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SHORT COMMUNICATION

NMR imaging of root water distribution in intact *Vicia faba* L. plants in elevated atmospheric CO_2

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ABSTRACT

The effect of elevated atmospheric CO_2 on water distribution in the intact roots of *Vicia faba* L. bean seedlings grown in natural soil was studied noninvasively with proton (^1H) nuclear magnetic resonance (NMR) imaging. Exposure of 24-d-old plants to atmospheric CO_2-enriched air at 650 $cm^3 m^{-3}$ produced significant increases in water imaged in upper roots, hypogeal cotyledons and lower stems in response to a short-term drying-stress cycle. Above ground, drying produced negligible stem shrinkage and stomatal resistance was unchanged. In contrast, the same drying cycle caused significant depletion of water imaged in the same upper root structures in control plants subject to ambient CO_2 (350 $m^3 m^{-3}$), and stem shrinkage and increased stomatal resistance. The results suggest that inhibition of transpiration caused by elevated CO_2 does not necessarily result in attenuation of water transport from lower root structures. Inhibition of water loss from upper roots and lower stem in elevated CO_2 environments may be a mitigating factor in assessing deleterious effects of greenhouse changes on crops during periods of dry climate.

Key-words: Vicia faba L.; broad bean; NMR imaging; roots; water transport; carbon dioxide (CO_2) effects; dry climate

INTRODUCTION

Increasing concern about the effects of elevated CO_2 levels on crops is a major consequence of the growing evidence for global changes in atmospheric CO_2 and 'greenhouse' climatic warming (Strain & Cure, 1985; Bolin *et al.* 1986; Warrick, 1988). In foliage, elevated CO_2 typically increases carbon fixation and inhibits water transpiration (Rogers, Thomas & Bingham 1983). While the latter may be fortuitous in warming climates, very little is known of the effect that increased CO_2 may have on the ability of root systems to transport water to meet the demands of increased fixation. This is due in part to the destructive nature of traditional techniques for studying root function.

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Recently, proton (^1H) nuclear magnetic resonance (NMR) imaging, a tool developed primarily for medical applications, was used to non-destructively probe water distribution and transport in plant root systems grown in natural and artificial soil media under normal and stress conditions (Bottomley, Rogers & Foster 1986; Brown, Johnson & Kramer 1986; Rogers & Bottomley 1987), and water in stems and other plant tissues (Johnson, Brown & Kramer, 1987; Connelly *et al.* 1987; Tamiya *et al.* 1988; Jenner *et al.* 1988). To see whether NMR imaging might contribute to the understanding of root water transport in plants exposed to elevated CO_2 environments, we used the technique to noninvasively monitor water distribution in the roots of *Vicia faba* L. (broad bean) seedlings growing in natural soil and exposed to elevated CO_2 during a short drying-stress cycle.

Vicia faba seedlings were grown at ambient CO_2 levels in a greenhouse in 0·15 m diameter plastic pots filled with either Eustis fine sandy loam (sandy, siliceous, thermic Psammentic Paleudults) or Kinston loamy sand (fine-loamy, siliceous, acid, thermic Typic Fluvaquents; Rogers & Bottomley 1987). These natural soil series were chosen because (1) they are transparent to conventional NMR imaging, (2) they do not significantly distort or interfere with root structures apparent in images which are accurately rendered as judged by comparisons of extracted and *in situ* plants, and (3) because soil water up to near saturation, which might otherwise be expected to overwhelm the NMR images, is rendered invisible in NMR images performed with standard spin-echo imaging sequences (Rogers & Bottomley 1987). This latter property is most likely due to the NMR relaxation properties of water in the soil matrix. Plants and their root systems grown in the two soils under identical greenhouse conditions showed no significant morphologic or NMR image differences.

Experiments were performed on healthy, turgid, undisturbed, 24-d-old plants 0·35 m high with root systems extending throughout the soil volume. The soil bulk densities were 1·2 and 1·1 $g cm^{-3}$, the soil water potentials were 0·52 and 0·43 MPa, and the soil gravimetric water contents were 4·8 and 9·3% for Eustis and Kinston, respectively, at the end of the study. Plants in elevated CO_2 ($n = 4$; 2 per soil type) were sealed in 48 dm^{-3} glove bags inflated to a slight positive pressure with dry bottled air (flow-rate, 24 $dm^{-3} min^{-1}$) and bottled CO_2 metered to maintain the

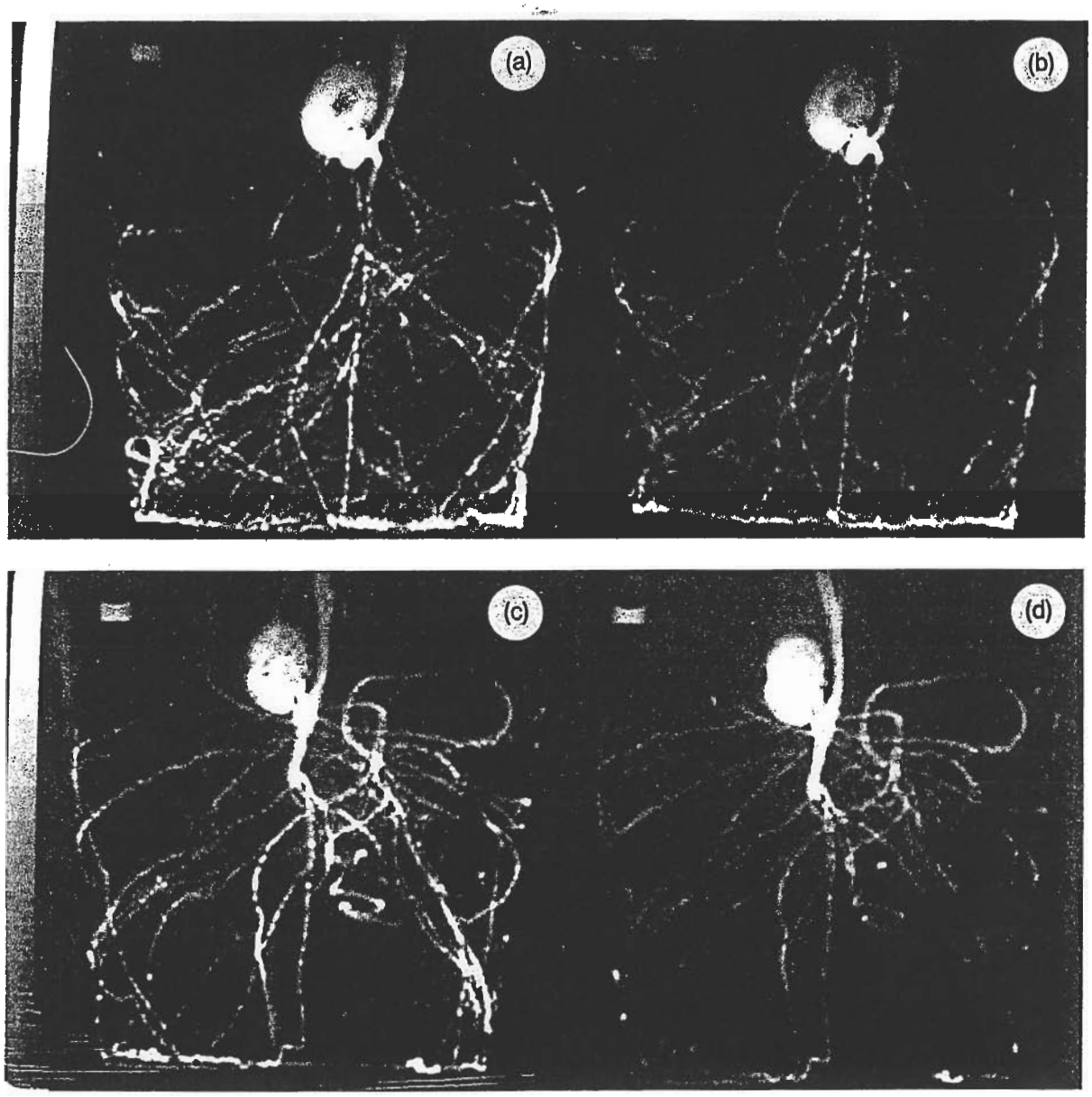

Figure 1. Root images showing mobile water distribution in *V. faba* seedlings in (a,b) control and (c,d) elevated CO_2 environments (a,c) before and (b,d) after a 2 h drying cycle. The small rectangles (a,c) are reference vials whose centers lie at the soil surface.

CO_2 level at 650 $cm^3 m^{-3}$ during the course of the drying-stress cycle and commencing at the initiation of the drying-stress cycle. Control plants (n = 4) were treated identically except that CO_2 was maintained at the ambient concentration of 350 $cm^3 m^{-3}$ from the same bottled air source. Light was supplied to each plant at an incident photon flux density of 960 μmol photon $m^{-2} s^{-1}$ by 850-W quartz-iodine lamps located outside of the glove bags. Air temperature was 25 ± 2 °C. The relatively strong artificial light and the dry air were sufficient to induce rapid water stress.

^{1}H NMR imaging of root systems was performed with a GE Medical systems (Milwaukee, WI, USA) *Signa* 1·5 Tesla whole-body medical scanner, and a custom 0·15 m diameter solenoid receiver coil. A 256×256 point two-dimensional imaging sequence with a 1 s repetition period and short (20 ms) spin-echo was employed to provide shadowgraphs of the integrated water distribution in the soil-root system in the third dimension (Rogers & Bottomley 1987; Bottomley *et al.* 1986) in image acquisition times of 8·5 min. NMR spin-lattice relaxation effects attenuated NMR signals from the root water by less than 10% (Rogers & Bottomley 1987; Bottomley *et al.* 1986). All plants were imaged immediately prior to the commencement of stress conditions. Plants were briefly removed from glove bags for NMR imaging during or at the end of the study, with the magnet bore kept dark. A sealed vial of $CuSO_4$-doped water on the soil surface served as a reference for NMR signal intensity.

The NMR image intensity relative to the reference is

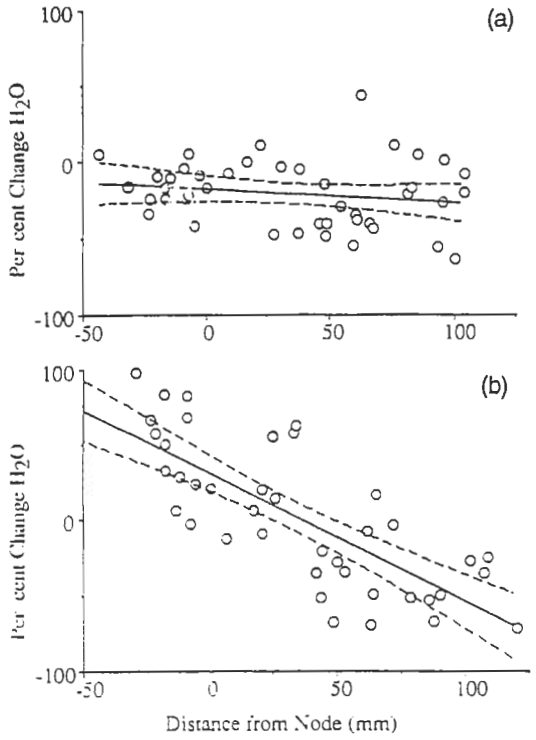

Figure 2. Typical changes in mobile water in the roots of seedlings in (a) control and (b) elevated CO_2 environments following a 2 hr drying cycle, as a function of distance from the node. Negative distances correspond to locations above the node, in the stem below ground level and within the cotyledon. Lines denote least squares fits (solid; correlation coefficients, $r = 0{\cdot}78$ and $0{\cdot}18$ in (a) and (b) respectively and confidence intervals (dashed; $P = 0{\cdot}95$).

proportional to the mobile water in the plant system (Rogers & Bottomley 1987; Bottomley *et al.* 1986; Brown *et al.* 1986; Johnson *et al.* 1987; Connelly *et al.* 1987; Tamiya *et al.* 1988; Jenner *et al.* 1988). Mean image intensity was measured on 44 points throughout each root system at distances $s \leq 120$ mm (delimited by the pot) from the node at the intersection of the cotyledon and taproot. Points in images of roots were relocated to within 1 mm on repeated image examinations during the course of the experiments. Changes in root image intensity relative to values recorded prior to initiation of water stress conditions reflect the effects of root water transport during exposure to the stress.

Figure 1 shows typical examples of root images from a control plant and a plant in elevated CO_2 before (a,c) and after (b,d) a 2-h drying cycle. In the control plant (a,b), the image acquired at the end of the stress shows that water has been depleted from the entire root system. Above ground, incipient wilting was apparent. The basal stem diameter shrank an average of 13±5% (SE) in control plants. Steady-state diffusive porometry of the most recently, fully expanded leaves at the end of the exposure periods yielded an average stomatal resistance of 6·3±1·6 s cm^{-1}. The image of the plant in elevated CO_2 (c,d) also shows water depletion in the lower roots following the water stress cycle, but the upper roots, hypogeal cotyledon and lower stem (below ground) show significantly increased hydration. Wilting and stem shrinkage (0±4%) were insignificant in plants in elevated CO_2, and the average stomatal resistance was higher at 12·6±2·7 s cm^{-1}, consistent with earlier findings (Rogers, Thomas & Bingham 1983; Dahlman, Strain & Rogers 1985; Wittwer 1985).

Figure 2 shows the percentage change in mobile water content versus distance from the node in a control plant (a) and in a plant in elevated CO_2 (b), as indexed by the NMR image intensity. Water is depleted on average 18±4 (SE)% throughout the control plant. In contrast, elevations in water content of 38±8% is evident in the CO_2 plant at depths of $s < 25$ mm, whereas depletion of 27±8% has occurred at $s \geq 25$ mm.

All plants in elevated CO_2 showed similar patterns of increased water around the cotyledon, lower stem, and upper taproot in response to water stress, while control plants typically showed water depletion in these regions. The responses were independent of the soil series. On average, the change in water content was −5%±25 (SD) for all points on all control plants at depths of $s < 25$ mm and mean depth of −10±17 (SD) mm, compared with +24%±29 at the same depths in plants subject to elevated CO_2 ($P < 0{\cdot}01$). However, in deeper roots there was no significant difference between plants in control and elevated CO_2 environments, although the mean root water content was lower in CO_2 plants during the drying cycle (change in water content was +16%±55 in control plants, versus -20% ±40 in CO_2 plants at a mean depth of 68±27 mm for $s \geq 25$ mm). These results suggest that increased CO_2 does not significantly attenuate water tension or water transport from these lower levels to the upper root structures.

The results from control plants are consistent with previous NMR imaging studies showing overall root and stem dehydration in response to a water stress cycle (Bottomley *et al.* 1986; Brown *et al.* 1986; Johnson *et al.* 1987; Tamiya *et al.* 1988). Thus, in control plants, water transported from the lower roots is depleted from upper root structures of *V. faba* by transpiration during the drying cycle.

While elevated CO_2 is known to inhibit water transpiration (Kimball & Idso, 1983; Dahlman *et al.* 1985; Wittwer, 1985), the present results from plants exposed to elevated CO_2 suggest that water transport from the lower roots to the upper roots and stem base continues at a substantial rate in response to stress, resulting in increased hydration of upper root and lower stem structures, at least for short-term exposures of *V. faba*. Thus, inhibition of transpiration caused by elevated CO_2 does not necessarily produce a corresponding inhibition in water transport from the lower roots. The possibility that water-transport from lower roots may be substantially unimpaired, resulting in an accumulation of a water reserve in upper roots and stem, or even the soil (Richards & Caldwell 1987), might represent a beneficial compensatory mechanism in times of 'greenhouse' climatic change, although it will be important to evaluate the effects over more extended periods of plant growth and larger sample sizes.

In conclusion, proton NMR imaging is a unique tool for providing non-invasive, non-destructive information about water distribution in root systems (Bottomley *et al.* 1986) and is ideally suited to elucidating plant response to environmental and experimental stimuli. Therefore, it may provide some useful insights into the effects of elevated CO_2 on root function.

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