Performance tests of Horiba B-751 Compact Calcium Ion Meter for suitability of measuring calcium concentrations in xylem sap of pea (*Pisum sativum*)

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Linearity of dilution curve for pea (*Pisum sativum* cv. Alderman) root xylem sap

This assay determines possible interference from other xylem sap compounds on calcium values from Horiba calcium electrode.

80 ul volume samples of pea root xylem sap, either undiluted or dilute to half or quarter strength with DI water or samples of just DI water were spiked with 20 ul of 0, 1, 5 or 10 mM $CaCl_2$ solution and measured for Ca with the Horiba ion selective electrode.

The linearity of the curves indicate that other compounds present in the sample matrix (xylem sap) do not interfere with the calcium reading given by the ion selective electrode.

Data points presented are means ± SE of 4 replicates with linear regressions fitted in SigmaPlot.

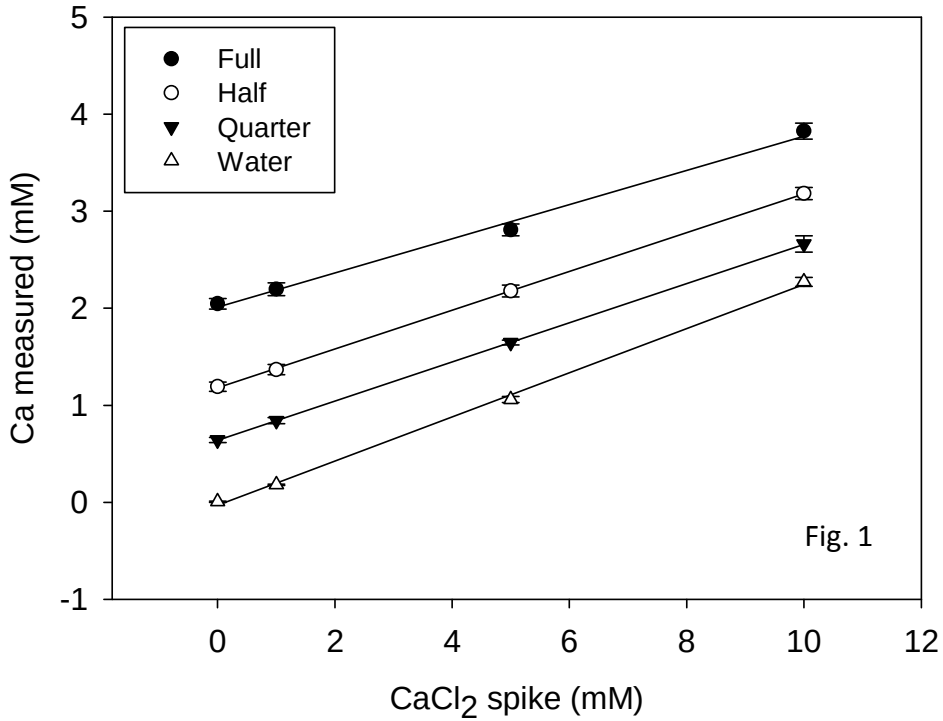
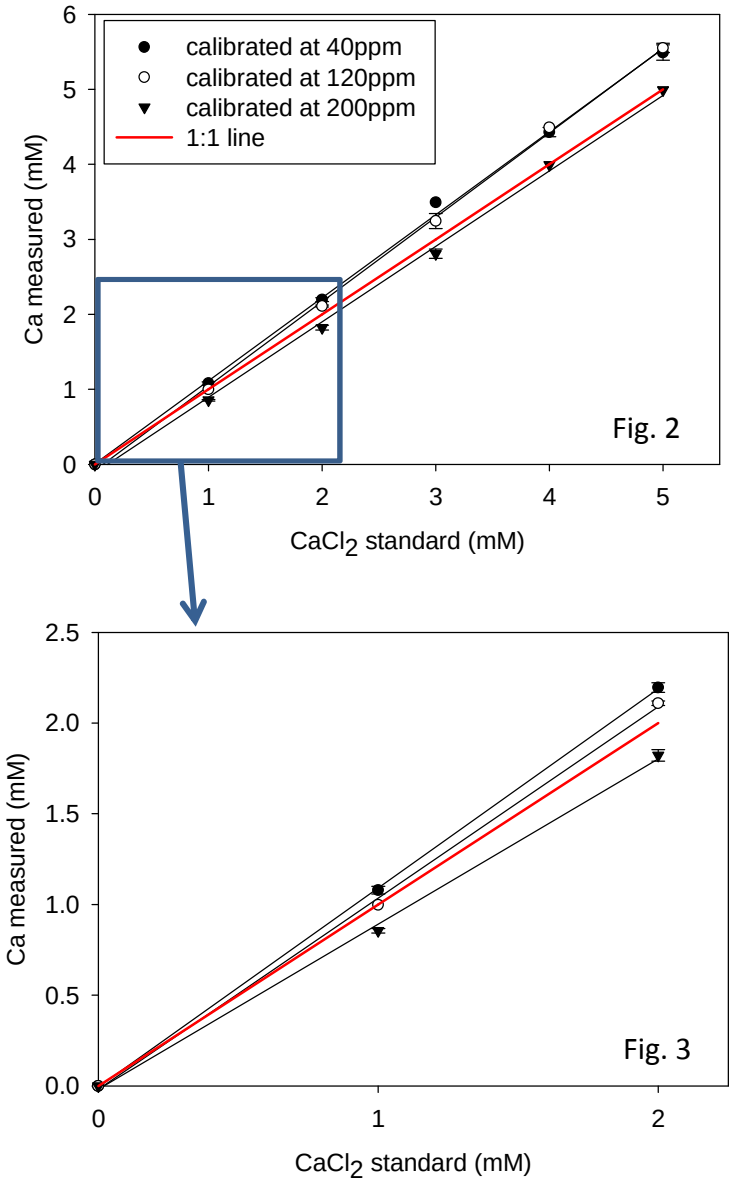


Fig. 1

Performance of electrode at differing single low calibration settings.



The electrode was calibrated at 40, 120 or 200 ppm (1, 3, 5 mM) and tested in a range of CaCl2 standards from 1 to 5 mM, a range expected in the xylem sap samples to be analysed.

At the higher end of the concentration range the 200 ppm calibration was most accurate with both the 40 and 120 ppm calibrations overestimating by approx 10% (fig. 2). However, at the lower concentration range the 200 ppm calibration underestimated the 1 mM standard by approx 20% and the 40 and 120 ppm calibrations were more accurate with the 120 ppm standard being exact at 1 mM (fig 3).

Considering these results and the discovery that xylem sap calcium concentrations were commonly below 1 mM a calibration setting of 120 ppm was adopted.

Data points presented are means ± SE of 4 replicates with linear regressions fitted in SigmaPlot.

Accuracy of the electrode below the stated 40 ppm (1mM) lower detection limit

The electrode was tested at a range of CaCl2 standards from 0.25 to 1 mM at both the 40 ppm and 120 ppm calibration settings.

Both calibration settings gave similar accuracies with the 40 ppm slightly overestimating and the 120 ppm slightly underestimating but having less variation in the recorded values (fig. 4).

These results suggest that the electrode is still accurate below the stated 40 ppm lower detection limit.

Data points presented are means ± SE of 4 replicates with linear regressions fitted in SigmaPlot.

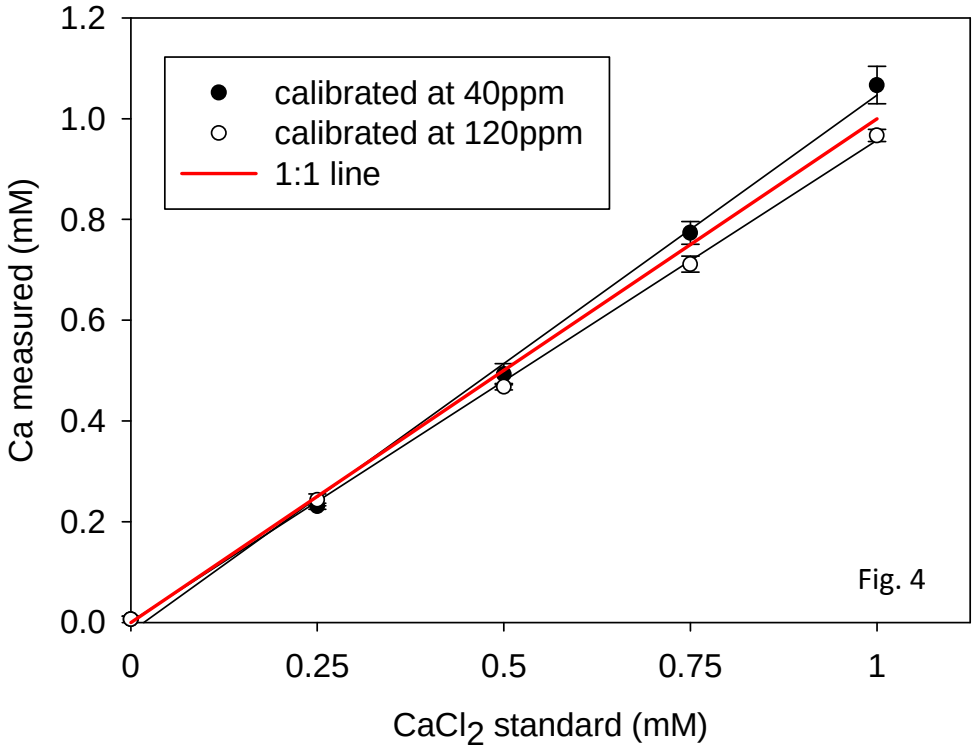


Fig. 4

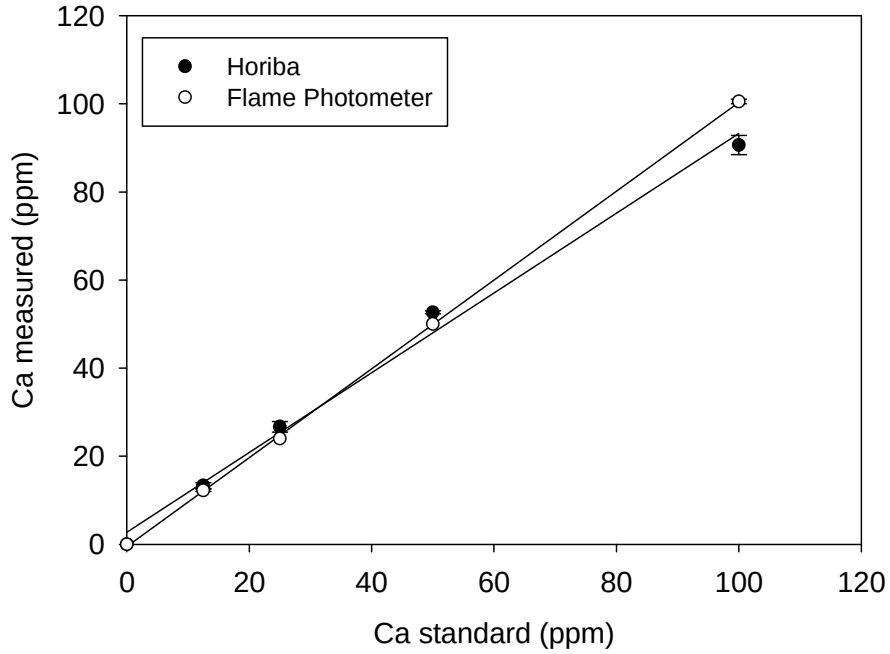
Comparison of Horiba Ca ion selective electrode with Flame Photometry

The electrode was calibrated at 50 ppm with a flame photometer standard and compared at a range of standard concentrations from 12.5 ppm to 100 ppm.

At the 12.5, 25 and 50 ppm concentrations the electrode compared favourably with the flame photometer. At the higher (100 ppm) concentration the electrode underestimated by approx 10%.

These results suggest that the electrode is accurate in its determination of calcium concentrations within the range of xylem sap concentrations encountered (see next slide).

Data points presented are means ± SE of 3 replicates with linear regressions fitted in SigmaPlot.



Example of xylem sap calcium concentration and delivery rate at different positions within the transpiration stream of pea (*Pisum sativum* cv. Alderman).

Sap was collected under induced excess root pressure and soil pore water was collected using a micro rhizon sampler (Van Walt Ltd, Haslemere, Surry UK) under vacuum. Delivery rates are calculated from calcium concentration and recorded rate of sap flow and are presented with measured concentrations in table 1 below. Fig 5 indicates where sap samples were collected.

Table 1.

	Conc (mmol)	Delivery rate (nmol s^{-1})
Soil	0.47	NA
Root	1.50	0.768
Stem	1.10	0.498
Petiole	1.12	0.083
Leaf	0.80	0.030

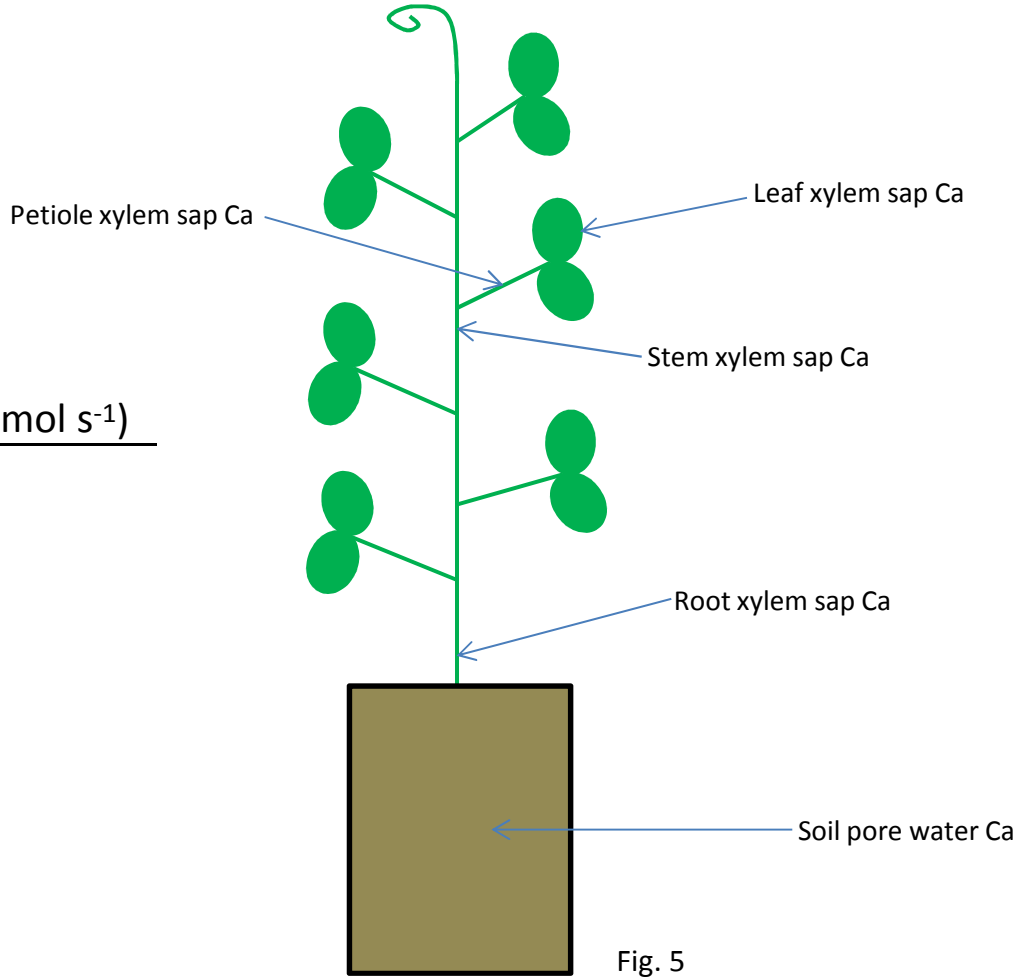


Fig. 5