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Enriched rhizosphere CO_2 concentrations can ameliorate the influence of salinity on hydroponically grown tomato plants

M.D. Cramer and S.H. Lips

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Our previous work indicated that salinity caused a shift in the predominant site of nitrate reduction and assimilation from the shoot to the root in tomato plants. In the present work we tested whether an enhanced supply of dissolved inorganic carbon (DIC, $CO_2 + HCO_3^-$) to the root solution could increase anaplerotic provision of carbon compounds for the increased nitrogen assimilation in the root of salinity-stressed *Lycopersicon esculentum* (L.) Mill. cv. F144. The seedlings were grown in hydroponic culture with 0 or 100 m*M* NaCl and aeration of the root solution with either ambient or CO_2-enriched air (5 000 μmol mol^{-1}). The salinity-treated plants accumulated more dry weight and higher total N when the roots were supplied with CO_2-enriched aeration than when aerated with ambient air. Plants grown with salinity and enriched DIC also had higher rates of NO_3^- uptake and translocated more NO_3^- and reduced N in the xylem sap than did equivalent plants grown with ambient DIC. Incorporation of DIC was measured by supplying a 1-h pulse of $H^{14}CO_3^-$ to the roots followed by extraction with 80% ethanol. Enriched DIC increased root incorporation of DIC 10-fold in both salinized and non-salinized plants. In salinity-stressed plants, the products of dissolved inorganic ^{14}C were preferentially diverted into amino acid synthesis to a greater extent than in non-salinized plants in which label was accumulated in organic acids. It was concluded that enriched DIC can increase the supply of N and anaplerotic carbon for amino acid synthesis in roots of salinized plants. Thus enriched DIC could relieve the limitation of carbon supply for ammonium assimilation and thus ameliorate the influence of salinity on NO_3^- uptake and assimilation as well as on plant growth.

Key words – Carbon dioxide, *Lycopersicon esculentum*, nitrate, nitrate reductase, phosphoenolpyruvate carboxylase, potassium, respiration, salinity, tomato.

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Introduction

Salinity stress results in changes in the physiology of a plant depending on the severity of the stress, environmental factors and the sensitivity of the plant to salinity. In tomato plants, ca 80% of nitrate reductase activity (NRA) is located within the shoots (Lorenz 1976), although the proportion of root reduction of NO_3^- varies with environmental factors and the concentration of NO_3^- supplied to the roots (Smirnoff and Stewart 1985, Andrews et al. 1992). In soybean, in vitro root NRA was higher in plants grown with 100 m*M* NaCl than in control plants, while leaf NRA was relatively unaffected (Bourgeais-Chaillou et al. 1992). In tomato plants one of the symptoms of salinity stress is an alteration in the primary site of NO_3^- reduction and assimilation within the plant from the shoot to the root (M.D. Cramer, A. Schierholt, Y. Wang and S.H. Lips, unpublished results). This alteration in the site of NO_3^- reduction and assimilation brought about by salinity may be related to the inhibition of NO_3^- uptake in the presence of salinity (Aslam et al. 1984) or the interference of salinity with the loading of NO_3^- into the xylem. Root reduction and assimilation of NO_3^- into amino acids, which is dependent on carbohydrate supply

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from the shoot, is energetically more costly to the plant than photosynthetically dependent shoot reduction and thus may impair plant growth (Blacquière 1987).

The translocation of NO_3^- may occur through the operation of a K^+-shuttle (Lips et al. 1970, Ben-Zioni et al. 1971). In this model, K^+-malate is translocated from the shoot to the root where malate is decarboxylated to yield HCO_3^-, which is exchanged for NO_3^- in the medium. This shuttle may serve to maintain the pH balance in the shoot by balancing the OH^- produced during shoot NO_3^- reduction through export of malate to the root (Raven and Smith 1976). The operation of the K^+-shuttle provides a link between the influence of NaCl on tissue concentrations of K^+ and the translocation of NO_3^- to the shoot. It is well known that Na^+ displaces other cations from the membrane surface (G.R. Cramer et al. 1987) and this interference with K^+ uptake may therefore inhibit NO_3^- translocation to the shoot through interference with the operation of the K^+-shuttle.

Salinity stress in tomato plants results in decreased incorporation in the root of dissolved inorganic carbon (DIC) and a shift in the products of this incorporation from organic acid to amino acids (M.D. Cramer, A. Schierholt, Y. Wang and S.H. Lips, unpublished results). Increased synthesis of amino acids utilizing the products of DIC incorporation has been observed previously with plants supplied with NH_4^+ nutrition in comparison to those supplied with NO_3^- nutrition (Cramer and Lewis 1993). Since NH_4^+ taken up by the root is almost exclusively assimilated within the roots, it is possible that increased anaplerotic provision of carbon from DIC incorporation for amino acid synthesis in the root is a feature of root assimilation of nitrogen (M.D. Cramer et al. 1993).

The nutrient solutions used for plant hydroponic culture are usually aerated with ambient air. However, due to root and microbiological respiratory activity, inorganic carbon in solution in the soil is usually higher than the CO_2 level of ambient air. Only in very porous and dry soils, lacking in organic material, is the DIC concentration likely to be low. In this investigation, we tested the possibility that elevated DIC could stimulate the provision of anaplerotic carbon for assimilation of nitrogen in the roots of plants grown with and without salinity. We determined whether this could influence growth, nitrogen metabolism, ion accumulation and the products formed from root DIC incorporation.

Abbreviations – DIC, dissolved inorganic carbon; LWR, leaf to plant weight ratio; NR(A), nitrate reductase (activity); $NRA_{exo(end)}$, nitrate reductase activity measured with exogenous (endogenous) NO_3^-.

Materials and methods

Plant material

Fourteen-day-old seedlings of *Lycopersicon esculentum* (L.) Mill. cv. F144 grown on vermiculite were transferred into hydroponic culture after carefully rinsing the roots with deionized water. The hypocotyls of the plants were wrapped with foam rubber and the plants inserted through holes in the lids of tanks (24 plants per tank) containing 130 l half-strength Long Ashton nutrient medium (Hewitt 1966), which was modified to contain 1 m*M* $NaNO_3$ and 60 mg l^{-1} Fe-sequestrene 138 (Ciba-Geigy, Petah Tikva, Israel) as the iron source. After transfer of the plants into nutrient solutions, the concentration of NaCl was increased at a rate of 50 m*M* day^{-1} to reach a final concentration of 100 m*M*. Controls had no added NaCl. The plants were utilized 10 to 15 days after transfer into hydroponics.

The pH of the medium (6.5) was adjusted on a daily basis with HCl, and the nutrient solution was replaced weekly. The concentration of NO_3^- in the nutrient solution was maintained at ca 1 m*M* by additions of NO_3^- every third day. The nutrient solution was strongly aerated and the temperature maintained at $20 \pm 1°C$ with aquarium heaters. The plants were grown in a greenhouse in April with a midday irradiance of ca 700 μmol m^{-2} s^{-1} and midday temperature ranging between 28 and 30°C with a minimum night temperature of 15°C. Relative humidity varied between 40 and 60% in the day and 70 and 90% at night.

Carbon dioxide was supplied at elevated levels (5 000 μmol mol^{-1}) by enriching ambient air with pure CO_2 using a peristaltic pump. The CO_2 concentration was monitored continuously with an APPA-3 infrared gas analyzer (Anarad Inc., Santa Barbara, CA, USA). Work with enriched rhizosphere CO_2 concentrations has been criticised because the possibility exists that the CO_2 may be released from the rhizosphere and may be assimilated by photosynthesis (Enoch and Olesen 1993). To prevent this artifact in these experiments, the CO_2 was flushed from below the lids of the containers with a high flow rate of ambient air and a fan was used to maintain air movement over the plant shoots. The air sampled routinely from above the lids of the hydroponic containers with an ADC LCA2 infrared gas analyser (Analytical Development Corporation, Hoddesdon, UK) was found not to differ from ambient CO_2 concentrations.

Biomass

Ten replicate plants of each treatment were harvested after 15 days growth in hydroponics. The roots of the plants were rinsed in running deionized water and the plants divided into leaf, stem and root components. The roots were blotted dry, the components weighed and the plant material oven dried at 80°C for 48 h and reweighed.

Tissue concentrations of NO_3^-, total N, K^+ and Na^+

The oven-dried plant material of each treatment was milled in a Wiley mill (A.H. Thomas, Philadelphia, PA, USA) using a 60 mesh screen. Samples of 50 mg material were digested in 35 cm long tubes with 4 ml 3.4% (w/v)

salicylic acid in 13.5 *M* sulphuric acid with 0.2 g selenium. The samples were digested at room temperature for 2 h, 200°C for 1 h, 270°C for 1 h and 370°C for 1 h. The digest was diluted and assayed for NH_4^+ (Solorzano 1969). Concentrations of K^+ and Na^+ in the digested samples were determined using a Corning 410 Flame Photometer (Halstead, UK).

For determination of tissue NO_3^- concentrations, a homogeneous sample of tissue of ca 0.3 g fresh weight was quenched in liquid N_2, suspended in 10 ml distilled water and extracted in a water bath at 80°C for 2 h. Each extract was mixed and sub-samples of 1 ml centrifuged at 1 300 *g* for 5 min; the NO_3^- concentration of the supernatant was determined according to Cataldo et al. (1975).

Nitrate reductase activity

Four plants of each treatment were harvested between 10.00 and 12.00 and the roots washed in deionised water. The plants were separated into root, stem and leaf. After weighing, the components were sliced into 1- to 2-mm sections and duplicate homogeneous samples of ca 0.3 g of tissue vacuum infiltrated in 10 ml 0.1 *M* Na-phosphate buffer with 1% (v/v) isopropanol with or without 100 m*M* KNO_3 (pH 7.2) until degassing occurred (ca 1 min). One of the pair of samples was killed and extracted by immersing the vials in boiling water for 2 h, immediately after vacuum infiltration, to provide a measure of the initial NO_2^- concentrations. The remaining samples were incubated anaerobically for 30 min in a shaking water bath at 30°C in the dark prior to killing and extraction. The samples were centrifuged at 1 300 *g* for 5 min and the supernatant assayed for NO_2^- according to Snell and Snell (1949). When 100 m*M* KNO_3 was included in the assay medium (NRA_{exo}) the assay was considered to reflect potential NRA not limited by the amount of NO_3^- available in the metabolic pool. Since the in vivo assay could have been limited by the availability of reductant, 100 m*M* concentrations of malate, glucose and sucrose were included in the assay medium as sources of reductant, but were found to have no effect on the rates of NO_2^- accumulation. The in vivo accumulation of NO_2^- does not provide a quantitative estimate of the potential rates of NRA because NO_2^- may be reduced to some extent in the dark (Soares et al. 1985). When KNO_3 was not included in the buffer and the assay depended on the utilization of endogenous NO_3^- (NRA_{end}), the assay was considered to represent an estimate of in situ NRA.

Nitrate uptake and transpiration rates

Four plants of each treatment were transferred into 250 ml fresh, aerated (12 l h^{-1} with either 360 or 5 000 μmol mol^{-1} CO_2) nutrient solution containing 10 m*M* MES (pH 6.5) and 2 m*M* $NaNO_3$, 9 h prior to the beginning of the experiment. Sub-samples (1 ml) of nutrient solution were taken from 6.00 onwards at regular intervals for 8 h. At each sampling time the volume of the nutrient solution remaining in the cylinders was determined gravimetrically for calculation of the transpiration rates by comparison with a suitable control (250 ml aerated nutrient solution without plant). The NO_3^- concentrations in the nutrient solutions were determined according to Cataldo et al. (1975) and the K^+ concentrations were determined using a Corning 410 Flame Photometer.

Collection and analysis of xylem sap

Four plants of each treatment were topped approximately 10 mm above the hypocotyl. The entire root system was then washed in deionised water and transferred into an Arimad 2 (Tel Aviv, Israel) pressure bomb. Sap was collected (ca 50 to 100 μl) at an applied pressure of 0.7 MPa for 10 min, temporarily stored on ice, and subsequently at −18°C. Xylem sap samples were analyzed for NO_3^- using the dissimilatory NR enzymatic method of McNamara et al. (1971), modified to use 0.6 *M* Na formate as a source of reductant (N. Savidov, personal communication). The reduced-N concentration was determined using the ninhydrin assay of Rosen (1957) with L-leucine as standard. Potassium concentrations in the xylem sap samples were determined using a Corning 410 Flame Photometer.

$NaH^{14}CO_3$ feeding and fractionation

The nutrient solutions were renewed 12 h prior to the experiment. A stock of fresh nutrient solution was aerated with air containing either 360 or 5 000 μmol mol^{-1} CO_2. At the commencement of the experiment (10.30), four plants of each treatment were transferred to sealed 250-ml containers with fresh nutrient medium at 20°C. To each container 1.4 μmol (2.8 MBq) $NaH^{14}CO_3$ was added. After 1 h the plants were removed from the nutrient solutions, the roots rinsed in deionized water and blotted dry. The plants were divided into leaf, stem and root, weighed, quenched in liquid N_2 and stored at −18°C. Extraction of the tissue and fractionation into carbohydrate, amino acid and organic acid components were performed according to M.D. Cramer et al. (1993).

Results

Biomass accumulation

Plants grown with salinity accumulated less biomass than non-salinized plants (Tab. 1). Enriched DIC resulted in greater biomass accumulation in leaves and roots of salinized plants than did ambient DIC. However, enriched DIC resulted in less biomass accumulation in leaves and roots of non-salinized plants than did ambient DIC. The salinized plants grown with ambient DIC had a significantly lower leaf weight ratio (LWR) than the plants of the other treatments (Tab. 1). Root weight ratios were lowest and shoot:root ratios highest in the non-salinized plants grown with enriched DIC, indicating that this treatment disadvantaged the roots.

Tab. 1. Dry weight, leaf and root weight ratios and shoot:root ratio of tomato plants grown in hydroponic culture with either 0 or 100 m*M* NaCl and aeration of the root solution with either ambient (AC, 360 μmol mol^{-1}) or enriched (EC, 5 000 μmol mol^{-1}) CO_2. The means ±SE are followed by letters indicating whether the salinity had a significant ($P<0.05$, Fisher's protected LSD) influence ($n=10$).

Parameter/Plant part	0 m*M* NaCl		100 m*M* NaCl	
	AC	EC	AC	EC
Dry weight (g)				
Leaf	0.73 ± 0.03d	0.65 ± 0.02c	0.40 ± 0.02a	0.50 ± 0.02b
Stem	0.31 ± 0.01b	0.30 ± 0.01b	0.18 ± 0.01a	0.21 ± 0.01a
Root	0.26 ± 0.01c	0.21 ± 0.01b	0.17 ± 0.01a	0.21 ± 0.01b
Plant	1.30 ± 0.05d	1.16 ± 0.03c	0.75 ± 0.03a	0.91 ± 0.03b
Weight ratio				
Leaf	0.57 ± 0.01b	0.56 ± 0.01b	0.53 ± 0.01a	0.55 ± 0.01b
Root	0.20 ± 0.00b	0.18 ± 0.00a	0.23 ± 0.01c	0.23 ± 0.01c
Shoot:root ratio	4.07 ± 0.07b	4.57 ± 0.10c	3.43 ± 0.14a	3.45 ± 0.16a

Tissue concentrations of NO_3^-, total N, K^+ and Na^+

There was a lower concentration of NO_3^- in the salinized than in the non-salinized plants but DIC had no significant influence on the NO_3^- concentrations in the tissue (Fig. 1). In plants grown with ambient DIC foliar NO_3^- concentrations were 2.4-fold higher in non-salinized than in salinized plants. Ambient DIC combined with salinity resulted in lower total N concentrations while enriched DIC reversed this influence of salinity to some extent (Fig. 1). Enriched DIC also had a positive influence on stem total N concentrations in non-salinized plants. In the salinized plants the differences between ambient and enriched-DIC treatments were largely due to changes in the reduced N concentration (difference between total N and NO_3^- concentrations).

Plants grown in salinity had higher Na^+ concentrations than non-salinized plants (Fig. 2), but DIC did not influence the concentrations of Na^+ in the tissues. The concentration of K^+ in the stems and roots was lower in salinized than in non-salinized plants treated with ambient DIC. The concentration of K^+ was significantly higher in leaf (2.8-fold) and root tissue of salinized plants grown with enriched DIC in comparison with salinized plants grown with ambient DIC. There were slightly higher concentrations of K^+ in stem and root tissue of non-salinized plants grown with enriched DIC compared to those grown with ambient DIC.

Nitrate and K^+ uptake rates

Nitrate and K^+ uptake rates were higher in non-salinized than in salinized plants (Tab. 2). Furthermore, enriched compared with ambient DIC increased the uptake of NO_3^- by salinized plants. Midday transpiration rates, expressed on the basis of leaf fresh weight, were ca 1.5-fold lower in salinized than in non-salinized plants, but DIC had little influence on the transpiration rates (Tab. 2). The

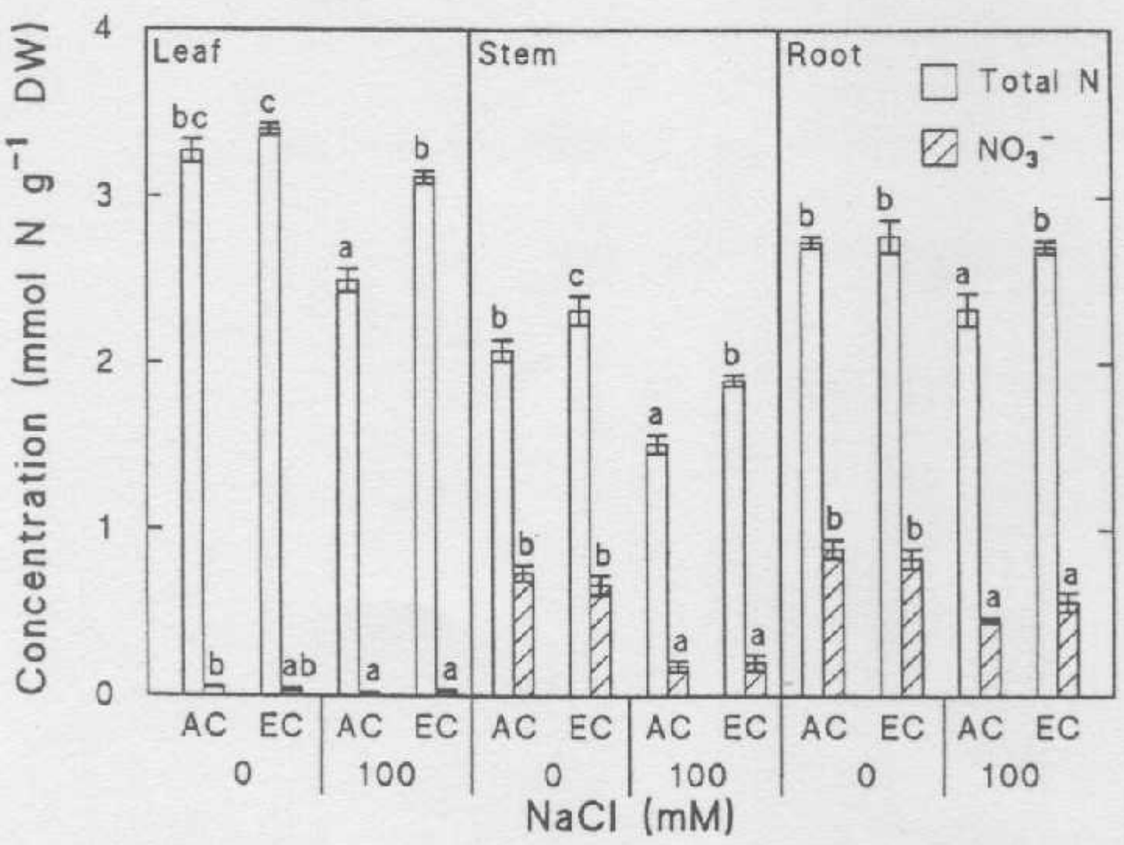

Fig. 1. Effect of 0 or 100 m*M* NaCl and aeration with either ambient (AC, 360 μmol mol^{-1}) or enriched (EC, 5 000 μmol mol^{-1}) CO_2 on the total N ($n=10$) and NO_3^- ($n=4$) concentrations in the tissue of tomato plants. The error bars represent the SE of the means. Dissimilar letters above of bars indicate significant ($P<0.05$) differences between means determined from analysis of variance followed by Fisher's protected LSD tests. The leaf, stem and root components were tested separately.

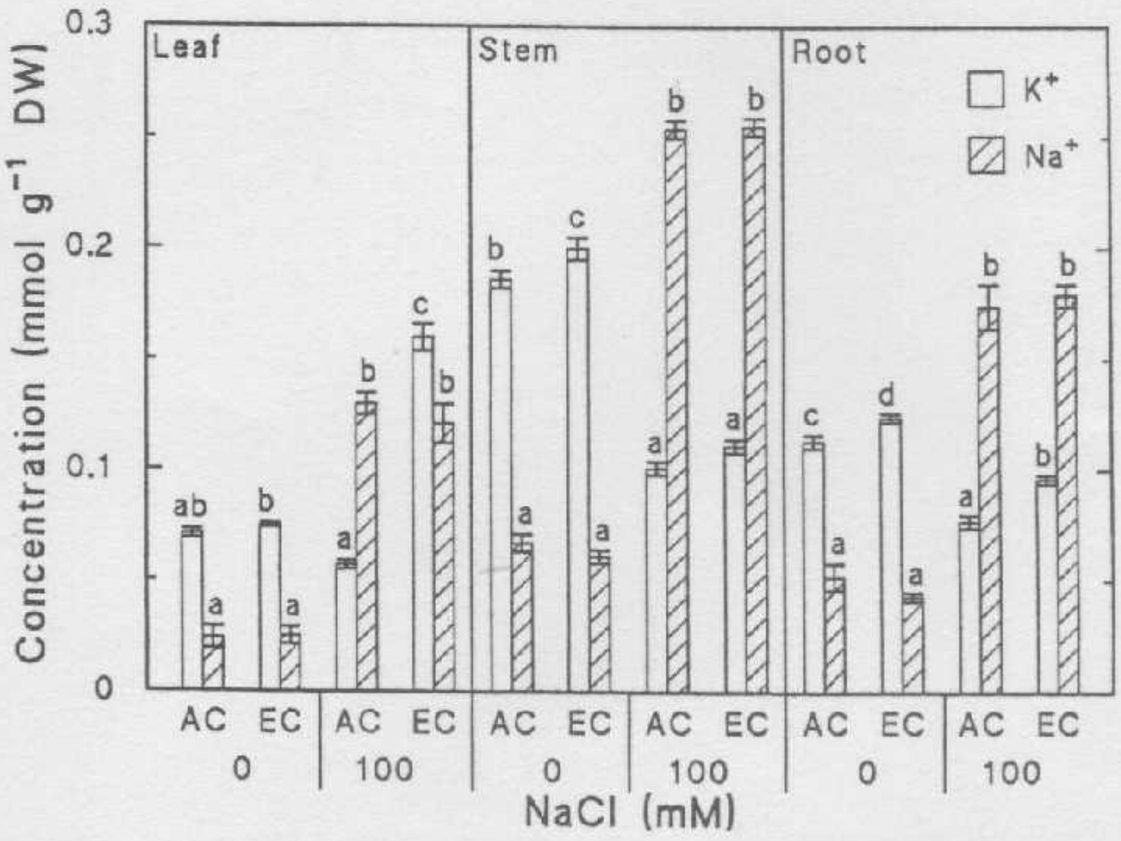

Fig. 2. Effect of 0 or 100 m*M* NaCl and aeration with either ambient (AC, 360 μmol mol^{-1}) or enriched (EC, 5 000 μmol mol^{-1}) CO_2 on the K^+ and Na^+ concentrations in the tissue of tomato plants ($n=10$). Statistics as in Fig. 1.

Tab. 2. Nitrate and K^+ uptake rates, rates of DIC incorporation (nmol g^{-1} root DW s^{-1}) into acid-stable products, transpiration rates (nmol g^{-1} leaf DW s^{-1}) and shoot:root ratios of ^{14}C distribution in tomato plants grown in hydroponic culture with 0 or 100 m*M* NaCl and aeration with either ambient (AC, 360 µmol mol^{-1}) or enriched (EC, 5 000 µmol mol^{-1}) CO_2 (means ± SE, n = 4).

Parameter	0 m*M* NaCl		100 m*M* NaCl	
	AC	EC	AC	EC
Transpiration	44 ± 1	40 ± 1	29 ± 1	29 ± 1
NO_3^- uptake	31.3 ± 3.2	34.1 ± 4.4	17.0 ± 2.7	26.5 ± 1.3
K^+ uptake	5.9 ± 0.4	7.0 ± 0.7	0.0 ± 0.5	1.2 ± 0.2
DIC incorporation	2.9 ± 0.2	26.4 ± 4.0	1.0 ± 0.1	11.0 ± 0.7
^{14}C shoot:root	0.08 ± 0.01	0.20 ± 0.05	0.05 ± 0.01	0.14 ± 0.02

rates of NO_3^- and K^+ uptake were thus not closely linked to transpiration in the experiments described here.

Nitrate reductase activity and composition of the xylem sap

Stem and root NRA_{exo}, measured in vivo as dark anaerobic accumulation of NO_2^- in the presence of 100 m*M* KNO_3, was unchanged by either DIC or salinity treatments (Fig. 3A). Leaf NRA_{end}, measured without addition of NO_3^-, was higher in salinized than in non-salinized plants and was also higher in salinized plants supplied with enriched DIC than in salinized plants supplied with ambient DIC (Fig. 3B). Stem and root NRA_{end} was unchanged by either salinity or DIC. Foliar NRA_{end} was correlated with foliar concentrations of NO_3^- (Fig. 1).

The fluxes of NO_3^-, K^+ and reduced N in the xylem are dependent on the rate of flow of sap in the xylem, which was higher in non-salinized than in the salinized plants. In salinized plants the flux of K^+ and NO_3^- in the xylem was much lower than in the non-salinized plants (Fig. 4). The fluxes of NO_3^-, reduced N and K^+ in the xylem sap were found to be higher in non-salinized plants grown with enriched DIC than in non-salinized plants grown with ambient DIC. In salinized plants grown with enriched DIC, fluxes of NO_3^- and reduced N in the xylem were higher than in salinized plants grown with ambient DIC.

Root incorporation of DIC

The rate of DIC incorporation was higher in non-salinized than in salinized plants and higher in enriched- than in ambient-DIC-grown plants (Tab. 2). After the 1-h pulse of dissolved inorganic ^{14}C the label was largely restricted to the roots, as shown by the low ^{14}C shoot:root ratios (Tab. 2). The ^{14}C shoot:root ratios were highest in the non-salinized plants supplied with enriched DIC, indicating that these plants translocated a greater proportion of the label to the shoots. The proportion of ^{14}C found in the shoots was not correlated with the transpiration rates (Tab. 2).

Differences between ^{14}C-C:^{14}C–N ratios (ratio of ^{14}C labelled organic acid + carbohydrate:amino acid frac-

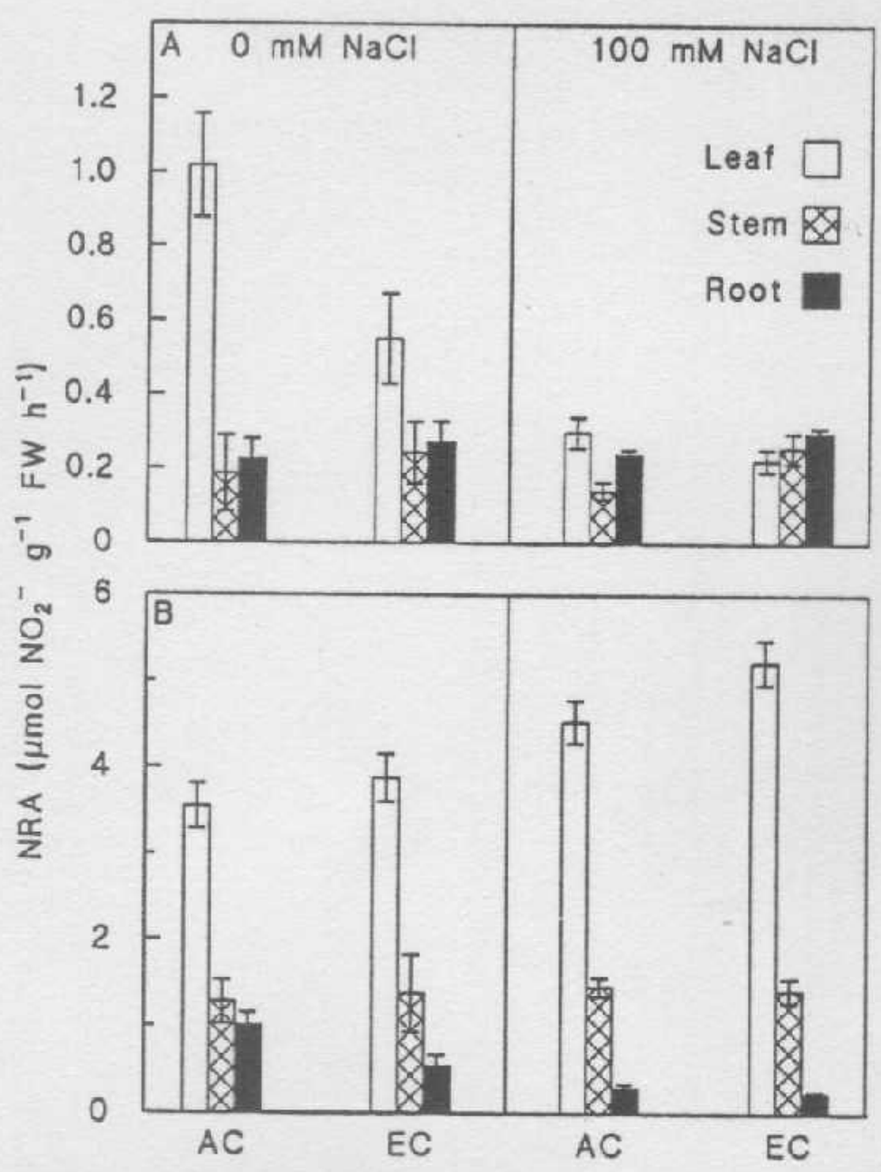

Fig. 3. Effect of 0 or 100 m*M* NaCl and aeration with either ambient (AC, 360 µmol mol^{-1}) or enriched (EC, 5 000 µmol mol^{-1}) CO_2 on the NRA in the tissue of tomato plants. NRA was measured in vivo as the accumulation of NO_2^- in the presence of 100 m*M* NO_3^- in the buffer (A) and in the absence of added NO_3^- in the buffer (B). The error bars represent the SE of the means (n = 4).

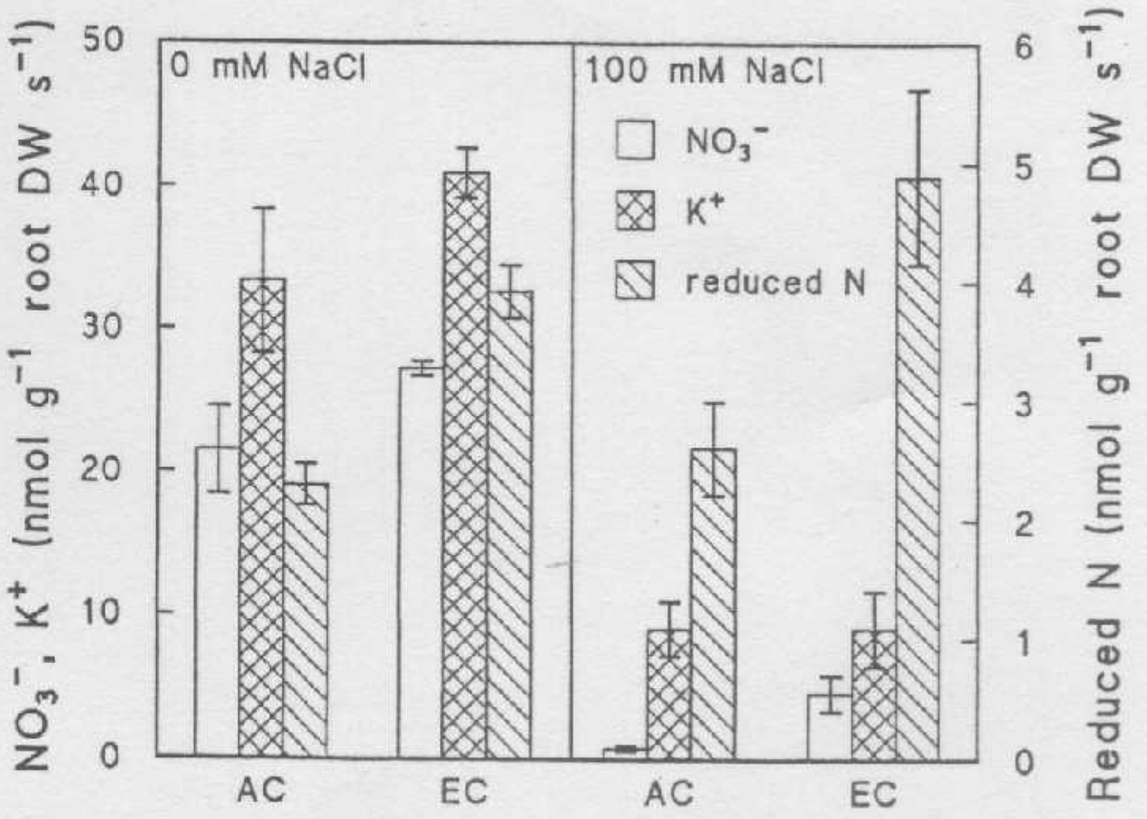

Fig. 4. The flux of NO_3^-, K^+ and reduced N in the xylem sap of tomato plants 12 days after transfer into hydroponic culture with either 0 or 100 m*M* NaCl and aeration with either ambient (AC, 360 µmol mol^{-1}) or enriched (EC, 5 000 µmol mol^{-1}) CO_2. The error bars represent the SE of the means (n = 4).

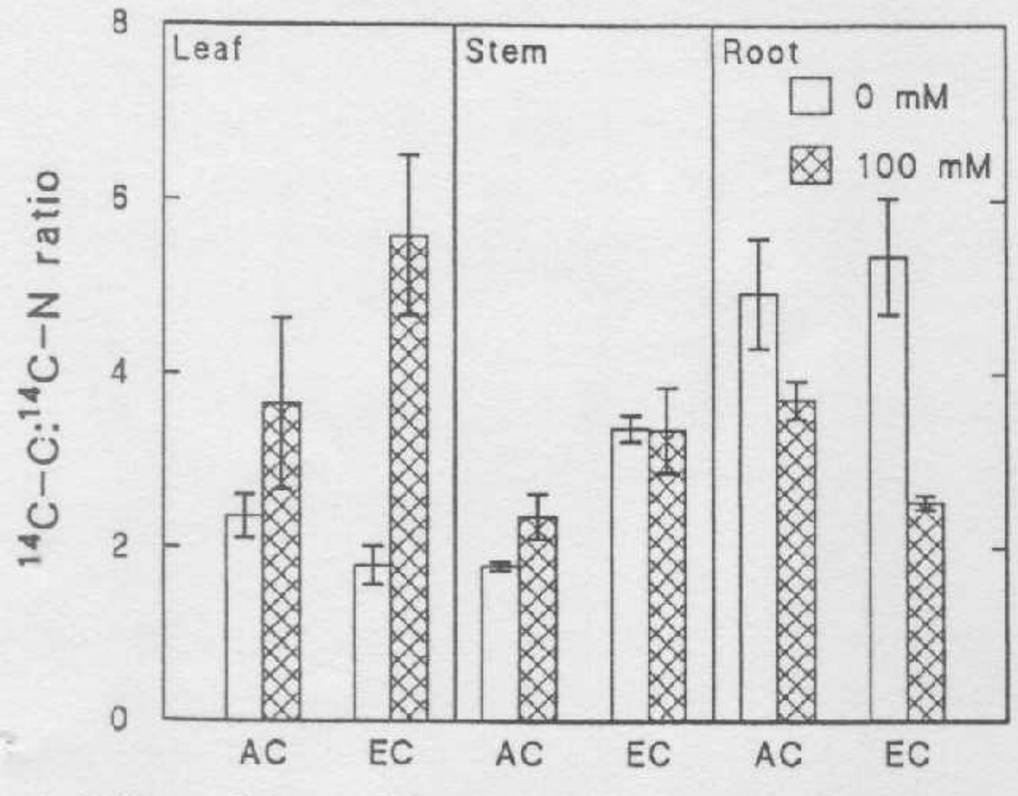

Fig. 5. Effect of 0 or 100 m*M* NaCl and aeration with either ambient (AC, 360 μmol mol^{-1}) or enriched (EC, 5 000 μmol mol^{-1}) CO_2 on the ^{14}C–C: ^{14}C–N ratios of the soluble ^{14}C-labelled products of root incorporation of dissolved inorganic ^{14}C by tomato plants. The error bars represent the SE of the means (n = 4).

tions) in the leaves, stems and roots of the salinized and non-salinized plants grown with ambient DIC were small (Fig. 5). In the leaves of salinized plants grown with enriched DIC, the ^{14}C-C:^{14}C–N ratios were more than 2-fold higher than in equivalent non-salinized plants. Salinization resulted in a greater allocation of ^{14}C to nitrogenous compounds in the roots of plants grown with enriched DIC. The absolute incorporation of label into all fractions, except for organic acid in salinized plants, was increased by enriched relative to ambient DIC (Fig. 6). In the non-salinized plants grown with enriched DIC there was a relatively large allocation of the ^{14}C to the organic acid fraction. Organic acids are the initial product of phosphoenol pyruvate carboxylation and thus may be expected to be highly labelled after a pulse of dissolved inorganic ^{14}C, as observed by Vuorinen et al. (1992). The equivalent salinized plants exhibited little incorporation of ^{14}C into the organic acid fraction, but the neutral and amino acid fractions were relatively highly enriched, despite the overall lower incorporation of ^{14}C in salinized than in non-salinized plants (Tab. 2).

Discussion

The significantly greater dry weight accumulated by the salinized plants supplied with enriched DIC than by equivalent plants supplied with ambient DIC indicates that root incorporation of DIC can have a significant influence on plant growth even over this relatively short growth period. Positive effects of DIC uptake on plant biomass accumulation have been found previously (Vapaavuori and Pelkonen 1985). In this experiment the positive effect of enriched DIC on biomass production was dependent on the presence of salinity stress. This influence of DIC on biomass accumulation of salinized plants was accompanied by changes in the LWR of the plants (Tab. 1). The LWR of the salinized plants supplied with enriched DIC was more similar to that of the non-salinized plants than to that of the salinized plants supplied with ambient DIC. LWR is an important determinant of growth because it reflects the proportion of photosynthetic assimilatory biomass relative to dissimilatory biomass (Poorter and Remkes 1990). Thus changes in LWR with DIC may have been partially responsible for changes in the biomass accumulation of the plants.

The controversial influence of enriched DIC on plant growth has been reviewed by Enoch and Olesen (1993), who concluded that DIC primarily acted as a plant hormone in promoting growth. This conclusion stems to some extent from the small contribution made by DIC taken up by the roots to the overall carbon budget of the plant. It was suggested that the transpiration stream may transport DIC taken up by the root to the shoot where it could be photosynthetically assimilated (Enoch and Olesen 1993). In the experiments reported here, however, the proportion of ^{14}C label from the 1-h pulse of $NaH^{14}CO_3$ located in organic products in the shoot was extremely small (Tab. 2). The reasons for the conflicting reports in the literature on the influence of enriched DIC on plant growth probably arise from the use of a variety of genotypes grown under a variety of environmental circumstances. We suggest that enriched DIC had a positive influence on growth of salinized plants because it provided a source of anaplerotic carbon for amino acid synthesis in the roots of plants forced to assimilate NO_3^- in the root by salinity. Thus, the influence of root DIC uptake and assimilation on plant growth may be greater than the contribution of DIC to the carbon budget of the plant due to changes in the carbon partitioning (Vuorinen et al. 1992).

Plants grown with salinity exhibited lower uptake of NO_3^- (Tab. 2) and correspondingly lower tissue NO_3^- concentrations (Fig. 1) than did non-salinized plants. The influence of salinity on NO_3^- uptake has been associated with anionic interference with NO_3^- translocation across

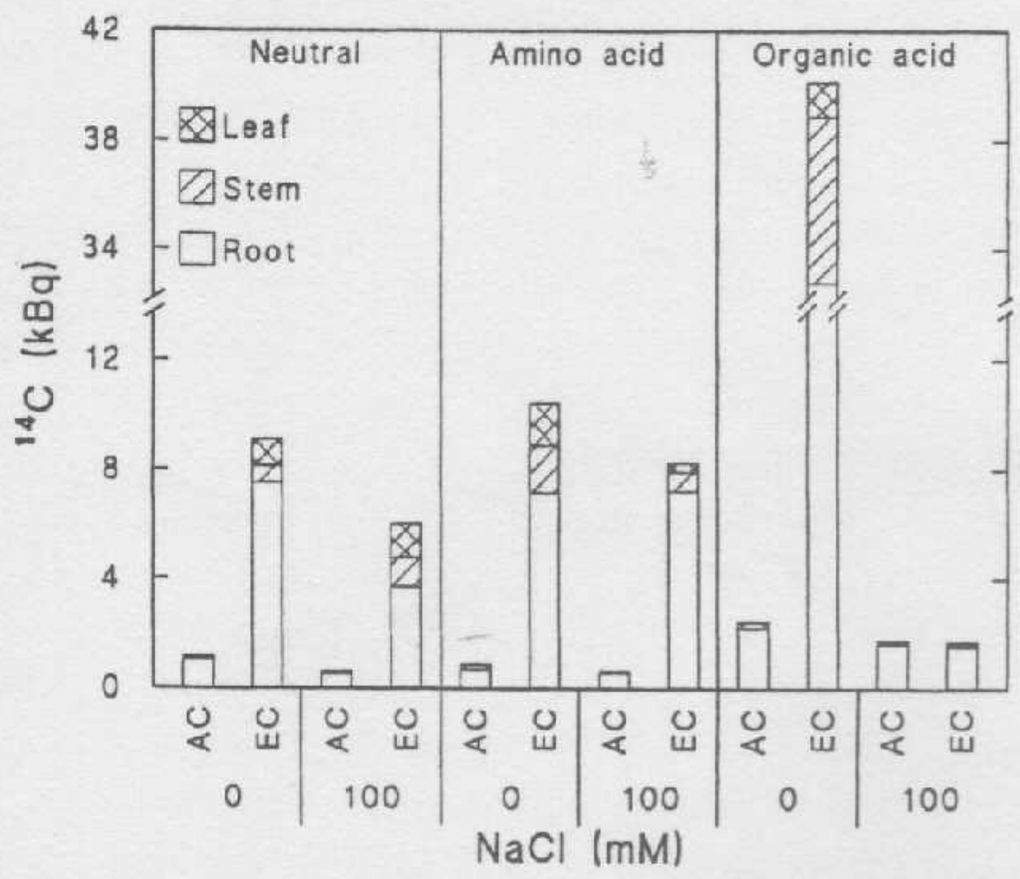

Fig. 6. Effect of 0 or 100 m*M* NaCl and aeration with either ambient (AC, 360 μmol mol^{-1}) or enriched (EC, 5 000 μmol mol^{-1}) CO_2 on the incorporation of dissolved inorganic ^{14}C into labelled ethanol-soluble acid-stable products by tomato plants (n = 4).

the root plasmalemma (Aslam et al. 1984). DIC had a significant influence on NO_3^- uptake by salinized plants. This may be due to the more rapid utilization of NO_3^- in the presence of an enhanced anaplerotic source of carbon in the salinized plants. The capacity of non-salinized plants for NO_3^- uptake was not strongly influenced by DIC, probably because these plants carry out reduction and assimilation of NO_3^- predominantly in the shoot (see below).

The low uptake of K^+ in salinized compared to non-salinized plants (Tab. 2) was associated with lower stem and root K^+ concentrations (Fig. 2) and lower flux rates of K^+ in the xylem. In salinity-treated plants the K^+-shuttle is likely to be less operative than in the non-salinized plants due to the relative lack of foliar NO_3^- reduction. The operation of the K^+-shuttle in the enriched-DIC- and salinity-treated plants may be further reduced because the root has additional capacity to assimilate the products of NO_3^- reduction as a consequence of the greater supply of anaplerotic carbon. Plants grown with salinity and enriched DIC had much higher K^+ concentrations in the leaves than did plants grown with salinity and ambient DIC, although the foliar K^+ concentrations must reflect a dynamic relationship between K^+ uptake and the cycling of K^+ between the shoot and root. The concentrations of NO_3^- and K^+ in the xylem sap were 23-fold and 4-fold higher, respectively, in non-salinized than in the salinized plants (Fig. 4). This indicates that salinity influenced the transport of these two ions independently and that there is no strict dependence of NO_3^- transport on the K^+-shuttle.

The proportion of NO_3^- reduced in the root has been reported to be negatively correlated with the NO_3^- concentration in the external medium in many plants (Smirnoff and Stewart 1985, Andrews et al. 1992). In these experiments, the plants were grown with a relatively low concentration of NO_3^- in the external medium (1 m*M*). Despite this low concentration of NO_3^-, salinization resulted in a large change in the proportion of NO_3^- and reduced N fluxing through the xylem (Fig. 4), indicating that salinity resulted in a greater proportion of NO_3^- reduction and assimilation in the root. If the flux of NO_3^- into the root is low, as in the case of salinized plants, it is likely that the root NR would reduce most of the incoming NO_3^-. This would explain the very low concentrations of NO_3^- found in the xylem sap of salinized plants (Fig. 4). Reduced N concentrations were higher in salinized plants grown with enriched DIC than in equivalent plants grown with ambient DIC (Fig. 1). This corresponds with the increased uptake of NO_3^- and increased provision of anaplerotic carbon (Tab. 2) for the assimilation of N in the roots of the salinized plants grown with enriched DIC.

The incorporation of HCO_3^- by the roots was ca 10-fold higher in plants supplied with enriched compared to those supplied with ambient DIC. This corresponds with the finding that CO_2-enriched atmosphere results in lower CO_2 release from shoot respiration (Bunce 1994, Thomas and Griffin 1994, Wullschleger et al. 1994, Ziska and Bunce 1994). Dark incorporation of HCO_3^- is likely to occur in most plant tissues due to the presence of phosphoenol pyruvate carboxylase which, in combination with carbonic anhydrase, has a high affinity for CO_2 (Edwards and Walker 1983) and may thus explain the apparent response of respiration to CO_2 concentrations.

Release of CO_2 from roots of tomato plants grown at ambient DIC with 0 and 100 m*M* salinity were previously found to be 86 ± 8 and 115 ± 7 nmol g^{-1} root dry weight s^{-1}, respectively (M.D. Cramer, A. Schierholt, Y. Wang and S.H. Lips, unpublished results). Root CO_2 release accounts for ca 10% of daily carbon gain in tomato plants. In plants grown with ambient DIC, the incorporation of DIC accounted for 3 and 1% of root carbon loss in the 0 and 100 m*M* salinity-treated plants, respectively. The corresponding figures for the plants grown with enriched DIC were 30 and 10% in the 0 and 100 m*M* salinity-treated plants, respectively. The presence of salinity was apparently required to enable the plants to utilize the enhanced supply of carbon to benefit growth. The enriched-DIC treatment may have perturbed the carbon allocation in the non-salinized plants by diverting carbon into organic acid synthesis. A large proportion of the labelled organic acid in these plants was found in the stem and leaf tissue, indicating that this carbon was translocated from the root to the shoot and representing a loss of carbon from the root, which may have resulted in reduced growth of the root.

The higher dissolved inorganic ^{14}C incorporation by plants supplied with enriched DIC than in those supplied with ambient DIC was associated with increases of label in all the fractions separated. The organic acid fraction was the most strongly affected of the soluble components by DIC in the absence of salinity. The enrichment of the organic acid fraction, which is readily translocated in the xylem sap, probably contributed to the high ^{14}C shoot:root ratios (Tab. 2) and to the high dry weight shoot:root ratios in these plants (Tab. 1). The low ^{14}C–C:^{14}C–N ratios of the roots of the salinized plants grown with enriched DIC compared to equivalent plants grown with ambient DIC (Fig. 5) indicates an increase in the proportion of ^{14}C allocated to amino acids. In these salinized tomato plants, which reduced and assimilated NO_3^- predominantly in the roots, enriched DIC increased root incorporation of DIC which provided carbon skeletons for amino acid synthesis, possibly ameliorating salinity stress.

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Alterations in photosynthesis and pigment distributions in pea leaves following UV-B exposure

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We compared photosynthetic and UV-B-absorbing pigment concentrations, gas-exchange rates and photosystem II (PSII) electron transport rates in leaves of pea (*Pisum sativum* mutant *Argenteum*) grown without UV-B or under an enhanced UV-B treatment (18 kJ m^{-2} biologically effective daily dose) in a greenhouse. We also compared the distribution of chlorophyll by depth within leaves of each treatment by using image analysis of chlorophyll autofluorescence. Ultraviolet-B treatment elicited putative protective responses such as an 80% increase in UV-B-absorbing compound concentrations (leaf-area basis), and a slight increase in mesophyll thickness (178 in controls compared to 191 μm in UV-B-treated leaves). However, photosynthetic rates of UV-B-treated leaves were only 80% of those of controls. This was paralleled by reductions in leaf conductance to water vapor (50% of controls) and intercellular CO_2 concentrations, suggesting that stomatal limitations were at least partly responsible for lower photosynthetic rates under the UV-B treatment. Total chlorophyll concentrations (leaf-area basis) in UV-B-treated leaves were only 70% of controls, and there was a shift in the relative distribution of chlorophyll with depth in UV-B-treated leaves. In control leaves chlorophyll concentrations were highest near the adaxial surface of the upper palisade, dropped with depth and then increased slightly in the bottom of the spongy mesophyll nearest the abaxial surface. In contrast, in UV-B-treated leaves chlorophyll concentrations were lowest at the adaxial surface of the upper palisade and increased with depth through the leaf. The most notable treatment difference in chlorophyll concentrations was in the upper palisade near the adaxial surface of leaves, where we estimate that chlorophyll concentrations in each 1-μm-thick paradermal layer were about 50% lower in UV-B-treated leaves than in controls. We found reduced electron transport capacity in UV-B-treated leaves, based on lower maximum fluorescence (F_m), variable to maximum fluorescence ratios (F_v/F_m) and quantum yield of PSII electron transport (Y). However, the above were assessed from fluorometer measurements on the adaxial leaf surface and may reflect the markedly lower chlorophyll concentrations in the upper palisade of UV-B-treated leaves.

Key words – Chlorophyll, flavonoids, fluorescence, leaf anatomy, leaf conductance, ozone depletion, pea, photosystem II, *Pisum sativum*.

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Introduction

In some plant species, photosynthesis appears to be sensitive to enhanced levels of ultraviolet-B radiation (UV-B; 280–320 nm; Teramura and Sullivan 1994). A commonly mentioned target of UV-B in the photosynthetic apparatus is photosystem II (PSII; Bornman 1989), which may be sensitive via reduced oxidative capacity (Renger et al. 1989), photoreduction of plastoquinone (Melis et al. 1992) and photodegradation of the D1 reaction-center protein (Jansen et al. 1993). Other suspected chromophores associated with photosynthesis include ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and chlorophyll, both of which can decline under en-

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