Journal of Tropical Ecology (2007) **23**:497–500. Copyright © 2007 Cambridge University Press
doi:10.1017/S026646740700421X Printed in the United Kingdom

SHORT COMMUNICATION

Visual assessment of wilting as a measure of leaf water potential and seedling drought survival

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(*Accepted 23 April 2007*)

Key words: method, mortality, performance, regeneration, water relations

Rainfall and soil moisture availability vary greatly both spatially and temporally. They are prime factors influencing species distribution patterns, diversity and habitat associations in a range of biomes, and limit primary productivity in many natural ecosystems, as well as in forestry and agricultural systems (Hawkins *et al.* 2003, Kozlowski & Pallardy 1997, Lieth 1975). Projections of drying trends, and increased frequency and intensity of drought events with climate and land-use changes (Hulme & Viner 1998, IPCC 2001) have fuelled an increased interest in the role of drought in determining the structure and function of natural and managed forest systems (Allen & Breshears 1998, Miles *et al.* 2004). Such projections accentuate the need to assess, understand and predict plant reactions to drought, as well as soil moisture variation at different scales.

In most tropical forests, dry-season droughts occur regularly once or twice per year, and especially severe droughts occur in association with El Niño climatic events (Walsh & Newbery 1999). They can lead to considerable drought stress in natural as well as managed tropical forests, with differential susceptibility to drought among species (Engelbrecht & Kursar 2003). Recent evidence indicates that, even in moist and wet tropical forests, low water availability plays a prominent role for plant population dynamics, local and regional distribution patterns, community composition and diversity and ecosystem function (Bongers *et al.* 1999, Bunker & Carson 2005, Chazdon *et al.* 2005, Engelbrecht *et al.* 2007). Furthermore, in agroforestry, a major factor determining the success of stand establishment in tropical reforestation efforts is known to be the careful matching of water availability and the moisture requirements of species (Evans 1996, Gerhardt 1993).

At the large scales required for comparative ecological or forestry studies it often is not possible to apply the difficult and costly techniques typically used to assess soil moisture variation, leaf water potentials, or plant responses to drought. The development of proxies that are easy to assess and that allow one to infer the spatial variation in plant and soil water status, as well as the variation in drought survival among a large number of species would significantly assist such studies.

Wilting under drought stress is a very commonly observed phenomenon, and indeed is probably the most widely used indicator of plant drought stress. This visual cue is used in agriculture and gardening, and has also been used to assess plant drought resistance in ecological studies (Bannister 1986). However, to our knowledge, no studies have explicitly examined the relationship between wilting and leaf water potentials or drought survival. The aim of this study is to quantitatively determine whether wilting, scored visually, provides a suitable proxy for assessing leaf water potentials as well as for comparing drought survival among species. We analysed data from (1) a screen-house experiment on drought tolerance of seedlings of five tropical woody species (Tyree *et al.* 2003), and (2) a comparative field experiment on drought effects on seedling survival of 28 species from 21 families, representing different life forms (shrubs and trees), phenologies (evergreen or deciduous) and seed sizes (Engelbrecht & Kursar 2003). Both experiments were conducted in the moist tropical forest of the Barro

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Table 1. Description of the categories used in the visual assessments of wilting. In the category 'nearly dead', A was used in the field experiment, B was used in the screen-house trials, since leaf water potentials cannot be measured in dead leaves.

Category	Wilting stage	Visual characteristics
1	Normal (not wilted)	*No signs of wilting* or drought stress
2	Slightly wilted	*Slight leaf angle* changes but no folding, rolling or changes in leaf surface structure
3	Wilted	*Strong leaf angle change* or *protrusion of veins on the leaf surface* but no cell death
4	Severely wilted	Very strong change of leaf angle or protrusion of veins on the leaf surface with *beginning necrosis*
5	Nearly dead	A. *All leaves dead, but stem alive*; distinguished by colour and elasticity
		B. *Most leaves necrotic*, some young leaves still green near the midrib, leaf angles mostly near 0°
6	Dead	*All above-ground parts dead*, no resprouting after rewatering at the end of the experiment

Colorado Nature Monument (BCNM) in Central Panama (Leigh 1999). Wilting was observed in all 28 species studied. In most species, leaves or leaflets drooped first, then rolled or folded. This was usually followed by macroscopic changes in leaf surface structure, namely protrusion of the minor leaf veins. However, in some species leaves moved upwards with progressing drought (e.g. *Cupania sylvatica* Seem.), and in one species (*Hymenaea courbaril* L.) no leaf angle changes were observed, but upon careful inspection, protrusion of the leaf veins could be detected even in this species. Even after all leaves were dead, many species were still clearly alive, having green stems and regrowing after rewatering (Table 1).

The relationship between wilting and leaf water potentials was analysed from a screen-house experiment with greatly varying drought survival in the field (see Figure 1 and below). Seedlings (28–39 per species) of equivalent size were experimentally dried out under moderate light conditions (5–10% full sun), and their wilting visually scored in five categories (Table 1, for photos of examples see Tyree *et al.* 2002, 2003) in the late morning. In each of the five different wilting categories, leaf water potentials were psychrometrically measured for a subset of plants, with 4–10 leaf punches taken per plant (see Tyree *et al.* 2003 for details).

Leaf water potentials (log-transformed) decreased in relation to the five categories of plant wilting. The correlation was highly significant within each of the five species examined (Figure 1). The slope of the relationship varied somewhat between species, but the differences were not significant. Leaf angle changes and other visual signs of drought stress became progressively more pronounced with advancing drought conditions and decreasing leaf water potentials, as was to be expected. However, various morphological, anatomical and physiological traits are involved in the visual expression of wilting. Additionally the study species included a full range of drought survival responses. Hence, we expected to find a rather complex relationship between wilting and leaf water potentials. We were thus surprised to find such strong relationships between leaf water potentials and our simple visual assessment of wilting stages.

To measure leaf water potentials, a variety of methods is available (e.g. pressure chamber, psychrometer, cell pressure-probe, reviewed in Boyer 1995). However, they are relatively expensive, time consuming and/or destructive for the plants, and require a thorough understanding of plant–water relations. A suitable, non-destructive alternative to the above methods would be advantageous, especially in studies requiring a large number of assessments, at sites that are difficult to access with equipment, or where funding is limited. The general relationship between visually assessed wilting stages and leaf water potentials reported here can be used to assess relative differences in leaf water potentials among individuals within a species. Once the relationship has been established for a specific plant species, it can also be used to assess absolute leaf water potentials. Because leaf water potentials and soil water status are intrinsically coupled under a wide range of conditions, variation of wilting stages within species (among similar-sized plants, preferably during the morning) will reflect spatial variation in soil moisture.

The relationship between the wilting behaviour of species and their drought survival was examined in a field experiment on first-year seedlings of 28 common woody plant species. Seeds were germinated and grown in the screen-house, and one seedling of each species was transplanted to 30 experimental plots in the understorey of a secondary forest at the end of the wet season. The plots (0.8 m × 1.0 m) were covered with transparent rain shelters in the 4-mo dry season. Wilting (in five categories, Table 1) and seedling survival were visually assessed in the late morning at regular intervals throughout the 22-wk experiment (for further details see Engelbrecht & Kursar 2003).

Both wilting behaviour and survival varied greatly among the 28 species (Figure 2, also see Engelbrecht & Kursar 2003). Across species, the percentage of plants that were visibly affected after short-term drought was negatively correlated with the percentage of individuals surviving the longer-term drought (Figure 2). The correlations were robust to different timings of the assessments, and to differences in the categorization of wilting (data not shown).

Although the observed correlations may seem obvious in hindsight, such a relationship could not necessarily be anticipated. For example, wilting has been interpreted as a mechanism to reduce transpiration rates and heat

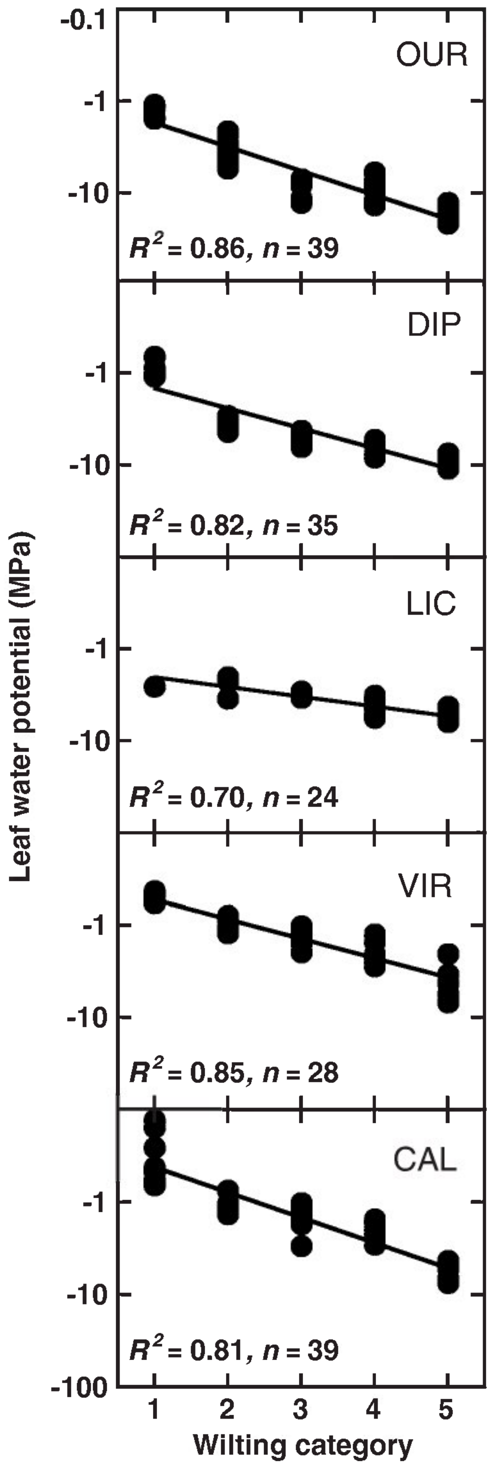

Figure 1. Relations of categorized plant wilting (Table 1) with leaf water potentials for five species. OUR: *Ouratea lucens* (Kunth) Engl. (97% drought survival after 22 wk), DIP: *Dipteryx panamensis* (Pittier) Record & Mell (73% survival), LIC: *Licania platypus* (Hemsl.) Fritsch (60% survival), VIR: *Virola surinamensis* (Rol. ex Rottb.) Warb. (20% survival), CAL: *Calophyllum longifolium* Willd. (20% survival). $P < 0.0001$ for all regressions, sample sizes n and R^2 values are given in the figure. Note that the y-axis is shown on a logarithmic scale.

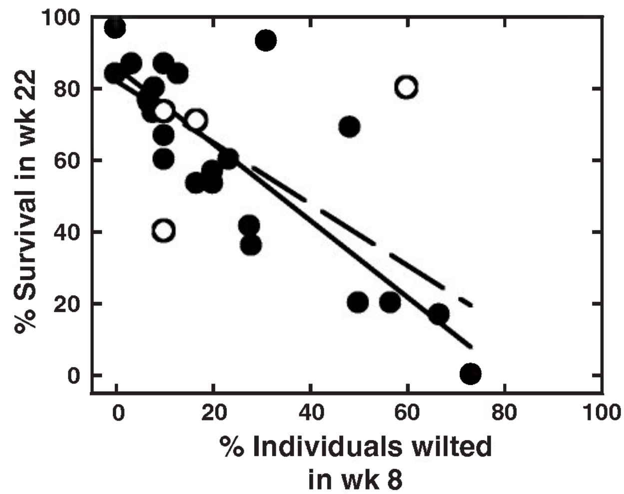

Figure 2. Correlation of per cent of individuals that were wilted or dead after 8 wk with per cent survival after 22 wk of drought for 28 species. Open symbols represent species that could a priori be defined as dry-season deciduous (from Croat 1978). For all species $r = -0.71$, $P < 0.0001$ (dashed line); for non-drought deciduous species $r = -0.84$, $P < 0.0001$ (continuous line).

damage, and thus drought stress, through reduced light interception (Chiarello *et al.* 1987). Early wilting should then lead to *higher* drought survival, which is contrary to our findings.

The correlations between the percentage of wilted plants and drought survival were improved, when species that were a priori known to be dry-season drought deciduous were removed from the correlation (Figure 2). Drought deciduousness is a well-known phenomenon reducing water-loss rates through reduction of leaf area, and should therefore also improve plant drought survival (Lambers *et al.* 1998). In systems with predominantly drought-deciduous species the correlation therefore may not hold.

Species drought survival can of course be assessed directly, but that approach requires repeated extensive, and therefore costly, censuses. Perhaps even more importantly, in many systems the crucial lethal conditions only occur during infrequent extreme drought events, and thus may not be reached within the time frame of a given study, rendering direct assessments unsuitable. A number of plant traits have been suggested as proxies for species susceptibility to drought (Hacke *et al.* 2001, Lloret *et al.* 1999). However, none of these correlated with quantitatively assessed species seedling survival in our study (wood density, seed or seedling size, root allocation or rooting depth; n = 19–48 species, all $P \gg 0.05$, unpubl. data) and therefore do not seem suitable in all systems, namely for tropical woody seedlings. Recently we showed that lethal leaf water potentials correlate tightly with drought performance of tropical seedlings (Tyree *et al.* 2002). But this approach is labour- and equipment-intensive, requiring seed collection, raising of seedlings and extensive physiological experiments.

Our data suggest that comparative visual assessment of plant wilting after relatively short durations of drought provides a suitable and robust ranking of species drought survival for longer-term and/or more severe droughts. The general approach can be applied for a wide range of species and conditions, and specific wilting categories can be defined as appropriate.

We conclude that fast and inexpensive visual assessments of wilting of plants may offer a suitable method in many studies (1) to assess local or regional variability of plant water status or soil water availability by comparing wilting stages within species across space, and (2) to rank drought survival of plant species by comparing the percentage of wilted individuals across species.

ACKNOWLEDGEMENTS

The project was funded by The Andrew W. Mellon Foundation, the University of Utah, and the USDA Forest Service.

LITERATURE CITED

ALLEN, C. D. & BRESHEARS, D. D. 1998. Drought-induced shifts of a forest-woodland ecotone: rapid landscape responses to climate variation. *Proceedings of the National Academy of Science USA* 95:14839–14842.

BANNISTER, P. 1986. Observations on water potentials and drought resistance of trees and shrubs after a period of summer drought around Dunedin, New Zealand. *New Zealand Journal of Botany* 24:387–392.

BONGERS, F., POORTER, L., VAN ROMPAEY, R. S. A. R. & PARREN, M. P. E. 1999. Distribution of twelve moist forest canopy tree species in Liberia and Cote d'Ivoire: response curves to a climatic gradient. *Journal of Vegetation Science* 10:371–382.

BOYER, J. S. 1995. *Measuring the water status of plants and soils.* Academic Press, San Diego. 495 pp.

BUNKER, D. E. & CARSON, W. P. 2005. Drought stress and tropical forest woody seedlings: effect on community structure and composition. *Journal of Ecology* 93:794–806.

CHIARELLO, N. R., FIELD, C. B. & MOONEY, H. A. 1987. Midday wilting in a tropical pioneer tree. *Functional Ecology* 1:3–11.

CHAZDON, R. L., BRENES, A. R. & ALVARADO, B. V. 2005. Effects of climate and stand age on annual tree dynamics in tropical second-growth forests. *Ecology* 86:1808–1815.

CROAT, T. B. 1978 *The flora of Barro Colorado Island.* Stanford University Press, Stanford. 943 pp.

ENGELBRECHT, B. M. J. & KURSAR, T. A. 2003. Comparative drought-resistance of seedlings of 28 species of co-occurring tropical woody plants. *Oecologia* 136;383–393.

ENGELBRECHT, B. M. J., COMITA, L. S., CONDIT, R., KURSAR, T. A., TYREE, M. T., TURNER, B. L. & HUBBELL, S. P. (2007). Drought sensitivity shapes species distribution patterns in tropical forests. *Nature* 447:80–82.

EVANS, J. 1996. *Plantation forestry in the tropics.* (Second edition). Clarendon Press, Oxford. 467 pp.

GERHARDT, K. 1993. Tree seedling development in tropical dry abandoned pasture and secondary forest in Costa Rica. *Journal of Vegetation Science* 4:95–102.

HACKE, U. G., SPERRY, J. S., POCKMAN, W. T., DAVIS, S. D. & MCCULLOH, K. A. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126:457–461.

HAWKINS, B. A., FIELD, R., CORNELL, H. V., CURRIE, D. J., GUEGAN, J. F., KAUFMAN, D. M., KERR, J. T., MITTELBACH, G. G., OBERDORFF, T., O'BRIEN, E. M., PORTER, E. E. & TURNER, J. R. G. 2003. Energy, water, and broad-scale geographic patterns of species richness. *Ecology* 84:3105–3117.

HULME, M. & VINER, D. 1998. A climate change scenario for the tropics. *Climate Change* 39:145–176.

IPCC. 2001. *Climate Change 2001: synthesis report.* Intergovernmental Panel on Climate Change 3rd Assessment report. Cambridge University Press, Cambridge. 21 pp.

KOZLOWSKI, T. T. & PALLARDY, S. G. 1997. *Growth control in woody plants.* Academic Press, San Diego. 336 pp.

LAMBERS, H., CHAPIN, F. S. & PONS, T. L. 1998. *Plant physiological ecology.* Springer, New York. 540 pp.

LEIGH, E. G. 1999. *Tropical forest ecology: a view from Barro Colorado Island.* Oxford University Press, New York. 262 pp.

LIETH, H. 1975. Primary production of the major vegetation units of the world. Pp. 203–231 in Lieth, H. & Whitthaker, R. H. (eds.). *Primary productivity of the biosphere.* Springer, New York.

LLORET, F., CASANOVAS, C. & PENUELAS, J. 1999. Seedling survival of mediterranean shrubland species in relation to root:shoot ratio, seed size and water and nitrogen use. *Functional Ecology* 13:210–216.

MILES, L., GRAINGER, A. & PHILLIPS, O. 2004. The impact of global climate change on tropical forest biodiversity in Amazonia. *Global Ecology and Biogeography* 13:553–565.

TYREE, M. T., VARGAS, G., ENGELBRECHT, B. M. J. & KURSAR, T. A. 2002. Drought until death do us part: a case study of the desiccation-tolerance of a tropical moist forest seedling-tree, *Licania platypus* (Hemsl.) Fritsch. *Journal of Experimental Botany* 53:2239–2247.

TYREE, M. T., ENGELBRECHT, B. M. J., VARGAS, G. & KURSAR, T. A. 2003. Desiccation tolerance of five tropical seedlings in Panama: relationship to a field assessment of drought-performance. *Plant Physiology* 132:1439–1447.

WALSH, R. P. D. & NEWBERY, D. M. 1999. The ecoclimatology of Danum, Sabah, in the context of the world's rainforest regions, with particular reference to dry periods and their impact. *Philosophical Transactions of the Royal Society London, Series B* 354:1391–1400.