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RESEARCH PAPER

Interactive effects of water stress and xylem-limited bacterial infection on the water relations of a host vine

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Abstract

***Xylella fastidiosa*, a xylem-limited bacterial pathogen that causes bacterial leaf scorch in its hosts, has a diverse and extensive host range among plant species worldwide. Previous work has shown that water stress enhances leaf scorch symptom severity and progression along the stem in *Parthenocissus quinquefolia* infected by *X. fastidiosa*. The objective here was to investigate the mechanisms underlying the interaction of water stress and infection by *X. fastidiosa.* Using the eastern deciduous forest vine, *P. quinquefolia*, infection and water availability were manipulated while measuring leaf water potentials (Ψ_L), stomatal conductance (g_s), whole shoot hydraulic conductance (K_h), per cent xylem embolism, and xylem vessel dimensions. No significant differences in any of the physiological measurements were found between control and infected plants prior to drought. Drought treatment significantly reduced Ψ_L and g_s at all leaf positions throughout the day in late summer in both years of the study. In addition, infection significantly reduced Ψ_L and g_s in the most basal leaf positions in late summer in both years. Whole shoot hydraulic conductance was reduced by both low water and infection treatments. However, per cent embolized vessels and mean vessel diameter were affected by drought treatment only. These results imply that the major effect of infection by *X. fastidiosa* occurs due to reduced hydraulic conductance caused by clogging of the vessels, and not increased cavitation and embolism of xylem elements. The reduced K_h caused by *X. fastidiosa* infection acts additively with the water limitation imposed by Drought stress.**

Key words: Drought, embolism, *Parthenocissus quinquefolia*, plant pathogen, vascular wilt disease, water relations, *Xylella fastidiosa*, xylem-limited bacteria.

Introduction

The current estimates of the economic cost of US crop losses to all plant pathogens are approximately $33 billion per year (Pimentel *et al*., 2000). In addition, pathogens can have substantial impacts on natural plant communities. For example, plant competition (Van der Putten and Peters, 1997) community succession (Van der Putten *et al*., 1993), reproduction (Levri and Real, 1998; Marr, 1997), and maintenance of species diversity (Mills and Bever, 1998) have been significantly affected by plant pathogens in natural communities. The loss of entire species can occur in severe cases, particularly if non-native pathogens are introduced into an ecosystem, (e.g. the American Chestnut, *Castanea dentata,* decimated by the fungus *Cryphonectria parasitica*) (Agrios, 1997).

Plant pathogens can affect the physiology of host plants in a variety of ways (reviewed in Goodman *et al*., 1986). The 'disease triangle', a conceptual model addressing the interaction between host, pathogen, and environment, is a seminal concept of plant pathology. However, physiological investigations addressing interactions between these three components have been neglected for many pathosystems, and more research is needed to assess how disease is modified by the response of the host or the pathogen to abiotic factors (Paul, 1990).

One plant pathogen of particular importance is *Xylella fastidiosa* (Wells *et al*., 1987). *X. fastidiosa* is a xylem-limited bacterial plant pathogen, that has a diverse and extensive host range encompassing at least 30 plant

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families (Sherald and Kostka, 1992). It is the causal agent of numerous scorching, scalding and stunting diseases worldwide. Economically important diseases caused by *X. fastidiosa* include citrus variegated chlorosis (CVC) (Chang *et al*., 1993; Hartung *et al*., 1994), Pierce's disease of grape (PD) (Davis *et al*., 1978), phony peach disease (Wells *et al*., 1983), alfalfa dwarf (Goheen *et al*., 1973), periwinkle wilt (McCoy *et al*., 1978), and leaf scorch of coffee (deLima *et al*., 1998), plum (Raju *et al*., 1982), pear (Leu and Su, 1993), almond (Mircetich *et al*., 1976), mulberry (Kostka *et al*., 1986), elm, oak, sycamore (Hearon *et al*., 1980), maple (Sherald *et al*., 1987), oleander (Grebus *et al*., 1996), and pecan (Sanderlin and Heyderich-Alger, 2000). The growing biological and economic significance of *X. fastidiosa* is further illustrated by being the first plant pathogen to have its genome entirely sequenced; a research consortium in Brazil recently completed the genome sequence for a *X. fastidiosa* strain that causes CVC (Simpson *et al*., 2000).

It has been known for some time that vascular wilt diseases that target the xylem induce water stress in their hosts by increasing resistance to water flow (Tyree and Sperry, 1989; Zimmermann, 1983). Reduced water flow results in leaf water deficits that can cause stomatal closure and lower transpiration (Saeed *et al*., 1999). Plant pathogens inhabiting the water stream under tension can promote the formation and spread of gaseous emboli (Newbanks *et al*., 1983; Ikeda and Kiyohara, 1995) or can physically clog the xylem conduits (Beckman, 1987). Historically, xylem dysfunction in diseases caused by *X. fastidiosa* has been attributed to the following factors: accumulation of bacterial polysaccharides, production of gels, gums, and tyloses by the host in response to infection, and/or accumulation of bacterial cell masses that physically clog the elements (reviewed by Hopkins, 1995; Kostka *et al*., 1986). Currently, the bacterial-cell clogging hypothesis is the most widely accepted by researchers studying this pathosystem. Similar to other xylem-limited plant diseases (Newbanks *et al*., 1983; Ikeda and Kiyohara, 1995), it was hypothesized that in addition to bacteria clogging the xylem conduits, embolism induced by the presence of *X. fastidiosa* significantly contributes to dysfunction in the water-conducting system of infected hosts.

A common response of plants to water stress is a reduction in the conductivity to water flow along the soil–plant–atmosphere water pathway. A portion of this reduction is due to increased resistance to flow in the shoot (Zimmermann, 1983). Reductions in shoot hydraulic conductivity can be caused by embolism (Tyree and Sperry, 1989) or by a reduction in the vessel diameter (Lovisolo and Schubert, 1998). Pathogen induced reductions in hydraulic conductivity in conjunction with water stress-induced reductions could promote extensive xylem dysfunction in plants subjected to both stresses concurrently.

Several researchers had suggested that leaf scorch symptoms caused by *X. fastidiosa* become severe only after some other stress is placed on the host (Hopkins, 1989, 1995; Sherald *et al*., 1983). Hearon *et al*. (1980) suggested that symptom development in mid to late summer in urban trees was associated with seasonal moisture and heat stress enhanced by the urban environment. Recent work has confirmed the idea that water stress enhances symptom severity and progression along the stem in *Parthenocissus quinquefolia* plants infected with *X. fastidiosa* (McElrone *et al*., 2001). Therefore, the objective here was to investigate the mechanism behind the interaction of water stress and *X. fastidiosa* infection in the eastern deciduous forest vine, *Parthenocissus quinquefolia*, in terms of leaf and shoot level water relations. The following hypotheses were tested: (1) water stress will decrease leaf water potentials, stomatal conductance and stem hydraulic conductivity in *P. quinquefolia*, and at severe levels will increase xylem embolism and cavitation; (2) infection by *X. fastidiosa* will decrease leaf water potentials, stomatal conductance and stem hydraulic conductivity in *P. quinquefolia*, and at severe levels will increase xylem embolism and cavitation; (3) the mechanism of the effect of *X. fastidiosa* infection on water relations will involve a combination of xylem plugging by the bacteria as well as enhancement of xylem embolism due to increased negative pressures in the xylem; (4) the effects of infection by *X. fastidiosa* and water stress will interact additively to reduce leaf water potentials, stomatal conductance and stem hydraulic conductivity of *Parthenocissus quinquefolia* shoots, and during periods of severe water stress this may lead to catastrophic collapse of the xylem water-conducting system.

Materials and methods

Plant species description

Parthenocissus quinquefolia (Virginia creeper), a deciduous liana native to North America, was found to be a symptomatic host of *X. fastidiosa* in habitats surrounding agricultural systems in Florida (Hopkins and Adlerz, 1988). 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 (Gleason and Cronquist, 1991). Its geographic range, combined with its common usage as an ornamental, provides ample opportunities in agricultural and urban systems where it can serve as a reservoir for transmission of *X. fastidiosa* to susceptible crops and trees by xylem-feeding insect vectors.

Growth conditions and experimental design

A 2×2 complete factorial design was used, with two pathogen treatments (non-infected, NI and infected, I) and two soil moisture treatments (high, HW and low, LW) to determine the response of *P. quinquefolia* plants to *X. fastidiosa*-infection and concurrent water stress in terms of leaf and shoot level water relations. This experiment was initially performed during the summer of 1999

and was repeated in the summer of 2000. Equal age *P. quinquefolia*, produced from cuttings of nursery-grown plants, were watered regularly and maintained in pots under ambient greenhouse conditions prior to use in the experiments. Continued growth throughout the season was managed by trellising with bamboo rods encircling each pot and suspended metal wires at several heights that extended the length of the greenhouse room. The apical shoot of the trellised vines were always oriented above horizontal so that all shoots grew upward. Plants were fertilized biweekly using standard liquid N-P-K fertilizer (20-10-20, Peters Fertilizer Products, Fogelsville, PA) at the full rate recommended for container-grown woody ornamentals.

Xylella fastidiosa *inoculations and treatment confirmation*

P. quinquefolia plants (approximately 10–20 cm in height) received a one-time inoculation with *X. fastidiosa* at the base of the stem just above the soil using a scalpel incision (Sherald, 1993). 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. This allowed a year for the bacteria to colonize and establish populations in the vascular tissues of inoculated hosts. A Pierce's disease strain of *X. fastidiosa* (strain 92-8 obtained from D Hopkins, University of Florida, Leesburg) was grown on a modified periwinkle wilt media, and was then transferred to phosphate-buffered citrate magnesium (PBCM) solution. The inoculum was standardized in PBCM at an optical density of 0.07–0.10 at 560 nm (10^7–10^8 cells ml^{-1}) with a Bausch & Lomb Spectronic 710 spectrophotometer (Sherald, 1993). Control plants also received scalpel incision inoculations, but with PBCM solution without bacteria.

Plant samples were tested for the presence of *X. fastidiosa* using an immunomagnetic separation and nested Polymerase Chain Reaction (PCR) technique (McElrone *et al*., 1999; Pooler *et al*., 1997). Samples consisted of xylem fluid extracted from 2–3 cm stem segments using a microcentrifuge or from plant sap obtained by macerating leaf petioles (McElrone *et al*., 1999). This assay involves immunomagnetic separation of the bacteria from host tissue, followed by a 2-step, nested PCR amplification using previously developed oligonucleotide primers specific to *X. fastidiosa*. Additionally, *X. fastidiosa* was re-isolated from all infected plants using liquid modified periwinkle wilt media. These cultures were then examined for diagnostic *X. fastidiosa* characteristics using phase contrast microscopy at 400× magnification.

Watering regime

Plants were watered to saturation once daily prior to the initiation of the water treatments. Plants were then randomly assigned to either a high (HW) or low (LW) 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 50% field capacity (determined gravimetrically; 50% soil moisture) 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 d and 68 d, respectively.

Physiological measurements

Stomatal conductance (g_s) and leaf water potential (Ψ_L) were measured on five replicate plants from each treatment using a Li-Cor 6400 photosynthetic system (Li-Cor, Inc. Lincoln, NE, USA) and a PMS pressure chamber (PMS Instruments Inc. Corvallis, OR, USA), respectively. g_s and Ψ_L were measured at five leaf positions along the stem in the 1999 experiment during the midday hours (11.00–13.30 h, EST) on 14 and 18 August. Ψ_L measurements were also made at predawn (05.00–06.00 h, EST) on 18 August. Since scorch symptoms in *P. quinquefolia* progress from the plant base towards the apex (McElrone *et al*., 2001), five leaf positions along the stem were chosen 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 towards the plant base, every fifth leaf was designated as a position until four additional positions were identified (5, 10, 15, 20), equalling a total distance encompassing 20 leaves. In the 2000 experiment, diurnal cycles of g_s and Ψ_L were measured at three leaf positions along the stem segments (most apically-located leaf [same as leaf position 0 in 1999] and two basally located leaves per replicate plant [same as leaf positions 15 and 20 in 1999]). Measurements were conducted on four different sampling dates in 2000 (20 June, 31 July, 27 August, and 24 September for Ψ_L; 20 June, 27 July, 21 August, and 20 September for g_s), and leaf positions sampled at each date were designated relative to the apex for each date (i.e. different leaves were used at each sampling date because of continued plant growth). Measurements for both parameters were taken on days with similar temperatures and clear sky conditions. During the sampling days, photosynthetically active radiation (*PAR*), air temperature, leaf temperature, and the leaf–air vapour pressure deficit (*VPD*) were collected and stored in a datalogger (CR-10X, Campbell Scientific, Logan, UT). *PAR*, air and leaf temperatures, and *VPD* were recorded using quantum sensors, thermocouples, and humidity sensors, respectively.

Hydraulic conductance and embolism

In the 1999 experiment, a vacuum infiltration technique (Kostka *et al*., 1986) was used to evaluate hydraulic conductivity. A 20 cm stem segment was excised at the most basal leaf position (leaf position 20). The basal end of this segment was placed in a graduated cylinder filled with sterile PBCM solution, and the apical end was sealed with tubing connected to a vacuum pump. A vacuum of 80 kPa was applied to the apical end and the amount of PBCM pulled through the stem over a 2 min interval was measured.

In the 2000 experiment, whole shoot hydraulic conductance was measured using the methods described in Kolb *et al*. (1996). Shoots from *P. quinquefolia* were harvested from experimental plants at midday, placed in plastic bags to prevent dehydration, and transported immediately to the laboratory for analysis. Leaves attached to the shoot were excised at the petiole using a razor blade while the whole shoot was submerged in water. The shoot segments were then recut under water to a size that included the 25 most apical leaf positions. Segments were recut under water to avoid the inclusion of vessels embolized during collection.

After preparation, the shoot segment was enclosed in a vacuum chamber with the basal end protruding. This basal end was sealed with tubing connected to a perfusing solution (sterile deionized water autoclaved and filtered through 0.22 μm filters) in a graduated cylinder. A vacuum was induced in the chamber holding the stem segment, initiating uptake of the perfusing solution and flow through the xylem. Vacuum pressure was increased stepwise, with flow rate being recorded for each pressure. Premeasurement hydraulic conductance (K_h) was determined from the slope of the flow rate versus pressure relationship. Maximal hydraulic conductances (K_m) were determined by increasing vacuum pressure to 150 kPa for 25–35 min, a procedure that removes all xylem emboli and refills all xylem elements. The percentage loss of hydraulic conductance due to embolism was then calculated using the relationship: $(1–K_h/K_m)\times 100$.

Total shoot leaf area (A_L) was measured on all leaves (25 node positions) excised from the shoot segments used for the K_h measurements using a leaf area meter (Li-cor model 3000A, Li-Cor, Inc., Lincoln, NE). The measured K_h was scaled by A_L, thus obtaining leaf specific conductivity, the K_h per unit leaf area (K_L).

Table 1. *Environmental variables measured on four different dates in 2000, corresponding with the days that physiological measurements were made*

Measurements were made in an unshaded greenhouse on the University of Maryland College Park campus.

Environmental variable	Date			
	20 June	27July	21 August	20 Sept
Total daily *PAR* (mol m^{-2})	37.1	37.5	41.3	33.6
Daily range of *PAR* (μmol m^{-2} s^{-1})	0–1310	0–1310	0–1520	0–1180
Average vapour pressure deficit (kPa)	2.89	1.96	2.60	2.85
Daytime range of *VPD* (kPa)	0.10–4.90	0.10–4.50	0.10–6.10	0.30–5.90
Average daytime air temperature (°C)	32.4	31.8	29.5	33.2
Daytime range of air temperature (°C)	19.8–38.5	16.7–39.1	20.5–42.7	20.0–42.2

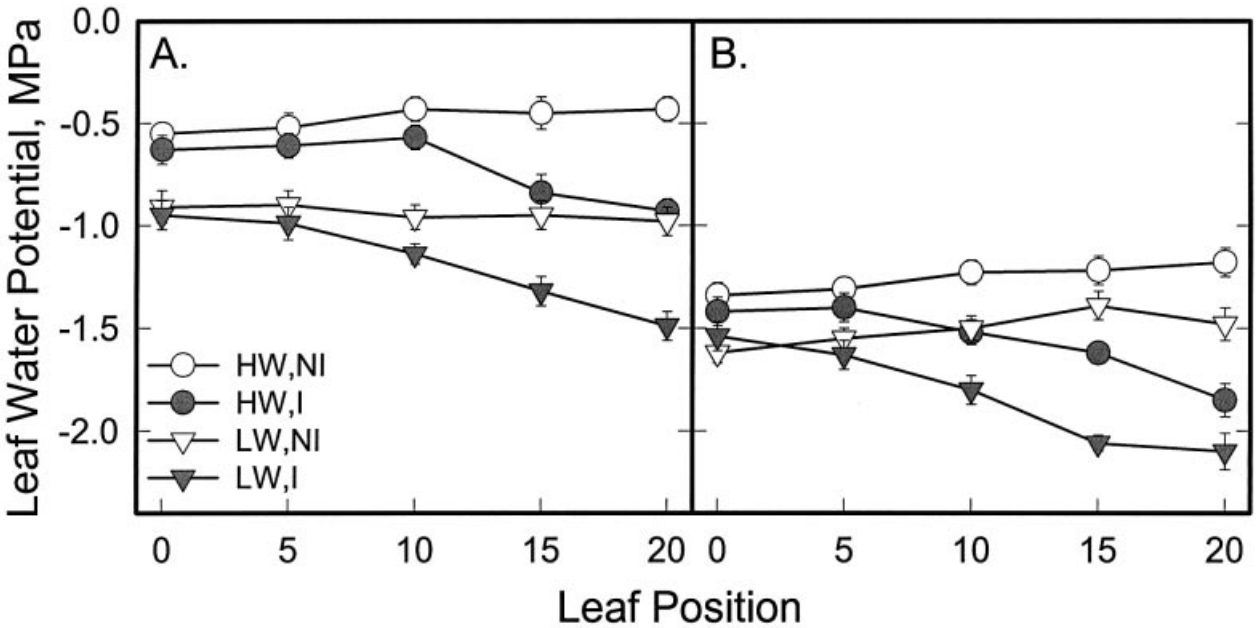

Fig. 1. Leaf water potentials for greenhouse-grown *P. quinquefolia* measured at (A) predawn, and (B) midday on 18August 1999 at five different leaf positions along the stem numbered basally from the most apical fully expanded leaf (leaf position 0=most apical; position 20=most basal). HW,NI=high water, non-infected treatment, HW,I=high water, infected with *X. fastidiosa* treatment, LW,NI=low water, non-infected treatment, LW,I=low water, infected with *X. fastidiosa* treatment. Data are the mean ±SE (n=5).

This calculation standardized measurements for the size of the shoot segment (Kolb *et al*., 1996).

Vessel anatomical measurements

Vessel dimension measurements were conducted using techniques similar to those of Hacke and Sauter (1996). Vessel diameters of *P. quinquefolia* cross-sections taken from the stem at the most basal leaf position (20) were determined using a high-power stereomicroscope (Zeiss, Inc.) fitted with a micrometer and attached to a projection screen. The diameters of all vessels in sectors reaching from the vascular cambium to the pith were measured on >120 vessels per segment using 10 μm diameter classes. The diameter of each vessel was taken between the interior walls in two directions (180° from each other) to calculate the average diameter of each vessel.

Vessel lengths were measured using the technique of Zimmermann and Jeje (1981). The basal end of stem segments were sealed by tubing attached to a gravity-fed paint solution (elevated to 2.0 m). Stem segments were perfused with paint for >48 h. After completion of the paint infusion, the stem was cut into segments, which were allowed to dry for subsequent counting of paint-filled vessels.

Statistical analysis

Analyses of variance (ANOVA) were computed using SAS system version 8.0 (SAS Institute Inc., Cary, NC, USA). Data transformations were performed as needed to meet ANOVA assumptions of normality and homogeneity of variances. All four possible treatment combinations were used with five replicates of each treatment in the 2×2 complete factorial design. For g_s and Ψ_L data, a repeated

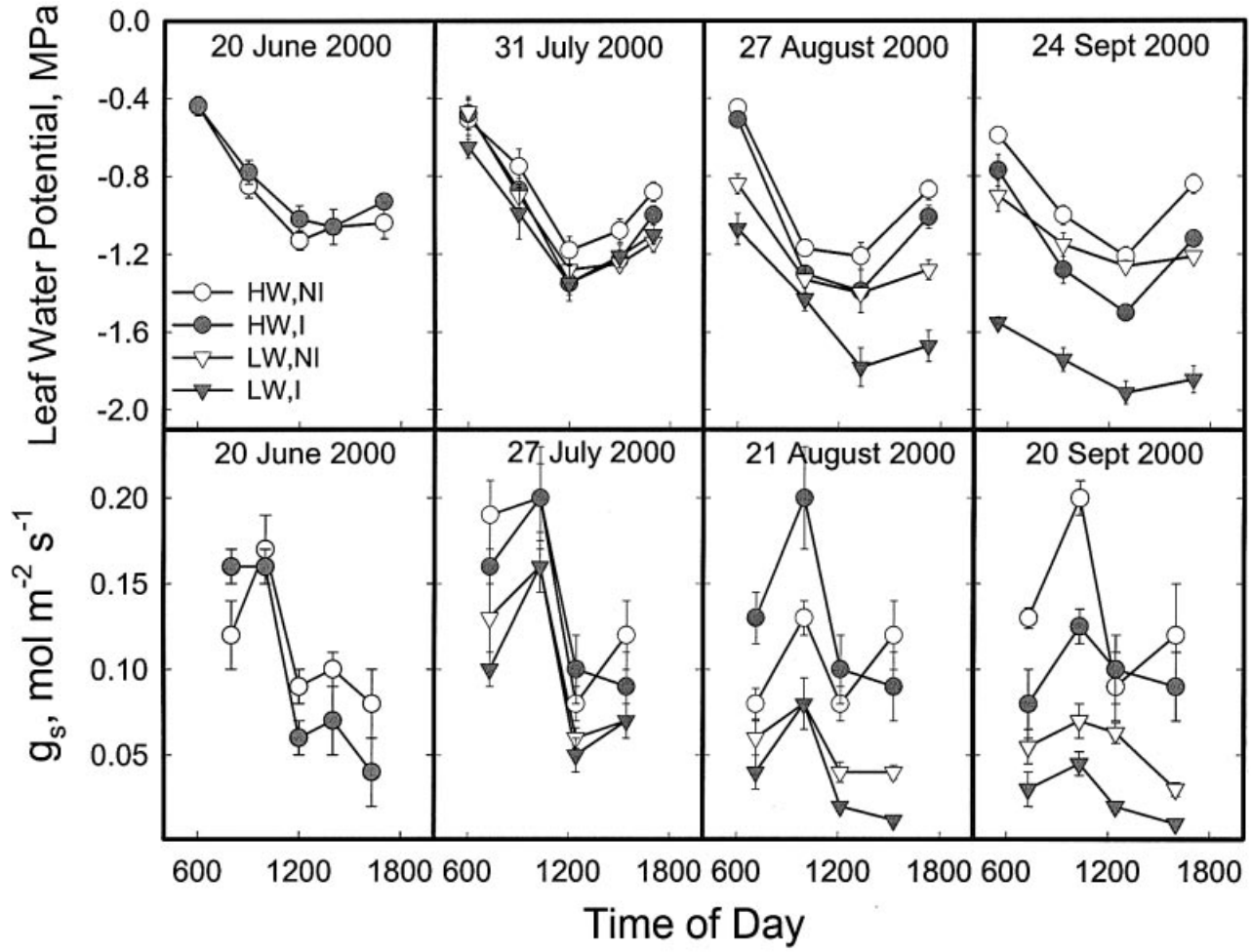

Fig. 2. Top panel. Leaf water potentials measured throughout the day on greenhouse-grown *P. quinquefolia* on four different sampling dates in 2000. Measurements were made on the 20th leaf located basally from the most recently fully-expanded leaf. Bottom panel. Stomatal conductances (g_s) measured throughout the day on four different sampling dates on leaf position 20 (numbered basally from the most recently fully-expanded leaf) in 2000. Data are the mean ±SE (n=5). Treatment abbreviations are as in Fig. 1.

measures ANOVA was used because multiple measurements (five leaf positions per vine) were made on the same experimental unit at several times for each sampling date. Vessel diameter and length distributions were analysed using an Atchison Log Ratio Model.

Results

Environmental conditions

In order to maximize the effects of bacterial infection and water stress on the physiological responses of the plants, plants were grown in an unshaded greenhouse on the College Park campus of the University of Maryland. This allowed the plants to be exposed to high light levels and ambient temperatures, similar to what they would experience in nature. On clear days during the course of the experiment, peak photosynthetically active radiation (*PAR*) in the greenhouse was lower than that typical of this latitude and season outside of the greenhouse (approximately 1800 μmol m^{-2} s^{-1}) (Table 1). However, total daily *PAR* within the greenhouse was within the range of vines found in high light habitats in the field (Table 1; Carter *et al*., 1989; Teramura *et al*., 1990). Air temperature and *VPD* followed diurnal patterns similar to those of *PAR*, with a displacement of approximately 2 h in the timing of peak values (14.00 h, EST). Air temperatures and *VPD*s were representative of summer conditions in Maryland, exceeding 40 °C and 6 kPa on some days (Table 1; Bell *et al*., 1988). *PAR*, air temperature, and *VPD* were highest for the August sampling dates, but overall the environmental conditions were similar over the experimental time period (Table 1).

Leaf water potentials

The effects of water treatment and infection by *X. fastidiosa* on leaf water potentials varied with leaf position and the date of sampling. The effect of leaf position is

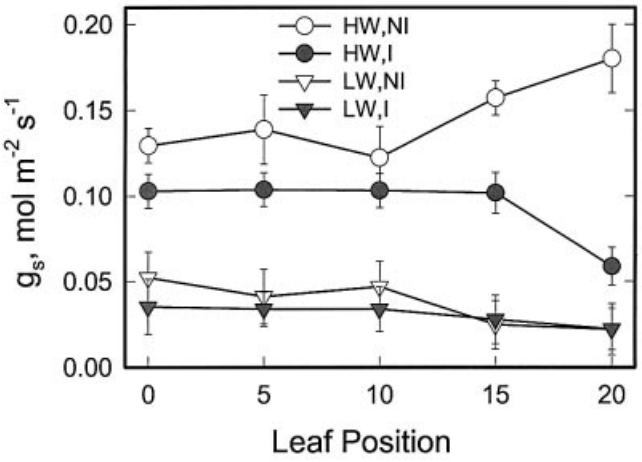

Fig. 3. Midday stomatal conductance (g_s) measured at five leaf positions numbered basally from the most apical fully-expanded leaf along the stem of individual *P. quinquefolia* plants on 14 August 1999. Data are the mean ±SE (n=5). Treatment abbreviations are the same as in Fig. 1.

illustrated by the 1999 data, where both midday and predawn Ψ_L sampled at the end of the experiment showed a significant water treatment effect (Fig. 1A, B). Plants receiving the HW treatment had higher values compared to plants in the LW treatment (P <0.001) (Fig. 1). There were also significant *X. fastidiosa*-infection effects, with I plants having lower predawn and midday Ψ_Ls than NI plants from leaf position 10 through 20 (P <0.001) (Fig. 1).

The data collected in 2000 illustrate the effects of date and time of day for leaves from leaf position 20. No significant difference in leaf water potential was found between NI and I plants at any time of the day on the first sampling date (June 12), prior to the initiation of drought (P >0.05)(Fig. 2, top panel). By the 31 July sampling date, LW plants had significantly lower midday Ψ_L (Fig. 2) than HW plants for apically-located leaves (data not shown (P=0.02)), however, no infection effect was found (P >0.05). The differences between the HW and LW plants were more pronounced for the August and September

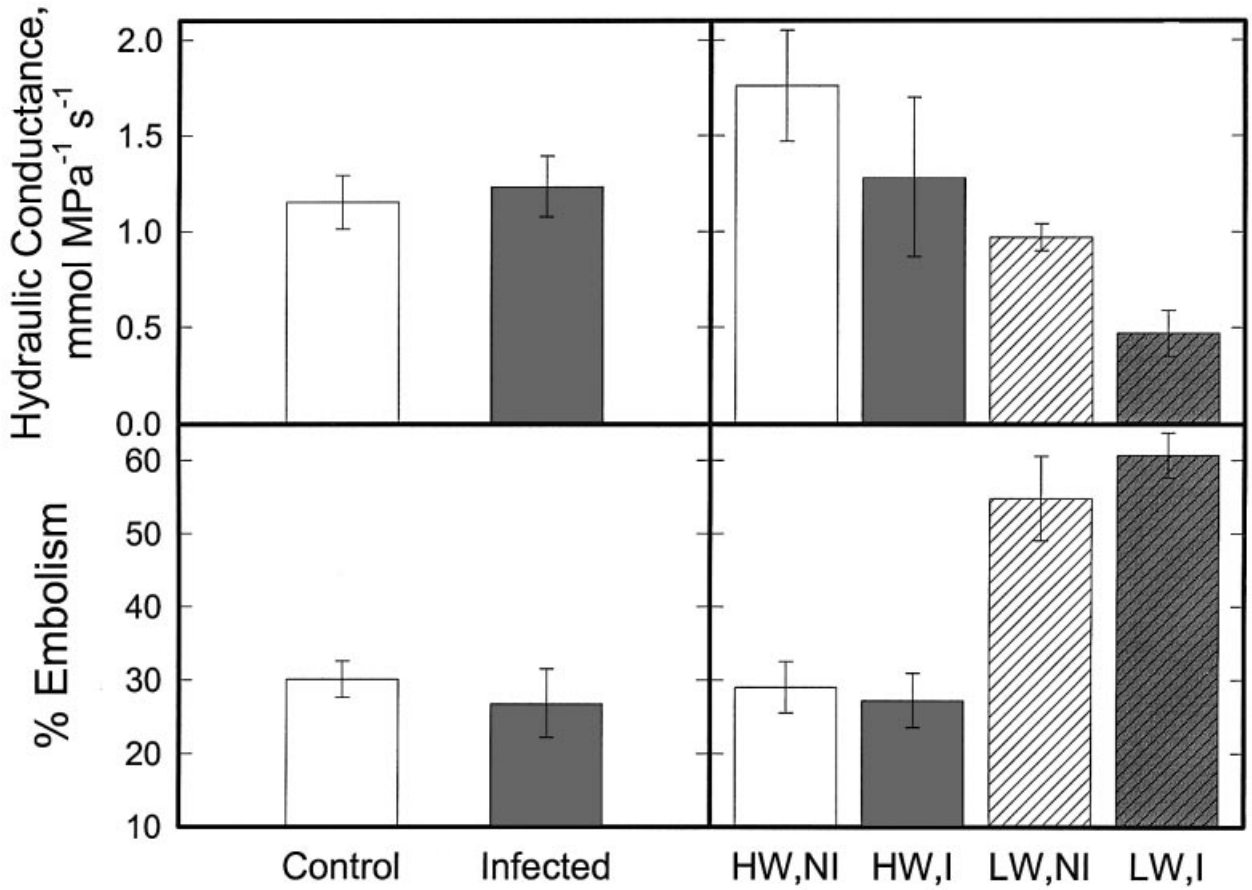

Fig. 4. (Top) Whole shoot hydraulic conductance of *P. quinquefolia shoots* measured prior to the imposition of drought in June 2000 (left panel), and in mid-September, approximately 65 d after the initiation of drought (right panel). (Bottom) Percentage embolism in shoots of *P. quinquefolia* measured in June 2000, prior to the imposition of drought (left panel), and in mid-September, 65 d after the initiation of the drought treatment (right panel). Data are the mean ±SE (n=5). Treatment abbreviations are the same as Fig. 1.

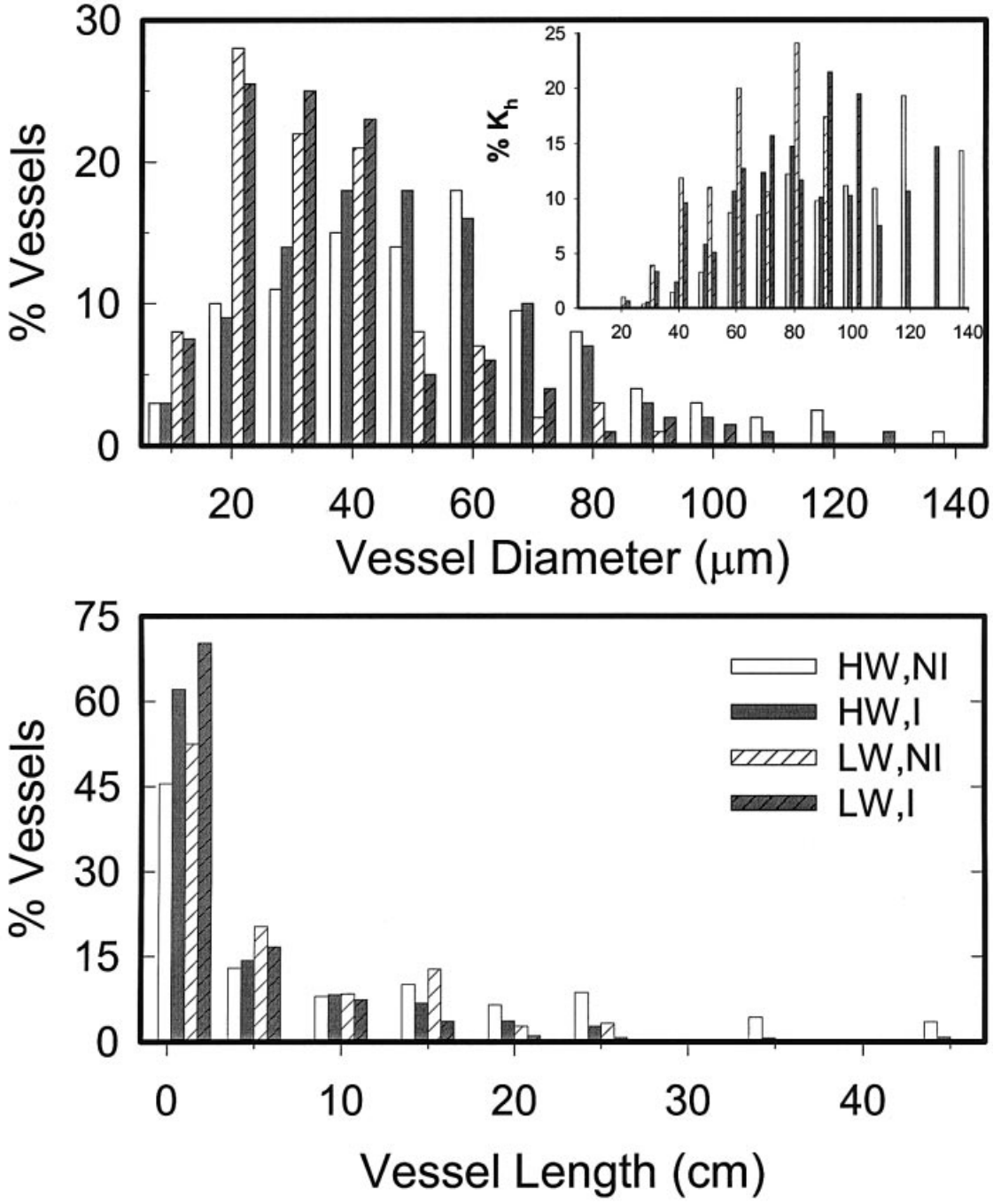

Fig. 5. Morphometric measurements of xylem vessel diameter (top) and length (bottom) for shoots of greenhouse-grown *P. quinquefolia* after 65 d of drought treatment in 2000. The vessel diameter distribution reflecting the contribution of each size class to total hydraulic conductance (K_h) (inset of top panel) is based on the Hagen–Poiseuille equation (Zimmermann, 1983). Morphometric measurements were taken from a vine section that started at leaf position 20 and continued towards the apex. Data are the mean of n=5. Treatment abbreviations are the same as Fig. 1.

sampling dates, with significantly lower leaf water potentials in the LW plants than the HW plants at all hours of the day and at all leaf positions (P <0.001) (Fig. 2). *X. fastidiosa*-infected plants also had significantly lower Ψ_L than non-infected plants in both August and September for basally-located leaves throughout the day (P <0.001) (Fig. 2), with the lowest water potentials being recorded in the LW,I treatment.

Stomatal conductance

By the end of the drought treatment in 1999, midday stomatal conductance to water vapour (g_s) varied with leaf

position, but was always higher for HW plants compared to LW plants (P <0.001) (Fig. 3). Additionally, at leaf positions 15 and 20, there were significant infection effects (P <0.001). Conductance in HW,I plants was significantly lower than HW,NI plants at these positions. Low water plants had low g_s at all leaf positions, and there was no significant difference between LW,I and LW,NI treatments (P >0.05).

During the year 2000 experiment, all four measurement days showed the highest g_s values in the mid-morning hours, with decreases from this peak throughout the day (P <0.001 for time effect) (Fig. 2, bottom panel). Significant water treatment effects were found by July 27 (P=0.05), 16 d after water treatments were imposed (Fig. 2). On the 21 August and 20 September sampling dates, LW plants had lower g_s than HW plants for all leaf positions and throughout the day (P=0.001) (Fig. 2). Prior to drought, NI and I plants had similar g_s values (P >0.05) (Fig. 2). On 20 September, HW,I plants had lower maximal (morning) g_s compared to HW,NI plants (P <0.05) (Fig. 2). LW,I plants had reduced g_s compared to LW,NI plants in the afternoon on 21 August and throughout the day on 20 September (P <0.05) (Fig. 2).

Hydraulic conductivity

At the end of the 1999 experiment, stem hydraulic conductivity was reduced by LW and I treatments (P <0.05, data not shown). In the 2000 experiment, no significant difference was found between NI and I plants prior to the initiation of the drought treatment in whole shoot specific hydraulic conductance (K_h) (P >0.05) (Fig. 4, top panel) or % embolism (P >0.05) (Fig. 4, bottom panel). However, in September (65 d after the initiation of the drought treatment), K_h was reduced in both LW and I treatments (P <0.05 for both effects) (Fig. 4, top panel). Similar results were found when the data were standardized for differences in shoot leaf area (data not presented). In contrast, the percentage embolism for whole shoot segments was increased by the LW treatment only (P <0.001), with no significant effect of infection (P >0.05) (Fig. 4, bottom panel).

Vessel size

As with the embolism results, vessel diameter distributions were affected by water treatment only (Fig. 5, top panel). Mean vessel diameter was reduced in LW plants relative to HW plants (P <0.001), but there was no significant difference between the mean vessel diameter for NI and I plants (P >0.05). Over 57% of all vessels were less than 5.0 cm in length (Fig. 5, bottom panel). Both *X. fastidiosa*-infection (P <0.05) and LW treatments (P <0.001) significantly reduced vessel lengths. *X. fastidiosa*-infected plants had a greater number of vessels less than 1.0 cm in length relative to NI plants (P <0.05). Low water plants had no vessels longer than 25 cm (Fig. 5, bottom panel). Nearly 23% of vessels in HW,NI plants were longer than 15 cm.

Discussion

The results of the physiological analysis of the interaction between water stress and *X. fastidiosa*-infection show that these factors act additively. As soil water availability decreases, *X. fastidiosa* infected plants develop earlier and more pronounced reductions in g_s, Ψ_L, and K_h than do non-infected plants. The effects of *X. fastidiosa* infection on the physiological responses of plants are similar to those of other vascular wilt pathogens (Beckman, 1987). For example, decreased K_h, g_s, and Ψ_L have been found in tomato, cotton, chrysanthemum, potato, and alfalfa infected by vascular wilt fungal pathogens from the genera *Verticillium* and *Fusarium* (Duniway, 1971; Tzeng and DeVay, 1985; MacHardy *et al*., 1976; Saeed *et al*., 1999; Pennypacker *et al*., 1991). Previous work on the *X. fastidiosa* pathosystem has also shown reduced Ψ_L in peach trees infected with phony peach disease compared to non-infected trees during late summer (Evert and Mullinix, 1983; Evert, 1987; Anderson and French, 1987). Kostka *et al*. (1986) found that stem hydraulic conductivity, the number of functional xylem vessels, and water potential were significantly lower in elm trees with leaf scorch disease than in healthy elm. Leaves on grapevines infected with a Pierce's disease strain of *X. fastidiosa* had lower Ψ_L and stomatal conductances compared to healthy control plants at similar leaf positions (Goodwin and Meredith, 1988; Goodwin *et al*., 1988).

Reduced g_s and Ψ_L are also common physiological responses of plants to water stress (Hsiao, 1973). In both years of the study, g_s and Ψ_L were reduced by lower soil water availabilities, and differences between HW and LW plants became larger over the course of the water treatment. *Parthenocissus quinquefolia* has been shown to have a greater stomatal sensitivity to low soil water conditions than other temperate vine species such as *Vitis vulpina* and *Lonicera japonica* (Bell *et al*., 1988). This sensitivity was apparent in the restriction of maximal g_s to the morning hours throughout both years of experimentation. A similar diurnal pattern was found in field-grown *P. quinquefolia* (Bell *et al*., 1988), and is typical for plants trying to avoid high transpirational water losses during the warmest parts of the day, especially when soil moisture is reduced. Despite reductions in maximal g_s and the temporal shift to earlier diurnal periods, *P. quinquefolia* was not able to prevent reductions in Ψ_L and K_h that accompanied the restricted water supply and bacterial infection.

Two primary mechanisms have been proposed to explain reductions in K_h due to water stress: (1) formation of emboli resulting in a loss of vessel function, and (2) reduction in the xylem vessel size (Sperry and Tyree, 1990;

Hacke and Sauter, 1996; Lovisolo and Schubert, 1998). The decrease in K_h due to vessel embolism can directly reduce water flow to the shoot by disrupting the continuous column of water between the shoot and the leaf and reducing the number of functional vessel elements (Schultz and Matthews, 1988). Since flow rate in capillary systems is proportional to the 4th power of the radius of the capillary (Hagen–Poiseuille law), any decrease in vessel diameter can cause considerable decreases in conductivity (Zimmermann, 1983). The results suggest that both mechanisms were operating during the experiments. Low water plants had a significantly higher percentage embolism in shoot systems relative to HW plants. In addition, water stress reduced the mean vessel diameter of *P. quinquefolia* shoots, thus combining with embolism to reduce the K_h through the shoot system.

Since vessel diameter and vessel length are positively correlated in angiosperms (Zimmermann and Jeje, 1981), a reduction in vessel length would be expected to accompany reduced vessel diameter in decreasing hydraulic conductance. Conversely, no reduction in vessel diameter due to *X. fastidiosa* infection was found and, therefore, no reduction in vessel length would be expected (Zimmermann and Jeje, 1981). However, xylem vessel length in *X. fastidiosa*-infected plants was reduced compared to NI plants. These results were interpreted to mean that the actual length of *P. quinquefolia* vessels was not reduced by infection, but that the functional length of vessels was reduced, most likely due to bacteria clogging the vessels and preventing the infused paint solution from reaching the true end of a vessel. A reduction in the functional length of vessels would force flow to reroute around non-conductive, plugged vessels, thus reducing the transport capacity and K_h in *X. fastidiosa*-infected plants.

Previous research with vascular wilt pathogens has found that xylem-limited micro-organisms can reduce hydraulic flow by inducing cavitation and subsequent emboli formation. For example, Newbanks *et al*. (1983) found evidence that embolism preceded any occlusion of vessels by other means in Dutch Elm disease. Similarly, emboli significantly reduced hydraulic conductivity and contributed to the Pine wilt disease symptoms caused by the pine-wilt nematode by modifying the surface tension of the water (Ikeda and Kiyohara, 1995). However, Pisante *et al*. (1993) found that inoculation of Douglas fir seedlings with two fungal xylem pathogens (*Phomopsis occulta* and *Diplodia pinea*) resulted in no increase in xylem cavitation in inoculated plants compared to controls. Whilst an increase in the formation of emboli due to bacterial infection was not found, the sampling method of only measuring percentage embolism on whole shoot systems may have prevented the finding of embolism in specific tissues, such as the petioles where, presumably, most of the blockage caused by *X. fastidiosa* in *P. quinquefolia* and grape occurs (Hopkins, 1981). Similarly, sampling at the beginning and end of the growing season may have meant that the period of time when the bacteria are inducing emboli formation in the xylem conduits was missed. When the bacteria first enter a newly colonized conduit, they may cause emboli formation by lowering the surface tension of the water (Tyree and Sperry, 1989) or by disturbing the pit membranes between adjacent vessels (Sperry and Tyree, 1988). Recent genomic analysis of *X. fastidiosa* has shown that the bacteria possess cellulases, a polygalacturonase, and other plant cell-wall-degrading enzymes, which may play a role in intervessel migration by degradation of the pit membrane (Simpson *et al*., 2000; Lambais *et al*., 2000). Any degradation of the pit membrane would probably embolize the vessel prior to the mass colonization of this new vessel.

Alternatively, *X. fastidiosa* may utilize another strategy to move between vessels. Zwieniecki *et al*. (2001) showed that the size of microchannels (pores) in the intervessel pit membranes change in response to solution ion concentration and pH. *X. fastidiosa* aggregates may locally alter the composition of xylem solution and create larger pores in the pit membrane. The increased pore size may be large enough for a polarly oriented bacterium to pass through to the new vessel as a colonist cell. In fact, some pit membrane pores may be large enough for bacteria to migrate between vessels even without an ion or pH-induced pore enlargement. Sperry and Tyree (1988) measured pit membrane pores as large as 0.4 μm in sugar maple (*Acer saccharum* Marsh.), albeit most pores were much smaller than 0.05 μm diameter. Typical cellular dimensions for strains of *X. fastidiosa* growing in culture are 0.25–0.35 by 0.9–3.5 μm (Wells *et al*., 1987). The majority of the pit membrane pores may be too small for a typical *X. fastidiosa* bacterium to pass through, but a few large pores may provide an opening large enough to accommodate a polarly oriented colonist bacterium. Host plant susceptibility and the speed with which *X. fastidiosa* move systemically may be related to the size and frequency of large pit membrane pores across plant species.

Classical water relations theory predicts that predawn plant water potential should be in equilibrium with the soil water potential (Nobel, 1991). However, recent work by Donovan *et al*. (1999) has shown that predawn Ψ_L for two cold-desert shrubs (*Chrysothamnus nauseosus* and *Sarcobatus vermiculatus*) was significantly more negative than the soil water potential, indicating a large predawn soil–plant disequilibrium. This study's results suggest that *X. fastidiosa* present in the xylem vessels can induce a predawn disequilibrium in basally-located leaves of *P. quinquefolia* (Figs 1, 2). All plants within a watering treatment received equal amounts of water added to the soil throughout the study. Therefore, plants within the same watering treatment, regardless of infection treatment, should have had similar predawn Ψ_L at similar leaf positions. The drought treatment induced a significant reduction in predawn Ψ_L; LW plants exhibited lower Ψ_L

than HW plants at all leaf positions for both 1999 and 2000 experiments. However, at basally-located leaf positions, *X. fastidiosa*-infected plants had significantly lower predawn leaf water potentials within a watering treatment, i.e. LW,I plants <LW,NI plants. It is suspected that *X. fastidiosa* plugs in the xylem vessels (Hopkins, 1981) disrupt the flow of water through the leaf petioles, thus inhibiting the plant's ability to recover overnight from daily transpirational water losses. Therefore, this study provides an undocumented mechanism that can contribute to predawn disequilibrium, and a mechanism to explain the additive interaction between water stress and *X. fastidiosa* infection in *P. quinquefolia* plants. Failure of leaves to rehydrate overnight will result in reduced leaf water contents the following day. This will make the leaves more susceptible to damage due to photoinhibition and high leaf temperatures. Compounding this sensitivity is the loss in evaporative cooling caused by reduced stomatal conductances, resulting in higher leaf temperatures for infected plants. Since photoinhibition and high temperature damage are functions of both the magnitude of light and temperature as well as the time of exposure, damage to infected leaves with blocked petioles should be much higher than that in non-infected leaves.

Reviews by Boyer (1995) and Schoeneweiss (1975) have illustrated that a predisposition to disease is often observed in host plants experiencing soil water deficits. For example, Suleman *et al*. (2001) recently found that date palm trees experiencing water stress are more susceptible to attack by two fungal pathogens, *Chalara radicola* and *Chalara paradoxa*. Previous work with the *X. fastidiosa* pathosystem has also shown increased leaf scorch symptom severity and progression along the stem during water stress (McElrone *et al*., 2001). A possible mechanism underlying this interaction in the *X. fastidiosa* pathosystem has been provided here. Individually, water stress and *X. fastidiosa* infection reduce K_h and leaf water potentials in *P. quinquefolia* by inducing embolism formation and reducing vessel diameter and length, or by clogging the xylem conduits and reducing functional vessel length, respectively. When combined, these reductions promote extensive xylem dysfunction as seen in K_h of LW,I plants (an additive reduction in K_h due to water treatment and infection combined). Since *X. fastidiosa* move from the base of the plant towards the apex in *P. quinquefolia*, the petioles on the most basally-located leaves are colonized earliest and eventually restrict all water movement into the leaf. Hopkins (1981) found, with serial sectioning of grape leaf petioles, that all vessels on severely scorched leaves are occupied by bacterial plugs. With a decrease in the soil water supply imposed by drought, combined with the plant's loss of ability to recover completely from transpirational water losses on a daily basis due to bacterial plugs, symptom onset is accelerated in drought-stressed plants.

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