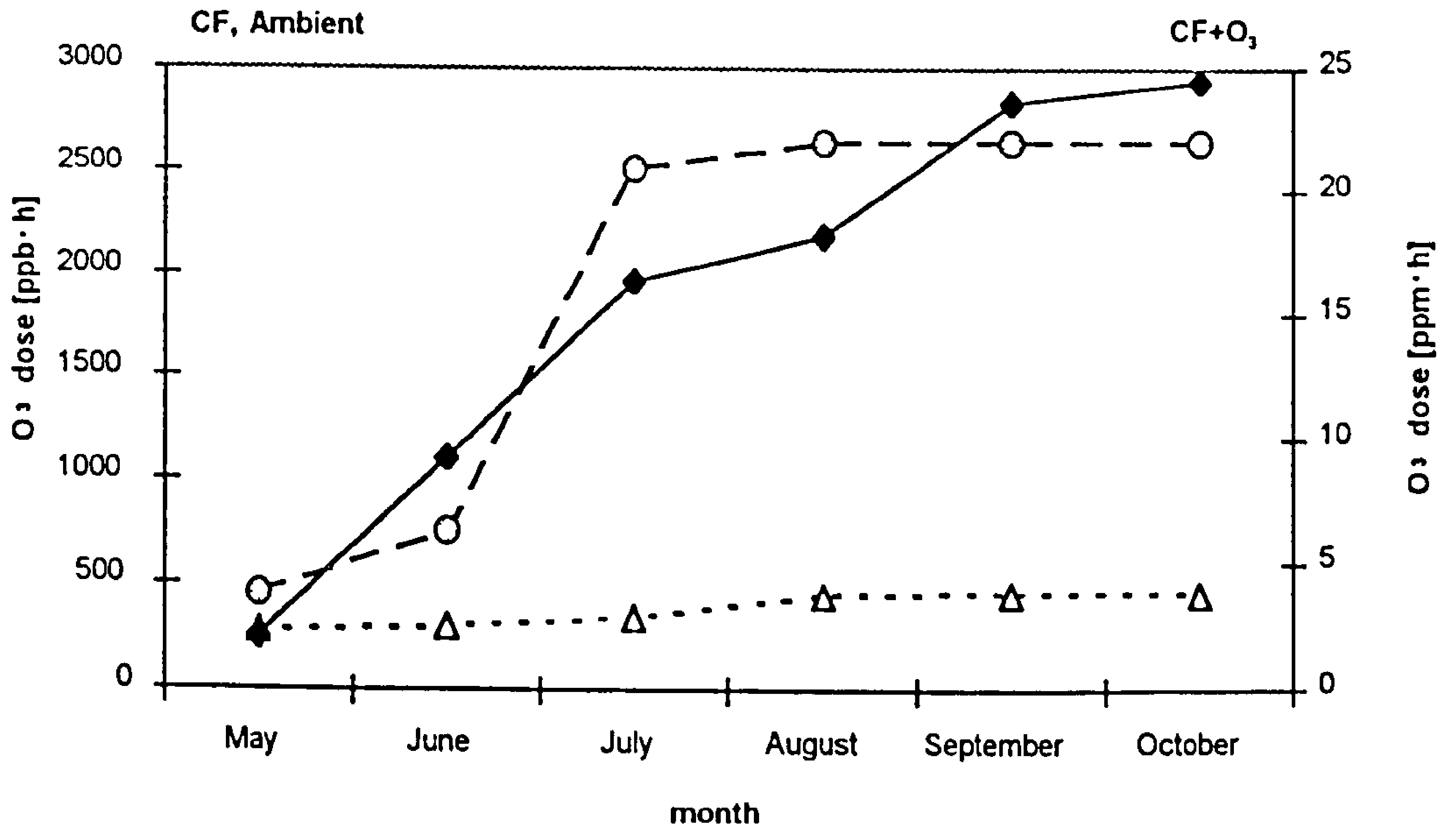

Fig. 8b Cumulative monthly AOT40$_{24}$, 1994.

For the CF+O_3 treatment, the $AOT40_{24}$ values (table 3) were similar for the two field seasons. However, for the Ambient and CF treatments, there were major differences between the $AOT40_{24}$ doses for the two years. Thus the plants of the CF treatment received a higher $AOT40_{24}$ in 1994 than did those of the ambient treatment in 1993. While the CF treatment in both years received its highest monthly $AOT40_{24}$ dose in May, the ambient treatment in 1993 received its highest dose in June and in 1994 the highest monthly $AOT40_{24}$ occurred in July. The 1994 peak was almost five times higher than that of the previous year.

Figures 8a, b give the cumulative curve of the $AOT40_{24}$ for 1993 (fig. 8a) and 1994 (fig. 8b). Due to the greater range of the $AOT40_{24}$ values, the values of the CF+O_3 treatment have been plotted on a second y-axis. From the graphs, the substantial differences between the treatments, as well as the different course of the increase of the ozone dose above 40 ppb become evident. The CF+O_3 treatment, which was determined by the exposure regime, showed a more continuous increase of the $AOT40_{24}$ throughout the season. On the other hand, the $AOT40_{24}$ for the CF treatment, which was similarly influenced by the exposure regime and received only filtered air during the exposures, increased only marginally from a low initial value in May of both years. While the development of the $AOT40_{24}$ of these two treatments showed a high level of continuity, the $AOT40_{24}$ of the ambient treatment increased erratically, with a big leap in either June 1993 or July 1994. This shows that higher levels of ambient ozone mainly occur during May, June and July and are very infrequent later in the season.

4.5 Soil water status

Soil water content of the pots of the two-year-old plants was determined at the end of each drought episode to determine the severity of drought and also to assess the difference between the droughted and the well-watered treatments.

The soil water contents measured at the end of the drought episodes (table 4) differed between the two years of the experiment. In 1993 the soil water content of the

1993		
	droughted	well watered
30 July	10 ± 2	13 ± 2
25 August	12 ± 3	15 ± 2
1994		
14 June	10 ± 1	20 ± 2
13 July	7 ± 1	20 ± 2
17 August	7 ± 1	17 ± 2

Table 4 Soil water content [in %] of pots of two-year-old saplings at the end of the drought episodes.

droughted treatment was higher on average than that measured for the same treatment in 1994. In contrast, the soil water content of the well-watered treatment was higher in 1994. This may have been due to the use of different substrata, with different pore sizes and thus different abilities to retain water.

Accordingly, the differences between well-watered and droughted treatments in 1994 were more pronounced than those in 1993, reflecting not only the difference in the substratum used, but also the more effective drought stress achieved in 1994.

5 Results

5.1 Stomatal conductance

In both years of the experiment, measurements of stomatal conductance were taken on the two-year-old potted saplings and on the field-grown trees. The assessments were made throughout each season. Measurements were taken in randomized order on plants from all three treatments (CF, Ambient, CF+O_3) and made possible a comparison between the three treatments. However, there were differences between the two years in the number and sequence of measurements.

Thus, in 1993, there were 19 assessments of stomatal conductance on the two-year-old saplings; these were between 22 May and 11 September (see appendix B for an overview) and 36 such assessments were taken between 11 June and 13 September 1994 (overview see appendix B). While in 1993 stomatal conductance was assessed regularly every five to ten days throughout the season, depending on weather conditions, assessments in 1994 concentrated on the exposure episodes and on those days preceeding and following them.

Besides these comparative assessments, measurements of diurnal variations of stomatal conductance in selected plots and measurements on healthy and visibly-injured mature leaves and on young leaves were taken in 1993.

On the field grown trees, 14 assessments were made between 22 May and 6 September 1993 (see appendix B for an overview) and 12 further measurements were taken between 18 July and 13 September 1994 (appendix B).

5.1.1 Course of stomatal conductance during the two field seasons

For both age groups investigated, the assessments made in 1993 (figs. 9, 10) started earlier in the season and thus only one exposure preceded the first measurements, while

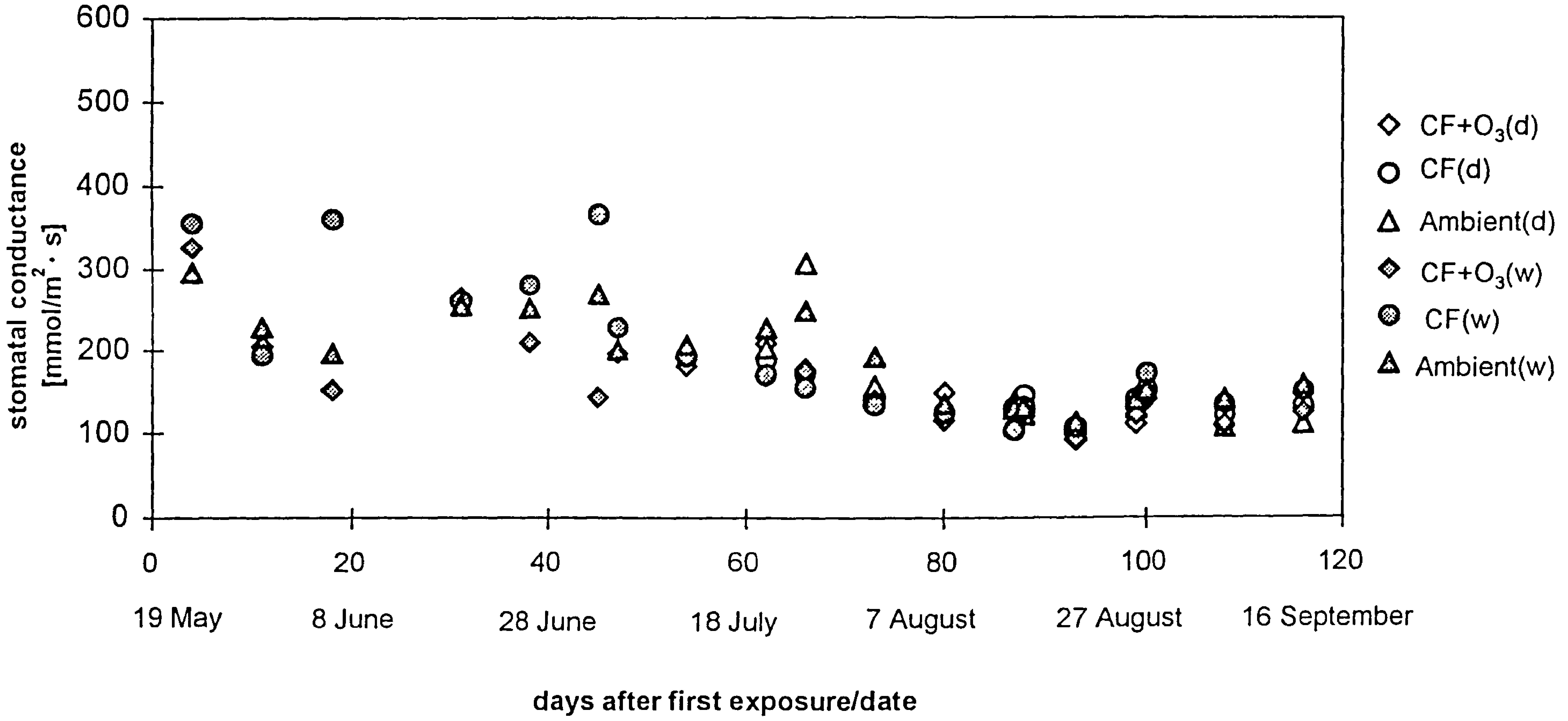

Fig. 9 Means of stomatal conductance of two-year-old saplings, 1993, showing the course of stomatal conductance across the season. From mid-July onward half of the plants from each treatment were droughted, so treatments split into a droughted and a well-watered treatment.

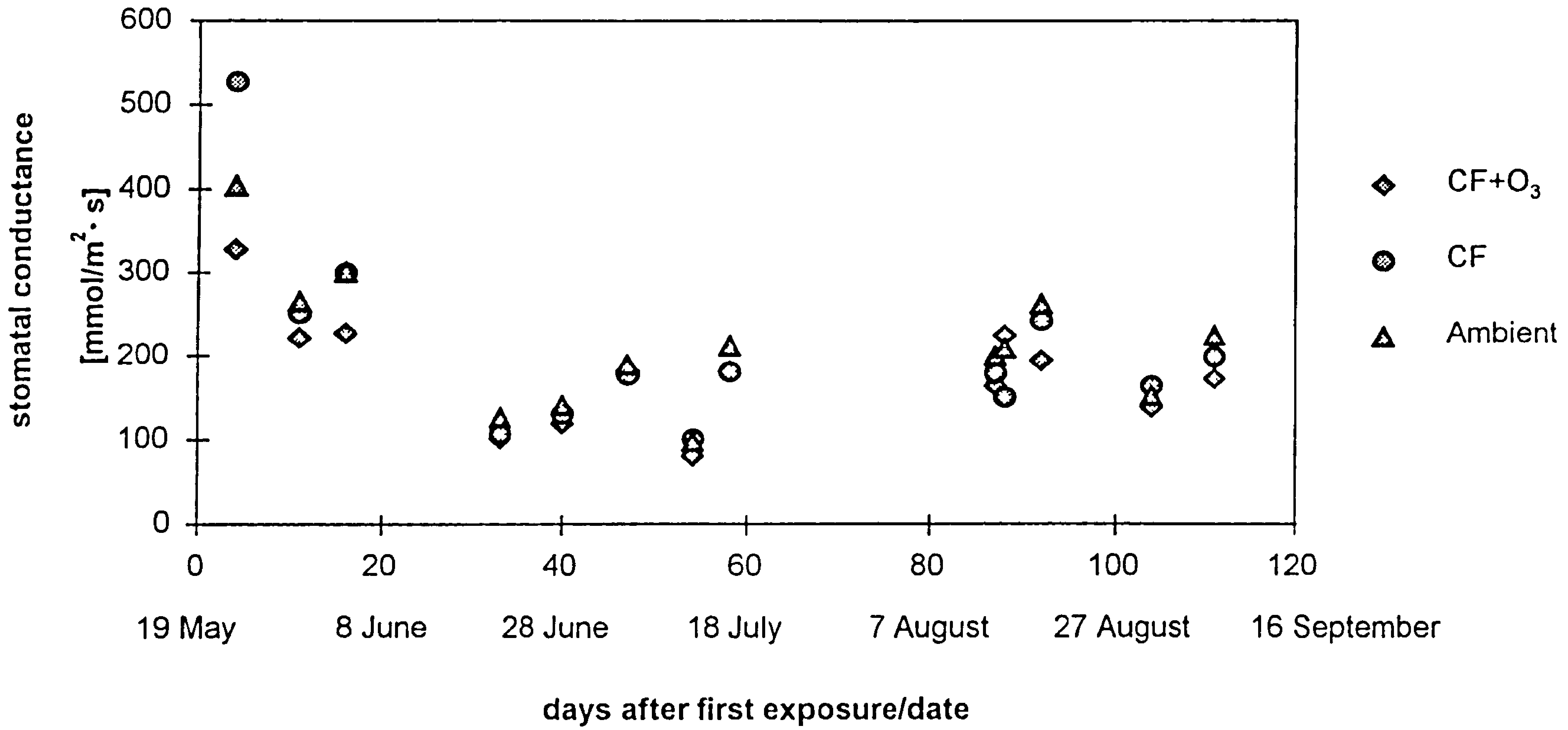

Fig. 10 Means of stomatal conductance of field-grown trees, 1993, showing the course of stomatal conductance across the season.

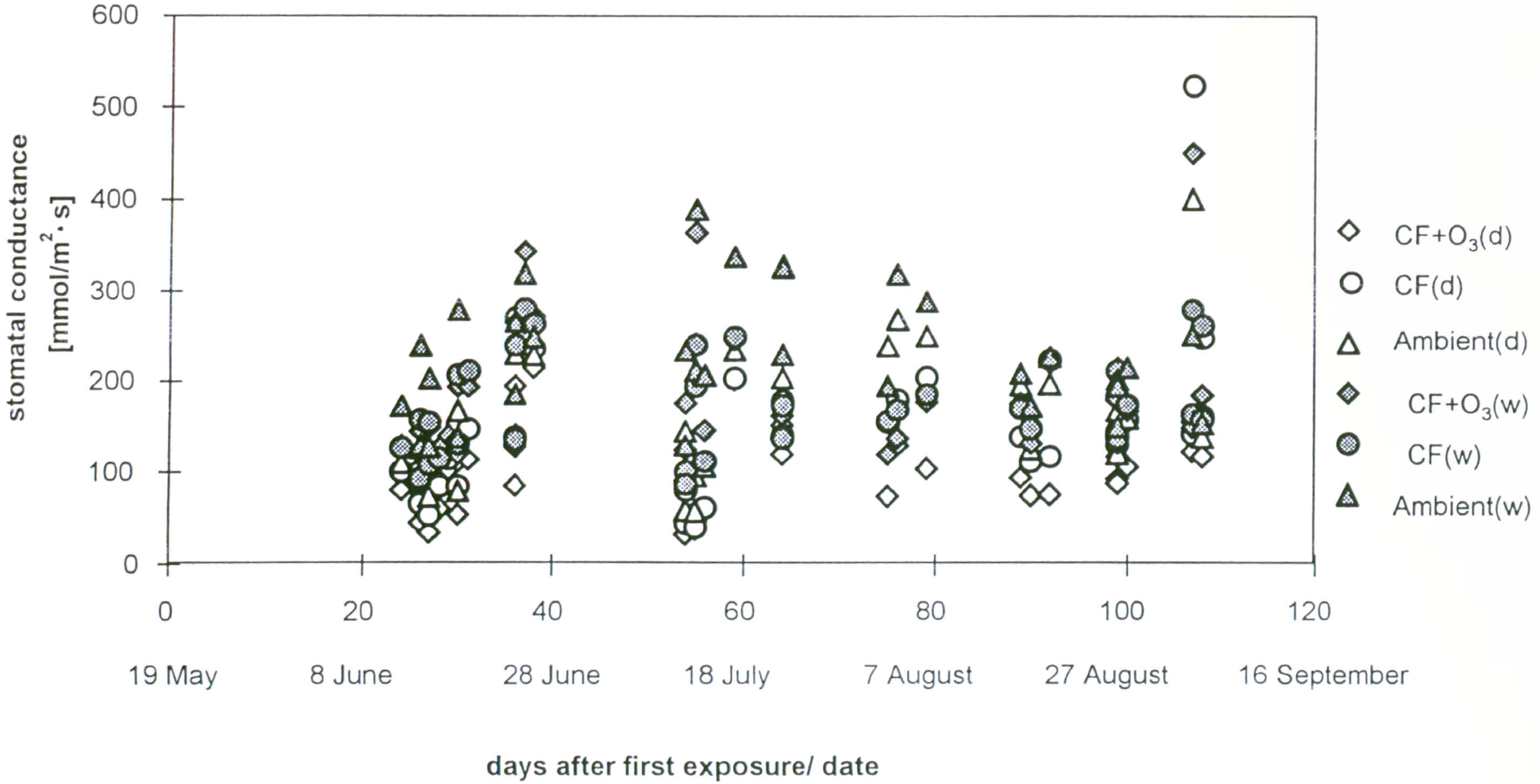

Fig. 11 Means of stomatal conductance of two-year-old saplings, 1994, showing the course of stomatal conductance for the six treatments.

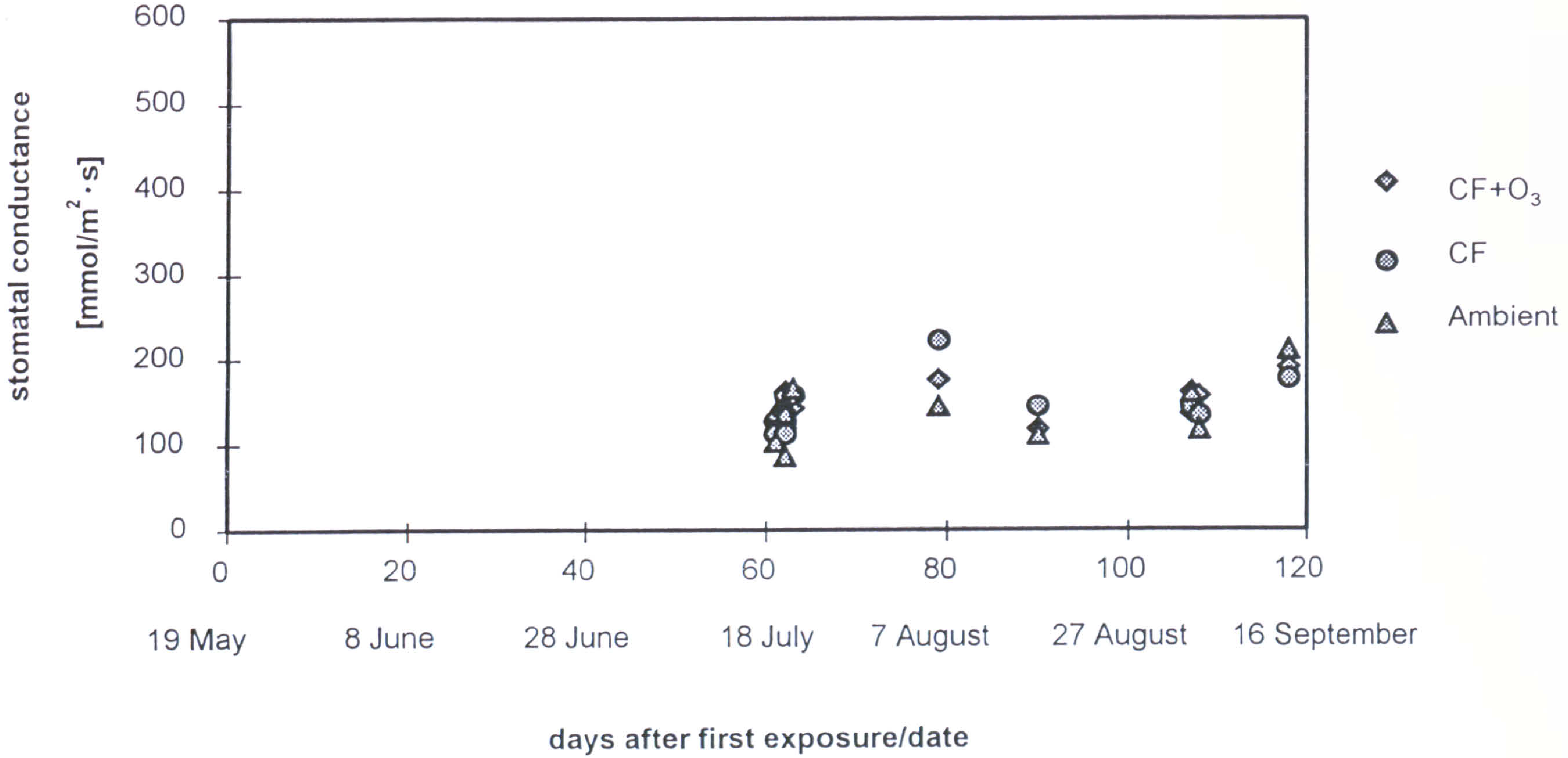

Fig. 12 Means of stomatal conductance of field-grown trees, 1994.

in 1994 (figs. 11, 12) the plants had already been subjected to three exposures before the start of the assessments. Thus the trends observed in the 1993 data exhibited a different seasonal course, with highest mean stomatal conductance during the first part of the season, an early decline and stabilisation of the conductance at lower levels.

5.1.1.1 Two-year-old saplings

At the beginning of the 1993 field season, stomatal conductance of the two year old potted saplings (fig. 9) reached values between 250 and 375 $mmol/m^2 \cdot s$. Stomatal conductance for all treatments declined after this initial phase, and from about the second half of July, stomatal conductance stabilized at a level of approximately 150 $mmol/m^2 \cdot s$.

From the beginning of the season until 15 July all plants received the same irrigation, thus until then there are only three treatments indicated in figure 9. From 15 July until the end of the season two different irrigation regimes were imposed, splitting the treatments into a well-watered sub-treatment and a droughted sub-treatment (fig. 9). However, since the favourable weather conditions prevalent during the first half of the season changed in July, a real drought stress could not be achieved, and accordingly values of stomatal conductance of the two irrigation regimes did not show different trends. Water vapour pressure deficits, shown in chapter 4.2.2, give an indication of the humid weather prevalent during the 1993 growing season.

Although the drought treatment, which had been imposed during a low water vapour pressure deficit during the second half of the season, was not effective (see also chapter 4.5 on soil water content, and chapter 4.2.2 on water vapour pressure deficit), the dry and sunny weather conditions during the first half of the season made the soil in the pots dry rapidly and on a few days mild drought stress was found for all plants.

Highly significant differences between pollution treatments could be found only for the third ($P < 0.001$), sixth ($P < 0.001$) and tenth ($P = 0.028$) assessment. On the first two of these dates, stomatal conductance of the $CF+O_3$ treatment was reduced to less than half of the value determined for the CF treatment. In both cases the Ambient treatment was intermediate. Both measurement periods coincided with dry weather conditions and mild drought stress, as mentioned above. The results from the ninth assessment were different, as were the environmental conditions - here the Ambient treatment showed by far the highest stomatal conductance, while the CF and CF+O_3 treatments differed only marginally from each other. All three assessment days followed an O_3 exposure period. Later in the season no further significant differences could be found, either on days coinciding with an exposure or on the following days.

In 1994 (fig. 11), the measurements started later in the season. The initial phase of high stomatal conductance and the following decrease to lower levels, found in 1993, is missing in the 1994 data. This may be due to the later start of the measurements or to differences in the environmental conditions at the beginning of the season.

Due to the much more effective drought stress, which in 1994 was imposed at intervals from the start of the season until mid-August, significant differences between the droughted and the well-watered treatment were found. In all three pollution treatments, the effect of drought led to a lower stomatal conductance as compared to the well-watered treatment. This drought effect already was from the first measurements ($P < 0.001$), since plants had been subjected to the first drought episode before the start of the assessments of stomatal conductance. However, after the drought episodes, the CF(d) treatment recovered more quickly to the level of stomatal conductance of the well-watered plants than did the CF+O_3(d) treatment. The plants of the droughted Ambient treatment [Ambient(d)] showed an intermediate behaviour. This better recovery of the CF(d) and the Ambient(d) treatments could be found particularly at the end of the season, when all plants were being well-watered from 18 August onwards. During this period, the CF(d) and the Ambient(d) treatments showed values of stomatal conductance in the range of the well watered plants or even greater, while stomatal conductance of the CF+O_3(d) treatment was still very low.

In 1994, stomatal conductances of all treatments were slightly higher than they had been in 1993 and levelled at about 200 mmol/m^2· s. Comparison of treatments showed the two unchambered Ambient treatments to have exhibited higher values of stomatal conductance than the chambered CF and CF+O_3 treatments throughout most of the season. This was particularly true for Ambient(w).

Because of the large weather-related variability in stomatal conductance values between days, differences between the treatment means from the CF(d), Ambient(w), Ambient(d), CF+O_3(w), CF+O_3(d) and CF(w) (which was considered the control treatment) were calculated for the two year old plants, giving a "relative stomatal conductance" (figs. 13, 14). Trends were fitted to these relative stomatal conductances by linear regression analysis (Excel 5).

For 1994, trends were fitted to the whole data set, since all treatments were imposed from the start of the season. In contrast, for 1993 only data from the second part of the season (after 15 July) were used to calculate the linear trends. Thus the trends are based on data from points in time when the drought treatment had been imposed and treatments had been split into a droughted and a well-watered sub-treatment.

In 1993 (fig. 13), the trends for all treatments were similar in direction and differed mainly in their slopes. This confirms the lack of pronounced differences between the droughted and the well watered treatments, as well as the observed smallness of differences between the three pollution treatments.

Compared to the control treatment [CF-(w)], all other treatments exhibited higher stomatal conductances at the beginning of the second part of the season, showing in the positive values of the relative stomatal conductances. Towards the end of the season stomatal conductance of all treatments decreased as compared to the control, and apart from the Ambient(w) treatment, all others exhibited lower stomatal conductance than the control.

In 1994 (fig. 14), there were considerable differences between the trends of the individual treatments, the most obvious being those between the droughted and the well watered treatments. Due to the early droughting, all three drought treatments showed negative relative stomatal conductances at the start of the assessments, while relative conductance values of the two well watered treatments were even slightly increased, as compared to the control, which in the diagrams is represented by the null-line.

While relative stomatal conductance values of the CF+O_3(d) treatment declined slightly over the season, those of the CF(d) and the Ambient(d) treatments increased, as can be seen from the decreasing differences to the control. The increase of CF(d)

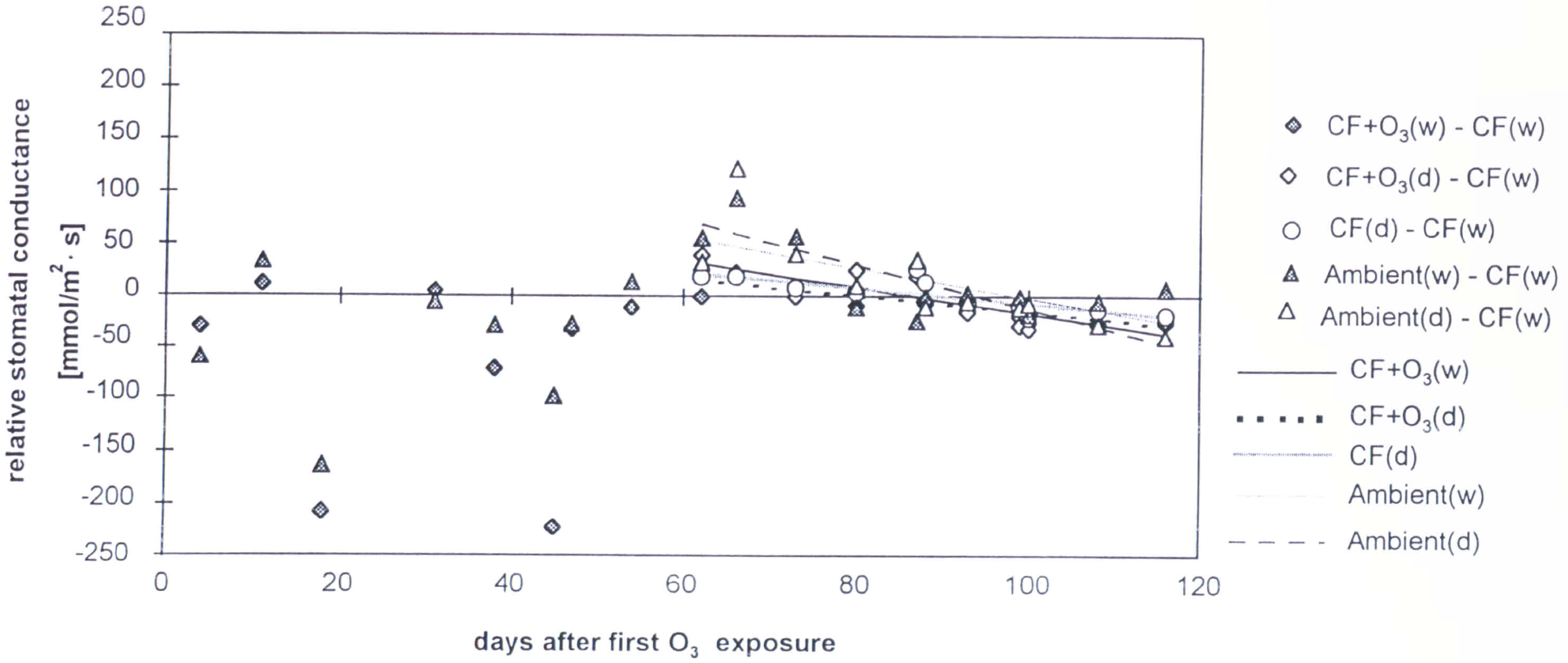

Fig. 13 Relative stomatal conductances for the two-year-old saplings, 1993, calculated as differences of stomatal conductance. CF(w) was used as control treatment and subtracted from all other treatments. Trends were fitted to the data by linear regression and cover only the second part of the season, when the drought treatment was imposed.

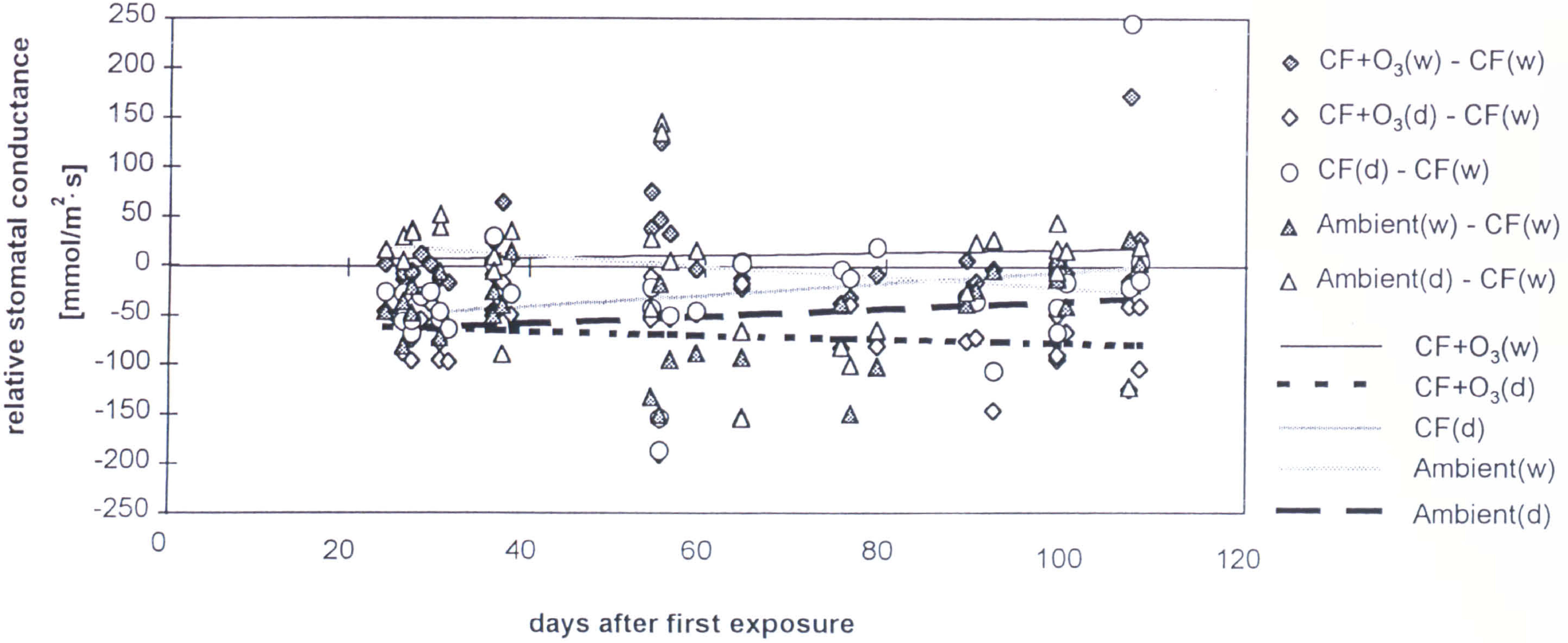

Fig. 14 Relative stomatal conductances for the two-year-old saplings, 1994. Trends were fitted to the data by linear regression analysis.

was stronger than that of Ambient(d) and at the end of the season relative stomatal conductance of the former had reached zero and thus had recovered to levels similar to those of the well-watered control. That of Ambient(d) was still considerably below zero, reaching about -40 $mmol/m^2 \cdot s$. These trends, which were determined by the recovery of the plants after the drought cycles rather than by the behaviour during drought, which was very similar for all droughted treatments, support the better recovery of the CF(d) plants observed above, as compared to those of the CF+O_3(d) and the Ambient(d) treatments.

5.1.1.2 Field-grown trees

The seasonal course of stomatal conductance of the field grown trees was similar to that of the two year old potted saplings.

From the 1993 data set (fig. 10 and for an overview see appendix B), the same initial phase of high stomatal conductance and the following decline and stabilisation at a lower level, already described for the two year old saplings, becomes evident. However, the initial values and the level where stomatal conductance stabilized were slightly higher than those of the potted saplings. Furthermore, the decrease of stomatal conductance was steeper, and stabilisation was already attained thirty days after the start of the pollution treatment, while in the case of the two year old saplings stabilisation was reached only about sixty days after the start of the treatment.

Differences between the pollution treatments were small and never significant. However, the CF+O_3 treatment exhibited the smallest values for most assessments.

In 1994 the measurements on the field grown trees (fig. 12) were started later in the season, wherefore, as for the two year old potted saplings, only the phase of stabilized conductance was recorded, its level being slightly lower than it had been the year before. Differences between the pollution treatments were more pronounced than they had been the year before. However, there was no specific trend for any treatment.

5.1.2 Stomatal conductance of different daytypes in relation to exposures

Data analysis comparing stomatal behaviour from different daytypes (= days in different relation to the O_3 exposures) was mainly performed on the 1994 data set, since measurements in 1993 were distributed more evenly across the season and certain daytypes were not replicated as they were in 1994.

The daytypes included in the analysis were:

1. Days where at least 6 days had elapsed since the last O_3 exposure (O),
2. Exposure days, where the measurements were taken during the exposures (E),
3. Days following an exposure day (E+1),
4. Days following two days after an exposure (E+2),

The analysis performed on the data was a split-plot analysis of variance, with the pollution treatments as the main treatments and the irrigation treatments as the subtreatments. The F-probability was calculated for data sets containing measurements from the Ambient treatments, as well as for data sets excluding such measurements. This was done to investigate the differences between the ambient plots and the open-top chambers that might have been due to chamber effects, as well as to find out about the possibility of interpreting the data from the elevated ozone treatments ($CF+O_3$) in the context of ambient climatic conditions.

5.1.2.1 Two year-old-saplings

In 1993 (fig. 15) data were only available for days without exposure (O) and for days following an exposure (E+1). For the analysis, three replicates of each of the two daytypes were used. There were no significant differences between the three treatments. However, differences between the two daytypes were highly significant ($P < 0.001$). This is probably due to differences in climatic conditions (see table 5).

For both daytypes the CF treatment showed the highest and the $CF+O_3$ treatment the smallest stomatal conductance. However, these differences between the two treatments were more pronounced on days following an exposure. The Ambient treatment exhibited intermediate values for both days.

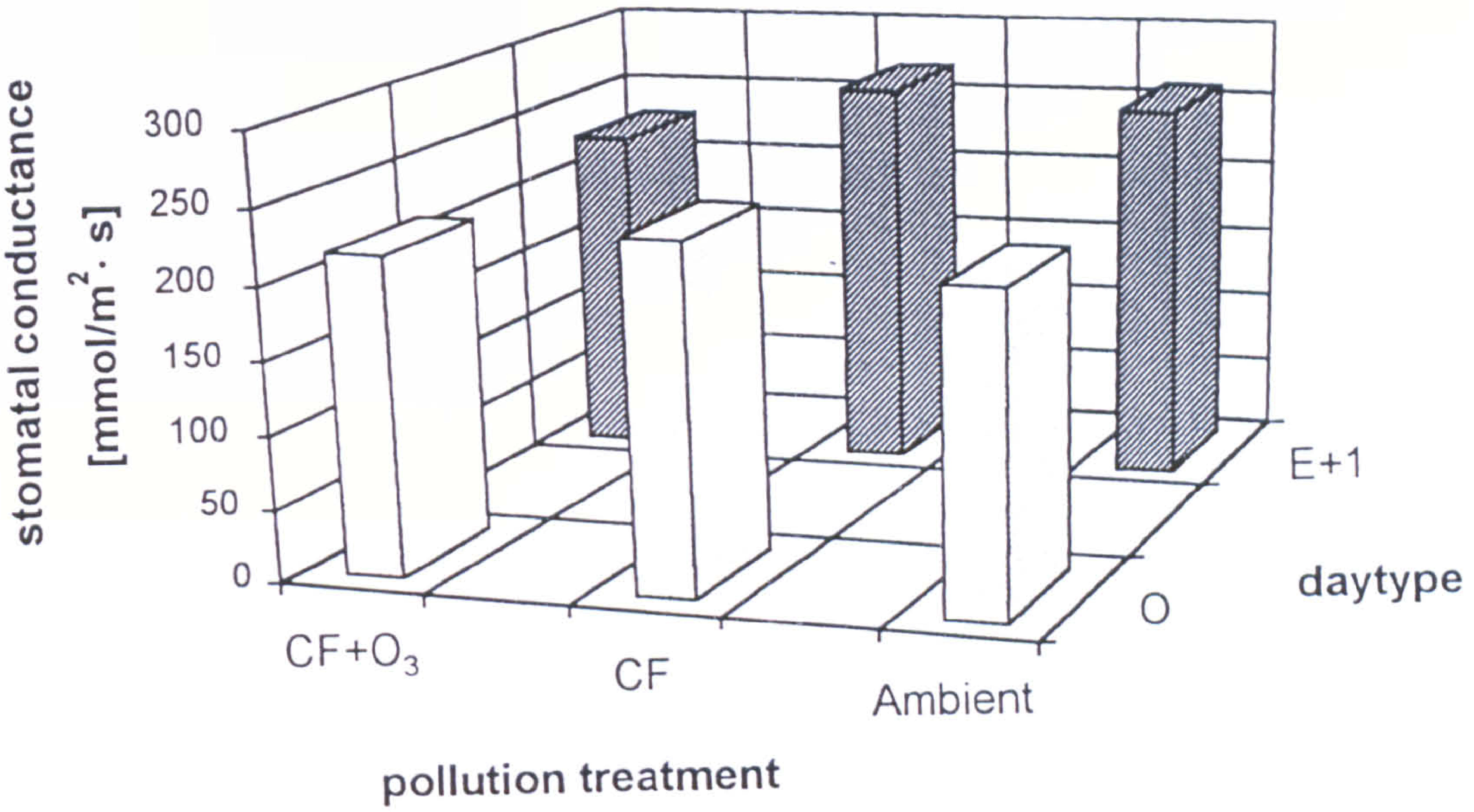

Fig. 15 Means of stomatal conductance of two-year-old saplings, 1993, for two different daytypes - days where at least six days had elapsed since the last exposure (O) and days that followed an exposure (E+1). Differences were not significant.

	PAR [$\mu E/m^2 \cdot s$]		Leaf temperature [^0C]	
	O	E+1	O	E+1
CF+O_3	217	228	18.9	22.6
CF	235	268	18.7	22.5
Ambient	215	259	18.8	22.7

Table 5 Means of PAR and leaf temperature measured concurrently with every measurement of stomatal conductance given in fig. 15. Temperature differences between the two daytypes were highly significant ($P < 0.001$).

The values of irradiation and leaf surface temperature (table 5), which were recorded simultaneously with the measurements of stomatal conductance, showed only marginal differences for irradiation levels between the two daytypes, while leaf surface temperatures were significantly different ($P < 0.001$). The lower temperatures of about 18.8 ^{0}C were found on days without preceding exposure (O), while on days following an exposure (E+1), temperatures around 22.7 ^{0}C were recorded.

Since in 1994 measurements were taken mainly about the time of the O_3 exposures, it was possible to perform split plot analysis on a number of different data sets, including two or four daytypes with a different number of replicates, depending on the number of measurements available for each daytype.

A comparison of exposure days (E) and days following an exposure (E+1), based on data sets with measurements on three leaves per plant and six replicates of each daytype is given in figure 16. In this analysis the Ambient treatments were not included.

The most striking effect was that of the drought treatment, causing a highly significant decrease in stomatal opening ($P < 0.001$) in both pollution treatments. While the effect of ozone was not significant, there was a significant interaction of ozone and drought ($P = 0.024$). This interaction showed in the stronger decline of stomatal conductance in CF+O_3(d), as compared to CF(d), while on the other hand the values of CF+O_3(w) were slightly higher than those of CF(w). As in the 1993 data set described above, there were differences between the two daytypes which were likely to be related to differences in climatic conditions.

Means of irradiation and leaf surface temperatures, which were measured concurrently, are shown in table 6. While for the individual daytypes both irradiation and leaf surface temperature were very uniform and similarly differences in irradiation between the two daytypes were negligible, the temperature difference between the daytypes was more than 5 ^{0}C and highly significant ($P < 0.001$), with the higher temperatures occurring on exposure days.

To find out about the recovery of the different treatments from the pollution and drought stress, a second comparison included all four different daytypes described above, with three measurements per plant and two replicates of each daytype.

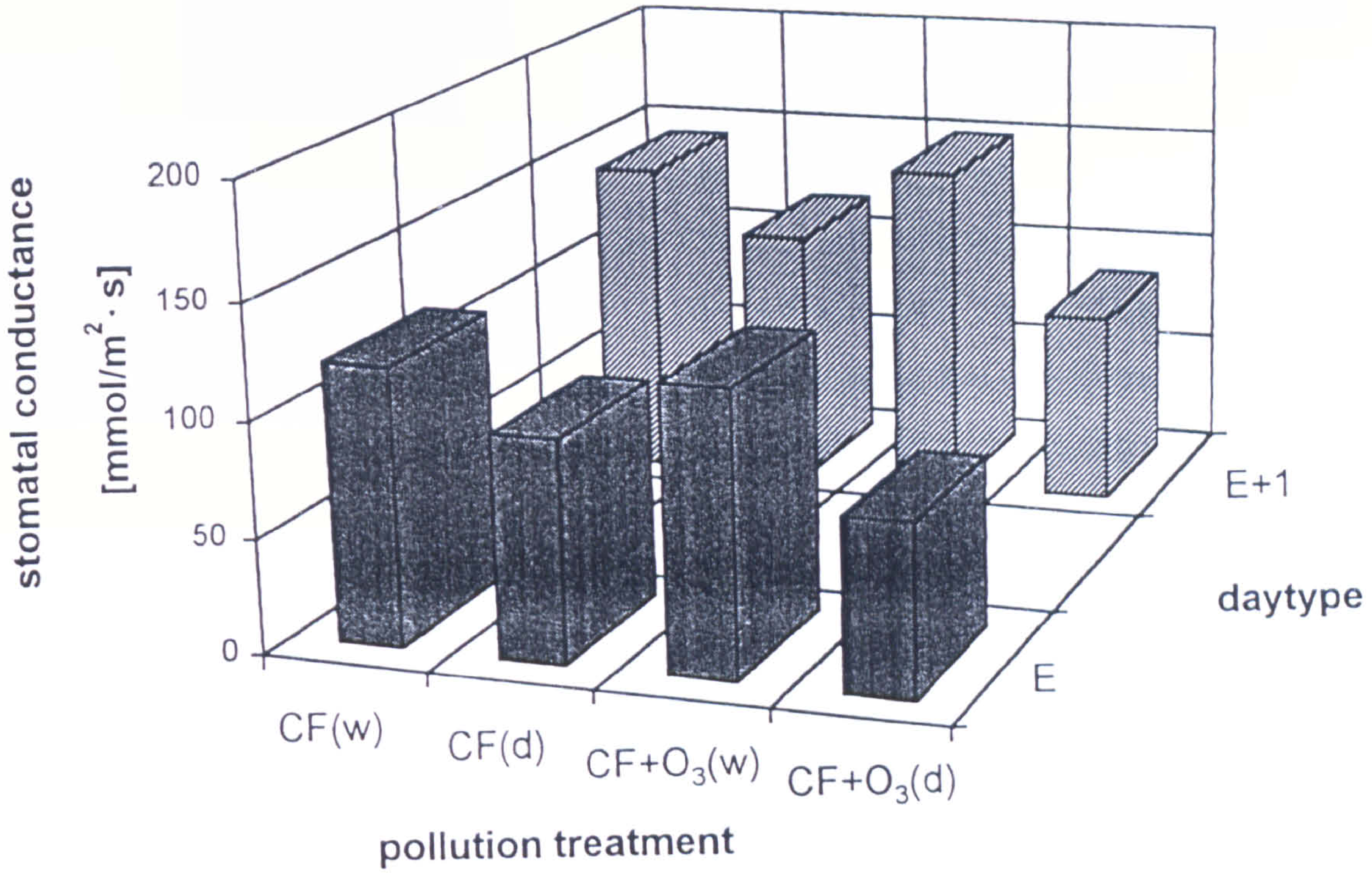

Fig. 16 Means of stomatal conductance of two-year-old saplings, 1994, for exposure days (E) and days following an exposure (E+1). Analysis of variance:

pollution treatment: $P = 0.166$

drought treatment: $P < 0.001$

pollution × drought: $P = 0.024$

	PAR [$\mu E/m^2 \cdot s$]		Leaf temperature [^0C]	
	E	E+1	E	E+1
CF+O_3(d)	330	335	25.8	20.4
CF+O_3(w)	342	299	25.5	20.5
CF(d)	307	276	25.8	20.3
CF(w)	332	247	25.7	20.2

Table 6 Means of PAR and leaf temperature measured concurrently with with every measurement of stomatal conductance shown in fig. 16. Highly significant temperature differences ($P < 0.001$) were found betwen the two daytypes.

Figure 17 shows the daytype means given by this analysis, including the Ambient treatments. For all four daytypes, Ambient(w) showed the highest values of stomatal conductance, while among the three droughted treatments Ambient(d) also exhibited the highest values, although the difference to the other droughted treatments was not as pronounced as that of Ambient(w) to CF(w) and CF+O_3(w). The drought effect, which significantly decreased stomatal conductance ($P < 0.001$), became evident particularly during exposure days (E) and during the days following exposure (E+1). On days without exposure (O) and on two days following an exposure there was little effect of the drought. This was due to the way the drought cycles were imposed, and particularly to the facts that the most severe drought coincided with the exposure episodes, and that plants had to be irrigated immediately at the end of the exposure episodes. Thus the drought on days without exposure was still very mild and two days after an exposure the plants had already been able to recover to some degree from drought stress.

However, statistical analysis revealed a highly significant drought effect ($P < 0.001$) for data sets including and excluding the Ambient treatments. Effects of the pollution treatment were only significant when the Ambient treatments were included in the analysis ($P = 0.001$), since for all daytypes the treatment means from the Ambient treatments, and particularly the Ambient(w) treatment, showed higher stomatal conductance than the chambered treatments. Similarly, the interaction of pollution and drought was only significant when Ambient(d) and Ambient(w) were included ($P = 0.048$), while if the two treatments were excluded from the analysis the interaction was no longer significant at the 95% confidence level ($P = 0.051$).

Similar microclimatic differences were found to those described earlier (table 7). The temperatures were highest on exposure days and least on days without exposure ($P < 0.001$). With the exception of days without exposure, irradiation was less variable between the daytypes but also significantly different ($P = 0.03$).

5.1.2.2 Field-grown trees

From the 1994 measurements, mean stomatal conductances on exposure days and days following an exposure were calculated (fig. 18). The analysis was based on data

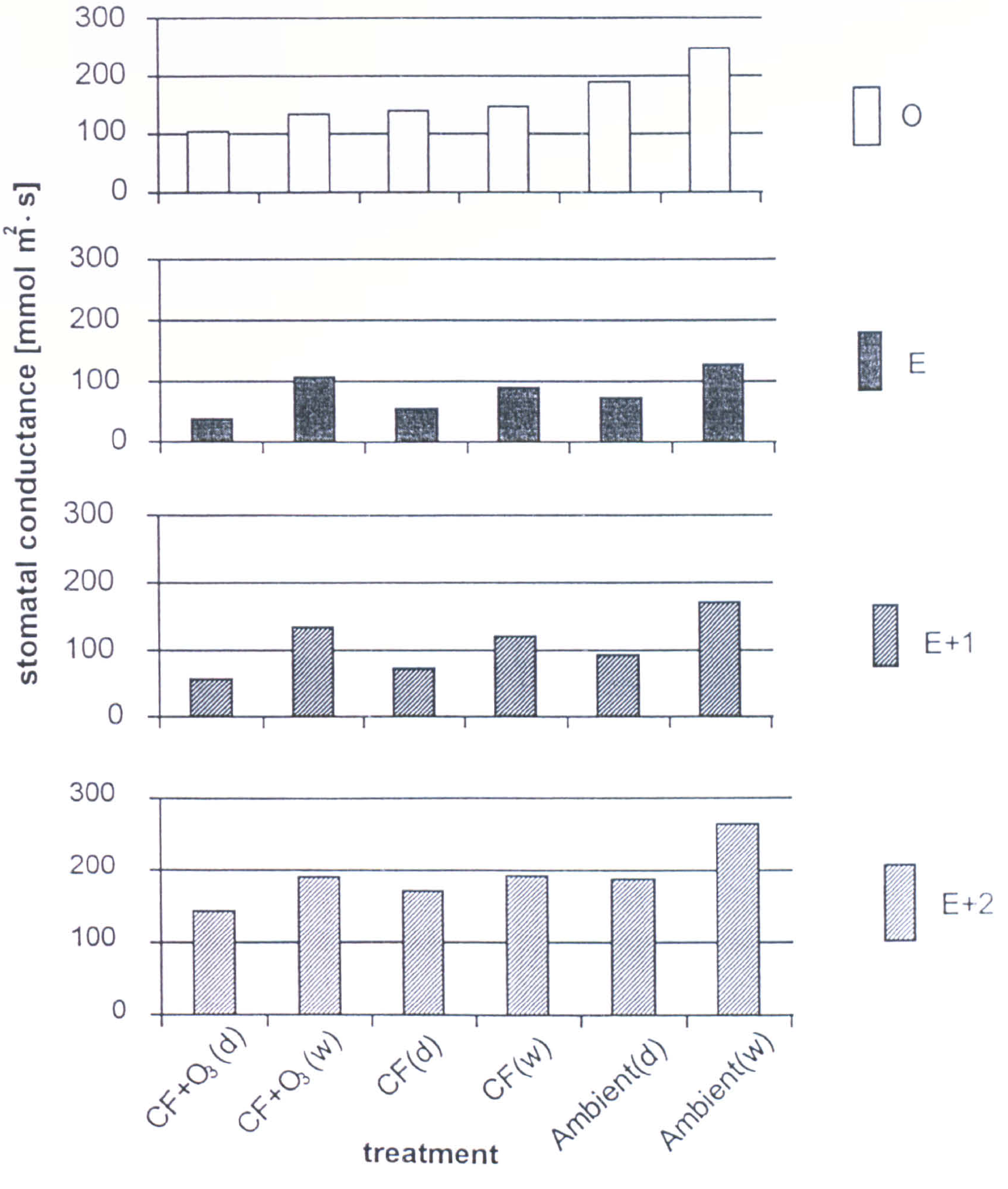

Fig. 17 Means of stomatal conductance of two-year-old saplings, 1994, for four different daytypes. Days not preceded by an exposure for at least six days (O), exposure days (E), days following an exposure (E+1), days following two days after an exposure (E+2).

Analysis of variance including all treatments: pollution treatment: $P = 0.001$; drought: $P < 0.001$; drought × pollution: $P = 0.048$

Analysis of variance including chambered treatments only: pollution treatment: $P = 0.281$; drought: $P < 0.001$; drought × pollution: $P = 0.051$

	PAR [$\mu E/m^2 \cdot s$]			
	0	E	E+1	E+2
CF+O_3(d)	488	333	240	386
CF+O_3(w)	427	369	241	370
CF(d)	509	349	238	358
CF(w)	484	333	243	409
Ambient(d)	681	465	299	678
Ambient(w)	548	487	296	525

	Leaf temperature [^{0}C]			
	0	E	E+1	E+2
CF+O_3(d)	22.0	28.3	23.3	24.6
CF+O_3(w)	21.2	28.1	23.4	24.3
CF(d)	21.1	28.6	23.2	24.5
CF(w)	21.6	28.5	23.1	24.6
Ambient(d)	21.6	27.2	23.4	24.7
Ambient(w)	21.2	27.3	23.5	24.7

Table 7 Means of PAR and leaf temperature measured concurrently with each measurement of stomatal conductance given in fig. 17. Temperature differences between the four different daytypes were highly significant ($P < 0.001$) and differences in irradiation were also significantly different ($P = 0.03$).

sets with 10 measurements per plant and three replicates of each daytype. Although there was a large difference between the Ambient treatment and the two chambered treatments, it was not significant. As for the two year old saplings, there were also differences between the two daytypes. However, while stomatal conductance was lower

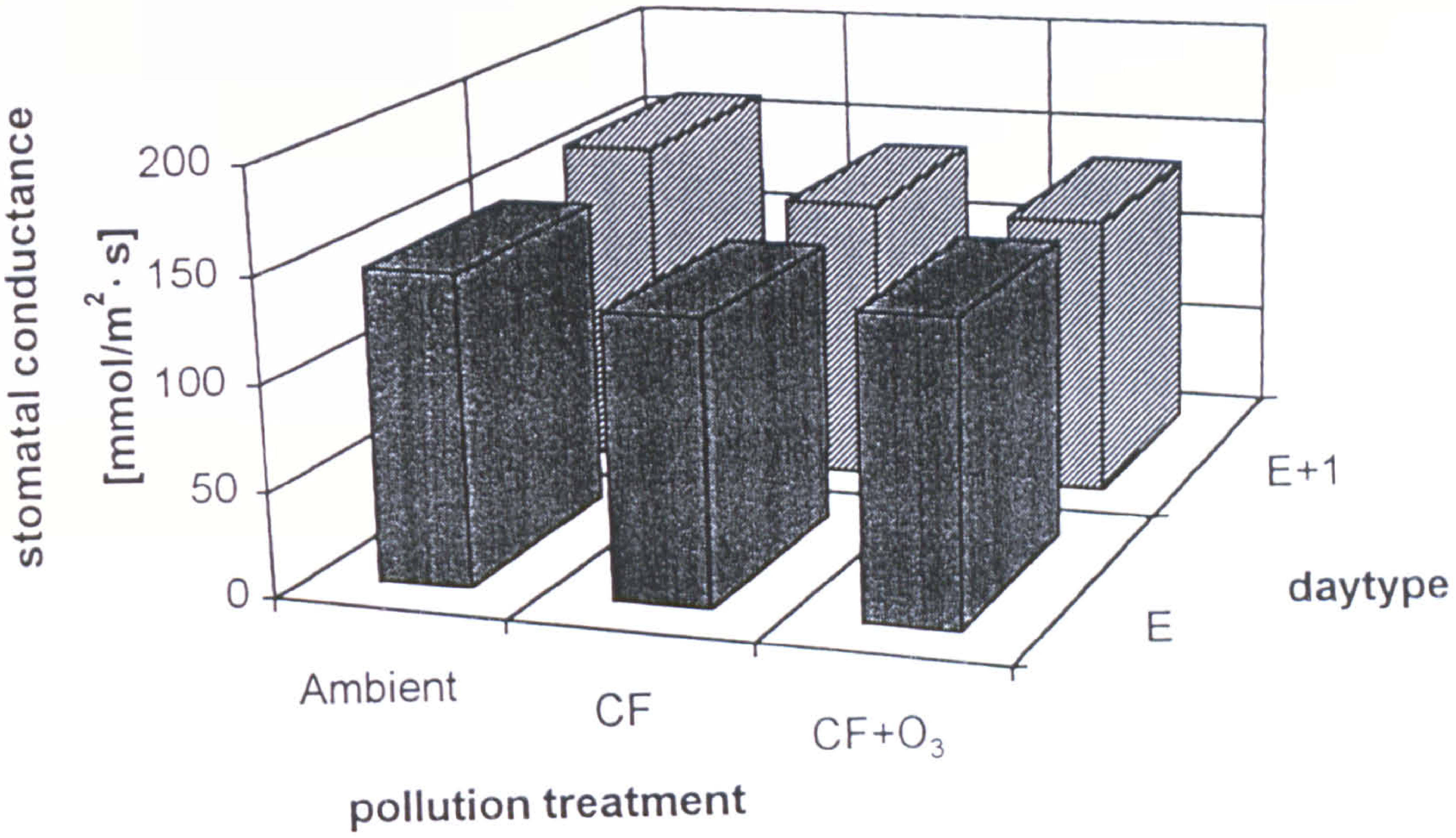

Fig. 18 Means of stomatal conductance of field-grown trees, 1994, for exposure days (E) and days following an exposure (E+1). Differences were not statistically significant.

	PAR [μE/m^2 · s]		Leaf temperature [^{0}C]	
	E	E+1	E	E+1
CF+O$_3$	171	133	22.7	19.1
CF	213	155	23.0	19.0
Ambient	191	158	22.4	18.4

Table 8 Means of PAR and leaf temperature measured concurrently with each measurement of stomatal conductance of the field grown trees given in fig. 18. Temperature differences between the two daytypes were significant (P = 0.006)

for the Ambient and CF treatments on exposure days, it was higher for the CF+O$_3$ treatment.

The means for irradiation and leaf surface temperature (table 8) showed both higher irradiation levels and significantly higher temperatures ($P = 0.006$) for the exposure days.

5.1.3 Diurnal variations of stomatal conductance

The diurnal course of stomatal conductance (fig. 19a) was assessed in individual plots on five days in June and on two days in August 1993. Measurements were taken between 6 and 16 h GMT; it was not possible to start the measurements earlier, since leaves were still moist from dew. The concurrent levels of irradiation and leaf temperature are given in figures 19b and 19c. The measurements on 19 August were taken during an exposure in a $CF+O_3$ plot, while all other assessments of diurnal variations were made in CF and Ambient plots.

The diurnal changes in stomatal conductance were highly variable between days. There were also seasonal differences, with lower stomatal conductance later in the season.

On most days, afternoon values of stomatal conductance were lower than those measured in the mornings. However, this was not true for 1 June and 19 August, although in both cases there were only minimal differences between morning and afternoon. The data show no indication of a midday low in stomatal conductance.

The diurnal courses of irradiation and leaf surface temperature also showed a high variability. Irradiation (fig. 19b) fluctuated very much but in general was highest around midday or in the early afternoon, while leaf surface temperature (fig. 19c) varied less and on most days rose steadily from morning to afternoon.

5.1.4 Comparison of stomatal conductance of different leaf groups

To investigate the differences in stomatal conductance that were due to leaf age and the leaves' state of health, assessments with four leaves of each of the following three leaf groups were made on every plant:

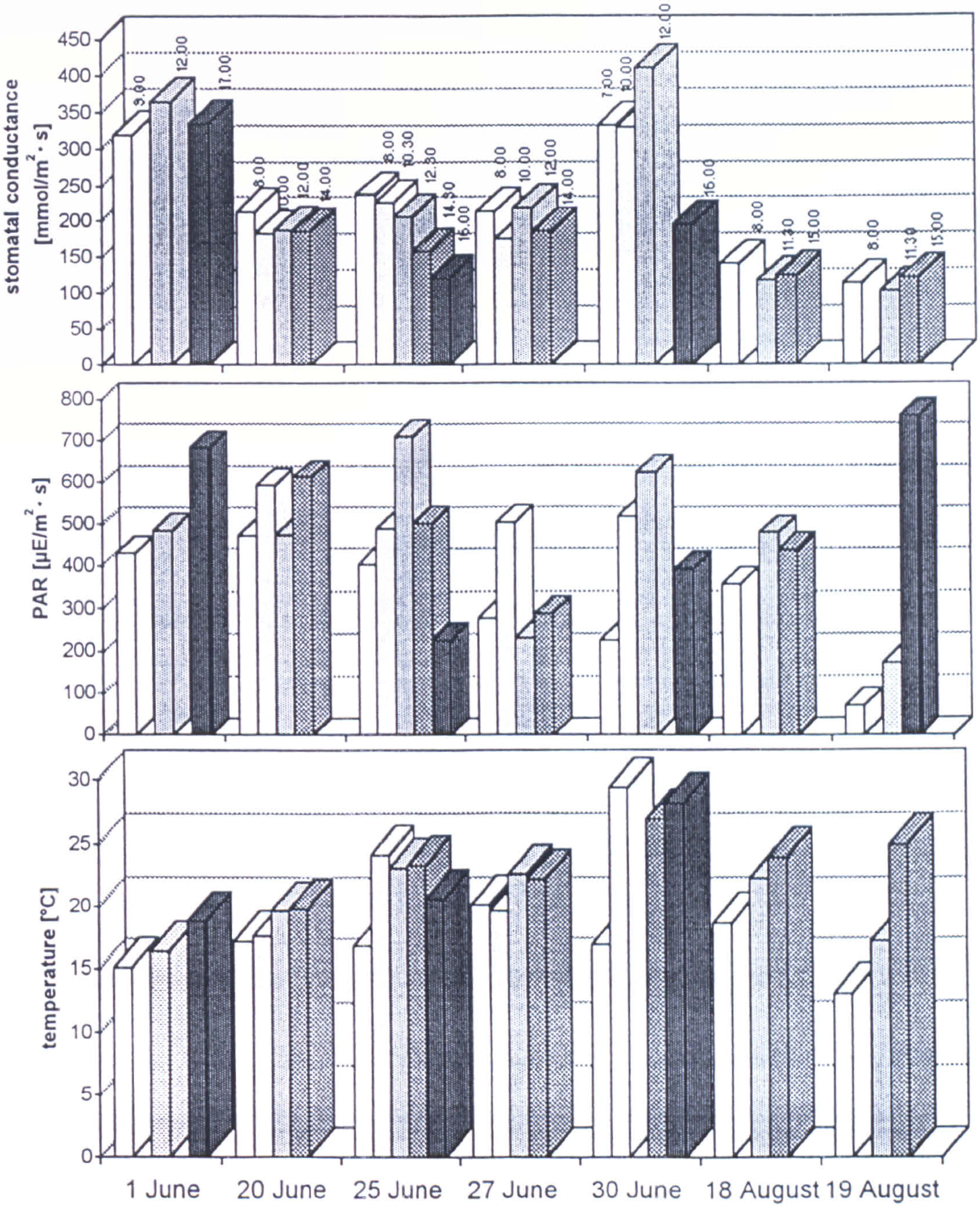

Fig. 19a Diurnal variations of stomatal conductance measured on 7 days in 1993. On each day shown measurements were taken on all plants from one selected plot, which had been chosen randomly. On 19 August measurements were taken in a CF+O_3 plot during a fumigation.

Fig. 19b Diurnal course of irradiance, measured concurrently with stomatal conductance. Hours when the individual measurements were taken are shown in fig. 19a which gives the data for stomatal conductance.

Fig. 19c Diurnal course of temperature measured concurrently with the measurements of stomatal conductance. Hours when measurements were taken are given in fig. 19a.

- mature and healthy
- mature and visibly injured
- young and healthy.

The treatment means for the leaf groups are given in figure 20. Assessments were made on 8 July 1993, two days after an exposure.

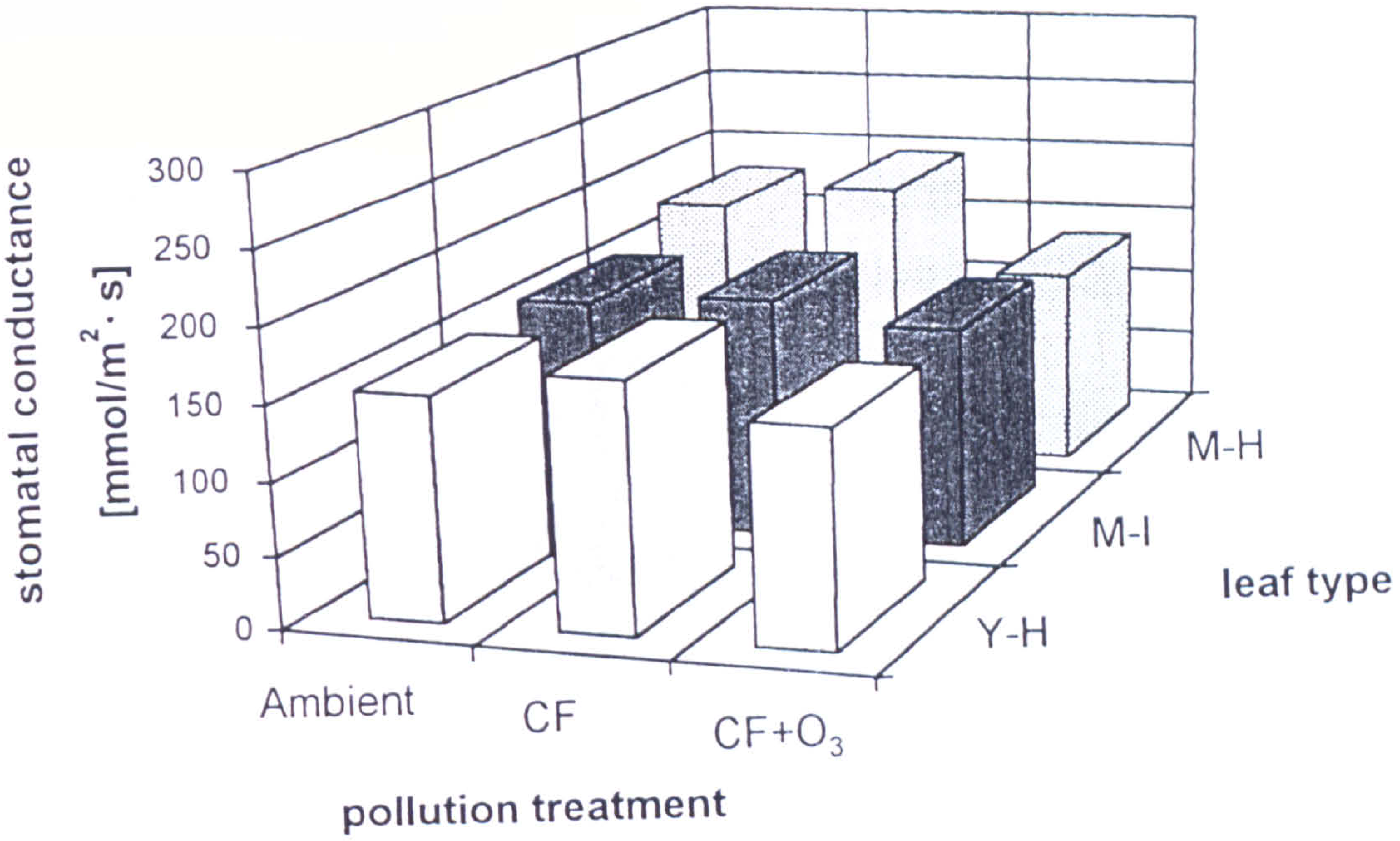

Fig. 20 Means of stomatal conductance of mature-healthy (M-H), mature-visibly injured (M-I) and young-healthy (Y-H) leaves 1993, showing the different response of these leaves to the pollution treatment. Differences were not significant.

There were no significant differences between the treatments for any of the three leaf groups. However, while young-healthy and mature-injured leaves responded similarly, with only minor differences between the three treatments, mature-healthy leaves showed a higher variability. The decrease in stomatal conductance in the CF+O_3 treatment, which was found for all three leaf groups, was strongest in the mature-healthy leaves, particularly since values for CF and Ambient leaves were higher than for the other two leaf groups. Thus, for the CF and Ambient treatments, mature-healthy leaves showed higher stomatal conductance than young-healthy and mature-injured leaves, but lower values for the CF+O_3 treatment.

5.1.5 Summary of stomatal conductance results

- In 1993, when assessments of stomatal conductance of both age groups started early in the season, values were high at the beginning, but declined soon and stabilized at lower values.
- In 1993 the drought treatment was not effective and did not cause any alterations to stomatal behaviour, but in 1994 a decrease in stomatal conductance was induced by drought stress. After the drought episodes were finished, only the CF(d) treatment recovered to a value of stomatal conductance similar to the well-watered control. In contrast, the CF+O_3(d) treatment showed a decrease in stomatal conductance.
- Comparison of chambered treatments only showed a highly significant decrease of stomatal conductance caused by drought and a further decrease, also significant, induced by the drought-pollution interaction.
- When all three pollution treatments were included in the analysis, the Ambient treatment was shown to have the highest stomatal conductances on all daytypes investigated.
- In both years the mature trees showed less variability between treatments than the two-year-old saplings.

5.2 Growth parameters

During both years of the study, assessments of extension growth, radial growth at the stembase and radial growth at the base of the new shoot were made on the two-year-old potted saplings. Measurements were taken regularly every second week throughout both seasons.

In the figures showing the growth increases over the season, the number of fumigations is plotted on a second y-axis.

Cumulative seasonal treatment means were calculated for each growth parameter as well as mean relative growth rates. The growth rates were determined on a seasonal basis, as well as for the first and the second half of the season.

Due to the ineffectiveness of the drought stress imposed in 1993, the analysis and presentation of the growth data does not include a distinction between the droughted and the well watered treatments, which in this case were considered as one.

5.2.1 Extension growth

5.2.1.1 Seasonal course of extension growth

Extension growth (figs. 21, 22a,b) was fastest at the beginning of the season due to the intense flushing in spring.

In 1993 (fig. 21), extension growth declined rapidly and remained low until the beginning of July, when it declined further. From August onwards extension growth was minimal. Apart from the measurements on 6 July, when extension growth of the ozone treatment was almost twice that of the other two treatments ($P = 0.014$), there were no major differences between the treatments during the 1993 season.

In 1994 there were marked differences between the well watered and the droughted treatments, and thus the data for the seasonal course of extension growth are shown in the two separate figures 22a,b. The general course of the growth increase was similar to that in 1993. However, for both treatments the slowing down of growth was later in the season than it had been the year before and accordingly extension growth lasted

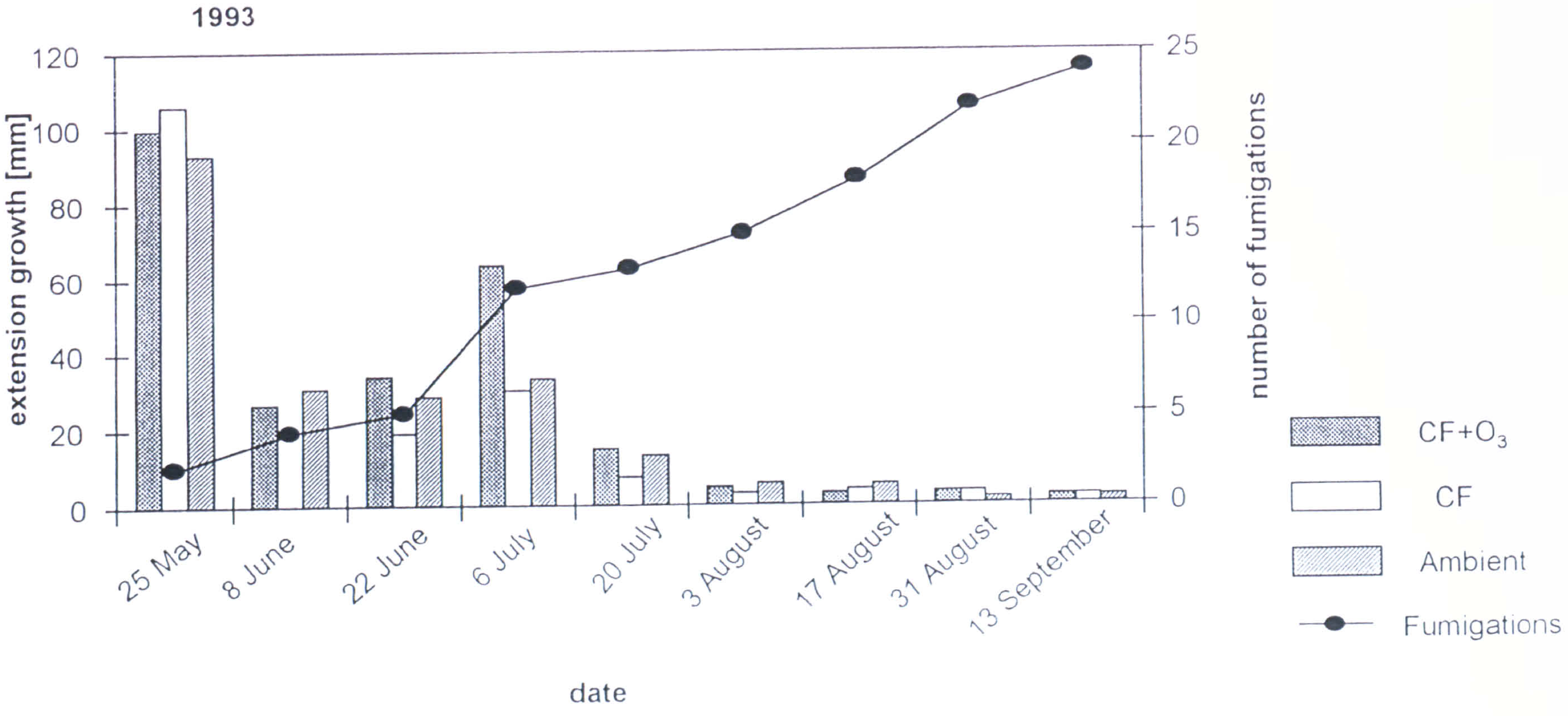

Fig. 21 Seasonal course of extension growth of two-year-old saplings, 1993 (left axis) and course of exposures (right axis)

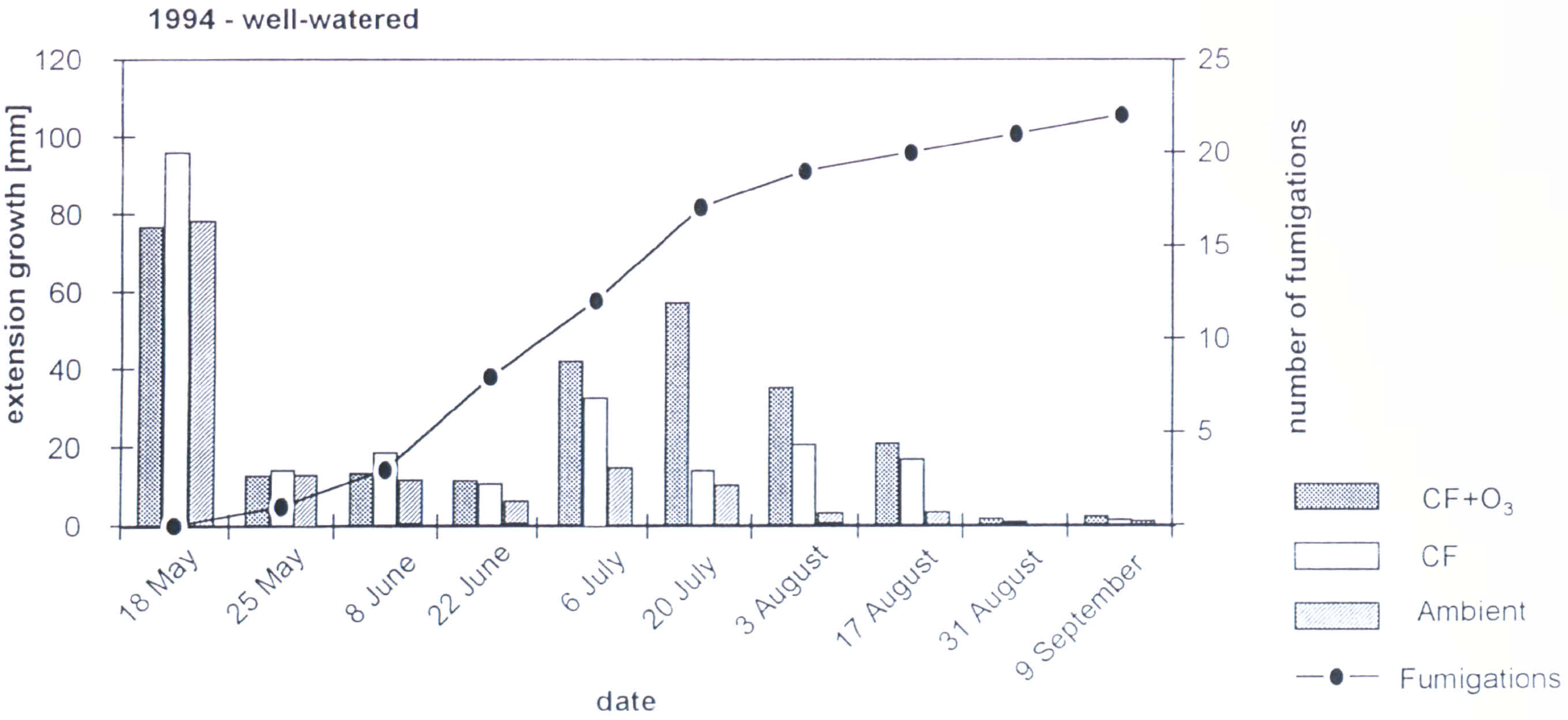

Fig. 22a Seasonal course of extension growth of well-watered saplings, 1994 (left axis) and course of exposures (right axis)

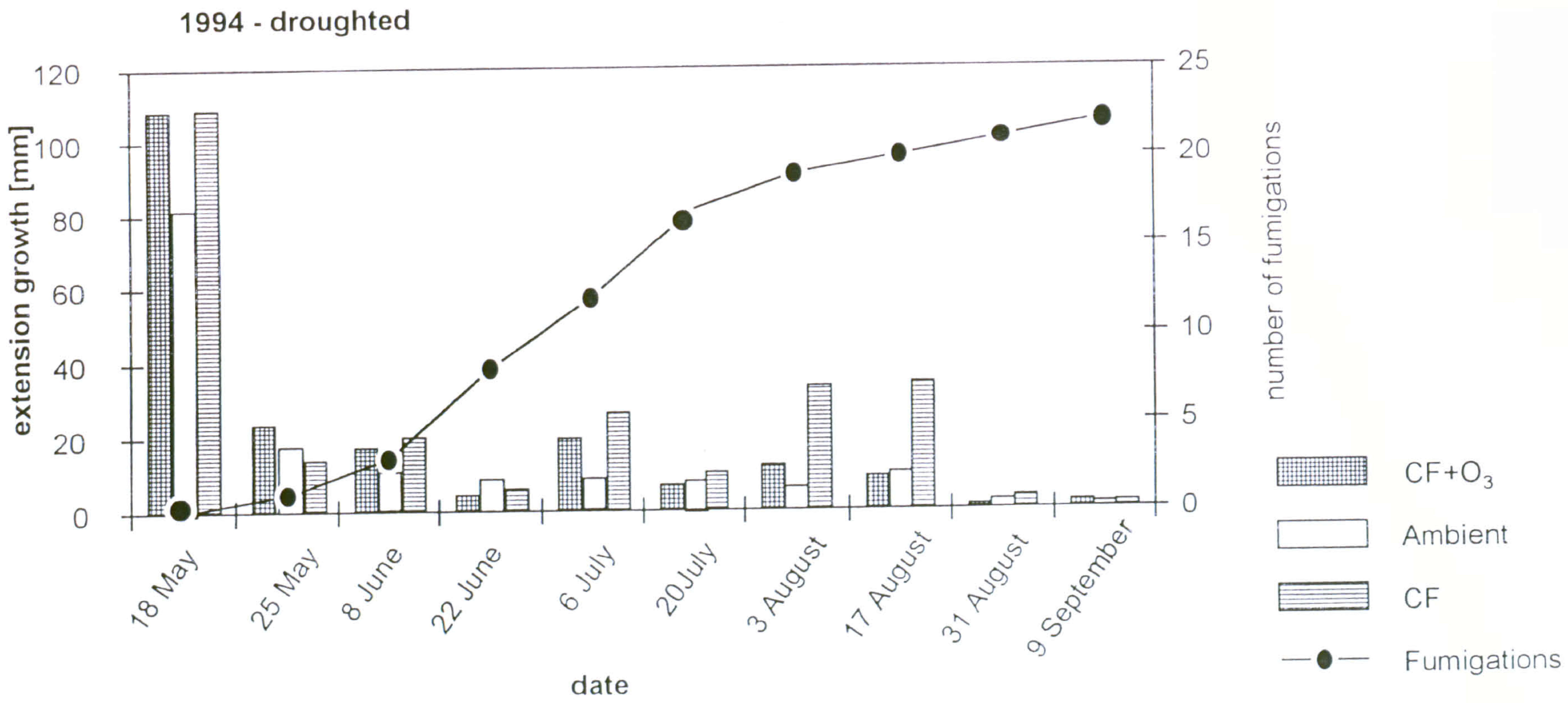

Fig. 22b Seasonal course of extension growth of droughted saplings, 1994 (left axis) and course of exposures (right axis)

until mid-August. Both the droughted and the well watered plants showed greater differences between treatments than had been found in 1993.

The strength of the flush in spring was slightly higher for the plants of the droughted treatment. This was probably due to endogenous factors, since neither the drought nor the pollution treatment had been started at that time. Until the drought treatment was imposed at the beginning of June, the growth increase was similar for plants from both treatments. After the drought stress was imposed, extension growth was reduced in this treatment as compared to that of the well watered plants.

Among the well watered plants (fig. 22a), the CF+O_3(w) and also the CF(w) treatment showed high values of extension growth during mid-summer, while those of Ambient(w) stayed at the low level they had dropped to after the initial growth flush. However, differences among treatments were only significant for 20 July ($P = 0.004$), and 3 August ($P = 0.03$).

For the droughted plants, no statistically significant differences between treatments could be found, although on 3 and 17 August growth of CF(d) was strongly increased, as compared to the other two droughted treatments.

During mid-summer, differences between the droughted and well watered CF+O_3 treatments were much more pronounced than those between the different irrigation treatments of CF and Ambient.

5.2.1.2 Cumulative seasonal means of extension growth

In general, values of mean seasonal extension growth were in the same range in both years of the experiment (figs. 23, 24), and furthermore in both years the Ambient treatment showed the least height increment.

In 1993 (fig. 23) extension growth of both chambered treatments was higher than that of the unchambered Ambient treatment. However, extension growth in the CF+O_3 treatment was still distinctly higher than that in the CF treatment, the mean difference between the two being almost 40 mm.

Statistical analysis showed this difference to be significant, not only when the

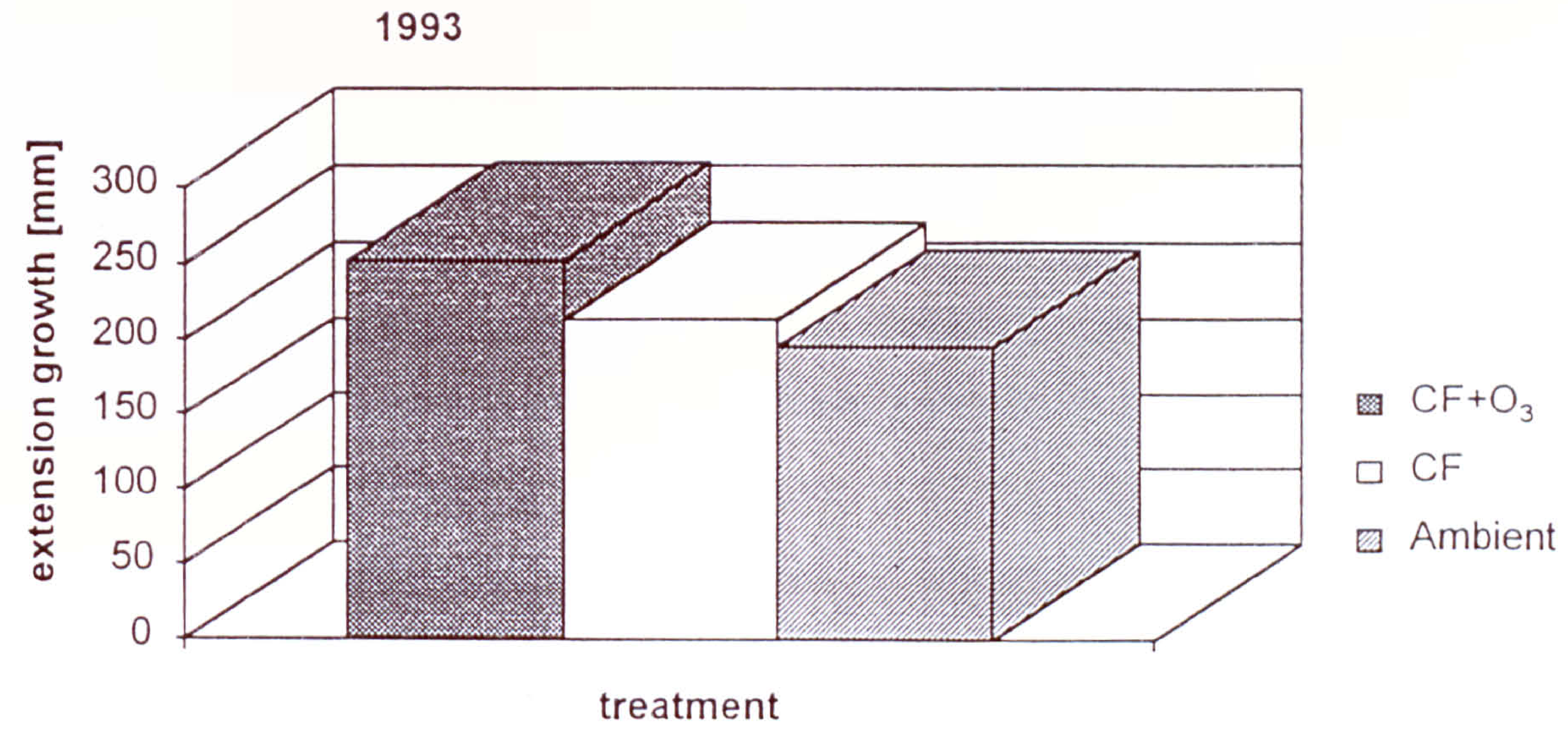

Fig. 23 Means of seasonal extension growth of two-year-old saplings, 1993. Statistical analysis: including all treatments: P = 0.001; excluding Ambient: P = 0.005.

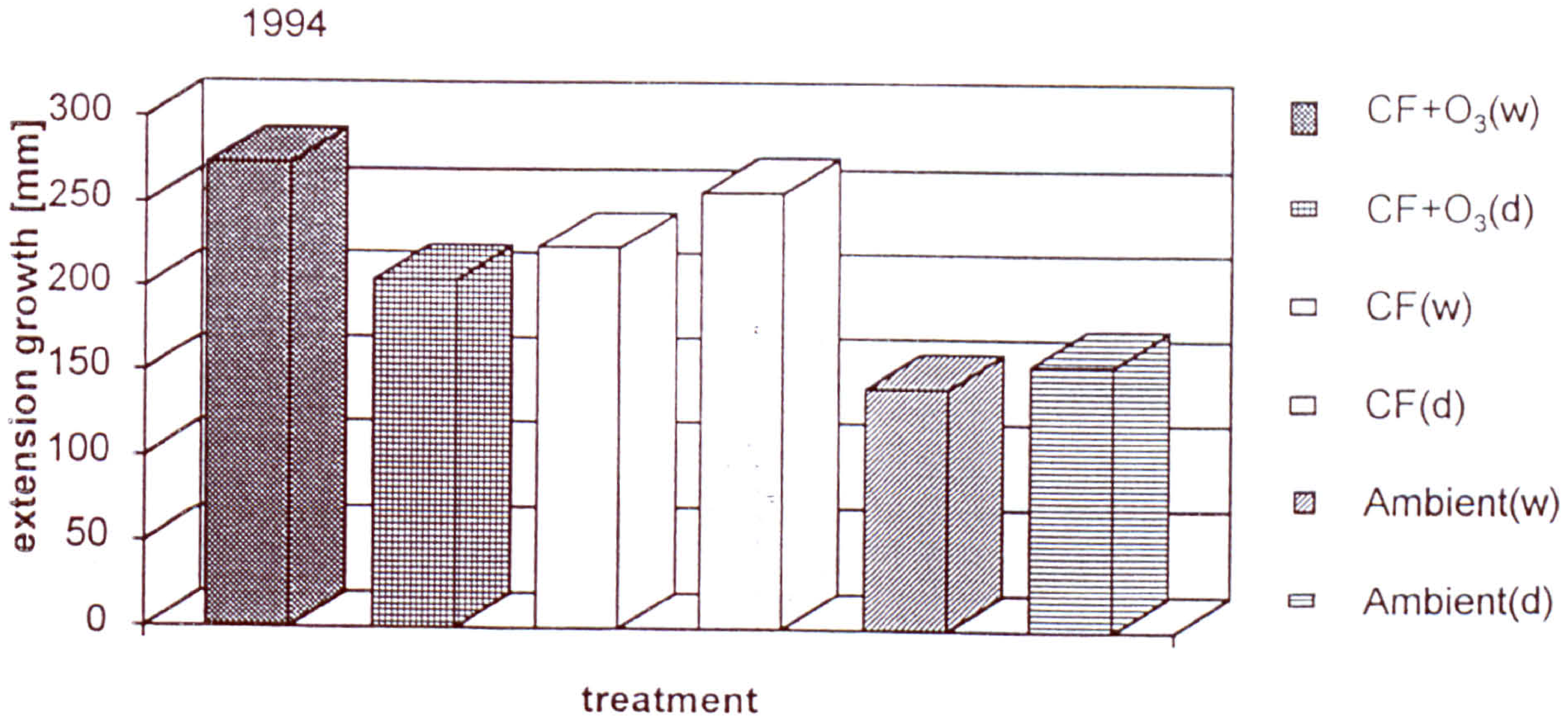

Fig. 24 Means of seasonal extension growth of two-year-old saplings, 1994. Analysis of variance including all treatments: P = 0.002.

Ambient treatment was included (P = 0.011), but even more so when it was excluded (P = 0.005), and only chambered treatments that had experienced similar microclimatic conditions were taken into account.

In 1994 (fig. 24), both Ambient treatments showed distinctly lower values of extension growth than those shown by the chambered treatments, the difference being almost 100 mm. Between Ambient(w) and Ambient(d) there was only a marginal difference, with Ambient(w) showing the lower growth.

The chambered treatments showed values of mean seasonal extension growth between 204 mm [CF+O_3(d)] and 274 mm [CF+O_3(w)]. While for the CF treatments CF(d) showed better seasonal growth than CF(w), CF+O_3(d) grew less than CF+O_3(w).

Of the described effects, only the pollution treatment difference between the chambered and the Ambient treatment was statistically significant (P = 0.002) in 1994.

5.2.1.3 Relative growth rates of extension growth

The mean relative growth rates of all plants for 1993 are given in figure 25 for the first and the second half of the growing season, as well as for the whole season.

During the first half of the season, the relative growth rates differed markedly between the treatments, and this also affected the relative growth rates calculated for the whole season. In contrast, differences between the lower growth rates found for the second half of the season were only marginal.

Statistical analysis that included all three treatments (CF, CF+O_3, Ambient) revealed significant differences only for data from the first half of the season (P = 0.031), while, when data from the Ambient treatment were excluded from the analysis, significant differences were found for the first half of the season (P = 0.017), as well as for the seasonal mean relative growth rate (P = 0.026).

From the 1994 data set, relative growth rates were calculated separately for the well watered and the droughted treatments. The means are given in figure 26.

Treatment means for both irrigation treatments were similar and did not show general trends for either the droughted or the well watered plants. During the first half

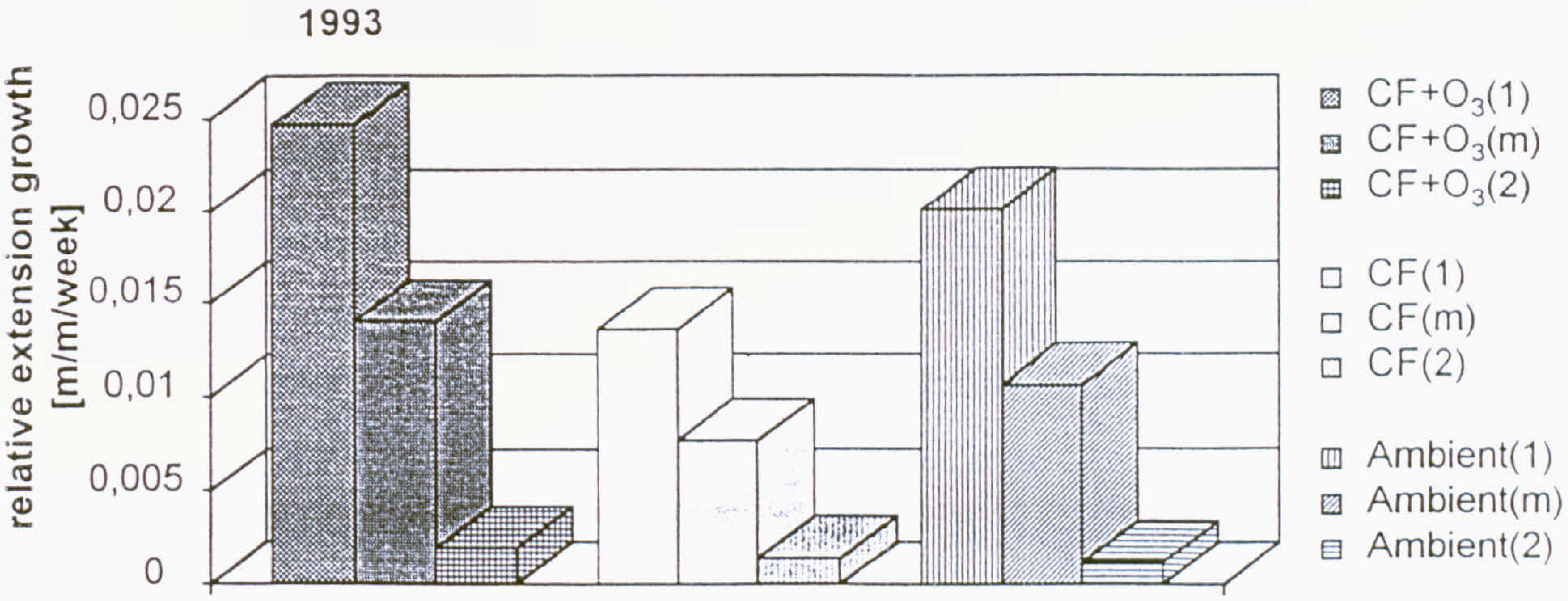

Fig. 25 Mean relative growth rates of extension growth of the two-year-old saplings for the first (1) and the second (2) part of the 1993 season and for the seasonal mean (m). ANOVA including all treatments: (1) pollution effect: P = 0.031; excluding ambient: (1) pollution effect: P = 0.017 and (m) pollution effect: P = 0.026

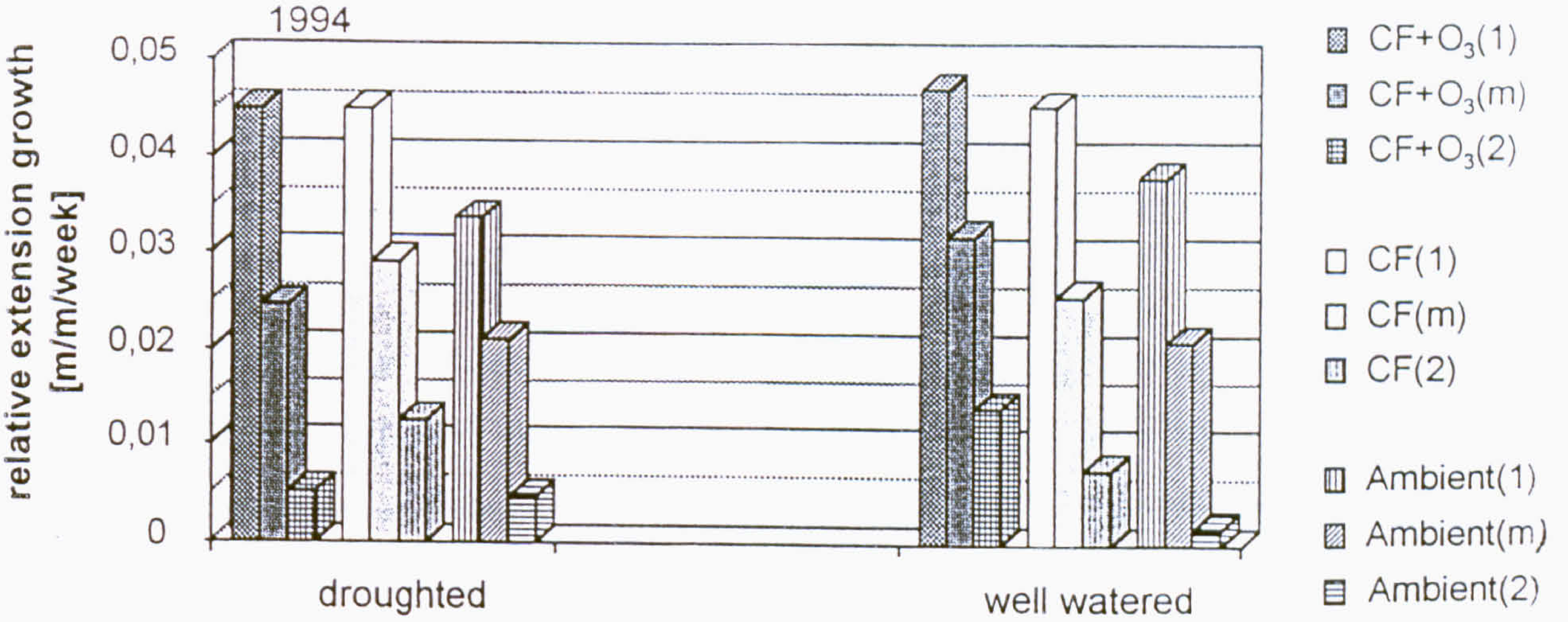

Fig. 26 Mean relative growth rates of extension growth of two year old saplings, 1994. ANOVA: All treatments: (1) pollution: P = 0.032; (m) pollution: P = 0.010; excluding Ambient: (m) pollution × drought: P = 0.035.

of the season, both Ambient treatments had lower relative growth rates than the CF and CF+O_3 treatments.

Although differences between treatments during the second part of the 1993 season were only marginal, those measured in 1994 were pronounced and strongly influenced the variability of the seasonal mean growth rates.

Due to the splitting into well watered and droughted treatments, statistical analysis gave information about the pollution treatment differences and on the irrigation differences, as well as on the interaction of the two factors.

If all three pollution treatments were included into the analysis, significant pollution treatment differences could be found for the first half of the season ($P = 0.032$) and for the seasonal mean relative growth rates ($P = 0.01$). However, when Ambient(w) and Ambient(d) were excluded from the split plot analysis, no further treatment differences could be found, but a drought × pollution interaction became evident ($P = 0.035$) for the seasonal mean growth rates.

5.2.2 Radial growth at the stembase

5.2.2.1 Seasonal course of radial growth at the stembase

The course of radial growth (figs. 27, 28a,b) differed from that of extension growth. After low spring values it peaked around mid-summer and decreased again to the lower level of growth found for the beginning of the season, and the differences between treatments were less pronounced than for extension growth.

In 1993 the highest values of radial increment were found for 6 and 20 July, after which date radial growth decreased again. Differences between the treatments were only significant on 20 July ($P = 0.033$), when CF showed the greatest and CF+O_3 the least increase.

Throughout most of the 1994 season, the treatment means showed lower radial growth for the droughted treatments (fig. 28a) as compared to their well watered counterparts (fig. 28b). Only before the start of the drought treatment at the beginning of June did both irrigation treatments exhibit the same level of growth.

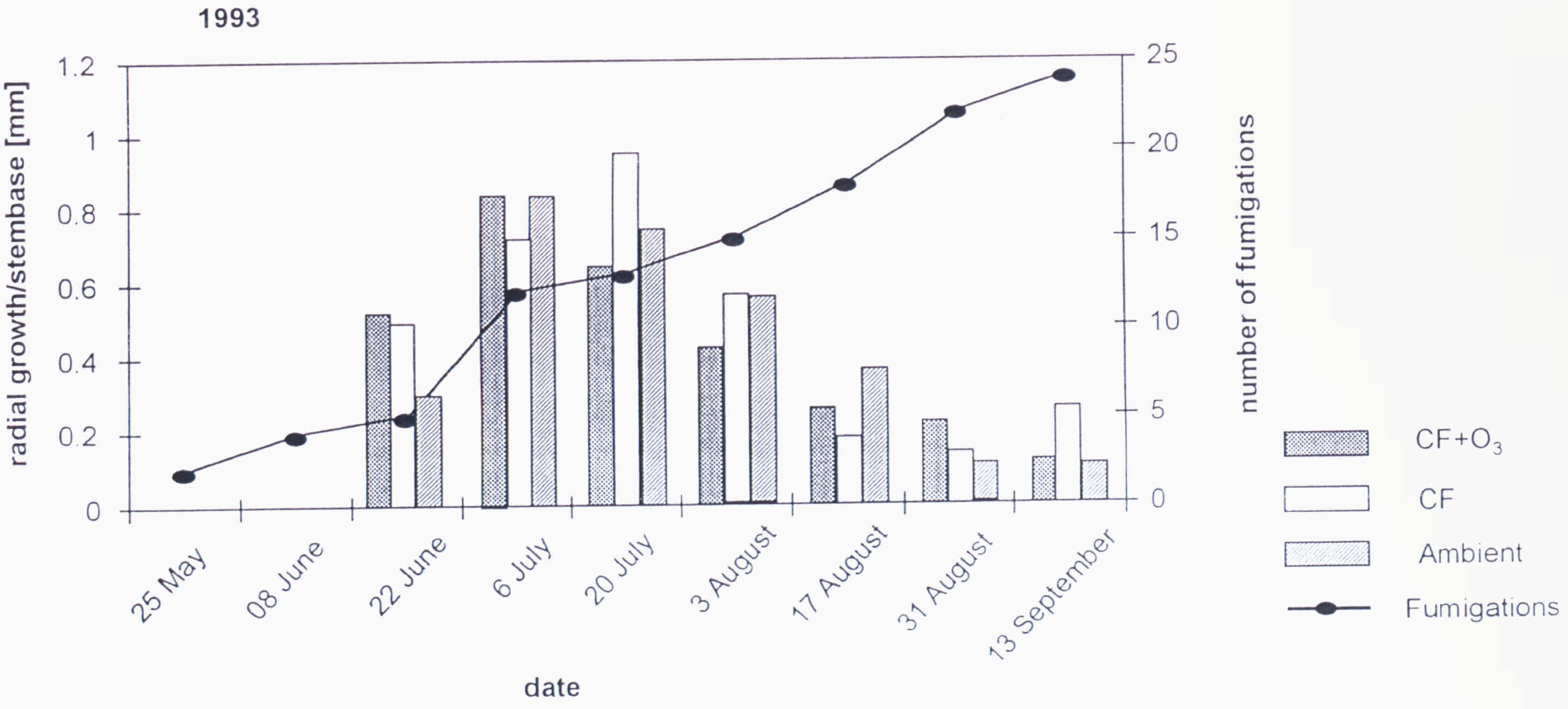

Fig. 27 Seasonal course of radial increment at the stembase of two-year-old saplings, 1993 (left axis) and course of exposures (right axis)

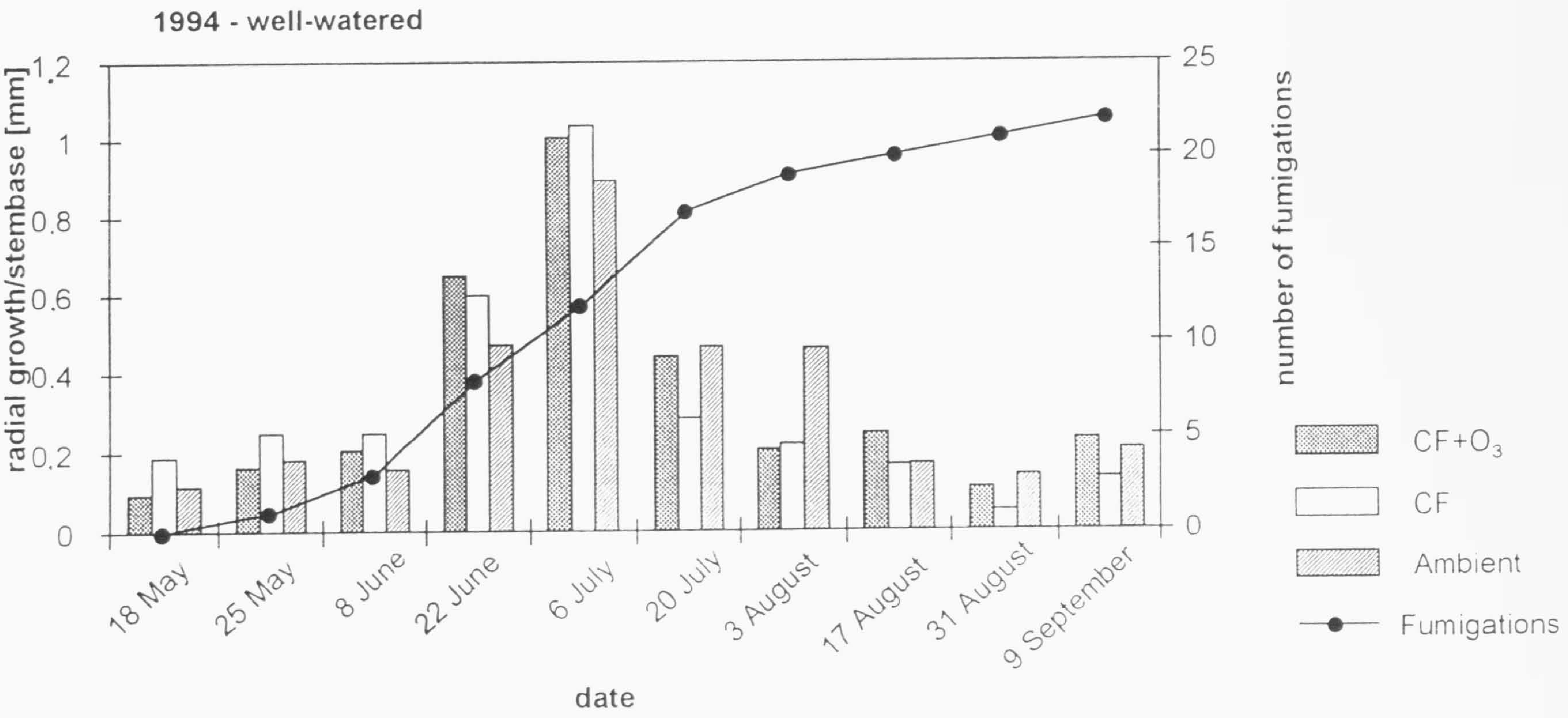

Fig. 28a Seasonal course of radial increment at the stembase of well-watered saplings, 1994 (left axis) and course of exposures (right axis)

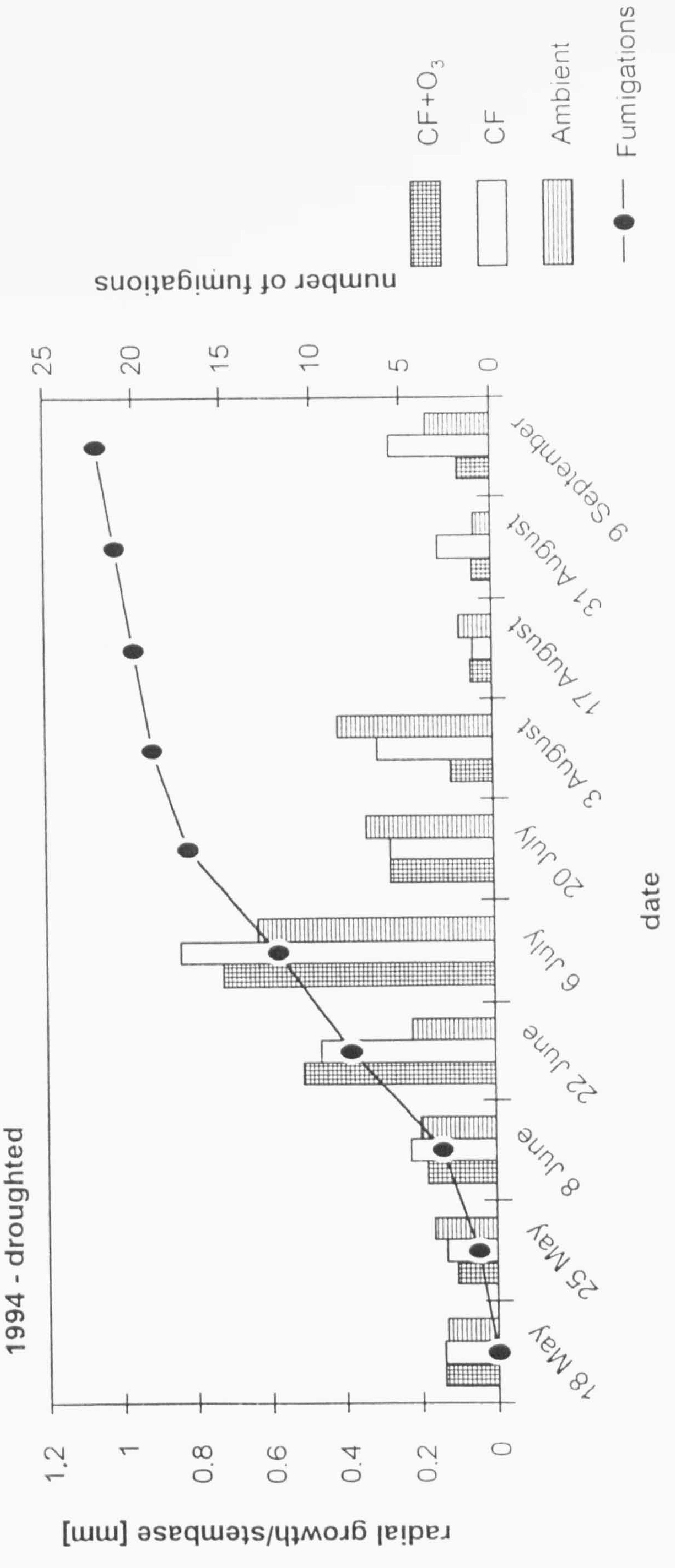

Fig. 28b Seasonal course of radial increment at the stembase of droughted saplings, 1994 (left axis) and course of exposures (right axis)

While at the beginning of the season, CF(w) showed the greatest radial growth among the well watered treatments, its growth later in the season was mostly lower than that of CF+O_3(w) and Ambient(w). Statistically significant differences were only found for 3 August, when the radial increment of Ambient(w) was more than twice that of the chambered treatments.

Among the droughted treatments, CF(d) exhibited a stronger radial growth throughout most of the season, and particularly at the end. However, statistically significant differences at the 95% confidence level were found only for 22 June, when the radial increment of Ambient(d) was decidedly lower than the values of CF(d) and CF+O_3(d). On 3 August and 9 September, radial growth of CF+O_3(d) was far lower, although not significantly lower than that of the other two droughted treatments (both: $P = 0.061$).

5.2.2.2 Cumulative seasonal means of radial growth at the stembase

The cumulative seasonal means of radial growth at the stembase (figs. 29, 30) showed similar trends for both years of the investigation. In 1993 the CF treatment exhibited the highest growth increment. In 1994, when the droughted treatments showed distinctly lower growth increments than their well watered control treatments, a similar pattern was found among the droughted treatments, while differences among the well-watered treatments were negligible.

In 1993 (fig. 29), no significant differences between treatments were evident. However, the seasonal radial increment of the CF treatment was markedly higher than those of Ambient and CF+O_3.

In 1994 (fig. 30), the seasonal radial growth of the well watered plants from all three pollution treatments was much greater than that of the droughted plants. However, while differences among the well watered treatments were only marginal, those among the droughted treatments were up to 0.6 mm and thus were about one fourth of the whole seasonal increment. Differences between the well watered and the droughted plants were highest in the CF+O_3 treatment, where CF+O_3(w) showed the highest

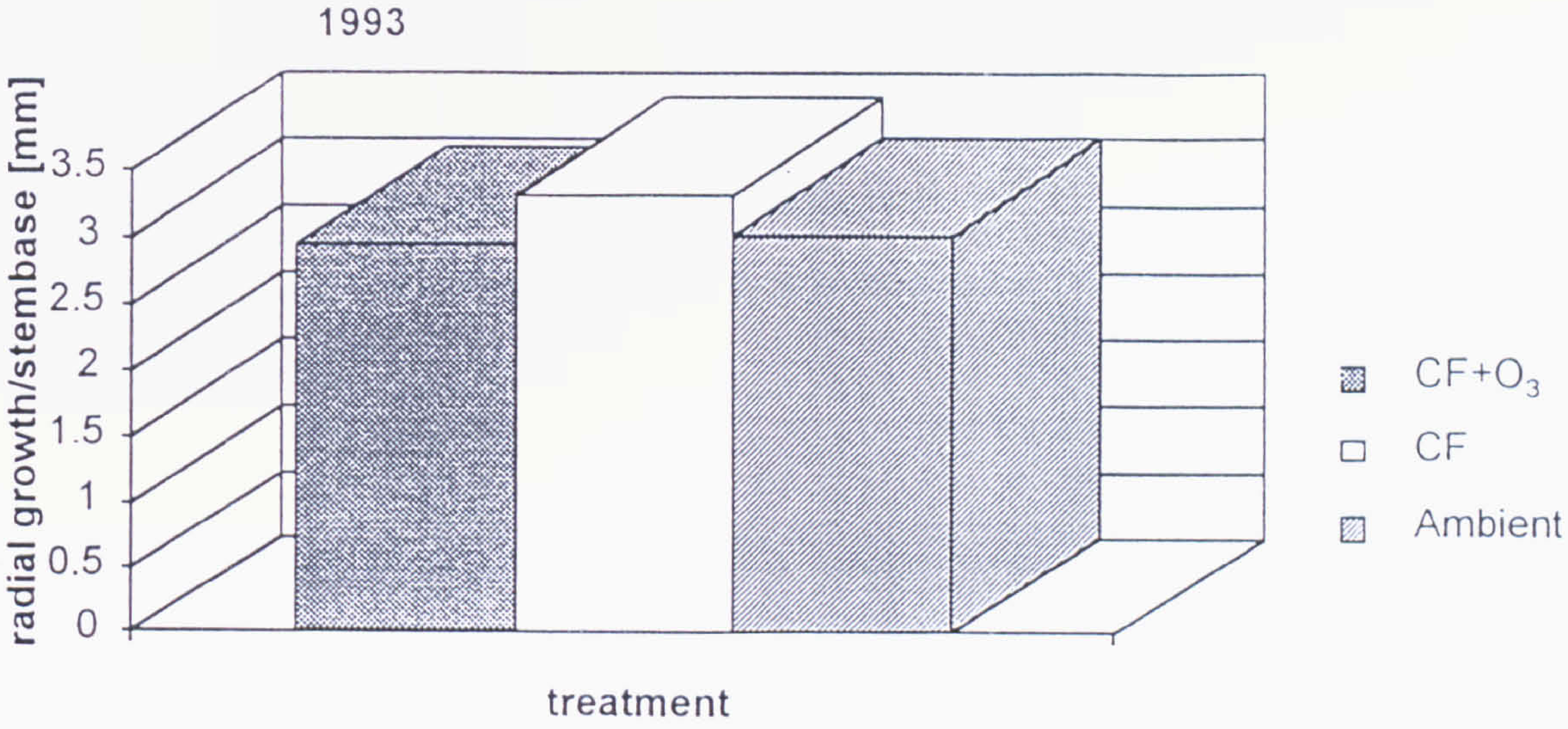

Fig. 29 Means of seasonal radial increment at the stembase of the two-year-old saplings, 1993. Treatment differences were not significant.

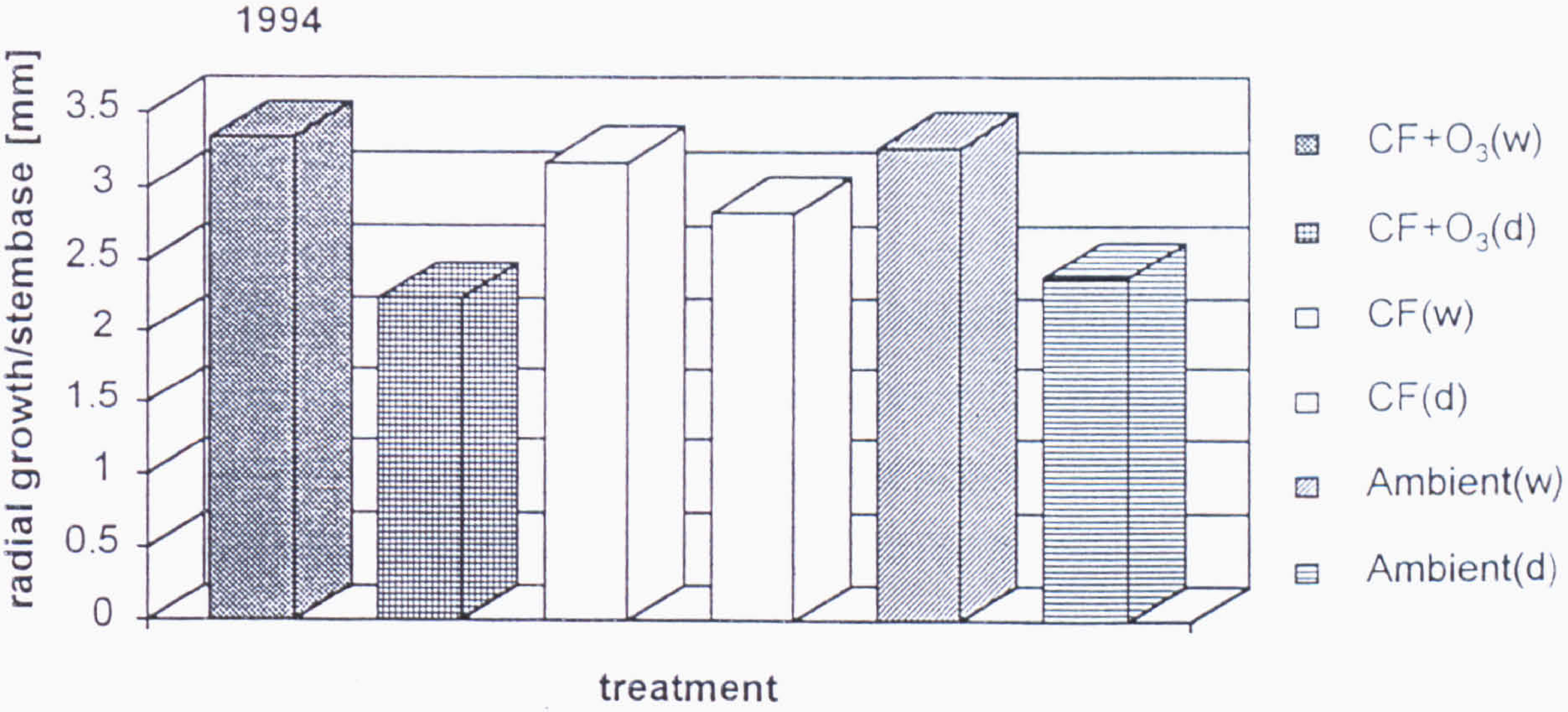

Fig. 30 Means of seasonal radial growth at the stembase of the two-year-old saplings, 1994. Analysis of variance including all treatments: drought effect: $P < 0.001$; excluding Ambient: drought effect: $P = 0.002$.

radial growth of all treatments, while CF+O_3(d) had the lowest radial increment. The opposite situation was found for the CF treatment, where CF(w) showed the lowest radial growth at the stembase among all well watered treatments, while radial growth of CF(d) was best of the droughted treatments.

Only the drought effect was statistically significant (incl. Ambient: $P < 0.001$; excl. Ambient: $P = 0.002$).

5.2.2.3 Relative radial growth rates at the stembase

Mean relative growth rates for radial growth at the stembase (figs. 31 and 32) were less variable than those found for extension growth.

In 1993 (fig. 31) the greatest variabilty between treatments was found for the first half of the season, while during the second half differences were less pronounced. Since during the second part of the season, differences between treatments were opposite in direction to those found during the first months, the seasonal mean relative growth rates were similar.

Significant differences between treatments became evident from statistical analysis if the Ambient treatment was excluded, and then only for the first part of the season ($P = 0.029$).

In 1994 (fig. 32), there was little variability between the well-watered treatments during the first half of the season and also between the seasonal means. However, during the second part of the season the relative growth rate of Ambient(w) was far higher than those of CF(w) and CF+O_3(w). Among the droughted treatments, radial growth at the stembase was more variable. Here the CF treatment performed best during the first part of the season, while during the second part CF(d) and Ambient(d) showed almost identical values and those of CF+O_3(d) were strongly reduced.

When all three treatments were included, statistical analysis of tha data revealed a highly significant drought effect for the first half of the season ($P = 0.003$) and for the seasonal mean ($P = 0.001$). When the Ambient treatments were excluded from the analysis, the drought effect found for the first half of the season ($P = 0.018$) and

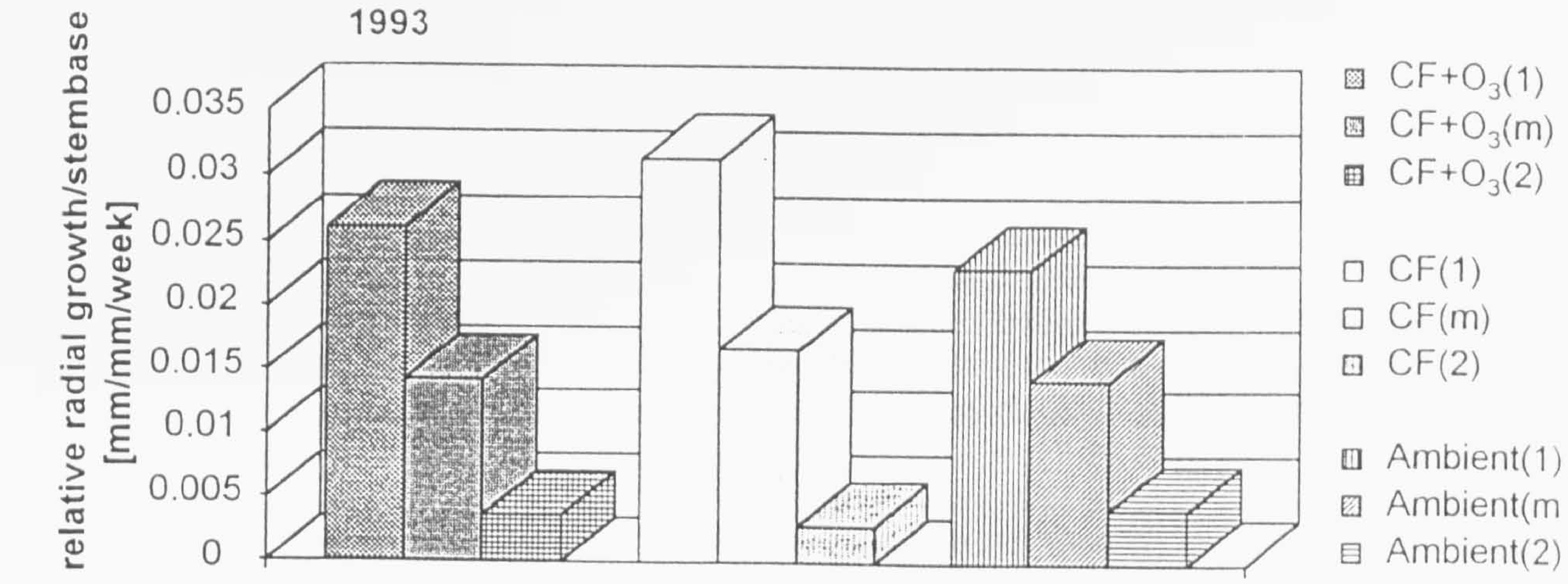

Fig. 31 Mean relative growth rates of radial growth at the stembase of two-year-old saplings, 1993, for the first (1) and the second (2) part of the season and for the seasonal mean (m). ANOVA excluding Ambient: (1) pollution effect: $P = 0.029$.

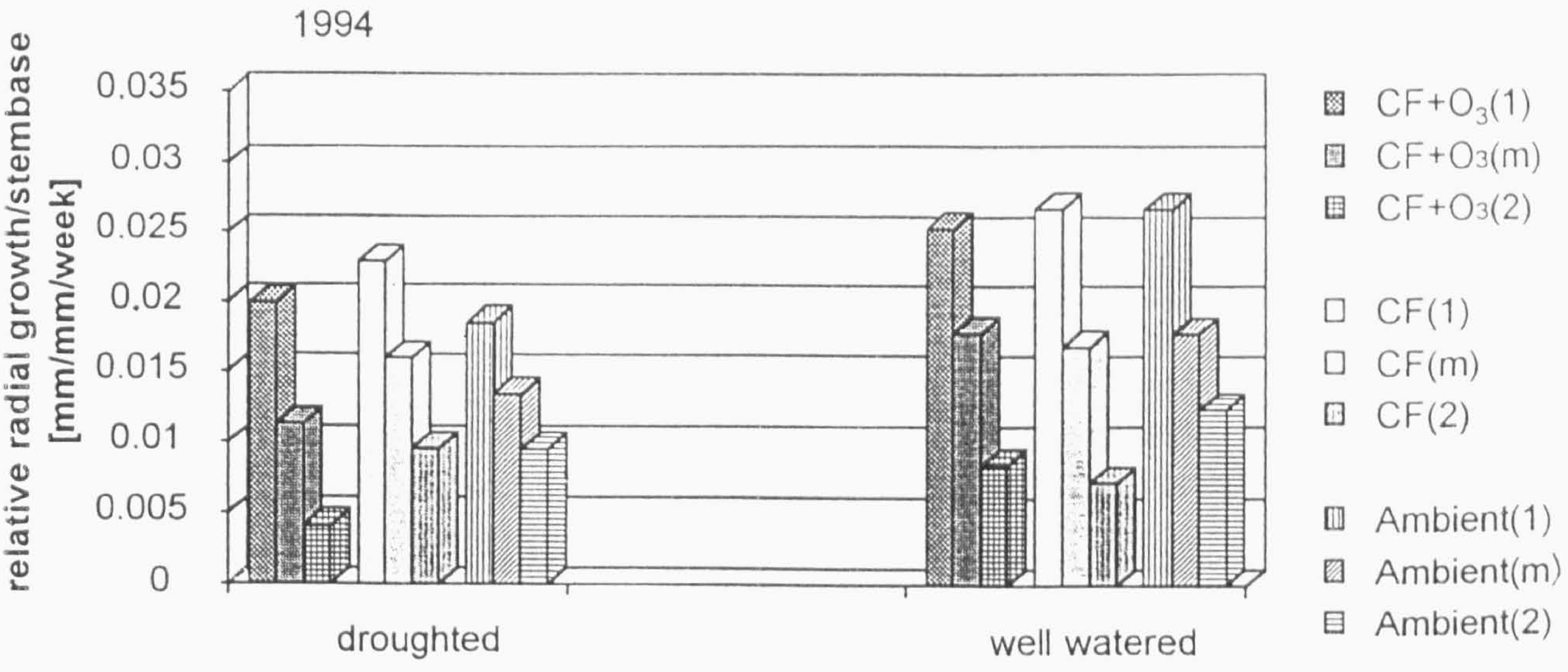

Fig. 32 Mean relative growth rates of radial growth at the stembase of two-year-old saplings, 1994. ANOVA: All treatments: (1) drought effect: $P = 0.003$; (m) drought effect: $P = 0.001$; (2) pollution effect: $P = 0.008$; excluding Ambient: (1) drought effect: $P = 0.018$; (m) drought effect: $P = 0.005$, pollution × drought: $P = 0.030$.

for the seasonal mean ($P = 0.005$) were still significant, and a drought × pollution interaction also became evident ($P = 0.03$).

5.2.3 Radial growth at the base of the new shoot

5.2.3.1 Seasonal course of radial growth at the base of the new shoot

For 1993, mean radial growth at the base of the new shoot (fig. 33) is shown only from 6 July onwards, as earlier measurements proved difficult. The first values taken were already the maximum radial increment found for the base of the new shoot in 1993. Values were little lower than 0.8 mm and thus did not reach the maximum growth increase found for the stembase in 1993. After 6 July, values dropped to almost half and then declined further towards the end of the season.

Statistically significant differences between treatments were found only for 17 August ($P = 0.025$). However, differences between treatments were more pronounced for all measurements at the end of the season than they had been earlier in the season.

In 1994, data on radial growth at the base of the new shoot (figs. 34a,b) were available from 22 June onwards. As in 1993, the maximum growth increase at this location on the plant stems was found on 6 July, with the exception of CF(d), which showed its maximum increment on 3 August. The growth increase between 22 June and 6 July was much more pronounced in the well watered plants than in the droughted ones.

In general, the droughted treatments showed reduced growth as compared to their well watered counterparts, although in this respect the values for CF(d) of 3 August also proved to be an exception, being almost 0.1 mm higher than those found for the well watered control.

Among the droughted treatments, CF(d) showed the highest growth for all but one assessment, while among the well watered treatments no overall pattern was found, although for the last three measurements CF+O_3(w) showed the best growth. However, differences were only marginal and never statistically significant.

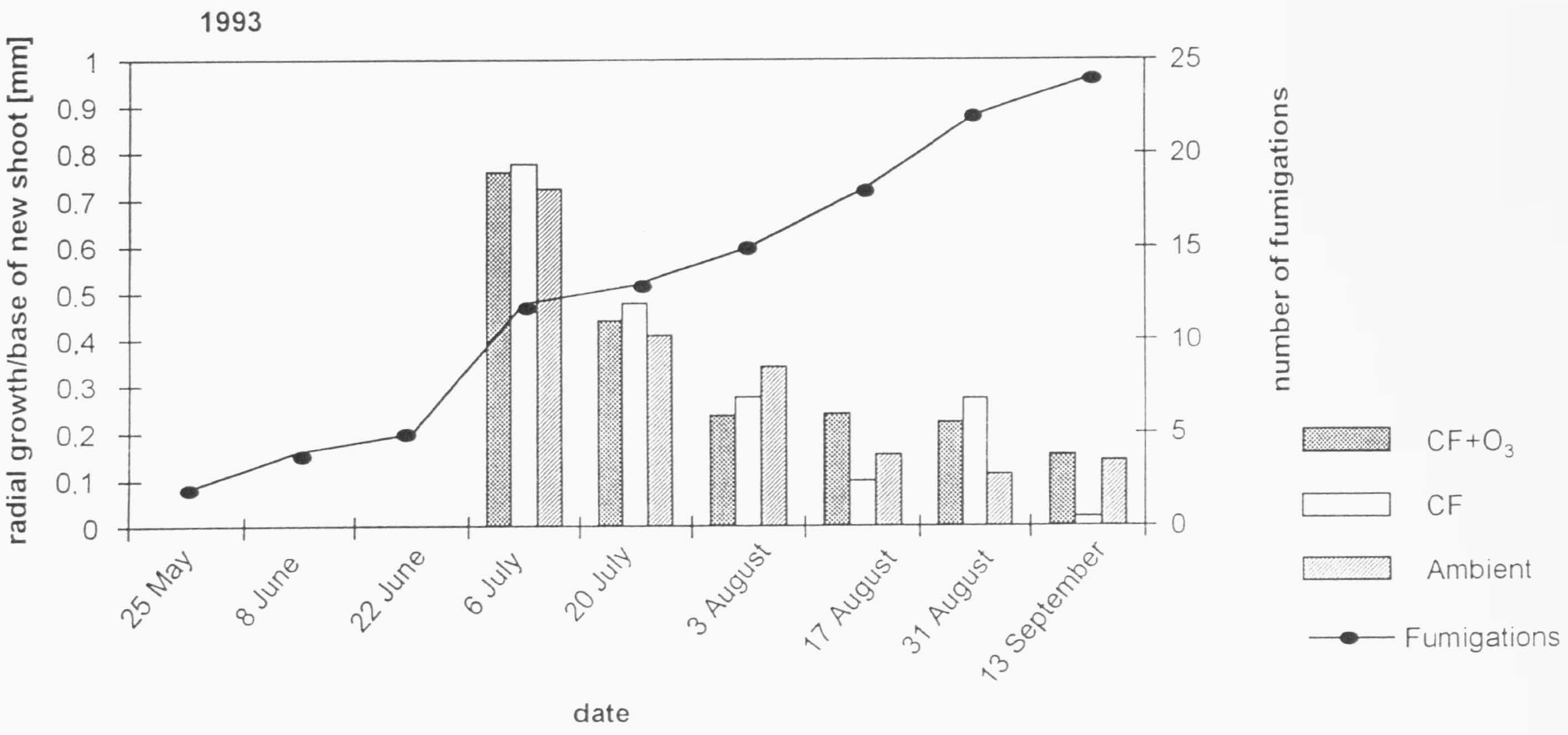

Fig. 33 Seasonal course of radial increment at the base of the new shoot of two-year-old saplings, 1993 (left axis) and course of exposures (right axis)

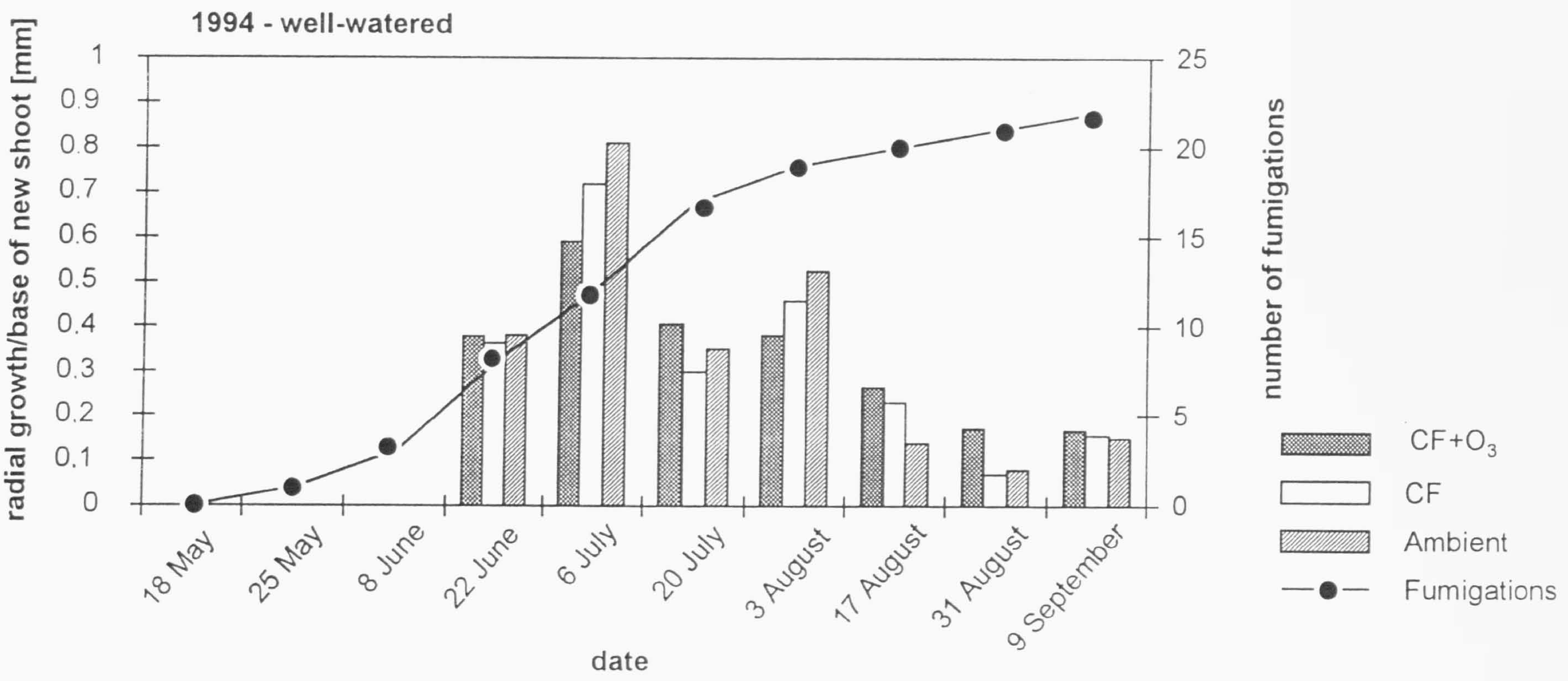

Fig. 34a Seasonal course of radial increment at the base of the new shoot of well-watered sapling, 1994 (left axis), and course of exposures (right axis).

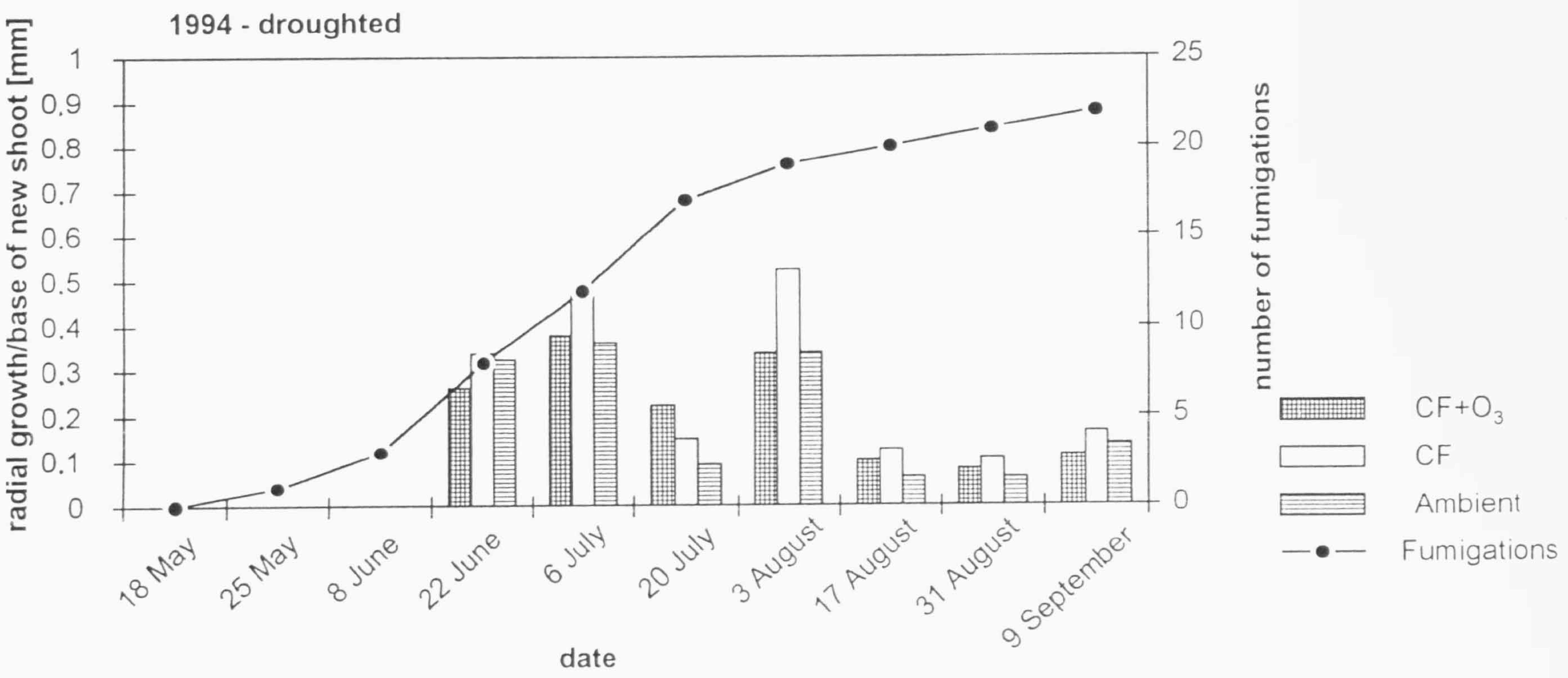

Fig. 34b Seasonal course of radial increment at the base of the new shoot of droughted saplings, 1994 (left axis) and course of exposures (right axis)

Due to the fall in values found for 20 July and the renewed increase of 3 August, radial increment at the base of the new shoot was found to be less regular than radial growth at the stembase.

5.2.3.2 Cumulative seasonal means of radial growth at the base of the new shoot

The seasonal means of radial growth at the base of the new shoot (figs. 35, 36) showed different trends for the two seasons. While in 1993 the CF treatment showed the highest radial increment at the base of the new shoot, it was the Ambient treatment which did so in 1994 for the well watered plants, while among the droughted treatments the CF treatment showed the highest cumulative radial growth.

In 1993 (fig. 35), there was no difference between the CF+O_3 and the Ambient treatment, and only the CF treatment showed higher radial growth at the base of the new shoot. This difference was significant when only the chambered treatments were considered in the analysis ($P = 0.036$); however, when the Ambient treatment was included there was no statistically significant difference.

In 1994 (fig. 36), the results also showed a marked difference between the well watered and the droughted treatments, the lower values being found for the droughted plants. Here the highest mean seasonal radial growth at the base of the new shoot was found for the Ambient(w) treatment, while the smallest increment was found for the Ambient(d) treatment. As with radial growth at the stembase, the difference between well watered and droughted plants of the CF treatment was smallest among the three pollution treatments.

Of the effects described, only that of drought was statistically significant (incl. Ambient: $P < 0.001$; excl. Ambient: $P = 0.006$).

5.2.3.3 Relative radial growth rates at the base of the new shoot

Relative radial increment at the base of the new shoot (figs. 37 and 38) was more uniform among treatments than radial growth at the stembase.

In 1993 (fig. 37), there were only marginal differences between treatments. This

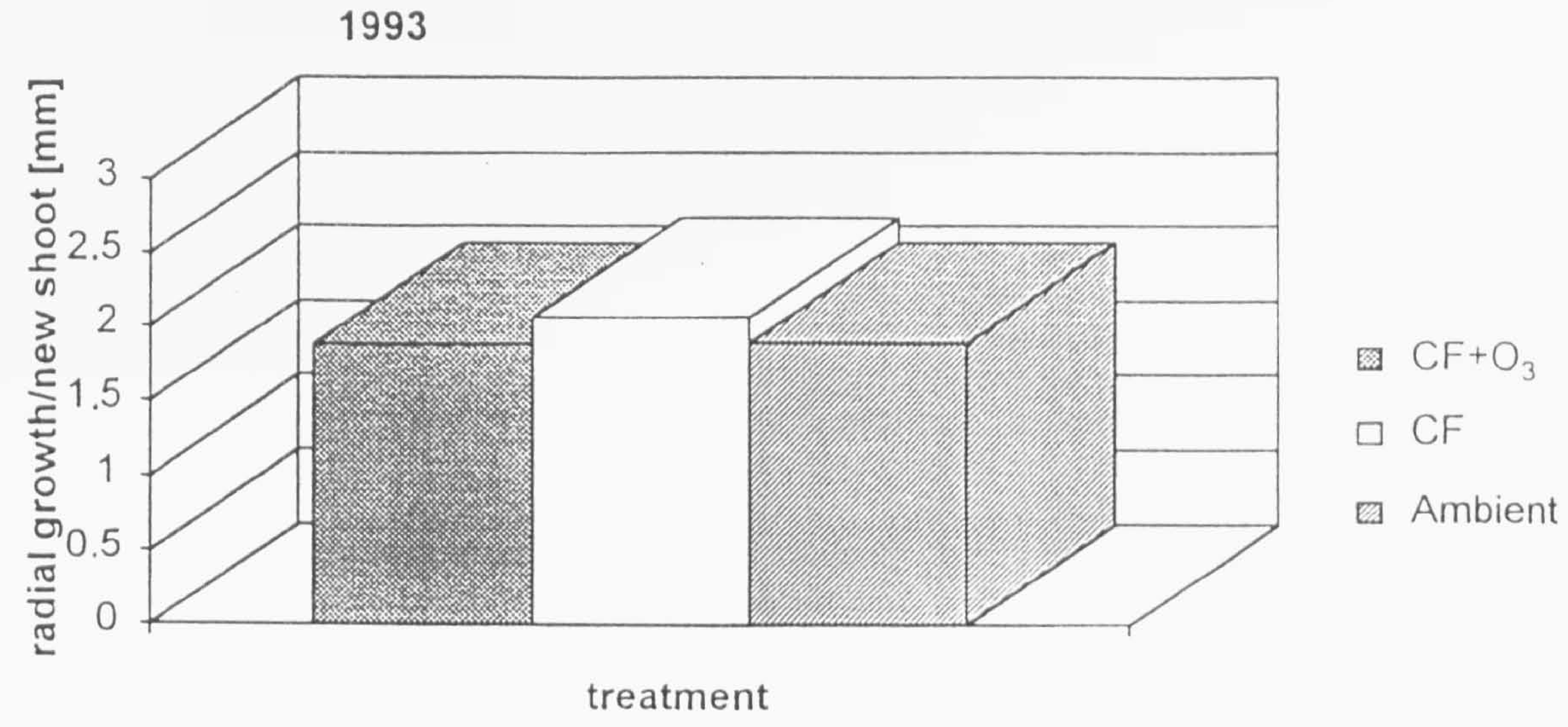

Fig. 35 Means of seasonal radial increment at the base of the new shoot of two-year-old saplings, 1993. Treatment differences were significant ($P = 0.036$) when the Ambient treatment was excluded from the analysis.

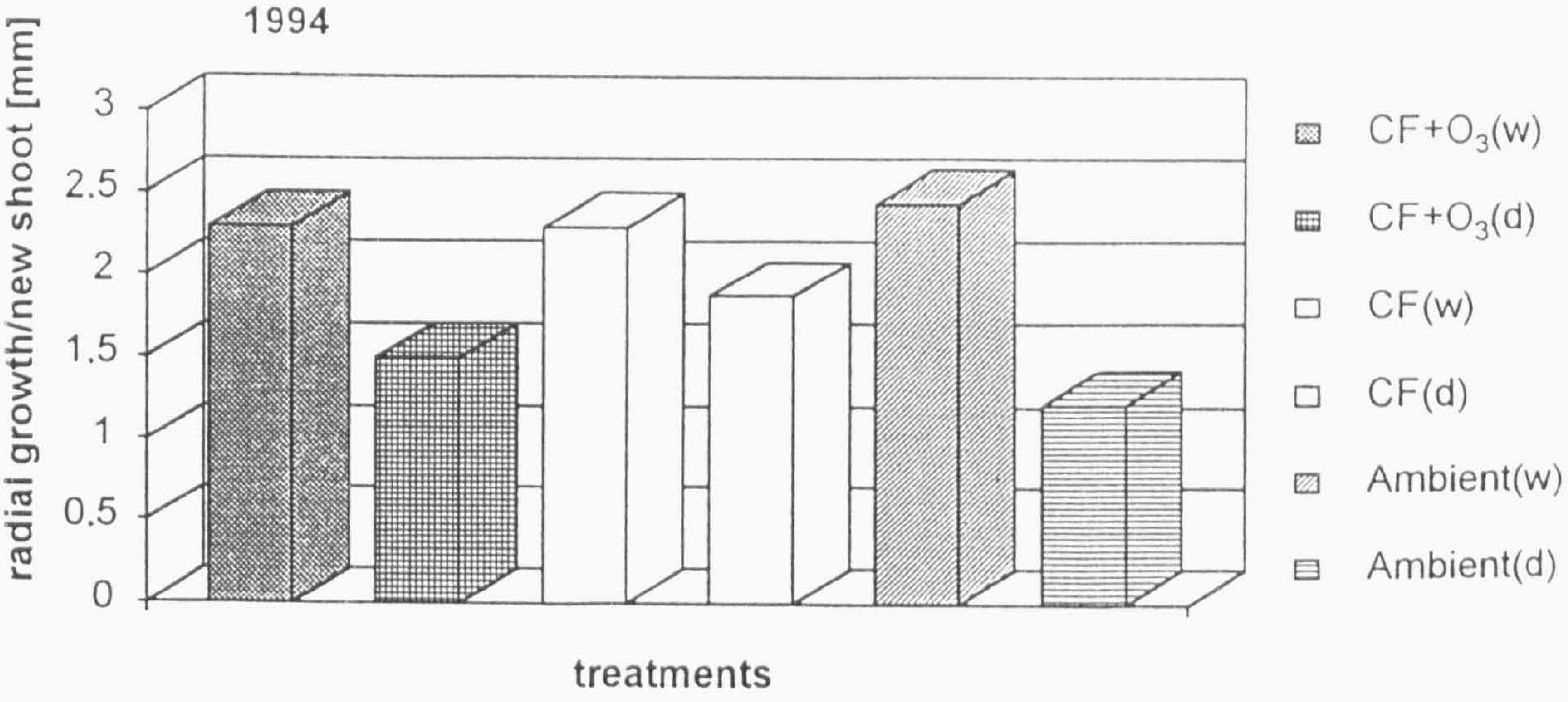

Fig. 36 Means of seasonal radial growth at the base of the new shoot of two-year-old saplings, 1994. Analysis of variance including all treatments: drought effect: $P < 0.001$; excluding Ambient: drought effect: $P = 0.006$.

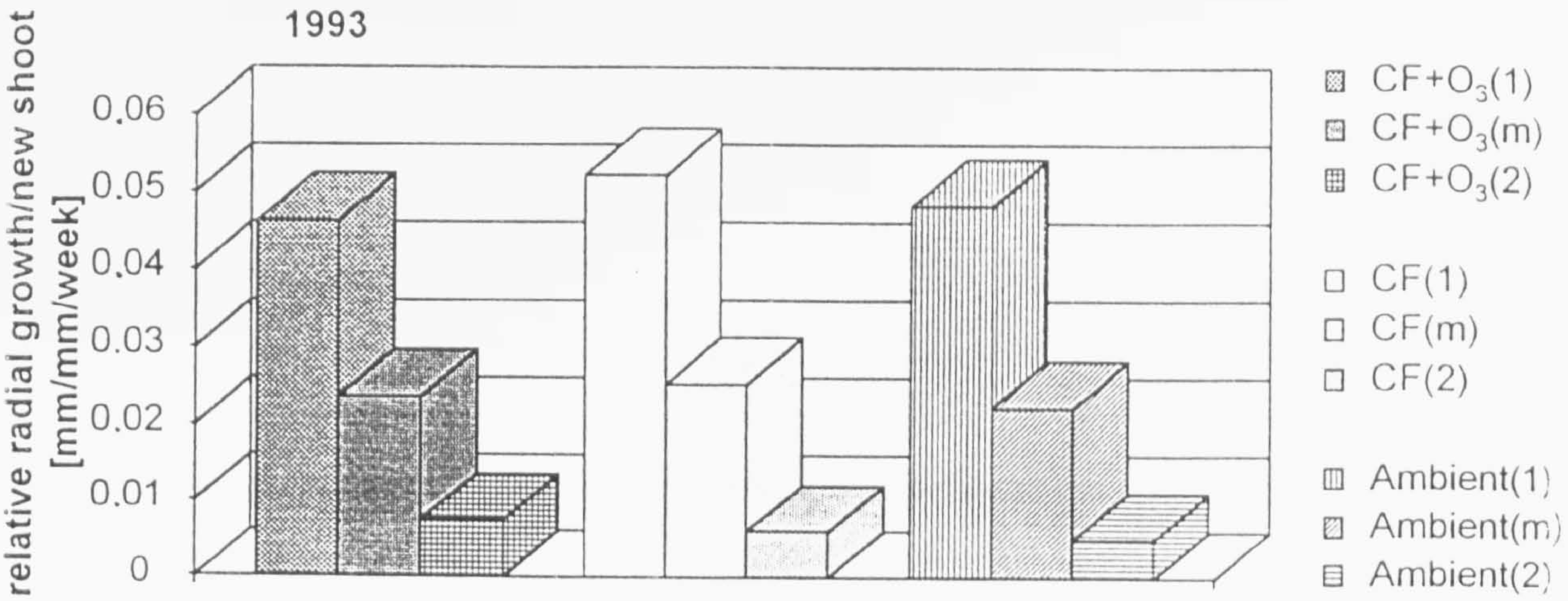

Fig. 37 Mean relative growth rates of radial growth at the base of the new shoot of two-year-old saplings, 1993. Treatment differences were not significant.

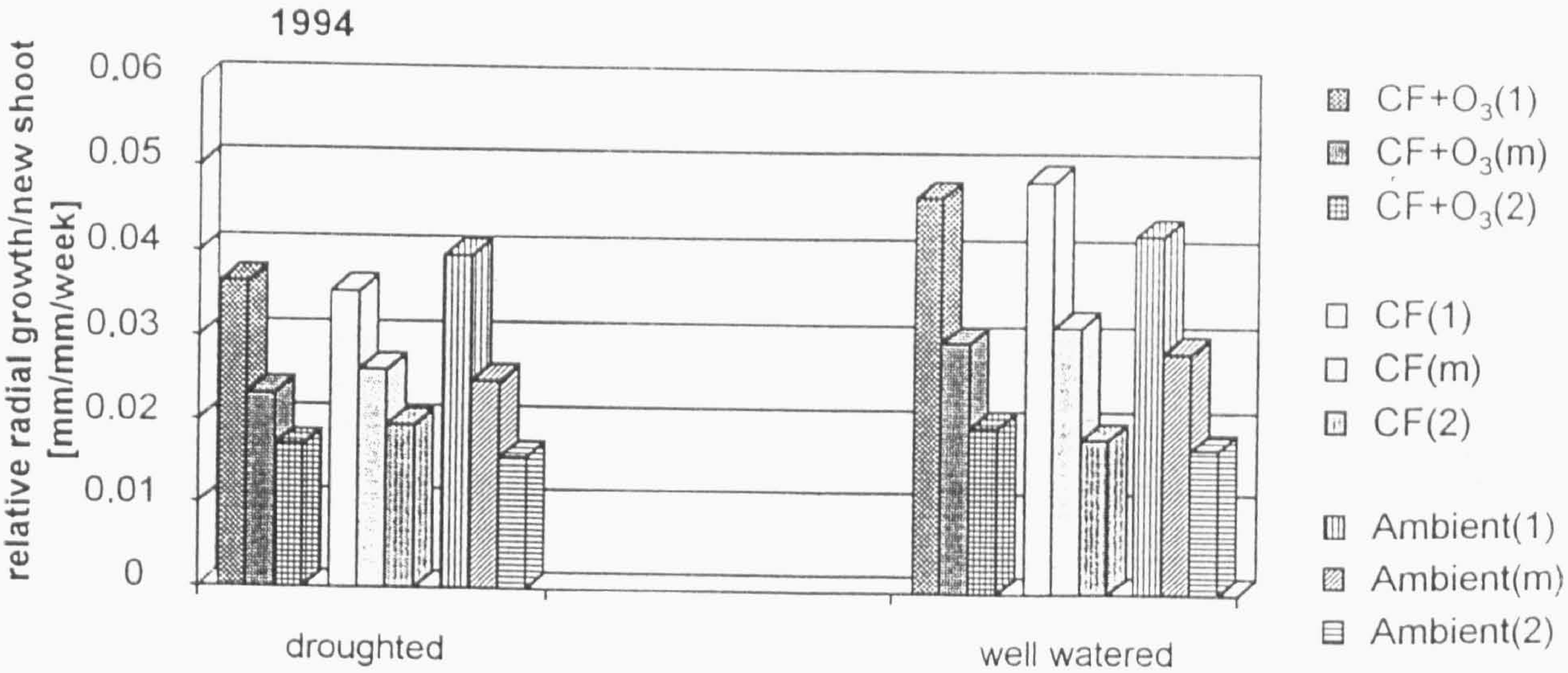

Fig. 38 Mean relative growth rates of radial growth at the base of the new shoot of two-year-old saplings, 1994. ANOVA: All treatments: (1) drought effect: P = 0.042; (m) drought effect: P = 0.026; excluding Ambient: (1) drought effect: P = 0.007; (m) drought effect: P = 0.017.

applied to the first, as well as to the second part of the season.

In 1994 (fig. 38), treatment means of relative growth rates were slightly more variable than they had been the year before. However, the most evident effect was that of drought, which reduced the relative growth rates of the droughted treatments, particularly during the first part of the season.

This drought effect during the first half of the growing season was statistically significant when all treatments were included ($P = 0.042$), as well as when the Ambient treatments were excluded ($P = 0.007$). Similarly, the drought effect on the seasonal relative growth rates ($P = 0.026$; Ambient excluded: $P = 0.017$) was significant.

5.2.4 Summary: Growth parameters

- The three growth parameters investigated, extension growth, radial growth at the stembase and radial growth at the base of the new shoot, were affected differently by the pollution treatment, while drought always caused a decrease of growth. The effects caused by the ozone stress were not always similar for the two years.
- For extension growth, which showed the highest values in spring, differences between treatments were more pronounced in 1994 than they had been in 1993. However, in both years the Ambient treatments showed the least growth.
- Radial growth at the stembase was highest during mid-summer. In 1994 a significant decrease of radial growth at the stembase was caused by drought and there was also a significant drought × pollution interaction, which led to a further decrease in growth. Where relative growth rates were concerned, differences were greater during the second part of the season, as compared to the beginning of the season.
- In contrast, relative radial growth at the base of the new shoot was more variable at the beginning of the growing season. Apart from a significant drought effect during the first part of the season, and for the seasonal means in 1994, growth at the base of the new shoot was less affected than at the stembase.

5.3 Leaf area of two-year-old saplings

5.3.1 Leaf area

The course of leaf area development (figs. 39 and 40), which was calculated from the regularly-assessed leaf numbers and the unit leaf areas that were determined for every plant individually at the final harvest, was different for the two seasons. In 1993 the maximum leaf area was attained around mid-season, while in 1994 it was reached only during the last third of the season. Furthermore, the maximum leaf area of 1993 was almost 50 % higher than that determined for 1994.

In 1993 (fig. 39), leaf area developed similarly for all three treatments. However, in mid-summer, at the time when leaf area reached its highest values, maximum differences between the treatments were also found; the differences, which were in the range of 0.2 m^2, were not significant. The largest leaf area was produced by the $CF+O_3$ treatment followed by the Ambient treatment and the CF treatment. The increase in leaf area in spring and the autumnal decline were similar for all treatments. Maximum leaf area was found on 20 July, followed by a slight decline at the beginning of August. After this decline the values stabilized again and only between 31 August and 13 September 1993 the decline found for the $CF+O_3$ treatment was slightly stronger (4 %) than that of the other treatments.

In all three pollution treatments, the drought stress imposed in 1994 also affected leaf area development (fig. 40), resulting in lower a leaf area for the droughted plants. As in 1993, the largest differences between treatments coincided with the time when all treatments but $CF+O_3$(d) had developed their maximum leaf area. For both the drought treatment as well as the pollution treatment, significant effects were apparent (drought: $P = 0.005$; pollution treatment: $P = 0.024$) when all treatments ($CF+O_3$, CF, Ambient) were included in the analysis, but only the drought effect was significant ($P = 0.002$) when the Ambient treatment was excluded. The largest within-pollution-treatment differences were found in the $CF+O_3$ treatment, where they reached up to 0.3 m^2 and where accordingly the well watered treatment showed a more than 50 % higher leaf area than the droughted treatment.

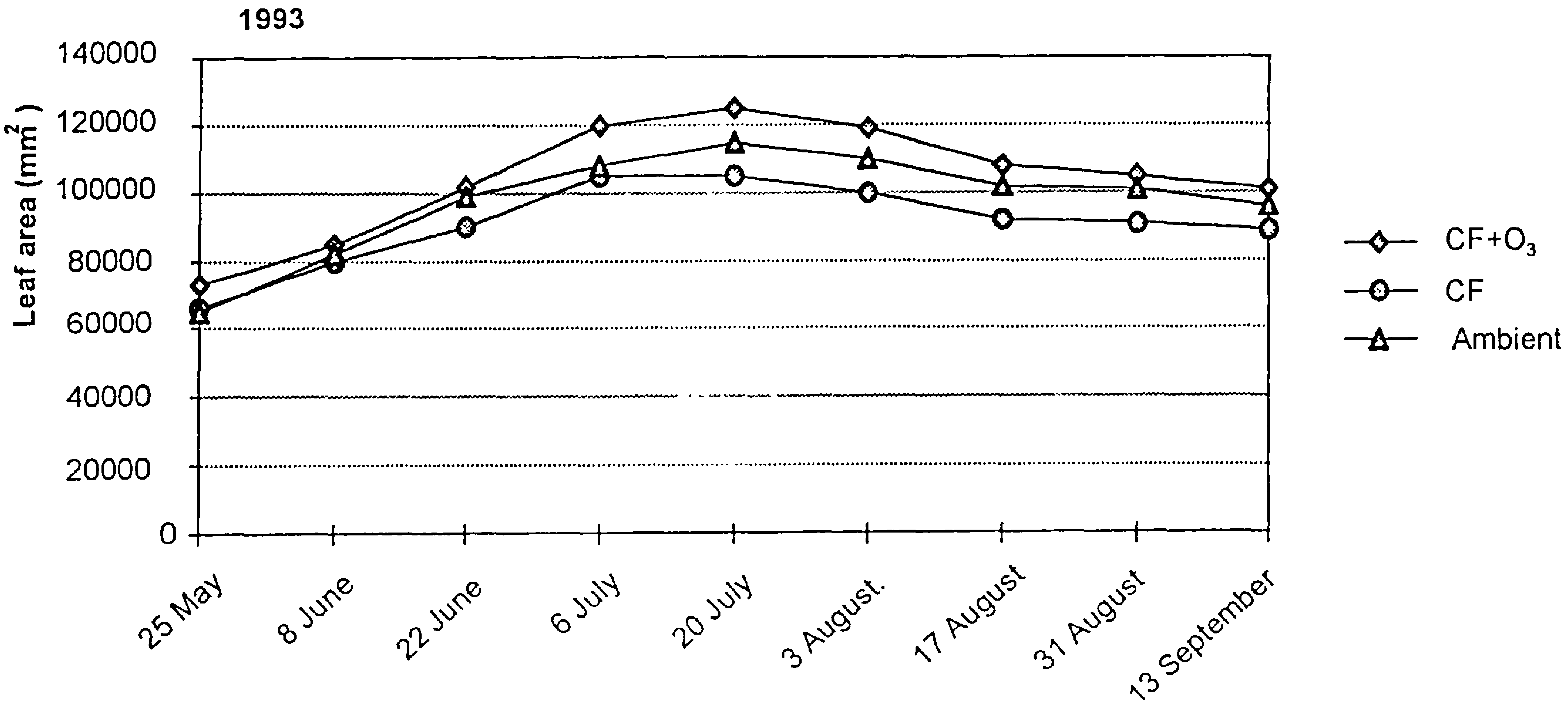

Fig. 39 Mean leaf area of two-year-old saplings, 1993.

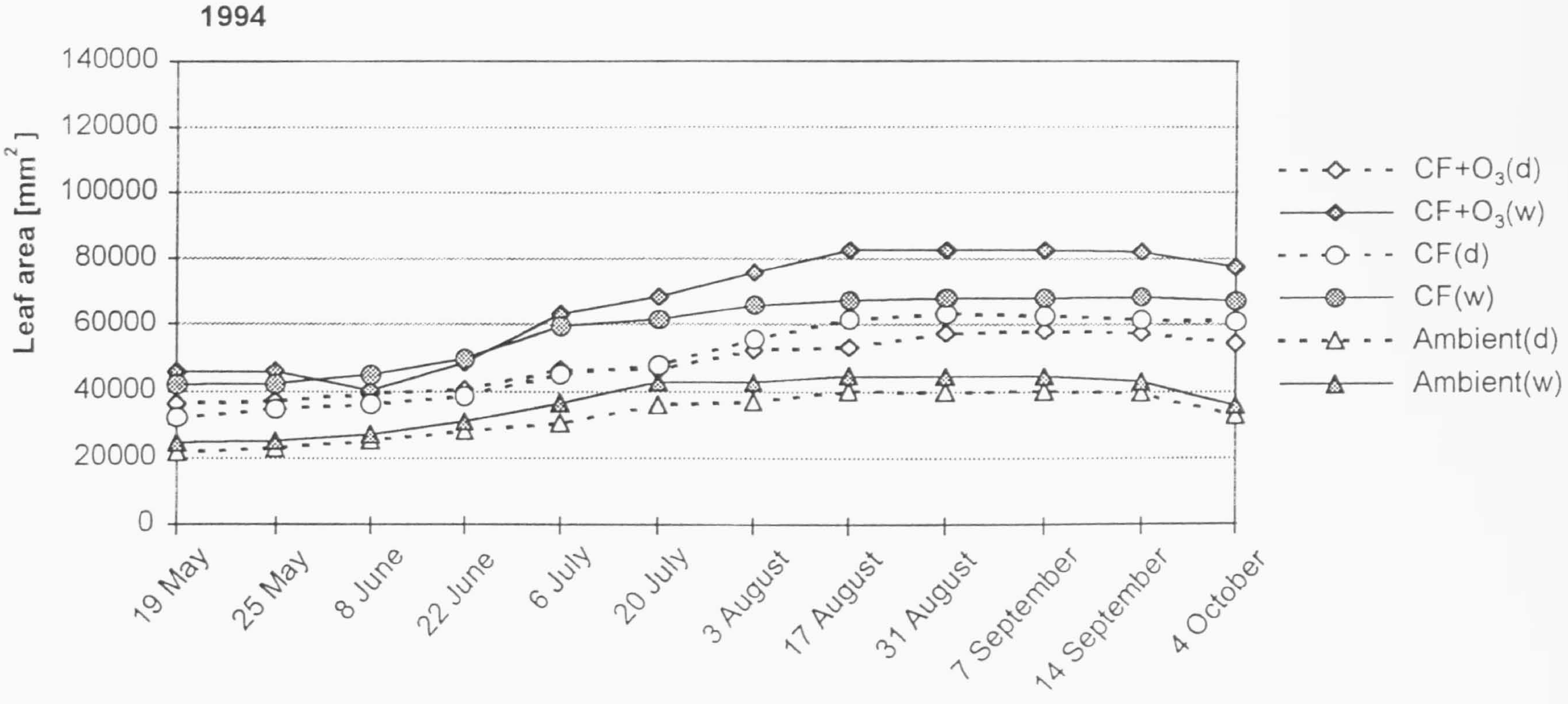

Fig. 40 Mean leaf area of two-year-old saplings, 1994.

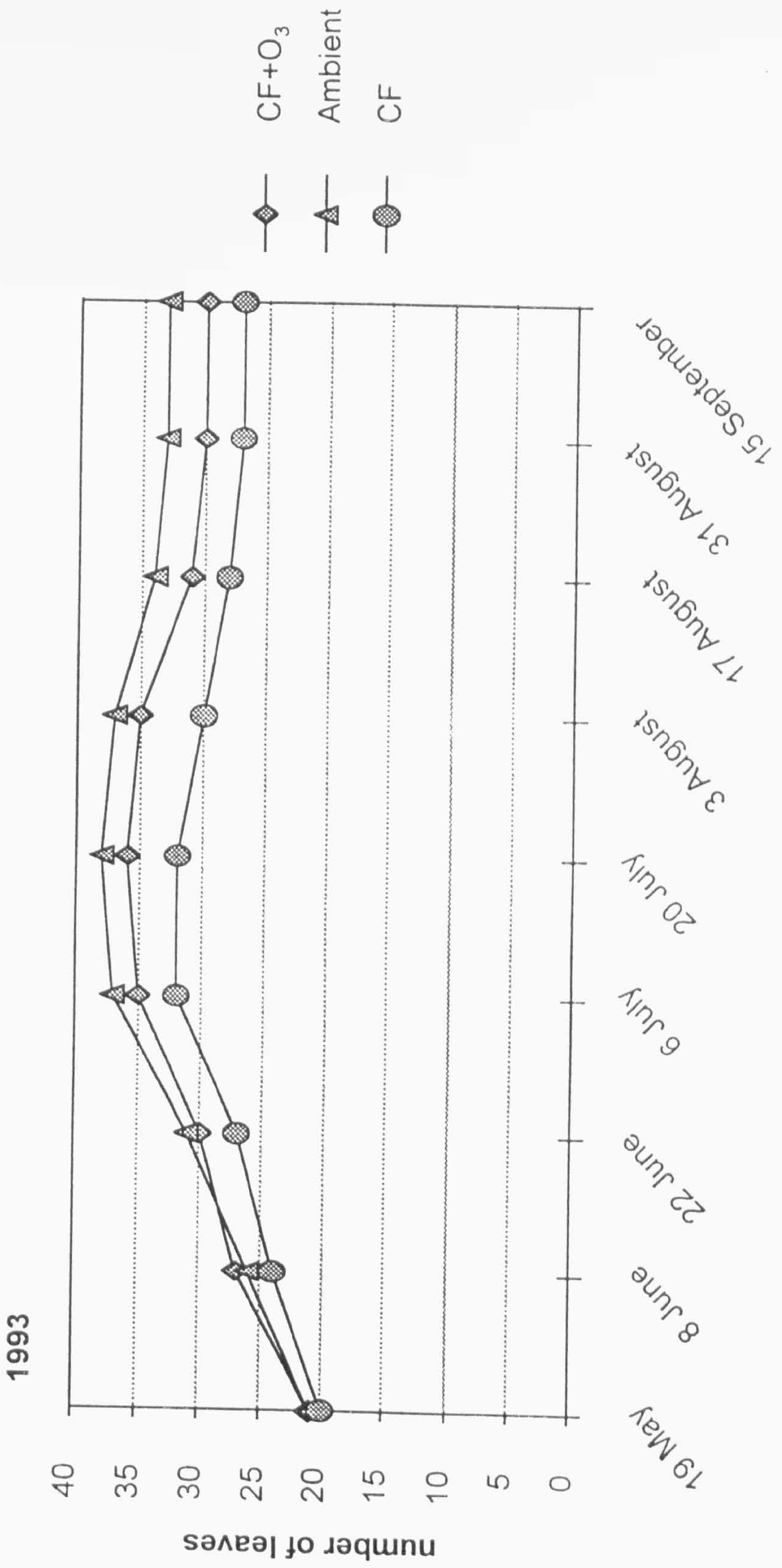

Fig. 41 Mean leaf number of two-year-old saplings, 1993.

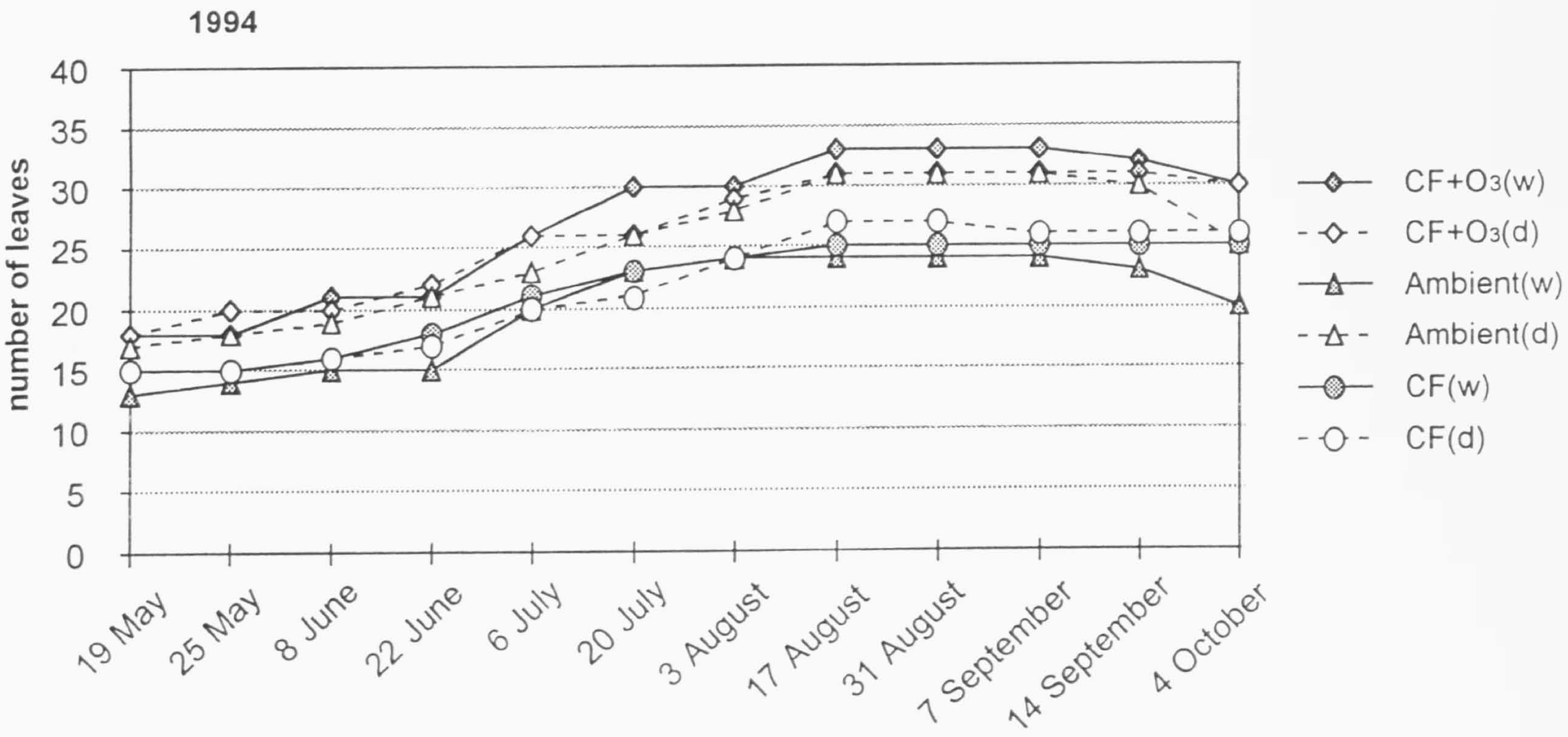

Fig. 42 Mean leaf number of two-year-old saplings, 1994.

Both Ambient treatments produced smaller leaf areas than the chambered treatments, which showed up to 0.5 m^2 higher values [CF+O_3(w) - Ambient(d)] and thus developed up to twice the leaf area produced by the Ambient treatment.

For all treatments, maximum leaf area in 1994 was attained only by mid-August after a continuous increase in the values. This was followed by a stabilization, which lasted until mid-September when leaf area of the Ambient and the CF+O_3 treatments began to decrease, while those of the CF treatments were still stable.

5.3.2 Leaf numbers

Leaf numbers that had been counted on the two-year-old potted saplings every second week throughout the two growing seasons are given in figures 41 and 42.

In 1993, leaf numbers (fig. 41) were highest during mid-summer and had already declined by mid-August. This was found for all three treatments, with Ambient showing the highest and CF the least leaf numbers and the maximum difference in mean leaf numbers between the treatments amounting to seven leaves.

In 1994 (fig. 42), all treatments reached their maximum leaf numbers by mid-August, after which the values started to decline again at the beginning of September. Leaf numbers of both CF+O_3 treatments were higher than those of the CF treatments. The maximum difference between the treatments with the highest [CF+O_3(w)] and the least [Ambient-(w)] mean leaf number was nine leaves, and was found when all treatments had reached their maximum leaf numbers.

In both years, the order of treatments with respect to leaf numbers differed from that found for leaf areas, indicating differences in leaf sizes among the treatments. Thus, in both years, plants from the Ambient treatment showed the smallest leaf sizes, and among the two chambered treatments CF+O_3 treatments had smaller leaves than the respective CF treatments.

1993	$CF+O_3$	CF	Ambient
Leaves	33.6	31.9	31.7
New shoot	11.3	8.9	9.1
Old shoot	55.1	59.2	59.2

Table 9 Relative biomass [in %] of individual plant organs of two-year-old saplings at autumn harvest 1993.

1994	$CF+O_3$(d)	$CF+O_3$(w)	CF(d)	CF(w)	Ambient(d)	Ambient(w)
Leaves	38.0	41.7	41.4	38.7	35.0	31.8
New shoot	12.8	17.9	17.2	17.3	11.9	16.9
Old shoot	49.2	40.3	41.3	44.0	53.2	51.3

Table 10 Relative biomass [in %] of individual plant organs of two-year-old saplings at autumn harvest 1994.

5.4 Aboveground biomass of two-year-old saplings

At the autumn harvests of the two-year-old saplings in 1993 and 1994, biomass of the aboveground plant organs was determined as dry weight. The results are shown in figures 43 and 44. While the amount of leaf and new shoot biomass was similar for both years, that of old shoot biomass differed considerably between the two seasons, and in 1994 old shoot biomass was only approximately 50% of that determined for plants harvested the year before.

For aboveground biomass, as for the growth parameters, only marginal, non-significant

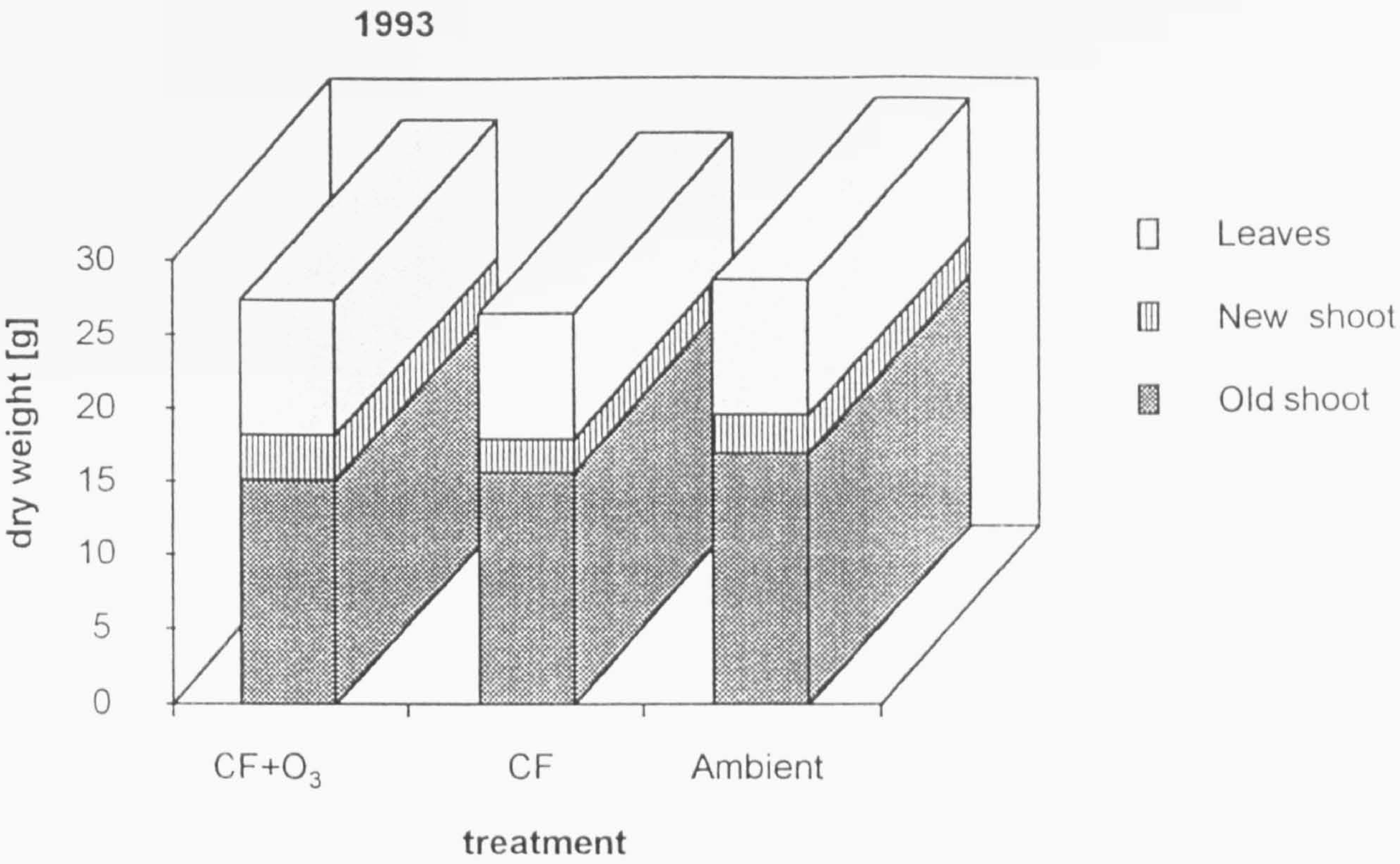

Fig. 43 Aboveground biomass of two-year-old saplings at autumn harvest 1993. Differences were not statistically significant.

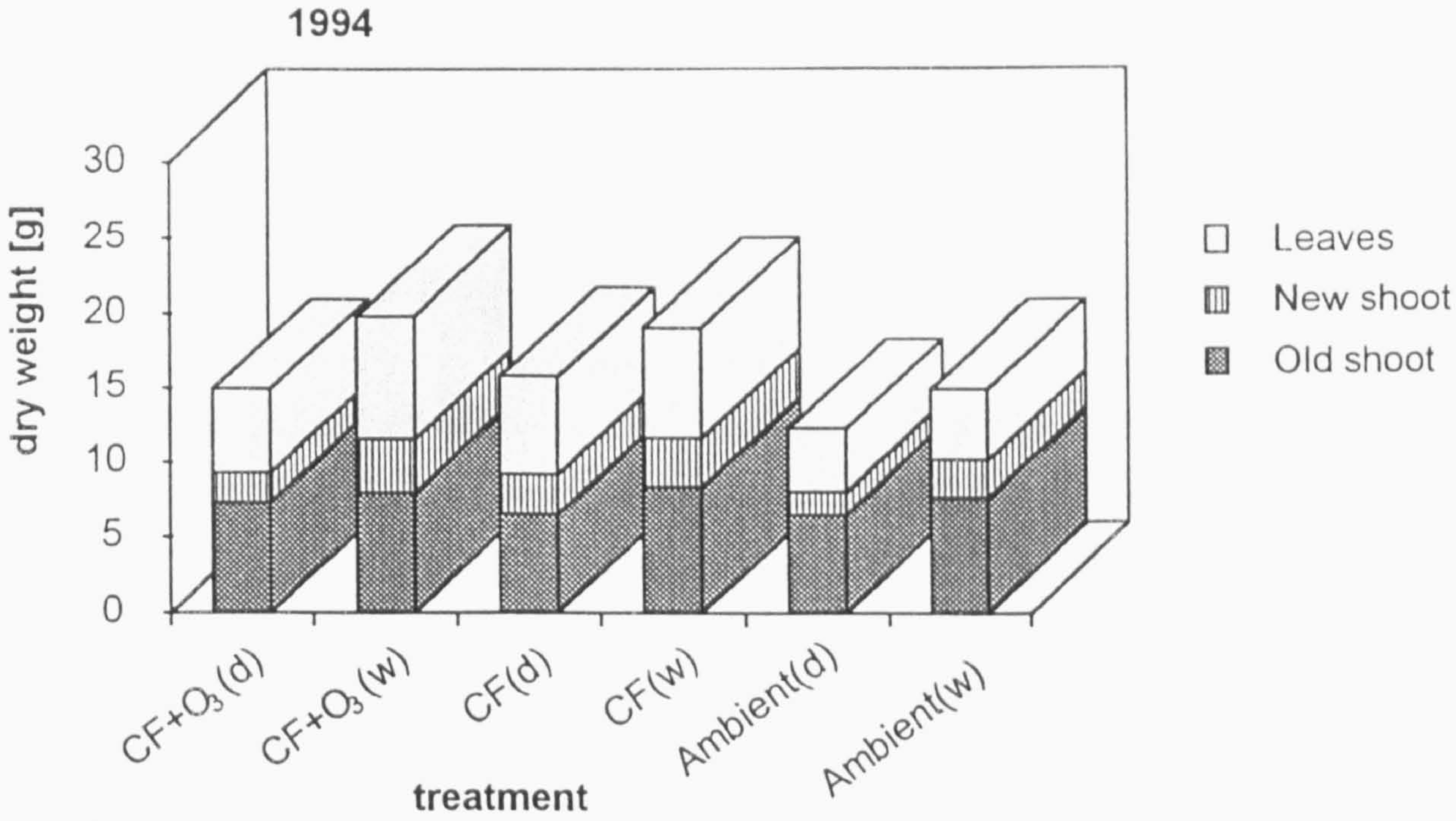

Fig. 44 Aboveground biomass of two-year-old saplings at autumn harvest 1994. Differences were not significant.

differences could be found between the treatments in 1993 (fig. 43). Total biomass differed by less than 2.5 g and was highest in the Ambient treatment and least in the CF treatment. The percentage biomass of the individual plant organs (table 9), i.e. old shoot, new shoot, and leaves, was similar for the CF and the Ambient treatments with 59%, 9% and 32% respectively, while the CF+O_3 treatment showed a different pattern with 55% old shoot, 11.5% new shoot and 33.5% leaf biomass.

In 1994 (fig. 44), differences between pollution treatments were more pronounced than they had been the year before and there were additional differences induced by the drought treatment. However, these differences were not significant. The drought caused a lower total biomass production in the affected plants as compared to their well watered counterparts and this difference between droughted and well watered plants was most marked in the CF+O_3 treatment. Values of total biomass of both the Ambient(w) and the Ambient(d) treatment were distinctly lower than those found for the chambered treatments.

Percentage biomass of the different plant organs (table 10) was also very variable among the treatments. Thus the highest percentage of leaf biomass was found for the CF+O_3(w) treatment (42%), immediately followed by the CF(d) treatment (41%) and the smallest percentage was that of the Ambient(w) treatment (32%). Similarly, the CF+O_3(w) treatment showed the highest percentage of new shoot biomass (18%), while in this case the Ambient(d) treatment had the smallest percentage (12%). The percentage of new shoot biomass determined for the CF+O_3(d) treatment was also in that low range (13%). Accordingly, the smallest percentage of old shoot biomass was determined for the CF+O_3(w) treatment (40%), followed by CF(d) (41%) and CF(w) (44%), while biomass of the Ambient(d) treatment showed the highest proportion of old shoot biomass (53%) among the treatments.

5.5 Annual rings of two-year-old saplings

After the harvests of the two-year-old plants, the width, number of cells and cell size in the annual rings produced during the relevant seasons were determined microscopically. In this analysis only the two chambered treatments CF and CF+O_3 were taken into account.

5.5.1 Ringwidth of annual rings

In general, the results from this analysis mirrored those found for radial growth at the stembase. The treatments showed similar trends in both years (figs. 45 and 46) with greater ringwidth for the CF treatment 1993 and the CF(d) treatment 1994.

In 1993 (fig. 45), ringwidth of the CF treatment was 22% greater than that of the CF+O_3 treatment and this difference was statistically highly significant ($P < 0.001$).

Drought decreased ringwidth in 1994 (fig. 46) for both pollution treatments. However, while the decrease found for the CF(d) treatment as compared to CF(w) was 12%, that determined for the CF+O_3(d) treatment as compared to CF+O_3(w) amounted to 34% and can be ascribed to the interaction of ozone and drought stress. Thus ringwidth was greatest for the CF+O_3(w) treatment and smallest for the CF+O_3(d) treatment.

The treatment differences caused by drought ($P < 0.001$), as well as by the drought-pollution interaction ($P < 0.001$) were both highly significant.

5.5.2 Cell number

Cell number of cell lines orientated with the pith rays (figs. 47 and 48) varied with treatments in the same way as ringwidth of the annual rings. However, while the values for ringwidth were of the same order in both years of the experiment, those for cell number were somewhat higher in 1994 than they had been in 1993.

As with ringwidth, cell number in the annual ring in 1993 was 23% higher in the CF treatment (fig. 47) than in the CF+O_3 treatment and this difference was also highly

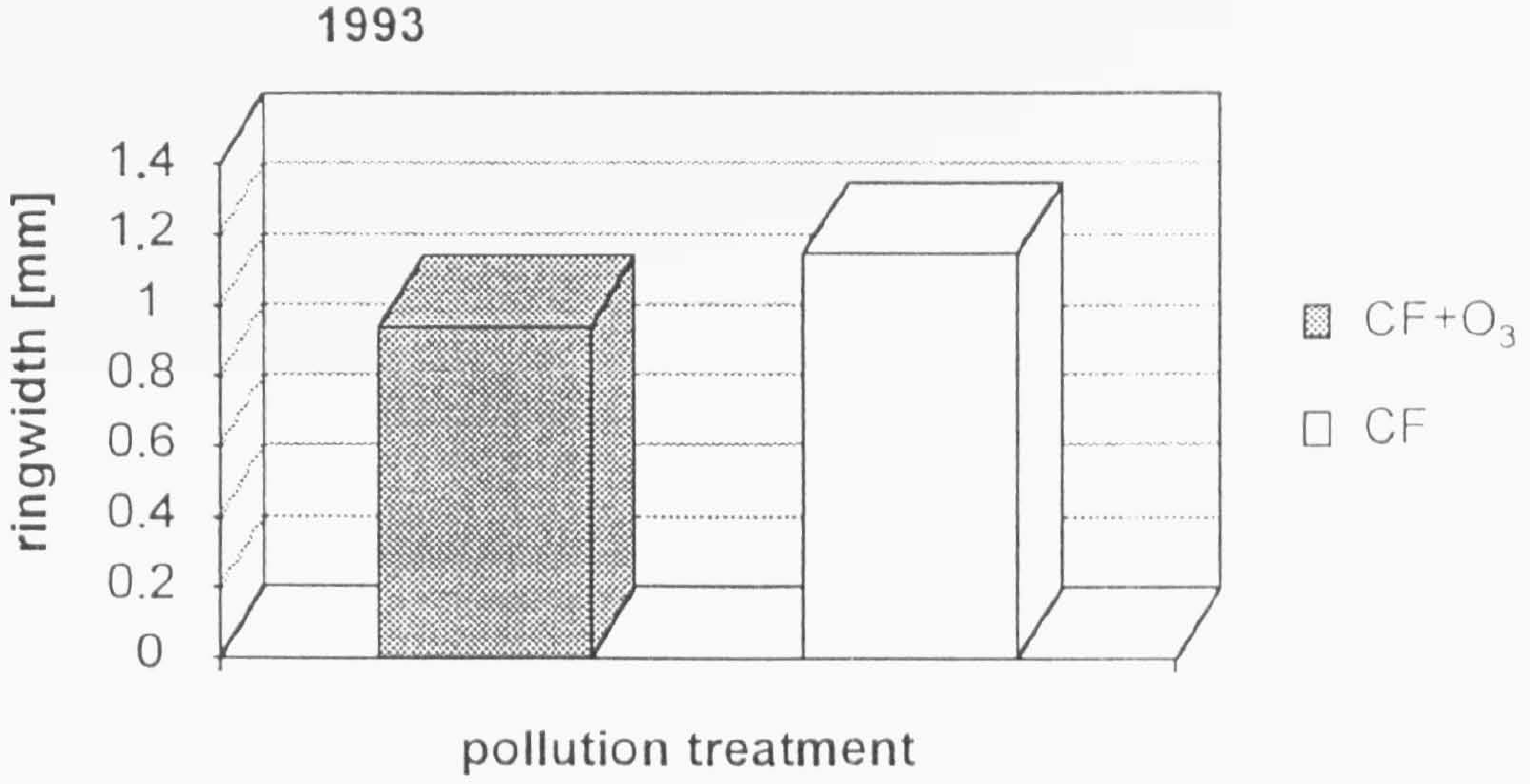

Fig. 45 Mean ringwidth of latest annual ring, 1993. ANOVA: pollution effect: $P < 0.001$.

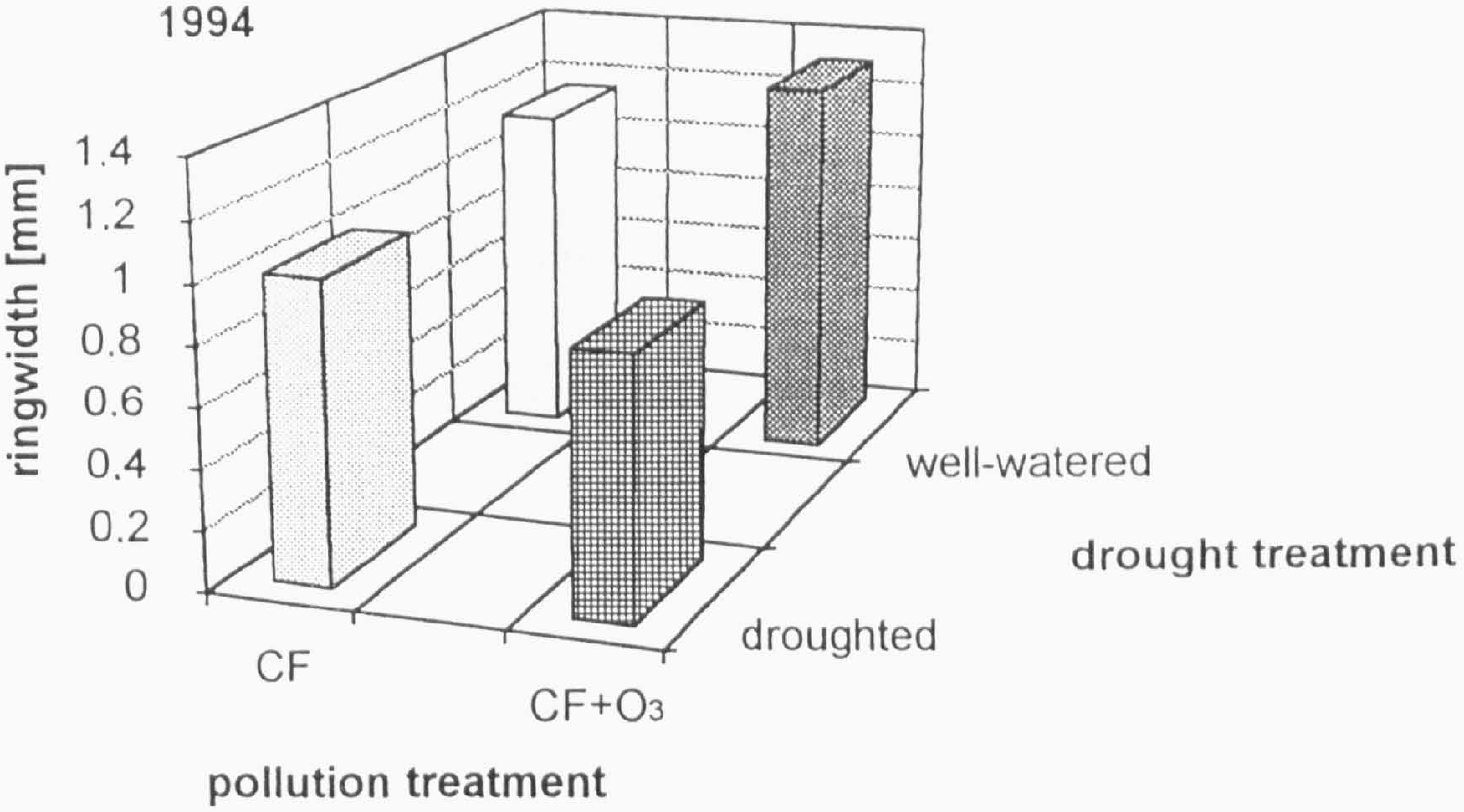

Fig. 46 Ringwidth of latest annual ring of two-year-old saplings, 1994. ANOVA: drought effect: $P < 0.001$; pollution × drought: $P < 0.001$.

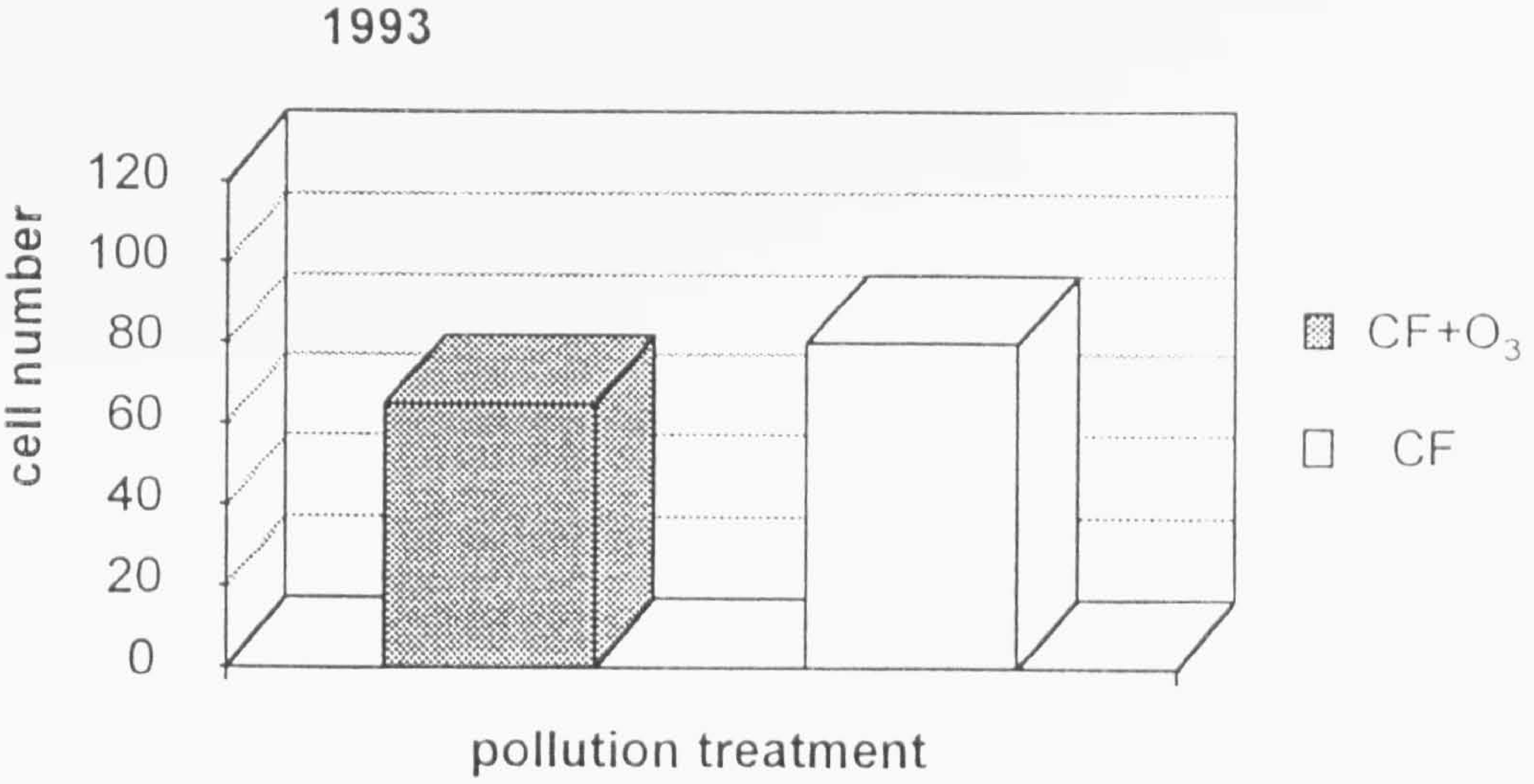

Fig. 47 Mean cell number of cell lines orientated with the pith rays in the latest annual ring of two-year-old saplings, 1993. ANOVA: pollution effect: $P = 0.001$

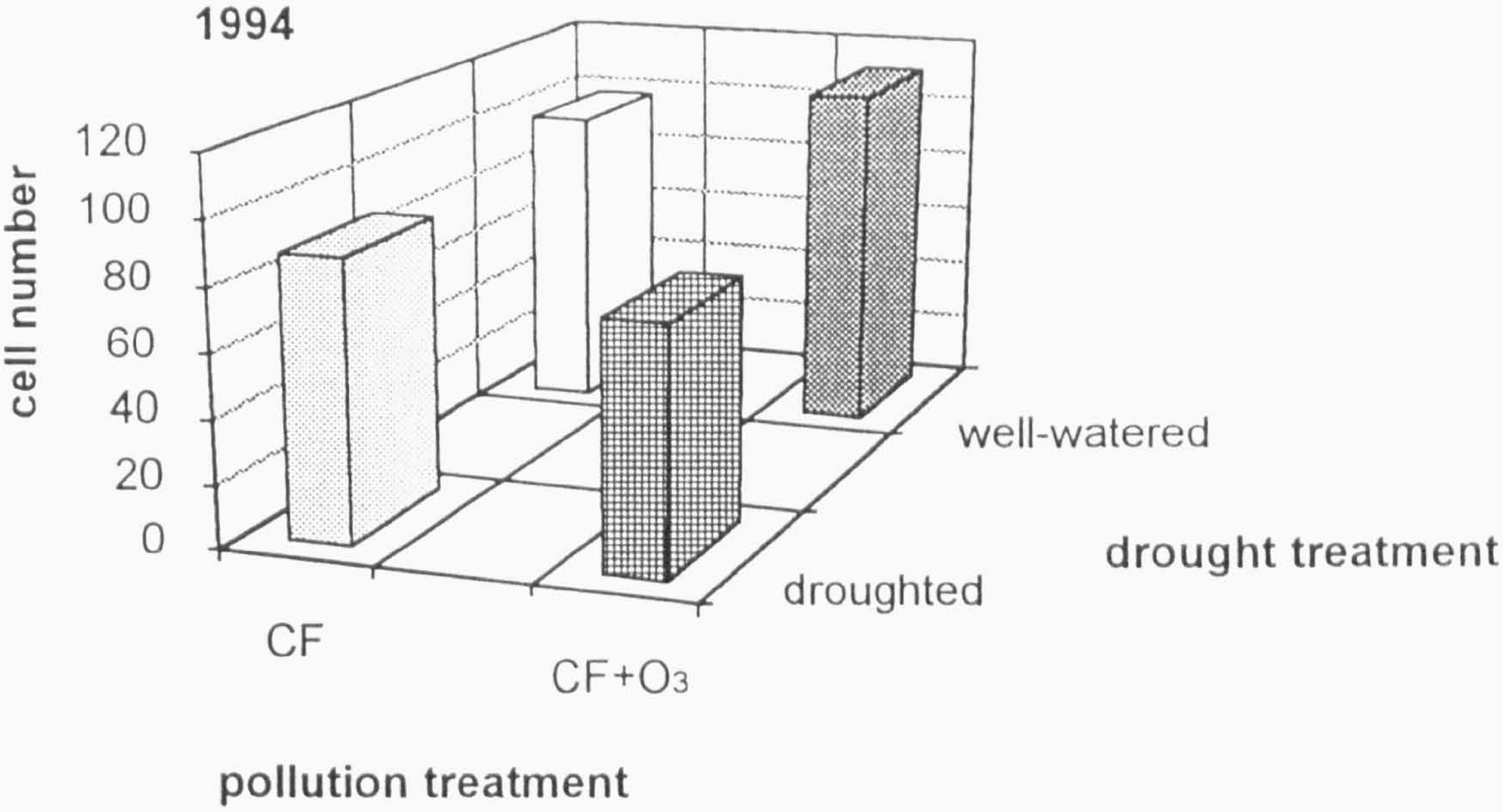

Fig. 48 Mean cell number of cell lines orientated with the pith rays in the latest annual ring of the two-year-old saplings, 1994. ANOVA: drought effect: $P < 0.001$; pollution × drought: $P < 0.001$.

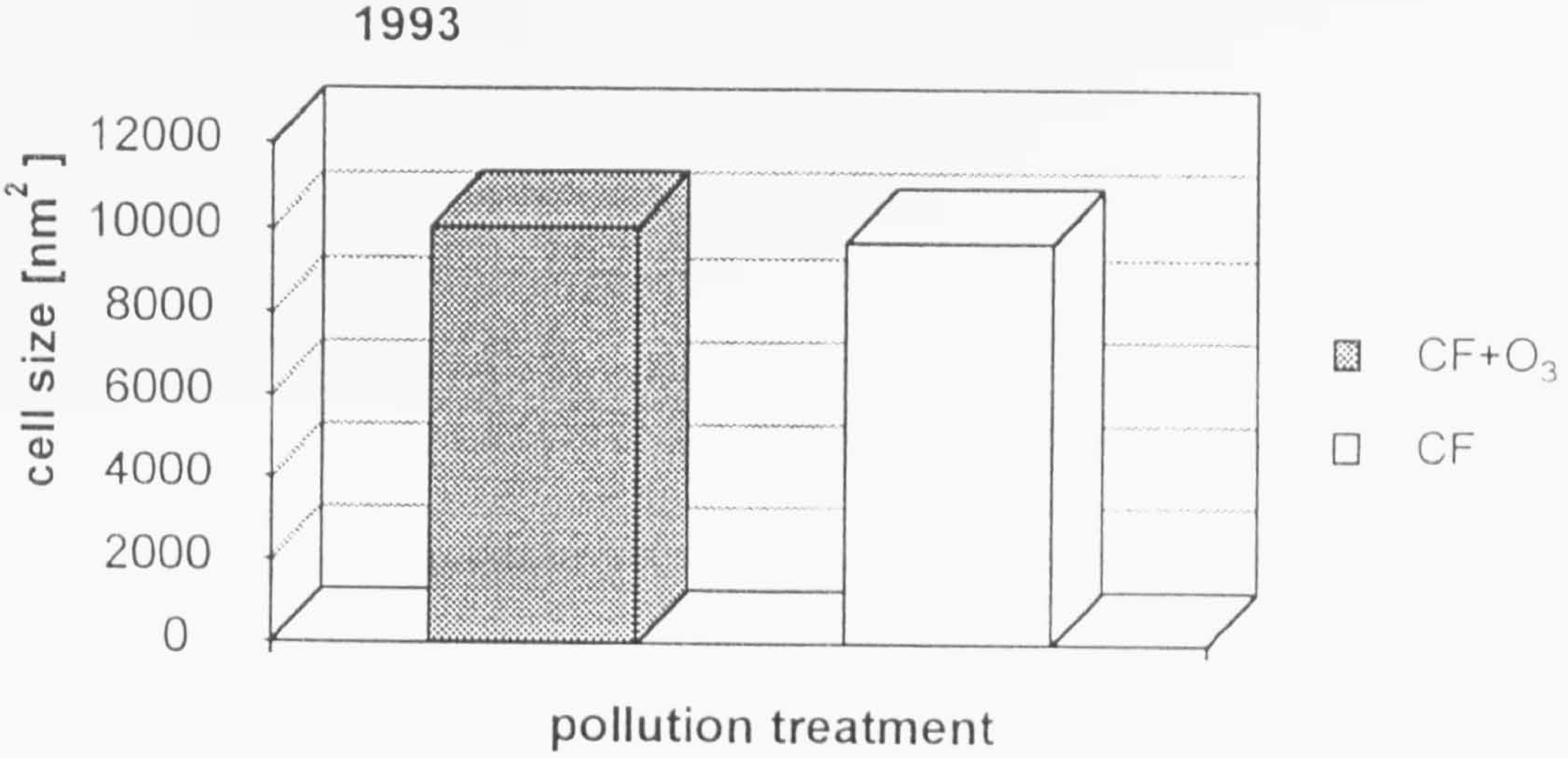

Fig. 49 Mean cell size of xylem cells in latest annual ring of two-year-old saplings, 1993. There were not statistically significant differences.

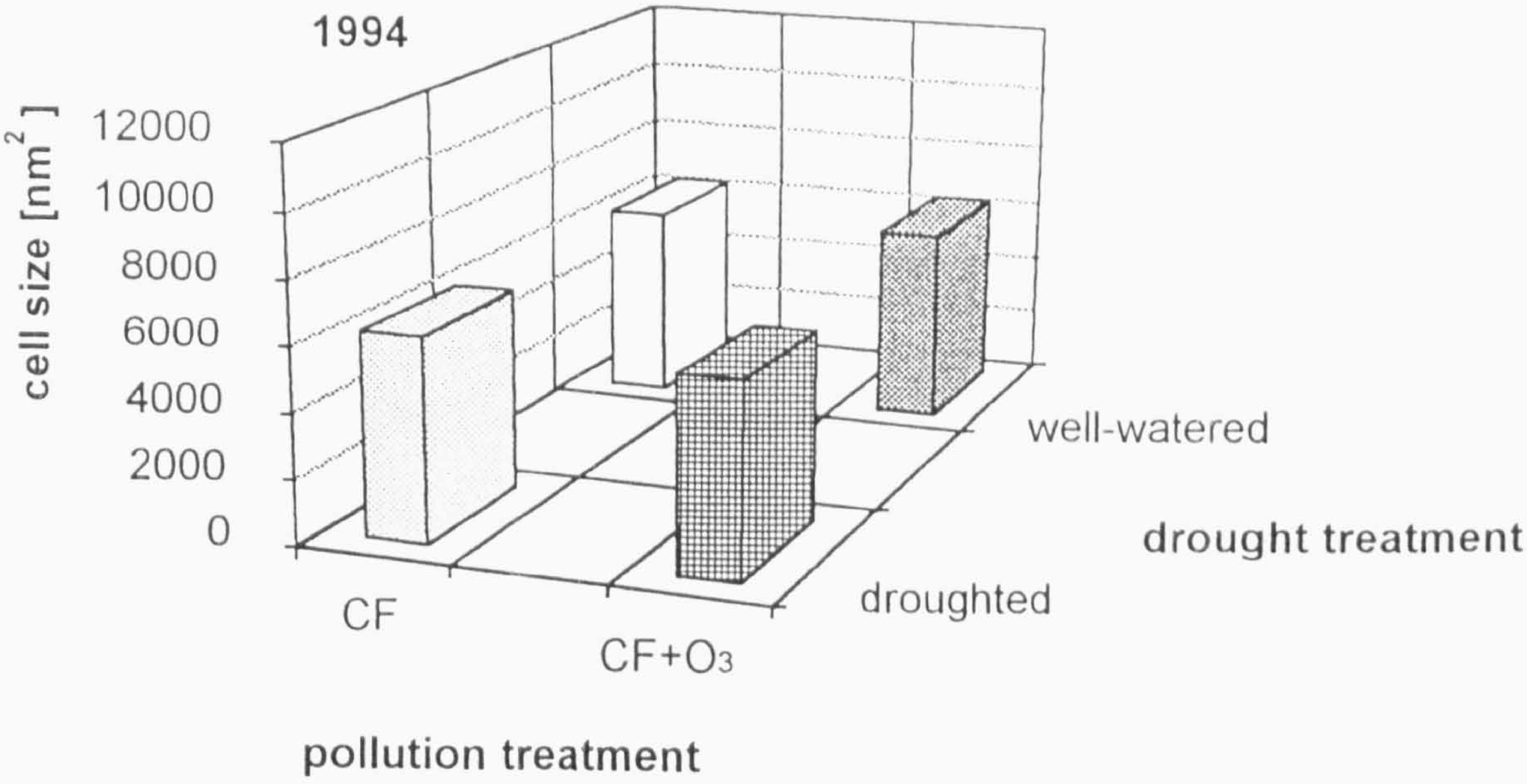

Fig. 50 Mean cell size of xylem cells in latest annual ring of two-year-old saplings, 1994. Differences were not statistically significant.

significant ($P < 0.001$).

In 1994 (fig. 48) the cell numbers also mirrored the results found for ringwidth of the annual rings, and correspondingly drought as well as the interaction of ozone and drought reduced the cell numbers in the annual rings. Both effects were highly significant ($P < 0.001$).

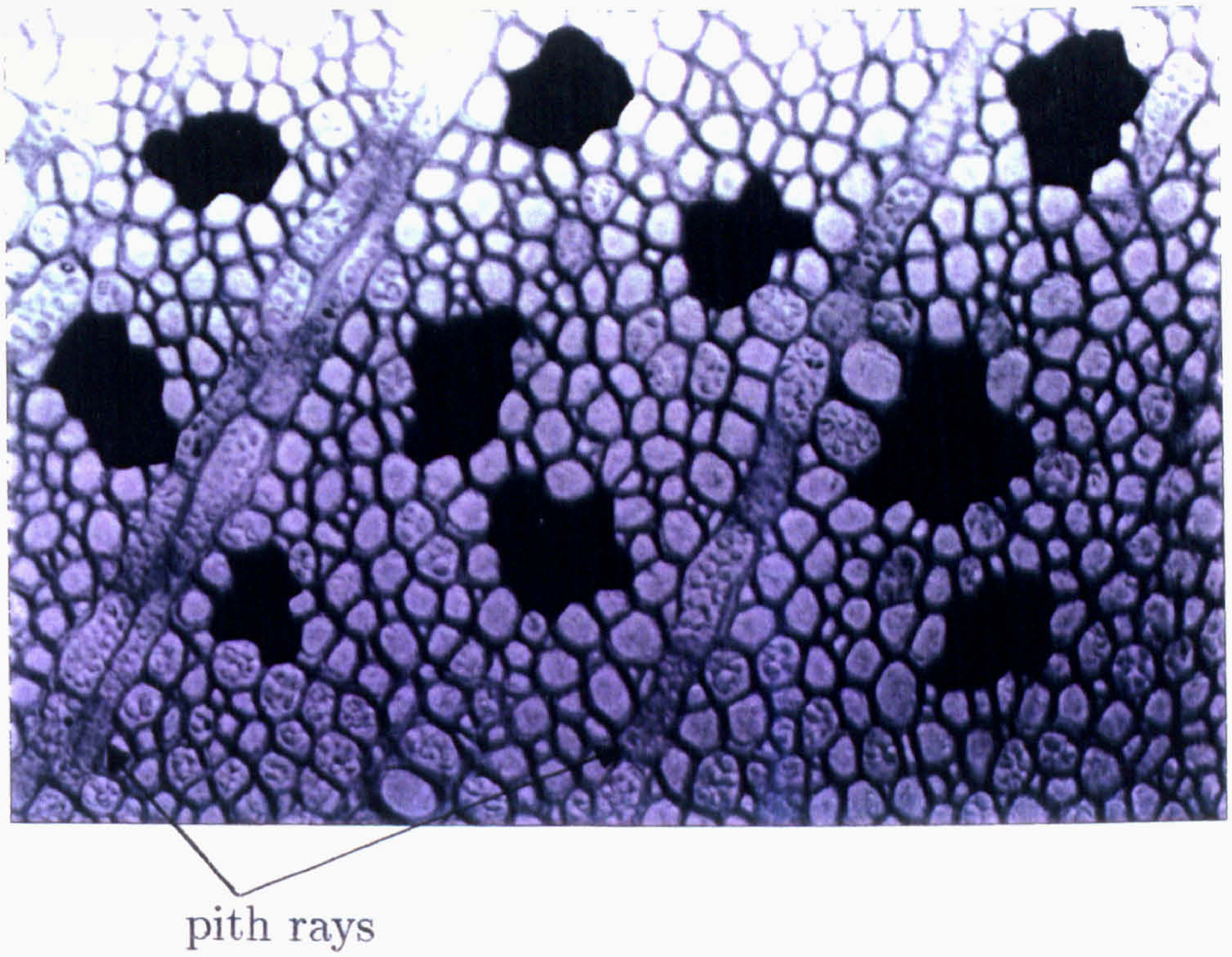

Fig. 51 Xylem of two-year-old ash saplings. Ten clusters of ten cells per section were blackened and cell size was determined from these clusters using an areameter. Two sections of every plant from the CF and CF+O_3 treatments were analysed.

5.5.3 Cell size

In contrast to cell number, cell size (figs. 49 and 50) determined from clusters of 10 cells from photographs (fig. 51) which had been taken from the stem sections did not show the same trends as ringwidth. Furthermore, cell size determined from the sections taken in 1993 (fig. 49) was considerably larger than that found in 1994 (fig. 50).

In 1993 there was little difference (4%) between the two treatments, with the CF

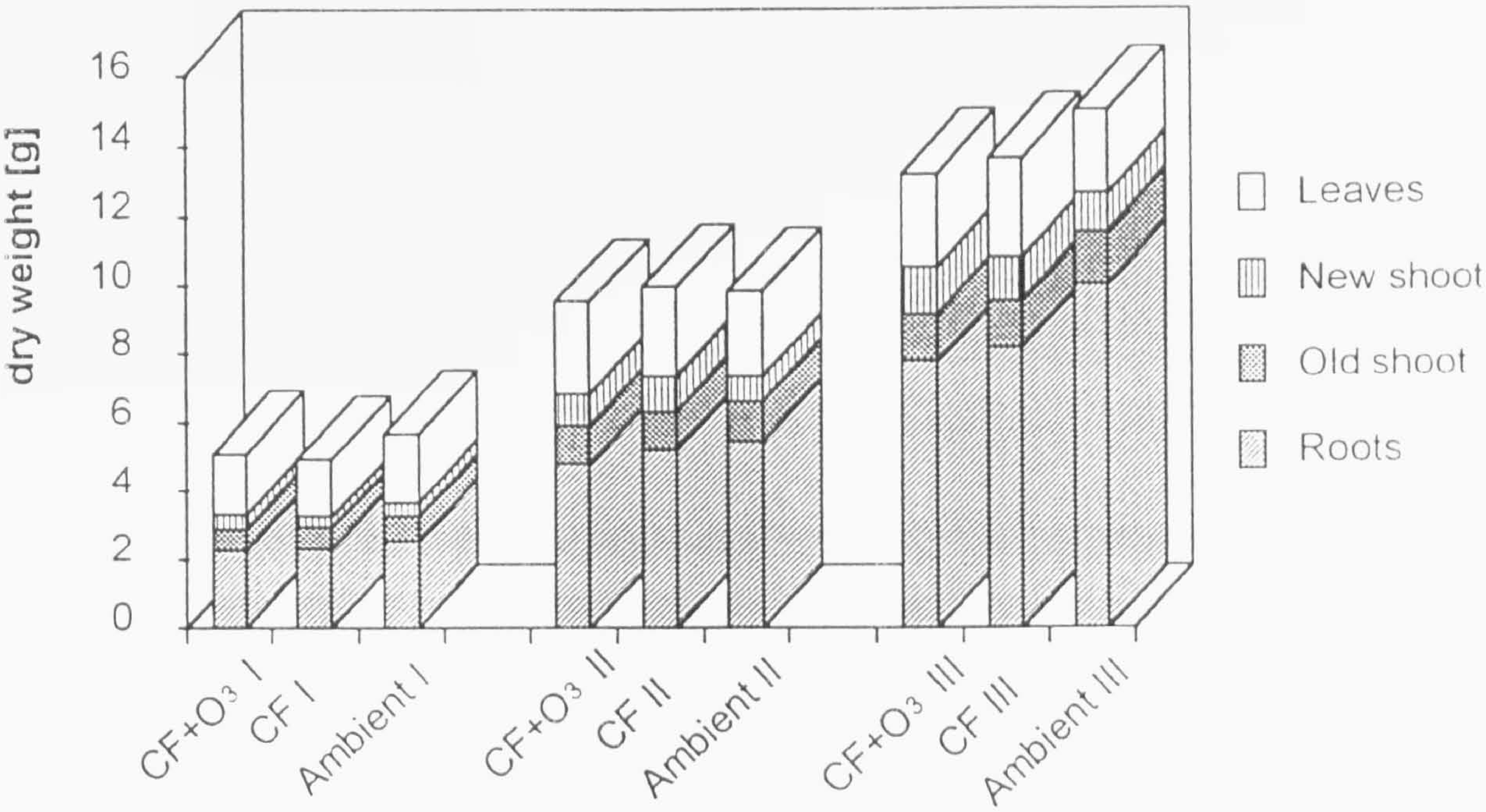

Fig. 52 Biomass of one-year-old seedlings at harvests I-III, 1994. Treatment differences were not statistically significant.

treatment showing the smaller cells. In 1994 the cell sizes of the treatments were very similar, with the exception of the CF+O_3(d) treatment, which showed markedly smaller cells.

5.6 Biomass of one-year-old seedlings

Biomass, given as dry weight of the individual plant organs from three harvests of the one-year-old seedlings in 1994, is shown in figure 52. Between the first harvest on 29 June - 2 July and the third one on 26 - 29 September, the biomass of the seedlings more than doubled. There were no major differences between the three pollution treatments at any of the harvests. Across the season, the relative biomass of the individual

Harvest I			
	$CF+O_3$	CF	Ambient
Leaves	35.2	34.3	35.2
New shoot	7.9	7.1	7.3
Old shoot	12.3	12.1	12.4
Roots	44.5	46.6	45.0
Harvest II			
	$CF+O_3$	CF	Ambient
Leaves	27.9	26.6	25.4
New shoot	9.8	9.6	7.3
Old shoot	11.9	11.6	12.0
Roots	50.3	52.2	55.3
Harvest III			
	$CF+O_3$	CF	Ambient
Leaves	20.3	20.9	15.6
New shoot	10.6	9.3	7.3
Old shoot	10.2	9.9	10.1
Roots	58.9	59.9	66.9

Table 11 Relative biomass [in %] of individual plant organs of one-year-old seedlings at harvest I-III, 1994.

plant organs changed (table 11).

While the relative biomass of the new shoot and of the roots increased over the season, that of the old shoot and the leaves decreased. However, the relative increase of new shoot biomass was only about 2% and so was the decrease of old shoot biomass. The relative increase in root biomass, as well as the relative decrease of shoot biomass, were much higher at about 15%.

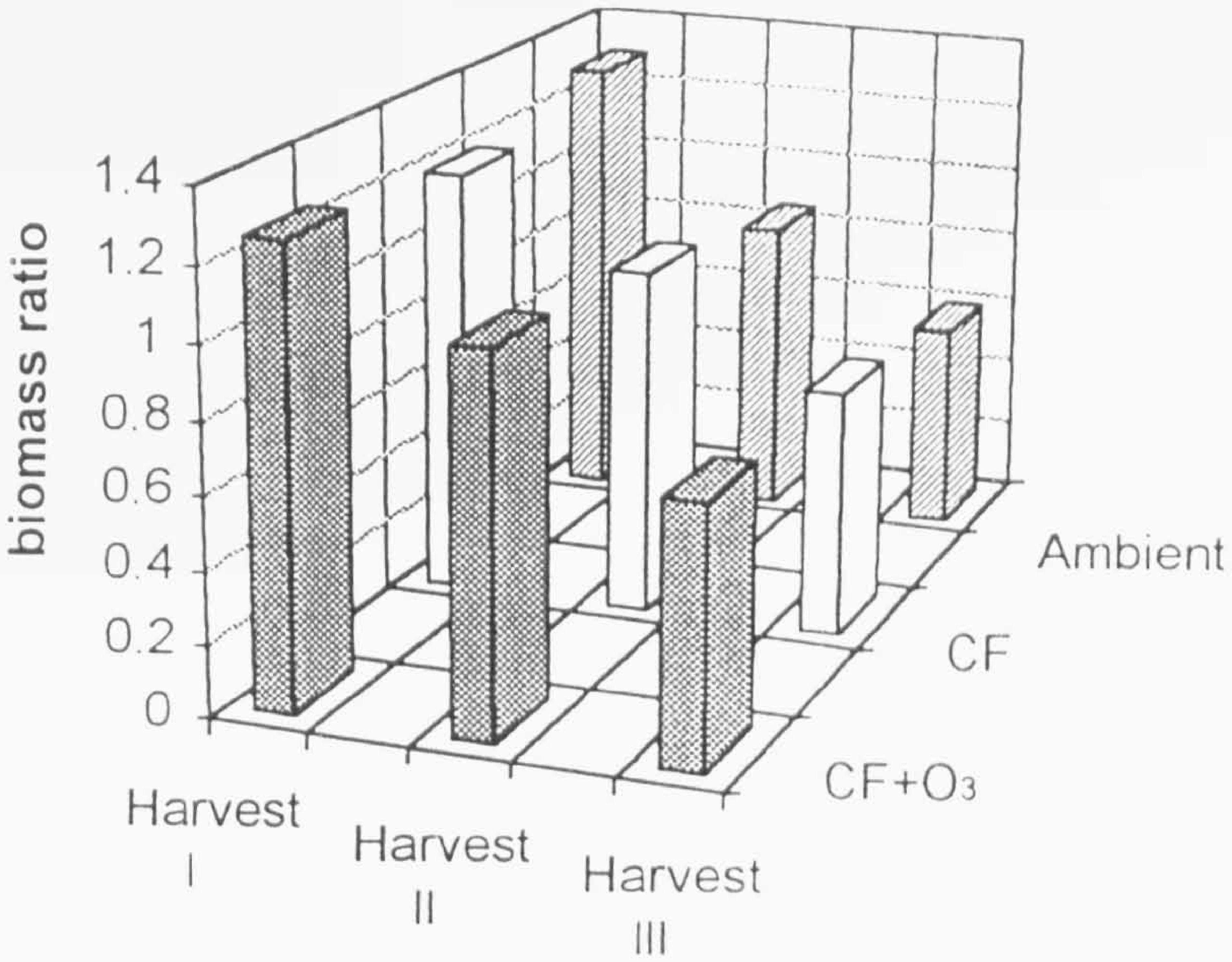

Fig. 53 Ratio of above:belowground biomass at the harvests of the one-year-old seedlings during the 1994 growing season. Treatment differences were not statistically significant.

Although at the third harvest, relative root biomass of the Ambient treatment was almost 7% higher than that of the chambered treatments, this difference was not statistically significant; neither was the 5% difference found for leaf biomass. Concomitant with the alterations in relative biomass of the individual plant organs, the ratios of aboveground : belowground biomass (fig. 53) also changed. For all three pollution treatments, a marked decline of the aboveground : belowground biomass ratio could be found, and while at the beginning of the season the ratio was > 1 for all treatments, it declined to app. 0.7 for the two chambered treatments and to 0.59 for the Ambient treatment.

5.7 Annual rings of one-year-old seedlings

As with the two-year-old saplings, annual rings of the one-year-old seedlings were investigated only for the CF and CF+O_3 treatments. The data shown derive from the second (4-7 August 1994) and third (26-29 September 1994) harvests of the one-year-old seedlings. Although there were almost 8 weeks between the two harvests, the differences between them were only marginal.

5.7.1 Ringwidth

While for the second harvest in August, ringwidth (fig. 54) of the CF treatment was higher than that of the CF+O_3 treatment, the situation was reversed at the third harvest, where ringwidth of CF+O_3 treatment was slightly higher than that of the CF treatment. However, on the second harvest the difference between CF and CF+O_3 was more pronounced.

The ringwidth found at the third harvest was similar to that found for the well-watered treatments of the two-year-old saplings in 1994 (fig 46) and thus higher than that of the droughted two-year-old plants.

5.7.2 Cell number

The results for cell number per ringwidth (fig. 55) mirror those found for the ringwidth of the annual rings, showing higher cell numbers for the CF treatment as compared to the CF+O_3 treatment on the second harvest, but lower ones on the third. On average, cell numbers per ringwidth were slightly higher than those found for the two-year-old plants in 1994 (fig. 48). However, they did not exceed those of the CF+O_3(w) treatment of the two year old saplings, which was the treatment with the highest cell numbers per ringwidth among those plants.

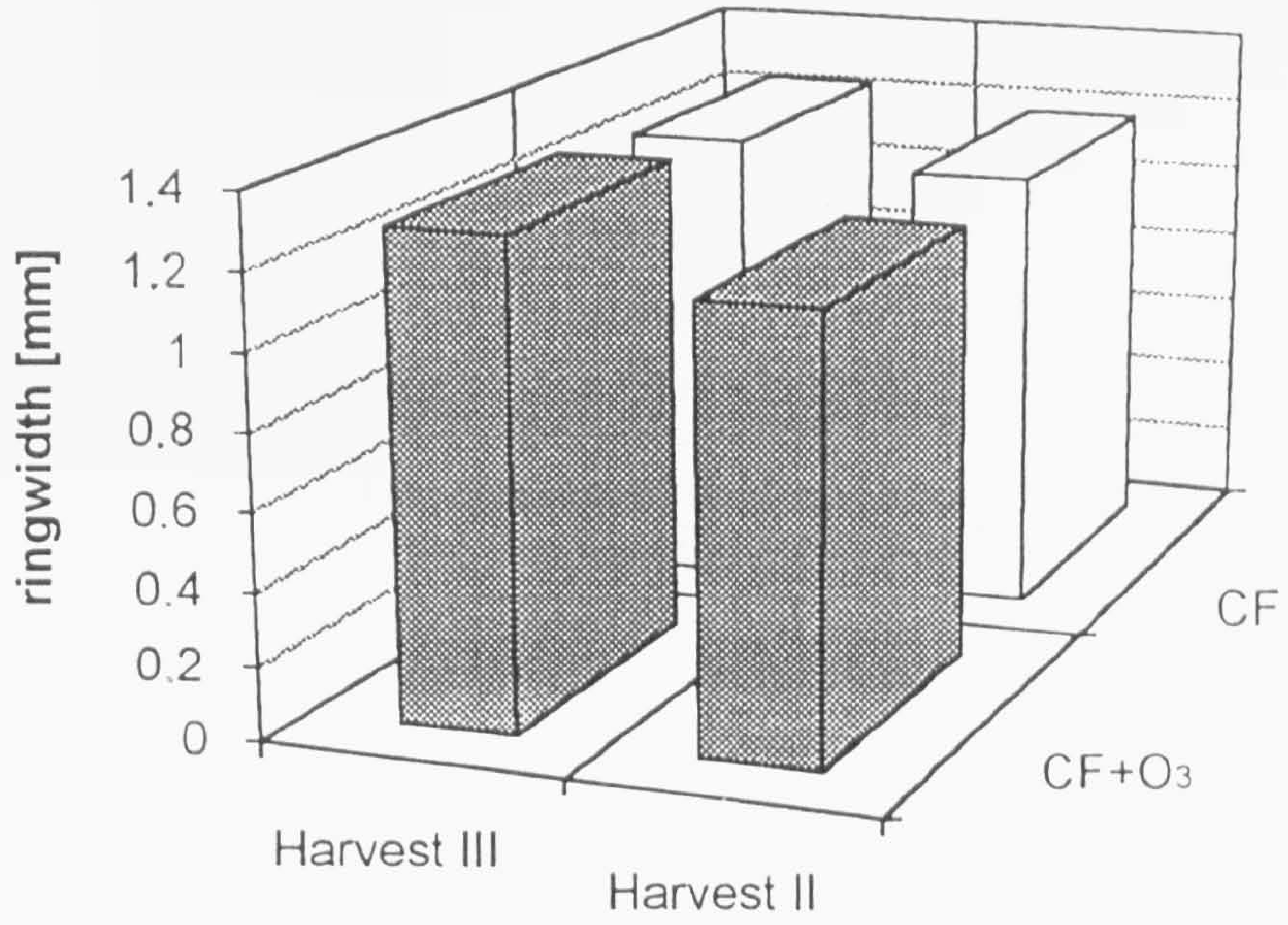

Fig. 54 Mean ringwidth of annual rings of one-year-old seedlings, 1994. Differences were not significant.

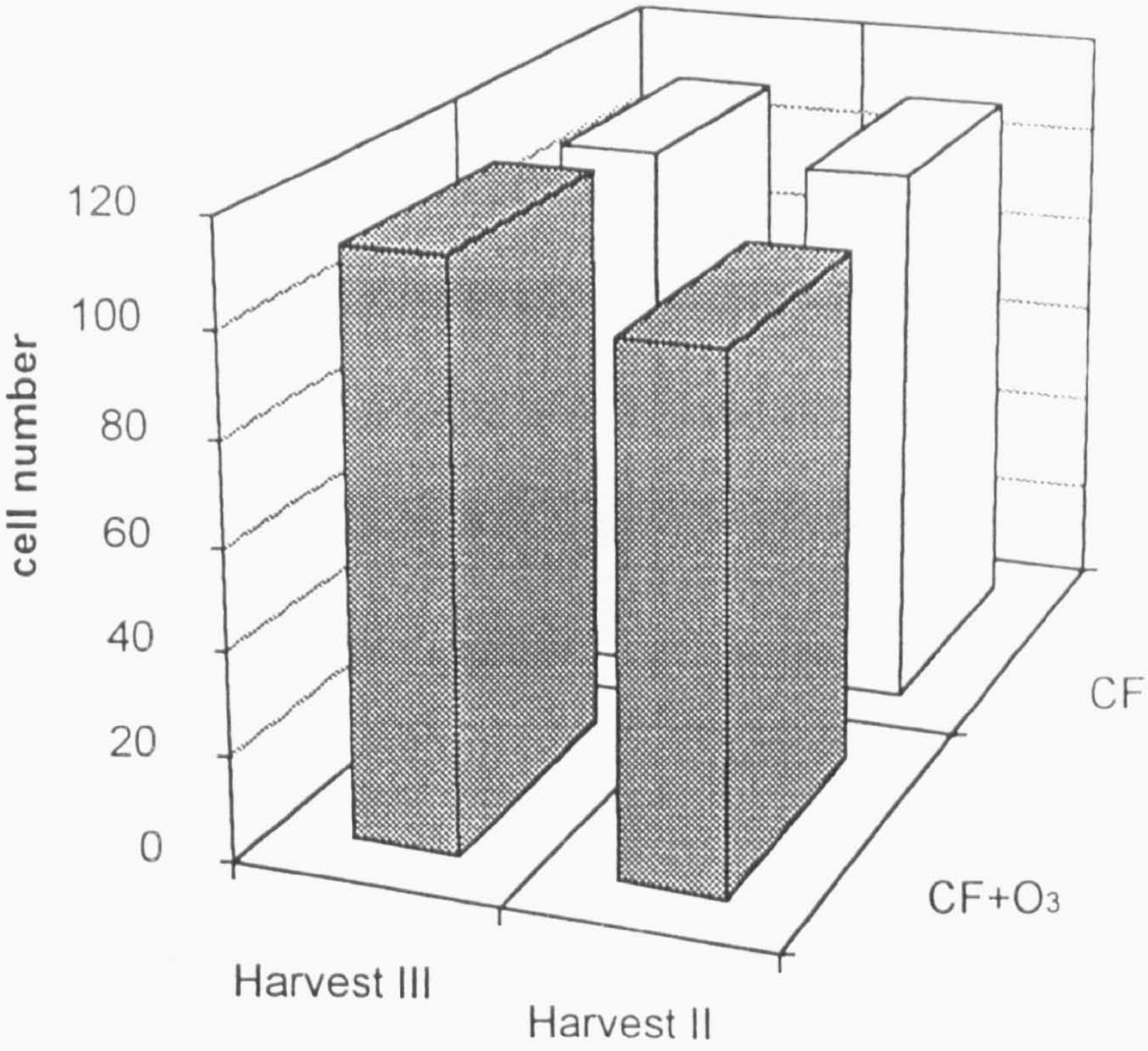

Fig. 55 Mean cell number of cell lines orientated with the pith rays in annual rings of one-year-old seedlings, 1994. Differences were not statistically significant.

5.8 Translocation of photosynthates

On the sixth day after the fumigation was started some plants of the 150ppb treatment showed small chlorotic areas. Those did not increase much until the termination of the treatment. Only two days after the fumigation first chloroses could be detected on the 75ppb treated plants. In no case did the chlorotic areas reach more than 10% of the leaf area. The control plants did not develop any chlorosis.

Forty-eight hours after the ozone fumigations were terminated (fig. 56), the distribution of the ^{14}C labeled photosynthates was altered for both ozone treatments as compared to the control. Figure 56 gives the percentage of labeled photosynthates per milligram fresh weight of the individual plant organs.

The amount of labeled biomass retained in the leaves was about 5% higher for the ozone treatments than for the control plants. While the percentage of labeled assimilates found in the shoots was very similar for all three treatments, the situation in the roots was different to that found for the leaves. Here the amount of labeled assimilate was about 3-3.5% higher for the control plants. Between the two ozone treatments of the experiment no real differences could be found.

The results of the ^{14}C-labeling which was conducted 120 hours after the ozone treatments were inaccurate, since the plants had assimilated only little $^{14}CO_2$. Thus the number of counts was very low and hardly exceeded the natural ^{14}C background of the scintillation cocktail.

One hundred and ninety-two hours after the fumigation, the ozone treatments and the control showed similar assimilate export rates from the leaves. In general, the values for translocated assimilate were lower than in the first experiment, which may be due to the unfavourable weather conditions on that day. The results for the assimilate translocation to shoots and roots of the 75ppb treatment did not fit into the otherwise homogeneous picture. Here translocation to the roots was slightly increased. The reason for this could be seen in the different distribution of biomass in these plants as compared to the other two treatments (fig. 57). The plants showed a lower root-shoot ratio. Possibly this was due to mechanical injuries of the root system, which had been inflicted already before the start of the experiments, when the plants were removed from

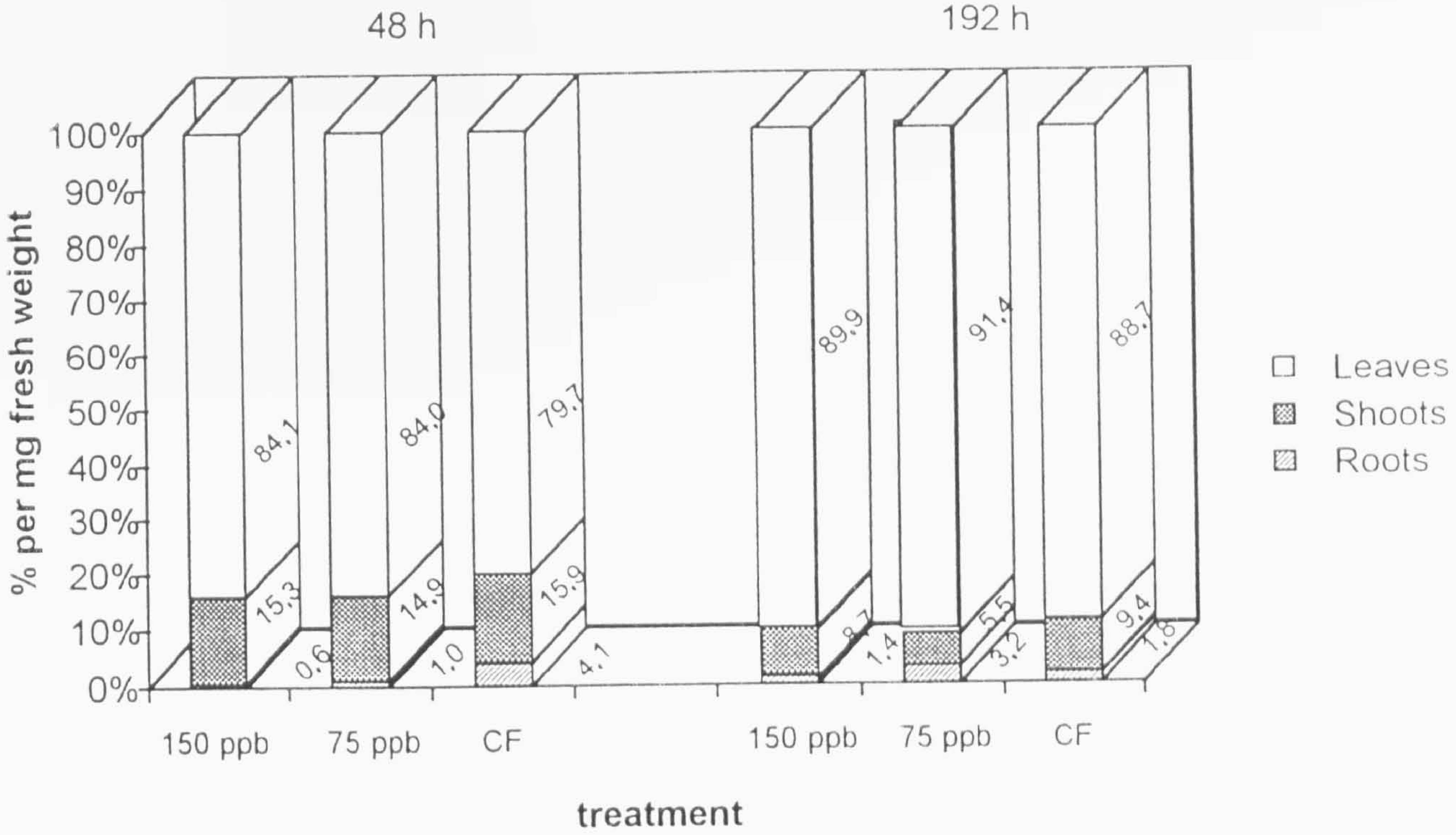

Fig. 56 Translocation of carbohydrates 48h and 192h after labeling.

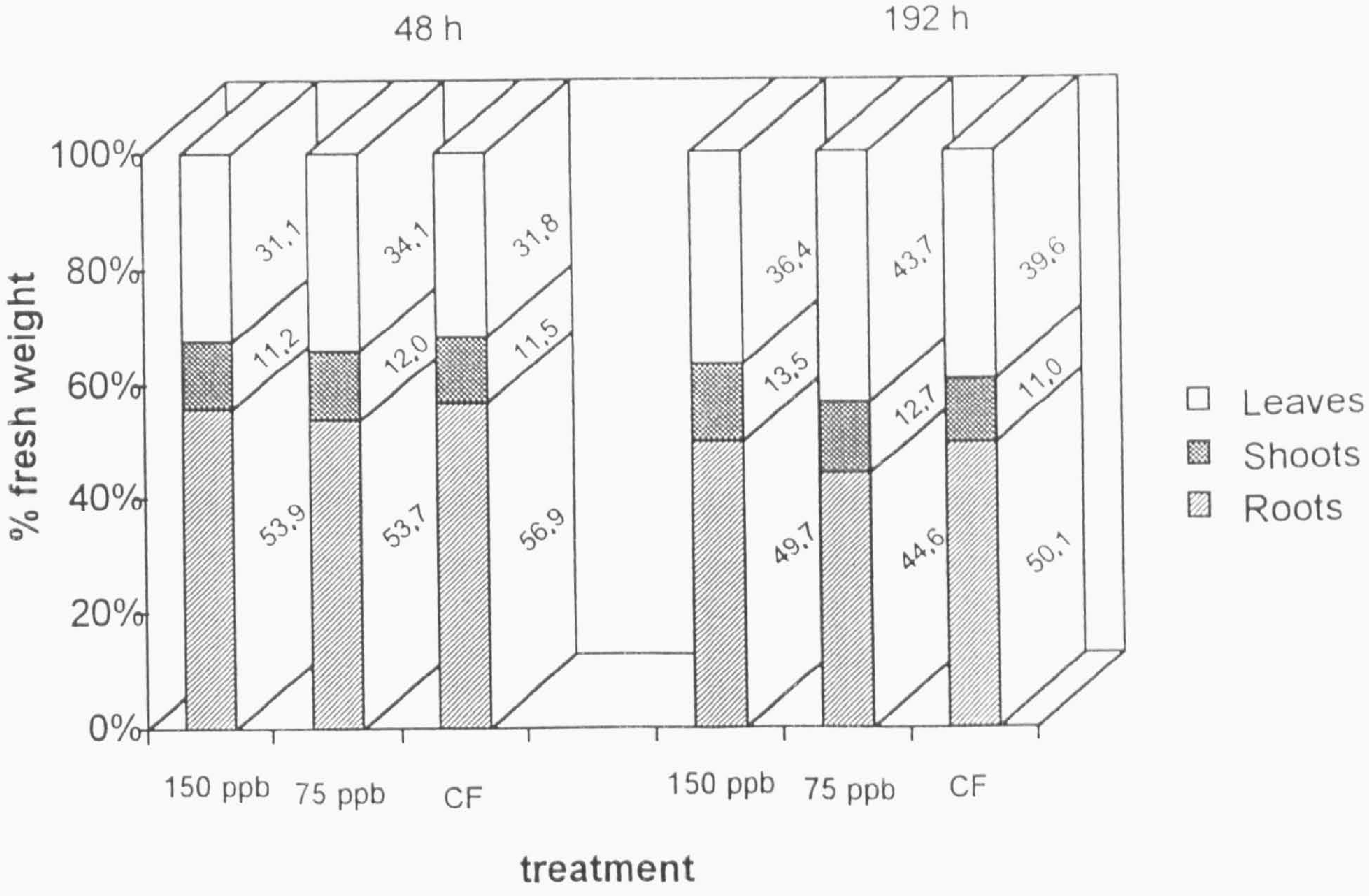

Fig. 57 Fresh weight of leaves, shoots and roots of plants harvested 48h and 192h after labeling with ^{14}C.

their pots and transferred into solution culture. The increased assimilate translocation to the roots could have worked as a compensatory mechanism. For the first experiment, conducted 48h after the termination of the ozone exposure, such differences in biomass of the individual plant organs did not exist.

6 Discussion

6.1 Stomatal response to ozone and drought stress

In this study on the response of ash to ozone and drought stress, ozone stress alone did not cause significant changes to stomatal conductance in either of the two age classes investigated, nor was there a clearly defined and uniform trend for all plants.

However, across the season, stomatal conductance of the field-grown trees, which were merely rainfed but nevertheless did not experience a real drought condition, tended to be lower in the CF+O_3 treatments than in the CF treatments and this was true for both years. This tendency, which was not statistically significant, was more pronounced in 1993. Decreased stomatal conductance and lowered transpiration rates in ozone stressed plants are frequently reported (Balaguer *et al.*, 1995; Eamus & Murray, 1991; Mikkelsen & Ro-Poulsen, 1994; Reiling & Davison, 1995). In the case of the two-year-old saplings, the described trend could be found only in 1993, while in 1994 stomatal conductance of the CF+O_3(w) was slightly increased as compared to the CF(w) treatment and this response was found particularly on exposure days and days following an exposure.

Where a decrease in stomatal conductance in response to the ozone exposures was found, it differed among leaves depending on their maturity or age and on their state of health. As the comparison of mature-healthy, mature-injured (with the term injured not referring to ozone injury in particular, but to injury in general, including that inflicted by insects developing on the leaves) and young healthy leaves showed, stomatal conductance decreased more strongly in mature-healthy leaves than in the other two leaf groups investigated. The decrease found in the young-healthy and in the mature-injured leaves was of the same magnitude, and so were the stomatal conductance levels for the different treatments. In contrast, the mature-healthy leaves showed distinctly higher stomatal conductances for the CF and the Ambient treatments. Accordingly, the variability in stomatal response was much higher in the mature-healthy leaves than in the immature or injured leaves. The stronger decline in stomatal conductance in the ozone-stressed mature-healthy leaves may have been caused partly by the higher uptake of ozone by these leaves, as compared to the other two leaf groups, due to their greater stomatal aperture when not affected by ozone, thus triggering a stronger response.

Differential physiological response to ozone of differently-aged leaves has also been reported for *Populus deltoides* x *Populus nigra* (Freer-Smith & Taylor, 1992) and several *Pinus* species (Barnes, 1972), with for example no effect of ozone exposure on photosynthesis of *Pinus strobus* seedlings bearing only primary needles, but a depression in secondary needles of older seedlings. A decline in both stomatal conductance and photosynthetic capacity with leaf age was shown for *Pinus ponderosa*, while, besides needle age, needle position also influenced ozone sensitivity (Clark *et al.*, 1995).

In contrast to the ozone exposures, the drought treatment alone caused a highly significant decrease in stomatal conductance in the two-year-old plants subjected to water stress in 1994. However, the drought applied in 1993 did not cause significant alterations in stomatal behaviour, suggesting that drought stress in 1993 was not as severe as in 1994.

Measurements of gravimetric soil water content in 1993 showed distinctly higher values for the droughted pots than were found in 1994. However, this does not necessarily imply that the availability of soil water to the plants was also higher in 1993, since the latter is determined by soil water potential, which depends on grain size and structure of the soil (Schaefer & Tischler, 1983) and thus on the soil type. Due to the mixing of sand into the soil in 1993, grain sizes and structure of the soil certainly differed from those in 1994; pore sizes will have been bigger in 1993. Hence it can be expected that, in addition to the higher soil water content, more water was available to the plants at a given soil water content, since in larger pores water is bound less strongly, and the water potential of the soil is less negative. Another factor that might have had an impact on the water stress was the different dessication behaviour of the soil-sand mixture as compared to the pure soil used in 1994.

Besides soil moisture stress, water vapour pressure deficit (VPD) of the air may have been another factor inducing the marked stomatal closure in 1994. During the months June-August, which were the main investigation period, VPD was considerably higher in 1994 than it had been in 1993 (cf. chapter 4.2.2) when high levels of rainfall occurred. Humidity control of stomatal opening has been described for *Prunus armeniaca* L. (Schulze *et al.*, 1975) for a range of soil water contents from field capacity to about 8%, which in their experiment corresponded to a soil water potential of -12 bar. On the other hand, Gollan *et al.* (1985) and Parker & Pallardy (1991) found stomatal movement during early stages of drought in *Nerium oleander* and *Juglans nigra* L. to be more closely related to soil water potential than to the diurnal leaf water status, which depends strongly on the water vapour pressure deficit of the surrounding air.

In 1994 stomatal response to drought differed for the three drought cycles, and although soil water status was very similar during the second and third drought cycle, stomatal response was decreased during the third drought cycle and reduction in stomatal conductance was smaller than it had been during the previous two cycles. This was observed for all three pollution treatments and it shows a decreasing sensitivity of the stomata to drought, as has been described also for *Picea mariana* (Stewart *et al.*, 1995). However, reports that stomatal sensitivity to drought increased with leaf age, especially in agricultural species, are more frequent (Stewart *et al.*, 1995).

Generally, polluted and unpolluted plants responded similarly to drought, but in 1994 an interaction between the pollution treatment and drought also became evident, causing stomata of ozone and drought stressed plants to close even more tightly than those of plants that were subjected to drought stress alone; on the other hand, the well watered but ozone stressed plants were showing slightly increased values of stomatal conductance. A corresponding response was found for *Picea abies* growing in air with different water vapour pressure deficits, but at identical soil moisture (Maier-Maercker & Koch, 1991); for yellow poplar seedlings, it was reported that ozone affected leaf diffusive resistance only in plants growing at 80% humidity, but did not cause any changes in seedlings that were kept at 40% humidity (Jensen & Roberts, 1986). These findings support the observation that trees growing in areas with a high humidity are more liable to ozone injury (Keller & Häsler, 1987) and that foliar injury in sensitive species is more widespread in humid years with lower ozone levels than in dry years with higher O_3 concentrations (Showman, 1991).

Besides increasing the ozone uptake of the plants, such stomatal opening could be expected to enhance water loss during periods of good water availability and possibly also during short periods of darkness (Keller & Häsler, 1984) or, since longterm ozone exposure can alter the diurnal pattern of stomatal conductance (Reich & Lassoie, 1985), also at night (Skärby *et al.*, 1987). Accordingly, water use efficiency would decrease (Freer-Smith & Dobson, 1989; Reich *et al.*, 1985; Wallin & Skärby, 1992; Yang *et al.*, 1983). This is not a unique feature of ozone-stress, but has been shown to be caused by other air pollutants, e.g. SO_2 and NO_2 as well (Lucas, 1990; Neighbour *et al.*, 1988). The increased water loss could further lead to more rapid depletion of soil water reserves and consequently to earlier and longer-lasting periods of drought. Since ash is adapted to a habitat with good soil water availability and naturally shows rather high values of stomatal conductance, compared to other woodland trees occurring in similar habitats (Körner *et al.*, 1979), ozone uptake can be assumed to reach high levels as well. This is particularly likely since, on a continuum of drought avoidance to drought tolerance, *Fraxinus* tends to tolerate drought and to sustain transpiration rates despite decreasing water potentials (Besnard & Carlier, 1990; Whitlow *et al.*, 1992). Thus a comparatively high level of stomatal conductance is maintained in the face of high water vapour pressure deficits that mostly occur in the early afternoon and are often correlated with the highest ozone concentrations. This behaviour also became evident from the diurnal courses of stomatal conductance measured in this study, where no midday depression was found, but conductances were maintained on a high level for most of the day or showed a more or less steady decline throughout the day. Due to this stomatal behaviour, ash is at comparatively high risk from ozone injury, as well as from air pollutant injury in general, and a significantly increased amount of foliar chlorosis in ambient air has already been reported for a site in south-eastern England (Ashmore *et al.*, 1985). Evidence that species with higher stomatal conductances are more likely to be affected by ozone stress has also been given by Reich & Amundson (1985).

A response corresponding with the drought-induced stomatal closure of ash, leading to partial protection from ozone damage, was found for *Pinus ponderosa* Laws. (Beyers *et al.*, 1992) and for *Picea abies* (L.) Karst. and *P. sitchensis* (Bong.) Carr. (Dobson *et al.*, 1990). A drought-dependent reduction of ozone injury was also reported for *Phaseolus vulgaris* and found to be related to the water-stress-induced stomatal closure (Tingey & Hogsett, 1985). Thus, withholding irrigation to reduce damage of ozone sensitive tobacco varieties was also described as a common practice of tobacco farmers, to whom ozone damage has been a well known problem since about 1955 (Dean, 1972).

However, Pearson & Mansfield (1993), in a similar experiment on beech, reported conflicting results with increased stomatal resistance for the well watered and ozone stressed plants and a decrease in stomatal resistance as the water stress developed. These differences in stomatal response are assumed to be related partly to the time of the application of the drought stress, which differed between studies. Thus Pearson & Mansfield (1993) subjected to water stress plants which had been pretreated with varying ozone concentrations for 39 days, while in this study on ash the plants had received only two ozone episodes before they were subjected to drought stress. Correspondingly, Lee *et al.* (1990) measured increased susceptibility to drought in *Picea rubens* seedlings exposed to ozone and acidic precipitation, when drought stress was imposed after the completion of the deposition treatment. Type, severity and time of application of resource stress, (for example water and nitrogen deficiency) have also been shown to be involved in determining the response of *Populus tremuloides* Michx. to ozone stress (Greitner *et al.*, 1994). Differences may also be due to species dependence of stomatal response to ozone at low and intermediate pollutant concentrations (Darrall, 1989), which, like the effective ozone uptake may be determined to some degree by the plant's undisturbed stomatal conductance level and stomatal behaviour; at a given soil moisture content, the latter, for example, can vary considerably between species', seemingly dependent on the species natural habitat. According to Roberts (1990), slight to moderate drought stress may enhance stomatal opening, resulting in higher CO_2 assimilation rates. Thus species adapted to a dry habitat often show comparatively high gas exchange rates during conditions of mild drought, while they exhibit comparatively low rates at high soil moisture (Ni & Pallardy, 1991).

Results for beech, analogous to those of Pearson & Mansfield, may illustrate the species dependence of the stomatal response. Thus, for example, Le Thierc *et al.* (1994) found that stomatal conductance was increased in ozone and drought-stressed beech during the morning, while it decreased below the levels of the well-watered plants later in the day, while Leonardi & Langebartels (1990) measured an increase in stomatal opening in response to low level ozone concentrations, which was then reversed with the lengthening of the exposure period. Eamus & Murray (1991) reported an ozone-induced decrease of stomatal conductance in non-drought stressed beech.

In comparison to their behaviour at low pollutant concentrations, when various stomatal responses are found, plants respond more uniformly with stomatal closure when exposed to high pollutant concentrations (Darrall, 1989).

For 1994, across-season analysis of the data for the two year old saplings showed that stomatal conductance of the ozone and drought stressed plants [CF+O_3(d)] decreased over the season, as compared to the control [CF(w)]. In contrast, plants subjected only to drought stress had shown an increase of stomatal conductance over the season, and while at the beginning of the season they had shown stomatal conductance levels that were as low as those found for the ozone and drought stressed plants, by the end of the season they had recovered to levels found for the well-watered control [CF(w)]. This tendency was due to the better recovery of the unpolluted plants from drought stress after the drought cycles were finished. That the ozone stressed plants' capacity for recovery from drought stress was less than that of the droughted control [CF(d)] also became evident from the comparison of stomatal conductance on the different daytypes. From the daytypes it became evident that the control plants [CF(d)] had almost recovered from drought stress two days after they had been watered again (this coincided with days following two days after an exposure), while recovery of the droughted and ozone stressed treatment was still lagging behind. On days with only very mild or no drought stress (coinciding with days where at least six days had elapsed since the last exposure) there was no longer any difference between CF(d) and the well watered plants, but there was still a considerable difference for the CF+O_3(d) treatment. Similar loss of the stomatal ability to rapidly adjust the transpiration rate to changes in climatic conditions was reported for *Picea abies* (Maier-Maercker & Koch, 1991). They found that healthy twigs of *Picea abies* immediately decreased stomatal opening when the flux-quotient of water uptake to water loss fell only minimally below 1.0 and, due to the control capacity of the stomata, it never fell below 0.9, implying that controls closed their stomata when water uptake was only minimally lower than water loss to the atmosphere. In contrast, twigs from ozone-pretreated trees closed their stomata only at flux-quotient values far below 1.0, and the irregular and delayed stomatal closure resulted in values of 0.5 or even lower.

For a stand of damaged spruce trees on a low mountain site in western Germany (Rhenania palatina), characterized by high ozone loads and poor magnesium availability, it has been shown that the plants no longer exhibited the otherwise typical midday reduction of transpiration on light-intensive and dry summer days. Thus the constant transpiration considerably reduced the water potential of the plants and caused water stress (Sabel, 1991). Such decreased responsiveness and reduced range of conductance may indicate accelerated ageing (Reich & Lassoie, 1984).

Besides the above differences between the treatments, there were also differences between the conductance values of the investigated daytypes, which were similar for all drought/pollution combinations. This variability may have been due to differences in atmospheric conditions at the time of the assessments, rather than specific effects due to changes in pollution climate surrounding the plants. Water vapour pressure deficit (VPD), which is accepted as one of the main determinants of stomatal conductance, even at decreased soil water content (Schulze *et al.*, 1975), is assumed to have differed for the respective daytypes. According to Wieser & Havranek (1993), VPD is the climatic factor most positively correlated with O_3 concentrations. Both ozone concentration and VPD are determined by irradiance and temperature. Since high leaf-air deficits lead to stomatal closure, this factor is assumed to reduce potential ozone stress. For ash, such mitigation of ozone stress by high leaf-air deficits may not apply unrestrictedly, since, as has been mentioned above, the diurnal course of stomatal conductance of well-watered plants seems to depend more on light intensity and midday depressions are not found. This behaviour has also been described for ash from an alluvial forest site (Besnard & Carlier, 1990).

The poor recovery of the ozone and drought-stressed plants indicates decreasing responsiveness of the stomata, as described for *Picea abies* (Keller & Häsler, 1984), *Populus deltoides* (Reich & Lassoie, 1984) and *Pinus sylvestris* (Skärby *et al.*, 1987). The results also demonstrate that stomatal responsiveness was unimpaired by prior drought cycles alone and that the decrease in stomatal conductance shown by the CF+O_3(d) plants must be attributed to the interaction of drought with ozone.

This decrease in stomatal conductance supports the results of Wiltshire *et al.* (1994) for four-year-old field grown ash trees; they reported a larger decrease of mean daily water use over the season, when plants had been exposed to ozone, while during the early part of the season the ozone-treated plants had been using more water than the control. Similarly Olszyk *et al.* (1991) detected small but consistent decreases in stomatal conductance and transpiration rates for 'Valencia' orange.

The differences in the plants' response to the imposed treatments, which were evident between the two year-old-plants from the two growing seasons and also between the field-grown trees and the well watered potted saplings used in 1994, may have been due to differences in either soil water status or air humidity. Since the soil water status of the field-grown trees was not monitored this cannot be proven in this case; however, from the results of the measurements of soil water contents in the pots of the two year old saplings, a lower water content became evident for the well-watered treatments in 1993, as compared to 1994 (see above).

Accordingly, it is possible that the amount of water available to the two-year-old saplings differed for the two years and that in 1993 water had been less abundant for the plants of the well watered treatment than it was the following year and that plants were at times mildly water stressed. This also may have been the case for the field-grown trees.

When results from the Ambient treatments are taken into account, the different microclimatic conditions as well as the ambient levels of ozone and other pollutants may have to be considered as the main factors contributing to the differences in stomatal behaviour, as compared to the chambered treatments.

During the summer months, means of water vapour pressure deficit were lower in the ambient plots than in the open-top chambers. There was very little difference in temperature between the ambient plots and the chambers, but here as well the ambient plots tended to show slightly lower values. Concerning ozone pollution, the ambient plots showed intermediate values for most months in both years of the investigation; however, they received a higher amount of low level background ozone, and since ambient levels of ozone did not increase and decrease as rapidly as in the CF+O_3 chambers, they received low level ozone for longer periods and to some degree also during the early hours of the night. Under natural conditions, these O_3 concentrations representing intermediate levels for a certain site are supposed to be very important to the plants. During the hours when peak ozone concentrations occur, CO_2 uptake does not reach its optimum (Krupa *et al.*, 1994), and thus stomatal conductance, which determines ozone uptake by the plant, is lower than earlier in the day. For ash this may be questionable since, as has been mentioned above, the plant's stomatal behaviour is mainly determined by irradiance and particularly in well watered plants no afternoon closure of stomata is found.

Apart from ozone, plants from the Ambient treatment also received a certain amount of SO_2, due to the proximity of the field site to Ratcliffe on Soar power station. Results from the typical exposure day (chapter 4.2.2) show that the peak concentrations of this pollutant also occurred during the afternoon hours. However, since variations in SO_2 concentrations depended on the working scheme of the power plant and also on the wind direction, the observed peaks were rather irregular and not connected to a certain time of the day. Peak concentrations ($>$ 40ppb) were found on approximately 30 % of days during the vegetation period (not shown). This additional impact of SO_2, to which all trees were exposed as long as the chambers were uncovered, but which was strongest on the always-exposed Ambient treatment, may have interacted with ozone. In general, it has been reported for mixtures of air pollutants that they reduce the threshold at which effects are first detected and increase the level of inhibitory responses (Darrall, 1989). According to Darrall, decreased stomatal conductance in response to ozone and SO_2 is reported in the majority of studies. A study of the impact of SO_2 and O_3 on stomatal behaviour of grapevines, however, showed that both pollutants individually, as well as their combination, decreased stomatal conductance in these plants. Although SO_2 injury to leaves was less when plants were exposed to both O_3 and SO_2, total leaf necrosis from SO_2 injury and oxidant stipple was greater for mixtures of the two gases (Rosen *et al.*, 1978).

In 1994, stomatal conductance of the two-year-old saplings from the Ambient treatment showed higher values than those from the two chambered treatments for 77% of the assessments, while in 1993 it did so in only 47% of measurements. Similarly, there was no specific tendency to higher or lower stomatal conductance of the Ambient treatment of the field grown trees. From their study on spruce and beech, Le Thierc *et al.* (1994) reported frequent reductions in stomatal conductance resulting from the impact of the open top chambers. They assumed that this was due to the ventilation in the chambers leading to decreased boundary layer resistance in chamber foliage as compared to foliage from the ambient plots. However, besides reducing stomatal conductance, increasing windspeed concomitantly leads to increased deposition of ozone onto leaves due to the reduced thickness of the boundary layer, where ozone concentrations tend to be lower than in the free air current (Janach & Imboden, 1989). Accordingly, a windspeed of 1 m/s leads to an increase of deposition velocity by a factor 4-5 as compared to still air. Unlike Le Thierc *et al.* (1994), who assumed the windspeed of chamber air to be higher than that of the unchambered plots, Janach & Imboden (1989) considered windspeeds applied in chamber experiments as often unrealistically low, particularly when plants from mountainous regions or from valleys in the Alps are investigated, since there thermally generated local winds are often much stronger than those found in the plains.

The lack of difference in stomatal conductance between plants from ambient plots and those from chambered plots does not imply that photosynthetic response is also similar. This was shown by Beyers *et al.* (1992), who found lower light-saturated photosynthetic rates in plants from chambered treatments that received non-filtered air as compared to those grown in the open. Since they found midday light levels comparable in both settings, they attributed this difference in photosynthetic capacity mainly to the consistently elevated midday temperature (3-5^{0}C) found inside the chambers, which obviously had no impact on concomitant stomatal conductance.

In this study on ash, the saplings from the ambient treatment subjected to the drought treatment recovered better from the drought stress than did those from the CF+O_3 treatment, but recovery was not as good as that of drought stressed plants from the CF treatment. This became evident from the across-seasonal trends of stomatal conductance, as well as from the mean stomatal conductances of the different daytypes. The better recovery indicates that stomatal functioning was not impaired as severely as in the plants from the CF+O_3(d) treatment.

It is evident that the results gained from the chamber experiments can be applied to ambient conditions only when great care is used, since besides the interaction with other air pollutants, in this study particularly with SO_2, chamber effects are playing a major role in determining the plant's response to the stressor investigated.

6.2 Impact of ozone and drought on growth and biomass accumulation

Stomatal conductance levels exert an important impact on growth, since besides determining water loss they also determine CO_2 uptake. Thus the differences in stomatal behaviour of the plants from the different treatments may be expected to affect growth as well. However, this is not a necessary consequence. In some studies, the light response of net assimilation was not affected by ozone, while stomatal conductance exhibited reduced values (Eamus & Murray, 1991; Freer-Smith & Taylor, 1992). In others, no clear impact on stomatal conductance became evident, while photosynthesis was enhanced, as for example in current year foliage of *Pinus ponderosa* Laws. (Beyers *et al.*, 1992). In the latter case, the enhanced photosynthetic capacity of the current year foliage was considered to be a mechanism that partly compensated for premature needle loss of the older age groups due to ozone injury, and thus did not necessarily result in higher amounts of biomass accumulation. Nevertheless, it shows that trends for stomatal conductance and photosynthesis do not always tally, nor do they necessarily lead to changes in growth or biomass accumulation, particularly since altered respiration also affects net carbon gain and thus biomass accumulation. This is true also for alterations induced by other stressors; for example, enhanced photosynthesis was reported for *Picea rubens* exposed to acidic precipitation, but did not result in an increase in seedling biomass, supposedly as a result of elevated rates of maintenance respiration during the night (Kohut *et al.*, 1990).

It has also been shown that, in plants subjected to both ozone and drought stress, stomatal closure in response to drought can be slowed down by the impact of ozone and thus the drought-induced decrease in photosynthesis may be slowed down as well Relatively high rates of photosynthesis may then occur for the ozone and drought stressed trees (Maier-Maercker & Koch, 1992).

From the measurements of total aboveground biomass during this study, no significant impact of the pollution treatment on well watered plants became evident from either of the two years investigated. However, in 1994 the Ambient treatment showed distinctly lower aboveground biomass than the two chambered treatments, while in the previous year its aboveground biomass slightly exceeded that of the CF and CF+O_3 treatments. This much smaller biomass accumulation of the Ambient treatment in 1994 does not tally with its higher stomatal conductance. Thus it can be assumed that the biomass allocation pattern of plants from the Ambient treatment differed from that found for the chambered treatments and that in the former more assimilates may have been allocated to the roots. Corresponding results gained in 1994 with the one-year-old seedlings support this thesis, since they showed the plants from the Ambient treatment to have higher percentages of root biomass and lower rates of leaf and new shoot biomass at the second and third harvests.

Such a difference in the biomass allocation pattern may be due partly to differences in the light climate between the open-top chambers and the Ambient plots, which existed particularly when the former were covered. Then irradiance levels within the chambers were distinctly (20-50%) lower than in the Ambient plots. The smaller leaf areas that were measured in 1994 for the plants from both Ambient treatments are in accordance with differences in the allocation pattern determined by irradiance.

The differences in leaf area were found from the beginning of the season and reflect the existence of a different light climate that will have had an impact on initial leaf differentiation as well as on further leaf development.

Differences in the temperature regime between the Ambient plots and the open-top chambers on days when the latter were covered may also have contributed to the possible alteration in the biomass allocation pattern (Minchin *et al.*, 1994). A corresponding temperature dependence of the relative effects of an air pollutant on roots versus shoots has been shown for SO_2 (Norby & Kozlowski, 1981). In this study, SO_2 fumigated seedlings of *Betula papyrifera* showed greater suppression of root than of shoot growth when grown at high temperatures, while shoot growth was more suppressed than root growth when plants were kept at low temperatures.

In 1993, when the Ambient treatment on average did not show higher values of stomatal conductance than the CF and $CF+O_3$ treatments, its aboveground biomass was marginally higher than that of the other two treatments. This difference was mainly due to the higher amount of old shoot biomass in the plants from the Ambient plots. However, it is not known whether these plants showed greater old shoot biomass from the beginning of the season, or if they allocated more biomass to the lower stems during the growing season. The results from the measurements of radial growth at the stembase suggest that, on average, radial stem growth at the stembase was increased in the Ambient treatment as compared to that in the CF and $CF+O_3$ treatments.

When considering only the chambered well-watered treatments, aboveground biomass production was higher in the $CF+O_3$ treatments of both years, although this difference was not statistically significant. For 1994, this finding was in accordance with the increased stomatal conductance values that had been measured in the $CF+O_3(w)$ treatment. Similarly, Billen *et al.* (1990) found increased dry matter production in *Fagus sylvatica, Picea abies* and *Abies alba* growing in ambient air, as compared to plants from filtered air treatments. This might also be attributed to the effect of ozone and some other air pollutants increasing stomatal conductance (Darrall, 1989; Lucas, 1990; Neighbour *et al.*, 1988). These findings for stomatal response mostly apply to low pollutant concentrations, while at higher concentrations stomatal closure is the response that is commonly observed.

In the study of Wellburn & Wellburn (1994), no information on biomass itself has been given, but the reported increases in height and in stem diameter of ozone-stressed *Pinus halapensis* Mill. indicate that aboveground biomass might have been increased as well. They also observed a significant drop in root:shoot ratios towards the end of the ozone exposures, caused by a decrease in photosynthate translocation to the roots of about 60%, due to a non-mobilizable starch component and most likely impaired phloem loading. Decreases in photosynthate translocation to the roots were also found in this study on ash, when translocation of ^{14}C labeled photosynthates was investigated in one-year-old *Fraxinus excelsior* L. seedlings. Small but consistent decreases were also found at the second and third harvest of the one year old seedlings exposed to ozone in the field experiment in 1994. The reduction in translocation 48 hours after completion of the ozone exposures amounted to 3-3.5%, while retention of labeled biomass in the leaves was about 5% higher than in the control treatment. The reductions were similar for plants from the high and the low ozone treatment, although the cumulative dose > 40 ppb in the low ozone treatment (3.2 ppm $\cdot$ h > 40 ppb) was only about one third of that of the high ozone treatments (10.1 ppm $\cdot$ h > 40 ppb).

Similar findings of alterations of the photosynthate translocation pattern were reported for tomato, which at 48 hours after termination of the ozone treatment retained about 15% more photosynthates in the leaves than the untreated control, and for *Phaseolus vulgaris* (Okano *et al.*, 1984) that showed a decreased translocation to the roots and stems of 53%, while translocation to the immature growing leaves was reduced by only 28%. Since, for most plants, recently matured leaves mainly translocate to the apex and to growing leaves, while older leaves translocate almost exclusively downward to the lower stems and roots (Larson & Dickson, 1986), these findings, besides showing an altered carbon allocation pattern, also may reflect the cumulative stress level of the different age groups of leaves (cf. Coleman *et al.*, 1995). The younger yet less affected leaves may be showing smaller reductions in CO_2-fixation or less need for assimilates that can be used for repair mechanisms and also less inhibition of translocation. Investigations into the response of *Populus tremuloides* foliage to ozone stress showed that photosynthetic rates of foliage from the base of the shoots of exposed plants were lower than those of plants grown in filtered air, while younger foliage exhibited elevated photosynthetic rates (Pell *et al.*, 1994). This indicates injury and accelerated senescence of foliage, but also a photosynthetic compensation of younger leaves for the reduced photosynthesis of older foliage. Early senescence and abscision of leaves is a response frequently reported for ozone-stressed plants (Jensen, 1973; Matyssek *et al.*, 1993; McLaughlin *et al.*, 1982; Pell *et al.*, 1994; Wiltshire *et al.*, 1993; Woodbury & Laurence, 1994), although a significant effect of ozone exposure on leaf longevity in the two-year-old ash saplings could not be found. However, ozone-exposed foliage does not only show earlier senescence, but reaches physiological maturity earlier as well, with O_3-treated young leaves behaving similarly to middle-aged leaves treated with filtered air (Greitner *et al.*, 1994). A corresponding response was described for *Populus tremuloides* Michx., in which mature leaves exposed to ozone showed a significant decrease in photosynthetic rate and translocated less carbon to roots, while recently mature leaves of these plants increased carbon allocation to the roots in a seemingly compensatory response (Greitner *et al.*, 1994).

Corresponding findings have been reported for both coniferous and broadleaf species; photosynthetic rates and the nitrogen content of current year foliage or the youngest leaves were increased when ozone treated (Beyers *et al.*, 1992; Pell *et al.*, 1994; Kelly *et al.*, 1993; Skeffington & Roberts, 1985; Temple & Miller, 1994). The increase in the nitrogen content of current year foliage was shown to be due to reallocation of N from senescent tissue to young, growing tissue (Manderscheid *et al.*, 1992; Rantanen *et al.*, 1994; Temple & Riechers, 1995).

According to the review of Lechowicz (1987), the overall impact of pollutant stress generally appears to be greater on roots than on shoots and root biomass decrease is more often apparent in response to either O_3 or SO_2 than to NO_2. However, the degree of the allocation response is highly dependent on the growth type of the species. In general, fast-growing species with smaller storage pools alter the pattern of allocation more readily in response to stress than do slow-growing plants with large storage reserves that can mostly meet the demands from their carbon reserves (Laurence *et al.*, 1994). Furthermore, slow-growing plants mostly exhibit smaller stomatal conductances resulting in lower O_3 uptake rates and thus less damage in the short term, while with the increased cumulative doses due to the longer exposure time, long term damage is equally found.

Experiments on *Pinus taeda* (Spence *et al.*, 1990), also showed reductions in photosynthesis, speed of phloem transport, phloem photosynthate translocation and total carbon transport towards the roots. For *Pseudotsuga menziesii*, Smeulders *et al.* (1995) reported a significant increase of 2.6 and 4.5% of ^{14}C retained in the needles respectively in autumn and pre-bud burst following ozone exposure, and these changes tally with the results found for the one-year-old ash seedlings. Besides considering the possibility of inhibited phloem loading, Smeulders *et al.* (1995) assumed that retention of assimilates in the needles may be caused by a reduction in sucrose content either by formation of other compounds or by incorporation of current assimilates into structural elements. Thus less carbon would be available in translocatable form. This assumption is supported by results from investigations into ^{14}C dynamics of mildly ozone-stressed *Pinus taeda*, which showed increased partitioning into organic acids, lipids and pigments - compounds that may be needed for injury repair - while on the other hand the formation of starch and proteins decreased (Friend & Tomlinson, 1992). Such a reduction in storage carbohydrates in stems and roots, besides decreasing root growth and regrowth of leaves, also increases susceptibility to cold temperatures in winter and to drought stress in summer (Dickson, 1989).

Similar alterations in the biomass allocation pattern were described in response to SO_2 (Noyes, 1980) and it has been shown for *Phaseolus vulgaris* that the effect on translocation was lasting longer than that on photosynthesis, since after the SO_2 exposures were terminated photosynthesis recovered within two hours, but the translocation rate did not recover to its pre-exposure rate during this time interval (Teh & Swanson, 1982). This longer lasting effect on translocation may be particularly important both under natural conditions and also under the exposure conditions that were used in this study on ash, since it may result in an altered carbon allocation pattern. Frequent ozone exposures might not allow recovery, although photosynthesis and stomatal conductance may have recovered to their normal and undisturbed level during the non-exposure intervals.

Significant changes in net retention of fixed carbon in aboveground biomass and subsequent reduction of biomass allocation to the root system were described for *Pinus taeda* (Kelly *et al.*, 1993), for which however no significant impact of O_3 on stem diameter, height or total biomass growth could be found. Decreases in root biomass were also reported for *Fraxinus americana* fumigated with ozone at concentrations of 50, 100 and 150 ppb (Chappelka & Chevone, 1986), and for ozone-treated *Fagus sylvatica* (Davidson *et al.*, 1992). Not all compartments of belowground biomass need to be affected to the same degree, as was shown by Skeffington & Roberts (1985) and by Braun & Flückiger (1995), who measured a reduction of fine root biomass only, while fine roots were found to be increased in *Pinus ponderosa* in combination with a decrease in coarse roots (Temple *et al.*, 1993). And besides biomass, the structure of roots may be altered as well, as reported by Taylor *et al.* (1989). They found thinner and longer roots in beech trees treated with ambient air, as compared to others grown in filtered air. In addition they reported a decrease in root biomass.

However, most studies investigating the impact of ozone on whole plant biomass or on total aboveground biomass indicate that exposure to the pollutant either reduces biomass production (Cooley & Manning, 1987; Edwards *et al.*, 1992, Jensen, 1981; Matyssek *et al.*, 1993; Pearson, 1995) or does not lead to significant changes in the amount of biomass produced (Kelly *et al.*, 1993). For birch, Matyssek *et al.* (1995) found that whole-plant biomass production and carbon allocation reacted more sensitively to night-time exposure. This may be important, particularly at higher elevation sites and in rural areas, where night-time ozone concentrations in general do not decline as rapidly as they do in the cities. This may explain why Braun & Flückiger (1995) reported a better correlation of their biomass data with the calculated 24 h-dose than with the daylight dose and they reported a 10% biomass reduction within three years when well watered beech plants had received a cumulative dose of 7 ppm · h > 40 ppb ($AOT40_{24}$) per growing season. In our experiment the plants received $AOT40_{24}$s of 21 ppm · h in 1993 and 24 ppm · h in 1994, while in the Ambient treatment in 1994 an $AOT40_{24}$ of nearly 3 ppm · h was attained. Nevertheless, no aboveground biomass reductions that would have been related to the ozone exposure dose could be measured. However, it has to be considered that the ash saplings used were exposed to the treatment for only one growing season, at the end of which they were harvested; thus, possible carry-over effects that might accumulate over the years were not taken account of.

In 1994, aboveground biomass production of the two-year-old plants from all pollution treatments was reduced by the impact of drought. The reduction, as compared to the aboveground biomass production of the respective well watered controls, was most pronounced in the CF+O_3 treatment. Among the droughted treatments, Ambient, as with the well watered plants, showed distinctly lower aboveground biomass production than the chambered treatments, but the difference between droughted and well watered plants was less pronounced for the Ambient than for CF and CF+O_3 treatments.

Such biomass reductions found for the aboveground plant organs are not necessarily representative for whole plant biomass, since shoot growth is generally more affected by drought than root growth, because more sensitive water deficits develop in transpiring shoots and they persist for longer periods (Kramer, 1983). Thus root growth tends to be maintained for longer in drying soil (Kozlowski *et al.*, 1991) and root:shoot ratios often increase in drought stressed plants; however, root development is restricted in drying soil since soil strength increases sharply as the soil dries and the resulting physical resistance to penetration by root tips limits growth irrespective of the deficiency of water for absorption (Kramer, 1980). Drought, however, not only restricts root growth, but also can modify it, for example by inducing plants to produce thickened root apices, which may result in an enhanced root volume, while root length is reduced (Rhizopoulou & Davies, 1993).

Contrasting results were reported for *Pseudotsuga menziesii* [Mirb] Franco seedlings (Gorissen *et al.*, 1994), which showed a reduction of total net uptake of carbon by low soil water contents, but were unaffected by ozone. In *Pseudotsuga*, reduced soil water availability also caused a decreased translocation of carbon from needles to roots, the decrease exceeding the reduction due to ozone exposure; eventually the translocation of carbon to the roots was additively affected by ozone and low soil water content.

In the *Fraxinus excelsior* saplings used in this study, an interaction between the pollution treatment and drought was also indicated in addition to the negative impact of drought on biomass accumulation. This became apparent from the even greater reduction of aboveground biomass accumulated in plants from the ozone and drought stressed treatment, as compared to the corresponding well watered treatment. This finding is in accordance with the drought × pollution interaction that has been found to have an impact on stomatal conductance, and which by lowering stomatal conductance may also have reduced carbon assimilation.

In this study on *Fraxinus excelsior*, in which aboveground biomass and different growth parameters - extension growth, radial growth at the stembase and radial growth at the base of the new shoot growth - were investigated, there were also different growth responses of the individual growth parameters.

The growth parameter most affected by the treatments was radial increment at the stembase. In 1993 the CF+O_3 treatment had the smallest seasonal increment, with values for the Ambient treatment only marginally higher. However, among the well-watered plants in 1994, radial growth at the stembase was highest for the CF+O_3 treatment. This difference was not significant, but it reflected the slightly increased values of stomatal conductance measured in these plants throughout the season. An increase in stem diameter has also been reported for *Pinus halapensis* (Wellburn & Wellburn, 1994). However, decreased radial growth (Edwards, *et al.*, 1992; Matyssek *et al.*, 1993; Temple & Miller, 1994) or no effects (Kelly *et al.*, 1993; Thornton *et al.*, 1992) are more frequently reported.

At the base of the new shoot, radial growth seemed to be slightly less affected by the pollution treatment in both years of the investigation. The trends of radial growth were the same as for the stembase, only they were attenuated. One of the reasons for the lesser growth response to pollution stress in the upper stem may be the above-mentioned differences in the degree of injury of differently aged leaves, that translocate to different sinks. Furthermore, cambial activity and xylem growth have been described as progressing from developing buds and branches to stem and to roots. Accordingly, radial growth of the lower stems takes place somewhat later in the season than at the upper stems (Dickson, 1989). From the seasonal overviews of radial growth of this study, however, this pattern did not become evident and the growth curves for upper and lower stems showed a similar course. Differences were most apparent for the absolute increase in diameter increment that was consistently smaller for the upper stem throughout the season.

In the two-year-old ash saplings, drought reduced the overall radial growth at the stembase for all three pollution treatments in 1994, but this reduction was far smaller in the CF treatment than in the CF+O_3 or the Ambient treatments, which showed intermediate growth. This water-stress-induced reduction of diameter growth was probably caused by the inhibitory effects of internal water deficits on cambial activity. For cell enlargement and cell wall deposition a certain level of turgor is required, which cannot be maintained during a more severe drought, and synthesis of growth hormones in the crown is also inhibited (Kramer & Kozlowski, 1979). Peterson *et al.* (1993) identified soil moisture supply as the dominant factor controlling interannual variation of basal area growth in *Pinus ponderosa*, and for *Fraxinus excelsior* L. Wardle (1962) reported that besides strong winds, soil dryness also can reduce the rate of height and diameter growth and the ultimate size of the tree.

During the second part of the season a significant interaction of ozone and drought became evident from the results of the four chambered treatments, showing in the larger difference between relative growth rates of the droughted and well-watered CF+O_3 treatments, as compared to the CF treatments. The reduced growth rate of the droughted CF+O_3 treatment during the second part of the growing season also led to a decrease of the seasonal mean radial increment. This large reduction in radial growth reflected the low level of stomatal conductance found for this treatment throughout the season. Interaction of ozone levels above 40ppb with low soil moisture and high temperatures leading to reduced stem expansion was also reported by McLaughlin & Downing (1995). However, they hypothesized that an alteration of stem water balance may have induced the slowing of stem growth, and that this might have been due to increased water loss and thus increased stomatal aperture of the plants, which would contrast with the results of this study.

The significant ozone × drought interaction was found only for the stembase of the plants, while radial growth of the upper stems was reduced significantly only by drought. Again this may reflect changes in partitioning or leaf injury in ozone-stressed plants, favouring growth of leaves and upper stems at the expense the lower stems and roots.

Wood production, which is mainly represented by radial growth, was found to be closely linked to leaf area development in poplar (Taylor *et al.*, 1992), which is not surprising, since, as has been mentioned above, radial growth is determined by currently fixed photosynthate. As long as only the chambered treatments are considered, this link between leaf area and wood production also seems to apply to ash. Thus leaf area development, stomatal responses and the allocation pattern are all assumed to have controlled wood production in ash. Besides determining radial growth, leaf area is an important factor controlling water loss, so plants subjected to drought stress frequently reduce the individual leaf sizes as well as leaf area in general. In ash, drought stress in 1994 also led to the formation of smaller leaf areas in plants from all three pollution treatments. Besides this effect on total leaf area, drought apparently also influenced leaf sizes of the plants, leading to a decrease for plants from all pollution treatments. Among the chambered treatments, a reduction of leaf size was also apparent in response to ozone. This can be seen from comparison of total leaf area and leaf number of plants from the CF+O_3 treatments as compared to those from the CF control. This reduction in leaf size was greater for the well-watered than for the droughted plants, respectively. As for total leaf area, leaf size of the Ambient treatments was least, but here the effect of a different radiation climate, rather than the ambient pollution, is considered to be the decisive factor that determines individual leaf size.

Decreases in leaf size in response to ozone exposure were also reported for poplar (Woodbury *et al.*, 1994), birch (Matyssek *et al.*, 1992; Pääkkönen & Holopainen, 1995) and white birch (Jensen & Masters, 1975), but conflicting results that showed increases in leaf size have also been reported (Oshima *et al.*, 1979; Taylor *et al.*, 1992).

Unlike diameter growth, extension growth of most species depends less on the current year's photosynthate, and more on stored assimilates (Dickson, 1989). In many temperate zone species, potential shoot growth is governed to a high degree by the number of anatomical stem units present in the unopened bud (Kramer & Kozlowski, 1979). Thus plant stress situations may affect the following year's extension growth rather than that of the current year.

Among the factors limiting plant growth, water availability is considered the most important both for growth of individual plants and distribution of plant species or vegetation as a whole (Larcher, 1984). This is true particularly for woody species, for which developing shoots, buds and leaves are at ever increasing negative hydrostatic pressures as they grow taller (Zahner, 1968). Thus trees growing on dry sites in general do not reach the height attained on mesic sites.

Extension growth tends to be decreased further by current water stress situations only in plants that exhibit free growth or recurrent flushing. So the effect of the water stress on tree growth depends strongly on the time of the year when the water stress occurs. In the temperate zone, soil drought usually develops later in the growing season, while in winter and spring soil water reserves tend to be replenished. Thus in many free growing or recurrently flushing species, extension growth may have terminated at the onset of drought, but the environmental conditions may still have an impact on bud formation and in this way control shoot length in the following year. In species with only a single flush at the beginning of the season, drought only has an impact on the following year's growth (Kramer & Kozlowski, 1979). When beech was subjected to drought stress, bud break was found to occur earlier in the next season, but flush length was reduced by 40% (Pearson & Mansfield, 1994). Substantial reductions in twig extension growth of beech were also found for the years 1976 and 1977 for naturally growing trees from several sites in southern England (Power, 1994). This indicated the effect of the severe drought that occurred in 1975 and 1976 throughout much of England.

Similarly, marked reductions of branch length developing in the year following treatments were also reported in response to ozone exposures (Matyssek *et al.*, 1993b; Pearson & Mansfield, 1994). These reductions reflect the decrease in stored assimilates determining the following year's growth that is caused by the impact of such stressors. Both studies showing the impact of ozone stress on the following year's growth were conducted with saplings, in which such carry-over effects become apparent more easily, since the young plants have only accumulated small carbon reserves. Swank & Vose (1990/91), who found ozone stress to induce premature senescence and leaf loss as well as reductions in wood increment and a decrease in net nutrient accumulation in a 28 year old plantation of *Pinus strobus*, reported that there were no carry-over effects of ozone on next year's growth in the plants investigated.

Decreases in terminal height growth caused by drought imposed on the plants during the season of the investigation were reported for *Picea rubens* (Roberts & Cannon, 1992), *Liriodendron tulipifera* (Jensen, 1985) and *Populus deltoides* Bartr. × *P. trichocarpa* Torr. & Gray (Jensen, 1981) and significant reductions in shoot length of beech and spruce exposed to ambient air were also observed by Billen *et al.* (1990).

In ash, vegetative buds open between the middle and the end of May, leaves in adult shoots then expand rapidly and the terminal bud re-assumes its winter form. In contrast, in shoots which are physiologically young, the buds continue to form leaves throughout the summer (Wardle, 1962). The two-year-old ash saplings used in this study showed two flushes in both 1993 and 1994, the second one being at the beginning of July. The second flush, however, developed better in the well watered treatments, and was rather weak for the droughted treatments in 1994. For the well watered plants, this second flush was much stronger in the ozone-treated plants of both years than in the other two treatments, but well developed also in the CF treatment. Thus relative growth rate for the second part of the season was distinctly higher for the well-watered ozone-stressed plants as compared to the other two well-watered treatments and this also led to a higher seasonal mean extension growth. Stimulation of height growth at twice ambient ozone concentrations has also been reported for *Pinus taeda* L. (Edwards *et al.*, 1992), and for ozone-treated *Pinus halapensis* (Wellburn & Wellburn, 1994). In *Liriodendron tulipifera* seedlings, height growth was stimulated at 50 ppb O_3, while at a concentration of 150 ppb it was no longer increased, but in contrast to other species investigated in the same study, no adverse effects were apparent either (Kress & Skelly, 1992).

Among the droughted chambered treatments in 1994, CF+O_3 showed lower seasonal mean growth than the CF treatment, which was mainly due to the much lower growth rate of these plants during the second part of the season. As with the radial increment, extension growth thus reflected the results from the stomatal conductance measurements, resulting in distinctly lower CO_2 uptake and consequently in less growth. As for aboveground biomass, both the droughted and the well-watered Ambient treatments exhibited by far the lowest extension growth in 1994. Corresponding results with reduced height growth of *Prunus serotina* plants growing in open plots, as compared to others growing in open-top chambers receiving ambient air, were reported by Neufeld *et al.* (1995).

A drought reduction of extension growth seems to have occurred only in the CF+O_3 treatment, since for CF and Ambient the mean seasonal growth of the droughted plants was even higher than that of the well watered counterparts, possibly due to better recovery from drought stress and therefore increased compensatory allocation to the growing shoot during the later part of the growing season, when all plants were being well watered again. The effect is thus considered rather an interaction of drought with ozone than a mere drought effect

In conclusion, comparison of the pollution effects on the individual growth parameters, on leaf area and on aboveground biomass, (which can be considered an integration of the other parameters), showed for the CF+O_3(d) treatment the most marked decrease of all parameters among the chambered treatments. When the ambient plots were also taken into consideration, the Ambient(d) treatment showed least growth for all parameters, except for radial growth at the stembase, which was less in the CF+O_3(d) treatment.

6.3 The effects of ozone and drought on the morphology of annual rings

From the analysis of stem sections taken from the two-year-old ash saplings and from the one-year-old seedlings it could be seen that xylem growth was the component which was significantly decreased by the impact of both stressors - ozone and drought - while phloem growth did not seem to be affected.

Reductions in xylem tissue growth in response to ozone treatment were also reported for *Populus* × *euramericana* (Landolt *et al.*, 1994) and for *Pinus strobus* (McLaughlin *et al.*, 1982). In *Populus* × *euramericana* (Landolt *et al.*, 1994), the decreased xylem growth was concomitant with a decrease of starch granules in the bark tissue. The latter also led to a reduced dry weight : fresh weight ratio of the stem bark, as compared to the control. Landolt *et al.* (1994) related this to an inhibition of phloem loading, since in addition they found increased numbers of starch granules along the minor leaf veins and an increased sucrose content in fumigated leaves. Besides reductions in radial growth, alterations in wood morphology have also been mentioned in the review of Pye (1988), who reported reduced wood density and tracheid length in controlled ozone exposures.

As the results from the second year of the investigation show, xylem tissue growth was not only affected by ozone, but even more so by drought. In his contribution to the biological flora of the British Isles, Wardle (1962) reported that width of annual rings of *Fraxinus excelsior* and monthly rainfall totals showed particular sensitivity to the amount of rainfall received in May and June. In 1994, the drought treatment was started early in the season, so this critical period was also included.

According to Kozlowsi *et al.* (1991), water availability affects both the quantity and the quality of wood produced; thus, in general, annual rings produced in years with abundant rainfall are wider and also contain a larger proportion of earlywood-containing xylem elements, characterised by larger cell diameters and thinner walls, than rings produced in dry years. Such drought-induced premature latewood formation is then associated with a measurable decline in seasonal growth of the crown, since the external climatic factors exert a direct influence on crown growth but only an indirect one on growth and quality of wood (Larson, 1962). Accordingly the proportion of latewood has been described as a function of the period of active crown growth and length of growing season (Albaugh *et al.*, 1991), since it only forms after active crown growth stops and then lasts until the end of the season. If active crown growth is prolonged the percentage of latewood decreases; this can be induced by irrigating the plants during the early part of the growing season (Larson, 1962).

For the ring-porous ash wood, it has been reported that most of the variation in the width of the annual rings due to different conditions of growth is made up of variations in the width of late wood (Wardle, 1962). However, Shumway *et al.* (1991) found that two-year-old seedlings from five populations of *Fraxinus pennsylvanica* subjected to drought stress responded to drought by producing less foliage, less earlywood and less latewood. Thus the effect of drought on the xylem structure of *Fraxinus pennsylvanica* became evident in a reduction in the number of xylem cells produced without marked change in average cell size and wood morphology. This finding is in accordance with the results of this study on *Fraxinus excelsior* which showed both stressors, drought and ozone, to induce a reduction in the number of xylem cells, while cell-size seemed unaffected. The only exception was the CF+O_3(d) treatment in 1994 that produced distinctly smaller cells. However, when plants were influenced only by one stressor - either drought or ozone - cell size was similar to that of the control.

A reduction of cell size in xylem tissue, as was found in the ozone and drought stressed treatment in 1994, leads to an increase in stem wood density. However, the literature mainly reports decreased stem wood density in response to ozone; thus reduced stem weight/stem length ratios were found for *Betula pendula* (Matyssek *et al.*, 1992) and the weight/length ratio of branches was also decreased in *Populus tremula* (Matyssek *et al.*, 1993). The authors related these findings to suppressed latewood formation, the suppression being due to a decline in CO_2 assimilation with ozone dose, so that at the time when latewood formation was initiated growth had already ceased. A comparison of stem volume with stem biomass suggested reduced tissue density in *Pinus taeda* (Edwards *et al.*, 1992), which was also assumed to be due to an earlier onset of dormancy and cessation of cambial activity induced by ozone. These studies investigated the impact of ozone on tissue growth in plants that were well watered, and thus the contrasting results are not considered conflicting with the increase in stemwood density found for the CF+O_3(d) treatment in this study. As mentioned above, drought decreases the period of active crown growth during which earlywood formation takes place, and from the measurements of extension growth in this study such an early reduction of crown development and extension growth becomes evident for the CF+O_3(d) treatment. This response can also explain the highly increased xylem tissue density with smaller latewood cells that were found for this treatment.

6.4 Comparison of the different age classes of ash

The three age classes of *Fraxinus excelsior* - one-year-old seedlings, two-year-old saplings and five/six year old field-grown trees - that were investigated in this study showed qualitatively similar responses for most parameters measured, although there were quantitative differences in the effects measured. Since only the two-year-old saplings were subjected to the drought treatment, comparison of the three age classes is restricted to a comparison of well-watered treatments.

Stomatal conductance of the well-watered two-year-old saplings and field-grown trees showed a similar response to the ozone treatment on exposure days, with increased levels in the CF+O_3 treatments of both age classes. However, the increase measured in the two-year-old saplings was higher than that found for the field-grown trees, and on days following an exposure this increase in stomatal conductance could still be found in plants from the CF+O_3(w) treatment, while the ozone-treated field-grown trees even showed a small decrease on those days. For both age classes, the Ambient treatment exhibited much higher stomatal conductances than the chambered treatments, which is attributed to chamber effects.

The general level of stomatal conductance differed only little between the two age classes. However, while in 1993 it was the field-grown trees that had shown the higher stomatal conductances, this was reversed in 1994, when the two-year-old saplings had the higher conductance. These differences in stomatal conductance may have been due to differences in the positions of leaves chosen for the measurements. In the two-year-old plants, all foliage was equally exposed to the light and differences in the stomatal conductance between the two seasons are assumed to be related to differences in water vapour pressure deficits between the two years and also to differences in the positioning of the plants within the chambers. In the field-grown trees, however, there were various levels of shading due to their more complex canopy structure.

This suggests that there may have been little difference in stomatal behaviour of mature foliage from the two age classes of trees when exposed to identical environmental conditions. However, it also shows that results gained on young saplings with simple crown structure cannot be applied uncritically to older trees with their more complex organisation, even if the qualitative responses are similar for the different age classes. Correspondingly, higher stomatal conductances of seedlings and upper crown leaves of canopy trees compared to other tree sizes and crown positions were reported for *Prunus serotina* Ehrh. (Fredericksen *et al.*, 1995) and for *Sequoiadendron giganteum* (Grulke & Miller, 1994). Such differences in stomatal conductance between leaves from different canopy positions correspond with the general sun and shade characteristics of leaves. Thus, high light intensity during leaf differentiation and growth increases stomatal frequency and, concomitantly with the increased stomatal pore area per unit leaf area, the maximum stomatal conductance also increases (Boardman, 1977). In contrast, Hanson *et al.* (1994) reported for *Quercus rubra* greater stomatal conductance and photosynthesis of mature tree foliage than seedling foliage at physiological maturity. Concomitantly, both photosynthesis and stomatal conductance were less affected in seedling leaves than in mature tree leaves.

An increase in stomatal conductance may lead to higher pollutant uptake and hence the risk of pollutant injury would also increase. For *Picea abies* an earlier limitation of ozone uptake was reported for leaves in the shade than in the sun crown (Wieser & Havranek, 1993). In accordance with this hypothesized elevated pollutant uptake of sun-exposed leaves, Fredericksen *et al.* (1995), found seedlings to have the greatest instantaneous rates of ozone uptake per unit leaf area of all tree size classes of *Prunus serotina*. Nevertheless, visible foliar symptoms were more prevalent on lower crown leaves than on the sun-exposed upper crown. The authors related this effect to differences in leaf morphology that might account for the greater sensitivity of the shade leaves, such as lower leaf thickness, a greater proportion of intercellular air space and a higher ratio of mesophyll cell surface to external leaf area (Björkman, 1981). In addition, the reduced light availability might have decreased the induction of antioxidant production, which is light dependent (Fredericksen *et al.*, 1995). Higher sensitivity of shade leaves and shade grown seedlings to ozone was also reported for *Acer saccharum* exposed to twice ambient concentrations of ozone (Tjoelker *et al.*, 1995).

In addition to the above-mentioned difficulties in applying results gained with seedlings and young saplings to mature trees, there are often differences in "stand density" between experimental studies and natural forest stands. Thus even plants from the same age class as investigated under experimental conditions may respond differently under natural conditions, and understory seedlings are often the ones differing most markedly from experimental plants (Reich, 1987).

In the case of ash, a tree commonly growing in woodlands as well as in the open and as isolated trees along hedgerows (Wardle, 1962), the results gained from the two-year-old saplings in this study may represent the response of open-growing plants of the same age or with similar canopy structure, but cannot be transferred to understory saplings growing in woodlands.

Besides these physiological differences between plants from different age classes, other factors, such as the ratio of metabolic to catabolic tissue and altered water and nutrient regimes, complicate the extrapolation from seedlings and saplings to mature trees (Pye, 1988).

With regard to growth and biomass accumulation, seedlings and saplings can thus be expected to show different responses than would mature trees. In general, seedlings are known to make better use of weak illumination and to assimilate and respire at higher rates than older plants (Larcher, 1969). For ash, persistent juveniles that survive for many years in the shade below a closed canopy, often producing just one pair of leaves, have been shown to be important for natural regeneration in the field (Tapper, 1993). The slow-growing juveniles maintain the ability to quickly and successfully respond to high light intensities as they occur in tree-fall gaps and can meet improved light conditions immediately by fast growth.

Besides such physiological differences, there may also be differences in phenology, carbon allocation and storage in plants from different age classes that will affect the growth response to the stressor. Thus seedlings and young saplings of *Fraxinus excelsior* showed concurrent flushing until much later in the season than the field-grown trees, whereby the cumulative exposure of the later leaves was reduced. Further, the translocation pattern from younger leaves is assumed to be different for differently aged leaves (Larson & Dickson, 1986). From the two age classes of ash - one-year-old seedlings and two-year-old saplings - for which total and total aboveground biomass were investigated respectively, no differences in the biomass allocation pattern to the aboveground parts could be found. This is probably due to the small difference in age and developmental stage, as well as to the fact that both age classes grew in pots, so that soil conditions, such as temperature, dessication behaviour and physical restriction to root growth due to the pots were similar. A possible promotion of root growth by warmer soil temperatures has also been discussed by Samuelson (1994) for *Quercus rubra* seedlings exposed in seedling chambers.

7 Conclusions

Among the factors determining the water relations of plants, stomatal behaviour is the one that controls water loss to the atmosphere, with the stomata being the interface between leaf and atmosphere.

Ozone affected the stomatal response of ash saplings of different water status differently, causing a slight increase in stomatal conductance in the well-watered plants and a decrease in the unwatered, drought stressed saplings. However, more important than the actual decrease in stomatal conductance in response to drought - a response also found for saplings from the CF treatment - was the reduced ability of the CF+O_3(d) treatment to recover from the drought stress and to adjust their transpiration rate to the changes in soil water and climatic conditions. This led to a long-term decrease in stomatal conductance in the droughted and ozone-stressed trees. Plants that may have been mildly water stressed, such as the field-grown rainfed trees and the two-year-old saplings in the first year of the investigation, showed a slight decrease in stomatal conductance in response to the ozone exposures.

The small increase in stomatal conductance that was found in the well-watered saplings might lead to a more rapid decrease of soil water reserves at times of sufficient soil moisture and thus could increase the duration and severity of following drought periods by leading to an earlier onset of a critical water deficit of the soil. This would be particularly important in drought years, when decreasing soil water reserves are not replenished. From the results, it cannot be predicted whether such plants that have increased stomatal conductance during times of good water availability would be able to adjust their stomatal conductance when soil water reserves were decreased. The response of stomatal closure of the drought-stressed trees cannot be considered as representative here, because these plants were subjected to the drought stress from the beginning of the growing season and thus did not experience an ozone-induced increase in stomatal conductance before the drought treatment commenced. If stomatal responsiveness was also decreased in the well-watered plants, as it was in the droughted plants, then a delayed stomatal closure could be expected in the face of drought. This might lead to very negative water potentials in the plants and to further prolongation of drought periods.

Besides stomatal conductance, the transpiring plant surface - and thus leaf area - determines water loss. Assuming that the density of stomata was constant among all treatments, the well-watered and ozone-treated ash saplings, with their greater leaf areas, should also be expected to have shown greater overall transpiration rates and this would further increase the propensity to water stress.

In contrast to this small but consistent increase in leaf area and biomass in the two-year-old well-watered saplings, the results gained from the one-year-old seedlings suggest that root biomass was slightly decreased and that, accordingly, plant susceptibility to drought might be increased even more, since the smaller amount of root biomass would reduce the plant's ability to take up soil water. This would result in a decrease in soil water availability, while concomitantly water demand by the well developed crown would be increased.

The differences in biomass that were found in this study were small and not significant and would not be expected to affect plant performance in the short-term; however, in the long-term such changes in the biomass allocation pattern could affect the plant's water relations and ability to withstand drought stress.

Unlike the well-watered plants with their contrasting trends in possible water uptake and water loss, the severely droughted and ozone stressed saplings greatly reduced water loss to the atmosphere by decreasing stomatal conductance as well as by reducing transpiring leaf area and biomass; they also showed a trend towards translocating assimilates to the lower stems of the plants and possibly also to the roots. Stomatal behaviour was reflected by a strong decrease of overall aboveground biomass and decreases of all growth parameters investigated. This is a characteristic response to drought stress, known to reduce crown development and to lead to an early onset of latewood formation, which here became evident from the reductions of cell size in the annual rings from the base of the stems of ozone and drought stressed saplings. The production of fewer and smaller xylem cells was found particularly at the stembase, while at the base of the new shoot differences in radial growth, as compared to the control treatment, were less pronounced. This could lead to restrictions of water transport to the crown.

No results on drought-stressed plants were available from the one-year-old seedlings, so it is not known how root biomass was affected in these plants. However, the trend of increased downward translocation of assimilates to the lower stems, found in the droughted and ozone-stressed two-year-old saplings, suggests that percentage root biomass may have been unaffected or even possibly increased. Thus ozone, which by decreasing stomatal conductance and responsiveness in the drought-stressed plants also led to a decrease of growth and biomass accumulation, most likely did not affect water relations in these plants as it did in the well-watered plants. Accordingly the stressors affected these plants negatively by reducing growth and biomass allocation: in a natural stand this would affect the plant's competitive ability.

A factor contributing to the different responses of well-watered and droughted plants to ozone stress may be identified in the sequence in which the different stresses were applied to the plants. The stress taking effect first was possibly the decisive factor for the plant's response to the subsequent second stress. Thus under field conditions the timing of drought and ozone episodes would have a major impact on the actual response of the plants to the stressors.

Accordingly, plants pre-stressed by ozone at times of good water availability, showing increased crown growth due to the impact of the pollutant, may be particularly affected by a following drought, since their demand for water would be increased. Due to their altered biomass distribution and to reduced stomatal responsiveness in the face of drought, water supply to the crown then might become limiting. Hence, the risk of a dieback of parts of the crown, as it has been observed in ash trees during drought years may be increased in such plants.

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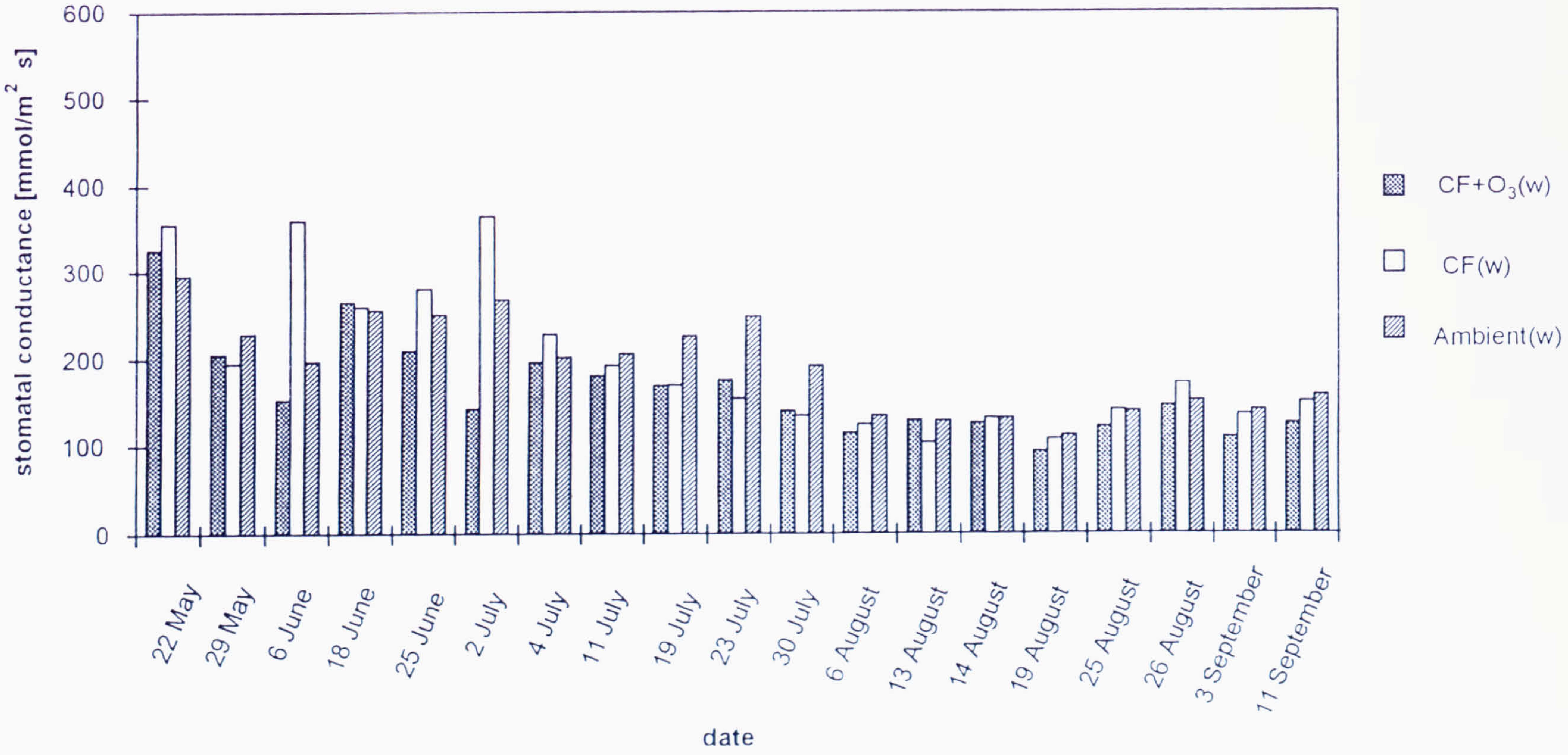
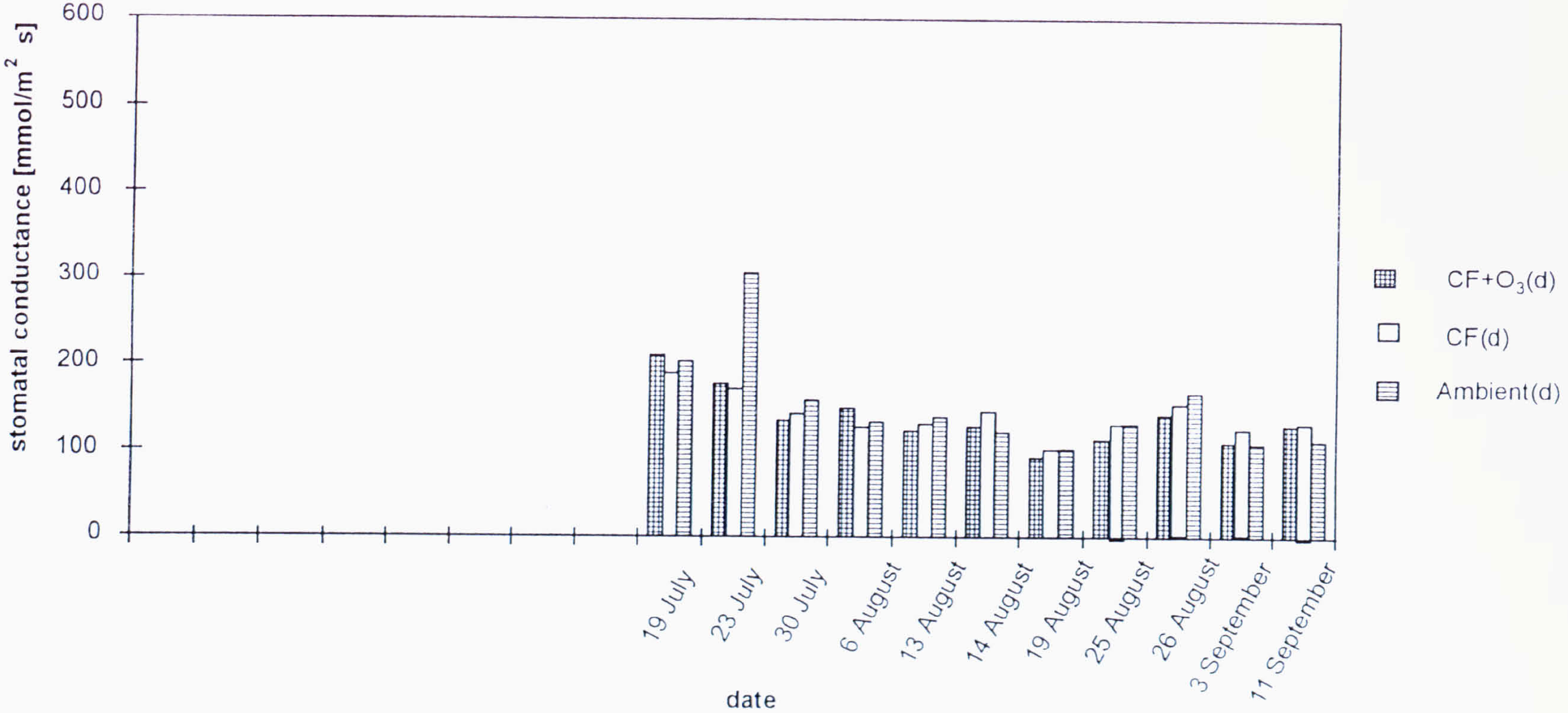
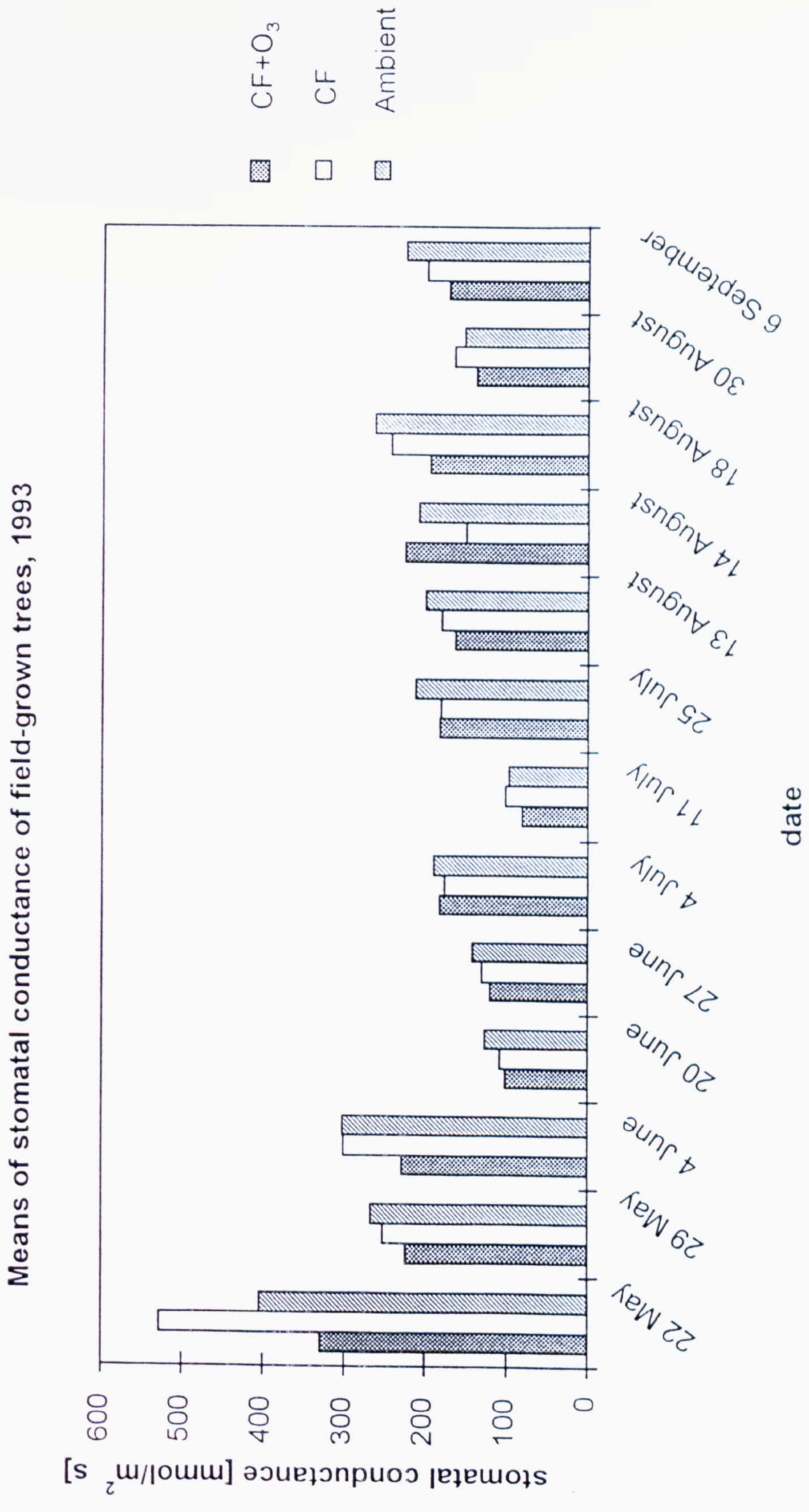
Composition of the modified Hoagland & Arnon solution:

Nutrient	mg/l culture solution
KNO_3	250.0
K_2PO_4	125.0
H_3BO_3	1.8
$(NH_4)_6Mo_7O_2 \times 4H_2O$	0.025
$MgSO_4 \times 7H_2O$	312.5
$MnSO_4 \times H_2O$	1.05
$ZnSO_4 \times 7H_2O$	0.2
$CuSO_4 \times 5H_2O$	0.1
$Ca(NO_3)_2 \times 4H_2O$	750.0
KCL	5.0
EDTA	21.6
Na_2CO_3	3.9
$FeCl_3 \times H_2O$	2.0

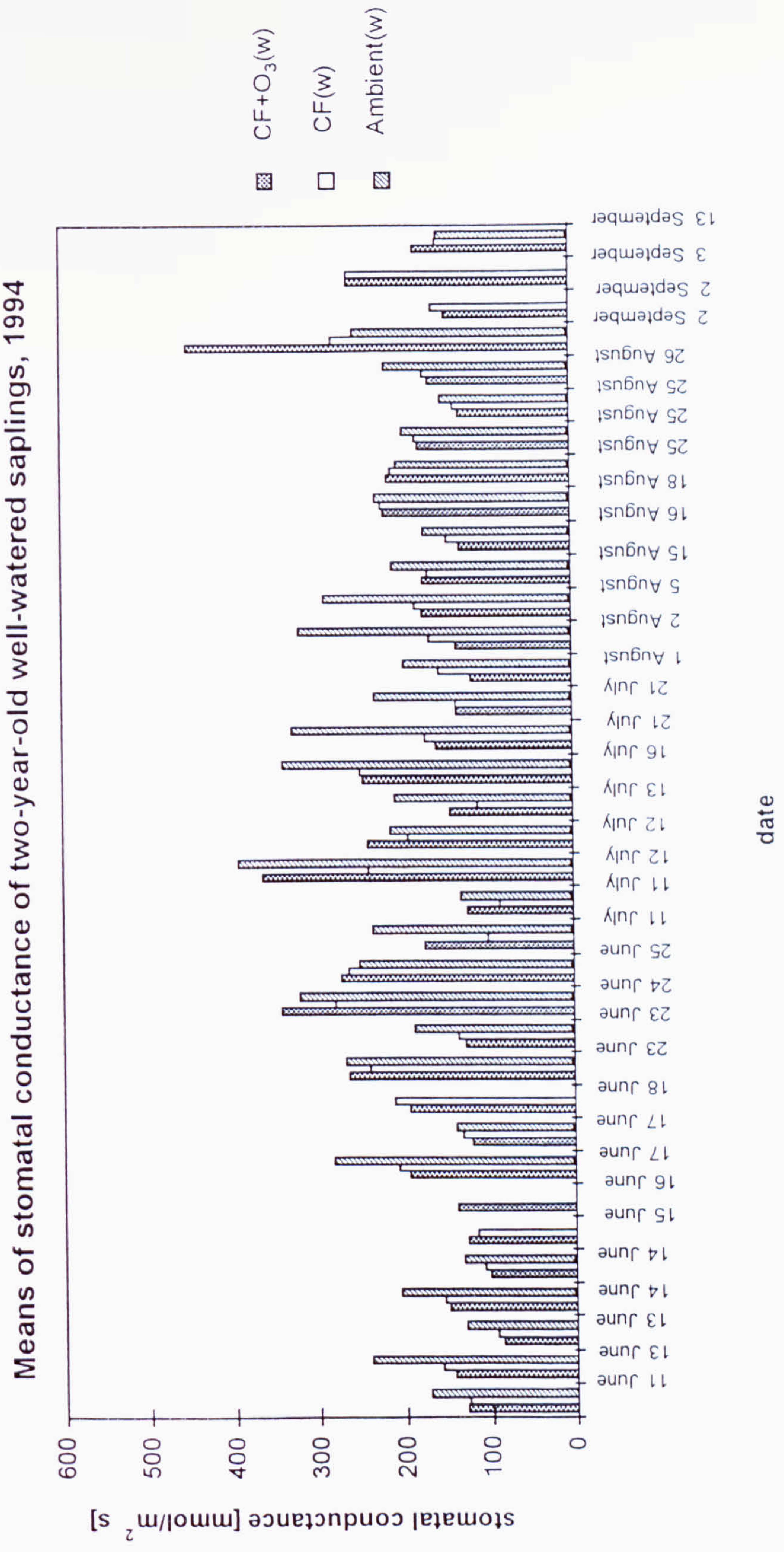


Means of stomatal conductance, two-year-old well watered saplings, 1993


Mean stomatal conductance of two-year-old droughted saplings, 1993



Means of stomatal conductance of field-grown trees, 1993


Means of stomatal conductance of two-year-old well-watered saplings, 1994
▩ CF+O$_3$(w)
☐ CF(w)
▨ Ambient(w)
stomatal conductance [mmol/m^2 s]
600
500
400
300
200
100
0
date
11 June
13 June
13 June
14 June
14 June
15 June
16 June
17 June
17 June
18 June
23 June
23 June
24 June
25 June
11 July
11 July
12 July
12 July
13 July
16 July
21 July
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15 August
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25 August
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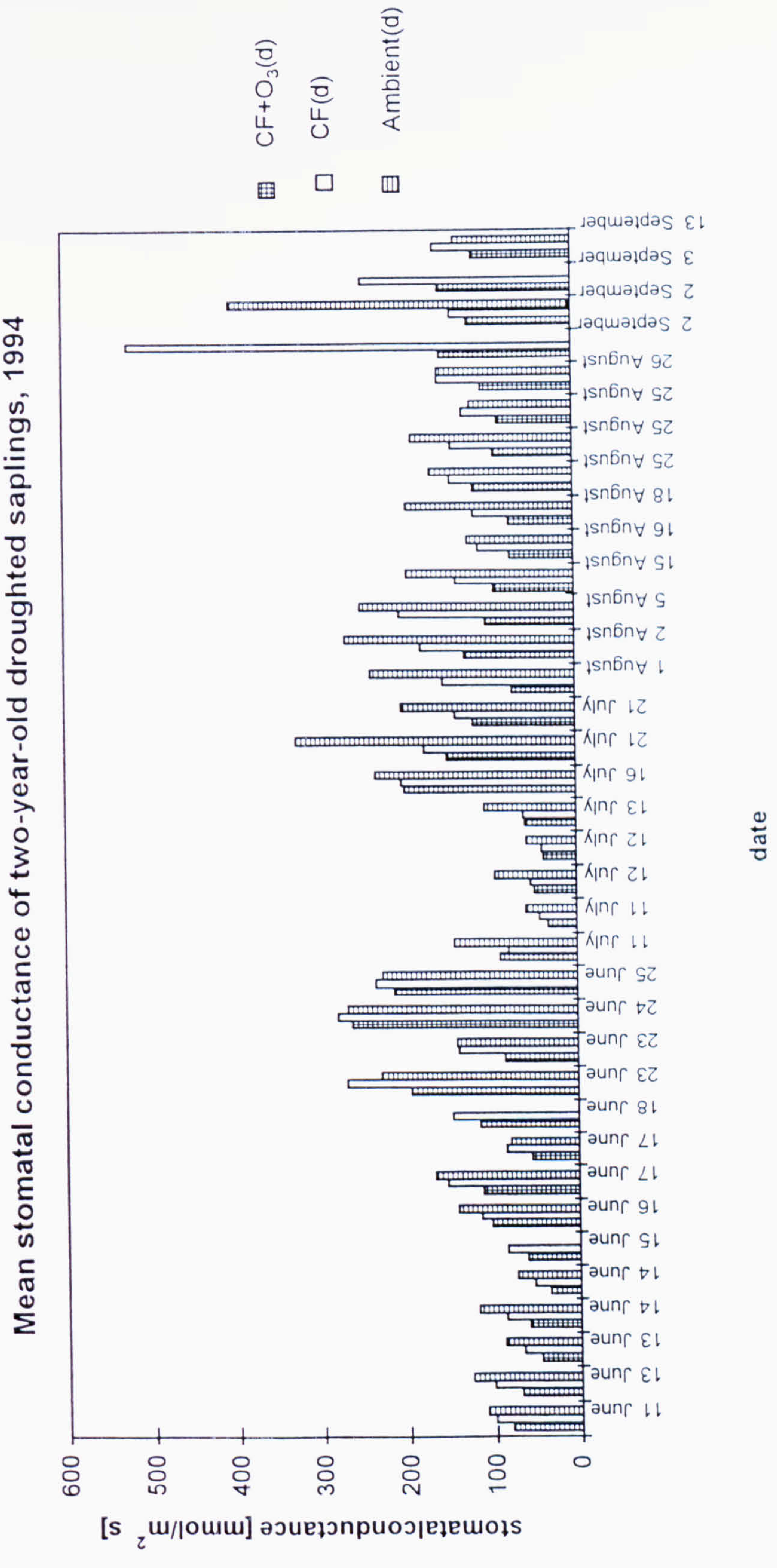
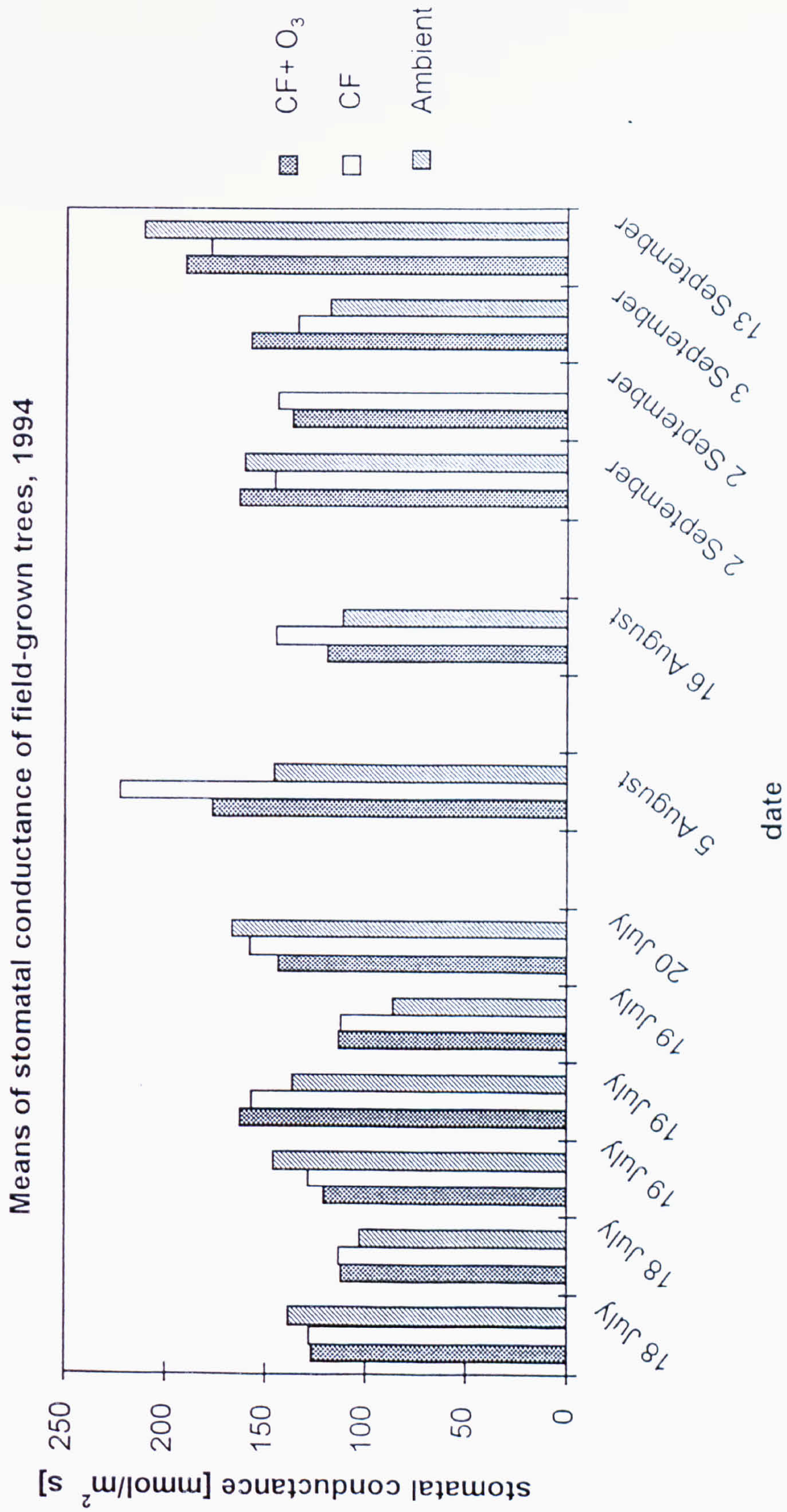
25 August
26 August
2 September
2 September
3 September
13 September


Mean stomatal conductance of two-year-old droughted saplings, 1994



Means of stomatal conductance of field-grown trees, 1994