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Measurement of plant water status in pineapple *(Ananas comosus* (L.) Merr. cv. Queensland Cayenne (clone 13))

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Summary

The paper describes techniques for the measurement of leaf water potential (ψ), leaf xylem pressure potential (P), relative water content (RWC), osmotic potential of xylem sap (π sap) and two techniques for the measurement of osmotic potential of cell sap (π leaf) in D leaves of pineapple.

A comparison of the Wescor C-52 hygrometer/psychrometer sample chambers automatically scanned to measure ψ and the pressure chamber to measure P, showed that P provides a slight overestimate of ψ.

If P is corrected for π sap, the relationship between ψ and P breaks down, since π sap varies with P.

The relationship between ψ and RWC is linearly closely correlated and preferred to P versus RWC.

A technique described for measuring π leaf, using a Wescor Osmometer is well correlated with π leaf as measured with a 16 channel automatic scanning Wescor dew point hygrometer using C.52 sample chambers.

INTRODUCTION

Pineapple, *Ananas comosus* (L.) Merr., has unusual anatomical and physiological modifications which enable the plant to survive extensive periods of water stress (Ekern 1965; Krauss 1948; Neales, Patterson and Hartney 1968). Sideris and Krauss (1928) report that pineapple plants are as hardy as cacti under drought conditions.

However, a number of workers who report pineapple growth responses to water application include Sideris and Krauss (1928), Py and Tisseau (1965), Huang and Lee (1969), Kadzimin (1975) and Aubert (pers. comm., 1978). In addition, Black (1962) reported that pineapples grown in south-east Queensland, a major production area, showed marked growth rate reduction during periods of low soil moisture. Coincidentally, Osmond (1975) points out that *Ananas* plants are most productive under irrigation with warm night temperatures.

While some of these papers identify the effects of applied water on plant growth, Kadzimin (1975) is the only work which attempts to relate plant growth to plant water status, and then only indirectly and for a part of the vegetative growth stage in pineapple.

To gain an understanding of the implications of plant water status on growth and floral initiation of pineapple, techniques for water status measurement have to be developed. This paper reports on techniques for measurement of leaf water potential (ψ), leaf xylem pressure potential (P), osmotic pressure (π) of both cell and xylem sap, and relative water content (RWC) of leaf tissues.

MATERIALS AND METHODS

Water relations measurements

Leaf water potential (ψ), leaf xylem pressure potential (P), osmotic potential (π), and relative water content (RWC) were measured.

Kadzimin (1975) used the dye technique of Shardakov (1953) as modified by Chapman (1970) to estimate total leaf water potential in pineapple, with mannitol as the osmoticum, and a 4 h equilibration time.

In the current work, we chose the pressure chamber unit after Scholander, Hammel, Hemmingsen and Bradstreet (1964) to measure leaf xylem pressure potential in pineapple because it offers a rapid result and can be readily adapted for field use.

The pressure chamber method was calibrated against ψ as measured with a 16 channel automatic scanning Wescor dew point hygrometer calibrated with a range of NaCl solutions. In addition, osmotic potential of the xylem sap (π sap), was measured during the calibration, with a Wescor osmometer, to comply with the suggestions of Ritchie and Hinckley (1975), where P measurements may require adjustment with respect to π sap.

Osmotic potential of the leaf (π leaf) was measured on each occasion along with ψ, P and πsap, using the Wescor osmometer or the Wescor C-52 unit. In addition, a comparison was made between the two techniques for measuring π leaf i.e. Wescor osmometer versus the automatically scanned C.52 sample chambers using different procedures.

RWC was determined concurrently with the above measurement using 8 mm diameter leaf disks, and a technique similar to that of Barrs and Weatherley (1962).

All measurements were made on Queensland Cayenne clone 13 pineapple plants, grown from crowns in a 50:50 sand/peat mixture in 9 L containers in a glasshouse for 2 months, and transferred to a controlled environment growth room (Gates 1970) for a further 2 months. Conditions in the room used were: 500μ/Em^2/S photosynthetic quantum flux for a 16 h day giving 824 J/cm^2/day of photosynthetically active radiation (400 to 700 nm); 32°C constant temperature; 50% RH=24 mb saturation deficit; 0.2 to 0.4 m/s directional windspeed. Carbon dioxide concentration was not controlled, but it was usually greater than 550 ng CO_2/m^3.

Initially, during the first two months in the growth room all plants were watered twice per week to field capacity. After this period some plants were all allowed to stress progressively for up to 12 weeks, to obtain the range in stress measurements required.

On each occasion when ψ, P, π sap, π leaf and RWC were measured, the D leaf (the longest most recently matured leaf; Sideris, Krauss and Young 1938) was the leaf sampled. Basal tissue of the D leaf (100 mm) was removed and the remainder immediately transferred to the pressure chamber (modified to accept a pineapple leaf) and P was measured.

Simultaneously, the basal 50 mm of leaf was cut off and snap frozen in a small polythene bag on dry ice for total acid determination at a later date. From the remaining 50 mm of leaf one 8 mm disk was cut and transferred to a Wescor C-52 sample chamber for ψ determination. Two further disks were cut and individually wrapped in polythene film (cling-wrap) and then in aluminium foil and snap frozen on dry ice. These latter disks upon thawing were transferred to a Wescor C-52 chamber or were crushed onto a filter paper disk and transferred to the Wescor osmometer for a π leaf determination.

Four other 8 mm disks were cut from the remaining leaf and after weighing were floated on distilled water in petri dishes for determination of RWC.

After 4 h, the disks were removed, blotted dry, reweighed and oven dried for 8 h at 70°C. They were then weighed again and RWC was calculated.

Values of ψ and π (bars) were determined from the chart recorder attached to the scanning Wescor dew point hygrometer by interpolation of calibration curves of microvolts vs. potential in bars for individual chambers.

All work was conducted in a constant temperature room at 25°C.

Additional technique details

The pressure chamber head used for measuring P on the pineapple leaves is described in Figure 1. This head is attached to a standard style chamber 280 mm high by 110 mm in diameter.

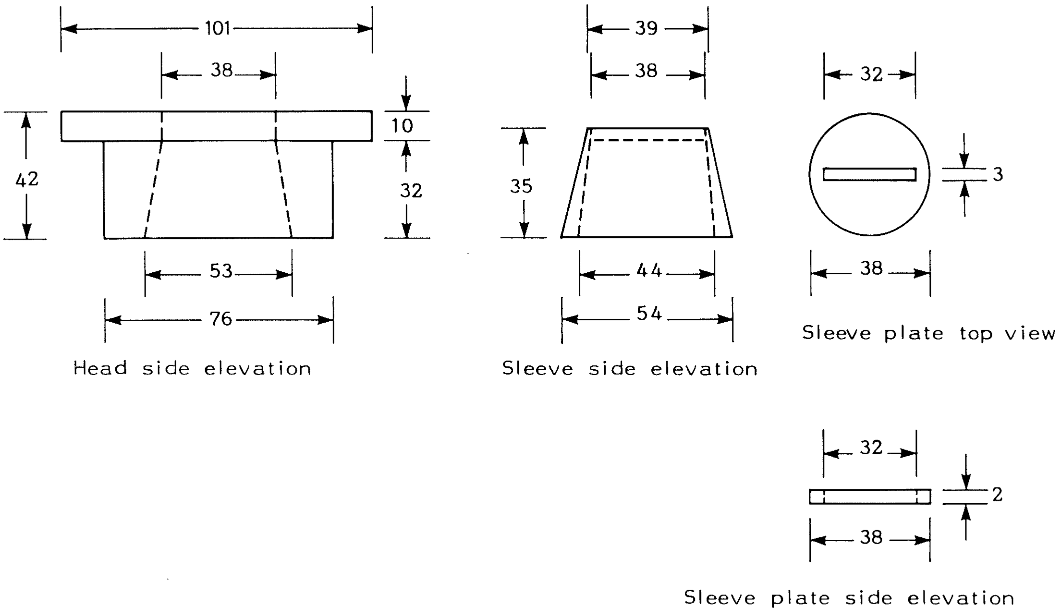

Figure 1. Details of modifications to the top plate of the pressure chamber unit to suit medium sized pineapple leaves (dimensions in mm).

Before applying pressure to the leaf in the pressure chamber the cut surface was blotted dry with filter paper.

A binocular microscope (x20) mounted over the cut surface of the leaf in the pressure chamber was used to determine the endpoint balance pressure. The endpoint was taken as that pressure where bubbling of sap occurred from the xylem tissue over a length of 10 mm along the cut surface.

The two surfaces of the rubber stopper which hold the pineapple leaf in place were coated with a very light covering of high pressure silicone grease to give a better seal against gas loss from the chamber.

A π sap sample was obtained at balance pressure by absorbing xylem sap onto a disc of filter paper and then quickly transferring the disc to the Wescor osmometer for determination of π sap in milliosmols.

When π leaf was determined using the Wescor osmometer, the leaf disc after thawing was placed on a filter paper disc and both enclosed in polythene cling-wrap film. The leaf disc was then pounded with a small blunt steel bar 2 mm in diameter by 100 mm long until the filter paper disc was quite moist. This disc was then quickly transferred to the Wescor osmometer for determination of π leaf.

The results of both π leaf and π sap were converted to bars as follows:

$$\pi \text{ (bars)} = 0.025159 \text{ mOs} - 0.1151$$

Data

The following data relationships were examined using linear regressions to establish correlation coefficients, the *y* intercept, slope and *t* values of *r*, namely:

1. Leaf water potential (ψ) vs. leaf xylem pressure potential (P).

2. Leaf water potential (ψ) vs. leaf xylem pressure potential (P) adjusted for leaf xylem sap osmotic potential (π sap) i.e. (−P+π sap).

3. Leaf xylem pressure potential (P) vs. leaf xylem sap osmotic potential (π sap).

4. Leaf water potential (ψ) vs. relative water content (RWC).

5. Leaf xylem pressure potential (P) vs. relative water content (RWC).

6. Leaf osmotic potential (π leaf) measured with the Wescor C-52 unit vs. leaf osmotic potential (π leaf) measured with the Wescor osmometer technique.

The minimum number of data sets for any comparison was 18 and in most cases exceeded 22.

RESULTS AND DISCUSSION

The relationship between P and leaf water potential (ψ) is shown in Figure 2. The linear relationship between P and ψ is close (r=+0.84, P=0.01). P overestimates the value of ψ as demonstrasted by the slope.

However, when P is adjusted for the osmotic potential of xylem sap (π sap), as has been suggested in the review by Ritchie and Hinckley (1975), the linear relationship with ψ breaks down (Figure 3). The reason for this is that πsap changes with P (Figure 4). As P varied between −10.73 and −16.35 bars, π sap varied from −2.07 to −8.38 bars. Boyer (1967) found this effect with yew and rhododendron, and Spomer and Langhans (1972) found even bigger changes with *Chrysanthemum moriflorum*, similar to those for pineapple.

Figure 5 presents the linear regression for ψ and RWC. The correlation coefficient (r=+0.93, P=0.01) is highly significant for this relationship. A 10% change in RWC corresponds to 2.82 bars of ψ.

For P xylem versus RWC (Figure 6) *r* is less (r=+0.83) and although highly significant (P=0.01) both the slope and the *y* intercept values differ considerably from ψ versus RWC. Also for a 10% change in RWC, P varies by 3.14 bars.

Therefore, if RWC is to be used to estimate water potential in the pineapple leaf, the relationship preferred is RWC versus ψ rather than RWC versus P. Kadzimin (1975) considered RWC to be an insensitive measure of water stress in pineapple, particularly when water deficits were not extremely severe. He arrived at this conclusion from a small number of measurements on different stressed plants where ψ was measured with the Shardakov technique, and RWC was measured in the same way as we have done.

Our data suggest that RWC is a reasonably sensitive measure of ψ, but we agree that under minimal stress situations, use of ψ is preferred.

Figure 7 presents data for π leaf determined, using the Wescor C-52 sample chambers ($\pi\ leaf_w$), versus π leaf determined with the Wescor osmometer ($\pi\ leaf_o$). Again, this linear relationship is a good fit as emphasised by the high value for *r* (r=+0.92, P=0.01). The advantage in using the osmometer is that readings can be obtained quickly, and the scanning Wescor C-52 can be kept free for ψ measurements. The osmometer readings can be made during the time taken for the Wescor C-52 sample chambers to come to equilibrium. The time taken to determine π leaf with the osmometer depends on the time required to freeze and thaw the leaf disks (30 to 60 min) and the time to obtain an osmometer reading on the filter paper disks, which is about 2 to 3 min.

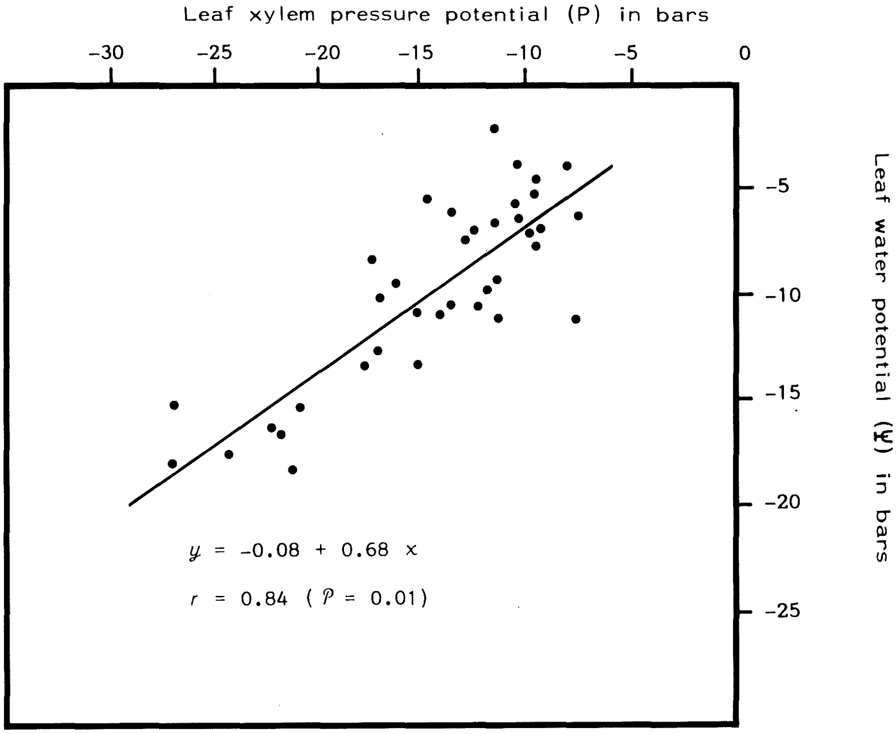

Figure 2. The relationship between leaf water potential (ψ) and leaf xylem pressure potential (P) in pineapple.

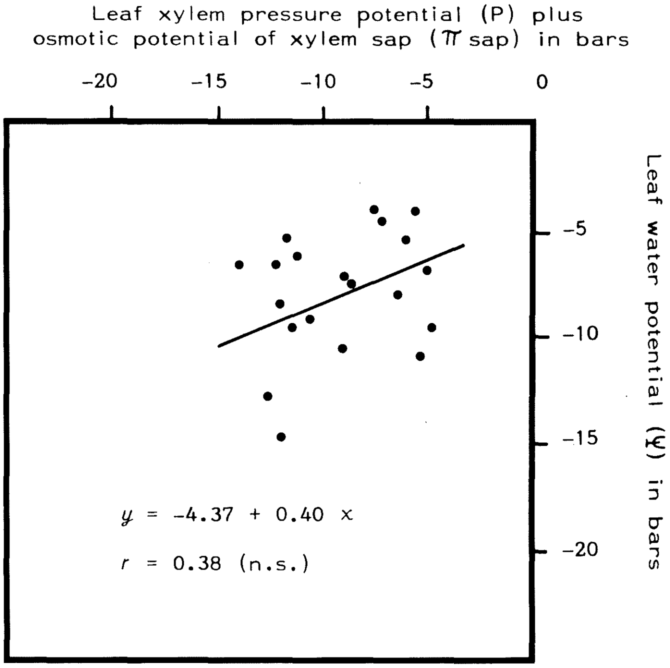

Figure 3. Leaf water potential (ψ) versus leaf pressure potential (P) corrected for osmotic potential of xylem sap (π sap) in pineapple.

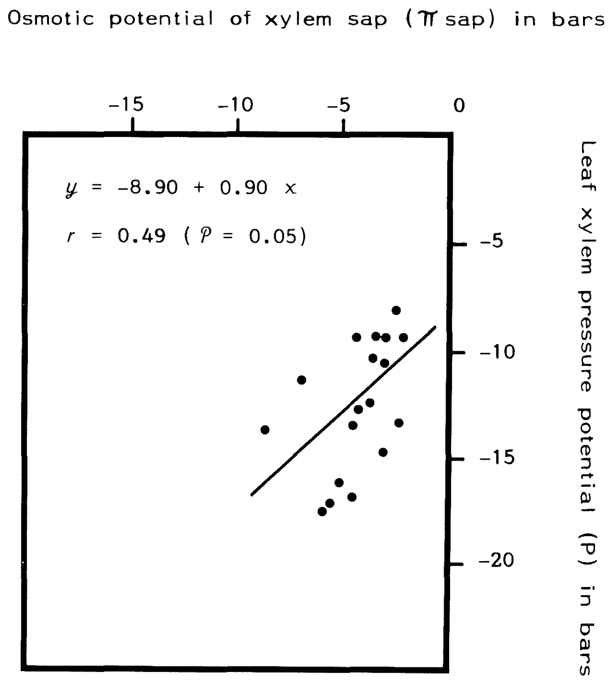

Figure 4. Variation in osmotic potential of xylem sap (π sap) with leaf xylem pressure potential (P) in pineapple.

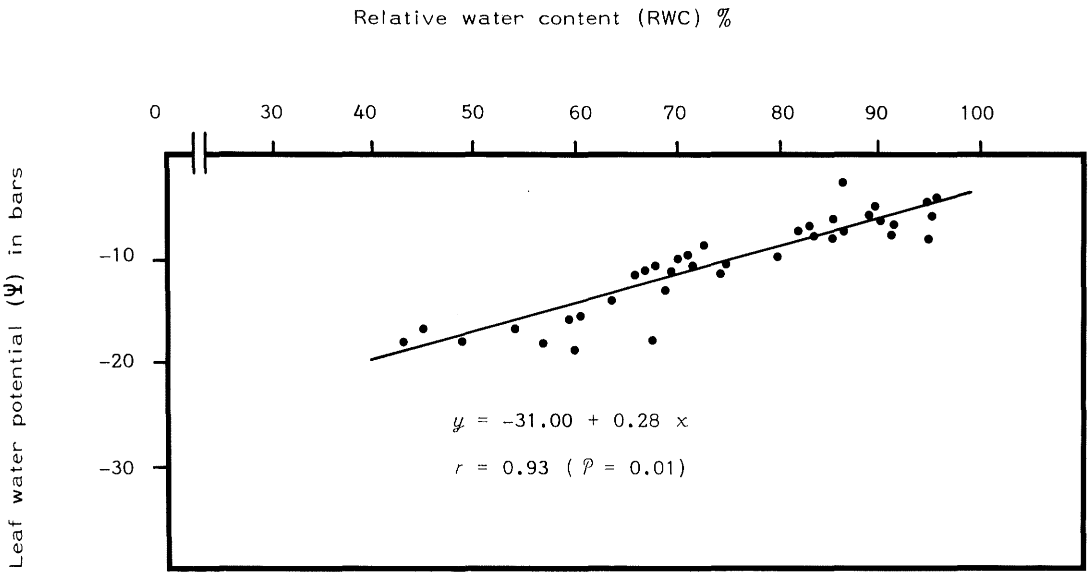

Figure 5. Relationship between leaf water potential (ψ) and relative water content (RWC) in pineapple leaves.

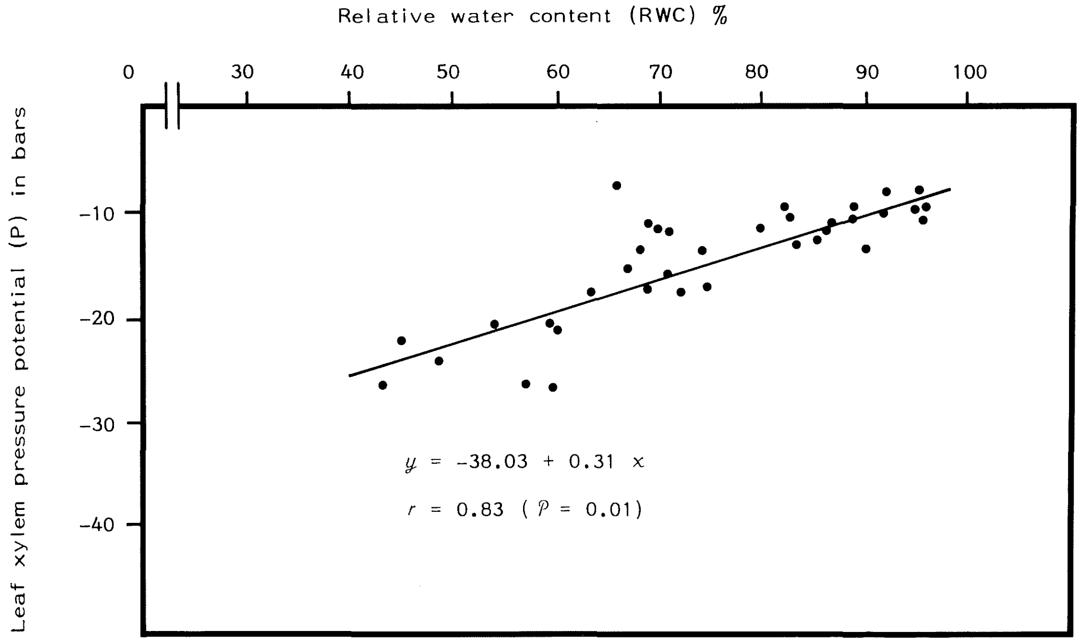

Figure 6. Leaf xylem pressure potential (P) versus relative water content (RWC) in pineapple leaves.

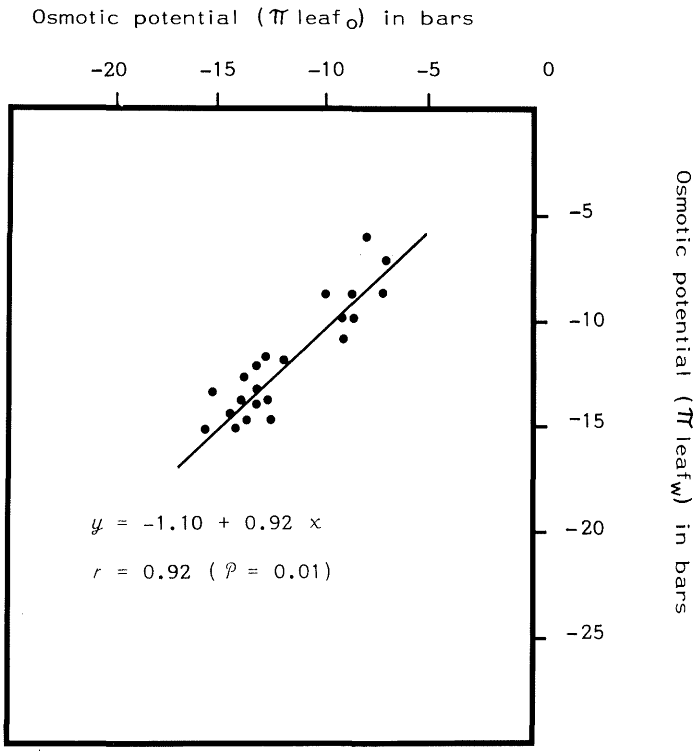

Figure 7. The osmotic potential of cell sap in pineapple as measured by two different techniques: π leafo, measured with Wescor osmometer; π leaf$_w$, measured with Wescor C.52 sample chamber.

CONCLUSIONS

Leaf xylem pressure potential (P) provides a reasonable measure of leaf water potential (ψ), but in general it overestimates the value of ψ. For comparative studies P would be quite acceptable.

Adjustment of P for osmotic potential of xylem sap (π sap) should not be attempted as π sap varies with P.

The relationship between ψ and RWC is linear and RWC is a reasonably sensitive measure of ψ, and is better than the relationship between P and RWC.

Both techniques developed for measuring osmotic potential of the leaf (π leaf) using either the Wescor osmometer or Wescor dew point hygrometer were rapid and highly correlated.

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