

Evaluation of chlorophyll fluorescence as an indicator of dehydration stress in American chestnut seedlings

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Introduction

American chestnut (*Castanea dentata* (Marsh.) Borkh.) used to be an important species for both timber and wildlife in the eastern US, but it was decimated by the chestnut blight (*Cryphonectria parasitica* (Murr.) Barr). Recent advances in breeding for resistance to the chestnut blight have increased interest in the silviculture and management of this species for deployment in afforestation programs. The American Chestnut Foundation has an agreement with the US Forest Service to work toward restoration of this species in its native range (Jacobs 2007).

During nursery production of hardwood seedlings, a 'dry-down' point is often used to identify the point at which seedlings need irrigation. Dry-down points are usually defined as a percentage of the saturated media weight. With the lack of information available to seedling growers on suitable dry-down points for American chestnut seedlings, it was proposed that chlorophyll fluorescence be assessed as an indicator of plant stress at different dry-down points given indications that chlorophyll fluorescence is an accurate means of measuring drought stress in deciduous plants (Percival and Sherriffs 2002).

Light energy that is absorbed by chlorophyll in plants can be a) used to drive photosynthesis, b) emitted as heat energy or, c) emitted as light (chlorophyll fluorescence). Dark-adapted leaves emit a minimum fluorescence (F_0) and when they are exposed to a saturating light, photosystem II receptors are closed. This excess energy increases fluorescence output for a short time period and this is the maximum fluorescence (F_m). The difference between maximum and minimum fluorescence is expressed as F_v . The maximum quantum yield of photosystem II can be expressed as F_v/F_m . F_v/F_m can be an indicator of the ability of a plant to tolerate stress and the amount of damage that has occurred to the photosynthetic capabilities of the plant (Maxwell et al. 2000).

The objective of the study was to examine the effects of dry-down on chlorophyll fluorescence. It was hypothesized that chlorophyll fluorescence will decrease with lack of irrigation, but will return to original levels following irrigation.

Materials and methods

Three-month old American chestnut seedlings that were grown in Styroblock™ containers (Beaver Plastics, Edmonton, AB) with a cavity volume of 340mL, were removed from operational growing conditions at the Pitkin Forest Nursery (Moscow, ID). Seedlings were approximately the same height (61.0 ± 0.8 cm) and root-collar diameter (5.62 ± 0.10 mm) across all treatments. Seedlings were allocated to dry-down treatments imposed via ambient drying. Treatments consisted of irrigation being withheld for 0, 24, 48, or 72 hours. Chlorophyll fluorescence measurements were taken after each irrigation. Similar sized and aged leaves were dark adapted for at least 20 min and were then exposed to a saturating flash ($> 7500 \mu\text{m}$) with a Li-Cor LI-6400 portable photosynthesis system with LI-6400-40 fluorometer (Li-Cor, Lincoln, NE). The maximum quantum yield, F_v/F_m , and weights of the individual seedlings were recorded for each sample.

Repeated measures analysis of variance was used to identify the main effect of day of measurement and the day of measurement \times irrigation regime interaction on seedling chlorophyll fluorescence. Piecewise regression analysis was used to identify the relationship between percent of saturated media weight and chlorophyll fluorescence. SAS (SAS Institute, Cary, NC) software was used for all data analysis.



Results

Repeated measures multivariate analysis of variance identified significant differences between day of measurement ($p < 0.0001$) and the day of measurement \times irrigation regime interaction ($p = 0.0017$). Least square means identified that these differences emerged only after the fourth day of measurement. Seedlings in Treatment 1 differed from those in Treatment 4 and seedlings in Treatment 2 differed from those in Treatments 3 and 4 (figure 1). Piecewise regression (figure 2) showed that when dry down weight is less than or equal to 30%, F_v/F_m increases by 0.07737 for every 1% increase in dry-down weight. When dry-down weight is greater than 30%, F_v/F_m increases by 0.00014 for every 1% increase in dry down weight.

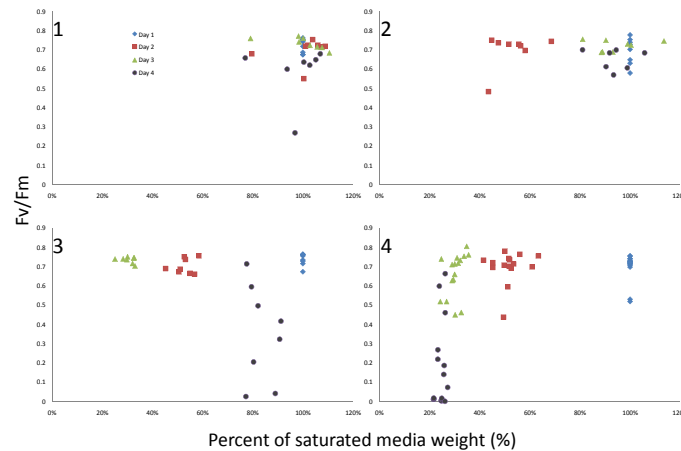


Figure 1. Changes in seedling chlorophyll fluorescence as represented by the ratio of F_v/F_m across four irrigation regimes and days of measurement. Points represent the same seedlings across four days of measurement where 1) no water was withheld; 2) water was withheld on day 2; 3) water was withheld on days 2 and 3; and 4) water was withheld on days 2, 3, and 4.

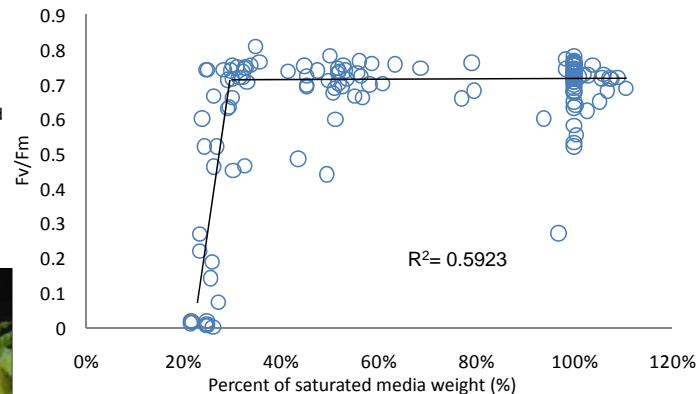


Figure 2. Piecewise regression based on all pre-recovery data points. When percent of saturated media weight was greater than 30%, F_v/F_m stayed relatively constant ($y = 0.68816 + 0.00014x$), but when percent of saturated media weight dropped below 30% F_v/F_m decreased dramatically ($y = -1.62874 + 0.07737x$).

Discussion

The lack of difference between Treatment 1 and 3 is likely spurious, as the F_v/F_m values of Treatment 2 are similar to Treatment 1 throughout the experiment. In Treatment 1, the F_v/F_m stayed constant throughout the experiment. Treatment 2 experienced dry-down for 24 hours, but this had little effect on F_v/F_m values. The critical point for dry-down occurred between 48 and 72 hours. F_v/F_m values dropped considerably during this time period. Treatment 3 showed some recovery after irrigation returned media to saturation. Treatments 3 and 4 both reached the permanent wilting point of their leaves. The drop in F_v/F_m along with the piecewise regression analysis indicated the critical dry-down point is around 30% of the saturated plug weight but the length of exposure that causes damage is not known. Some seedlings in Treatment 2 reached a dry-down point of less than 50% of the saturated plug weight and this did not have any effect on F_v/F_m . The dramatic drop in F_v/F_m shows that the resolution of the measurement is not high enough to be useful as a stress indicator for dehydration. This lack of correlation between F_v/F_m and drought stress corresponds to the findings of Ögren (1990) pertaining to *Salix*, and Duan et al. (2005) in *Picea asperata*.

Conclusions and future directions

This study showed that dry-down affected chlorophyll fluorescence in American chestnut seedlings treated with different irrigation regimes. Seedlings in Treatment 3 did not recover fully during the measurement period in this study, and most leaves passed the permanent wilting point. Treatments 3 and 4 both showed severe damage to the leaves. Given these results, chlorophyll fluorescence does not appear to have the resolution needed to be a viable method for determining dry-down points beyond 50% of saturated media weight or assessing seedling dehydration stress. Repeating this study on younger seedlings may allow us to determine the variability in this measure over a growing season, and the more gradual dry-down might provide more resolution. Furthermore, research should include hybrid chestnut seedlings that will represent those used in restoration efforts to better understand implications in seedling production.

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Literature cited

- Duan, B., Lu, Y., Yin, C., Junttila, O., Li, C. 2005. Physiological responses to drought and shade in two contrasting *Picea asperata* populations. *Physiologia Plantarum*. 124:476-484
- Jacobs, D.F. 2007. Toward development of silvical strategies for forest restoration of American chestnut (*Castanea dentata*) using blight resistant hybrids. *Biological Conservation* 137:497-506
- Maxwell, K., Johnson, G.N. 2000. Chlorophyll fluorescence- a practical guide. *Journal of Experimental Botany*, Vol 51, No. 345:659-668
- Ögren, E. 1990. Evaluation of chlorophyll fluorescence as a probe for drought stress in willow leaves. *Plant Physiology* 93:1280-1285
- Percival, G.C., Sherriffs, C.N. 2002. Identification of drought-tolerant woody perennials using chlorophyll fluorescence. *Journal of Arboriculture* 28:215-223

