Leaf thickness to predict plant water status

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Abstract

Plant-based techniques to measure crop water status offer advantages over soil-based methods. The objective of this study was to quantify the relationship between leaf thickness measurements, as a promising plant-based technique, with leaf relative water content (RWC) and assess the model across different species and leaf positions. The relationship between RWC and relative thickness (RT) was determined on corn (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), soybean (*Glycine max* (L.) Merr.), and fava bean (*Vicia faba* L.). RWC was calculated as measured leaf water content/leaf water content at full turgor, and RT as measured leaf thickness/leaf thickness at full turgor. Two leaves from the top, middle, and bottom of five plants of each species were collected at 60 days of age. Leaf samples brought to full turgor were left to dehydrate in a lab. Leaf thickness was measured using a magnetic field sensor and water content using weight loss. The RWC-RT relationship showed a distinct breakpoint, which we hypothesize coincides with the turgor loss point. Linear piecewise modelling was used to regress RWC versus RT, resulted in models explaining 86-97% of the variations. The precision was improved by including leaf position on the plant in the model. The piecewise model parameters were related to salt tolerance of the species, which is also an indicator of drought resistance. Generally, the species with greater drought and salinity tolerance had a larger RT at the breakpoint.

Keywords: leaf thickness sensor; water stress; plant water status; drought tolerance; leaf structure; piecewise model.

1 Symbols and abbreviations

2 *Nomenclature*

a	The intercept of the piecewise linear model of leaf relative water content versus leaf relative thickness for where relative thickness is equal or smaller than the breakpoint
b_1	The slope of the piecewise linear model of leaf relative water content versus leaf relative thickness for where relative thickness is greater than the breakpoint
b_2	The slope of the piecewise linear model of leaf relative water content versus leaf relative thickness for where relative thickness is equal or smaller than the breakpoint
c	The leaf relative thickness at the breakpoint of the piecewise linear model of leaf relative water content versus leaf relative thickness
EC	Soil electrical conductivity
TLP	Turgor loss point
RT	Leaf relative thickness
RWC	Leaf relative water content

3

4 **1. Introduction**

5 Improvements in water use efficiency can be achieved by precision irrigation timing to avoid
6 early or late irrigation, which may lead to water or yield loss. Ideally continuous measurements
7 of plant water status would be the optimum means to determine the best irrigation timing.
8 Common methods of plant water status estimation for practical applications tend to range from
9 the simple visual wilting approach to measurement intensive evapotranspiration models, or soil
10 moisture measurements (Jones, 2004). Irrigation scheduling based on plant measurements merits
11 consideration as an alternative to soil measurements and/or water balance computations. There
12 are plant-based alternatives for estimating plant water needs, such as pressure chamber,
13 psychrometer, thermal sensing, and sap-flow sensors (Jones, 2004). We propose a simple
14 measurement method based on leaf thickness as suggested by Seelig, Stoner, and Linden (2011).

1 Instead of improving soil moisture measurement methods or evapotranspiration models, we
2 suggest it may be better if the critical water stress points at which irrigation should start could be
3 directly measured on the crops grown. Plant-based approaches may offer a more reliable plant
4 water status estimation by reducing the need for complex calculations and data sets associated
5 with soil-plant-atmosphere water relationship (Jones, 2004; Kramer & Boyer, 1995). In this
6 study, we set out to determine if leaf thickness could be a reliable indicator of water status of a
7 plant.

8 Bachmann (1922) was the first to report that leaf thickness decreases upon dehydration and
9 increases upon rehydration. Subsequently, Meidner (1952) determined that there is a strong
10 correlation between leaf thickness and relative water content (RWC). Búrquez (1987) reported a
11 strong correlation between RWC and leaf thickness in *Brassica napus* L., *Mirabilis jalapa* L.,
12 *Phaseolus vulgaris* L., and *Impatiens parviflora* DC. These researchers measured leaf thickness
13 using different types of calipers and micrometers. Meidner (1952) used a gear-wheel micrometer
14 and Búrquez (1987) used a spring-loaded gear-wheel. These devices were bulky and hard to
15 automate to enable continuous plant water stress sensing. Transducer-based techniques were
16 explored to enable automated leaf thickness measurement (Dongsheng, Manxi, Huijuan, &
17 Ziqian, 2007; Li & Song, 2009; Malone, 1993; Marengo, Antezana-Vera, & Nascimento, 2009;
18 McBurney, 1992; Rozema, Arp, Diggelen, Kok, & Letschert, 1987; Syvertsen & Levy, 1982;
19 Vile et al., 2005; White & Montes-R, 2005). Most of these sensors are relatively bulky linear
20 variable displacement transducers (LVDT).

21 Sharon and Bravdo (1996) and Seelig et al. (2011) developed tiny leaf thickness sensors to
22 optimize irrigation scheduling. Sharon and Bravdo (1996) compared irrigation scheduling using
23 continuous leaf thickness monitoring with four conventional drip irrigation regimes based on
24 timetables and water depletion. In this 4-year study, the sensor-based drip irrigation treatment
25 resulted in the highest yield and greatest water use efficiency of grapefruit cv. Oroblanco (*Citrus*
26 *x paradisi* Macfad). Similarly, Seelig et al. (2011) were able to improve water use efficiency of
27 irrigated cowpea 25 to 45% by an automated irrigation system based on a leaf thickness sensor
28 compared with timed irrigation scheduling. These studies show that irrigation scheduling based
29 on the automated leaf thickness sensing has the potential to improve water use efficiency and
30 conserve irrigation water.

1 The relationship between leaf thickness and RWC depends on plant and leaf characteristics and
2 is affected by environmental variables. Leaf thickness is determined by plant anatomy, including
3 the number, size, and arrangement of leaf cells that differ among species (Carpenter & Smith,
4 1979; Nicotra et al., 2011; Taiz & Zeiger, 2006; Vogelmann, Bornman, & Yates, 1996). Giuliani
5 et al. (2013) have reported a significant difference in leaf anatomy among species of the genus
6 *Oryza*. There is a significant variation in the structure of leaf mesophyll even between leaves
7 located at different positions on the same plant (Eames & MacDaniels, 1925). The number and
8 arrangement of the palisade parenchyma, as well as the overall morphology of a leaf, vary
9 between species. They also depend on environmental variables such as light exposure,
10 temperature, age, and irrigation regimes (Abrams & Kubiske, 1990; Búrquez, 1987; Carpenter &
11 Smith, 1979; Gausman, Allen, Cardenas, & Richardson, 1970; Gausman, 1974; Hanba,
12 Miyazawa, & Terashima, 1999; Nicotra et al., 2011; Taiz & Zeiger, 2006).

13 Leaf thickness changes not only as a result of RWC fluctuations but also due to environmental
14 and physiological factors (Blum, 2011; Scoffoni, Vuong, Diep, Cochard, & Sack, 2014; Taiz &
15 Zeiger, 2006). Some of these are rapid. For example, leaf thickness shows a diurnal-nocturnal
16 dynamic. Under well-hydrated conditions, leaf thickness is almost constant during night hours
17 but decreases during the day (Búrquez, 1987; Meidner, 1952; Rozema et al., 1987; Seelig et al.,
18 2011; Syvertsen & Levy, 1982). Leaf thickness was negatively correlated with air temperature
19 and light and positively with ambient relative humidity (Búrquez, 1987; Rozema et al., 1987;
20 Syvertsen & Levy, 1982). These environmental factors affect leaf thickness variations through
21 their role in transpiration (Búrquez, 1987; Giuliani et al., 2013; Rozema et al., 1987). Vapour
22 loss and water supply as the discharge and charge sources of the leaf water content result in
23 varying leaf thickness (Búrquez, 1987). Soil salinity increases leaf shrinkage during the day and
24 the time of thickness recovery at night (Rozema et al., 1987). This may be explained by the water
25 shortage-induced condition which causes plants to reduce water uptake from a saline medium
26 (Blum, 2011; Parida & Das, 2005; Rozema et al., 1987). On the other hand, wounding, for
27 example by insects, causes rapid leaf swelling (Alarcon & Malone, 1994).

28 Leaf thickness has been shown to affect photosynthesis (Smith, Bell, & Shepherd, 1998; Taiz &
29 Zeiger, 2006). Varieties with thicker leaves show enhanced photosynthesis, a character trait used
30 by plant breeders to improve yields (Peng & Ismail, 2004; Takai et al., 2013). Physiological

changes that affect leaf thickness can have a long-lasting effect. Smith et al. (1998) found that a decrease in precipitation and an increase in leaf inclination were associated with thicker leaves. In addition, there is a positive correlation between leaf thickness and total daily light exposure (Abrams & Kubiske, 1990; Carpenter & Smith, 1981; Eames & MacDaniels, 1925; Nobel & Hartsock, 1981; Smith et al., 1998; Yun & Taylor, 1986). Nobel and Hartsock (1981) reported a positive correlation between leaf thickness and leaf age.

A number of researchers have worked on the relationship between leaf thickness and leaf structural and functional traits (Giuliani et al., 2013; Patakas & Noitsakis, 1997; Sack & Scoffoni, 2012; Scoffoni et al., 2014). Leaf thickness was positively correlated with leaf hydraulic conductivity (Scoffoni et al., 2014) and elastic modulus (Aasamaa, Niinemets, & Söber, 2005; Scoffoni et al., 2014). Drought results in cell wall hardening and larger elastic modulus which helps the tissue maintain turgor pressure and avoid water stress (Blum, 2011; Martinez, Silva, Ledent, & Pinto, 2007). Salinity decreased the epidermal and mesophyll thickness of mangrove *B. parviflora* leaves (Parida, Das, & Mittra, 2004). The tight relationship between leaf thickness and leaf water potential (ψ_{leaf}) was shown in several studies (Búrquez, 1987; McBurney, 1992; Neumann, Thurtell, Stevenson, & Beadle, 1974; Scoffoni et al., 2014; Syvertsen & Levy, 1982).

In summary, earlier work suggests leaf thickness measurement to be a feasible technique for estimation of plant water status. However, the earlier studies did not quantify the relationship between leaf thickness and RWC, which is often employed as a measure of plant water condition (Jones, 2007; Seelig, Wolter, & Schröder, 2015). In spite of this, Jones (2007) recommended use of RWC as a measure of plant water status for irrigation scheduling purposes. In practical applications (e.g. irrigation timing), using leaf thickness measurements as a means for estimation of plant water status requires a quantified relationship to determine when to irrigate.

In addition, as mentioned earlier, leaf thickness is a function of leaf structural traits which may vary by species and leaf position on the plant. Therefore, another question is whether species and leaf position on the plant influence the leaf thickness - leaf water content relationship.

Therefore, the objective of this study was to determine the relationship between RWC and leaf relative thickness (RT). The species were selected from dicotyledons and monocotyledons with

different drought tolerances to study their differences in the RWC-RT relationship. This study also considered the extent to which leaf location on a plant impacted the relationship.

2. Materials and methods

2.1. Plant cultivation

Corn (*Zea mays* L., hybrid DKC53-45), sorghum (*Sorghum bicolor* (L.) Moench, cv. AF7101), soybean (*Glycine max* (L.) Merr., cv. HS 2766), and fava bean (*Vicia faba* L., cv. Vroma) were grown under controlled conditions in a greenhouse at the Pennsylvania State University, University Park, Pennsylvania. Fifteen plants of each crop were grown in eight-litre pots filled with a loamy soil, under a mixture of natural and artificial light to generate a photoperiod of 15 hours, day/night temperatures of 27/18 °C, relative humidity of 60% ($\pm 20\%$), and were irrigated every three days to maintain field capacity – a set of environmental conditions favourable for all the crops grown to provide non-stress growing conditions. No fertilizer or pesticide was applied.

2.2. Leaf sampling

Sixty days after seeding, the pots were irrigated to saturation eight hours before collecting the leaves. Two leaves were selected from top, middle and bottom of five randomly selected plants of each species (five replications). The petioles of the cut leaves were immediately submerged in distilled water and the entire leaves and the container holding the water were enclosed in dark polyethylene bags. These bags were stored in the dark at 7 °C for eight hours in a refrigerator to ensure that leaves reached full turgidity (Búrquez, 1987).

Two 225 mm² samples were taken from the centre of a leaf (avoiding the edges and major veins) under lab conditions (25 °C and relative humidity of 40 $\pm$ 5%). One sample was used for continuous leaf thickness measurement and the other sample was used for continuous leaf weighing. This method was chosen because it was not possible to simultaneously weigh the sample with the sensor attached to it due to wiring attaching the sensor to the data logger. In addition, the aim was to not remove the leaf thickness sensor to prevent possible leaf damage, loss of data continuity, and spikes or drops in thickness measured due to repositioning the sensor. The preliminary data confirmed the made assumption for this method that the rate of water loss

over time was not significantly different for two leaf pieces dehydrating under the same conditions in the lab.

2.3. *Leaf thickness and water content measurement*

Leaf thickness was measured by a developed leaf thickness sensor using a magnetic field sensor and two permanent magnets that were fixed on a clear rigid clip (Fig. 1). One of the magnets and the magnetic field sensor were fixed on opposite sides of the clip. The voltage output of the magnetic field sensor varied based on the distance from this magnet enabling continuous leaf thickness measurement. The second magnet was fixed under the magnetic field sensor with the opposite polarity to the other magnet to reduce the offset magnetic field. This magnet helps to avoid sensor saturation for very small thicknesses while still allowing high resolution. Sensor calibration using paper sheets of known thickness showed a coefficient of determination of 0.99 with an accuracy of 4 µm and a precision of better than 1 µm. Sensor output was recorded at one-minute intervals using a DT85M data logger (DataTaker, Melbourne, Australia).

RWC was calculated as follows:

$$RWC = 100 \times \frac{\text{actual leaf weight} - \text{dry weight}}{\text{turgid leaf weight} - \text{dry weight}} \quad (1)$$

where actual leaf weight is the weight of the sample placed on the balance, turgid leaf weight is the weight of the leaf sample at full turgor, and dry weight is the weight of the leaf sample dried in an oven at 60 °C for 24 hours.

At the start of a measurement period, two pieces were taken from a leaf: one piece was inserted in the thickness sensor for leaf thickness measurement and the other piece was put on a balance for weighing under the same environmental conditions.

The samples for RWC measurements were weighed every 5 minutes from full turgidity to 60% RWC. Since the time interval between RWC measurements was greater than that between leaf thickness measurements, the missing RWC data were calculated by linear interpolation. The result was one-by-one RWC and leaf thickness data at intervals of 1 minute.

Leaf thickness among leaves varied and therefore RT was used, which was calculated as follows:

$$RT = \text{leaf thickness measured} / \text{leaf thickness at full turgor} \quad (2)$$

2.4. Statistical analysis

Minitab 16.0 was employed for ANOVA while the nonlinear procedure of SAS 9.3 was used for piecewise linear regression of RWC-RT as described by Ryan and Porth (2007). The following equation shows the applied piecewise parametric model utilized to represent the RWC-RT relationship:

$$RWC = \begin{cases} a + c(b_2 - b_1) + b_1 RT, & RT > c \quad (\text{phase 1}) \\ a + b_2 RT, & RT \leq c \quad (\text{phase 2}) \end{cases} \quad (3)$$

where a is the intercept with the y-axis (RWC) of the regression line below the breakpoint, c is the RT at the breakpoint, b_1 is the slope of the line at $RT > c$, and b_2 is the slope of the line at $RT \leq c$. Parameter estimates are shown in Tables 1 and 2.

3. Results and discussion

3.1. RWC-RT relationship

The RWC-RT relationship of each species averaged by leaf location is shown in Fig. 2. The RWC-RT curve showed a sudden change in slope at a point we refer to as the “breakpoint”. To reflect this non-linear behaviour and the distinct breakpoint, piecewise linear regression modelling was used to regress RWC vs. RT (equation 3).

The slope of the regression line at the early stages of dehydration was much larger than the slope of the line at the later stages of dehydration (Fig. 2). This means that RT changed much with a given change in RWC at early stages of dehydration, while RT changed little with a given change in RWC at later stages of dehydration. This result is in agreement with the results of Sancho-Knapik, Alvarez-Arenas, Peguero-Pina, Fernández, and Gil-Pelegrín (2011) on *Quercus muehlenbergii* Engelm. They reported that a breakpoint occurred at the turgor loss point (TLP) in the relationship of RWC and leaf thickness such that the changes of RWC for a given decrement in leaf thickness from full turgor to TLP was small in comparison with that below TLP. TLP can be considered as a critical water stress since it represents the point that leaf turgor pressure is equal to zero bar and the tissue transitions from a turgid to a flaccid state (Taiz & Zeiger, 2006).

This breakpoint has also been observed in the relationship between RT and leaf water potential in other studies (Begg et al., 1964; Lenz, Wright, & Westoby, 2006; Neumann et al., 1974; Sanchez-Diaz & Kramer, 1971; Scoffoni et al., 2014; Stoyanov, 2005). Neumann et. al. (1974) and Scoffoni et. al. (2014) reported a distinct breakpoint in the dynamics of leaf thickness versus ψ_{leaf} which occurred at TLP. These results imply that the observed breakpoint in our study occurred at TLP and represents a critical water status threshold.

Seelig et al. (2015) stated that the leaf thickness seems to change more rapidly compared to RWC in the early stage of leaf dehydration, similar to the observed contrast between the early and late slopes of the RWC-RT relationship in the leaf dehydration process in our study. In an effort to explain such a behaviour by a physiological property, they hypothesized that leaf thickness is related to turgor pressure of leaf cells. They investigated this relationship using bean plants (*Phaseolus vulgaris* L.). They observed strong correlations between leaf thickness and leaf water potential for each individual leaf. However, they could not find a general relationship between leaf thickness and either leaf turgor potential or leaf water potential. However, they reported that this result could be caused by errors in their method for estimating leaf turgor potential.

McBurney (1992) observed a strong non-linear relationship between leaf water potential and leaf thickness of Brussels sprout plants (*Brassica oleracea* var. *gemmifera* D.C. cv. Gabion). However, the pattern of this relationship varied across the leaves of different plants such that the results did not allow them to draw a general relationship between leaf water potential and leaf thickness of Brussels sprout plants.

In contrast, our data (Fig. 2) shows that the relationship between RWC and RT followed a consistent non-linear pattern that could be explained by a piecewise linear model.

3.2. Estimated model parameters by species

Table 1 shows the estimated model parameters for each species, averaged over the leaf locations. Large differences were observed among the estimated model parameters of the species. The slope of the regression line in phase 1 (b_1) was greatest for sorghum, followed by soybean, fava bean and smallest for corn. In phase 1, in sorghum, RT changed least with a given change of RWC, while it changed most with a unit change of RWC in corn. The slope b_1 of soybean was

1 similar to that of sorghum while b_1 of fava bean was similar to that in corn. The breakpoint c
2 occurred at the largest RT in sorghum, followed by corn, soybean, and fava bean. The slope of
3 the line in phase 2 (b_2) was largest for sorghum, followed by corn, soybean, and smallest for fava
4 bean.

5 The results show that the relationship between RWC-RT varied by species. The relationship may
6 also vary across different varieties of a species. This question requires further examination.

7 The dynamics of the RWC-RT relationship can possibly provide information about species'
8 drought tolerance. The greatest differences between the estimated slopes and breakpoint occurred
9 between sorghum and fava bean. Sorghum had the greatest slopes in both phases and the highest
10 RT at the breakpoint c . Fava bean, on the other hand, had the smallest b_2 , the smallest c , and the
11 second smallest b_1 . Sorghum is among the most drought tolerant cultivated crops (Sabadin et al.,
12 2012). Fava bean, on the other hand, is known for its lack of drought tolerance (Multari, Stewart,
13 & Russell, 2015). Therefore, this suggests that a drought-sensitive crop might have a smaller c
14 while a drought-tolerant species would have a larger c . This interpretation implies that the
15 drought tolerance of corn and soybean is intermediate between sorghum and fava bean.

16 Blum (2011), Giuliani et al. (2013), and Patakas and Noitsakis (1997) have recognized that there
17 is a relationship between cell rigidity and drought tolerance by maintaining cell structure at lower
18 water potentials. A larger RT at the breakpoint suggests that the leaves transitioned from an
19 elastic to a more rigid behaviour sooner, exhibiting an overall more rigid behaviour throughout
20 the dehydration process. This implies that RT at the breakpoint is related to the crop drought
21 tolerance. These interpretations are in agreement with Scoffoni et al. (2014) who reported that
22 the thickness of leaves of moist habitat species decreased more from full-turgor to TLP
23 (breakpoint) compared with the leaves of dry habitat species better adapted to drought.

24 3.3. *Monocotyledons versus dicotyledons*

25 RT at the breakpoint c and the slope b_2 were larger for the monocot (monocotyledon) species
26 than for the dicot (dicotyledon) species, implying that the leaves of monocots had overall less
27 elastic behaviours. These differences are in agreement with the leaf anatomical differences,
28 specifically bulk leaf rigidity, between monocots and dicots.

1 In contrast to the dicots, the monocot leaves contain dense longitudinal main veins and silica
2 deposition providing mechanical strength to their long leaves (Read & Stokes, 2006). Thicker
3 cell walls, greater lignin content in the vascular tissues (Myburg & Sederoff, 2001; Sack &
4 Scoffoni, 2013) and higher silica content (Read & Stokes, 2006), result in a more rigid bulk leaf
5 blade in the monocots than in dicots. Therefore, the larger c and b_2 for the monocot leaves may
6 be explained by the more rigid leaf structure of these species compared to that of dicots.

7 3.4. *RWC-RT model and salt tolerance*

8 Salt tolerance of a species is characterized by a threshold EC (soil electrical conductivity) below
9 which crop yield is not affected (Hoffman, Rhoades, Letey, & Sheng, 1990; Maas & Hoffman,
10 1977). Since drought and salt tolerance mechanisms of plants are closely related (Jones, 2014;
11 Kramer & Boyer, 1995; Taiz & Zeiger, 2006), the relationship between threshold EC levels for
12 the crops and model parameters were investigated.

13 The slope b_1 was strongly correlated with the salinity thresholds (Fig. 3A). Also, the crops with
14 higher thresholds tended to have greater RT values at the breakpoint c , and greater b_2 slopes (Fig.
15 3B and Fig. 3C). These results suggest that larger b_1 , b_2 , and c may be indicators of a more salt-
16 tolerant crop.

17 Cell wall rigidity is one of the salt-tolerance mechanisms of crops to maintain cell structure at
18 low water potentials (Gall et al., 2015; Morgan, 1986; Zagorchev, Kamenova, & Odjakova,
19 2014). Therefore, if a larger breakpoint and smaller rates of RT variations in response to
20 dehydration (larger b_1 , and b_2) imply a more rigid structure, the effect of cell wall rigidity may
21 explain the positive correlation between the piecewise parameters and the salinity threshold.

22 Salinity reduces soil water osmotic potential which impedes water uptake, increasing drought
23 stress (Taiz & Zeiger, 2006). Crops share some similar tolerance mechanisms for salt and
24 drought stresses (Jones, 2014; Kramer & Boyer, 1995; Taiz & Zeiger, 2006). These results
25 suggest that a larger breakpoint c and larger slopes b_1 and b_2 might reflect a greater salt tolerance
26 and accordingly, a greater drought tolerance of a crop.

3.5. Effect of leaf location on the RWC-RT relationship

Table 2 shows the estimated piecewise parameters for different leaf locations by the species. Leaf location had a significant effect on most parameters of the RWC vs. RT relationship of all four species (Table 3). Leaf location significantly affected b_1 for all the species, the breakpoint c for corn and sorghum, and b_2 and a for soybean and fava bean. McBurney (1992) also observed that the slope of the leaf thickness vs. leaf water potential for higher position leaves differed from that of lower position leaves in Brussels sprout plants.

Evidently, the effects of leaf position on model parameters varied by species. Leaf position reflects leaf age and light exposure which affect leaf structural traits. Sylvester (2001) reported a significant effect of age on the leaf anatomy of corn, rice, and bluegrass. Aging causes cell wall thickening and increased elastic modulus (Lambers, Chapin, & Pons, 2008; Patakas & Noitsakis, 1997). The penetration of sunlight decreases from top to bottom of a canopy (Jones, 2014). In our study, the plants were spaced sufficiently close together such that upper leaves shaded lower leaves. Exposure to sunlight has a strong influence on the leaf structure (Smith et al., 1998). Vogelmann et al. (1996) showed that sun-leaves (leaves directly exposed to sunlight) of *Thermopsis montana* had longer palisade cells and were much thicker than shade-leaves (leaves that do not receive direct sunlight). The cell walls of sun-leaves have higher lignin content and greater rigidity than shade-leaves (Gall et al., 2015).

The breakpoint c of monocots was smaller for the top leaves than for the bottom leaves while this trend was reversed for the dicots. The growth pattern of monocots implies that the older leaves are at the bottom while younger leaves are at the top. Since age affects cell wall rigidity (Nobel, 2009; Patakas & Noitsakis, 1997), the lower leaves of the monocots are expected to have more rigid bulk structure than the higher leaves. This rigidity may help explain why lower leaves of the monocots had a larger c . McBurney (1992) observed that the hardened leaf cells of droughted plants shrank more laterally and less longitudinally (in the direction of the leaf thickness) compared to the non-hardened leaf cells of non-droughted plants, suggesting that the hardened cells had more longitudinal rigidity.

On the other hand, the growth pattern of dicot species does not ensure the vertical age distribution of leaves. The plant grows vertically along the main stem and laterally by branching. Therefore, in dicots, the leaf location does not assure the order of leaf age along the main stem.

However, for the dicots, the leaf location may reflect the light distribution. Light exposure of leaves of monocot species at lower levels in the canopy may not decrease as much as in dicots because the helical leaf arrangement and a smaller horizontal leaf angle allow more light penetration in monocots (Read & Stokes, 2006). It is known that the cell wall lignin content and the bulk elastic modulus of sun-leaves are greater than shade-leaves (Gall et al., 2015). Therefore, it appears that the leaves near the top of the dicots received more light and therefore had more rigid cells, resulting in more tissue rigidity and larger c values.

4. Conclusion

This study shows that leaf thickness can be used to measure leaf relative water content. We observed an abrupt increase of the slope of the RWC-RT curve as leaves dried out. Piecewise linear regression resulted in models that explained 86-97% of the variation of RWC-RT relationship of the four species studied. We suggest that the parameter c in these models represents the RT at the TLP. The relationship between RT and RWC varied by species and leaf location. To use leaf thickness as an estimate of leaf water content, it is, therefore, necessary to develop models that vary by species, leaf location, and probably variety.

It is recognized that model calibrations will change as leaves age - a top leaf may become a middle leaf later. Additionally, environmental factors such as light, temperature, relative humidity, disease, and pest pressure may affect leaf thickness as well. We recognize that the results were impacted by growing the plants in pots in a greenhouse rather than in a field. Some of these differences may include root distribution, nutrient availability, and variability of the soil structure that may affect plant water uptake, growth characteristics, and leaf physiological and anatomical structure, which may impact the leaf thickness and water content relationship.

Additional work building on this research accounting for some of these environmental factors would strengthen the development of more reliable models. We suggest that the RWC-RT curves can provide information about drought and salt tolerance of species. If it is true that the breakpoint c represents TLP, the breakpoint implies a critical water stress level which leads to a dramatic change in the leaf physiology. This information could be used to screen species and varieties for drought and salinity tolerance. In addition, this knowledge may be applied to develop leaf thickness sensors to monitor plant water stress for irrigation scheduling and drought

1 monitoring. Since plant water potential is widely adopted as an indicator of plant water
2 condition, further work to determine a relationship between leaf thickness and leaf water
3 potential components may help develop a better understanding of the relationship between leaf
4 thickness variations and plant water status.

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Tables

Table 1 - Estimated parameters for the piecewise regression models of leaf relative water content (RWC) vs. leaf relative thickness (RT) for different species. c is the RT at the breakpoint of the piecewise model, b_1 and b_2 are the slopes of the model for respectively $RT > c$ and $RT \leq c$, and a is the model intercept for $RT \leq c$.

Species	b_1	c	b_2	a	R^2	P-value	Estimated RWC at c
Corn	0.166 ± 0.113	0.672 ± 0.024	1.050 ± 0.051	0.208 ± 0.025	0.86	<0.001	91%
Sorghum	0.348 ± 0.075	0.750 ± 0.013	1.156 ± 0.032	0.032 ± 0.020	0.97	<0.001	90%
Soybean	0.322 ± 0.063	0.653 ± 0.024	0.935 ± 0.044	0.247 ± 0.022	0.93	<0.001	86%
Fava bean	0.192 ± 0.064	0.517 ± 0.029	0.885 ± 0.063	0.407 ± 0.021	0.89	<0.001	86%

Note: The margins of error are calculated based on a 95% confidence interval.

Table 2. Estimated parameters for the piecewise regression models of leaf relative water content (RWC) vs. leaf relative thickness (RT) across leaf locations of the species. c is the RT at the breakpoint of the piecewise model, b_1 and b_2 are the slopes of the model for respectively $RT > c$ and $RT \leq c$, and a is the model intercept for $RT \leq c$.

Species	Leaf location	b_1	c	b_2	a	R^2	P-value	Estimated RWC at c
Corn	Top	0.229 ± 0.150	0.557 ± 0.025	1.435 ± 0.107	0.070 ± 0.056	0.99	<0.001	87%
	Middle	0.169 ± 0.036	0.635 ± 0.007	1.220 ± 0.021	0.142 ± 0.010	0.99	<0.001	92%
	Bottom	0.223 ± 0.022	0.662 ± 0.005	1.334 ± 0.029	0.015 ± 0.013	0.90	<0.001	90%
Sorghum	Top	0.452 ± 0.059	0.694 ± 0.007	1.115 ± 0.021	0.075 ± 0.013	> 0.99	<0.001	85%
	Middle	0.380 ± 0.036	0.728 ± 0.006	1.247 ± 0.020	-0.020 ± 0.012	> 0.99	<0.001	89%
	Bottom	0.276 ± 0.022	0.766 ± 0.007	1.353 ± 0.018	-0.110 ± 0.010	> 0.99	<0.001	93%
Soybean	Top	0.289 ± 0.045	0.650 ± 0.016	0.976 ± 0.066	0.248 ± 0.033	0.98	<0.001	88%
	Middle	0.282 ± 0.025	0.637 ± 0.008	1.038 ± 0.021	0.209 ± 0.010	> 0.99	<0.001	87%
	Bottom	0.379 ± 0.049	0.611 ± 0.017	1.118 ± 0.033	0.116 ± 0.016	0.98	<0.001	80%
Fava bean	Top	0.239 ± 0.022	0.487 ± 0.011	1.236 ± 0.056	0.242 ± 0.016	0.91	<0.001	84%
	Middle	0.211 ± 0.042	0.425 ± 0.013	1.314 ± 0.078	0.289 ± 0.024	0.97	<0.001	85%
	Bottom	0.257 ± 0.082	0.394 ± 0.031	1.100 ± 0.166	0.374 ± 0.061	0.99	<0.001	81%

Note: The margins of error are calculated based on a 95% confidence interval.

Table 3. The significance of leaf location as a factor in the variability of the piecewise model parameters of leaf relative water content vs. leaf relative thickness (RT). c is the RT at the breakpoint of the piecewise model, b_1 and b_2 are the slopes of the model for respectively $RT > c$ and $RT \leq c$, and a is the model intercept for $RT \leq c$.

Species	P-value			
	b_1	c	b_2	a
Corn	<0.01	0.01	0.51	0.66
Sorghum	<0.01	0.01	0.20	0.21
Soybean	0.04	0.80	<0.01	<0.01
Fava bean	0.01	0.49	<0.01	0.01

Figure captions

Fig. 1. Schematic of the thickness sensor.

Fig. 2. Fitted piecewise models of leaf relative water content (RWC) vs. leaf relative thickness (RT) for corn (A), sorghum (B), soybean (C), and fava bean (D). The solid lines show the piecewise regression lines. The grey points display the observations.

Fig. 3. The relationship between linear piecewise model parameters of leaf relative water content-relative thickness (RT) with soil electrical conductivity (EC) threshold (maximum EC that does not result in yield loss) for corn (▲), sorghum (■), soybean (○), and fava bean (●). c is the RT at the breakpoint of the piecewise model, and b_1 and b_2 are the slopes of the model for respectively $RT > c$ and $RT \leq c$. A) b_1 vs. threshold ($y = 0.035x + 0.126$; $R^2 = 0.95$; p-value = 0.02), B) c vs. threshold ($y = 0.028x + 0.542$; $R^2 = 0.54$; p-value = 0.26), C) b_2 vs. threshold ($y = 0.027x + 0.903$; $R^2 = 0.33$; p-value = 0.42). The references for the EC threshold data of the crops are cited in the text.

Figures

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