

Improving Drought Tolerance of Trees: Theoretical and Practical Considerations

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Abstract

Selecting trees for improved drought tolerance has long been of interest for those involved in landscape horticulture and urban forestry. However, there are several pitfalls that need to be addressed in designing studies for selecting drought tolerant trees. Standardizing and quantifying the amount of drought to which trees are subjected is a major problem both in field studies and in pot studies. In field studies, variation in rainfall can limit the ability to apply a known amount of drought stress. In container studies, different growth rates among the genotypes can result in different levels of drought stress even at comparable levels of irrigation. In this paper, I discuss some of the theoretical and practical limitations of various drought-screening methods, and provide suggestions for methods that may be used to effectively identify trees with superior drought tolerance. I present examples of two techniques that have been effective in identifying variation in drought tolerance in seedling trees; stress-bed drought screening and the target water potential concept. Both of these techniques may be useful for screening a relatively large number of genotypes for drought tolerance. A second major constraint for screening trees for drought tolerance is determining the appropriate response variables to measure. Researchers have had varying degrees of success using single point-in-time measurements such as osmotic potential or gas exchange in identifying drought adapted genotypes. I present examples of the application of carbon isotope discrimination as an integrated measure of water use efficiency in studying genotypes of *Pinus sylvestris*, *P. ponderosa* and other conifers. I also discuss theoretical and practical limitations of using carbon isotope discrimination and other methods to identify drought-adapted genotypes.

INTRODUCTION

Water is the principle factor limiting survival and growth of trees, particularly in urban and suburban landscapes (Clark and Kjelgren, 1990; Kramer, 1987). Drought stress is exacerbated in urban environments due to soil factors (Craul, 1985) as well as elevated temperatures associated with urban heat islands (Cregg, 1995; Cregg and Dix, 2001; Whitlow and Bassuk, 1988; Whitlow et al., 1992). The detrimental impacts of water stress may be mitigated through irrigation. However, watering restrictions are becoming more common throughout the United States. This suggests that selecting trees with improved drought tolerance and reduced water use may be the best strategy to improve survival, growth, and health of trees in urban and suburban landscapes.

On the surface, screening trees for improved drought tolerance based on greenhouse or field trials seems like a straightforward proposition. However, there are a number of key factors that must be addressed, each with their own subtleties and pitfalls. These include method of applying drought and method of assessing drought. In this paper I will review several studies that have been conducted in this area and summarize the key insights we have gained.

APPLYING DROUGHT STRESS

Withholding Water

Drought is defined as a meteorological occurrence characterized by below normal rainfall. The most obvious means to select for improved drought tolerance is to withhold water or reduce irrigation and compare the response of various genotypes. However, the shortcomings of this technique can be significant, both in field trials and in controlled environments.

In field plots variation in rainfall can make the imposition of consistent drought stress difficult, particularly in regions prone to summer thunderstorms. A novel approach for studying the long-term effects of drought on woody plants is the Throughfall Displacement Experiment (TDE) developed at the Oak Ridge National Laboratory (Wullschlegel et al., 1998). In the TDE, one third of the study area is designated as 'dry' plots. In the 'dry' plots troughs cut from PVC pipe cover one-third of the ground surface area, reducing effective precipitation by one-third. The rainfall collected in the troughs is applied to the 'wet' plots, which receive ambient + 1/3 rainfall. The TDE system has been used to effectively study drought responses on several hardwood species in natural upland sites (Gebre et al., 1998; Gebre and Tschaplinski, 2002; Tschaplinski et al., 1998). The system could have additional application for drought screening studies by overlaying the system on existing provenance tests or other long-term genetics trials.

In studies of container-grown plants, dry-down responses are often confounded with plant size (Graves et al., 2002). Therefore volumetric water content and plant water potential may decline more rapidly for larger genotypes than smaller one, regardless of their relative drought tolerance.

Stressed Screening

One means to obviate the problems of differential pot drying is grow seedlings in a large, common container so that all seedlings have access to the same soil volume and soil moisture. In controlled environments this may be accomplished by growing seedlings in large sand-filled 'stressbeds' (Chambers et al., 1988). The stressed technique is well-suited for screening relatively large numbers of genotypes based on gross characteristics such as survival and growth. For example, Cregg (1994) identified significant variation in survival and relative growth rate among diverse populations of ponderosa pine (*Pinus ponderosa* Dougl. Ex Laws.) in a large stressbed trial (Fig. 1). However, stressbed screening may not be suited for differentiating physiological responses to drought among genotypes. If the stressbeds are filled with a sand-based medium, soil matric potential and plant water potential decline rapidly as the soil dries (Fig. 2), making it difficult to characterize subtle differences in physiological processes that may occur at intermediate stress levels. Drought studies involving stressbeds also require intensive and time-consuming measurements of plant water potential in order to document the severity and duration of the stress applied. If large stressbeds are used, investigators may have difficulty accessing seedlings near the center of the stressbeds for gas exchange measurements.

Polyethylene Glycol (PEG)

Soil osmotic potential and, consequently, plant water potential may be effectively manipulated using solutions of known concentration of osmoticum (Sikurajapathy et al., 1983). Although many compounds are effective osmotica, researchers have favored polyethylene glycol (PEG). The principle advantage of PEG is that it allows precise control of soil and plant water potential. However, the use of PEG for long-term studies of drought tolerance is problematic due to toxicity and reduced oxygen diffusion (Lawlor, 1970; Mexal et al., 1975). One approach to reducing plant uptake of PEG is to enclose the root system in a semi-permeable membrane and then immersing the roots in a high molecular weight PEG (e.g., MW >8000) (Zur, 1966; 1967). However these systems require intensive maintenance, and growth of microorganisms in the semi-permeable membranes is a serious problem (Weigrefe, personal communication).

Target Water Potential

The target water potential method is a two-phase process for comparing the response of containerized seedlings of numerous genotypes to a known level of water stress for a growing season or longer (Olivas-García, 1995). In the first phase, plant and pot weight (W) and pre-dawn water potential (Ψ_{predawn}) are monitored on each seedling during a dry-down cycle and the data are plotted on a chart (Fig. 3). From the chart, the investigator can determine the W that corresponds to a given level of Ψ_{predawn} . Subsequently, each seedling is watered to run-off and allowed to dry until W reaches the value associated with target water potential. Because weighing pots is simple and quick a relatively large number of seedlings may be subjected to a standard level of water stress. While spot-checks of Ψ_{predawn} are recommended, the method also eliminates issues associated with frequent removal of leaves from small plants for pressure chamber measurements.

The target water potential method represents a simple, effective method to apply a known level of water stress that is appropriate for drought screening trials. However, there are several factors that investigators should consider. The method requires significant initial effort to develop the W - Ψ relationships. For example, a moderately-sized trial involving 12 genotypes and 8 replications will require a series of paired measurements of W and Ψ on 96 seedlings. The technique assumes that the investigator has *a priori* knowledge of the drought response of the species to select an appropriate target water potential. For example, from studies with ponderosa pine Zhang et al. (1997) found that stomatal closure occurred as Ψ approached -2.0 Mpa. Olivas-Garcia et al. (1995) selected this level as the target water potential and were able to maintain a steady separation in net photosynthesis, and carbon isotope discrimination between well watered and drought stressed seedlings (Fig. 4).

ASSESSING RESPONSE TO DROUGHT

In comparing relative drought tolerance among populations, investigators must decide which parameter(s) are most appropriate as a selection criteria. Investigators may compare relative drought tolerance based on survival, growth, or morphophysiological responses.

Survival

Mortality of seedlings of a given population under prolonged drought typically follows a sigmoidal response. This response can be readily modeled using standard statistical software such as SAS PROC LIFEREG. The response may also be linearized using a logistical transformation and modeled using a standard least squares approach (Neter et al. 1985). An LD50 (days without water to cause 50% mortality) and associated confidence intervals may be estimated using inverse estimation techniques allowing a simple and straightforward test of differences in drought tolerance among populations. Using this technique Cregg (1994) found that LD50 of seedling populations of ponderosa pine from diverse geographic origins ranged from 40 to 58 days without water. Pre-conditioning the seedlings by exposure to cyclic drought stress before the final drought cycle increased the LD50 range to 47 to 78 days without water. The LD50 of Scots pine seedlings from diverse seed sources ranged from 65 to 85 days (Cregg and Zhang, 2001).

Morpho-Physiological Responses

Because seedling survival and growth under drought are mediated by physiological responses, considerable attention has been focused on understanding and comparing the physiology and morphology of drought tolerant genotypes (Teskey et al., 1987; Van Buijtenen et al., 1976). Jones et al. (1981) identified two broad mechanisms of drought tolerance that apply to trees: drought tolerance at high tissue water potential and drought tolerance at low tissue water potential. Drought tolerance at high tissue water potential or drought stress avoidance involves morphological and physiological mechanisms that maintain water uptake or reduce water loss. Drought tolerance at low

tissue water potential or drought stress tolerance is usually associated with osmotic adjustment, a decrease in osmotic potential that results in the maintenance of turgor pressure as the plant dehydrates. Both forms of drought tolerance occur in trees and investigators have found significant genotypic variation for each in trees.

Drought Tolerance at High Tissue Water Potential

Cregg (1994) observed significant genotypic variation in allocation patterns to roots and shoots in ponderosa pine seed sources that differed in their ability to survive imposed drought. This study indicated seed source that had the highest capacity to survive drought were intermediate in their allocation to roots and shoots. Seedlings from seed sources that allocated relatively high amounts of biomass to shoots versus roots survived poorly, presumably due to excessive transpiration relative to absorption. Seedlings from seed sources that allocated relatively high amounts of biomass to roots also had low survival under imposed drought. While the latter observation is counter-intuitive, it may be that, because of their low leaf area, these seedlings were unable to produce sufficient carbohydrate reserves to meet their maintenance respiration needs.

In addition to optimizing allocation to roots to increase water uptake many tree species have leaf adaptations that reduce water loss and increase drought tolerance: Genetic variation in leaf morphology has been observed for a number of species including *Cercis canadensis* L. (Abrams, 1988), *Acer* spp. (St. Hilaire and Graves, 2001) *Pinus taeda* (Bilan et al., 1974), and *Fraxinus pennsylvanica* (Abrams et al., 1990). Needle length and stomatal density of 1-year-old foliage of 25-year old ponderosa pine trees grown in southeastern Nebraska differed significantly among four diverse seed sources suggesting differences in their relative ability to tolerate drought (Cregg, 1994). Abrams et al. (1990) found that specific leaf area and stomatal density decreased in sources of *Fraxinus pennsylvanica* along a longitudinal and decreasing precipitation gradient from New York to South Dakota. *Cercis canadensis* L. seedlings from a xeric Kansas prairie source had significantly lower specific leaf areas than seedlings from a more mesic Indiana source; however, stomatal density was greater for the Kansas source (Abrams, 1988).

In general, physiological adaptations to tolerate drought at high tissue water potential are related to water conservation through low stomatal conductance (g) resulting in increased water use efficiency or rapid stomatal closure under stress (Jones et al., 1981). Such adaptations have been found for a number of tree species (Bennett and Rook, 1978; Blake et al., 1984; Kelliher and Tauer, 1980). Seasonal gas exchange patterns of 25-year-old ponderosa pine indicated that trees from a South Dakota source had relatively low rates of net photosynthesis early in the growing season when soil moisture was high but were able to maintain higher rates later in the growing season as soil moisture declined (Cregg, 1993). Bennett and Rook (1978) observed that the transpiration rate (E) of a *Pinus radiata* D. Don clone that survived poorly in the field was twice that of a clone that had high field survival. Similarly, g , of the poor-surviving clone was much higher than that of the other clone. Increased water use efficiency (ratio of carbon gain to transpiration) of *Populus* clones was partially attributed to reduction of water loss due to earlier stomatal closure (Blake et al. 1984). Bilan et al. (1977) found that E of loblolly pine seedlings from the xeric “lost pines” source was significantly lower than E of seedlings from a mesic east Texas seedlings at the end of a seven-day dry-down cycle. The percent of open stomata also was lower for the “lost pines” source. Differences in g of progeny from crosses of coastal and interior varieties of ponderosa pine (*ponderosa x scopulorum*) and coastal varieties (*ponderosa x ponderosa*) were highly significant ($p < 0.0001$) (Monson and Grant, 1989). Low g and E resulted in increased water use efficiency in the *ponderosa x scopulorum* progeny.

In addition to analyzing drought tolerance through gas exchange measurements, water use efficiency may also be compared using carbon isotope discrimination (Δ) or isotopic carbon ratio ($\delta^{13}C$). Recently several studies have demonstrated significant genotypic variation in carbon isotope discrimination (Δ) in crops and forest trees. The

isotopic ratio of ^{13}C to ^{12}C in C_3 plants varies mainly due to discrimination during diffusion and enzymatic processes (Farquhar et al. 1989). As A increases or g decreases (increasing A/g), discrimination against ^{13}C decreases. The amount of discrimination against ^{13}C in photosynthesis has been shown to be highly negatively correlated with instantaneous water use efficiency (WUE_i) from gas exchange measurements (Condon et al., 1990; Condon et al., 1992; Donovan and Ehleringer, 1994; Hubick et al., 1986; Wright et al., 1994).

The extent of intra-specific variation in trees has been analyzed for species including Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) (Zhang et al., 1993), black spruce (Flanagan and Johnsen, 1995), boxelder (*Acer negundo* L.) (Dawson and Ehleringer, 1993), and western larch (*Larix occidentalis* Nutt.) (Zhang and Marshall, 1994; Zhang et al., 1994). Zhang and Cregg (1996) observed significant intra-species variation in Δ in several *Pinus* species including *P. ponderosa*. Cregg and Zhang (2001) recently studied the carbon isotopic composition of Scots pine (*Pinus sylvestris* L.) seedlings from diverse seed sources representing a longitudinal transect across the range of the species. They found significant variation among seed sources. Moreover, the $\delta^{13}\text{C}$ was significantly correlated with precipitation of the seed source, indicating Siberian seed sources had higher water efficiency than European seed sources

Advantages of Δ as a Selection Criterion for Improved WUE

Carbon isotope discrimination has several conceptual and logistical advantages to screening for drought tolerance based on instantaneous water use efficiency (WUE_i) (Farquhar et al., 1989). Carbon isotope discrimination integrates A/g over the time the sampled tissue was formed. In contrast, WUE_i measured by gas exchange provides 'snapshots' of A/g and may not be representative of overall WUE . One particular advantage of using isotope analysis in trees is that isotope discrimination can be determined on annual rings from increment cores (Livingston and Spittlehouse, 1993; MacFarlane et al., 1999). Thus, Δ can be determined across the range of climatic conditions that may have occurred over the life of the tree (e.g., drought versus wet years). In addition to sampling across years using increment cores, the effect of varying weather patterns on isotope discrimination can be assessed on evergreen trees that retain multiple year's of leaves. For example, in a study of ponderosa pine growing in two range-wide provenance tests, Cregg et al. (2000) determined Δ of needles formed in four years in one location and three years at the second location. Age:age correlations are generally high for isotope discrimination indicating a high degree of reproducibility in values and low genotype x environment ($G \times E$) interactions associated with variation in precipitation (Hall et al., 1994). Age:age correlations in leaf Δ of *Picea mariana* trees varied from 0.70 to 0.84 (Johnsen et al., 1999). Cregg et al. (2000) analyzed $G \times E$ interaction in Δ among four seed sources of ponderosa pine grown in Oklahoma and Nebraska. They found that within a location, year x source interactions were non-significant ($P > 0.05$), indicating that seed source rankings were stable across years of varying rainfall.

Drought Tolerance at Low Tissue Water Potential

In contrast to the physiological adaptations associated with drought tolerance at high water potential, drought tolerance at low tissue water potential is generally indicated by maintenance of g , E , and other physiological processes as plant water potential declines. Drought tolerance at low tissue water potential is usually achieved by osmotic adjustment, whereby positive turgor pressure is maintained at lower bulk water potential than without adjustment. The benefits of osmotic adjustment to the plant are: 1, maintenance of cell elongation; 2, maintenance of stomatal opening; 3, maintenance of photosynthesis; 4, survival of dehydration; and 5, exploration of greater soil volume for water (Turner and Jones 1980). Numerous studies have documented genotypic differences in osmotic adjustment and maintenance of physiological processes under drought.

Variation in growth among families of black spruce between wet and dry sites was

related to the ability of the families to maintain turgor pressure (Johnsen and Major, 1999). Maintenance of turgor was dynamic and related to both osmotic adjustment and changes in cell wall elasticity (Major and Johnsen, 2001). *Eucalyptus camaldulensis* Dehn, adapted to a dry site in Victoria, Australia maintained greater productivity under drought than a less adapted source from Western Australia (Grunwald and Karshon, 1982). The Victorian source reached a lower water potential while maintaining a higher relative water content than the Western Australian source. Results from Blake and Yeatman (1989) suggest that outcrossed families of *Pinus banksiana* Lamb, had faster growth rates than selfed families due, in part, to maintenance of stomatal conductance and photosynthesis later in the growing season. Heth and Kramer (1975) determined that *P. radiata* seedlings were more tolerant of drought than seedlings of *P. taeda* or *P. echinata* Mill. in dry-down experiments. The difference in survival could not be explained through water conservation as *P. radiata* had the greatest *g* and *E* of the three species. Heth and Kramer noted that *P. radiata* seedlings maintained a higher turgor potential than the other species for several weeks following cessation of watering. Abrams et al. (1990) observed that *g* and net photosynthesis of *Fraxinus pennsylvanica* seedlings from a xeric site in South Dakota were higher than that of seedlings from a more mesic site in New York. Seedlings from South Dakota also were found to have lower osmotic potential at full and zero turgor than the New York source. Abrams (1988) found a similar trend in *Cercis canadensis*, as seedlings from a xeric Kansas site had significantly lower osmotic potentials than seedlings from a more mesic Indiana source. Additionally, leaf osmotic potential was determined to be significantly different among three species of *Quercus* examined by Parker and Pallardy (1988b). They observed that leaf osmotic potential of *Q. stellata* Wang, was consistently lower than that of *Q. alba* L. or *Q. macrocarpa* Michx. Parker and Pallardy (1988a) also observed significant differences in leaf and root osmotic adjustment among several sources of *Juglans nigra* L. Genotypes with a relatively high capacity to osmotically adjust (i.e., decrease osmotic potential in response to drought stress) may be better able to maintain photosynthesis and other physiological processes during drought than those types that lack the ability to osmotically adjust. Thus, it is possible that the ability to osmotically adjust and maintain turgor pressure during drought may serve as a criterion for drought tolerance selection programs.

SUMMARY

Selecting trees for improved drought tolerance requires careful consideration of both the method used to apply drought stress and the criteria used to evaluate drought response. Field-based drought studies are often difficult due to variation in rainfall, though innovative approaches such as throughfall displacement may be useful for long-term evaluations. In controlled environments, stressbed screening is useful for initial evaluations of relatively large numbers of genotypes based on easily measured characteristics such as survival and growth. For detailed characterizations of physiological responses to drought, controlled drought methods based on target water potential or target soil water content can reduce problems associated with differential dry-down on container-grown plants. Numerous morphophysiological traits have been associated with improved drought tolerance. These include leaf morphology, water use efficiency, and allocation of biomass to roots, maintenance of cell turgor via osmotic adjustment, abscisic acid accumulation (Aasamaa et al., 2002; Chen et al., 1997), and hydraulic architecture (Jackson et al., 1995; Tognetti et al., 1997). In all likelihood, drought tolerant genotypes will possess more than one mechanism (Aussenac et al., 1989); therefore investigators should consider a suite of adaptations rather than focusing on a single trait.

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Figures

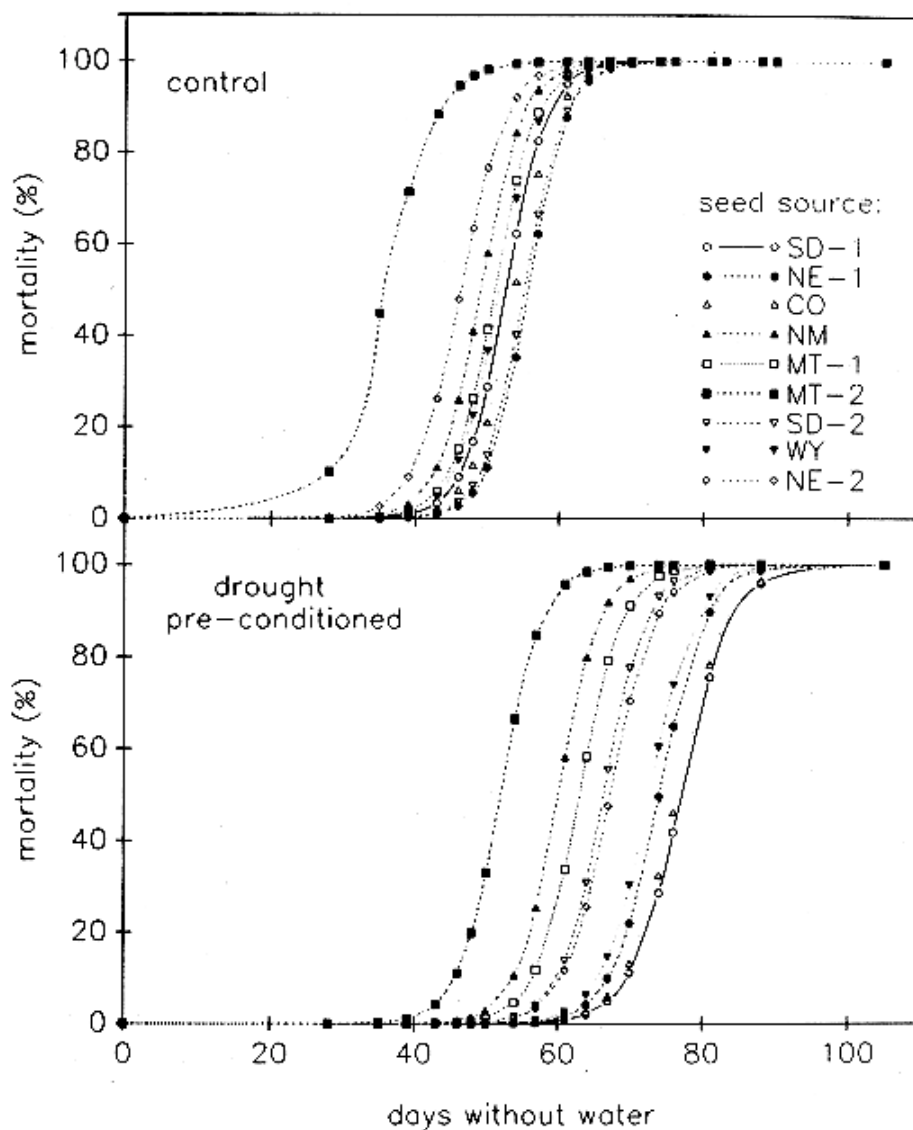


Fig. 1. Mortality of seedlings from nine diverse seed sources of ponderosa pine subjected to imposed drought. Seedlings were well-watered before the imposition of drought (top) or pre-conditioned by cyclic drought before the final dry down (bottom). From: Cregg (1994) *Tree Physiol.* 14:883-898, © 1994 Herron Publishing, used by permission.

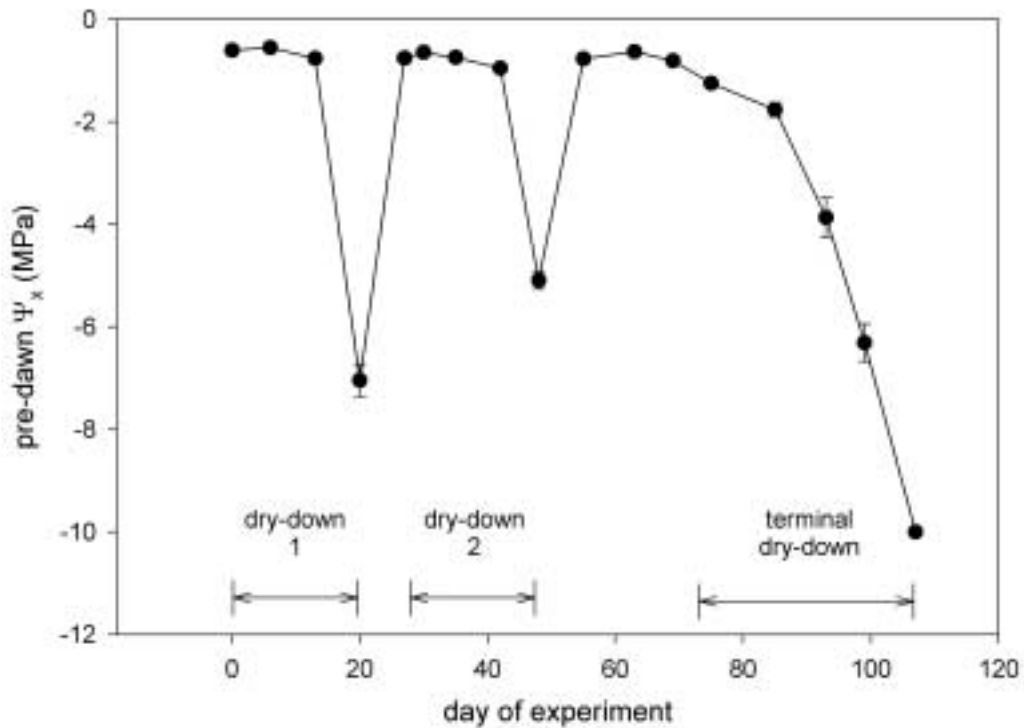


Fig. 2. Pre-dawn needle water potential of Scots pine seedlings during three dry-down phases in sand-filled stressbeds. From Cregg and Zhang (2001) *For. Ecol and Mgmt.* 154: 131-139. © Elsevier Science B.V., used by permission.

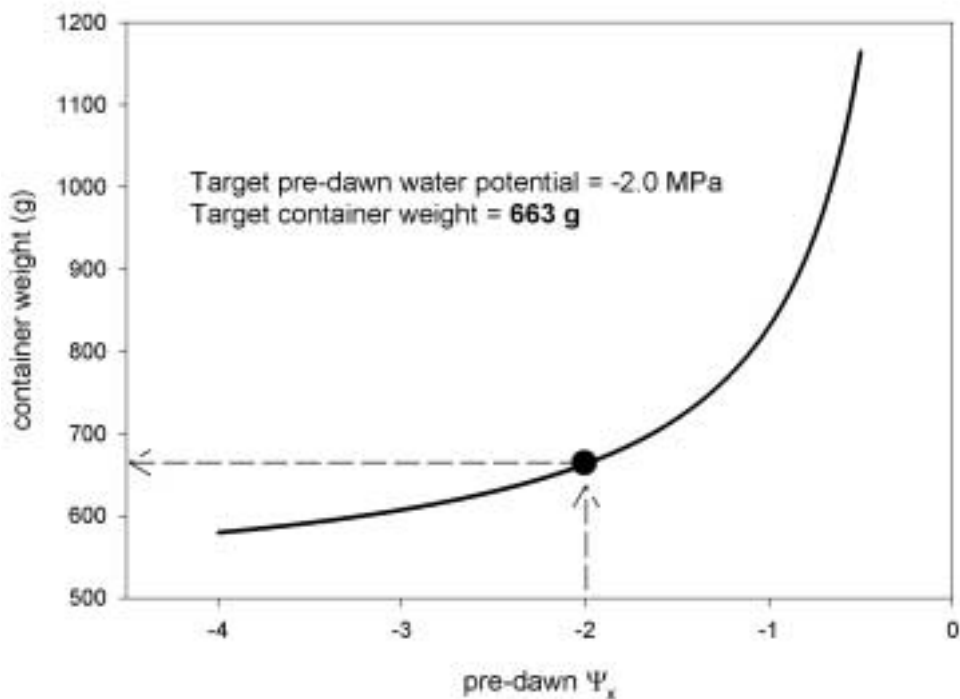


Fig. 3. Determining target container weight from the relationship between pre-dawn water potential and container weight. Adapted from Olivas-García (1995).

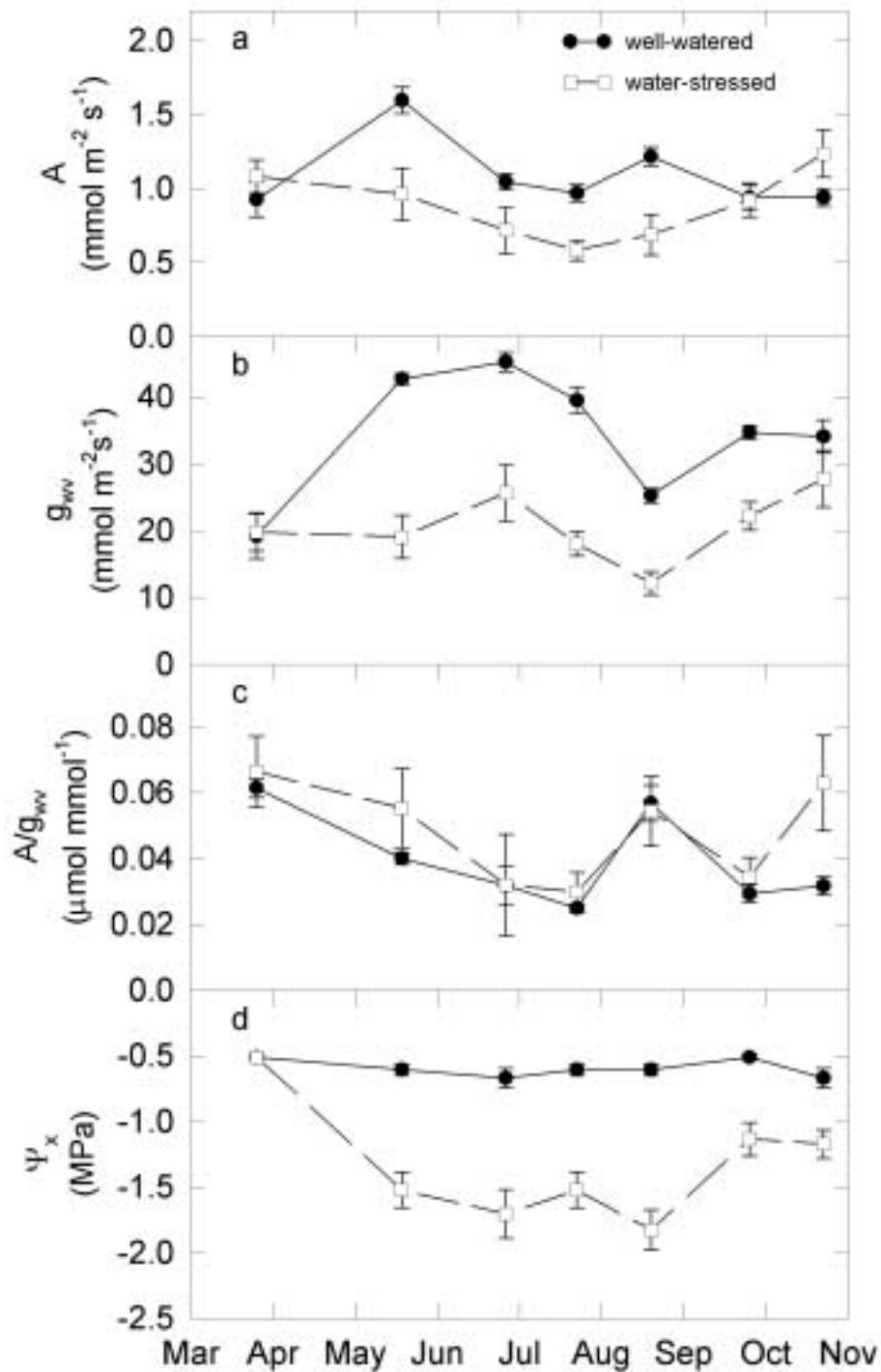


Fig. 4. Net photosynthesis (A), needle conductance to water vapor (g_{wv}), water use efficiency, and pre-dawn needle water potential of ponderosa pine seedlings under two levels of water stress. From Olivas-García et al. 2000. *Can. J. For. Res.* 30:1581-1590. © NRC Canada, used by permission.