

# Effects of Water Stress on Symptomatology and Growth of *Parthenocissus quinquefolia* Infected by *Xylella fastidiosa*

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## ABSTRACT

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A greenhouse study was conducted to test the hypothesis that bacterial leaf scorch symptoms, caused by *Xylella fastidiosa*, are more severe during periods of drought stress. A two-by-two complete factorial design with two pathogen treatments (control and infected) and two soil moisture levels (high and low) was used with *Parthenocissus quinquefolia* vines in 1999 and 2000. In each year, a high percentage of *P. quinquefolia* plants inoculated with *X. fastidiosa* expressed typical bacterial leaf scorch symptoms, with the outer scorched portion of the leaf separated from green tissue by a chlorotic halo. *X. fastidiosa* was detected in all symptomatic plants using an immunomagnetic capture and nested polymerase chain reaction technique, and was reisolated and cultured in modified periwinkle wilt liquid media. In both years, symptoms progressed further along the stem and were more severe at corresponding leaf positions in low-water-infected plants compared to high-water-infected plants. Total leaf area, shoot length, and number of nodes on the longest shoot per plant were all reduced due to drought and *X. fastidiosa* infection. This study is the first to verify the hypothesis that bacterial leaf scorch symptoms are enhanced during drought stress. Maintaining plant vigor with regular watering can be used to sustain plants infected by *X. fastidiosa*, particularly during periods of water stress.

Additional keywords: bacterial leaf scorch, drought, liana, Virginia creeper, xylem-limited bacteria

*Xylella fastidiosa* (37) is a xylem-limited bacterial plant pathogen that has a diverse and extensive host range encompassing at least 30 plant families (34). It is the causal agent of numerous scorching, scalding, and stunting diseases worldwide. Economically important diseases caused by *X. fastidiosa* include citrus variegated chlorosis (2,11), Pierce's disease of grape (3), phony peach disease (38), alfalfa dwarf (8), periwinkle wilt (21), and leaf scorch of coffee (4), plum (29), pear (20), almond (23), mulberry (19), elm, oak, sycamore (12), maple (36), oleander (9), and pecan (30).

When present in agricultural and urban ecosystems, *X. fastidiosa* often occurs at high incidence and can be particularly

devastating. In Brazil, where citrus variegated chlorosis limits citrus production, an estimated 68 million trees were diseased in 2000 (Bergamin-Filho, University of Sao Paulo, *personal communication*). On the National Mall in Washington, D.C., Sherald et al. (35) surveyed approximately 600 elms (*Ulmus americana*) and found that 30% of these trees were infected with *X. fastidiosa*. Additionally, Pierce's disease has long been considered the primary factor limiting development of the grape industry in Florida and other southeastern states in the United States (14).

In addition to the economically important hosts, numerous symptomatic and asymptomatic reservoir host plants have been identified in natural habitats surrounding crops (5,16,22,28). The list of alternative hosts continues to grow as awareness of bacterial leaf scorch increases and as more precise molecular techniques are developed to detect *X. fastidiosa*. Transmission of bacteria from alternative reservoir hosts to susceptible, economically and aesthetically valuable hosts is considered a common occurrence in many agricultural and urban areas (27,34). This transmission of *X. fastidiosa* from plant to plant is accomplished by xylem-feeding insects, including sharpshooter leafhoppers and spittlebugs (26).

No adequate control of infected plants is currently available; therefore, the removal of alternative hosts near susceptible crops is used as a management technique. For example, in California vineyards, alternative host plants growing along riparian zones and irrigation ditches are removed and subsequently replaced with "nonhosts" to reduce the spread of *X. fastidiosa* to grapevines (24). Although, in some habitats, the removal of alternative hosts is a viable option, in other areas this may not be a feasible management technique. For example, in the eastern United States, many alternative hosts are desirable ornamentals or native species endemic to the surrounding habitat (16,22). Additionally, *X. fastidiosa* has such an extensive host range that it is unlikely that all potential hosts would be removed. Therefore, knowing how economically significant and alternative reservoir hosts are affected by *X. fastidiosa* is essential to developing a complete understanding of the disease dynamics and in guiding disease management.

*Parthenocissus quinquefolia* (L.) Planchon, (Virginia creeper), a deciduous liana native to North America, was found to be a symptomatic alternative host of *X. fastidiosa* in habitats surrounding agricultural systems in Florida (16). The natural distribution of *P. quinquefolia* ranges from Florida to as far north as southern Canada and west to Iowa, and it is generally abundant throughout this range (6). Its geographic range, combined with its common usage as an ornamental, provides ample opportunities in agricultural and urban systems for it to serve as a reservoir for transmission to susceptible crops and trees.

Several aspects of *X. fastidiosa*-caused diseases have received little attention experimentally. One such area is whether abiotic stress affects disease development. Several researchers have suggested that leaf scorch symptoms caused by *X. fastidiosa* become severe only after some other stress is placed on the host (14,15,33). Hearon et al. (12) suggested that symptom development in mid- to late summer in urban trees was associated with seasonal moisture and heat stresses enhanced by the urban environment. Although studies have suggested that drought stress favors symptom development in bacterial leaf scorch, water limitation has never been used as a treatment in a con-

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trolled experiment. Therefore, we used a greenhouse study to test the hypothesis that leaf scorch symptoms caused by *X. fastidiosa* infection are more severe during periods of drought stress.

## MATERIALS AND METHODS

We used a two-by-two complete factorial design, with two pathogen treatments (inoculated and control) and two water treatments (high and low), to determine the effect of drought stress and *X. fastidiosa* infection on growth and leaf scorch symptom development on *P. quinquefolia*. This experiment was initially performed during the summer of 1999 and was repeated in the summer of 2000. *P. quinquefolia* vines of equal age, produced from cuttings of nursery-grown plants, were watered regularly and maintained in pots under ambient greenhouse conditions prior to use in the experiments. Plants were fertilized with a standard liquid N-P-K fertilizer (20-10-20; Peters Fertilizer Products, Fogelsville, PA) recommended for container-grown woody ornamentals.

*P. quinquefolia* plants (approximately 10 to 20 cm in height) were inoculated with *X. fastidiosa* at one location near the stem base just above the soil using a scalpel incision method (31). Plants used in the summer 1999 experiment were inoculated in mid-July 1998, and plants used in the summer 2000 experiment were inoculated in mid-July 1999. A Pierce's disease strain of *X. fastidiosa* (strain #92-8 obtained from Don Hopkins, University of Florida, Leesburg) was grown on a modified periwinkle wilt media and was then transferred to phosphate-buffered citrate magnesium (PBCM) solution. Inoculum was standardized in PBCM at an optical density (OD) of 0.07 to 0.10 at 560 nm ( $10^7$  to  $10^8$  cells/ml) with a Bausch & Lomb Spectronic 710 spectrophotometer (31). Control plants also received scalpel incision inoculations but with sterile PBCM solution.

Plant samples were tested for the presence of *X. fastidiosa* using an immunomagnetic separation and nested polymerase chain reaction (PCR) technique (22,25). Samples consisted of xylem fluid extracted with a microcentrifuge from 2- to 3-cm stem segments or from plant sap obtained from macerated leaf petioles (22). This assay involves immunomagnetic separation of the bacteria from host tissue, followed by a two-step, nested PCR amplification using previously developed oligonucleotide primers specific to *X. fastidiosa*. This method is more reliable and sensitive than previous approaches (e.g., enzyme-linked immunosorbent assay), and allows for the detection of as few as five bacteria per sample (25). Additionally, *X. fastidiosa* was reisolated from all infected plants using liquid modified periwinkle wilt media. These cultures then were examined for diagnostic *X. fastidiosa* using phase contrast microscopy at  $\times 400$  magnification.

Plants were watered to saturation once daily prior to the initiation of water treatments. Plants were then randomly assigned to either a high (H) or low (L) soil moisture treatment. High-soil-moisture plants continued to be watered to saturation once daily throughout the experiment. Low-soil-moisture plants were watered to half of field capacity (determined gravimetrically) daily, except on cloudy days, when reduced transpiration alleviated the need for watering. Water treatments were initiated on 24 June in the summer of 1999 and on 11 July in the summer of 2000, and were sustained for 52 and 68 days, respectively.

Evaluation of plant responses included leaf scorch symptom analysis and several growth measurements. On 15 and 16 August 1999, leaf area and the percentage of leaf area exhibiting scorch symptoms (including the chlorotic halo) were assessed for plants inoculated in 1998 using a photographic archive of leaves from each treatment. Scorch symptoms in *P. quinquefolia* progress from the plant base towards the apex (A. J. McElrone, *personal observation*); therefore, five leaf positions along the stem were chosen for disease assessment in order to span a basal to apical range. The most recent, fully expanded leaf was the most apical leaf sampled and corresponded with leaf position 0. Moving toward the plant base, every fifth leaf was sampled, encompassing a total distance of 20 leaves. Photographs of leaves were made with a camera at a standard distance (approximately 0.5 m) from the leaves. Leaves were positioned on engineering graph paper to facilitate calculating the area precisely while controlling for slight differences in camera position.

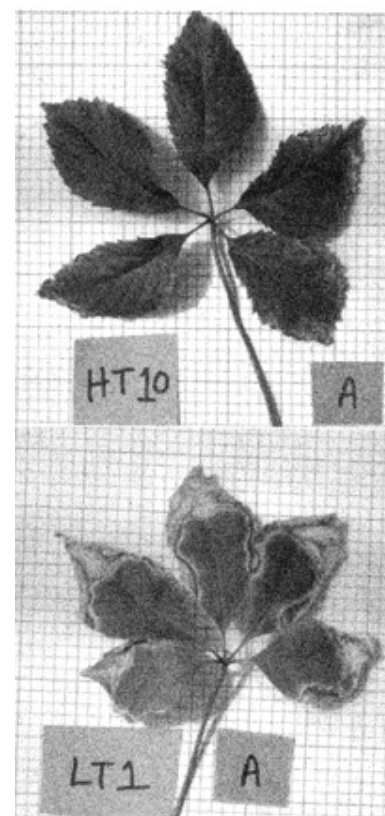
In the second trial on 17 September 2000, percentage of leaf area exhibiting scorching and growth parameters (total shoot leaf area, number of nodes, internode length, and total season shoot length) were measured for harvested *P. quinquefolia* plants. Leaf area for individual leaves at the same leaf positions (as described in the first trial) was measured using a Licor leaf area meter (model LI 3000A; Licor Inc. Lincoln, NE). Symptomatic sections of these leaves then were excised and measured with the leaf area meter. Percentage of leaf area scorched was calculated using (scorched area/total leaf area)  $\times$  100. Total shoot leaf area, total seasonal shoot length, and the number of nodes were measured on the most prolific shoot on each plant at the conclusion of the experiment. Mean internode length was calculated by dividing the total seasonal shoot length by the number of nodes on this shoot for each of five replicates.

Analysis of variance (ANOVA) was computed using the SAS system (version 8.0, (1999; SAS Institute, Inc., Cary, NC). A repeated measures ANOVA was used to analyze the percentage of leaf area

scorched to account for multiple measurements (five leaf positions on the same vine) on the same experimental unit. Data for each year were analyzed separately. Differences in means were analyzed using least-squares means (LS means) analysis for all parameters. All four possible treatment combinations were used with five replicates of each treatment in the two-by-two complete factorial design.

## RESULTS

In each year, a high percentage of *P. quinquefolia* plants inoculated with *X. fastidiosa* expressed leaf scorching symptoms the following summer (46 out of 55 plants inoculated in 1999 exhibited symptoms in 2000; specific data not recorded for 1998 inoculations, but most inoculated plants exhibited symptoms). The outer scorched portion of the leaves was separated from the green tissue by a chlorotic halo typical of bacterial leaf scorch diseases caused by *X. fastidiosa* (Fig. 1). Symptoms were most severe on older leaves near the plant base and decreased in severity on more recently developed leaves near the plant apex. All *X. fastidiosa*-inoculated plants used in the experiments tested positive using the immunomagnetic and nested PCR detection technique. No



**Fig. 1.** Photographs of basally located leaves (position 20) used to calculate percentage of leaf area scorched from *Parthenocissus quinquefolia* infected by *Xylella fastidiosa* and concurrently drought stressed in the 1999 experiment. HT = high-water-infected (top photo); LT = low-water-infected (bottom photo).

control plants tested positive for *X. fastidiosa*. Additionally, *X. fastidiosa* was reisolated and grown in modified periwinkle wilt liquid media from all infected plants exhibiting symptoms. The reisolated and cultured bacteria were observed under

×400 phase contrast microscopy and matched the typical characteristics of *X. fastidiosa* grown in culture.

Leaf scorch symptoms were more severe on low-water-infected (LI) plants in both the 1999 and 2000 experiments (Figs. 1

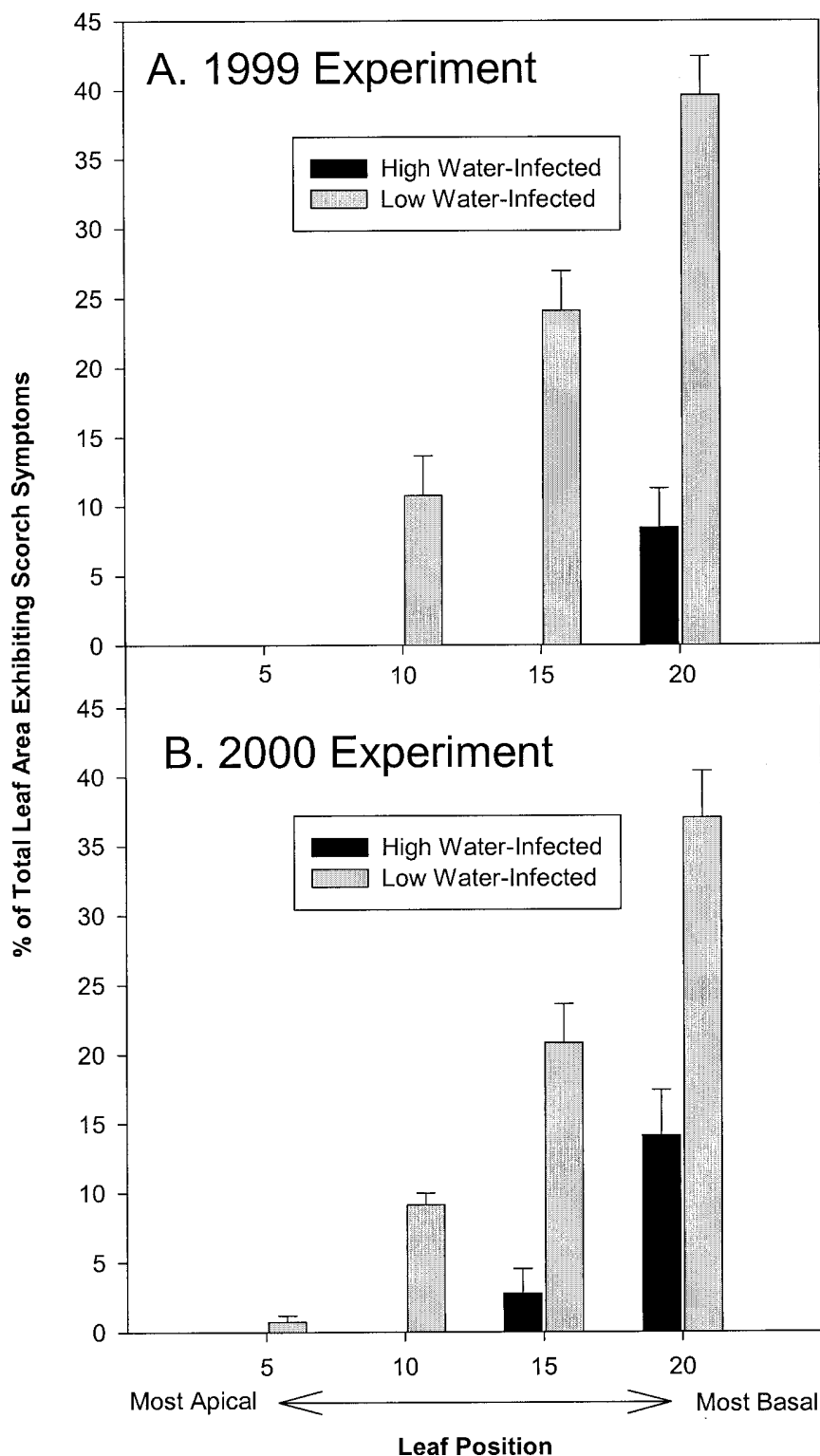
and 2). In 1999, nearly 40% of LI leaf area exhibited scorching at the most basal leaf position (20), compared with only 8% for high-water-infected (HI) plants at this same leaf position ( $P < 0.0001$ ; Figs. 1 and 2A). Additionally, there was a significant soil moisture treatment–leaf position interaction ( $P < 0.0001$  from repeated measures analysis), with symptoms progressing further along the stem (scorching up to leaf position 10) in LI as compared with HI plants (no scorching at leaf positions 10 and 15). Similar results were found in 2000, with LI plants exhibiting more severe symptoms and symptoms progressing further along the stem compared with HI plants (Fig. 2B). Scorching was slightly higher in 2000 compared with 1999 for the HI plants, with 15% scorching at leaf position 20. Also, symptoms advanced an additional leaf position more apically for both HI and LI plants in 2000. No *P. quinquefolia* control plants, inoculated with PBCM only, showed symptoms.

In 1999, the water treatment had a significant impact on total leaf area (healthy and scorched) across all leaf positions with H plants having a greater area than L plants ( $P = 0.02$ ; Fig. 3). No differences were found between control (C) and infected (I) treatments when total leaf area was analyzed ( $P > 0.05$ ). When only healthy leaf area (unscorched) was analyzed within each of the five positions, it was significantly reduced by the infection treatment at leaf position 20 for the 1999 experiment ( $P = 0.05$ ; data not shown).

In the 2000 experiment, all leaves from the largest shoot per plant were combined to calculate the total leaf area. This total shoot leaf area was significantly reduced by both L ( $P = 0.001$ ) and I ( $P = 0.03$ ) treatments (Table 1). Similarly, the total season shoot length and number of nodes were significantly reduced by L ( $P = 0.001$  and  $P = 0.01$ , respectively) and I ( $P = 0.004$  and  $P = 0.05$ , respectively) (Table 1). Internode length was not significantly different for any of the treatments ( $P > 0.05$ ), and there were no significant interaction effects for any of the parameters measured.

## DISCUSSION

Hopkins and Adlerz (16) previously described *P. quinquefolia* as a symptomatic alternative host of *X. fastidiosa* in Florida; however, it had not been used in any experimental studies. Our study was the first attempt to mechanically inoculate *P. quinquefolia* and subsequently reisolate *X. fastidiosa* from symptomatic plants. Inoculations were successful approximately 84% of the time, resulting in symptoms typical of infections caused by *X. fastidiosa* in other hosts and reported on *P. quinquefolia* in Florida. The ease with which the host was infected suggests that *P. quinquefolia* may be highly susceptible and, because of its wide distribution, may be an important



**Fig. 2.** Percentage of leaf area exhibiting scorch symptoms at five leaf positions for *Xylella fastidiosa*-inoculated plants only for the **A**, summer 1999 and **B**, summer 2000 experiments. No scorch symptoms were seen in control plants at any leaf position or in inoculated plants at leaf position = 0 (most apical). Data are the mean ± S.E.

reservoir. It is not surprising that *P. quinquefolia* (family *Vitaceae*) is particularly susceptible to a Pierce's disease strain of *X. fastidiosa* because of its genetic relatedness and close similarities in growth form and habit with members of the genus *Vitis* (6). The ease and success of inoculation also show the potential of *P. quinquefolia* as an experimental host.

Several researchers (12,14,15,33) had suggested that drought stress enhances symptom severity and progression in *X. fastidiosa*-infected hosts; however, no studies to date have empirically tested this hypothesis. To our knowledge, this is the first controlled experimental study to support this hypothesis. In both years of our study, leaf scorching symptoms of LI plants were more severe and progressed further along the stem compared with HI plants, suggesting that the abiotic water stress imposed by the low-water treatment adds to the biotic water stress induced by the presence of the bacteria in the xylem conduits. Recommendations commonly made to growers experiencing problems with bacterial leaf scorch have included keeping the plant vigorous with regular watering and fertilization (10,32,34); however, until this study, these recommendations have not been based on experimental evidence. No effective curative therapy is yet available; therefore, maintaining well-watered conditions, particularly when moisture is limiting, may help to slow the progression of the disease, reduce symptom severity, and extend the life of infected plants. This practice should be of particular importance in agricultural and urban settings where the plants are of value both economically and aesthetically.

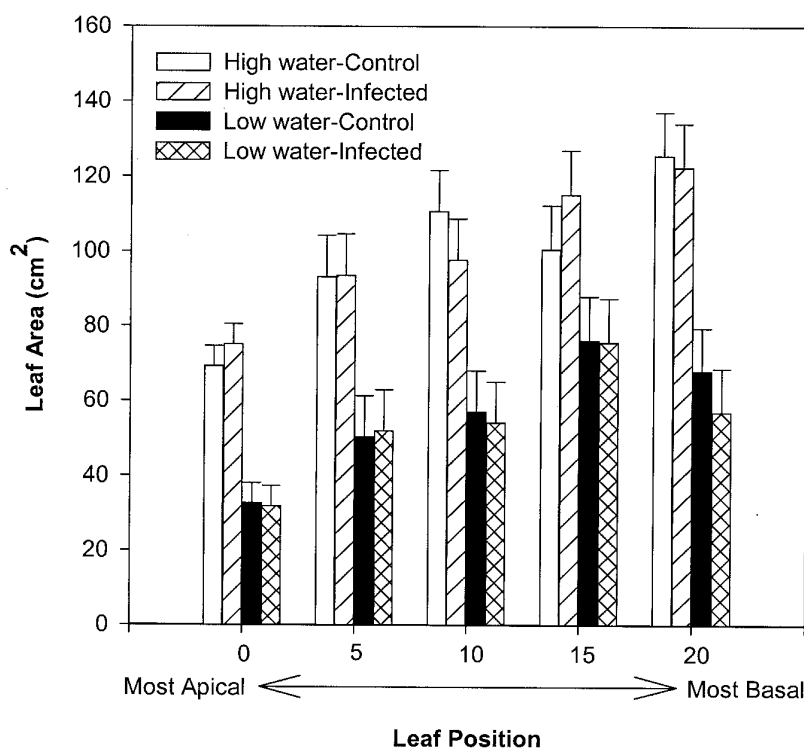
Although results from both years were qualitatively similar, there were quantitative differences. Symptoms progressed further along the stem for both LI and HI plants in the 2000 experiment. We suspect that the extended duration of the drought treatment (65 days in 2000 compared with 52 days in 1999) allowed the symptoms to progress further along the stem due to an extended period of plant stress. Additionally, the end of the experiment in 2000 fell in mid-September compared with mid-August in 1999, which put the plants closer to the beginning of natural senescence. Changes in the hormonal balance of plants nearing the end of the growing season have been implicated in promoting bacterial leaf scorch symptom development. Hopkins (13) showed that changes in the hormonal balance of the plants, particularly increases in abscisic acid, can enhance symptom development in grapes.

In the 1999 experiment, leaf area for individual leaves was reduced by drought but not by *X. fastidiosa* infection. Kostka et al. (18) found similar results with American elm trees in which leaf size was not affected by *X. fastidiosa* infection. However, in both studies, the amount of healthy tissue per leaf was reduced for

leaves with significant scorching. Additionally, when we analyzed the total leaf area for the whole shoot in the 2000 experiment, it was reduced by both drought stress and *X. fastidiosa* infection. The growth habit of *P. quinquefolia* puts a premium on internode length, presumably to aid in climbing and finding trellis support, and to space leaves and reduce self-shading, and we found no significant differences in this parameter. By maintaining internode length, even when shoot lengths are reduced, the resultant shoots produce fewer nodes. *P. quinquefolia* typically produces one leaf per node, and thus the reduction of total leaf area that we found resulted primarily from fewer nodes being produced per shoot.

Goodwin et al. (7) showed that symptomatic leaves on grapevines infected with Pierce's disease had reduced stomatal conductance and photosynthesis, which is also

a common response of water-limited plants (17). For *X. fastidiosa*-infected plants, the further reduction in photosynthate production (reduced leaf area and stomatal closure) induced by drought probably impairs the plant's ability to defend against or continue growth ahead of the advancing bacteria. Boyer (1) proposed the following two mechanisms to explain how water stress increases the susceptibility of plants to attack by pathogens: (i) reduced photosynthate production induced by drought eliminates the plant's ability to produce defensive compounds or (ii) plant growth is reduced without reducing the pathogen's ability to reproduce, thus allowing further progression and increased symptom severity in the host (1). Whether one or both of these mechanisms form the basis of the observed enhancement of disease symptomology and progression in our system is yet to be determined.



**Fig. 3.** *Parthenocissus quinquefolia* leaf area for one leaf per plant at each of the five positions along the stem. Data are from the 1999 experiment and include tissue exhibiting scorching as well as healthy tissue. Data are mean  $\pm$  S.E.

**Table 1.** Total leaf area for whole shoot, total season shoot length, mean internode length, and mean number of internodes on the longest shoot for *Parthenocissus quinquefolia* infected by *Xylella fastidiosa* from the 2000 experiment<sup>z</sup>

Treatment	Leaf area (cm <sup>2</sup> )	Shoot length (cm)	Internode length (cm)	Number of nodes
High water, control	10,994 a	643 a	7.1 a	92 a
High water, infected	7,876 b	532 a,b	7.5 a	71 b
Low water, control	4,625 b,c	455 b	7.2 a	64 b,c
Low water, infected	2,144 c	331 c	6.4 a	52 c

<sup>z</sup> Data are the mean of five reps. Within a parameter column, means followed by the same letter are not significantly different ( $P = 0.05$ ) based on the least-squares means (LS means) test.

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