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# PLANT WATER STATUS OF APPLE TREES AND ITS MEASUREMENT IN THE FIELD. 4. STOMATAL APERTURE, DETERMINED BY INFILTRATION SCORING, AS AN INDEX OF LEAF WATER POTENTIAL

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SUMMARY

**Stomatal aperture has been used by a number of workers as a physiological indicator of plant water status. With apples, early work shows that stomatal aperture may provide a useful and sensitive measure of plant responses to applied water and the onset of stress conditions.**

**Apertures were assessed in the field with an infiltration technique using liquid medicinal paraffin in preference to a diffusion porometer, which presented construction difficulties. Infiltration scores at almost complete stomatal closure (which was assessed microscopically for three apple varieties) were recorded as an approximation of permanent wilting point. Values for the three varieties differed, this being attributed to the relative pubescence of the ventral leaf surfaces.**

**The relationship between stomatal aperture and leaf water potential was established for three apple varieties, since all plant water status measurements should be related back to water potential. The scoring technique for stomatal aperture was somewhat less accurate than the dye method for assessing leaf water potential; however, the technique was faster and more sensitive than the relative water content method.**

**Problems of reduced light intensity, increased wind speed, time of day for testing and the evaporative environment are discussed in relation to the use of infiltration scoring for stomatal aperture of apple trees in the Stanthorpe district of south-eastern Queensland.**

**It appears that while infiltration scoring for apertures may be somewhat less accurate, the fact that stomatal aperture responds quickly to the onset of stress may partly compensate for this inaccuracy, and makes the method suitable for field irrigation indexing.**

## I. INTRODUCTION

As early as 1930 Magness and Furr, working on stomatal behaviour of apple leaves, showed that the measurement of stomatal aperture provides a useful tool for observing a response to applied water. They found that stomata opened with light in the morning, and with decreasing soil moisture supply this morning

phase of opening was shortened to 2 hr. During the third hour after sunrise, a pronounced reduction in stomatal aperture was noted, and finally when permanent wilting occurred, stomata were open for a short period very early in the morning and then remained closed until next day. Oppenheimer and Elze (1941) reported similar behaviour in citrus.

Further studies by Magness, Degman and Furr (1935) showed that the first measurable effect of a reduced moisture supply on the apple tree was an earlier closing of stomata. These workers considered that stomatal aperture was a much more sensitive indicator of plant water deficits in the apple tree than fruit growth rate, which has since been used by a number of workers, including Till (1957). In view of the sensitivity of aperture to water deficits, it seemed likely that a close relationship may exist between stomatal aperture and water potential of apple leaves. This latter relationship is essential to the success of the technique as an index of plant water stress, and as such the relationship should be established at the outset of soil studies.

The first problem of assessing stomatal aperture was to find a satisfactory technique which could be developed for field use. Magness and Furr (1930) used direct microscopic examination of epidermal strips of apple leaf tissue. However, this technique is not suitable for field use. Portable porometers and infiltration methods are obviously more suitable for field application, but for the orchardist infiltration techniques are probably better.

Apple leaves which have stomata only on the lower surface presented difficulties when viscous air flow porometers after Alvim (1965) were considered. The use of diffusion porometers originally designed by Wallihan (1964) had to be deferred because of construction difficulties.

An infiltration technique was then developed, using kerosene and later paraffin. The time for infiltration of the liquid was recorded and a scoring technique was developed. Paraffin was the more useful liquid, since its slower penetration increased the accuracy of timing rapid infiltration, when stomata were fully open.

In this paper a description of the scoring technique developed is given, and the relationship between stomatal aperture and leaf water potential is presented to evaluate the usefulness of the former for estimating water potential.

An additional section is included for irrigation studies, where it was essential to obtain an infiltration score at almost complete stomatal closure which approximated permanent wilting point. This was done microscopically and with infiltration scoring.

## II. MATERIALS AND METHODS

*General method.*—Liquid medicinal paraffin was used as the test liquid. In the field a leaf was taken from the plant, or left attached. One drop of paraffin was applied to the ventral surface with an eyedropper pipette, and the time taken for a confluent infiltration "grease ring" to form around the edge of the paraffin drop was recorded. Initially it was necessary to record the time in seconds with a stop watch, but with practice it was possible to count seconds accurately without the watch. In most cases it was easier to remove the leaf from the tree, apply the drop to the ventral surface, hold the leaf up to the light with the ventral surface uppermost and observe infiltration by examining the lower dorsal surface.

Exposed, fully expanded, terminal spur leaves, 5 ft above ground level, were selected from each tree. All readings were made approximately 3 hr after sunrise. When cloud cover was heavy, readings were deferred because of the effect of reduced light intensity on stomatal aperture.

*Determination of near stomatal closure.*—One group of five leaves from each of eight replicate trees of each of three varieties was sampled, brought to the laboratory, and the groups were placed in beakers of water illuminated by incandescent and fluorescent lamps, whose intensity of illumination approached that of full daylight. The three apple varieties were Delicious, Jonathan and Granny Smith. Five leaves at a time were removed from the light source and allowed to dry out in the laboratory. At 5 min intervals, strips of epidermal tissue removed from the leaves and mounted dry on a slide were examined under the microscope. Initially, all stomata were fully open and then later they began to close, probably as a result of both water loss and removal from the light, and produced increased infiltration values. At near closure of stomata as observed microscopically, infiltration scores were made on four leaves with paraffin. Near closure was determined by observation of two microscopic fields on each epidermal strip and was defined as that condition in which no stomata were seen open. The eight groups of five leaves were recorded in a similar fashion for each variety.

*Relationship between stomatal aperture and leaf water potential.*—For each of three apple varieties (Delicious, Jonathan and Granny Smith), four replicates of three trees were used to examine the relationship between stomatal aperture and leaf water potential. Samples taken on a number of occasions provided a range of stress values. Leaf water potential was measured with the dye technique (Chapman 1970*a*). Four leaves on each tree were scored for stomatal aperture and the mean for three trees provided the replicate value. From the data, regression lines were fitted, relating water potential to stomatal aperture, scored with paraffin, for each variety.

## III. RESULTS

*Almost complete stomatal closure.*—Table 1 shows infiltration scores for paraffin at almost complete stomatal closure for three apple varieties.

**TABLE 1**

INFILTRATION SCORE WITH PARAFFIN FOR THREE APPLE VARIETIES AT NEAR STOMATAL CLOSURE

| Variety | Infiltration Score (sec) | L.S.D. | |
|---|---|---|---|
| | | 5% | 1% |
| Delicious .. | 14·25 | | |
| Jonathan .. | 19·44** | 2·71 | 3·76 |
| Granny Smith .. | 13·40 | | |

Paraffin penetrated the stomata of the Delicious and Granny Smith varieties much more quickly than those of the Jonathans at near closure. Rates of infiltration were similar for the Delicious and Granny Smith varieties.

*Stomatal aperture and leaf water potential.*—Figures 1-3 present data on the relationship between stomatal apertures and leaf water potential for the three apple varieties.

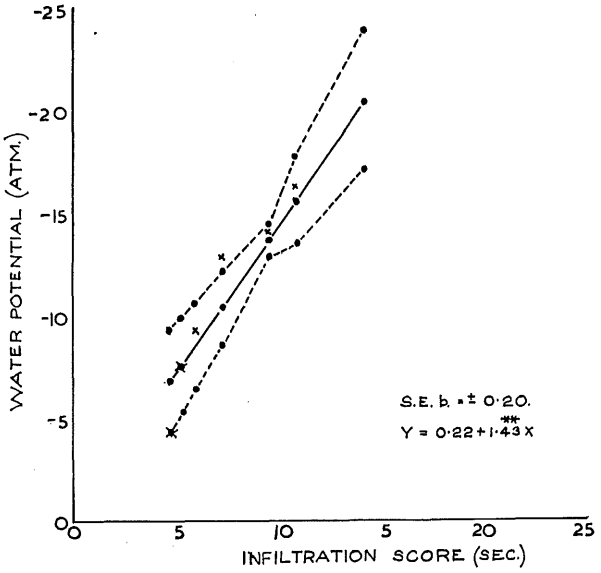


Fig. 1.—Relationship between water potential and paraffin infiltration score for Delicious.

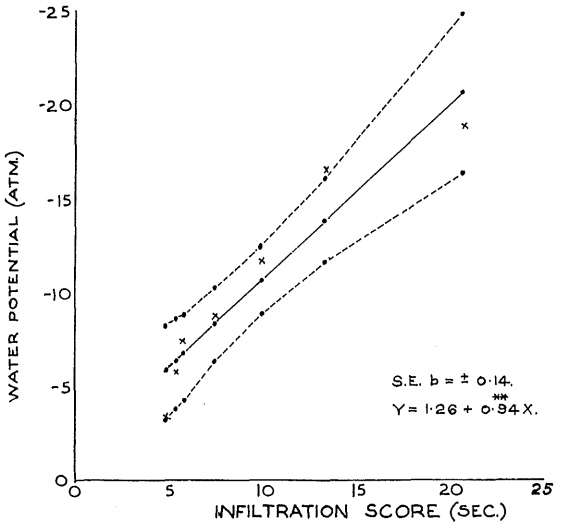


Fig. 2.—Relationship between water potential and paraffin infiltration score for Jonathan.

The regressions were highly significant for all varieties and regression equations are shown. Regression coefficients (b values) for the three varieties were not significantly different, but there were differences between the positions of the lines. Fiducial limits are presented at $P = 0{\cdot}05$ on each of three varieties, and show the limits in which the true relationship is likely to lie.

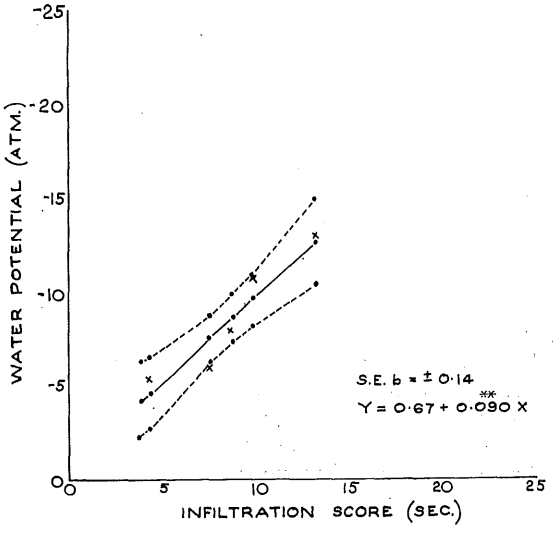


Fig. 3.—Relationship between water potential and paraffin infiltration score for Granny Smith.

## IV. DISCUSSION

At almost complete stomatal closure, the slower infiltration rate of paraffin with the Jonathan variety was attributed to the more pubescent ventral leaf surface of this variety. This pubescence retarded the access of the viscous paraffin to the epidermal surface of the leaf, and consequently increased score values.

Some care is therefore needed with leaves of different varieties where paraffin is used as the liquid for assessing stomatal aperture.

Stomatal aperture determined by infiltration scoring with paraffin is a rapid technique for field stress measurement and should be suitable for irrigation indexing. The scoring technique developed is somewhat less accurate than the dye technique (Chapman 1970*b*) for the field measurement of plant water stress. However, the infiltration technique was faster and more sensitive than the relative water content method, tested in the course of these studies. This, together with its simplicity, makes infiltration scoring suitable for use in the field by an orchardist.

A number of workers, including Oppenheimer and Elze (1941), Shmueli (1953) and Alvim (1960), have used infiltration scoring techniques as physiological indicators of plant water stress. However, few workers have related stomatal aperture to the water potential of the leaves concerned. Such a relationship should be established at the outset of such studies. This is because plant water stress is best measured by water potential (Kozlowski 1965; Kramer 1963) and any other measurement should be related back to this parameter.

Leaf position, leaf age, exposure and height were standardized at the outset, since water potentials were found to vary with age, position and height of insertion in earlier studies (Chapman 1970*c*), and so presumably will stomatal aperture. With regard to exposure, this effect was not evaluated in earlier studies, so fully exposed leaves were sampled to standardize procedures and eliminate any effects of variable exposure upon stomatal aperture.

Some problems arise from the direct effects of reduced light intensity and increased wind speeds, which can reduce stomatal apertures (Pasternak personal communication). As the day proceeds, cumulative transpiration rates, wind speed and cloud cover may vary widely and exert an important influence over stomatal apertures. However, by selecting a constant time of day for scoring, the third hour after sunrise, cumulative transpiration levels were minimal and exerted only small effects on leaf water stress and hence stomatal aperture (unpublished work of the author). This period, 3 hr after sunrise, was predominantly influenced by soil moisture stress and not the evaporative environment. Cloud and wind which may markedly influence stomatal apertures are not serious problems in the Stanthorpe district of Queensland at the time of day selected for testing. Tests may be deferred on the odd occasions when cloud and wind conditions are unfavourable. From other studies made by the author it appears that leaves are more sensitive to changes in plant water stress than are fruits. This may explain the results of Magness, Degman and Furr (1935) who found that when an apple tree was subjected to water stress, fruit growth was one of the last processes to suffer, while stomatal aperture was the first to respond. Therefore, perhaps the lower accuracy of the infiltration technique for irrigation indexing will be partially compensated for by the rapid response of stomata to the onset of water stress.

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