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IMPROVEMENT OF PRESSURE CHAMBER MEASUREMENTS OF TWO LEGUMES BY CONSTRICTION OF STEMS

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Key words

Plant water potential Pressure chamber Stylosanthes

Summary

A valid determination of the balancing pressure end-point was not possible with *Stylosanthes hamata* (L.) Taub. cv. Verano and *S. scabra* Vog. using a sealing stopper of the Waring and Cleary type. An exudate, apparently from the pith, occurred at low pressures even in stressed plants. The problem did not occur in a chamber fitted with a compression gland. A modification of the Waring and Cleary chamber which caused greater stem constriction proved successful.

Since the publication by Scholander *et al.* in 1965, the pressure chamber has become the most widely used method for assessing internal water status of plants in the field. We have been using the method described by Waring and Clearly[4] in studies of adaptation of exotic tropical legumes sown into native woodland pastures in northern Australia. Assessment of xylem water potential is difficult with *Stylosanthes hamata* and *S. scabra* due to exudation of liquid and profuse bubbling from the cut stem prior to attainment of balancing pressure in the chamber. Others have encountered this problem with other herbaceous dicots, *e.g.* cotton[1]; tobacco, sunflower, and cotton (N. Turner, personal communication); and *Macroptilium atropurpureus* (M. Fisher, personal communication). Ritchie and Hinkley[2] reported that 'experience and reduced rates of pressure increase seemed to enhance end-point recognition' in the cotton study of Jordan[1]. With the *Stylosanthes* species, however, determination of the true end-point was so difficult that we considered the method unacceptable without the improvements in techniques described in this note.

During periods when the soil was wet, shoots of *Stylosanthes hamata* and *S. scabra* consistently produced a droplet of liquid on the cut surface immediately following excision. More liquid was expressed as pressure was imposed in the chamber and at 0.1 to 0.2 bars pressure, bubbling occurred. Initially this situation was interpreted as a balancing pressure of zero. However, when this phenomenon persisted as plants became visibly stressed, comparisons of pressure chamber values (P) and leaf water potentials (ψ_L) were made. The latter were measured with a Wescor dewpoint hygrometer on punched leaflet discs. Samples were equilibrated for 35 minutes with the chambers in an insulated box and at ambient temperatures. It was found that droplets formed on cut surfaces with ψ_L as low as -16 bars. When ψ_L dropped to -19 to -20 bars, no droplets formed, but liquid was expressed with P values of 0.1 to 0.3 bars.

To make certain that this liquid (hereafter referred to as first sap) was not xylem contents, a comparison was made of its osmotic potential (ψ_S), with that of the liquid expressed when chamber pressure was raised above that equivalent to measured leaf water potentials (ψ_L), *i.e.* second sap. Firstly, leaf water potentials were determined as described above. From the same plant and surrounding ones shoots were cut to obtain first sap. This sap was blotted onto a small filter paper. When the paper was saturated (requiring several shoots) it was placed in the sample chamber of the dewpoint psychrometer and osmotic potential was measured as with leaves except that equilibration could be achieved in 10 minutes. Additional neighbouring shoots were cut and placed in the pressure chamber for expression of second sap; each shoot produced sufficient sap to saturate the filter paper. The results are shown in Table 1. Since ψ_S xylem are generally greater values than -3 bars[2], it appears highly unlikely that the first sap was xylem contents. The second sap appears to have been xylem contents but with, possibly, some contamination with first sap.

We were not able to determine from what tissue the first sap came when forced out under pressure. However, it was clear that the bubbles which developed as the flow of liquid slowed originated at the outer margin of the pith. It was also found that although a plant might exude a droplet (without pressure) when excised 10–20 cm from the tip, no exudation occurred when the stem was excised near ground level. At this level in the stem secondary xylem was developed and pith was nearly absent.

Although the information in Table 1 demonstrated the consequences of accepting a 'false end-point', there remained the problem of detecting the true end-point obscured by a froth (Fig. 1) which usually could not be controlled sufficiently by persistent blotting. In a study of comparative water relations of a number of species including *S. hamata* and *S. scabra*, a colleague, C. J. Gardener, experienced no such problems. He used a pressure chamber with a manual compression gland seal[3]; we used a PMS Model 1000 (PMS, Corvallis, Oregon) in which the seal was effected by the chamber pressure[4]. N. Turner (personal communication) observed that problems with several herbaceous dicots, particularly cotton, seemed to be less with the Scholander-type seal.

When we compared the two instruments using shoots of both *Stylosanthes hamata* and *S. scabra* which had been grown in the field for several months, profuse bubbling occurred at low pressures with the PMS chamber, but not with the Scholander chamber. When a

Table 1. Comparison of the sap osmotic potentials (bars) with total water potential of leaf tissue of *S. hamata* shoots

Date	Hour	ψ_L			First Sap ψ_S			Second Sap ψ_S		
		$\bar{x}$	S.E.	n	$\bar{x}$	S.E.	n	$\bar{x}$	S.E.	n
20/5	0730	−7.30	1.70	3	−8.30	2.10	3	−2.90	.90	3
	0830	−10.00	1.60	3	−12.00	1.40	3	−2.90	1.30	3
	0930	−10.00	3.20	3	−12.80	2.70	3	−2.10	1.30	3
25/5	0730	−10.90	.30	21	−11.20	.60	6	−2.50	1.00	3
	1300	−14.60	.60	6	−20.90	.50	5	−1.00	.70	3

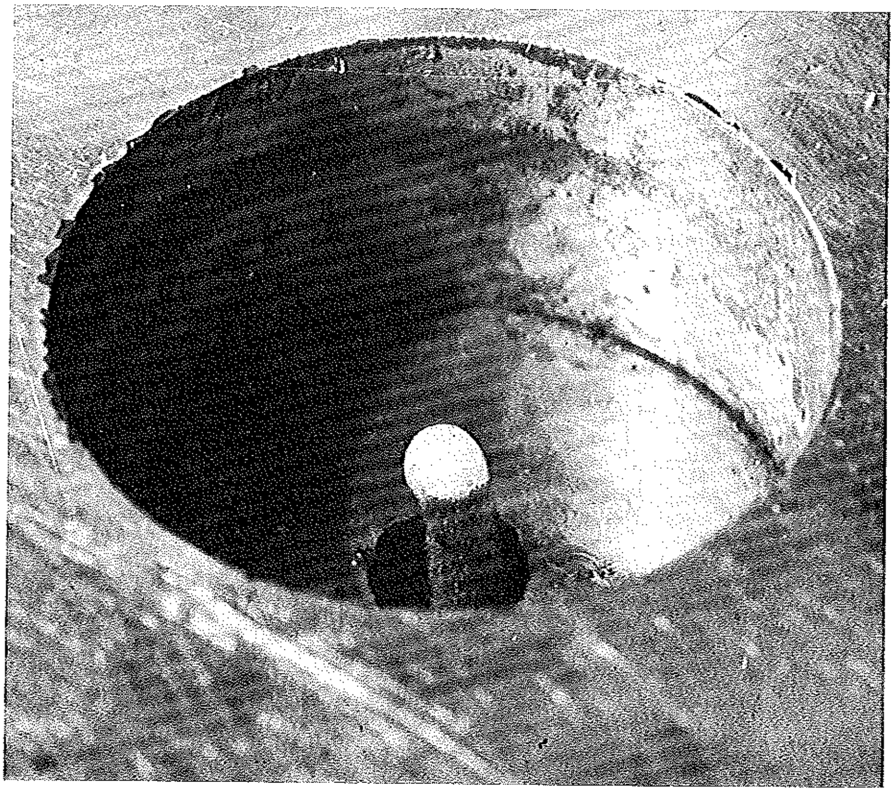

Fig. 1. Froth on stem in pressure chamber produced at pressures about one-fifth of the true balancing pressure.

shoot was inserted in the Scholander-type gland but tightened just enough to prevent gas leakage, the result was identical to that with the PMS chamber. The exudation and bubbling could be stopped during a run simply by tightening the studs on the gland assembly.

Many commercially-manufactured pressure chambers are of the Waring and Clearly type. We have replaced the seal of one of these, the PMS Model 1000, with a compression gland with satisfactory results. The cover of the chamber is shown in Fig. 2 with a cover insert and rubber stopper. Cover inserts vary in size and shape of orifice to accommodate different types of plant material and are easily interchanged. After the cover aperture is enlarged slightly, the compression gland assembly is interchangeable with cover inserts.

Conclusions

When shoots of many herbaceous dicots are pressurized in a chamber, gas forces liquid from non-vascular tissue prior to the return of the menisci to the cut ends of the xylem.

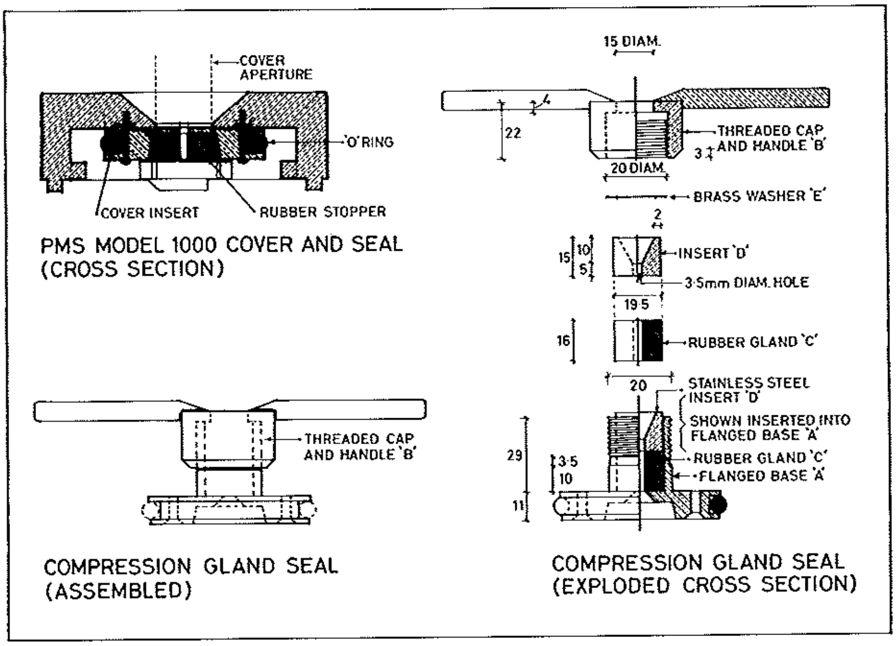

Fig. 2. Modification of the PMS model 1000 pressure chamber to provide a compression gland seal. (All dimensions in mm).

Possible errors due to this phenomenon are:

1. Mistaking this bubbling as the end-point.
2. Inaccurate observation of the true end-point due to the presence of a froth.
3. Under-estimation of the osmotic potential of the xylem sap due to contamination by the non-xylem exudate.

Although our studies show that radial pressure can overcome the problem, elucidation of the mechanism requires further work. It seems likely, however, that the radial pressure compresses parenchymatous tissues sufficiently to prevent flow, but is insufficient to do the same to xylem elements.

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