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Rapid Estimates of Relative Water Content

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ABSTRACT

Relative water content may be accurately estimated using the ratio of tissue fresh weight to tissue turgid weight, termed here relative tissue weight. That relative water content and relative tissue weight are linearly related is demonstrated algebraically. The mean value of r^2 for grapevine (*Vitis vinifera* L. cv. Shiraz) leaf tissue over eight separate sampling occasions was 0.993. Similarly high values were obtained for maize (*Zea mays* cv. Cornell M-3) (0.998) and apple (*Malus sylvestris* cv. Northern Spy) (0.997) using a range of leaf ages. The proposal by Downey and Miller (1971. Rapid measurements of relative turgidity in maize (*Zea mays* L.). New Phytol. 70: 555–560) that relative water content in maize may be estimated from water uptake was also investigated for grapevine leaves; this was found to be a less reliable estimate than that obtained with relative tissue weight. With either method, there is a need for calibration, although this could be achieved for relative tissue weight at least with only a few subsamples.

The relative water content technique, formerly known as relative turgidity, was originally described by Weatherley (9, 10) and has been widely accepted as a reproducible and meaningful index of plant water status (see literature cited by Barrs [1]).

Leaf tissue is most commonly used for RWC[3] determination, measured as follows. A composite sample of leaf discs is taken and the fresh weight is determined, followed by flotation on water for up to 4 hr. The turgid weight is then recorded, and the leaf tissue is subsequently oven-dried to a constant weight at about 85 C. RWC is calculated by

$$\text{RWC} = \frac{(\text{fresh weight} - \text{dry weight})}{(\text{turgid weight} - \text{dry weight})}$$

In other words, RWC is a ratio of the amount of water in the leaf tissue at sampling to that present when fully turgid. Although originally defined as a percentage, RWC is now commonly expressed in decimal form (5, 11), and this convention is followed here.

A major disadvantage of the RWC technique is the considerable time lag between sampling and obtaining the result. Further, the four weighing operations required (one for tare weight) are time-consuming and monotonous. These objections could be partly overcome if the oven-drying operation and subsequent weighing operation could be eliminated. However, this would require that RWC could be reliably estimated from the prior weights: sampling weight, turgid weight, or the difference, water uptake. Downey and Miller (3) have determined an empirical relationship between RWC and water uptake for maize, using small discs of constant area.

A second indirect estimate of RWC is introduced here. This index, termed relative tissue weight, is calculated as the ratio tissue fresh weight to turgid weight. The same ratio has been used before (4, 7, 8), but in all three instances it has been termed erroneously relative turgidity.

MATERIALS AND METHODS

Grapevines (*Vitis vinifera* L. cv. Shiraz) growing in an irrigation trial at the Griffith Viticultural Research Station, N.S.W. provided a range of leaf material of varying water status. Two replicates of eight treatments were sampled on each of 2 consecutive days on eight occasions throughout the growing season of 1968–69. The youngest fully expanded leaf was used. Four leaf samples were taken from each plot of four adjacent vines between 6 AM and 6:30 AM EST, sealed in plastic bags, and taken to the laboratory. There an entire disc of 8.4 cm diameter was cut from each leaf. Discs of this size were found to give considerably less variable RWC values than samples of 20 discs of diameter 0.8 cm, as recommended by Barrs (1); RWC values for the larger discs were, on the average, 1% lower. After fresh weight determination, the discs were floated in the sampling can in distilled water for 3 hr at room temperature (about 15 C) with no illumination. Following surface drying with absorbent paper toweling and turgid weight determination, the discs were oven-dried at 85 C overnight and reweighed.

Corn (*Zea mays* cv. Cornell M-3) was grown at Ellis Hollow, Ithaca, N.Y. during 1971 in waterproof boxes providing a range of soil moisture contents. RWC samples were taken during the ear-filling stage over a 4-day period using leaves of varying ages. The apple data were obtained from 6-month-old *Malus sylvestris* cv. Northern Spy seedlings. The plants were grown in a greenhouse at Ithaca and transferred to artificial illumination in the laboratory. RWC measurements were made on four plants as they passed through a drying and subsequent recovery period.

RWC estimates were made using portions of the lamina for

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[3] Abbreviations: RWC: relative water content; RTW: relative tissue weight.

corn and the entire leaf for apple. The tissue was floated for 4 hr to achieve turgidity and then oven-dried at 80 C for 24 hr. Since a constant leaf area was not used for apple and corn, it was not possible to check relationships of uptake with RWC.

THEORETICAL CONSIDERATIONS

The following parameters are defined for unit leaf area: s, the weight of water present in leaf tissue at sampling; t, the weight of water in leaf tissue when fully turgid; u, the weight of water taken up by tissue during flotation $(t - s)$; d, dry weight of tissue at sampling. Following Barrs and Weatherley (2), it is assumed that dry weight does not change appreciably during flotation for a short period. Weatherley's (10) definition of RWC in decimal form, is:

$$\text{RWC} = s/(t) = s/(s + u) \quad (1)$$

For s constant and u variable, the form of equation 1 indicates that the relationship between RWC and uptake is not linear as is suggested by Downey and Miller (3). The departure from linearity at low RWC presented by Downey and Miller is not as large as would be expected from considerations of equation 1. This fact, plus results to be subsequently presented for grapevine leaf tissue, suggest that a linear relationship is a good approximation to the RWC-uptake curve. For this to be so requires $(s + u)$ or t in equation 1 to be constant; then, algebraic manipulation produces the following linear relationships

$$\text{RWC} = 1.00 - u/t \quad (2)$$

The similarity of equation 2 to Downey and Miller's (3) equation (RWC = $0.995 - 0.0793u$) should be noted where $t = 1.00/.0793 \text{ mg cm}^{-2} = 12.62 \text{ mg cm}^{-2}$.

RWC may be alternatively predicted with the ratio of tissue fresh weight to tissue turgid weight, termed here relative tissue weight.

$$\text{RTW} = (s + d)/(t + d) \quad (3)$$

Hewlett and Kramer (6) claim that such a ratio is not related to the normally determined RWC; however, simple algebraic manipulation yields the following linear relationship between RWC and RTW:

$$\text{RWC} = (1.0 - 1.0/\beta) + \text{RTW}/\beta \quad (4)$$

where β is defined as:

$$\beta = t/(t + d) \quad (5)$$

That is, β equals the ratio of the weight of water present at turgidity to the total tissue turgid weight. Note that the form of equation 4 leads to the fact that for RWC equals 1.0, then RTW = 1.0. Since dry weight is a relatively minor component of leaf tissue turgid weight, β is relatively invariant. Equations 2 and 4 indicate that relationships between RWC and uptake, and RWC and RTW, are respectively unique for each value of t and β.

RESULTS

Grapevine Leaf Tissue. For each 2 consecutive days' samples, a total of 32 points was available for regression analysis. Results of correlations of RWC on uptake and RTW are presented in Tables I and II, respectively. All values of r^2, slope, and intercept were significant at 0.1%. Correlation of RWC with uptake was lower than with RTW on each occasion.

Comparisons of the equations in Tables I and II with equations 2 and 4 yield estimates of the constants t and β for each sampling date. In fact, β may be separately estimated from the slope and intercept, although the two values agree within 0.5%. Figure 1 shows the seasonal course of t and β values calculated from the equations. Separate estimates t' and β' were also calculated as means for each sampling date, using the raw data. Especially early in the season, t' is lower than t, while β' and β are generally similar. The coefficient of variation was calculated for each estimate over the season; appropriate values are t, 10.8%; t', 6.9%; β, 3.2%; and β', 3.2%.

The gradual decline in both t and β over the season is due to an increasing proportion of dry weight in the leaf tissue. Figure 2 shows a plot of the proportions of s, u, and d to tissue turgid weight throughout the season. Mean values of t and β were also calculated for each sampling occasion for treatments receiving the maximum and minimum amounts of irrigation. The results, not presented, indicate no consistent effect of previous or current water status on either t' or β'.

Corn and Apple Leaf Tissue. Twenty five samples of corn leaves were taken for RWC and RTW determinations, covering the range of RWC from 0.77 to 1.00. The linear regression of RWC on RTW was

$$\text{RWC} = -0.357 + 1.355 \text{ RTW}, r^2 = 0.998 \ (\beta = 0.738)$$

Similarly, 34 points were available for apple leaf tissue, spanning the range from 0.78 to 0.98 RWC. The fitted linear regression was of the form,

$$\text{RWC} = -0.465 + 1.466 \text{ RTW}, r^2 = 0.997 \ (\beta = 0.682)$$

Table I. *Linear Regressions of RWC on Uptake* (*mg* cm^{-2})

Sampling Date	r^2	Regression Equation
Nov. 8–9, 1968	0.966	RWC = 1.014 − 0.061u
Nov. 13–14, 1968	0.924	RWC = 0.998 − 0.049u
Nov. 20–21, 1968	0.888	RWC = 0.985 − 0.046u
Nov. 25–26, 1968	0.928	RWC = 1.004 − 0.050u
Dec. 11–12, 1968	0.976	RWC = 0.994 − 0.050u
Dec. 18–19, 1968	0.989	RWC = 1.003 − 0.055u
Jan. 2–3, 1969	0.963	RWC = 1.005 − 0.052u
Jan. 21–22, 1969	0.970	RWC = 0.997 − 0.062u

Table II. *Linear Regressions of RWC on RTW*

Sampling Date	r^2	Regression Equation
Nov. 8–9, 1968	0.998	RWC = −0.325 + 1.32 RTW
Nov. 13–14, 1968	0.997	RWC = −0.266 + 1.27 RTW
Nov. 20–21, 1968	0.975	RWC = −0.230 + 1.23 RTW
Nov. 25–26, 1968	0.971	RWC = −0.285 + 1.29 RTW
Dec. 11–12, 1968	0.999	RWC = −0.307 + 1.31 RTW
Dec. 18–19, 1968	0.999	RWC = −0.337 + 1.34 RTW
Jan. 2–3, 1969	0.998	RWC = −0.299 + 1.30 RTW
Jan. 21–22, 1969	0.998	RWC = −0.357 + 1.36 RTW

DISCUSSION

One of the prime disadvantages of using RWC to assess plant water status is the considerable time requirement involved. The two methods of estimating RWC evaluated here obviate the need for oven drying and oven-dry weight determination. One method, the linear correlation of RWC with water uptake during flotation, was proposed as an empirical linear relationship by Downey and Miller (3). This relationship, when rigorously analyzed, indicates that a truly linear

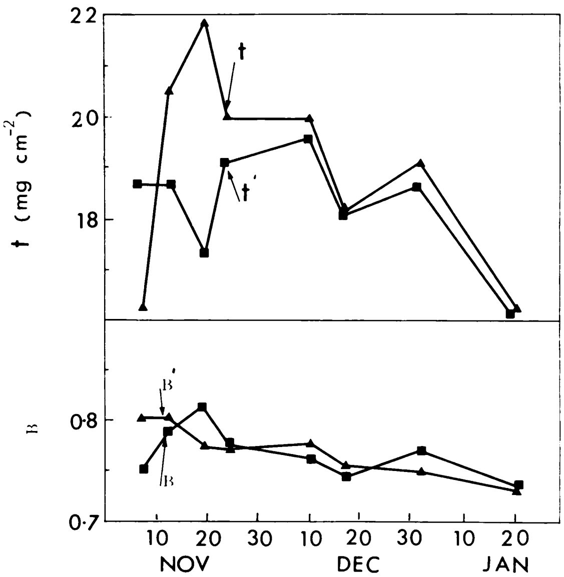

FIG. 1. Seasonal variation in t and β for grapevine leaf tissue, determined from linear regression equations and from raw data (t', β').

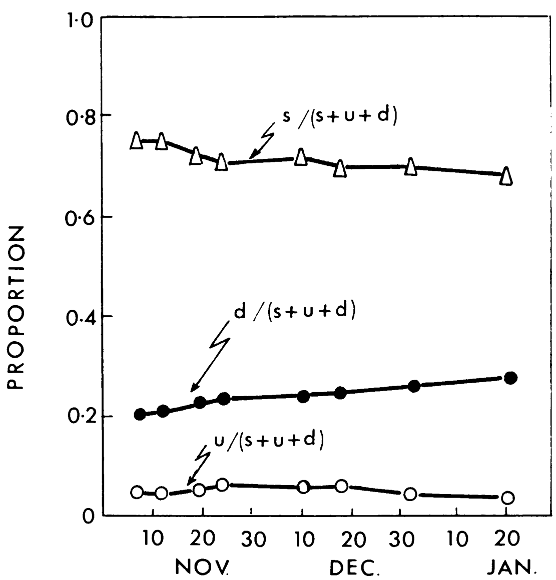

FIG. 2. Seasonal variation in the proportions of sampling water content (s), dry matter (d) and uptake (u). Each point in the mean of 32 samples.

correlation is anticipated only when the turgid water content per unit area (t) is constant. This parameter was demonstrated to be subject to seasonal variation for grapevine leaf tissue of uniform morphological age, although there was no apparent effect of prior or current moisture stress.

The second, and more precise method, is based on correlation of RWC with the ratio termed relative tissue weight. This ratio, calculated as tissue fresh weight/tissue turgid weight is linearly related to RWC. Data for grapevine leaf tissue of uniform morphological age and varying moisture status supported this contention with r^2 values ranging from 0.971 to 0.999 from eight sampling occasions (mean value 0.993). Similarly high correlation coefficients were found for apple and corn. The relationship between RWC and RTW contains a constant (β) equal to the ratio of turgid water content/turgid tissue weight. This parameter showed variation between species tested but was relatively constant within species. For grapevine leaf tissue, there was a gradual decrease in β over the growing season, though the change was less than for t.

A further advantage of using RTW to estimate RWC is that β is a dimensionless ratio and hence the relationship between the two is unaffected by variation in sample size. This may occur, for instance, when small leaf discs compose the subsample. An error in counting discs would introduce a proportional error if RWC was estimated from water uptake.

As demonstrated algebraically, the precision of using either uptake or RTW to estimate RWC depends upon sample to sample variation in the proportions of sampling water, turgid water, and dry weight in turgid tissue weight. Both methods require calibration, in that coefficients t and β need to be determined before RWC can be calculated. In view of the low variation in β reported here within three species, this parameter could likely be estimated from a small number of subsamples for most plants.

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