Water Uptake Patterns in Blighted Citrus Trees

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Abstract. Sweet orange cultivars, Citrus sinensis (L.) Osbeck, in several stages of blight (young tree decline) were studied for characteristics of waterflow or uptake. Many small and major roots and the trunk on moderately blighted trees had restricted waterflow or uptake capabilities. Some small and major roots on the blighted side of early-stage or sectored trees also had restricted waterflow or uptake capabilities, but the healthy side roots functioned similarly to those on healthy trees. Dye uptake patterns confirmed that the waterflow or uptake was restricted in the diseased portions of the trees. On moderately blighted trees, young xylem appeared to be more functional for water movement than older xylem. Necrotic roots were found on the blighted side of early-stage sectored trees and moderately blighted trees, but not on the healthy side of sectored trees or on healthy trees.

The sectoring type of early blight expression affords a model system for study whereby both blighted and apparently healthy tissues occur on the same tree.

Wilt diseases are common in plants, including agricultural crops, and can be caused by either fungi or bacteria (4, 10, 20, 25). Vascular wilt pathogens invade water conductive vessels, release metabolites, cause cell breakdown, pollute the transpiration stream, induce phenolic and aromatic compounds to accumulate, and restrict water movement from the roots to the leaves (2, 4, 10, 12, 25). In many cases, the organism produces a toxin which affects cell membrane permeability, stomate opening, and other processes (20, 24, 25).

Citrus blight (young tree decline, sandhill decline, and rough lemon decline) is a wiltlike disease of unknown cause which is characterized by a xylem dysfunction (5, 15, 17, 18, 26, 27), restricted water movement (7, 11, 28), and phenolic accumulation (28). Visual symptoms of the disease have been described (5, 6, 7, 23). Tree age is a factor in disease expression in that symptoms do not appear until trees are at least 6 to 7 years old, thus eliminating studies on small plants. Blight causes losses of at least 500,000 trees annually in Florida and has been found on the commonly grown citrus cultivars and rootstocks (7, 11, 28). Sweet orange cultivars on rough lemon rootstock appear to be most susceptible (23).

Most of the physiological differences reported between healthy and blighted citrus trees have been from studies on trees exhibiting moderate to severe symptoms. Few studies have been made with trees in the earliest stages of the disease where physiological changes may offer the best insight into the cause of the disease. We evaluated waterflow patterns in citrus trees exhibiting varying degrees of blight with special emphasis on trees in early stages.

Materials and Methods

Plant materials. Citrus used in these studies were 'Valencia' orange, Citrus sinensis, on rough lemon rootstock, C. limon (L.) Burm. f., and 'Hamlin' orange, C. sinensis, on sour orange, C. aurantium (L.), and on Carrizo citrange, Poncirus trifoliata (L.) Raf. × C. sinensis. The ages and locations of the trees were: 'Valencia' oranges, 9 to 11 years old near Leesburg and Winter Garden, and 18 years old near Leesburg; 'Hamlin' oranges, 10 years old near Clermont, and 25+ years old near Citra.

We selected trees with 3 stages of blight symptoms: healthy, sectored, and moderate. On sectored trees, 1/4 to 1/3 of the canopy exhibited typical early symptoms. The sectored portion of the trees was confined to 1 or 2 major scaffold limbs located adjacent to each other. Sectored trees selected exhibited the earliest recognizable visible symptoms. Moderately blighted trees had typical symptoms throughout the canopy. All trees selected had exhibited blight symptoms less than 1 year, and at least 50% of the leaves remained, even on trees with moderate symptoms.

Waterflow measurements. Waterflow through detached roots

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2 Plant Physiologist and Plant Pathologist, respectively. The authors gratefully acknowledge the assistance of Mrs. Eldora Waldon, Biological Laboratory Technician, and Dr. Heinz Wuscher, Research Horticulturist.

Literature Cited

7 to 14 mm in diam was accomplished by the vacuum method reported by Garney and Young (11). The ability of roots to conduct water was measured by connecting root pieces 15 cm long to a vacuum source and to a calibrated water reservoir. The end of the root closest to the trunk was connected to the vacuum supply so that water movement was in the direction of normal flow. A 3/4 atmosphere vacuum was applied by an electric pump. Care was taken to avoid mechanically damaged roots. Most roots were taken within 29 cm of the surface. Seven to 10 detached roots were measured per tree, except on sectored trees. In one experiment, 30 roots were taken randomly from around the sectored trees, while in the other experiments, 7 to 10 roots each were taken from the blighted and from the healthy sides of the sectored trees. Roots were detached in the afternoon, kept damp and cool (11.1°C) until morning, warmed to room temp, and waterflow determined. Results are reported in flux density units as ml waterflow per cm² xylem cross-sectional area per min (ml/cm² per min).

Water uptake was measured by gravity injections between 11 AM and 2 PM by the method of Cohen (7). Holes, 6 mm x 40 mm, were drilled into either roots or trunks and metal injectors were tapped into the holes 1 cm. Calibrated polyethylene tubing was attached to the injectors and filled with water to measure water uptake. We compared our 3-hr water uptake results with a 24-hr uptake period used by Cohen, and found that trends in differences between healthy and blighted trees were the same, although there was a greater difference between rates for the 24-hr period. Injections were made into 8 to 11 major roots, 5 to 8 cm in diam, and 4 trunk sites, 15 cm above and below the bud union, per tree. Two healthy trees and 2 trees with sectored and moderate symptoms were evaluated for detached-root waterflow and major root- and trunk-water uptake in April and July 1976. Two sectored trees were evaluated in October 1975. Because the trends were similar, the results from all dates were combined. In another experiment, single injection sites 15 cm above and below the bud union were used on each tree. Results of gravity injections in all tests were expressed as ml water uptake/hr.

Dye-uptake studies. Crystal violet (0.1% in water) was used for dye-flow studies because it was easily detected. A preliminary study indicated movement in citrus xylem was similar for crystal violet and for acid fuchsin and several other dyes tested. A 19-liter reservoir of dye was connected by rubber tubing to tapered, metal inserts tapped into the root- or trunk-injection sites. In another test, rubber tubing was attached to the tree side of several 7-mm-diam roots. Dye was fed by gravity (pressure head approximately 60 to 70 cm) for 3 days to 1 set of trees (healthy, sectored, and moderate) for each sampling date. To determine the pattern of dye movement, we either sectioned the trees or took trunk cores with an increment borer.

Leaf-resistance and trunk-zinc analyses. Leaf-resistance was measured, with a leaf-resistance meter (1), on 3 leaves per tree at 1:00, 2:00, and 3:00 PM on the southwest or southeast sides of the tree.

Sectored trees were selected with the blighted sector on the southwest or southeast sides of the tree so that leaf resistance measurements of the healthy-appearing part of the tree could be made on the south to southeast side of the tree, while leaf resistance of the blighted portion could be made on the southwest side. Weather and soil conditions varied among sampling dates, but at each date, 1 tree of each disease category was sampled the same day. Zinc was determined by standard digestion procedures and quantified by atomic absorption spectroscopy. One sample from the first outer cm of trunk xylem was taken from each tree for analysis.

Results and Discussion

Waterflow and water uptake studies. Waterflow through detached roots was about 40% less for moderately blighted trees than for healthy roots (Table 1), which confirmed previous observations (11). However, the average waterflow rate through randomly selected roots was not significantly different for sectored blighted trees than for healthy trees. The percentage of roots with waterflow less than 5.0 ml/cm² per min was least for healthy trees, greatest for moderately blighted trees, and intermediate for sectored trees. Standard deviations of mean waterflow for roots from sectored (19.4) and moderately blighted (17.2) trees was statistically greater (95% level of confidence) than deviations for healthy roots (12.9). This explained why percentage of roots with waterflow rates below 5.0 was greater on sectored than on healthy trees while the mean rate was equal. The exact location of slow-flow roots on the trees was not identified. Roots with waterflow rates 5.0 ml/cm² per min or less probably had more xylem tissues which were nonfunctional than roots with flow rates above 10.0 ml/cm² per min.

To establish the location of these nonfunctional roots on sectored trees, we undertook a detailed study. Waterflow through detached roots, and water uptake through major roots and the trunks of healthy, sectored, and moderately blighted trees were measured. Care was taken to measure separately the detached roots and injection sites on the sectored and healthy side of sectored trees (Table 2). Data clearly indicate that waterflow was impaired in small roots, large major roots, and in the trunks of moderately blighted trees. These results confirm previous observations (7, 11). Waterflow also was impaired in the roots on the sectored side of blighted trees. Water uptake also tended to be impaired in trunks on the sectored side of blighted trees. These differences were not significant because of the low water uptake during the 3-hr measurement period. This indicated that in the early stage of blight, such as on sectored trees, the waterflow in roots may be impaired on only a small portion of the tree. Blight symptoms on sectored trees eventually will encompass the entire tree. In addition, early blight symptoms are not always confined to a sector on a tree, but instead affect the entire canopy. Restricted water movement has been reported in wilt-diseased oak (19, 29), Dutch elm (13, 14), and tomato (9).

Water uptake by trunks of trees on 3 rootstocks with injectors placed either above the bud union in the trunk or below the bud union in a major root was much less in blighted than in healthy trees (Table 3). The poor water uptake into diseased trunks above the bud union indicates that the disease is not a bud-union disorder on commercial rootstocks. Cohen's (7) conclusion was similar from measurements on trees on rough lemon rootstock.

Leaf resistance and trunk-zinc analyses. Leaf-resistance measurements indicated that water stress affected the canopy of moderately blighted trees and the blighted side of sectored trees (Table 4). These results were expected, because of the

| Tree blight condition | No. of trees | No. roots per tree | Waterflow through detached roots (ml/cm² per min) | Roots with water flux density <5.0 ml/cm² per min (%)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>11</td>
<td>7</td>
<td>26.6 ± 1.8X</td>
<td>2.9</td>
</tr>
<tr>
<td>Sectored</td>
<td>4</td>
<td>23</td>
<td>23.2 ± 2.4</td>
<td>17.8</td>
</tr>
<tr>
<td>Moderate</td>
<td>11</td>
<td>7</td>
<td>16.2 ± 2.3</td>
<td>43.0</td>
</tr>
</tbody>
</table>

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Table 1. Waterflow² through detached roots from 'Valencia' orange trees² on rough lemon rootstock with different degrees of blight.

²Waterflow measured in root sections 15 cm long and about 7 to 14 mm in diam under 3/4 atm vacuum (equivalent to 775 cm waterhead).

²Results are a composite of data taken from 3 trees in groves located near Leesburg and Winter Garden, Florida.
waterflow impairment in the trunk xylem tissues (Table 2), and, on moderately blighted trees, were similar to published results (1, 3). Our limited leaf-resistance measurements verified the water stress phenomenon in the leaves.

Smith (22) and Wutscher et al. (28) demonstrated that zinc accumulates in the trunks of moderately blighted trees, but zinc-deficiency symptoms often occur in the leaves. We found that zinc accumulated in trunks of sectored and moderately blighted trees, including both sides of the sectored trees (Table 4). This accumulation of zinc in the xylem tissues may distinguish between citrus blight and other wiltlike diseases, since it has, to our knowledge, been reported only in citrus blight. Accumulation on the apparently healthy side of sectored trees, even though watering had not yet become impaired, may be an early physiological effect of the disease.

**Dye-movement patterns.** After watering and uptake data were taken on trees (Table 2), 3 trees each of healthy, sectored, and moderate blight were allowed to take up crystal violet dye through all the major root injectors for 3 days. Trunk cross sections 15 cm above the bud union were made to show the dye-flow patterns on one set of trees (Fig. 1). The healthy tree had a series of rings of stained xylem tissues located 5 to 10 mm from the cambium, extending inward and encompassing about half the xylem area of the trunk. The center portion of the trunk was devoid of dye. The sectored tree had fewer stained xylem tissues than the healthy tree, and the rings of stained tissues were not continuous. The sectored side (Fig. 1B, upper left portion) was almost devoid of dye. This coincided with the area of reduced water uptake on that side of the tree (Table 2).

Restricted dye movement in wilt-diseased portions of oak trees was also reported (19, 29). The moderately blighted tree had few stained xylem tissues which were scattered and exhibited less tendency than the healthy tree to have rings of stained tissues. Dye movement up the trunk and into the scaffold limbs was related directly to the patterns shown in Fig. 1. Dye moved freely into the scaffold limbs of the healthy tree and of the healthy side of the sectored tree, but moved poorly and irregularly into scaffold limbs on the blighted side of the sectored and moderately blighted tree. Dye also moved downward in the major roots which were functional, such as those on the healthy tree and on the apparently healthy part of the sectored tree. Dye was found as far as 3 m from the injection the down into roots 5 mm in diam. These patterns of dye movement were typical of those in the other 2 sets of trees from which cores were taken, and the trees were not sacrificed. In this series of trees, dye did not move in the young, outer centimeter of xylem tissues.

Vertical sections of healthy and sectored tree trunks through the bud union indicated that dye moved across the bud union, except on the blighted side of the sectored tree (Fig. 2B, right side) where dye never reached the bud union (Fig. 2). This was consistent with the water uptake patterns discussed in Table 3, which indicated that a bud union incompatibility was not involved in the disease. Cohen (7) found acid fuchs in dye uptake by healthy and blighted trees to be proportional to the uptake of distilled water, but did not report the qualitative differences in dye movement that we observed.

The lack of dye movement into the younger xylem tissues (Fig. 1) apparently was caused by blocking of the young vessels by the injector inserts, so that the dye was forced to move only in the old xylem tissues of the major roots and the trunks. To further clarify whether our injection technique blocked dye movement in young xylem, we used several dye injection techniques on 2 moderately blighted trees; Fig. 3 shows the results from 1 tree. In positions 1 and 3, slotted injectors were tapped 1 cm horizontally into the trunk about 18 cm above the ground line (Fig. 3A). Dye was absorbed into the older xylem, as in previous studies, and was absent in the young xylem next to the cambium. Staining rapidly became less concentrated above the point of injection, and was poorly defined (Fig. 3B), indicating slow flow. In positions 2 and 4, slotted injectors were slanted upward at a 45° angle into the trunk 1 cm deep and about 18 cm above the ground line. Dye was absorbed into both young and old xylem. Staining again became less concentrated above the injection point, but more dye than for positions 1 and 3 remained in the young tissues. In position 5, a small, young root (7 mm diam), growing directly out of the trunk at the ground line, was attached to the dye reservoir through a tube connected to a cut end of the root. Dye moved up the trunk to the scaffold limbs and downward into adjacent roots by way of the young, outer xylem. In positions 6 and 7, injectors were tapped into major roots 1 cm deep, and the dye-flow patterns were

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**Table 2. Water uptake by trunk injection and waterflow through detached roots of 'Valencia' orange trees on rough lemon stock with different degrees of blight.**

<table>
<thead>
<tr>
<th>Tree condition</th>
<th>No. of trees</th>
<th>Major roots (ml/hr)</th>
<th>Trunk (ml/hr)</th>
<th>Waterflow through detached roots (ml/cm² per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>4</td>
<td>20.6 ± 4.0 w</td>
<td>16.0 ± 2.8</td>
<td>29.4 ± 1.4</td>
</tr>
<tr>
<td>Sectored</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy side</td>
<td>6</td>
<td>17.2 ± 2.2</td>
<td>14.8 ± 3.5</td>
<td>36.6 ± 6.2</td>
</tr>
<tr>
<td>Blighted side</td>
<td>6</td>
<td>9.4 ± 1.4</td>
<td>8.0 ± 1.8</td>
<td>11.8 ± 2.9</td>
</tr>
<tr>
<td>Moderate</td>
<td>4</td>
<td>8.1 ± 0.8</td>
<td>11.2 ± 0.8</td>
<td>8.5 ± 3.5</td>
</tr>
</tbody>
</table>

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**Table 3. Water uptake by trunk injection above the bud union and major root injection below the bud union of budded trees with and without blight.**

<table>
<thead>
<tr>
<th>Sdron/rootstock</th>
<th>No. of trees</th>
<th>Healthy Trunk (ml/hr)</th>
<th>Healthy Root (ml/hr)</th>
<th>Blight Trunk (ml/hr)</th>
<th>Blight Root (ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valencia/rough lemon</td>
<td>5</td>
<td>10.7 ± 1.2 w</td>
<td>32.3 ± 18.7</td>
<td>7.0 ± 1.4</td>
<td>4.7 ± 0.9</td>
</tr>
<tr>
<td>Hamlin/Carrizo</td>
<td>5</td>
<td>36.0 ± 15.3</td>
<td>56.9 ± 20.8</td>
<td>6.5 ± 2.8</td>
<td>5.8 ± 1.9</td>
</tr>
<tr>
<td>Hamlin/sour orange</td>
<td>5</td>
<td>7.4 ± 1.9</td>
<td>14.1 ± 2.4</td>
<td>0.2 ± 0.1</td>
<td>1.0 ± 0.5</td>
</tr>
</tbody>
</table>

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2Valencia trees located in Winter Garden; Hamlin/Carrizo located near Clermont; and Hamlin/sour orange located near Citra, Florida.

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Fig. 1. Dye patterns 15 cm above bud unions in Valencia orange tree trunk cross sections: A) healthy, B) sectored with blight, and C) moderate blight. Sectored portion in trunk denoted in 1B as sec. Trunk cross sections were approximately 35 cm from injection point in major roots.

Fig. 2. Dye patterns in Valencia orange tree trunk vertical sections: A) healthy, and B) sectored with blight. Arrows indicate bud union. Sectored portion in trunk denoted in 2B as sec.

Similar to those in previous studies. De Villiers (8) demonstrated that old xylem in citrus roots was connected directly with the old xylem in the trunk. In healthy trees, his dye movement patterns were similar to ours, although he used methylene blue and different injection techniques. When we injected dye into the trunks of healthy trees with the injector slanted upward at a 45° angle, dye moved readily in young xylem vessels (results not shown). Thus, our injection technique appeared to favor water and dye movement in old xylem.

In moderately blighted trees water may move better in young, outer xylem than in older xylem. However, previous findings indicated that obstructions were present in both

Table 4. Leaf resistance and zinc content of trunks of 'Valencia' orange trees² on rough lemon rootstock with different degrees of blight.

<table>
<thead>
<tr>
<th>Tree blight condition</th>
<th>No. of trees</th>
<th>Leaf resistance (see cm⁻¹)</th>
<th>Zn content trunk (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>4</td>
<td>$8.1\pm2.0$</td>
<td>$10\pm3$</td>
</tr>
<tr>
<td>Sectored</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy side</td>
<td>6</td>
<td>$7.4\pm2.4$</td>
<td>$27\pm11$</td>
</tr>
<tr>
<td>Blighted side</td>
<td>6</td>
<td>$14.1\pm3.9$</td>
<td>$20\pm5$</td>
</tr>
<tr>
<td>Moderate</td>
<td>4</td>
<td>$19.8\pm4.3$</td>
<td>$28\pm2$</td>
</tr>
</tbody>
</table>

²Trees located in a grove near Winger Garden, Florida.

degree of water movement in vessels of different ages and in relation to vessel obstructions from the disease.

**Root necrosis and blight.** We found 1 to several black or necrotic large roots on all our moderately blighted trees, which confirmed a published observation (5). We usually found at least 1 major root and smaller subtending roots which were necrotic on the blighted side of sectored trees, but did not find any on the healthy side or on the limited healthy trees examined. Feeder roots on the sectored trees were visually indistinguishable from roots on healthy trees (11). Waterflow or uptake in necrotic roots was greatly reduced or nil. The significance of the necrotic roots in the disease is unknown, and attempts to culture blight-inducing fungi or bacteria from these roots were not successful (5, 16). We suspect, however, that the necrotic roots result from physiological breakdown caused by blight and subsequent invasion by secondary fungi or bacteria.

**Conclusion.** Visual symptoms, restricted water uptake or movement, xylem obstructions, and phenolic accumulation in citrus trees with blight are similar to the symptoms of other wilt diseases of plants, but the causal organism is unknown. Electron microscopic examination of diseased trees to date has not revealed the presence of a virus, mycoplasma-like agent, or bacterium associated with the disease (21), and repeated attempts to transmit the disease by budding or grafting have been unsuccessful (23).

Our work with the sectoring type of early blight expression affords a model system for study whereby both blighted and apparently healthy tissues occur on the same tree. We are using this model system to study the physiology and biochemistry of the disease.

**Literature Cited**


Fig. 3. Dye patterns in a moderately blighted Valencia orange tree trunk cross section: A) about 18 cm above, and B) about 35 cm above the major root injection sites. Numbered areas indicate where dye was introduced as follows: 1) trunk injection with injector inserted straight into trunk; 2) trunk injection with injector inserted upward at a 45° angle; 3) same as No. 1; 4) same as No. 2; 5) dye reservoir attached to 7 mm root; 6) and 7) injector inserted into major roots.
Effect of Moisture Stress on Leaf Anatomy and Water-use Efficiency of Peas

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Additional index words. stomata, transpiration, Pisum sativum

Abstract. Soil moisture levels maintained at 100 to 80%, 80 to 60%, 60 to 40%, and 40 to 20% of field capacity were examined from the third node stage to maturity for influence upon water-use efficiency and development of water-conducting and -controlling elements of peas (Pisum sativum L.). Water-use efficiency was positively correlated with moisture regime, plant height, leaf area, and seed yield. Thickness of the leaf blade was significantly less in plants grown at 100 to 80% and 40 to 20% than in those grown at 80 to 60% and 60 to 40% of field capacity, and appeared to be regulated by the size of the palisade and spongy mesophyll cells of the leaf. Intercellular air spaces and substomatal cavities of plants grown at 40 to 20% of field capacity were much reduced and had less xylem area and fewer xylem elements in the primary vascular bundle than plants in the other treatments. The cross sectional diameter of the xylem was correlated with water-use efficiency. Stomatal density increased with increasing soil moisture stress. Upper leaflets in the plant canopy had more stomata per unit area than did the lower leaves. Within a leaflet the abaxial surface had more stomata per unit area than did the adaxial surface and stomatal density did not vary between the tip, middle or base positions.

Because water is lost to the atmosphere from the plant primarily through leaf stomata (2, 11), the “efficiency” of stomatal pores as pathways for gaseous diffusion depends essentially on pore size and frequency. Van de Roovaart and Fuller (20) found that cereals had a lower stomatal frequency under optimal moisture conditions than they did under stress conditions. Cell enlargement follows cell division; therefore, water stress at different stages of leaf development reduced cell size and leaf growth. They found that under adverse climatic conditions compared to optimal conditions normal epidermal cells were small and that the ratio of the number of stomata to that of epidermal cells under both conditions remained almost constant (8, 20). Because stomatal resistance is related to photosynthesis and transpiration, stomatal density could be an important criterion for modifying stomatal resistance and improving water-use efficiency (6, 18).

Studies on water-use efficiency in other crops have shown that increased availability of water usually increases water usage, above-ground biomass (3, 7), and stomatal density (5, 14, 21). Size of transpiring area (leaf area and number of leaves per unit area) also plays an important role in water loss, and is modified by water availability during a plant’s active growth (8, 15).

Limited research has been done on how soil moisture stresses affect the morphological and anatomical development of peas. We studied the influence of available soil moisture on water-use efficiency (WUE) defined as:

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WUE = \frac{\text{grain yield or total dry matter yield (g)}}{\text{water used (kg)}}
\]

Leaf thickness, foliage density, morphological and anatomical development of stomata, numbers of xylem elements, cross sectional area of xylem, palisade and spongy mesophyll cells, intercellular air space, substomatal cavities, and epidermal...