Water Relations and Related Leaf Characteristics of Healthy and Blight Affected Citrus Trees[1]

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Abstract. Citrus blight or young tree decline, is a wilt-like disease of unknown etiology which is characterized by restricted water movement and an upset in normal zinc distribution patterns. Diurnal leaf and fruit water potentials and leaf stomatal conductances of sweet orange *Citrus sinensis* (L.) Osbeck leaves on trees in various stages of decline were characterized to determine the progression of this disorder. All blight affected trees, regardless of severity of tree condition, had similar diurnal water relations. Blight affected trees have fewer and smaller leaves, less leaf area per tree, lower stomatal conductances, and lower diurnal transpiration rates than healthy trees. These differences did not result in any apparent changes in specific leaf weight, leaf osmotic potentials or in the critical leaf water deficits at which leaf turgor was lost. At equivalent transpirational fluxes, leaf water potential was much lower in blight affected trees than in healthy trees. Therefore, the water stress symptoms associated with blight are related to increased resistances in the water transport system and are not a result of lost stomatal function or changes in water relations characteristics of leaves that remain on blight affected trees.

The citrus disease called blight or young tree decline, probably has been present in Florida for a century (16). Blight is a wilt-like citrus disease that has been characterized as a trunk xylem dysfunction which causes restricted water movement, decreased leaf water potentials, and decreased stomatal conductance (3, 4, 7). Similar water relations changes in other plants have been correlated with increased resistance to water transport caused by phytophthora root rot disease (17) and can result in reductions in photosynthesis and yield (11). Increased water stress attributed to blight has been correlated with xylem vessel blockage in the wood of both roots and stems from declining trees (20, 21, 22). The earliest symptoms are a delayed spring flush or total lack of new growth and frequent wilting or leaf curl. Usually, these water stress symptoms progressively worsen over 2 to several seasons until the blight affected trees have extensive canopy thinning and twig dieback. Such trees are not economically productive. Blight has been correlated with unusually high accumulations of elemental zinc and phenolic-like compounds in the trunk portions of blighted trees while their leaves sometimes display typical zinc deficiency patterns (21, 22). The cause or causes of blight have never been transmitted or propagated (6, 10) and it is not known if the water stress symptoms are caused by the presence of a pathogenic organism or by some toxic substance that affects membrane permeability or stomatal function. Because no cause-effect relationship is presently known, measurement and analysis of physiological changes related to blight will likely provide useful insights into its etiology. If this disease affects membrane permeability or stomatal function, then blight affected leaves should have stomatal responses to environmental and plant conditions that differ from healthy leaves. These experiments were designed to test that hypothesis.

Allen and Cohen (3) have previously reported that blight affected citrus trees took up less water, had lower diurnal leaf water potentials and lower stomatal conductances than healthy

trees. Although their study incorporated data from trees which were in various stages of decline ascribed to blight, these data were reported in a way that does not permit evaluation of the progression of this disorder. The purpose of this study was to characterize the leaf and fruit diurnal water relations of trees in various stages of decline in an effort to understand changes in plant responses to the environment and disease condition. In addition, since it is not known whether these water relations changes can be correlated with any changes in leaf characteristics, we evaluated total tree leaf area, specific leaf weights, leaf osmotic potentials, and water deficits at which leaf turgor became zero on healthy and blight affected trees.

Materials and Methods

Data reported here are from 13-year-old 'Valencia' orange trees, on rough lemon rootstock (*C. limon* (L.) Burn. f.) that are located in Central Florida. Trees were selected that were apparently healthy or in each of 3 stages of blight using visible wilt symptoms and zinc analyses. Trees were rated as follows: 0 for apparently healthy, with no visible wilting or zinc deficiency symptoms; 1 for general lack of new flush or having visible symptoms in one sector of the canopy; 2 for moderate overall symptoms; and 3 for severe symptoms. Trees of various ratings were selected for study by their proximity to each other in an effort to minimize differences in soil characteristics. Trees were well watered in all cases.

Pressure chamber estimations (12, 15) of leaf water potential (Ψ_Q) were made using 6 individual sun-exposed leaves detached from 4 trees, one tree in each of the 4 rating categories, on a clear day in the fall (September) 1978. These leaves were from the previous spring flush. In the spring (April, 1979), fruit water potentials (Ψ_f) and Ψ_Q were estimated using 6 individual fruit or leaf samples, from the previous year's flush, from both sun-exposed (southeast) and shaded (north) fruit and leaves on a clear day. Fruit water potentials were estimated by placing whole fruit into the pressure chamber (13, 18). Leaves and fruit from 2 trees rated 0 and 2 trees rated 2 were used on the spring sampling day. Stomatal conductances (k_s, cm s^{-1}) were measured on the same trees as Ψ_Q and Ψ_f during the spring and fall. Abaxial leaf surface k_s (calculated from the inverse of the stomatal resistance) were measured with a Li-cor diffusion Autoporometer.

All Ψ and k_s data presented are means of 6 replicate determinations. Leaf surface temperatures were measured using a

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Barnes Instatherm infrared thermometer which has an accuracy of ± 0.2°C at 20°C. Vapor pressure deficits (VPD, kPa) were calculated from a wet-dry bulb psychrometer and used to calculate the water vapor concentration ($[H_2O]$, in μg cm^{-3}) of the air. Transpiration rates (E, in μg H$_2$O cm^{-2} s^{-1}) were estimated by setting:

$$E = \Delta[H_2O] k_s$$

where $\Delta[H_2O]$ is the difference in water vapor concentration between the leaf (assumed saturated at air temperature) and ambient air and k_s is the measured leaf conductance (9). Since sun-exposed leaf surface temperatures exceeded air temperature, only mean k_s from the shaded position were used for calculating E. Leaf-air temperature differences from the shaded position never exceeded ± 0.5°C. All Ψ and k_s data were analyzed using factorial split plot in time analyses of variance.

The total tree canopy volume was estimated using a method developed by Albrigo et al. (2). Total leaf area per tree was estimated for a typical healthy and a blight affected tree (rated 2) from the leaves harvested from within a 0.6 × 0.6 m sampling frame which extended from the canopy surface to the trunk. The frame was placed into each canopy 4 times, once in each of the 4 cardinal directions, at a height of 2 m. The volume sampled was approximately 1 m^{-3}. The total leaf area from each volume sampled was measured using a Li-cor leaf area meter. The mean leaf area from the 4 replicate samples per tree was used to extrapolate to the total leaf area within the canopy. Mean specific leaf weight (SLW) was estimated from the leaf dry weight/area of 30 leaves for both a healthy and a blight affected tree rated 2.

Zinc concentration was determined by standard digestion procedures and quantified by atomic absorption spectroscopy (21). One sample was taken from the first outer cm of trunk xylem from each tree used in this study.

Osmotic components of water potentials (Ψ_π) and leaf water deficits (WD) at zero turgor were estimated using at least 4 replicate leaves by pressure volume relationships (PV) (5, 14, 15, 19). Estimating the Ψ_π in this manner equates Ψ_π to the total non-turgor component potential which includes any contribution of matrix forces. Since the x-intercept of the PV represents total water expressed at infinite pressure, this value estimates the fraction of leaf water available for osmotic exchange (OF). The leaf water potential at incipient plasmolysis (Ψ') and its corresponding WD (WD'), correspond to the pressure and the fraction of total leaf water lost at which the pressure volume relationship becomes non-linear. These values quantitatively estimate the point at which leaf turgor pressure becomes zero and the leaf begins to wilt.

Results

There were no significant differences (P > 5%) among the Ψ_Q (expressed in units of MPa = 0.1 bar) of sun-exposed leaves on blight affected trees rated 1, 2, and 3 (Table 1). Except for the predawn (0600 and 0630 hr) and 0800 hr sampling times, the blight affected trees had lower Ψ_Q than the healthy trees (rated 0). The afternoon recovery rate, as estimated from the rise of approximately 0.5 MPa from 1330 to 1500 hr in the spring, appears to be similar for both healthy and blight affected trees. Though not as striking as the sun-exposed Ψ_Q data, the Ψ_Q from the shaded exposures (Table 2) show the same relative pattern. The Ψ_Q and Ψ_f from trees rated 2 are significantly lower (P < 5%) than those from the healthy trees rated 0 (Table 2).

Although k_s were highly variable, the k_s from the spring sampling day were generally lower than k_s in the fall (Table 3). This is partly because the sampled leaves in the spring were 7 months older and more hardened than those in the previous fall. Except for the shaded exposures in the spring, all disease ratings and exposures appear to exhibit a mid-day decrease in

Table 1. Leaf water potentials (Ψ_Q) from healthy (rated 0) and disease trees (rated 1, 2 or 3) in the fall and spring. Each value is the mean (± 1 SD) of 6 replicate leaves removed at the indicated time of day (Eastern Standard time). All leaves were samples from southeast (sun exposures).

Season and	Ψ_Q (MPa)			
time of day	Disease rating			
	0	1	2	3
Fall				
0630 hr	−0.4 ± 0.1	−0.5 ± 0.2	−0.5 ± 0.1	−0.4 ± 0.1
0800	−0.8 ± 0.3	−1.2 ± 0.3	−1.1 ± 0.2	−0.8 ± 0.3
0930	−1.2 ± 0.2	−1.6 ± 0.2	−1.7 ± 0.2	−1.7 ± 0.3
1200	−1.8 ± 0.1	−2.2 ± 0.2	−2.6 ± 0.2	−2.6 ± 0.1
1330	−2.0 ± 0.2	−2.4 ± 0.2	−2.7 ± 0.1	−2.6 ± 0.2
Spring				
0600	−0.3 ± 0.1		−0.4 ± 0.0	
0900	−1.3 ± 0.2		−1.9 ± 0.3	
1130	−1.4 ± 0.6		−2.3 ± 0.3	
1330	−1.7 ± 0.5		−2.6 ± 0.2	
1500	−1.2 ± 0.2		−2.1 ± 0.2	

Table 2. Leaf (Ψ_Q) and fruit (Ψ_f) water potentials from healthy (rated 0) and diseased (rated 2) trees in the spring. Each value is the mean (± 1 SD) of 6 replicate leaves of fruit removed at the indicated time of day. All samples were from north (shade) exposure.

	Ψ_Q (MPa)	Ψ_f	Ψ_Q	Ψ_f	
	Disease rating				VPD
Time	0	0	2	2	(kPa)
0600 hr	−0.3 ± 0.0	−0.3 ± 0.1	−0.3 ± 0.1	−0.5 ± 0.2	0.1
0900	−0.8 ± 0.1	−0.7 ± 0.3	−1.1 ± 0.1	−0.5 ± 0.1	0.7
1100	−1.1 ± 0.2	−1.0 ± 0.4	−1.9 ± 0.2	−1.5 ± 0.5	1.7
1300	−1.3 ± 0.3	−1.1 ± 0.5	−2.2 ± 0.4	−1.9 ± 0.7	2.0
1500	−1.2 ± 0.2	−1.3 ± 0.5	−2.2 ± 0.1	−1.3 ± 0.5	1.9

Table 3. Stomatal conductance from healthy (rated 0) and diseased trees (rated 1, 2 or 3) in the fall and spring. Each value is the mean (± 1 SD) of 6 replicate leaves which were evaluated at about the time of day (Eastern Standard time) indicated. Each disease rating is followed by direction of exposure: SE = sun, N = shade.

Season and	Stomatal conductance (k_s cm s^{-1})			
time of day	Disease rating (exposure)			
	0 (SE)	1 (SE)	2 (SE)	3 (SE)
Fall				
0900 hr	.80 ± .57	.67 ± .49	.33 ± .09	.37 ± .31
1100	.47 ± .38	.19 ± .05	.09 ± .04	.14 ± .09
1300	.27 ± .17	.11 ± .04	.12 ± .04	.10 ± .04
1500	.13 ± .06	.07 ± .02	.10 ± .05	.08 ± .02
Spring	0 (SE)	0 (N)	2 (SE)	2 (N)
0840	.13 ± .05	.05 ± .02	.15 ± .05	.05 ± .01
1110	.13 ± .19	.04 ± .01	.05 ± .04	.03 ± .01
1330	.12 ± .07	.05 ± .02	.07 ± .02	.04 ± .01
1515	.03 ± .01	.04 ± .02	.03 ± .01	.03 ± .01

k_s. In the fall, the k_s from the blight affected trees (1, 2, and 3) are not significantly different, but collectively the blight affected trees had significantly lower (P < 5%) k_s than the healthy tree. In the spring, the shaded leaves (healthy and diseased combined) had significantly lower (P < 5%) k_s than the sun-exposed leaves. The variations in the spring data obscure any
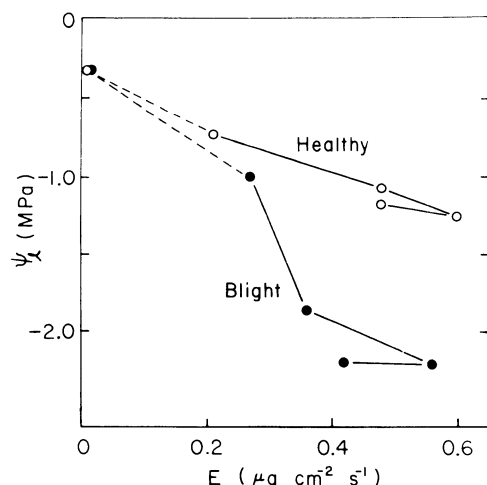
Fig. 1. The relationship of leaf water potential (Ψ_ϱ) to transpirational flux (E) of leaves from healthy (○) and blight affected (●) trees. Predawn Ψ_ϱ are paired with E values shown to be near zero.

significant variation in k_s that can be attributed to blight. The measured VPD (Table 2) and the mean k_s from the shade exposed leaves in the spring (Table 3) were used to calculate the estimated transpiration rate (E) in Fig. 1. Mean Ψ_ϱ values were plotted against the appropriate value of E (Fig. 1). Except in the early morning, leaves from blight affected trees had consistently lower Ψ_ϱ at equivalent E than those from the healthy trees. This relationship is likely due to increased resistances to water transport (17) in diseased trees. The apparent reduction in E did not result in any measurable differences in leaf surface temperatures between blight affected and healthy trees. Shaded leaf surface temperatures never varied from air temperature more than ± 0.5°C; leaf surface temperatures from sun-exposed leaves varied from 1-4°C above air temperature with no consistent relationship to blight.

Blight affected trees (rated 2) had much higher trunk Zn concentration than healthy trees (Table 4). A blight affected tree had about one-half the total leaf surface area (TA) of a healthy tree. The blight affected tree was somewhat smaller and had fewer and smaller leaves than the healthy tree. There were no significant differences between SLW, leaf osmotic potential (Ψ_π) or in the amount of leaf water available for osmotic exchange (OF) that could be related to blight. In addition, blight affected and healthy leaves had comparable WD (0.08) and Ψ_ϱ (ca. −2 MPa) at plasmolysis. Leaves from blight affected trees reach critical Ψ_ϱ (Ψ') sooner in the day and remain below these stress levels for longer periods of time (Tables 1 and 2).

Discussion

The lack of significant differences among the diurnal Ψ_ϱ and

k_s from trees in various stages of decline due to blight can be partially explained by the marked thinning of the canopy and decreased leaf areas associated with the progression of blight. As the disease progresses, either leaf abscission rates increase or the rate of new leaf production decreases in response to increased water stress over healthy trees. The similarity of Ψ_ϱ responses within the same age class of leaves from the blight affected trees in both the fall and spring, indicates that variations in Ψ_ϱ associated with this disorder apparently are not greatly affected by leaf senescence. The similar spring afternoon Ψ_ϱ recovery rates for both healthy and blight affected trees along with similar predawn Ψ_ϱ for all trees show that at least under these conditions, blight does not affect the tree's capability of recovering from daily WD (Table 1).

The Ψ_π is important since it represents the maximum turgor pressure that a leaf can develop assuming there is not concomitant change in cell wall elasticity. The OF represents the actual volume of water that is available for exchange as the leaf water content fluctuates in the physiological range of WD. Taken together, Ψ_π and OF determine the slope of the linear portion of PV and hence, the response of Ψ_π to daily or seasonal changes that occur in Ψ_ϱ. The specific leaf weight, Ψ_π and OF were not affected by blight. Although there were fewer and smaller leaves on blight affected trees, the leaves that remained did not have greater specific leaf weights nor did they develop lower Ψ_π in response to increased water stress. In addition, Ψ' and WD' were similar for both healthy and blight affected leaves. Taken together, these data lend credence to the idea that although this water stress related disease lowers daily Ψ_ϱ and k_s, the water relations characteristics of individual leaves from blight affected trees do not differ significantly from healthy leaves. Thus, these data show that blight affects stomatal function but there is no evidence that the relationship of stomatal response to environmental and plant conditions differs between healthy and blight affected trees.

Blight affected leaves reach critical Ψ_ϱ (Ψ') sooner and remain below Ψ' for longer periods of time during the day. This water stress results in a loss of leaf turgor and assumably normal leaf function which affects the size and number of leaves and thus, the total leaf area per tree. The consistent relationship that exists between the leaves and fruit on trees with blight along with the water uptake data from Allen and Cohen (3), indicate that this disease affects tree water relations physiology through increased resistance to water transport. The lower Ψ_ϱ at similar E of blight affected trees also indicates increased water transport resistance and provides additional evidence to support that conclusion.

The lower mean k_s associated with shade leaves on blighted trees resulted in lower E than that of healthy trees. Although Edwards and DuCharme (8) reported no significant effects of blight on whole canopy temperatures, we thought that the decreases in k_s and E might have resulted in differences in individual leaf temperatures. The differences in E of shaded leaves did not result in any measurable differences in leaf surface temperatures. Any differences may have been obscured by variations in k_s.

Table 4. Summary of the various leaf and tree parameters that correspond to healthy (rated 0) and blight affected trees (rated 2). Mean values are followed by ± SD. Listed are zinc (Zn) concentration in parts per million (ppm); TA = total leaf area per tree, SLW = leaf dry weight per area, Ψ_π = leaf osmotic potential at 0 water deficit, OF = osmotic water fraction, Ψ' = water potential at plasmolysis, and WD' = leaf water deficit at plasmolysis.

Rating	Zn (ppm)	TA (m²)	SLW (mg cm⁻²)	Ψ_π (MPa)	OF	Ψ' (MPa)	WD'
0	2.3 ± 1.2	138.5	12.3 ± 2.4	−1.8 ± 0.2	0.62 ± 0.13	−2.0 ± 0.2	0.08 ± 0.01
2	18.0 ± 3.5	58.9	11.7 ± 1.2	−1.8 ± 0.1	0.57 ± 0.13	−2.1 ± 0.2	0.08 ± 0.02

The spring k_s from both shade- and sun-exposed leaves were generally lower than the k_s from the more recently expanded leaves in the previous fall. Part of this difference was due to the natural "hardening off" of leaves during stomatal and cuticle development (1). The mid-day decrease in k_s of all trees in the fall was also apparent in the sun-exposed leaves during the next spring. Though more frequent hourly data would be illustrative, it appears that the lower Ψ_Q was associated with the decreased k_s of blight affected and senescent leaves. It is possible that the restricted water movement through the plant may hasten natural leaf senescence. The decreased water movement and consequent decreased Ψ_Q and k_s apparently result in canopy thinning but not in changes in specific leaf weight, leaf osmotic potentials, or in the critical leaf water deficits at which leaves wilt. Whether these responses are specific to the disorder known as blight or young tree decline or typical of citrus responses to water stress in general, remains a question.

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Sampling Strategies for Estimates of Cluster Weight, Soluble Solids and Acidity of 'Concord' Grapes[1]

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Abstract. Variability of cluster weight, soluble solids, and titratable acidity of 'Concord' grapes (*Vitis labruscana* Bailey) was quantified with respect to several fixed and random factors. All 3 measurements were affected by cluster position while only cluster weight and soluble solids were affected by sunlight exposure. Estimates of variance components indicated that the greatest percentage variability was among vines. Variance components were used to examine efficient sampling plans that would detect a specified difference at a selected level of Type I error. Graphs illustrate various schemes of sample allocation which would achieve the desired level of precision.

Variation from a number of sources is known to exist within grape vineyards. Differences in vine size, crop load, cluster position and exposure to sunlight can affect the sugar content of berries (8, 13) and the number and size of clusters (7). This variation is important for several reasons. Yield and ripeness estimates are frequently made in order to determine whether cultural options are economically feasible and whether the crop has ripened to the degree required by a particular processor. Research efforts are hindered when variation masks the difference between 2 applied treatments. Without a knowledge

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