Desiccation Tolerance of Five Tropical Seedlings in Panama. Relationship to a Field Assessment of Drought Performance

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Studies of the desiccation tolerance of the seedlings of five tropical trees were made on potted plants growing in a greenhouse. Pots were watered to field capacity and then dehydrated for 3 to 8 weeks to reach various visual wilting stages, from slightly wilted to dead. Saturated root hydraulic conductance was measured with a high-pressure flowmeter, and whole-plant hydraulic conductance was measured by a vacuum chamber method. Leaf punch samples (5.6-mm diameter) were harvested for measurement of leaf water potential by a thermocouple psychrometer method and for measurement of fresh and dry weight. In a parallel study, the same five species were studied in a field experiment in the understory of a tropical forest, where these species frequently germinate. Control seedlings were maintained in irrigated plots during a dry season, and experimental plants were grown in similar plots with rain exclusion shelters. Every 2 to 4 weeks, the seedlings were scored for wilt stage and survivorship. After a 22-week drought, the dry plots were irrigated for several weeks to verify visual symptoms of death. The field trials were used to rank drought performance of species, and the greenhouse desiccation studies were used to determine the conditions of mesophyll plants correlated with field assessment of drought performance for the five species ($p^2 > 0.94$).

Spatial and temporal variation in the composition of tropical forest plant communities has fascinated researchers for a long time, and understanding the causes of this variation remains a prominent topic of study. The factors that best correlate with the distribution of individual species and with diversity gradients in tropical forests are the annual amount and seasonality of rainfall (e.g. Gentry, 1988; Conditt, 1998; Veenendaal and Swaine, 1998; Bongers et al., 1999). Wet forests typically have many more species than dry forests, and most species occur only over a limited range of rainfall. Tropical forests also undergo dramatic temporal changes in species abundance and distribution, on scales of decades as well as thousands of years (e.g. Flennery, 1998). Again, these shifts in species composition seem to be associated with changes in moisture availability. Because tropical forest plants experience drought and water stress (Tobin et al., 1999; Walsh and Newberry, 1999; Nakagawa et al., 2000), moisture availability in tropical forest soils may be one of the main factors influencing plant growth (Chandrasekhar et al., 1998; Ito et al., 2000; Poorter and Hayasida-Oliver, 2000), mortality (Cox et al., 1995; Veenendaal et al.,

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cell survival at low water content (ψ) and low water potentials (Ψ), increased resistance of xylem to embolism, and ability of cells (especially mesistems) to remain alive at low ψ or Ψ.

Although species differences in drought resistance has been hypothesized to explain differences in species distribution in rainfall gradients, we know of no published systematic studies attempting to measure drought resistance in terms of survivorship and to see if drought survival can be explained by either desiccation tolerance or desiccation delay. This paper focuses on desiccation tolerance properties and relates them to survivorship in the field. In field experiments, we compared performance of watered plants with plants in rainout shelters, and we monitored will state and survivorship over a 22-week period coincident with the natural dry season in central Panama. In parallel greenhouse experiments, we correlated visual symptoms of wilt with changes in leaf water content (ψ), leaf water potential (Ψ), and whole-stem hydraulic conductance (i.e. the whole shoot with leaves removed [kₑ])

We also quantified desiccation tolerance, how dry plants can become and still survive after rewatering, and compared this with measurements of drought performance under field conditions. We argue that to understand the role of the commonly studied mechanisms of drought resistance, one must quantify drought performance and desiccation tolerance. Unless we can quantify whole-plant responses that integrate complex, interacting mechanisms (e.g. desiccation tolerance and drought performance), our understanding of specific mechanisms will be reduced to the limited insight of comparative physiology (see “Discussion”).

RESULTS

The dry season of 2000 to 2001 started and ended about 3 weeks later than normal (based on 71 years of weather records) but had average precipitation for a dry season. The rainout shelters were relatively ineffective at keeping out moisture once the rainy season returned, but during the dry season gravimetric water content averages about 20% below that of the wet plots.

Examples of wilting states are shown in Figure 1 from greenhouse experiments, and these are representative of wilting states in field experiments. Survival values and wilting states on the field census dates are shown in Figure 2. Even slight wilting was rare in the wet plots; therefore, wilting states are given only for the dry plots. Mortality in the dry plots was significantly higher than in the wet plots after 22 weeks for C. longifolium, V. surinamensis, and L. platypus (ANOVA, n=102) but not significantly different for O. lucens and D. pyrifera panamensis (ANOVA P > 0.5), both of which were thought to be tolerant of the average drought in Barro Colorado Island, Panama.

In greenhouse experiments, in contrast, all species were desiccated until death was noted. The survival figures for plants rewatered from the last three wilt states are shown in Table 1. Moribund plants might be defined as plants in the driest wilt state with 50% mortality; however, we believe that replication of rewatered plants was not sufficient to determine the moribund wilt state with statistical certainty. However, even if we could determine the moribund wilt state, we are unlikely to get accurate values of leaf water potential (Ψ) and water content (ψ) because most leaves are dead. In all species except O. lucens, the ψ of necrotic (discolored) tissue was significantly less than green tissue (data not shown) for plants in any given wilt state. In the “nearly dead” state, buds may be alive, but nearly all leaves are necrotic; hence, few samples of living leaf tissue can be found. A biologically significant variable for desiccation tolerance would be the lowest values of ψ and Ψ associated with living leaf tissue. Values of ψ and Ψ versus wilt state are shown in Figure 3.

The hydraulic conductance of whole stems (without leaves) generally fell with wilt state in C. longifolium, L. platypus, O. lucens, and D. panamensis (Fig. 4A; see Table II for abbreviations). V. surinamensis stem conductances were discarded because they were much lower than the other species, apparently because of a pink latex that exudes from cut petioles. The whole-stem hydraulic conductances are expressed as percentage change from unstrressed controls (percentage native conductance), and the conductance of controls is given in the caption of Figure 4. In all species, whole-plant conductance declined with wilt state except for occasional increases in the dead state, which also corresponded with stem splitting. The hydraulic conductance of roots (approximately 0.5 h after rehydration) was generally not significantly different (Fig. 4B). Root hydraulic conductance (scaled to leaf area at the start of the experiment) of O. lucens increased with wilt states 1 and 3 for possible reasons to be discussed below.

DISCUSSION

For many years, researchers have studied drought-induced xylem dysfunction. Vulnerability curves relate how stem segment hydraulic conductance declines with stem water potential (Ψₑₑₑₑ), and these have been measured on >40 species of plants, e.g., see the literature survey of Tyree et al. (1994). More recently, a vacuum chamber technique has been used to measure vulnerability curves on whole shoots of arid zone species (Kulb et al., 1996). Many species have been compared in terms of the Ψₑₑₑₑ needed to induce 50% loss of hydraulic conductance (Ψₑₑₑₑ), 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 Ψₑₑₑₑ, respectively. The presumption is that the plants

Figure 1. Wilt state of Calophyllum longifolium (A–F) and Ouratea lucens (H–O) are shown for representative plants. Wilt stages are: normal (A and H), slightly wilted (B and I), wilted (C and K), severely wilted (D and L), nearly dead (E and M), and dead (F and N). Also shown are plants before and 60 d after rewatering for CAL53 (severely wilted → alive, D and G) and OUR28 (nearly dead → alive, O and P).
Figure 2. Shown are field survey results of survival and wilt state for *C. longifolium* (CAL), *Licania platypus* (LIC), and *O. lucens* (OUR). Black and white symbols, Survival in wet and dry plots, respectively. Results for CAL were very similar to those of *Virola surinamensis*, and OUR were similar to *Dipteryx panamensis* (DIP). The recovery of wilt states of living plants after week 18 was due to resumption of rain events and lateral movement of water to the rain-out shelters from the wetter regions, perhaps by lateral hydraulic lift facilitated by the mature trees.

Tend to die when stems lose too much hydraulic conductance; hence, species with xylem more resistant to cavitation are presumed to be more drought resistant. However, to our knowledge, there are no studies of whole plants that demonstrate what values of $w$, $\Psi$, or $K_{stem}$ correlate with plant death. There is, however, a growing body of evidence that loss of stem hydraulic conductance correlates with branch dieback in the shoots of some temperate plants (Rood et al., 2000; Davis et al., 2002; Sperry and Hacke, 2002). There have been studies of leaf tissue viability after desiccation (Peace and McDonald, 1980; Buckley et al., 1981; Loesch, 2001), but our field experience has revealed that many plants are still alive even after most or all leaf tissue is dead.

One objective of our work was to monitor loss of conductance of whole stems (without leaves) of seedlings as they approach death. Whole-shoot conductance fell to a critical percent of native state (controls) of about 15% to 20% (i.e. 80%–85% loss conductance) in severely wilted and nearly dead plants (Figs. 4 and 5). Plants with good drought performance fell to a critically low conductance at much more negative $\Psi$ values than *C. longifolium*, which has poor drought performance. Hence, our limited data set is consistent with the notion that vulnerability to cavitation determines desiccation tolerance and that the critical value of whole-stem conductance for survival of seedlings might be around 15% to 20% of unstressed plants (Tyree et al., 1994). In many species, the cavitation of xylem in roots might have a larger impact on desiccation tolerance than in shoots. More work is needed to determine if this is generally true.

The effect of drought on whole-root hydraulic conductance has been studied in Crassulacean acid metabolism plants (e.g., Nobel and Sanderson, 1984; North et al., 1992) but less commonly in non-Crassulacean acid metabolism species. The only example we know of is a study on seedlings of olive (*Olea oleaster*). Root conductance of irrigated seedlings was compared with root conductance of droughted seedlings measured immediately after rewatering the seedlings. LoGuolo et al. (1998) found that drought reduced root conductance, and this reduction was correlated with morphological changes to the root in response to dry soil, i.e. the formation of corky layers and shedding of fine roots. Recovery of root conductance after a drought event correlated with the emergence of new fine roots.

Rather more work has been done on loss of conductance in woody root segments (Kolb et al., 1996; Tsuda and Tyree, 1997; Hacke et al., 2000; Sperry and Hacke, 2002), but it is difficult to extrapolate from root segments to whole roots without resorting to some kind of model. The hydraulic conductance of whole roots is likely to be limited more by fine root conductance than woody root conductance for reasons discussed in detail by Tyree and Zimmermann.

Table 1. Survival data in greenhouse desiccation experiment

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<th>Species</th>
<th>Seperately Wilted</th>
<th>Nearly Dead</th>
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<td>CAL</td>
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<td>OUR</td>
<td>7 Nearly</td>
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<td>3 Nearly</td>
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<tr>
<td>DIP</td>
<td>5 Nearly</td>
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Table II. Survival data in greenhouse desiccation experiment

<table>
<thead>
<tr>
<th>Species</th>
<th>Seperately Wilted</th>
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(2002); hence, an 80% loss of conductance observed in a large woody root segment is likely to have 3- to 5-fold lower impact on loss of whole-root conductance. Our technique for measuring root conductance is likely to dissolve most embolisms in the vessels of roots; hence, it measures only the effect of drought on morphological changes such as loss of fine roots and growth of cortex in thicker roots.

At one extreme, L. platyphyllos behaved like oak seedlings, but at the other extreme, O. lactea increased root conductance > 200% when in the wilted state versus controls (Fig. 4B). This increase in root conductance in response to drought might be a new mechanism of desiccation delay not reported previ-
ally high desiccation tolerance of O. laciniata (see below), but more work needs to be done to resolve the issue.

Quantifying Drought Performance and Drought Resistance

We introduced the classical definition of "drought resistance" in the introduction, where drought resistance is defined in terms of its possible physiological mechanisms. Although we have a notion of what drought resistance represents, we do not have any way of measuring it and this is a major conceptual obstacle that must be overcome if we ever hope to understand drought resistance at a mechanistic level. In contrast, we understand the mechanisms of electrical circuits in part because we have an instrument to measure electrical resistance; similarly, we understand the hydraulic architecture of plants because we have an instrument to measure hydraulic resistance (or conductance) of whole plants or plant parts. Without a quantitative measure of drought resistance, we cannot decide the relative importance of the various measures of desiccation tolerance and desiccation delay in determining drought resistance.

Without a measure of drought resistance, we are confined to the limited insights gained by comparative physiology. Comparative physiology methods might reveal the species A has higher cuticular resistance than species B, and species A is less vulnerable to cavitation than species B and because species A grows in a drier ecosystem than species B. Therefore, we might think A is more drought resistant than B, and this difference might be explained by the cuticular resistance and the vulnerability to cavitation. The weakness of this kind of comparative study is that we usually take A as "given" that A is more drought resistant than B without experimental proof, and, hence, we cannot evaluate if the physiological traits reported even correlate with the superior growth or survival of A versus B. Furthermore, such comparative studies provide no information on the relative importance of traits of desiccation delay and desiccation tolerance.

Survival data similar to that in Figure 2 might ultimately provide measures of drought resistance or drought performance, which are different concepts as explained below. If we perform a pristine experiment on a number of species in which drought is the only controlled stress, then there will be a singular plant response to the single stress that will be manifested in terms of differences in growth or survival of drought-stressed plants versus the controls. Such differences in growth and survival might be used to measure drought resistance. On the other hand, if we perform an experiment with multiple stresses from, say, drought, pathogens, and insects, the plant could well have a very complex response. The stress response mechanisms might interact with each other such that the stress of drought might make an attack of a pathogen more serious or lethal, whereas the attack of the pathogen alone might reduce growth, but not increase mortality (Schoeneewiss, 1986). Alternatively, some types of stress may actually precondition the plant to withstand drought better. In most field-based experiments and in many greenhouse experiments, we usually have control over one or more stresses but there may be other uncontrolled stresses changing the growth and survival responses, which we might call measures of drought performance.

In an ecological setting, measures of drought performance might be more important than measures of...
drought resistance. However, if the objective of an experiment is to evaluate the relative importance of the mechanisms of drought resistance (desiccation tolerance and desiccation delay), then single-stress experiments might be more appropriate.

How can we use survival as an initial measure of drought performance? One measure might be the differential survival at the end of the 22-week drought period in Figure 2, i.e. $D_{S_2}^* = S_2 - S_0$ where $S_0$ is the percentage survival of the irrigated plants and $S_2$ is the percentage survival of droughted plants. However, we have some mortality in irrigated plants hence, multiple stresses were present. In some cases, the non-drought stress might act independently of drought and occur equally in both irrigated and dry plots. In this case, we might want a measure $D_{S_2} = 100(S_0 - S_2)/S_0$ evaluated at 22 weeks. A minor disadvantage of $D_{S_2}^*$ and $D_{S_2}$ is that plants with good drought performance (e.g. O. lucens in Fig. 2) have values of nearly 0% for $D_{S_2}$ and $D_{S_2}^*$ whereas we might want a better performer to have a bigger number. This is resolved by defining new parameters of drought performance as:

$$D_{S_2} = 100 - D_{S_2} = 100 - (S_0 - S_2)$$

or

$$D_{S_2} = 100 - D_{S_2}^* = 100 - 100(S_0 - S_2)/S_0 = 100(S_2/S_0)$$

The parameters defined in Equations 1 or 2 might be appropriate for drought performance in a time-limited measure of the effects of drought, but these measures are inherently limited because: (a) The values computed will change with the duration of the drought, and (b) it is not good at evaluating differences in species at the extremes of low and high drought performance. To be most useful, the survivor data will have to be evaluated when as many species as possible in the trial lie between the two extremes of complete survival and complete mortality. A better measure might be based on time integrals of the survivorship curves in an experiment carried out over a time interval large enough to cause complete mortality of all species. In that case, plants with low drought performance will have a smaller integral value than plants with good drought performance, but these issues are beyond the scope of this paper. For now, we will use the values of $D_{S_2}$ and $D_{S_2}$ evaluated at 22 weeks (Fig. 2) to see if either measure of drought performance is related to some measure of desiccation tolerance.

Is Drought Performance Correlated with Desiccation Tolerance?

Two possible measures of desiccation tolerance are the leaf Ψ or leaf relative water content ($R_w$) just before death. In Figure 6, we plotted values of $D_{S_2}$ versus the mean Ψ or mean $R_w$ evaluated in severely wilted plants. The r² values are >0.9, and a significant correlation is found. Values of $D_{S_2}$ and $D_{S_2}$ differed from each other only a few percent in the five species of our study; therefore, r² values are also >0.9 for the $D_{S_2}$ parameter. Other combinations of plots with $D_{S_2}$ or $D_{S_2}$ versus Ψ and $R_w$ values for nearly dead plants also have r² > 0.9 (data not shown), but mean values of Ψ and $R_w$ are based on quite small data sets in nearly dead plants because little living tissue was found for measurement of Ψ and $R_w$.

To our knowledge, our study is the first in which quantitative measures of drought performance are correlated with quantitative measures of desiccation tolerance. We tentatively conclude that most of the differences in drought performance of the five species in our study are explained by differences in desiccation tolerance. Much more work is needed to see if this relationship holds for more species. Also,
more robust measures of drought performance must be sought. If the correlation is less strong with a regression involving 15 or 20 species, then residuals from the regression might serve as a basis for evaluat- ing through a statistical model the impact of para- meters that provide measures of desiccation delay.

MATERIALS AND METHODS

Five species from central Panama were selected for this study. Two were thought to be drought resistant (Yucca sarothrae and Cattleya loricata), two were thought to be drought resistant (Hotive Lycium and Diplasterospermum), and one was intermediate (Lycoris ADVISED; see Table 5). Selection of species was based in part on suggestions of likely drought performance from field observations by plant ecologists who have worked at Barro Colorado Island, Smithsonian Tropical Research Institute, in central Panama.

Field Trials for Withholding States and Soilsurvivability

Within five days after collection, each xeromorph was seeded into germination in the greenhouse under moderately low light conditions (5%-10% full sunlight). They were then transplanted and grown in pots (0.3-0.5 cm) according to seeding size in the greenhouse with root-repelling potting soil. Seedlings were transplanted to plots in the field from September through November 2001. For all seedlings, the dry season 2000-2001 was the low-gp year expected. Due to time of transplanting and germination, the seedlings were between 9 and 2 months old at the start of the experiment. Our intention was to work with seedlings at the age naturally occurring at the onset of their first dry season. Seedling size varied between species, with an average seedling height of the species of 60 to 250 mm and average leaf area of 27 to 265 mm^2 (Table 3).

The seedlings were exposed to two treatments, wet and dry, for 22 weeks in the dry season of 2000 to 2001 (December 19, 2000-July 12, 2001). Sixty plots (0.2 x 0.4 m) were established in the understory. Light conditions in the plots were assessed with hemispherical photographs (model Grichcopic-90, Nikon, Melville, NY) and analyzed with Hemiscan 2.1 (Delta-T Devices, Cambridge, UK). Hot-probe positions were chosen among the established tables with a minimum distance of 1 m between plots, and all plots were situated near large trees to allow for root competition for water. All plots were caged with wire mesh (1.1 x 1.3 x 0.5 m) to exclude vertebrates (and some invertebrate herbivores) and to minimize damage through leaf, twig, and branch fell. The species were randomly assigned to positions within the plots. One seedling of each species was transplanted to each plot, and their growth and survival followed during the course of the experiment. To accommodate some degree of light variation from both substrates, the plants were potted by light conditions as predicted by the Hemiscan analysis, and one of each plot was randomly assigned to each treatment.

Dry Xets were covered with rain-out shelters made from transparent plastic to protect them from any dry season rains. Light intensities (photometric photon flux density) decreased by approximately 75% due to the plastic cover (assessed by quantum sensor measurements, LI-COR, Lincoln, NE). Wet plots were watered regularly with water from the Lake Gatun. Initially, 35 mm (1.3 L) of water was applied with watering cans three times a week, equivalent to 105 mm of monthly rain. Later, in the dry season, the amount of water applied had to be increased because competition from neighboring plots decreased relative soil water content even in the wet plots. The amount of water applied was increased individually for the different according to the number of the seedling. Cumes of greenhouse soil were not added to the water content, seed survival and plant survival were initially conducted monthly, later biweekly throughout the experiment.

Greenhouse Trials for Desiccation Tolerance

Methods for seedling desiccation tolerance trials were developed for L. corymbosa, and the procedures for the other species are described elsewhere (Tyree et al. 2002). In brief, we did the following for all species.

- Seedlings were grown under moist conditions but simulated drought conditions (15%-20% photosynthetically active radiation measured above the fan canopy) for 20 months. Plants were grown in unsterilized forest soil collected from the site of our field trials above. Sola had high clay content. A total of 20 seedlings were grown in 20 months. Plants were then transplanted in water for a few minutes to stimulate root development. Measurements were made at 6 month intervals. Plants were destructively harvested (fully wilted, wilted, severely wilted, nearly dead, and dead). Because we could not determine if mortality was attributable to drought stress or the death of plants from the last three states were not observed and for an additional 3 months.

The following measurements were performed on all the above states (category) (a) tissue water content (measured by thawing-hermaphroditic psychrometers on 6 to 10 leaf pods per plant, b) leaf fresh and dry weight of the samples used for the thermocouple psychrometers, c) whole-leaf hydraulic conductivity measured by a high-performance fiber optic probe system, and d) whole-leaf conductance measured for a high-performance flow meter, and e) whole-leaf conductance measured for a high-performance flow meter, and f) whole-leaf conductance measured for a high-performance flow meter. Although leaf conductance measured for a high-performance flow meter, and f) whole-leaf conductance measured for a high-performance flow meter, and f) whole-leaf conductance measured for a high-performance flow meter, and f) whole-leaf conductance measured for a high-performance flow meter, and f) whole-leaf conductance measured for a high-performance flow meter, and f) whole-leaf conductance measured for a high-performance flow meter, and f) whole-leaf conductance measured for a high-performance flow meter, and f) whole-leaf conductance measured for a high-performance flow meter, and f) whole-leaf conduc