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PLANT WATER STATUS OF APPLE TREES AND ITS MEASUREMENT IN THE FIELD. 1. THE DYE TECHNIQUE FOR MEASUREMENT OF LEAF WATER POTENTIAL

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SUMMARY

A suitable field index of plant water stress was required for studies involving water usage by apple trees. A dye technique, developed after Shardakov's technique, and modified for use with leaf discs, is described. An assessment of the effects of disc size and equilibration time on measured water potentials is made for three apple varieties under different stress conditions.

The larger disc size (16 mm diam.) and an equilibration time of 4 hr were chosen to minimize early "time" effects and contamination errors, which are discussed in relation to measured potentials.

I. INTRODUCTION

Apple trees in the Stanthorpe district of Queensland experience soil moisture deficits in almost every growing season, with the result that growth and cropping are restricted. Since water available for irrigation is in short supply there is a need for efficient plant water use. Initially a suitable field index of plant water stress was required for investigational purposes.

An examination of the literature indicated that only a limited number of techniques are suitable for field use. These include the dye technique (Shardakov 1953), the refractometric technique (Ashby and Wolf 1947), and Boyer's (1967) pressure chamber method for direct measurements of leaf water potential, which is considered to be the best measure of plant water stress (Kozlowski 1965). The dye technique was chosen for modification on the basis of simplicity, speed and the fact that a more representative sample of an area of trees could be taken using this method.

Other, indirect methods were also used and these are discussed in other papers in this series (Chapman 1970*a, b, c*).

The dye technique for the measurement of water potential has errors, some of which are inherent while others are dependent on the plant species being studied. The addition of methylene blue to the sucrose solution can, according to Kramer and Brix (1965), result in a small detectable change in density of the solution. This may produce errors in water potential estimates where water exchange between leaf and solution is small, under low stress conditions.

Minimum amounts of methylene blue are dictated by the limits of detection of the human eye. These workers further point out that while errors arise from this source they are likely to be small and unimportant.

The dye technique used was modified for leaf discs rather than whole leaves, firstly to utilize less material, as the technique is destructive, and secondly to shorten the equilibration time. There are a number of problems which arise with the use of cut leaves. Firstly, exudation of solutes or cell sap can occur from cut surfaces into the external solutions, altering the density and the osmotic pressure of these solutions and leading to errors in water potential estimates. A measure of these errors is obtained by varying the ratio of cut surface to surface area of discs and observing the effect on water potential. Secondly, a "time effect" may arise from the cutting of leaf tissue which produces initially much lower water potentials but disappears with a longer equilibration time (Kramer and Brix 1965). Thirdly, where there are membranes which are not strictly semi-permeable, mass solute transfer can take place and lead to errors by reducing the apparent osmotic pressure of the external solution.

In this work a description of the dye technique used is given, the errors involving exudation and equilibration time are elucidated, and disc sizes and equilibration times are discussed for apple leaves of three varieties.

A measure of mass solute transfer obtained by a comparison of the dye technique with the vapour equilibration method, after Slatyer (1958), is given in paper 2 of this series (Chapman 1970*a*).

II. MATERIALS AND METHODS

General method.—Sucrose solutions, seven in all, were prepared to provide a range of osmotic pressures in approximately 2 atm steps. They were placed in phials 5 cm x 2 cm diam., with two series of seven solutions for each determination to be made, one series to receive leaf discs and the other the test solutions. The series of solutions which were to receive leaf discs were dyed with methylene blue to a medium blue colour. This was carried out on the bulk solution before the phials were filled. Test phials contained approximately 8 ml and the other phials contained sufficient solution (2–3 ml) to cover four leaf discs. After filling, all phials were stoppered with corks and placed in holes made in polystyrene foam. The polystyrene provided insulation to keep the temperature constant and also served as a phial stand.

Leaves were brought to the laboratory in tightly sealed plastic bags from which most of the air had been excluded. Leaf discs were cut with a very sharp cork borer on a soft pine board. Large veins were avoided, any discs with large veins being discarded. Four discs were then quickly placed in each phial, submersed in the dyed sucrose solution and the phials restoppered. After equilibration, the discs were removed with a pair of fine-pointed forceps and discarded. Phials were restoppered and either sealed over the cork with hot paraffin wax for storage at low temperature, when testing of solutions was to be deferred, or left unsealed and tested immediately.

After mixing, a portion of dyed solution was extracted with a Pasteur pipette. The pipette was then inserted into the test solution at its midpoint of depth, a drop of dyed solution ejected, and the behaviour of the drop observed. Where the drop fell at one concentration and rose at the next the water potential was assumed to lie midway between those two concentrations.

Disc size and equilibration time—Method.—The effect of two disc sizes representing two cut-surface:surface-area ratios were examined for two apple varieties (Jonathan and Delicious) on two different occasions, and for another variety (Granny Smith) on one occasion. The six times for equilibration were 0·5, 1·0, 2·0, 3·0, 4·0 and 6·0 hr. In the first instance, with the Jonathan variety, 1·5 hr was included, but this was later eliminated. The two different occasions were chosen in an attempt to determine equilibration times for low and high water stress.

Leaf samples were taken so that 14 leaves from each of three trees provided material for a single replicate. Three such replicates were used for each variety on each occasion. Leaves sampled were fully expanded, exposed spur leaves close to the terminal bud and are termed terminal spur leaves for the purpose of discussion. These leaves were taken from a height of 5 ft above ground level. Leaves were collected in the field, placed in plastic bags and brought to the laboratory, where four 16 mm discs and sixteen 8 mm discs were cut from each leaf and transferred to phials of dyed sucrose solutions. Four 16 mm discs or sixteen 8 mm discs were placed in each phial. After the equilibration times had elapsed, discs were removed and solutions either tested immediately or sealed with paraffin wax and tested at a later time.

III. RESULTS

Figures 1–3 show results for the effect of disc size and equilibration time on the water potentials of apple leaves of the Delicious, Jonathan and Granny Smith varieties, respectively.

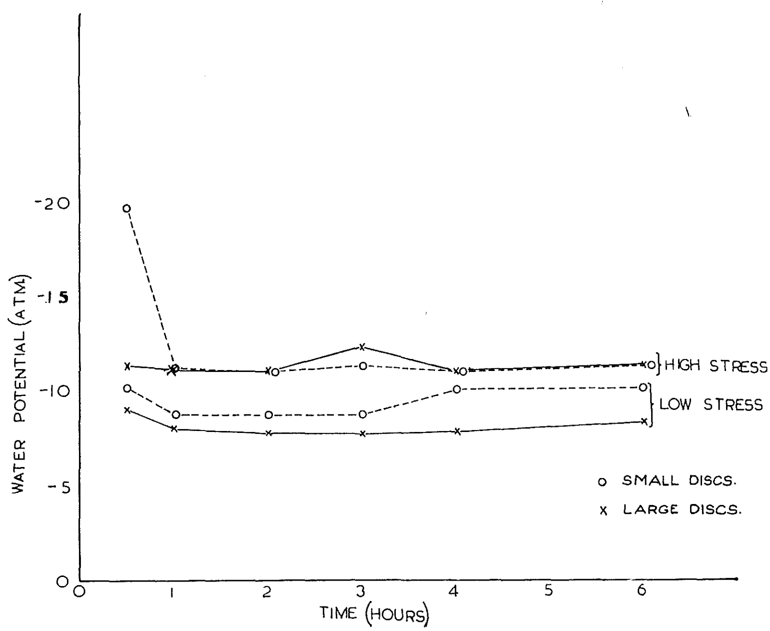

Fig. 1.—Effect of disc size and equilibration time on water potential of Delicious apple leaves at two stress levels.

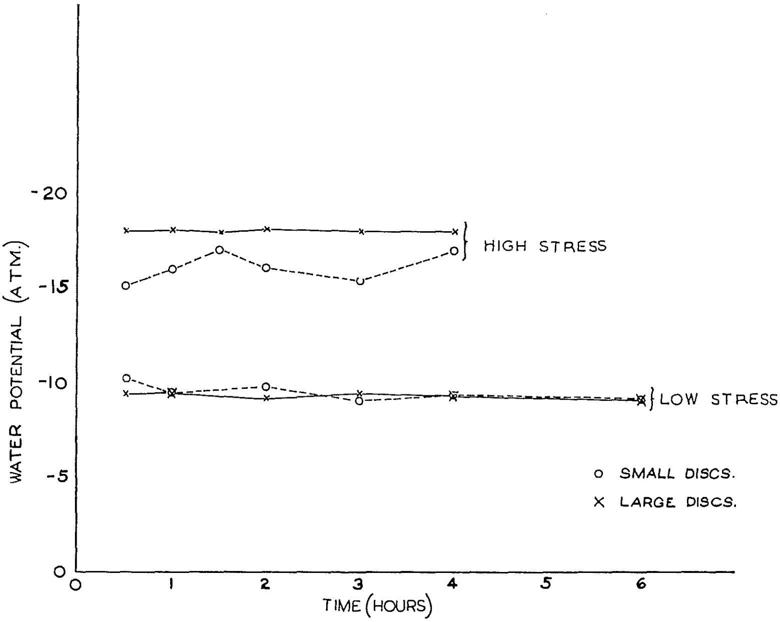

Fig. 2.—Effect of disc size and equilibration time on water potential of Jonathan apple leaves at two stress levels.

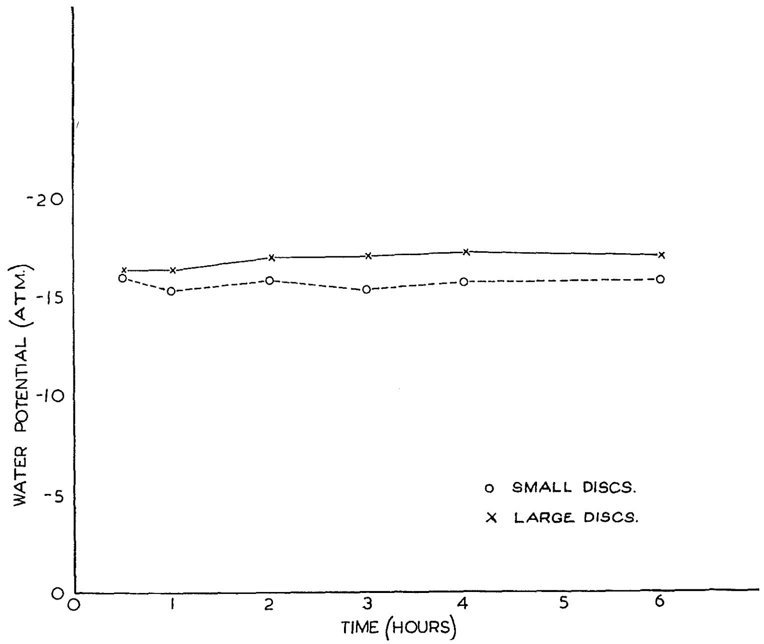

Fig. 3.—Effect of disc size and equilibration time on water potential of Granny Smith apple leaves at high stress level.

For Delicious at low stress there was no interaction and water potential increased with equilibration time up to 3 hr, after which there was a slight decrease. Water potentials for the small discs were significantly lower than for the larger discs. At the higher stress there was an interaction between disc size and equilibration time. The effect of disc size on water potential was confined to the 0·5 hr observation, where the estimated water potential for small discs was lower than for large discs. An effect of equilibration time was only noted for small discs, where at 0·5 hr the water potential was lower than at all other times. With large discs this effect was absent.

With Jonathan at the lower stress, there was no interaction and equilibration time had an effect on water potential, but this effect was confined to the 0·5 hr observation. Disc size did not influence water potential at the low stress condition. At the high stress there was an interaction between disc size and equilibration time. The effect of time was only noted for the smaller discs and there were no disc effects after 4·0 hr.

For Granny Smith at the high stress there were no significant differences in water potential with the different equilibration times for either disc size. However, large discs had lower potentials than those of small discs. Data are not available at low stress.

IV. DISCUSSION

The difference in estimated water potential, with Delicious at the higher stress, for large and small discs is explained by the difference in cut surface area, with the small discs having twice the cut surface area of the larger discs. This difference was attributed to exudation of solutes from the cut edges of discs, which caused contamination of solutions during the early period of immersion. The subsequent leakage of low-density contaminants from the leaf tissue according to Knipling (1967) probably helps to compensate for the initial density changes, so the initial effect disappears with time of immersion. This early "time" effect could not be attributed to the adsorption of water onto leaf surfaces since the effect was absent with the larger discs, which had the same surface area as those of the smaller discs.

For Delicious at the lower stress condition, there was some evidence of an early "time" effect which disappeared with longer immersion times but reappeared in the core of the smaller discs. Here, the adsorption of water onto leaf surfaces may have been responsible for this early "time" effect, as potentials were influenced irrespective of disc size.

With Jonathan at the lower stress, a small initial "time" effect was noted for the smaller discs; it conformed to the general pattern for Delicious but was of a much smaller magnitude. Disc size effects on water potential were absent. At the higher stress, differences in water potential for the two disc sizes were absent after 4·0 hr had elapsed. With larger discs no significant differences in water potential occurred with the different equilibration times. However, for the small discs there were some fluctuations in potentials and it appears that there were contamination errors throughout the equilibration period. These errors probably arose from exudation of sap which had a lower density than that of the solutions concerned, although uptake of solutes by the leaf tissue may have also contributed to the total error.

For Granny Smith, no differences in water potential were noted for either disc size with the equilibration times employed. However, disc size differences were evident, with the larger discs having lower potentials than the smaller discs. This again was attributed to the exudation of sap of a lower density than that of the solutions concerned.

For all three varieties the relative magnitude of water potential in relation to disc size appears to alter with the stress level. The effect of solute exudation from the leaves on water potential recorded depends on the density of the sucrose solution, the density of the cell sap exudate and the amount of exudation which has occurred. Where the sucrose solution has the same density as the cell sap exudate, exudation will produce a rise in density at concentrations below this, in the series, and a fall in density at concentrations above, therefore altering the null point (Gaff and Carr 1964). On this basis, therefore, exudation tends to decrease water potential at the low stress levels, and with the smaller discs, where exudation is greater, this would tend to produce measured potentials less than those for larger discs. At the higher stress conditions, the converse will apply. However, at the lower stress when water exchange between solution and leaf is small this effect of exudation may be accentuated. These arguments seem to be supported by data in Figures 1–3.

Errors due to contamination according to Knipling (1967) are likely to be larger for woody species, where relatively small changes in relative water content occur per unit change in water potential. This is because final contamination errors depend on the extent to which osmotic water gain or loss from the leaf tissues compensates for the contamination density changes during complete equilibration.

Therefore on the data available for the apple tree 4 hr was chosen as the equilibration time, and large discs rather than the smaller ones were chosen for further studies. These two precautions will minimize the errors likely to be encountered with apple leaf tissue.

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