Title
Calcium translocation and whole plant transpiration: spatial and temporal measurements using radio-Strontium as tracer

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INTRODUCTION

Calcium (Ca) in plants has essential roles affecting tissue mechanical strength and tolerance to biotic and abiotic stresses (*Hirschi, 2004*). Understanding Ca translocation and partitioning to the different plant parts with time and the factors affecting it has a high agronomic and economical value as it will allow improving Ca nutrition practices to give higher quality end products.

Ca was shown to accumulate mainly in transpiring organs in a process affected by various environmental conditions at both the canopy and root level, and is considered to be coupled to water movement driven by transpiration although controversies still arise in that relation (*Atkinson et al., 1992*). Furthermore, as Ca moves mainly in the xylem, a transport conduit under negative pressure, any attempt to sample it en-route will cause cessation of flow. As a result, the use of cumbersome destructive methods, which has limited research scope due to time and space constraints, has brought only fragmented and/or circumstantial evidence (*Schurr, 1998*). For example, using pressurized stem exudation and leaf bleeding *Siebrecht et al. (2003*) showed either diurnal pattern or spatial distribution but not both together. Looking into various nondestructive methods it was found that Ca nuclides are either incompatible or inapplicable. As Strontium (Sr) was found to behave in similar ways in plants (*Herren and Feller, 1997*) as well as in the more complex environment of human clinical research (*Wasserman, 1998*), it was chosen to serve as Ca tracer. Having a high energy gamma emitting nuclide (^{85}Sr) that can be detected outside the plant, remote sensing became feasible.

MATERIALS AND METHODS

Tomato plants (*Lycopersicon esculentum* L., Var. 870, Hazera Genetics, Israel) were grown in the phytotron of the Hebrew University (Israel) under controlled climate of day/night temperatures of 28/18°C and RH of 40/65% respectively. Each plant was grown in a 5 L container containing half-strength modified Hoagland solution (thereafter referred to as nutrient solution) and was continuously aerated. After three months, reaching approximately a height of 1.60 m and having three fruit bearing trusses, eight plants were transferred each to a 2 L cylinder filled with nutrient solution and moved to a growth room subjected to temperature of 24/16°C and RH of 40/80% during the day and night respectively. Air temperature and RH at plants vicinity were recorded continuously and VPD was calculated according to *Lowe (1997*). Light was supplied between 08:00 to 20:00 by two cool mercury lamps at 400 µmole m^{-2} s^{-1} PAR. Plants were arranged in four pairs with the 1st plant of each pair placed on a weighing lysimeter and monitored continuously with momentary whole plant transpiration derived from weight loss. The 2nd plant was installed with an array of five gamma radiation detectors (RP-11; Rotem Ind., Israel), each with a custom-made lead shield. The shielded detectors were mounted on a moveable platform positioned to target the following locations: 1) main stem below the 1st fruit truss; 2) main stem below 2nd fruit truss; 3) main stem below 3rd fruit truss; 4) first fruit of 2nd truss; 5) leaf petiole adjacent to 2nd fruit truss. The detectors were connected to a PC via a custom-made communication device (Rotem Ind., Israel) and radiation activity was measured continuously. More details of the system can be found in *Wengrowicz et al. (2008)*. Radiation readings were resampled to one minute and filtered (MATLAB, The MathWorks, Inc., MA, USA) in parallel to the transpiration data to eliminate noise. After three days of acclimatization, radio-Sr solution (as $^{85}SrCl_2$; Perkin-Elmer, MA, USA) with an activity of 0.25 mCi was diluted in 10 mL of distilled water containing 4 mM Sr (as $^{88}Sr(NO_3)_2$; Merck, Germany) and added to the nutrient solution of the 2nd plant around noon. The cylinders were refilled with nutrient

solution to full capacity every second day. Every few days the radiation measuring system was detached and moved to the 2nd plant of the next pair.

RESULTS

An example of radiation readings from one plant on the day of application is shown in Fig. 1. Within 30 minutes after adding the mixed Sr and radio-Sr solution to the nutrient solution, a sharp increase in radioactivity was noticed in the lower-most stem detector (SB). A similar pattern yet with about half the rate was observed 30 minutes later in the middle stem detector (SM) and another 30 minutes took the radio-Sr to reach the upper-most stem detector (ST) with half the rate of the previous. Starting at the top of the plant root system and accounting for the distances between the detectors along the stem, radio-Sr velocity is estimated to be 0.154 mm s^{-1}, 0.143 mm s^{-1} and 0.125 mm s^{-1} at the 1st, 2nd and 3rd stem detectors respectively. Fruit and leaf petiole detectors (FM and LM respectively) showed a slow radiation increase and as no clear arrival time was seen, velocity could not be defined.

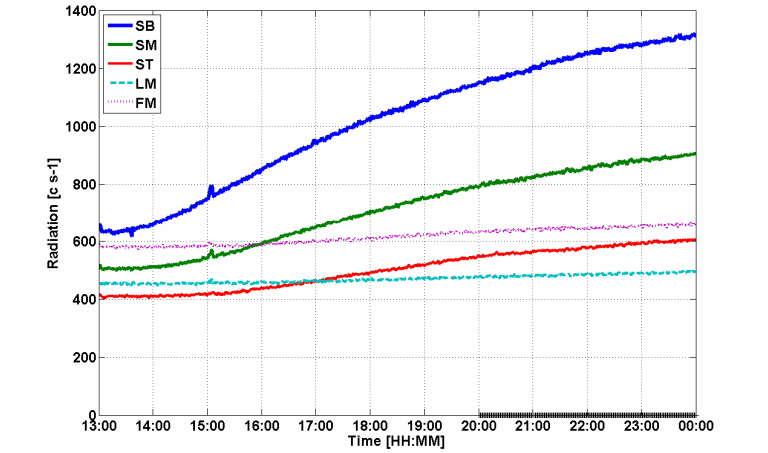

Figure 1. Radiation readings [c s^{-1}] of the detectors array on day of Sr application (incl. background levels). Legend: SB – main stem below 1st fruit truss, SM – main stem below 2nd fruit truss, ST – main stem below 3rd fruit truss, LM – Leaf petiole adjacent to 2nd fruit truss, FM – 1st fruit at 2nd fruit truss. Dark period is marked at charts bottom.

To emphasize changes in radiation activity, and omit background levels, time derivative of radiation readings were calculated. On the day following application (Fig. 2), radiation rate increased already before lights were switched on (dark period is marked at charts bottom), starting at the low stem detector and followed by middle and top stem detectors around 03:10, 04:20 and 05:30 respectively. Fruit and leaf petiole radiation rate increased around the same time however with a much lower rate. Initial daily rate was highest at the lower-most stem detector and decreased the further the stem detector was from the source, with fruit and leaf petiole the lowest. Maximum rates were achieved around 10:00 following the same order of both timing and rates, excluding the fruit detector which showed a 2-fold rate compared to leaf petiole. Thereafter radiation rates dropped quickly only to show a 2nd smaller wave peaking towards 18:00 and subsiding towards evening. A third wave was clearly observed at the three stem locations after lights were switched off, with rates decreasing the further the detector is from the source.

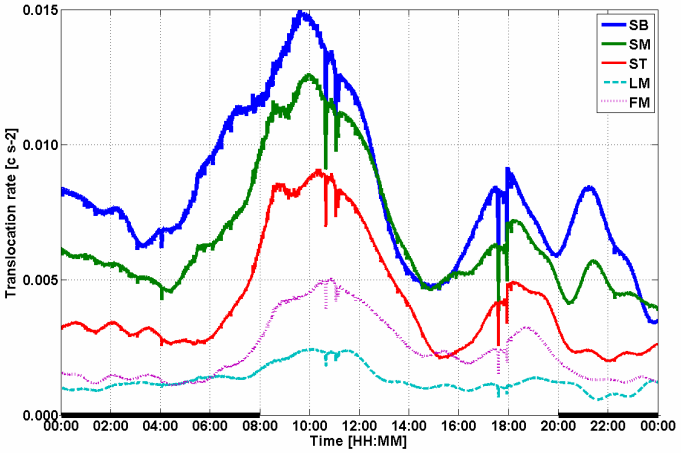

Figure 2. Radiation rate [c s^{-2}] on the 1st day after Sr application. Legend: SB – main stem below 1st fruit truss, SM – main stem below 2nd fruit truss, ST – main stem below 3rd fruit truss, LM – Leaf petiole adjacent to 2nd fruit truss, FM – Stalk of 2nd fruit truss

Transpiration rate pattern of a neighbor plant (Fig. 3a) showed low rates during dark periods (see marking at charts bottom) except from a noticeable swell starting around 03:30. During light hours, a rate increase with three distinct peaks can be seen which correlated nicely with room VPD (Fig. 3b). It should be noted that transpiration rate correlated with radiation rate patterns only until the 10:00 peak, suggesting thereafter a more complex relationship between sap transport and radio-Sr translocation.

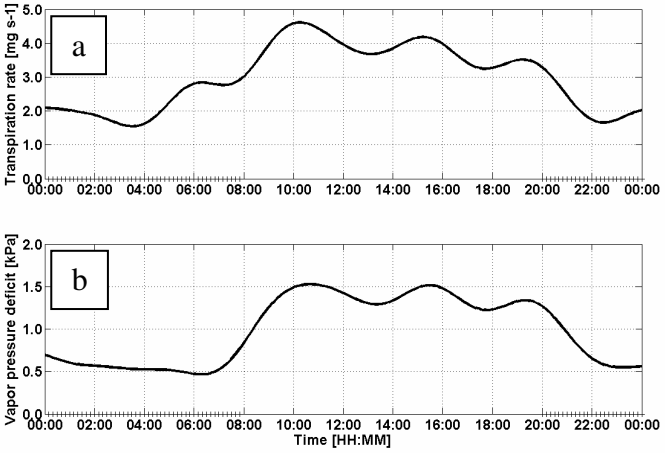

Figure 3: a. Neighbor plant transpiration rate [mg s^{-1}]; b. VPD [kPa] at plants vicinity

As time passed, radiation readings at the top-most stem, fruit and leaf petiole detectors increased. The middle stem detector showed in-large a saturation curve pattern, while the lower-most stem sensor, which measured the first few days the highest radiation increase, showed later a decline to level lower then those detected at above stem positions (not shown). To shed some light on the accumulative patterns, radiation rates on the 10th day after application are presented in Fig. 4. Lower-most stem detector exhibited negative predawn and morning rates yet a morning peak (of negative value) was still present. Rates climbed slowly towards zero during light hours and proceeded with an after-dark positive peak. The middle stem detector showed a similar pattern

although being positive till the predawn drop to later "surface" above zero in the afternoon. The top-most stem as well as fruit and leaf petiole detectors showed positive rates throughout the day with a similar pattern as the other stem detectors.

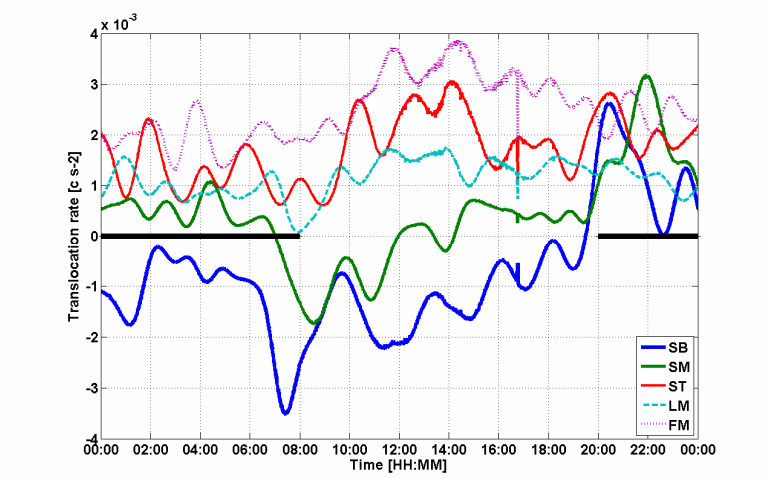

Figure 4. Radiation rate [c s^{-2}] ten days after Sr application. Legend: SB – main stem below 1st fruit truss, SM – main stem below 2nd fruit truss, ST – main stem below 3rd fruit truss, LM – Leaf petiole adjacent to 2nd fruit truss, FM – Stalk of 2nd fruit truss

DISCUSSION

The sequential arrival of root applied radio-Sr to stem locations on the day of application clearly maps its flow path, whereas its decreased velocity along it suggests sap loss as it is being directed towards side organs as leaves and to a probably lesser extent, fruit trusses. As radio-Sr translocation rates were also reduced along the path, it is assumed that Sr was embedded in plant tissue, absorbed on cation exchange sites, and/or unloaded off the xylem causing sap Sr dilution.

Throughout the following days, daily transpiration rate showed predawn increases with a possible link to circadian stomata opening (*Hotta et al., 2007*) resulting in a sap flush within the plant. Predawn translocation rate pattern depended on time that passed from application and detector location. On the first days, when Sr was still accumulating on available cation exchange sites within the stem tissue, translocation rates exhibited significant increase at all locations. On the following days however, when exchange sites at the lower part of the stem were assumingly saturated and the amount of radio-Sr in the feed declined due to solution topping and dilution, "wash out" of radio-Sr was demonstrated by negative rates at the lower stem detectors with positive rates at the upper parts indicating continuous accumulation at the flow terminals.

Each morning, a sharp increase in both transpiration and translocation rates was seen, which implies coupled transport of water and Sr ions. Within hours however, translocation rates declined although transpiration rate was relatively stable. A possible dilution effect resulting from unloading activity as well as adsorption dynamics to tissue exchange capacity could explain such phenomena. As the day advanced and transpiration rate decreased, translocation rates peaked and subsided suggesting a 2nd balance shift. The 3rd and last daily wave observed after dark can be attributed to the low night transpiration rate and a possible desorption and/or loading of Sr into the sap which needs further studies (*Bell and Biddulph, 1963*).

A relative high rate daytime inflow of radio-Sr was registered at the fruit detector which contradicts with the assumed night sap filling of xylem-borne ions due to fruit expansion. A possible explanation is that the existing mature fruits having low daily volume change coupled

with a high sink term result in day-fill patterns. In regard to the relative high fruit radiation rates as compared with the leaf petiole detection rates, it should be noted that fruit radiation shield geometry was different than all other shields and allowed more radiation interception at the detector as compared to that of the petiole and stem detectors. It is likely that related normalization would reduce relative fruit radiation levels.

CONCLUSIONS

Using high resolution time measurements of spatially located sensitive gamma detectors enabled studying the transport and partitioning patterns of root applied radio-Sr, used as a Ca tracer, in a whole plant. Simultaneous indicative transpiration measurements showed early morning coupled transport which was later disrupted due to probable adsorption/desorption dynamics as well as loading/unloading activity. As days passed, radio-Sr moved and accumulated downstream at the terminal organs (fruit, leaf petiole and upper stem parts) presenting a wash-out pattern from the lower stem sections. Fruit inflow pattern showed day-time inflow which contradicts the accepted fruit night-fill of xylem-borne ions. Fruit maturity is suggested as a possible explanation.

ACKNOWLEDGMENT

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