DETECTING STRESS IN SOUTHERN LIVE OAK (QUERCUS VIRGINIANA) AND SAND LIVE OAK (Q. VIRGINIANA VAR. GEMINATA)
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Abstract. Walt Disney World (Florida, U.S.) arborists evaluated three affordable and easy-to-use measures of tree stress—leaf chlorophyll concentration, leaf temperature, and water potential—for use in detecting water and physical injury stress in live oak. The evaluation included observations of trees being relocated, and experiments of girdled trees and trees subjected to various watering regimes. Leaf chlorophyll concentration was largely unresponsive to stress and unlikely to be useful as a stress indicator in sand live oak and southern live oak. Both water potential and leaf temperature were responsive to imparted stressors and have potential as indicators of stress in sand live oak and southern live oak. Water potential exhibited a predictable and consistent response to both water stress and physical injury. Inadequately watered live oaks, and trees subject to relocation, had lower water potentials than trees with an adequate water regime or in situ. By contrast, live oaks injured by girdling had higher water potential than nongirdled trees. Leaf temperature was less sensitive and less robust at detecting stress than water potential, but still potentially useful. Leaf temperature was greater for a nonirrigated relocated tree than an irrigated relocated tree, and for relocated trees when rootballed and immediately after relocation. Leaf temperature was largely unresponsive to girdling or mild water stress.

Key Words. Girdling; leaf chlorophyll concentration; leaf temperature; relocation; sand live oak; southern live oak; tree stress; water potential.

Water surplus or deficit, air pollutants, temperature extremes, mechanical wounding, nutrient deficiency, insects, and disease can stress trees. Stress can be detected by attention to phytohormone, protein and chlorophyll synthesis; foliage temperature, appearance and fluorescence, growth and repair rates, water potential, nutrient uptake, photosynthetic and respiration rates, and ultrasonic acoustical emissions (Hsiao 1973; Kimmerer and Kozlowski 1982; Sanford and Grace 1985; Reicosky et al. 1985; Schreiber and Bilger 1993).

Oak borers (Paranthrene simulans) attacked several Walt Disney World southern live oak (Quercus virginiana) specimen trees. Walt Disney World arborists responded by seeking a stress indicator that would facilitate early detection and remediation. An initial review of the literature suggested three indicators that were affordable, relatively easy to use, and had a potential for success: (1) leaf chlorophyll concentration, (2) leaf temperature, and (3) water potential. Leaf chlorophyll concentration is reportedly a physiological indicator of cumulative stress that does not fluctuate daily, but does respond to long-term stress (Stutte and Stutte 1992). Baldy et al. (1996a, 1996b) found that the yield from phylloxera-infested grapevines was correlated with early to midseason leaf chlorophyll concentration. The University of Nebraska Cooperative Extension, noting the relationship between nitrogen levels and leaf chlorophyll concentration, recommend farmers use chlorophyll level to establish fertilizer application rates (Peterson et al. undated). In both instances, leaf chlorophyll concentrations were estimated using handheld chlorophyll meters.

Leaf temperature has been recognized as an indicator of water availability (Gates 1964; Wiegand and Namken 1966; Begg 1980). Leaf temperatures of water stressed plants are typically 2°C to 4°C higher than nonstressed plants, but differences as great as 15°C have been recorded (Drake 1976; Bucks et al. 1984; Mattson and Haack 1987). Regressing the difference between canopy and air temperature against vapor pressure deficit produces a crop water stress indicator (Jackson et al. 1981; Sammis et al. 1988; Sammis and Jernigan 1992). Reicosky et al. (1985) also found that water surpluses can increase leaf temperatures, and suggested that canopy temperature measurements may be sensitive enough to indicate types of stress in addition to water deficits. Leaf temperatures can be easily measured using a handheld infrared thermometer.

Water potential integrates the soil moisture tension in the root zone, the resistance to water movement within the plant, and transpiration demands. A well-hydrated plant at steady state attains a water potential only a few bar less than water potential in the fully turgid state (Ψ = 0, Mohr and Schopfer 1995). A loss by transpiration in excess of water uptake from the soil causes water potential to fall, leading to water stress. The critical level of water potential, the permanent wilting point, varies with species.

Over the course of 3 years, we evaluated leaf chlorophyll concentration, leaf temperature, and water potential as indicators of stress in sand live oak (Q. virginiana var. geminata) and southern live oak. Sand live oaks relocated to accommodate resort construction provided the opportunity for two observational studies. One instance involved a sand live oak that was relocated and intentionally not irrigated (sacrificial tree) to facilitate evaluation of gross responses to water stress. Trees relocated, maintained, and then re-relocated (relocated trees) were observed to evaluate detection of stress from typical relocation activity.
We also had the opportunity to conduct two experiments. Sand live oaks scheduled for removal during future development activities were girdled to evaluate their response to physical injury (girdled experiment). Finally, containerized live oaks were subjected to varying water regimes to evaluate response to water stress (water experiment).

**METHODS**

The study was conducted at the Walt Disney World Resort in Lake Buena Vista (Orlando), Florida, U.S. Lake Buena Vista is within Zone 26 of the Sunset National Garden Plant Climate Zones, and the average minimum winter temperature ranges from –9°C to –1°C (16°F to 30°F). In this area, new oak leaves begin to emerge in May and share the tree with old leaves through July. Senescence is evident in November, and abscission occurs December through February. Buds swell in March and begin to open in April.

All leaf chlorophyll concentration, leaf temperature, and water potential measurements were taken on the south side of trees between 1200 and 1500 hours on sunny days. Leaf chlorophyll was measured using a Minolta SPAD-502 meter. The meter converts leaf transmission of 940 and 650 nm to SPAD units. Measurements were accomplished by positioning an in situ leaf within the meter. Ten separate leaves were measured on each tree, and a mean value was calculated.

Leaf temperatures were measured with an Omega OS520 handheld IR thermometer with the emissivity set at 0.98. The thermometer was held at an angle of about 30 degrees from the horizontal about 1 cm (0.4 in.) from the leaf surface. Ten leaves per tree were measured for the sacrificial and relocated tree studies and for the girdle experiment. Five leaves per tree were measured for the water experiment. A mean value was calculated for each tree.

Water potential was measured with a Plant Moisture Stress Model 600 pressure bomb. Values ranging from 0 to –4.0 Mpa are measurable. Measurements were made on five different twigs for each tree in the sacrificial tree study, relocated tree study and girdle experiment, and on three different twigs for each tree in the water experiment. Values were calculated as the average of readings for a tree. Standard errors were typically less than 0.1 Mpa. Wet and dry bulb temperatures were measured proximal to the sample location with a psychrometer, and the values used to calculate ambient vapor pressure deficit.

**Sacrificial Tree**

Two 10 cm (4-in.) diameter (dbh) sand live oaks were spaded, rootballed, and relocated to accommodate development on 22 June 1999. Both the original and relocation sites were oak scrub habitat, which is characterized by well-drained, infertile, sandy soils. After relocation, one tree was irrigated directly to the root ball using a low-volume system at a frequency intended to keep the root ball moist. The other tree was not irrigated.

Leaf chlorophyll concentration, leaf temperature, and water potential were measured on six occasions between 24 June and 14 July 1999. The experiment was terminated when all of the leaves on the nonirrigated tree were chlorotic and brittle.

**Relocated Trees**

Ten sand live oak trees to be preserved for future use were spaded, rootballed, and relocated about 1 km (0.6 mi) distant. The trees, ranging from 15 to 20 cm (6 to 8 in.) dbh, were heeled in and irrigated directly to the root ball using a low-volume system at a frequency intended to keep the root ball moist. Leaf chlorophyll concentration, leaf temperature, and water potential of each tree were measured twice in situ (1 and 15 October 1998), and once while the trees were rootballed (18 November 1998). Leaf chlorophyll concentration, leaf temperature, and water potential were measured eight times between December 1998 and October 1999, at which time seven of the trees were re-relocated to their final location. Three trees were moved to a separate location, two in August 1999, and one in October 1999, and were eliminated from the study. Leaf temperature and water potential of the seven re-relocated trees were measured 11 times between October 1999 and January 2000. Leaf chlorophyll concentration measurements were terminated October 1999 because no response to stress had been detected during sacrificial tree observations, 10 months observation of the relocated trees, or 15 months of the girdle experiment (see below).

**Girdled Experiment**

Ten pairs of trees, each pair proximally located and as similar in size and form as possible, were selected for leaf chlorophyll concentration, leaf temperature, and water potential measurements. Three baseline measurements were taken in August 1998, and then one tree of each pair of trees was girdled about 30 cm (12 in.) above ground level. Girdling removed the epidermis, cortex, and phloem for the entire circumference of the tree. Water potential was measured 36 times, leaf temperature 27 times, and leaf chlorophyll concentration 25 times after girdling between September 1998 and July 2001. Measurements of leaf chlorophyll concentration were terminated after 15 months because no response to stress had been detected during sacrificial tree observations, 10 months observation of the relocated trees, or this experiment.

**Water Experiment**

Thirty 57 L (15 gal) live oak trees in plastic, aboveground containers were obtained from a local nursery in mid April 2001 and randomly assigned to one of three groups: control, overwatered, and underwa tered. All trees received 1.9 L (0.5 gal) of water per day through 3 May 2001, when leaf temperature and water potential were measured. Thereafter, the
control trees received 1.9 L of water per day until 28 June, 3.8 L (1 gal) per day from 29 June until 3 July, and then 7.6 L (2 gal) per day from 4 July until the experiment was terminated 8 October 2001. Overwatered trees received twice as much water as the control trees, and underwatered trees received half as much water as the control trees. Beginning 15 June 2001 (between weeks 6 and 7), we attempted to recover the underwatered trees by increasing their water to match that of the control trees.

**STATISTICAL ANALYSIS**

Data from the sacrificial tree and relocated tree observations, and from the water experiment, lacked homogeneity of variance and were not normally distributed. Consequently, nonparametric statistics were used to evaluate leaf chlorophyll concentration, leaf temperature, and water potential data (Statsoft 1994). Friedman’s ANOVA ($\chi^2$) was used to test for variation in response within treatments across time. The Wilcoxon’s Signed Rank Test (Z) was used to compare sacrificial tree observation irrigated and nonirrigated values on each measurement date, and date-to-date values of the relocated tree observations. Kruskal-Wallis ANOVA by ranks (H) was used to compare water experiment control, overwatered, and underwatered trees on individual dates. Differences among water experiment treatments were tested using Mann-Whitney U-Test (Z*).

Data from the girdle experiment satisfied the assumptions for parametric statistics. However, leaf fall precluded measurement of several girdled trees at the end of the experiment, which precluded effective application of a Repeated Measures ANOVA. Consequently, the T-Test for Dependent Samples (t) was used to compare leaf chlorophyll concentrations, leaf temperatures, and water potentials among control and girdled trees on each measurement date.

The level of significance is 0.05 for all statistical tests. Significant differences between treatments are indicated with an asterisk (*) on the applicable figures.

**RESULTS**

**Sacrificial Tree**

The water potential of the irrigated tree ($\chi^2_{10,10} = 17.38, P = 0.004$) and nonirrigated tree ($\chi^2_{10,10} = 22.09, P = 0.0005$) varied throughout the course of the study (Figure 1). Irrigated tree water potential was relatively invariable through 8 days after relocation, and then increased 12 and 20 days after relocation. Nonirrigated tree water potential decreased to the lower measurement limit of –4.0 Mpa 8 days after relocation. Nonirrigated tree water potential was significantly lower (1 to 2 Mpa) than the water potential of the irrigated tree on all measurement dates (Wilcoxon Matched Pairs Test Z, all $P < 0.05$).

The leaf temperatures of the irrigated tree ($\chi^2_{10,10} = 46.43, P < 0.0001$) and the nonirrigated tree ($\chi^2_{10,10} = 42.19, P = 0.0001$) also varied throughout the study (Figure 1). Both the irrigated tree and nonirrigated leaf temperatures were about 2°C warmer than the air temperature immediately after relocation. Nonirrigated tree leaf temperatures increased throughout the study, peaking at 7°C greater than the air temperature. By contrast, irrigated tree leaf temperatures were generally less than air temperature (mean –1.8 ± 2.5 s.d.). The leaf temperature of the nonirrigated tree was significantly greater than the leaf temperature of the irrigated tree after the initial reading (Wilcoxon Matched Pairs Test Z, all $P < 0.05$).

Nonirrigated tree leaf chlorophyll concentrations declined significantly during the last observation 20 days after relocation ($\chi^2_{10,10} = 12.54, P = 0.03$). No change in irrigated tree leaf chlorophyll concentration was observed ($\chi^2_{10,10} = \ldots$

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**Figure 1. Water potential, leaf temperature, and leaf chlorophyll concentration (mean ± s.d.) of relocated irrigated and nonirrigated sand live oaks. Significant differences between irrigated and nonirrigated trees are indicated by an asterisk.**
4.97, \( P = 0.42 \)). Leaf chlorophyll concentrations did not differ consistently between irrigated and nonirrigated trees. However, leaf chlorophyll concentration was greater for the irrigated tree than the nonirrigated tree 6 days after relocation (\( Z_{10} = 2.49, P = 0.01 \)), and at the end of the study when nonirrigated tree leaves were achlorotic (\( Z_{10} = 2.29, P = 0.02 \)). There were no significant differences in irrigated and nonirrigated tree leaf chlorophyll concentrations at other times during the study.

**Relocated Trees**

Water potential varied throughout the study (\( \chi^2_{5,21} = 48.59, P = 0.0006 \)), and ranged from –0.9 Mpa to –1.5 Mpa (Figure 2). Water potential was significantly higher during the baseline period and several months after relocation and re-relocation than when the trees were rootballed, had recently been relocated, or during the summer prior to re-relocation (Wilcoxon Matched Pairs Test \( Z_{10} \), all \( P < 0.05 \)).

Leaf temperature varied widely, ranging from 7.2°C less than air temperature during the baseline period to 0.1°C more than air temperature 8 months after relocation (\( \chi^2_{7,21} = 91.61, P < 0.00001 \), Figure 2). Temperatures were highest when the trees were rootballed and after relocation or re-relocation, and coolest when in situ and acclimated to their new location (Wilcoxon Matched Pairs Test \( Z_{10} \), all \( P < 0.05 \)). Relatively warm leaf temperatures prior to re-relocation reflect high ambient temperatures and reduced watering.

Leaf chlorophyll concentrations did not vary significantly throughout the study (\( \chi^2_{7,8} = 3.05, P = 0.93 \)). Values averaged 45.6 ± 0.8 s.d SPAD.

**Girdle Experiment**

There were no differences in water potentials between the pairs of trees prior to girdling, and no differences between control and girdled trees until 29 September 1998 (T-Test for Dependent Samples \( t_9 \), all \( P > 0.05 \), Figure 3). From 29 September 1998 until 13 May 1999, with the exception of one measurement episode on 13 October 1998, the girdled trees had higher water potential than the control trees (\( t_{19}, \) all \( P < 0.05 \)). Girdled tree water potential was again higher than the control trees from 10 July 1999 until 26 January 2000 (\( t_{19}, \) all \( P < 0.05 \)). At that time, one of the girdled trees had lost all of its leaves. Two more girdled trees had lost their leaves by 6 June 2000, and two more (totaling five) lost their leaves by 14 September 2000. Trees that lost their leaves never produced new leaves. The water potential of remaining pairs was apparently different from 6 June 2000 through 26 July 2001, but the number of pairs was too small to reliably test with statistics.

Leaf temperature did not differ between the control and girdled trees on most dates (\( t_{19}, \) all \( P > 0.05 \), Figure 3). However, girdled tree leaves were warmer than control tree leaves 14 August 1999 (\( t_{19} = 2.45, P = 0.02 \)) and 25 September 1999 (\( t_{19} = 4.87, P = 0.0001 \)).

Leaf chlorophyll concentrations did not differ significantly between the girdled and control trees on any dates (\( t_{19}, \) all \( P > 0.05 \), Figure 3).

**Water Experiment**

Significant differences were detected among control, overwatered, and underwatered water potentials for some dates (Kruskal-Wallis ANOVA by ranks \( H_{2,15} \), all \( P < 0.05 \), Figure 4). There was no difference in water potential among the treatments during the baseline period and during the first 2 weeks of the experiment. Beginning 8 May 2001, 2 weeks after treatments were applied, the underwatered trees had lower water potentials than the control trees (Mann-Whitney
U-Test, $Z^*_{5,5} = -2.62$, $P = 0.009$). These differences persisted ($Z^*_{5,5}, \text{ all } P < 0.05$) through 7 August 2001, 14 weeks after the treatments were applied, despite a return to a normal watering regime for the under watered trees 7 weeks earlier. Differences in under watered and control water potentials ranged from 0.2 Mpa in week 9 to 1.5 Mpa in week 7. Water potentials for the under watered and control trees were similar throughout the remainder of the experiment ($Z^*_{5,5}, \text{ all } P > 0.05$).

There were no consistent differences between control and over watered water potentials. Over watered trees water potential was less than the control water potential 12 June 2001 ($Z^*_{5,5} = -2.22$, $P = 0.03$) and 3 July 2001 ($Z^*_{5,5} = -2.61$, $P = 0.009$), and was higher than the control tree water potential 17 July 2001 ($Z^*_{5,5} = -2.61$, $P = 0.009$).

Significant differences were detected among control, over watered, and under watered leaf temperatures for some dates (Kruskal-Wallis ANOVA by ranks $H_{2,15}$, all $P < 0.05$).

DISCUSSION

Prior studies indicated that leaf chlorophyll concentrations respond to water deficits, pest infestations, and nitrogen levels (Virgin 1965; Hsiao 1973; Alberte et al. 1975; Ayres 1984; Baldy et al. 1996a, 1996b). However, leaf chlorophyll concentration was not a useful indicator of direct water stress, stress associated with tree relocation, or stress from girdling in sand live oak and southern live oak trees in our study. Leaf chlorophyll concentrations were largely invariable when trees were relocated and irrigated. Moreover, differences in leaf chlorophyll concentration could not be detected between an irrigated and a non-irrigated tree until the leaves of the latter tree had become chlorotic and dry. Some variability in leaf chlorophyll was observed in the girdle experiment; however, values did not differ between girdled and control trees. Therefore, we conclude this variation in leaf chlorophyll is related to seasonal factors rather than stress.

Canopy temperature is commonly used to detect stress from water deficits in crop plants (Jackson et al. 1981; Sammis et al. 1988; Sammis and Jernigan 1992). Reicosky et al. (1985) also found that water surpluses can increase leaf temperatures, and suggested that canopy temperature measurements may be sensitive enough to indicate types of stress in addition to water deficits. In our study, leaf temperature was indicative of severe water stress and stresses associated with relocation (e.g., water, injury from root pruning). The leaves of the nonirrigated sand live oak were consistently warmer than both air temperature and the leaves of the irrigated sand live oak. Also, the leaves of relocated and irrigated sand live oaks were warmest when rootballed and immediately after relocation. As an indicator, leaf temperature may reliably integrate stress associated with relocation, although further study is required.

By contrast, leaf temperature was an ineffective indicator of mild water stress in southern live oak, or physical injury.
(i.e., girdling) to in situ sand live oaks. No differences were detected in leaf temperature between under-watered and control southern live oak. Only two significant differences in leaf temperature were detected between girdled and nongirdled sand live oaks in 27 sampling events. The significant differences occurred midway in the experiment and do not suggest a pattern.

We anticipated that sand live oak and southern live oak water potential would decrease in response to a water deficit (Mohr and Schopfer 1995). This response was clearly observed in under-watered trees in the water experiment and the nonirrigated tree in the sacrificial tree experiment. Responses were apparent within days (sacrificial tree) or weeks (water experiment). The water experiment also indicated that water stress could be detected early enough to allow recovery of stressed trees.

Water potential was also responsive to stress imparted by relocation and girdling. Trimming of peripheral roots, rootballing, and transport likely induce temporary water stress during tree relocation. As might be expected, water potential was lower during these activities than in situ. By contrast, girdled sand live oak had higher water potentials than nongirdled individuals. We did not investigate the reason for this phenomenon, which suggests that water absorption by the roots had increased.

In summary, Disney arborists investigated three affordable, easy-to-use measures of stress in live oak and sand live oak. Both water potential and leaf temperature demonstrated a consistent and predictable response to stress that make these measures useful for managing sand live oaks and southern live oaks. Water potential of sand live oaks and southern live oaks decreased in response to reduced watering and to stress induced by relocation activities. Water potential was sensitive enough to detect mild water stress in southern live oaks in time to effect recovery. By contrast, water potential increased in response to injury (i.e., girdling). Our studies suggest that leaf temperature is sensitive to severe water stress and stress associated with relocation activities. However, leaf temperature was less sensitive and less robust an indicator of stress in sand live oak and southern live oak than water potential.

**LITERATURE CITED**


Acknowledgments. Thanks to Lee Johnson of NASA Ames for early discussion of SPAD for leaf color in grape leaves (GRAPES project), Ed Gilman of the University of Florida for design suggestions, and two anonymous reviewers.

Résumé. Les arboriculteurs de Walt Disney World ont évalué trois mesures économiques et abordables—concentration foliaire en chlorophylle, température foliaire, potentiel en eau—pour la détection des stress en eau et ceux causés par les dommages physiques chez les chênes verts. L'évaluation a inclus des observations sur des arbres relocalisés ainsi que des expériences à partir d'arbres strangulés et d'arbres soumis à divers régimes d'arrosage. La concentration foliaire en chlorophylle ne réagissait que peu au stress et donc n'était pas un bon indicateur de stress chez le chêne vert des sables et le chêne vert du Sud. Le potentiel en eau et la température foliaire réagissaient aux stress causés et constituaient donc de bons indicateurs de stress chez ces deux mêmes espèces de chênes verts. Le potentiel en eau présentait un réponse prédictible et solide à la fois au stress en eau ainsi qu'aux dommages physiques. Des chênes verts mal arrosés ou encore ayant été l'objet d'une relocalisation avaient un potentiel en eau plus faible que ceux adéquatement irrigués ou n'ayant pas été déplacés. Par contre, les chênes verts étranglés avaient un potentiel en eau plus élevé que ceux qui n'étaient pas dans cette situation. La température foliaire variait de manière moins sensible et donc était moins sûre que le potentiel en eau, mais elle pouvait quand même s'avérer utilité. La température foliaire était plus élevée pour un arbre relocalisé et non irrigué que pour un arbre relocalisé et irrigué, de même que pour des arbres relocalisés lorsqu'ils avaient été mis en motte immédiatement avant leur relocalisation. La température foliaire ne variait que peu suite à une strangulation ou à un stress plus modéré en eau.

Resumen. Arboristas de Walt Disney World evaluaron tres formas económicas y prácticas de medir el estrés de los árboles—concentración de clorofila en las hojas, temperatura de la hoja y potencial hídrico—para usarse en la detección de daños físicos por estrés hídrico en encinos. La evaluación incluyó observaciones de árboles trasplantados y experimentos de árboles cinchados y otros sometidos a diferentes regímenes de riego. La concentración de clorofila en la hoja no fue responsable en gran parte del estrés y no sería útil como indicador de estrés en el encino. Tanto el potencial hídrico como la temperatura de la hoja prometen como indicadores de estrés en los encinos. El potencial hídrico presentó una respuesta consistente y predecible tanto para estrés hídrico como para daño físico. Los encinos inadecuadamente regados y los trasplantados tienen potenciales hídricos más bajos que los encinos que cuentan con un adecuado régimen de agua o los in situ. En contraste, los encinos con daño por cinchado tuvieron más altos potenciales hídricos que los árboles no cinchados. La temperatura de la hoja fue menos sensible y menos robusta para detectar el estrés que el potencial hídrico, pero aún así puede ser útil. La temperatura de la hoja fue mejor para árboles trasplantados no irrigados que para árboles con riego, y para árboles trasplantados cuando fueron arpillados e inmediatamente después trasplantados. La temperatura de la hoja no respondió al cinchado o estrés hídrico.