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Drought until death do us part: a case study of the desiccation-tolerance of a tropical moist forest seedling-tree, *Licania platypus* (Hemsl.) Fritsch

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Abstract

Studies of the desiccation tolerance of 15-month-old *Licania platypus* (Hemsl.) Fritsch seedlings were performed on potted plants. Pots were watered to field capacity and then dehydrated for 23–46 d to reach various visible wilting stages from slightly-wilted to dead. Root hydraulic conductance, k_r, was measured with a high-pressure flow meter and whole-stem hydraulic conductance, k_{ws}, was measured by a vacuum chamber method. Leaf punches were harvested for measurement of leaf water potential by a thermocouple psychrometer and for measurement of fresh- and dry-weight. *L. platypus* was surprisingly desiccation-tolerant, suggesting that most species of central Panama may be well adapted to the seasonality of rainfall in the region. The slightly-wilted stage corresponded to leaf water potentials and relative water contents of –2.7 MPa and 0.85, respectively, but plants did not die until these values fell to –7.5 MPa and 0.14, respectively. As desiccation proceeded k_r and k_{ws} declined relative to irrigated controls, but k_{ws} was more sensitive to desiccation than k_r. Values of k_{ws} declined by 70–85% in slightly-wilted to dead plants, respectively. By comparison, k_r showed no significant change in slightly-wilted plants and fell by about 50% in plants having severely-wilted to dead shoots.

Key words: Desiccation tolerance, drought resistance, hydraulic conductance, *Licania platypus*, water potential.

Introduction

Mechanisms of drought resistance can be divided into two classes: desiccation-delay and desiccation-tolerance. Desiccation-delay involves traits that increase access to water and reduce water loss. These include deep roots, early stomatal closure, low cuticular conductance, water storage in plant organs, and leaf shedding. Desiccation-tolerance is promoted by physiological traits that permit continued water transport, gas exchange or cell survival at low water content (*WC*) and low water potentials (Ψ), such as osmotic adjustment, decreased vulnerability of xylem to embolism, and the ability of cells (especially meristems) to remain alive at low *WC* or Ψ.

This paper focuses on desiccation-tolerance properties. Visible symptoms of wilt are correlated with changes in leaf water content (*WC*), leaf water potential (Ψ), whole-stem hydraulic conductance (i.e. the whole shoot with leaves removed, k_{ws}), and whole-root hydraulic conductance (k_r). The aim of this study was to develop experimental protocols that might allow comparative studies in the future that relate survivorship to the visible wilt stage and quantitative measures of some important water-relations parameters.

For many years researchers have studied drought-induced xylem dysfunction. Vulnerability curves relate how stem segment hydraulic conductance declines with stem water potential, Ψ_{stem}, and these have been measured on >60 species of plants, for example, see the literature survey in Tyree *et al.* (1994). More recently, a vacuum chamber technique has been used to measure vulnerability

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curves on whole shoots of arid zone species (Kolb *et al.*, 1996). Many species have been compared in terms of the Ψ_{stem} needed to induce 50% loss of hydraulic conductance, Ψ_{50PLC}, and there has been a satisfying general correlation in which species preference to wet versus dry sites correlates with less negative to more negative values of Ψ_{50PLC}, respectively. The presumption is that the plants tend to die when PLC approaches 100%, but, as far as is known, there are no studies of whole tropical or temperate plants that demonstrate what values of *WC*, Ψ or k_{ws} correlate with plant death. There have been studies of leaf tissue viability after desiccation (Buckley *et al.*, 1980; Peace and Macdonald, 1981; Lösch, 2001), but field experience has revealed that many plants are still alive even after most or all leaf tissue is dead.

Materials and methods

Licania platypus (Chrysobalanceae) is an old-forest tree 10–30 (rarely 50) m tall with buttressed trunks of 0.75 m diameter (measured above the buttresses). It ranges from the east and west coasts of Mexico through Central America to Colombia.

Seeds were collected when ripe in April 2000 and immediately germinated and grown in a screened exclosure designed to exclude most herbivores while permitting maximum ambient airflow. *L. platypus* generally germinates in old-forest understorey where daily PAR values are 5–7% of PAR above the forest canopy. Hence plants were grown on covered benches with shade cloth sufficient to simulate old-forest understorey PAR levels. Plants were first germinated in shallow trays and then transplanted to pots holding approximately 1.5 l of unmodified forest soil and allowed to establish for 6 months before irrigation was discontinued. By this stage the plants had a mean height 32 cm (range 19–53) and leaf area of 258 cm^2 (range 41–623) and were 15-months-old.

Drought exposure

Experiments were performed on 50 potted seedlings. All leaves were numbered on every plant and leaf length and width (cm) were recorded at the beginning of the experiment. Leaf areas were estimated from a previously determined regression for the species:

$$A=0.66\times L\times W-0.5495 \ (R^2=0.995 \ N=20)$$

where area (A) is in cm^2. Seven well-watered control plants were harvested for measurements described below. At the start of the experiment 42 pots were moved to a shaded growth bench, which was also covered with plastic sheets to exclude rain, and allowed to dry. Pots were brought to field capacity by immersing them in water for ~10 min and weighed after water drainage ceased 2 d later. Plants were dehydrated for 23–46 d to reach the various wilting stages described below. Plants were harvested at visible wilt stages as shown in Fig. 1, i.e. slightly-wilted, wilted, severely-wilted, nearly-dead, and (presumed) dead. Additional plants that were in the last three categories were rewatered and kept irrigated for 2–3 weeks to determine if they were still alive and then harvested for the measurements described below.

The descriptions for the wilt stages were as follows. (1) Slightly-wilted: leaves green but leaf angled slightly towards the ground compared to controls. (2) Wilted: leaves green with leaf angles near 45° and leaf blades have begun to fold (curl) inward parallel to midrib and with very limited necrosis (grey-green to grey-brown). (3) Severely-wilted: leaves green, most leaf angles near 90°from horizontal and extensive curling of leaves, more extensive necrotic zones (grey-green to grey-brown) mostly near leaf margins or leaf tips. (4) Nearly-dead: most leaves necrotic, more extensive curling, leaf angles mostly near 90° from horizontal. Some young leaves still green near the midrib. (5) Dead: necrosis on all leaves, extensive curling, leaf blades brittle, leaf angles mostly near 90° from horizontal.

The visible wilt stages were developed in an independent experiment during field census measurements conducted every 2 weeks over a 22 week drought period, hence the visual condition of dead plants was based on extensive experience involving 28 species in a study to be reported elsewhere. When plants in the nearly-dead and dead categories were rewatered in the field most of the nearly-dead plants were alive after 6 weeks and most of the dead category plants showed no signs of life. All plants that survived drought grew new leaves and all had some green pliable leaves that were retained throughout the drought period.

Leaf water potential and water content

Leaf water content and water potential, *WC* and Ψ, respectively, were measured on leaf punches 5.6 mm diameter using Merrill leaf-cutter psychrometers (Merrill Instruments, Logan, UT). Leaf punches excluded regions with large leaf veins and crisp-dry-necrotic zones, because leaves do not dry uniformly and the main interest was in measurements of *WC* and Ψ were made in living portions of dehydrating leaves that were still alive. In addition, leaf punches were taken from necrotic zones of totally dead leaves. Typically 4–10 psychrometer samples were collected from each plant with usually one leaf disc per psychrometer, but two discs per psychrometer were used for dry leaves. Leaf punches were taken on every other leaf from apex to base (or a minimum of four leaves in small plants). *WC* at full hydration changed with leaf age so a range of leaf ages was needed to get a representative mean *WC* for a whole plant. The psychrometers were equilibrated in an insulated water bath at room temperature for >5 h and then Ψ was measured by the psychrometric mode using an automated multi-channel microvoltmeter (Model CR7, Campbell Scientific, Logan, UT). The CR7 actually made water potential measurements every 40 min with 15 s thermocouple cooling currents and stable readings were usually achieved in 3 h and always within 5 h. A final set of readings were made with 45 s cooling currents and this permitted more accurate reading when Ψ was <–3 MPa.

The psychrometers were frequently calibrated and all were capable of measuring Ψ down to –7.5 MPa. When Ψ of leaves or standards were <–7.5 MPa, the CR7 reported incorrect Ψ values usually >–3 MPa.

After Ψ was measured the leaf disc was removed from the sealed psychrometer chamber and immediately weighed to the nearest μg on a model MC 210 microbalance (Sartorius AG, Goettingen, Germany). The discs were then dried for >48 h in an oven at 60 °C and weighed again. *WC* was computed from $(W–D)/D$, where W=initial weight and D=dry weight. *WC* values made it possible to distinguish valid readings of Ψ <–2.5 MPa from invalid readings since valid readings consistently had much higher *WC*'values than invalid reading (see results). Values of Ψ <–7.5 MPa could be extrapolated from the trend-lines of standard water potential isotherm analysis, i.e. plots of $-1/\Psi$ versus *WC* (see results).

Soil water contents

One of the main interests was in relating wilting stage to some measure of soil water content, and a method was required that would be compatible with future needs to harvest roots from pots. Harvesting roots requires washing soil away hence it was not possible simply to measure fresh and dry weghts of the soil. Volumetric methods were also inappropriate in the clay soils

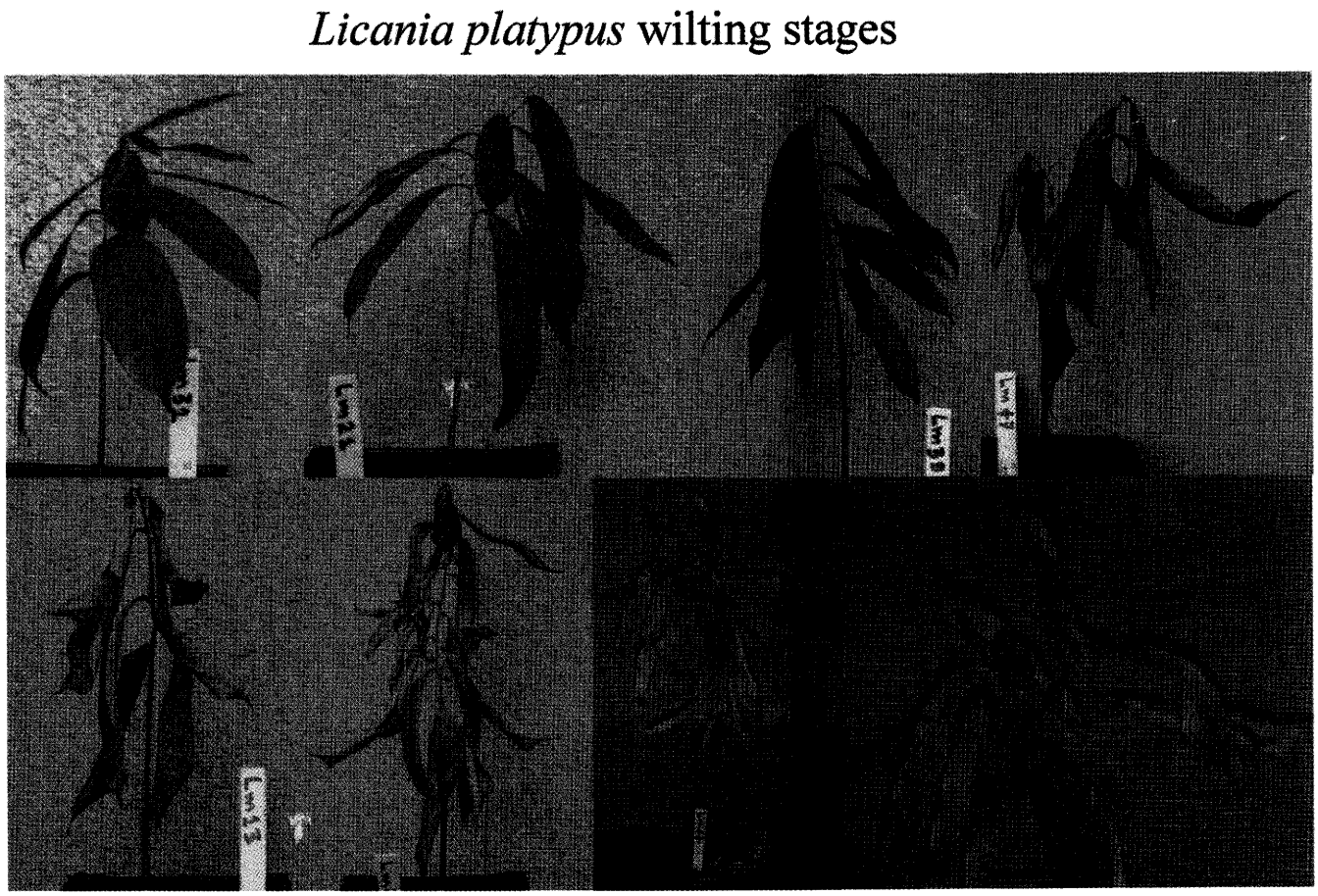

Fig. 1. Visible wilting stages of *Licania platypus*. From top left: Normal, slightly-wilted, wilted, severely wilted. From bottom left: nearly-dead, dead, nearly-dead plant rewatered for 3 weeks, close-up of previous picture.

because they shrank and cracked upon desiccation. A measurement of relative soil water content analagous to relative leaf water content, R_{swc}=(g soil water at harvest)/(g soil water at field capacity) was necessary, and this measure had to be made with reasonable accuracy with the plant in place. The following method was devised.

First the water content at field capacity was measured in representative pots. Pots were weighed 2 d after the immersion of pots in water for 10 min. After 2 d the water drainage had ceased and the weights equalled the soil+pot+plant weight at field capacity. The average pot weighed 55.6 g and by difference the soil+plant was 1626 g. Five pots with plants at field capacity were weighted (W_{fc}) and then dried in an oven to get the dry weight (W_D). The value of $W_o=(W_{fc}-W_D)/W_{fc}$ was 0.4074±0.0069 (mean ±sem). With this value of W_o it was possible to estimate R_{swc} of experimental pots from weights at field capacity (W_{fc}) and at harvest (W_h). The water loss as a fraction of weight at field capacity was then calculated ($W_l=(W_{fc}-W_h)/W_{fc}$). Hence the relative soil water content, R_{swc}, could be estimated from

$$R_{swc}=1-W_l/W_o=1-(W_{fc}-W_h)/(W_{fc}-W_D)=(W_h-W_D)/(W_{fc}-W_D).$$

It was estimated that the maximum error in the R_{swc} due to plant weight was <1%.

Whole stem and whole root hydraulic conductance

The entire pot and shoot were immersed in water and the shoot was excised 2 cm above soil level with a new razor blade. The leaves were immediately excised with the razor blade and the base of the shoot was connected under water to a compression fitting and peek tubing of the type supplied with a high-pressure flowmeter (Dynamax Inc, Houston TX). The peek tubing was passed through the tight fitting hole of a rubber stopper, sized to fit in the top of a 2.0 l vacuum flask. The whole shoot was sealed in the vacuum flask and the peek tubing was connected to a ULFM (ultra-low flowmeter) described below. Water was sucked through the whole stem system by applying partial vacuum pressures in the sequence of 0, 23, 26, 70, 59, 35, 12, and 0 kPa. This sequence of partial pressure permitted checking for hysteresis in the flow versus applied suction pressure. Hysteresis rarely occurred, but when noted the data were discarded and the measurement was repeated.

This method was very similar (except for physical scale) to that described by Kolb *et al.* (1996) and was selected as the method least likely to displace air in embolized vessels during measurement. The flow rates, however, were too small to measure very quickly using a digital balance, but this problem was solved with the ULFM. The slope of flow versus applied vacuum pressure gave the whole stem conductance, k_{ws}.

Whole root conductances were measured on rewetted pots within 20 min of excising the shoots under water. The HPFM technique was identical to that reported by Tyree *et al.* (1998). In a previous study on severely dehydrated olive seedlings LoGullo *et al.* (1998) found a semi-permanent loss of whole root conductance that lasted for days after soil was rewatered, so it was expected that similar effects would be found in *L. platypus*.

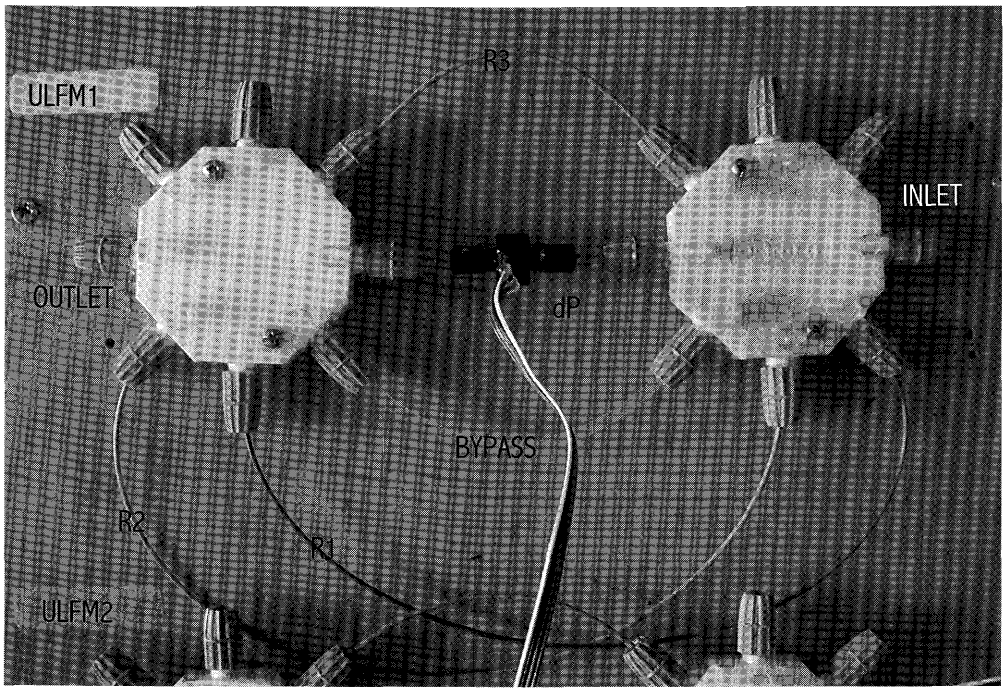

Fig. 2. Pictured is one of two Ultra-Low Flowmeters (ULFM) mounted on plywood. Water flows from the INLET to the OUTLET through two Omnifit 8-way values (catalogue number U-06473-12, Cole-Parmer, Vernon Hills, IL). Valves are adjusted so water flow is through one of three ranges determined by the ID and length of PEEK tubing (i.e. tubes range from R1 through R3: R1=0.13 mm ID by 28 cm long, R2=0.18 mm ID by 28 cm, R3=0.18 mm ID by 9.5 cm) and the pressure drop across the range tube is measured with a differential pressure transducer (dP=PX26-005DV, Omega Engineering, Stamford, CT). The BYPASS is 1.5 mm ID hard nylon tubing used for rapid filling of the system with water and for zero dP pressure checks. The four wires to dP provide power and send the signal to a datalogger connected to a computer. Although the pressure range of dP is 0–35 kPa, only the lower 0–5 kPa is used. During flow calibration dP was found to be reproducible to ±0.005 kPa, a long-term zero drift of about 0.05 kPa d^{-1} has been observed, but has little impact on measurements which typically required 10–20 min for seven measurements of flow at different suction pressures. The outlet tubing is 1 mm ID PEEK tubing connected to a compression fitting with custom cut rubber seals (catalogue number U-06473-07, Cole-Parmer). a PX26-001DV transducer with a dP range of 0–7 kPa was also used, but the zero drift and repeatability is about the same as the PX26-005DV and is more easily damaged by over-pressurization.

Ultra-low flowmeter (ULFM)

The ULFM (Fig. 2) uses the same principle for measuring water flow as the HPFM (Tyree *et al.*, 1995), i.e. it measures the pressure drop, *dP*, across a standard peek capillary tube which has been calibrated to quantify the linear relationship between flow rate and *dP*. In the ULFM the objective is to measure small flows with very small pressure drop across the peek tubing, because it was required that maximum *dP* be small compared to the maximum vacuum pressure applied (i.e. ≡70 kPa). The lowest flow range in the ULFM was 0–0.15 mg s^{-1} at a maximum *dP* of 5 kPa. This is low compared to the lowest range of a HPFM, which is typically 0–0.6 mg s^{-1} at a maximum *dP* of 100 kPa. The ULFM collected flow readings every 2 s and the response time for stable flow measurement in the ULFM was typically 20 s at the lowest flow range and 4 s at the highest. Although a digital balance readable to ±0.1 mg could be used for these measurements, reading intervals had to be typically 3–5 min per point to get standard deviations on measured values similar to those of the ULFM.

Results and discussion

Water potential isotherms

Water potential isotherms are shown in Fig. 3. When leaf-water content (*WC*) fell below 0.275 g H_2O g^{-1} dry weight (solid squares, Fig. 3A), the psychrometer could no longer read the correct value of Ψ because the cooling current could no longer condense water on the thermocouple junction. A standard isotherm analysis (Tyree and Jarvis, 1982) was performed on Ψ values when *WC* >0.275 and this is shown in Fig. 3B. The turgor loss point appears to occur at a *WC*≈1.25, which corresponds to a Ψ≈–2.6 MPa. A linear regression of (–1/Ψ) values between *WC* of 0.275 and 1.25 was performed with a few outliers removed at *WC* >1.0. The regression values were used to estimate Ψ values on leaf discs with *WC* <0.275.

The regression line is non-ideal because the *y*-intercept is >0. Readers are referred to Tyree and Jarvis (1982) for a discussion of the factors that cause non-ideal water potential isotherms. Briefly the non-ideal behaviour is probably caused by: (1) Osmotic coefficients that are ≠1 and changeable with concentration as osmolality of the cell contents increases during desiccation. (2) Cell lysis as cells die. The concentrated cell contents would be released into apoplastic water space, i.e. water in the cell walls and non-embolized vessels, which would cause further non-ideal changes in osmotic pressure. (3) At extreme desiccation

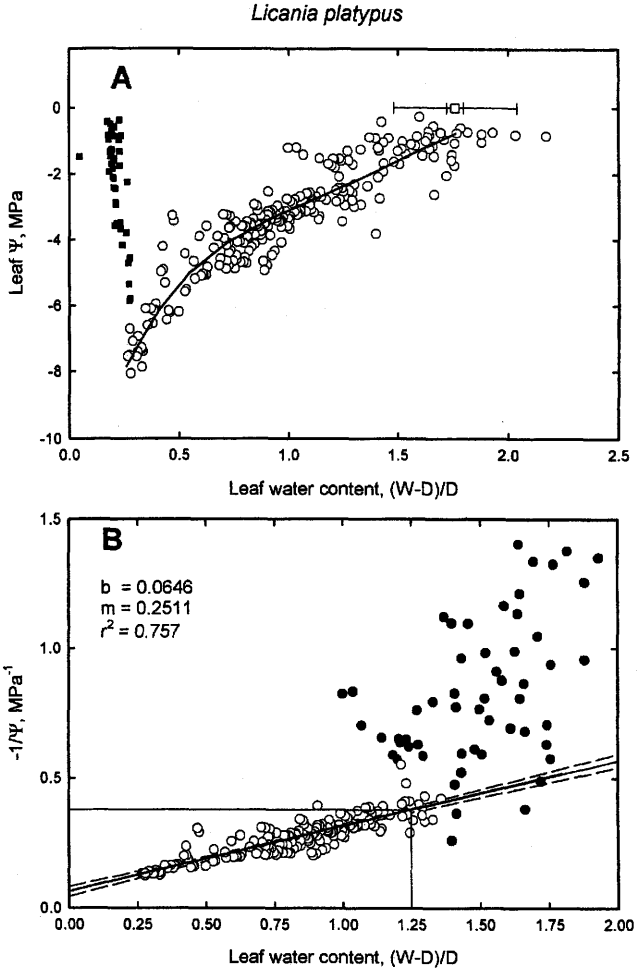

Fig. 3. Water potential isotherms of leaf tissue discs (5.6 mm diameter). (A) Psychrometer reading of leaf disc water potential (Ψ) versus leaf disc water content = $(W–D)/D$ where W=weight of disc after removal from the psychrometer and D=oven dry weight. Solid squares are invalid data and open symbols are valid data as explained in the text. The open square symbol at Ψ=0 is the water content of plants hydrated to zero water potential. The horizontal error bars are the SD and SEM (N=57). The high SD is not due to measuring error but is due to variation in WC with leaf age. The oldest leaves had WC=1.3 gradually increasing to 2.0 for the youngest leaves (data not shown). (B) Valid data from (A) replotted as $-1/\Psi$ versus leaf disc water content. Open symbols are valid data in the linear region (where turgor pressure is zero) and closed symbols are valid data where turgor pressure is >0. A solid line is linear regression of open symbols where m=slope and b=y intercept. Dashed lines are 95% confidence intervals on the linear regression. The horizontal and vertical fine-lines indicate the x-, y-values at the presumed turgor loss point, i.e. the last cluster of points on the straight line.

some precipitation of solutes would be expected. Extrapolation of Ψ values become less certain as WC falls below 0.275 because of non-ideality, but in this study extrapolations were over a fairly narrow range of Ψ from –7.5 to –9.1 MPa, i.e. WC from 0.275 to 0.193.

Leaf water isotherms and visible wilting stage

The bar histograms in Fig. 4A and 4B show how WC and Ψ of leaf punches, respectively, change with visible wilt

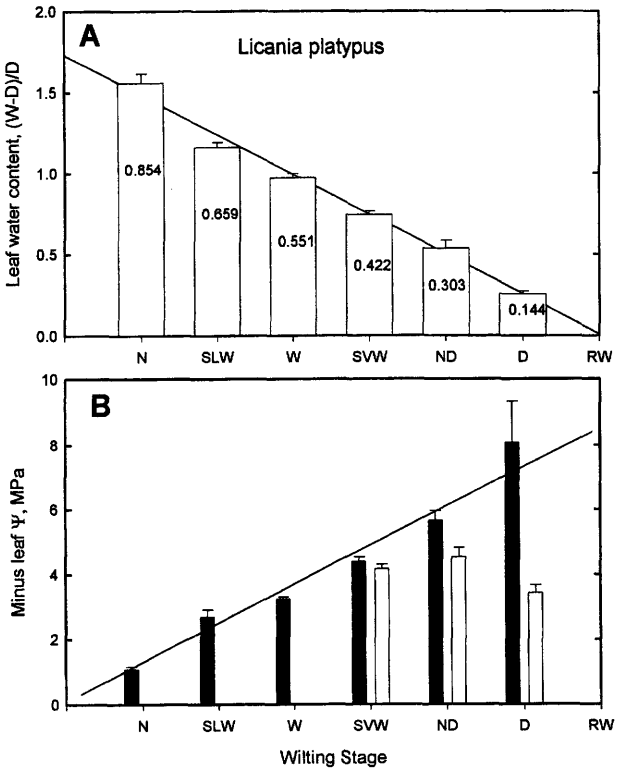

Fig. 4. Leaf water relations versus visible wilting stage as in Fig. 1. *N*, normal; *SLW*, slightly-wilted; *W*, wilted; *SVW*, severely-wilted; *ND*, nearly-dead; *D*, (presumed) dead. (A) Shows leaf disc water content versus wilting stage; water content is expressed as a fraction of disc dry weight. N was significantly different from SLW (P=0.05) and all others were different with P <0.002. Numbers in bars are computed relative water content values = (WC at harvest)/(WC at Ψ=0) from Fig. 3A. (B) Shows leaf disc water potential versus wilting stage. Solid bars give means of corrected psychrometer data, where the regression in Fig. 3B is used to compute disc water potential when WC <0.275, and white bars give raw psychrometer means of water potential. The raw values are shown only when the correction makes a difference. All error bars are SEM with n=20–48 leaf discs (corresponding to samples from 5–9 plants). All corrected water potentials means were significantly different from each other (P <0.001).

stage. The water potential of slightly-wilted plants was –2.67 MPa ±0.24 (SEM, N= 38), and was not significantly different from the turgor loss point inferred from the water potential isotherm in Fig. 3B.

Each wilting stage had a leaf-water content (Fig. 4A) that was significantly different from every other (p <0.05) and the water content varied nearly linearly with wilting stage. Leaves of *L. platypus*, like many tropical species in these field trials, desiccate very unevenly with dry-brittle leaf areas being confined mostly to leaf margins while the surviving leaves have green pliable areas of leaf blade near the midrib. This is especially evident 3 weeks after nearly-dead plants have been rewatered. Three severely-wilted and four nearly-dead plants were irrigated for 3 weeks and all plants survived. Old dead leaves tended to shed (Fig. 1E) whereas younger leaves with green areas around the midrib were retained (Fig. 1F) and new leaves usually