

Specific leaf weight as an indicator of juvenile leaf toughness in Tasmanian bluegum (*Eucalyptus globulus* ssp. *globulus*): implications for insect defoliation

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Summary

The influence of watering regime, provenance and leaf-age were studied in relation to specific leaf weight (abbreviated SLW; inversely correlated with percentage leaf water) of juvenile leaves of *Eucalyptus globulus* subspecies *globulus*. SLW provides a physiological estimate of leaf "toughness" relevant to studies of insect herbivory. Leaves from saplings given continuous access to water had lower SLWs and higher water contents than leaves from saplings that were drip irrigated. Saplings of the King Island provenance produced leaves of consistently lower SLW compared with leaves from saplings of the Geeveston provenance irrespective of watering regime. Furthermore, leaves from the King Island provenance saplings exhibited less variation with respect to water content than leaves from the Geeveston saplings irrespective of watering regime. Generally, new leaves exhibited lower SLWs and higher water contents than old leaves irrespective of watering regime or provenance. The importance of leaf "toughness" (as indicated by SLW) to insect herbivory, and therefore its relevance to plantation managers, is discussed.

Introduction

Eucalypts produce sclerophyllous leaves that are tough (i.e. high leaf strength), high in fibre, low in nitrogen and high in secondary compounds. Consequently, the leaves of eucalypts are considered of low nutritive value, or food quality, to herbivores (Morrow 1983). Because the nutritional value of leaves is thought to influence the level of herbivory, it is important that plantation managers have a simple (but meaningful) method for gauging the suitability of the leaves produced by their trees to insects.

Differences in the level of herbivory by insects have often been attributed to qualitative differences in the composition of secondary compounds in the leaves (Schoonhoven *et al.* 1998). However, the physical properties of leaves (notably their toughness and the dilution of essential nutrients post ingestion by the cell wall fraction) may be of equal significance in this regard (Hochuli 1996). The composition of secondary compounds is not readily determined, because of the limited availability of the necessary analytical equipment and the financial cost of having such analyses performed. Furthermore, the host specificity of some supposedly eucalypt-specific insects, usually attributed to the internal composition of plant secondary compounds, appears variable (Steinbauer and

Wanjura 2001). Such evidence suggests that so long as hosts possess a particular suite of secondary compounds (e.g. most species of *Eucalyptus*) they might be assessed from a distance by the insect to be of similar quality to one another. Consequently, although it may be possible to quantify the composition of plant secondary compounds it is more difficult to quantify the respective role(s) of these chemicals in determining the final level of herbivory a plant will experience. Given these limitations, might it be possible to compare the quality of leaves using some other leaf characteristic?

Plant physiologists have for many years used specific leaf area (SLA, area (mm²)/dry weight (mg)) to compare the thickness and/or density of leaves. This is because SLA is primarily influenced by plant species (Cunningham *et al.* 1999), environment (e.g. the availability of soil moisture/nutrients and light, Li *et al.* 2000) and changes in photosynthetic capacity (Pinkard and Beadle 1998 (canopy size), Reich *et al.* 1998 (between species)). Variation in SLA with leaf longevity (i.e. evergreen versus deciduous) probably reflects different cost-benefit ratios associated with leaf production. For example, evergreen leaves (e.g. those of eucalypts) are considered more expensive to produce but are productive for longer than those of deciduous species (Eamus and Prichard 1998, Eamus *et al.* 1999). Differences in SLA between leaf-types within a species (e.g. juvenile *versus* adult foliage) may also be related to differences in photosynthetic capacity as well as the scaling of mechanical support associated with tree support (King 1999). Typically, juvenile leaves have higher SLAs than adult leaves. Reasons potentially associated with these differences in *E. globulus* ssp. *globulus* are discussed in James *et al.* (1999) and James and Bell (2000). Clearly, by citing a value for SLA it is implicit that a particular leaf exhibited a specific suite of traits.

Landsberg (1990a, b, c) used the inverse of SLA, namely specific leaf weight (SLW in mg/mm²), as an indicator of leaf toughness in her studies of insect herbivory and eucalypt dieback. SLW has subsequently become more widely used in this context (e.g. Stone and Bacon 1994, 1995, Landsberg and Gillieson 1995, Steinbauer *et al.* 1998, Abbott *et al.* 2000). Choong (1996), who measured leaf biomechanical properties, reported that leaf toughness was the main deterrent to consumption in a suite of tree-feeding caterpillar species. Leaf toughness appears, increasingly, to be a significant plant characteristic for eucalypt-feeding insects also (e.g. studies by Steinbauer *et al.* 1998, Howlett *et al.* 2001, Nahrung *et al.*

2001). Hence, it is important to adopt a standard, meaningful parameter to quantify leaf toughness. Concomitant with the estimation of SLW is the potential to determine leaf water content using the same plant material. Water content is a leaf characteristic crucial to the nutrition of herbivorous insects (Scriber and Slansky 1981). Scriber and Feeny (1979) also suggested that tree-feeding lepidopterous larvae were more sensitive to the water contents of host leaves than forb-feeding lepidopterous larvae.

A precise definition of foliar toughness (a biomechanical trait) is "the amount of work needed to fracture a leaf". Whilst leaf toughness has often been reported to influence the performance of herbivorous insects, the techniques and units of quantification cited (in particular from studies that used penetrometers) in the literature have often been fallacious estimates of leaf toughness (Choong *et al.* 1992, Choong 1996). Choong (1996) used an instrumented scissor technique to quantify lamina toughness (also termed fracture toughness and measured in Joules/m²). Choong (1996) found that leaf toughness was created "by the cell wall volume fraction of component tissues" combined with the "neutral detergent fibre content". Scissoring techniques are not readily employed if researchers have few laboratory facilities and limited time. Consequently, the issue becomes "how close an approximation of leaf toughness (*sensu* Choong 1996) is SLW?" Choong *et al.* (p. 605, 1992) found that leaf toughness was correlated with leaf dry matter content. Specifically, leaf toughness exhibited a significant, negative correlation with SLA ($r^2 = -0.453$, $P = 0.01$). This finding suggests that SLW, by itself, is a good indicator of leaf toughness.

This study comments upon the validity of using SLW as an indicator of leaf toughness and illustrates the ease with which SLW and leaf water content can be determined. In addition, this study illustrates how some factors can influence these two leaf characteristics. When plantation managers provide forest entomologists with clearly defined estimates of leaf quality, as well as estimates of insect population size, it will be possible to exchange and compare observations. In addition, by routinely determining SLW and leaf water content researchers develop a better understanding of the phenology of tree growth, agents influencing changes in tree vigour and factors influencing the quality of leaves as insect food.

Methods

Plants

Eucalypts used in this study were grown from seed obtained from the Australian Tree Seed Centre (ATSC), Yarralumla, A.C.T., in 1998. Seeds were sown in a potting mixture that comprised eight parts hammer-milled and composted pine bark, two parts coarse river sand and one part peat; the fertiliser used was Aquasol™. Seeds were left to germinate in a glasshouse that maintained a mean air temperature of 23.1°C (max. 35.9°C & min. 10.6°C), mean relative humidity of 53.5% (max. 92.9% & min. 26.3%) and ambient photoperiod. Seedlings were grown in original trays until large enough to be transplanted into individual pots.

Two provenances of *E. globulus* ssp. *globulus* were selected for this study, namely: locality "south of Geeveston" (43°12'E 146°54'S, 250 m ASL), seedlot number 16476; and locality

"King Island" (39°56'E 143°52'S, 40 m ASL), seedlot number 17608.

Twenty (10 of each provenance) 12 month-old saplings were cut back to a height of 18 cm before being assigned to a watering regime on the 17 May 1999. Ten saplings (5 of each provenance) were randomly assigned to either drip irrigation (2 min. (approximately 300 mL) every 2 days administered by a "Gardena Computer 1060") or water *ad libitum* (trays continually supplied with water). Saplings were kept in the glasshouse in which they were germinated (see above) for the duration of the study. All saplings were kept in the same sized pots for the duration of the study and were not supplemented with fertilisers. All saplings were pruned to approximately the same size at various times during the course of the study to prevent saplings from falling over as well as outgrowing the moisture retention capacity of the soil in each pot.

Estimating SLW and water content

Ten leaves from each sapling were harvested on the 4 July 2000. Leaf-age, whether new (expanding) or old (fully expanded), was noted at the time of harvest. Leaves were transferred immediately to individual, sealable plastic bags that were kept in an insulated box above ice packs until all leaves were harvested. In the laboratory a hole-punch (diameter 5.54 mm) was used to take a leaf disc from the middle of the leaf lamella. Fresh leaf discs were weighed before being placed in individual aluminium foil cups and placed in an oven for drying. Leaf discs were dried at approximately 70°C for 48 h before being re-weighed.

SLW and water content were calculated as follows: $SLW = \text{dry weight of leaf disc in mg/area of hole-punch in mm}^2$ and $\text{water content (\%)} = (\text{leaf fresh weight} - \text{leaf dry weight})/\text{leaf fresh weight} * 100$.

Statistical analyses

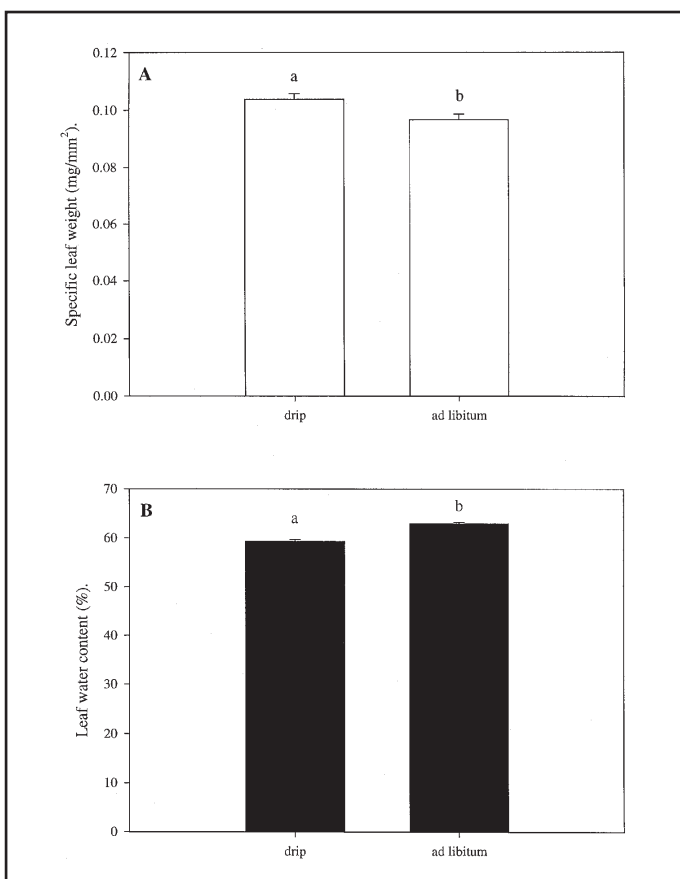
Analysis of variance (ANOVA) was used to analyse the data. Watering regime (2 levels), provenance (2 provenances) and leaf-age (2 classes) were used as factors in the analysis. Watering regime is crossed with provenance whilst leaf-age is nested within watering regime and provenance. Leaf-age is considered nested within watering regime and provenance because leaves belonging to different classes were harvested from the same trees. To compare SLWs and leaf water contents, the factors were modelled as: watering regime; provenance; watering regime * provenance; leaf-age(watering regime, provenance) (see Results). Post-hoc one-way ANOVAs were conducted for each statistically significant factor.

Results

At the time leaves were harvested, saplings were approximately the same height. All the variables used as factors in the ANOVA had highly significant influences upon SLW (Table 1). In contrast, provenance did not have a statistically significant influence upon leaf water content (Table 1), however the interaction between watering regime and provenance was highly significant suggesting that closer investigation of individual means is necessary. As a consequence, the influence of each factor on SLW and leaf water content is considered individually below.

Table 1. ANOVAs of SLW and leaf water content in relation to watering regime, provenance and leaf-age

Factor	df	MS	F-value	P
SLW				
Watering regime	1	0.0019	11.83	0.001
Provenance	1	0.0090	54.98	<0.001
Watering regime * Provenance	1	0.0028	16.92	<0.001
Leaf-age(watering regime, provenance)	4	0.0039	23.98	<0.001
Error	152	0.0002		
Total	159			
Water content				
Watering regime	1	517.93	63.13	<0.001
Provenance	1	24.40	2.97	0.087
Watering regime * Provenance	1	190.65	23.24	<0.001
Leaf-age(watering regime, provenance)	4	96.28	11.73	<0.001
Error	152	8.20		
Total	159			

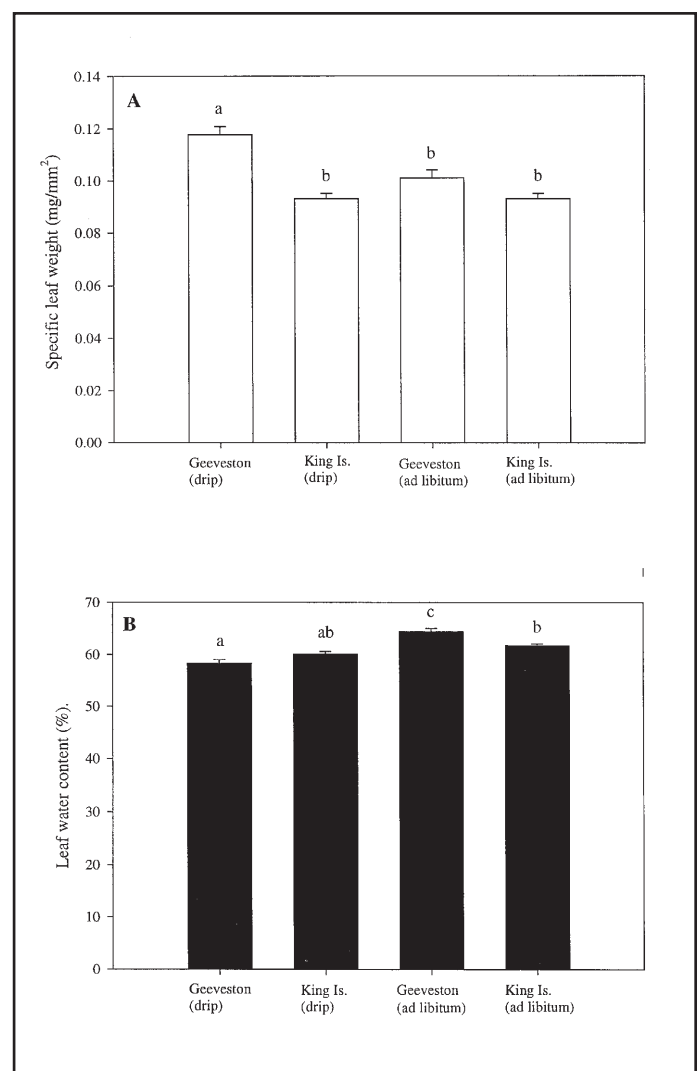
Figure 1. Leaf characteristics of *Eucalyptus globulus* versus watering regime, namely (A) SLW (□) and (B) water content (■). Data are means ± s.e. Different letters above bars indicate means are significantly different (one-way ANOVA); (A) $F_{1, 159} = 5.85$, $P = 0.017$ and (B) $F_{1, 159} = 44.30$, $P < 0.001$.

Watering regime

Drip irrigation resulted in the production of leaves with higher SLWs (Fig. 1A) and lower water contents (Fig. 1B) than those produced by trees given water *ad libitum* (SLW drip *versus ad libitum*: 0.104 ± 0.002 mg/mm² *versus* 0.097 ± 0.002 mg/mm² and water content drip *versus ad libitum*: $59.3 \pm 0.4\%$ *versus* $62.9 \pm 0.3\%$).

Provenance

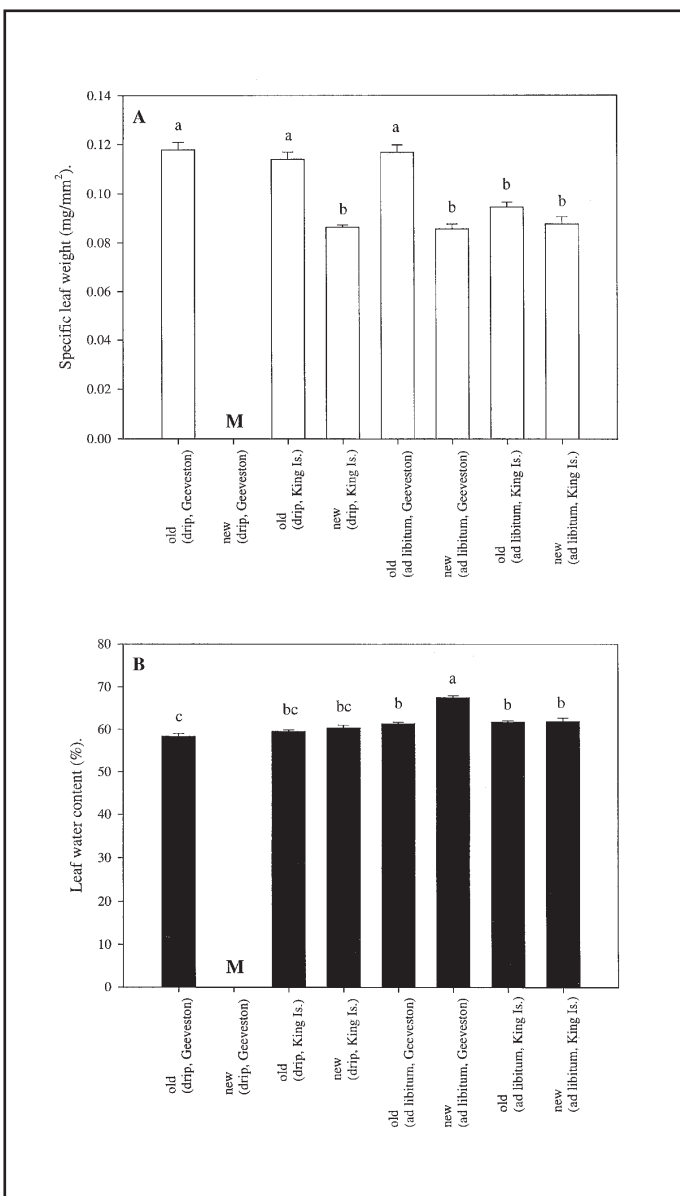
Saplings of the Geeveston provenance produced leaves with consistently higher SLWs than those produced by trees of the King Island provenance (0.108 ± 0.003 mg/mm² *versus* 0.093 ± 0.001 mg/mm², respectively) (Fig. 2A). This difference was statistically significant for leaves produced by saplings given drip irrigation. Surprisingly, Geeveston provenance leaves were not of consistently lower water content despite having high SLWs ($61.8 \pm 0.6\%$ *versus* $61.0 \pm 0.3\%$, respectively). The water content of Geeveston provenance leaves was more variable ($\pm 6\%$ drip cf. *ad libitum*) than that of King Island provenance leaves ($\pm 2\%$ drip cf. *ad libitum*) (Fig. 2B). This provides an explanation for the significant interaction term in Table 1.

Figure 2. Leaf characteristics of *Eucalyptus globulus* versus provenance according to watering regime, namely (A) SLW (□) and (B) water content (■). Data are means ± s.e. Different letters above bars indicate means are significantly different (one-way ANOVA); (A) $F_{3, 159} = 17.56$, $P < 0.001$ and (B) $F_{3, 159} = 23.35$, $P < 0.001$.

Leaf-age

Old leaves, irrespective of watering regime and provenance, had higher SLWs than new leaves ($0.108 \pm 0.002 \text{ mg/mm}^2$ versus $0.086 \pm 0.001 \text{ mg/mm}^2$, respectively) (Fig. 3A). In only one case, that is King Is. saplings in the *ad libitum* watering regime, was the difference between old versus new leaves not statistically significant. Although differences were sometimes slight and statistically not significant, old leaves had lower water contents than their same provenance new leaf counterparts ($60.4 \pm 0.3\%$ versus $63.0 \pm 0.6\%$, respectively) (Fig. 3B). The largest observed difference according to leaf-age was for old leaves versus new leaves from the *ad libitum* watering regime of the Geeveston provenance. In this instance, old leaves had greater specific leaf weights, approximately $\pm 0.03 \text{ mg/mm}^2$ (drip cf. *ad libitum*), than new leaves and were approximately $\pm 6\%$ (drip cf. *ad libitum*) lower in water content than new leaves.

Figure 3. Leaf characteristics of *Eucalyptus globulus* versus leaf-age according to watering regime and provenance, namely (A) SLW (□) and (B) water content (■). M = missing data (no new leaves being produced). Data are means \pm s.e. Different letters above bars indicate means are significantly different (one-way ANOVA); (A) $F_{6, 159} = 30.14, P < 0.001$ and (B) $F_{6, 159} = 22.86, P < 0.001$.



Discussion

It is clear from this comparatively simple data-set that the expression of leaf toughness, as approximated by SLW, and water content are dependent upon genetic and environmental factors. Whilst these factors can alter leaf quality, the significance of such changes upon the insects that subsequently feed on the leaves requires further study. It is possible that many of the differences in SLW and leaf water content presented herein are unlikely to be "biologically significant" (cf. statistically significant). That is, all of the estimates of SLW presented herein are considered unlikely to drastically reduce the performance, much less the survival, of defoliating insects such as the autumn gum moth. This is because species such as the autumn gum moth only appear to be highly susceptible to differences in SLW once some insect-specific threshold has been exceeded. For example, neonate autumn gum moth larvae appear to be susceptible to values of SLW above 0.236 mg/mm^2 (associated leaf water contents $\leq 56.5\%$) (M.J. Steinbauer unpubl. data). Such high values for SLW are attained by eucalypts such as *E. globulus* when the phase-change to adult foliage occurs. In contrast, other species of eucalypt (e.g. *E. occidentalis*) produce leaves with high SLWs (e.g. 0.311 mg/mm^2 , leaf water content 43.5%) at the juvenile stage. Perhaps significantly, coupes of *E. occidentalis* can remain completely foliated even though they co-occur in plantations where outbreak numbers of autumn gum moth larvae have largely defoliated neighbouring eucalypts (namely *E. grandis* and to a lesser extent *E. camaldulensis*) (M.J. Steinbauer pers. obs).

As mentioned in the Introduction, leaf or fracture toughness is correlated with leaf dry matter content (Choong *et al.* p. 605, 1992). In addition to a significant negative correlation with SLA, these authors also reported that leaf toughness exhibited a very significant, positive correlation with leaf density (i.e. dry weight/unit volume, $r^2 = 0.519, P \leq 0.001$). Consequently, SLW might be further improved as an indicator of leaf toughness if, instead of only calculating the area of the dried leaf disc (mm^2), we determined its volume (mm^3), i.e. area * lamina thickness (as determined using an eye-piece graticule or micrometer gauge, for example). Such a parameter might be called the specific leaf density (SLD, units mg/mm^3). The variation between SLW and its precision as a measure of leaf toughness might be partially explained by leaf venation. Veins are tougher than the leaf lamella (Choong 1996). This suggests that the smaller the size of the hole-punch, that is the more veins avoided, used to take leaf discs, the closer the correlation with lamina toughness as predicted by morphometric and chemical techniques (Choong 1996). This prediction does indeed appear to hold. For example, the SLW of adult (old) leaves of *Corymbia eximia*, as estimated using a 5.54 mm diameter hole-punch, was 0.165 mg/mm^2 (leaf water content 48.7%). In contrast, the SLW of similar leaves, as estimated using a 21.00 mm diameter hole-punch, was 0.188 mg/mm^2 (leaf water content 49.4%) (SLW for 5.54 mm versus 21.00 mm, $t_{0.05(2), 27} = 2.05, P(\text{lt} \geq 9.20) \leq 0.001$) (M.J. Steinbauer unpubl. data). These data suggest that more study is needed to determine the hole-punch diameter that best approximates the leaf toughness of most biological significance. The latter associations will be unique to the insect-eucalypt system under investigation. For example, because autumn gum moth instars I through to III avoid leaf venation and scrape lamina only, SLW should be estimated using a small diameter hole-punch. In

instar larvae, which consume whole leaves, should quote values for SLW determined using a hole-punch of large diameter or possibly even whole leaf estimates. It should be noted however that whole leaf estimates of SLW may over estimate leaf toughness because they include the mid-vein which is usually avoided even by late instar autumn gum moth larvae (M.J. Steinbauer pers. obs).

Conclusion

Studies of insect herbivory cannot rely solely upon knowing the provenance (usually meant to imply that the genetically regulated composition of secondary compounds in the leaves is somehow unique to that provenance) of the hosts to explain observed differences in defoliation. Such studies are common in the forestry literature but, given the lack of information supplied about the physical and/or chemical composition of the leaves, these studies provide no clue to the mechanism underlying the patterns of defoliation observed or facilitate comparisons between similar studies. Hence, provision of estimates of SLW and water content will improve the interpretation of such findings. Consequently, my objective was to increase the adoption of SLW and leaf water content as measures of foliar quality by colleagues attempting to better understand insect-eucalypt interactions. However, one-off estimations of SLW and water content cannot possibly hope to illuminate intimate fluctuations in the relationship between insect and tree. Consequently, a better understanding of patterns of herbivory will be possible when researchers routinely supply estimates of SLW and water content that reflect changes in the condition of the tree.

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