Journal of
Experimental
Botany

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 micro-voltmeter (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/Ψ 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

Licania platypus wilting stages

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_1=(W_{fc}−W_h)/W_{fc}). Hence the relative soil water content, R_{swc}, could be estimated from

$$R_{swc}=1-W_1/W_o=1-(W_{fc}-W_h)/(W_{fc}-W_D)=(W_h-W_D)/(W_{fc}-W_D).$$

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*.
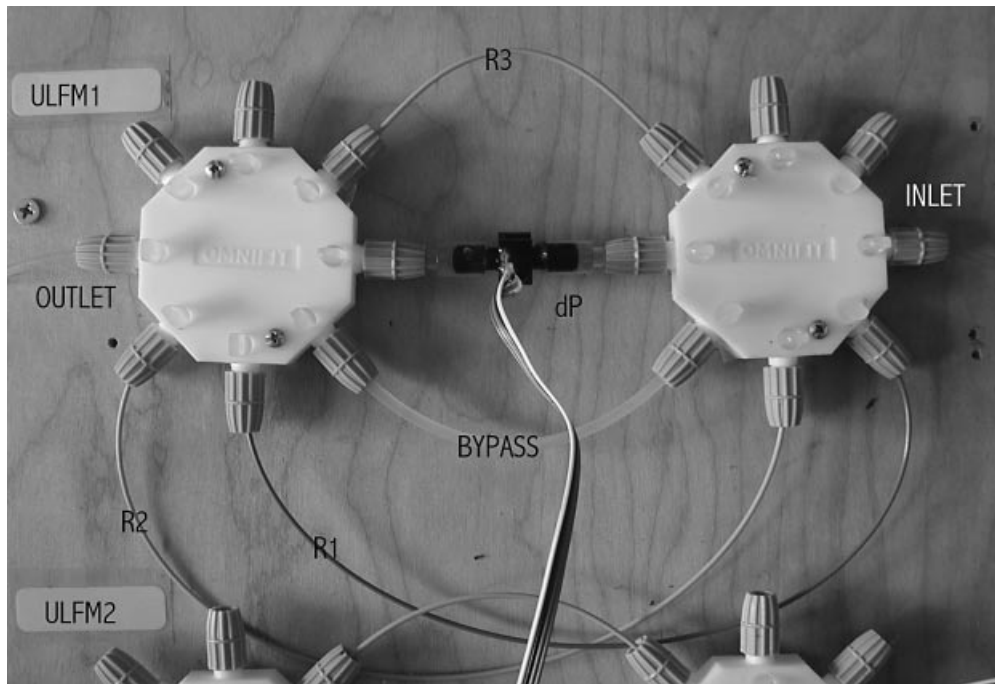
Fig. 2. Pictured is one of two Ultra-Low Flowmeters (ULFM) mounted on plywood. Water flows from the INLET to the OUTLET through two Omnifit 8-way values (catalogue number U-06473-12, Cole-Parmer, Vernon Hills, IL). Valves are adjusted so water flow is through one of three ranges determined by the ID 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$_2$O 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

Licania platypus
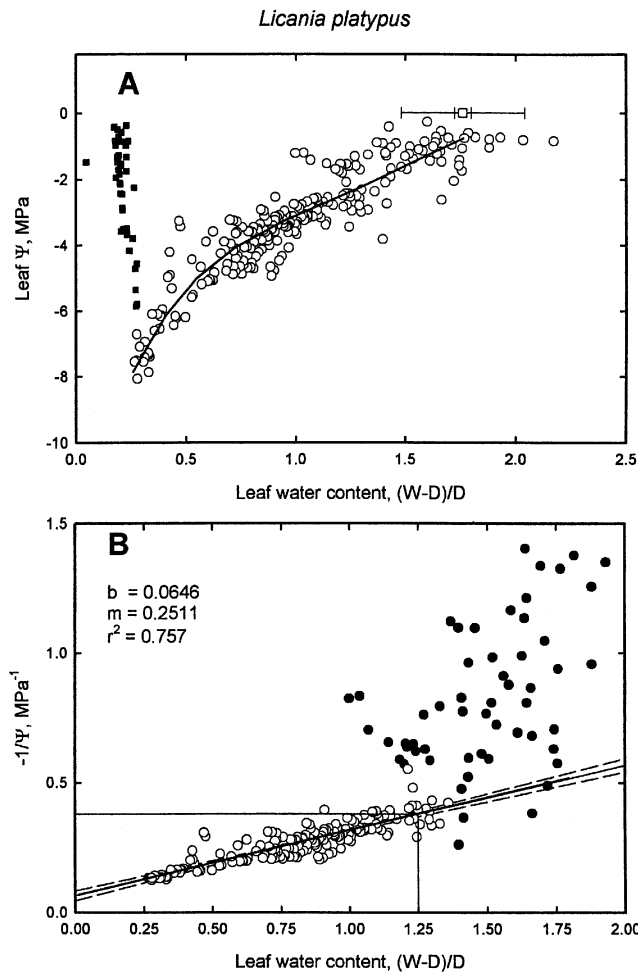
Fig. 3. Water potential isotherms of leaf tissue discs (5.6 mm diameter). (A) Psychrometer reading of leaf disc water potential (Ψ) versus leaf disc water content $= (W\text{–}D)/D$ where W=weight of disc after removal from the psychrometer and D=oven dry weight. Solid squares are invalid data and open 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
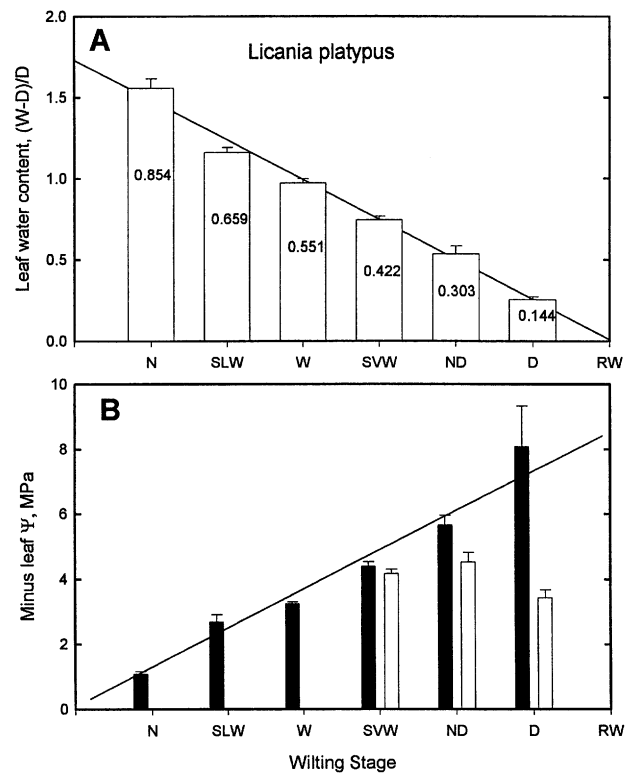
Fig. 4. Leaf water relations versus visible wilting stage as in Fig. 1. *N*, normal; *SLW*, slightly-wilted; *W*, wilted; *SVW*, severely-wilted; *ND*, nearly-dead; *D*, (presumed) dead. (A) Shows leaf disc water content versus wilting stage; water content is expressed as a fraction of disc dry weight. 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
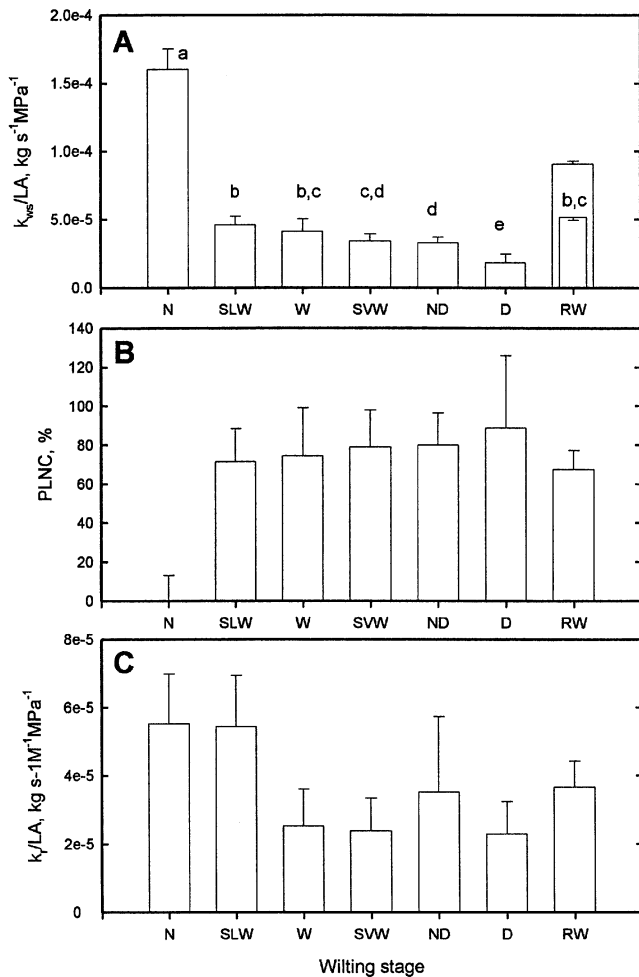
Fig. 5. Shown are root and whole stem hydraulic conductance data versus visible wilting stage. RW, state of rewatered plants that survived from the SVW or ND stages. See Fig. 4 for *x*-axis abbreviations. (A) Bars are mean whole-stem conductance per unit leaf area. For the first six stages, leaf area is leaf area at the start of the desiccation experiment for RW the leaf area is the area on the plant after 2–3 weeks when plants were harvested. Means that are significantly different $P < 0.05$ have different letters. The smaller bar in RW is the mean based on the initial leaf area before dead leaves were shed. (B) Bars are means of per cent loss native conductance, i.e. $PLNC=100 \times (1 - k_{ws}/k_n)$, where k_{ws} is the whole stem conductance measured at the wilting stage in (A) and k_n is the conductance at the native state. Error bars are estimates of how SEM would propagate based on SEM values (A, C) Bars are mean root conductance normalized to leaf area to make them comparable to values in (A). All error bars are SEM based on $n=5–9$ plants.

emerged. It is important to remember that all leaf discs were cut from the healthiest portions of living leaves. By contrast, two out of three plants in the dead category showed no signs of life after 3 weeks of irrigation.

Whole-stem and whole-root hydraulic conductance versus wilting stage

Hydraulic conductance of the whole-stem and whole-root, k_{ws} and k_r respectively, were approximately equal for unstressed *L. platypus* seedlings, but k_{ws} values declined much faster than k_r values as desiccation progressed (compare Fig. 5A–C). Whole-stems suffered a 70% loss of hydraulic conductance relative to the native state (PLNC, Fig. 5B) by the time they were slightly-wilted, i.e. when leaf Ψ was –2.6 MPa, which is in line with previous observations of vulnerability curves of several Panamanian species (Tyree *et al.*, 1994). What is unusual is that *L. platypus* retains a surprising large fraction (20–25%) of its native k_{ws} until it is dead. Rewatered plants (*RW*) irrigated for 3 weeks, which remained alive, recovered some hydraulic conductance, but the recovery was not significantly different from slightly-wilted plants. When it is taken into account that rewatered plants shed some of their initial leaf area it can be concluded that few if any of the vessels made dysfunctional during desiccation recovered their ability to conduct water. This interpretation is consistent with dye perfusions of stems that showed no more stained and conducting vessels in slightly wilted plants than in rewatered plants (data not shown).

No significant change in whole-root conductance per unit leaf area, k_r/LA, was found between visible wilting stages. Values of k_r/LA had a high standard error of the mean, hence a change of 50% of the native state would not be detectable. One of the main contributors to the high SEM in this study had to do with the mechanical properties of the compression fitting used for stems 4–7 mm OD. The non-ideal elastic behaviour of the rubber seal caused a non-zero slope on the transient measurement of conductance when a solid metal rod was used in place of a root. This technical problem has been known for years and is described in the technical manual provided by Dynamax. The typical error in conductance due to the compression fitting was $3.3 \times 10^{-7} \pm 1.5 \times 10^{-7}$ kg s^{-1} MPa^{-1}. This value was subtracted from all the raw values of root conductance, but the raw values were often only slightly bigger than the correction. In order to improve statistical power the root conductance values of N+SLW classes are pooled and compared to the pooled values of W+SVW+ND. The pooled means were 5.59×10^{-5} and 2.78×10^{-5} kg s^{-1} m^{-2} MPa^{-1}, respectively, and were significantly different ($P=0.02$).

Soil water content and wilting stage

L. platypus can extract approximately 70% of the soil water content from the clay soils of BCI before the SLW stage (Fig. 6). SLW to D stages were observed over a fairly narrow range of relative soil water contents (R_{swc}) of 0.30–0.15. Plants in soils with $R_{swc} < 0.15$ were dead, which corresponded to a gravimetric water content of 0.11 g water g^{-1} dry weight of soil.

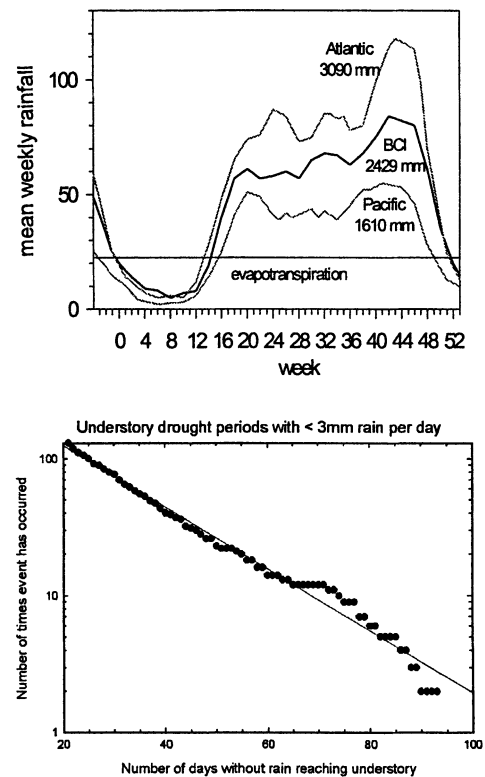## A history of drought events near Barro Colorado Island

From the above information it was concluded that *L. platypus* is quite desiccation-tolerant. In terms of drought
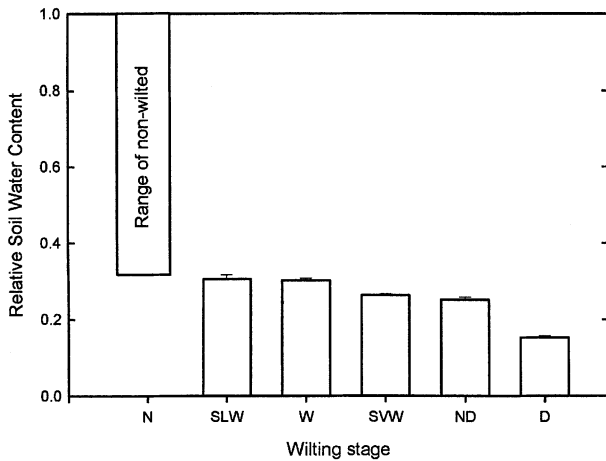
Fig. 6. Relative soil water content (R_{swc}) is plotted versus visible wilting stage. R_{swc} is defined as $(W_h-W_D)/(W_{fc}-W_D)$ where W_h=weight of pot plus plant at time of harvest, W_{fc}=weight of pot plus plant at field capacity, and W_D=dry weight. The bar for normal plants shows the range of water extraction that can occur before first signs of wilting.


Fig. 7. The upper curve is weekly average precipitation from three sites across the Panama Canal region based on data from Condit *et al.* (1998) for 27 years of rainfall data. Lower curve is an analysis of rainfall data for BCI from 1929 to 2000. A program was written to examine daily total rainfall. When rainfall exceeded 3 mm in one day, the number of days to the next rainfall event >3 mm was tabulated. The presumption is that >3 mm rain is needed for moisture to reach the forest floor; when precipitation is less it is all intercepted by the canopy and evaporates from there without reaching the ground. The *y*-axis is the number of times in 71 years that no rain reached the forest floor for the number of days (or more) on the *x*-axis. The line is the linear regression of the log-transformed data.

performance of 28 species in field trials *L. platypus* ranked in the lowest 33 percentile (9 out of 28) in terms of overall survivorship of a 22 week drought under field conditions (unpublished data).

People unfamiliar with the water relations of tropical plants are prone to assume that desiccation-tolerance strategies may be poorly developed. In equatorial regions where there is no pronounced dry season this may indeed be true for land plants, but probably not true for epiphytes, because even a few days without rain poses a serious drought for epiphytes. However, in tropical latitudes north and south of the equator, the intensity of seasonal droughts increase with latitude. So in the Panama Canal region (9° N latitude), there is a marked dry season, which is more pronounced on the Pacific than the Atlantic side (Fig. 7, upper). At the study site rainfall records are available for a >70 year period. During the 8 month wet season the monthly rainfall averages about 250 mm, but during the dry season it averages <50 mm per month (Fig. 7, upper). Moreover, there are many cases of prolonged periods with no rain. In three cases there has been no rainfall for a 50 d period or more, i.e. in 1962, 1966 and 1974–75. But the situation is often worse for the understorey because, in general, 3–5 mm of rain must fall on the forest canopy before significant quantities reach the ground. Rainfall events of <3 mm are generally intercepted by foliage and evaporate from there without ever reaching the ground. This analysis of rainfall data reveals that there is a log-linear relationship between the number of times rain has not reached the understorey and the length of the understorey drought period (Fig. 7, lower) for understorey droughts of >20 d duration. Since 1929 there have been 100 instances in which no precipitation has reached the

forest floor for 25 d or more or 23 instances for 50 d or more etc. Hence seedlings in the understorey of this study site are used to long periods without precipitation and soils can become quite dry in the upper 50 cm where most seedlings must extract their water.

Desiccation-tolerance in Licania platypus*: likely physiological strategies*

Desiccation-tolerance is believed by many (Tyree *et al.*, 1994; Tyree and Zimmermann, 2002) to be related, in part, to vulnerability curves. Vulnerability curves are often symmetrical in shape (upper thin-line in Fig. 8), but sometimes are asymmetrical (lower thick-line in Fig. 8). Vulnerability curves are usually constructed by measuring the initial hydraulic conductivity of stem segments, K_i, and then removing all embolisms by some means to get maximum potential conductivity, K_{max}. Per cent loss hydraulic conductance is then calculated from
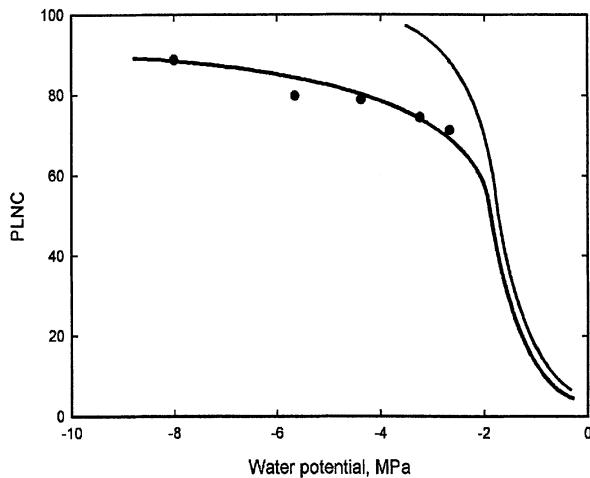
Fig. 8. Shows the typical shapes of vulnerability curves. Imposed on the thick-lined (lower) curve are points showing actual data values of PLNC from this study. See text for more details.

$PLC=100\times(1-K_i/K_{max})$. It was not possible to measure PLC in *L. platypus* because the usual methods of restoring hydraulic conductance failed; so *PLNC* (per cent loss native conductance) has been reported. It is felt that *PLNC* is ecologically more important than *PLC*, because plants in their native state can have *PLC* values anywhere between 0% and 60% (see, for example, Sperry *et al.*, 1991). It is considered that the maximum conductance which might be attained under artificial conditions is ecologically irrelevant. For desiccation-tolerance studies, what is most important is $PLNC=100\times(1-k_{stressed}/k_{native})$, i.e. how much whole shoot conductance falls under water stress compared to the value under 'normal' stress levels.

Although there is not a vulnerability curve for *L. platypus*, it is thought that the curve is probably shaped more like the thick- than the thin-lined curve in Fig. 8. Curves of this shape have been noticed in desiccation-tolerant plants (Tyree *et al.*, 1994). Vulnerability curves of this shape develop in conifers because small diameter latewood tracheids remain conductive after larger, more-efficient earlywood tracheids have embolized. Part of the desiccation-tolerance strategy of *L. platypus* appears to be to retain a relatively constant residual whole shoot conductance (20–30% of the native state) down to very negative water potentials ($\approx$ –6 MPa) and to maintain relatively high root conductivities even in dry soil, i.e. when leaf Ψ is –6 MPa and relative soil water content is 0.15. This is in contrast to olive root systems that lose 83% of their hydraulic conductance when predawn leaf Ψ falls to –3 MPa (=1.6 times Ψ at the turgor loss point), at which time the roots have developed corky layers. By pooling values of root conductance per unit leaf area, a significant decline of root conductance of about 50% in *L. platypus* could be resolved, i.e. plants at –3 MPa to –5 MPa had about half the root conductance of plants at 0 to –2.6 MPa.

In olive, the recovery of hydraulic conductance takes days after rewatering and is associated with the emergence of new roots (LoGullo *et al.*, 1998).

Another strategy of *L. platypus* is the 'programmed' death of older leaves before the younger leaves. Totally dead leaves are shed after soils again become wet, but leaves with some living tissue near the midrib are retained.

Surprisingly, *L. platypus* is not able to reverse the loss of whole stem conductance after soils become wet. *L. platypus* seedlings tend to have small-diameter vessels (20 μm) and capillarity alone might be expected to draw water to a height of 0.7 m in well-watered non-transpiring plants. The permanent loss of whole stem conductance may be due to sealing of vessels by tyloses or gums (Tyree and Zimmermann, 2002) but anatomical work will be needed to test this hypothesis.

The methods used in this preliminary study show promise for future work. The objective is to do similar studies in other species selected from the field trials of 28 species. In more desiccation-tolerant plants it might be expected to find (a) less drought-induced dysfunction in stem xylem, (b) better recovery of xylem conductance after rewatering, and (c) survival of living tissues to lower water contents and water potentials.

L. platypus died at –7 to –8 MPa (Fig. 4B), but has relatively low drought-survivorship (ranking 9 out of 28) and has a positive association with the relatively wetter slope soils (Harms, 1997). If this ranking of survivorship reflects mostly desiccation-tolerance rather than desiccation-delay, then many tree seedlings of the tropical moist forest may have surprisingly high tolerance to desiccation.

Many studies in the past have focused on comparing the vulnerability curves between species or between genotypes within a species. It has generally been assumed that plants with low vulnerability to loss of hydraulic conductance are necessarily drought-tolerant. However, there are very few studies that actually correlate survivorship after a drought event to measures of desiccation, for example, PLNC, *WC*, Ψ, relative soil water content, and visible wilting stage. Studies such as this are needed to evaluate the relative importance of desiccation-tolerance and the physiological components of desiccation-tolerance to plants' survival of drought.

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