

Department of Plant Biology, University of Maryland, USA

Photosynthetic Responses of a Temperate Liana to *Xylella fastidiosa* Infection and Water Stress

A. J. McELRONE^{1,2} and I. N. FORSETH²

Authors' addresses: ¹Current address: Department of Biology, 133 Biological Sciences, Duke University, Durham, NC 27708, USA; ²Department of Plant Biology, University of Maryland, College Park, MD 20742, USA (correspondence to A. J. McElrone, E-mail: mcelrone@duke.edu)

With 9 figures

Received May 28, 2003; accepted October 5, 2003

Keywords: bacterial leaf scorch, chlorophyll fluorescence, gas exchange, *Parthenocissus quinquefolia*, photoinhibition, vascular wilt, *Xylella fastidiosa*

Abstract

Xylella fastidiosa is a xylem-limited bacterial plant pathogen that causes bacterial leaf scorch in its hosts. Our previous work showed that water stress enhances leaf scorch symptom severity and progression along the stem of a liana, *Parthenocissus quinquefolia*, infected by *X. fastidiosa*. This paper explores the photosynthetic gas exchange responses of *P. quinquefolia*, with the aim to elucidate mechanisms behind disease expression and its interaction with water stress. We used a 2 × 2-complete factorial design, repeated over two growing seasons, with high and low soil moisture levels and infected and non-infected plants. In both years, low soil moisture levels reduced leaf water potentials, net photosynthesis and stomatal conductance at all leaf positions, while *X. fastidiosa*-infection reduced these parameters at basally located leaves only. Intercellular CO₂ concentrations were reduced in apical leaves, but increased at the most basal leaf location, implicating a non-stomatal reduction of photosynthesis in leaves showing the greatest disease development. This result was supported by measured reductions in photosynthetic rates of basal leaves at high CO₂ concentrations, where stomatal limitation was eliminated. Repeated measurements over the summer of 2000 showed that the effects of water stress and infection were progressive over time, reaching their greatest extent in September. By reducing stomatal conductances at moderate levels of water stress, *P. quinquefolia* maintained relatively high leaf water potentials and delayed the onset of photosynthetic damage due to pathogen and drought-induced water stress. In addition, chlorophyll fluorescence measurements showed that *P. quinquefolia* has an efficient means of dissipating excess light energy that protects the photosynthetic machinery of leaves from irreversible photoinhibitory damage that

may occur during stress-induced stomatal limitation of photosynthesis. However, severe stress induced by disease and drought eventually led to non-stomatal decreases in photosynthesis associated with leaf senescence.

Introduction

Xylella fastidiosa (Wells et al., 1987) is a xylem-limited bacterial plant pathogen that has a diverse and extensive host range encompassing at least 30 families of monocotyledonous and dicotyledonous plants (Sherald and Kostka, 1992). Leaf scorching or scalding is the most typical symptom of *X. fastidiosa* caused diseases. Leaf symptoms appear in mid- to late summer and become progressively worse until fall dormancy (Raju and Wells, 1986; Blanchard and Tattar, 1987). Long-term effects of infection are delayed leaf emergence, reduced reproductive output, root system decline, dwarfing and eventual plant death (Blanchard and Tattar, 1987). Economically important diseases caused by *X. fastidiosa* include: citrus variegated chlorosis, Pierce's disease of grape, phony peach disease, alfalfa dwarf, periwinkle wilt, and leaf scorch of coffee, plum, pear, almond, mulberry, elm, oak, sycamore, maple, oleander and pecan (da Silva et al., 2001). The growing biological and economic significance of *X. fastidiosa* is further illustrated by it being the first plant pathogen to have its genome completely sequenced (Simpson et al., 2000). Recent genomic analyses (e.g. Bhattacharyya et al., 2002) in addition to physiological studies will advance our understanding of symptom development and progression for future management of this devastating plant pathogen.

The interaction of abiotic stressors with *X. fastidiosa* infection has received little attention experimentally. Several researchers have suggested that leaf scorch

symptoms caused by *X. fastidiosa* become severe only after some other stress is placed on the host (Hearon et al., 1980; Sherald et al., 1983; Hopkins, 1989, 1995). Our previous work confirmed the idea that water stress enhances symptom severity and progression along the stem in *Parthenocissus quinquefolia* (a deciduous forest vine common throughout the eastern United States) infected with *X. fastidiosa* (McElrone et al., 2001, 2003). However, additional physiological studies are still needed to elucidate the mechanism underlying this interaction between water stress and *X. fastidiosa* infection.

Gas exchange studies of vascular wilt pathosystems have been useful in determining factors involved with disease progression (Bowden et al., 1990; Pennypacker et al., 1990; Saeed et al., 1999). Disease-induced reductions in photosynthesis can be caused by water stress-induced stomatal closure, which limits CO₂ supply to chloroplasts, or by direct effects on the biochemistry of photosynthesis (Bowden et al., 1990). These two mechanisms can be distinguished by an analysis of the response of photosynthesis (A) to the calculated partial pressure of CO₂ inside the leaf (C_i) (A/C_i response curves). Analysis of A/C_i response curves allows the determination of stomatal vs. non-stomatal reductions in net photosynthesis, as well as what components of photosynthesis are most sensitive to infection and water stress, i.e. electron transport, ribulose biphosphate (RuBP) regeneration, and/or carboxylation efficiency (CE) (Farquhar and Sharkey, 1982). Measurements of chlorophyll fluorescence from leaves are also used to examine the functioning of the photosynthetic apparatus (Balachandran and Osmond, 1994). Chlorophyll fluorescence measurements provide direct probes of the photochemical efficiency of photosynthesis, and have been used extensively to examine how plants respond to excess light energy, especially when other stresses, such as water stress and plant pathogens reduce photochemical dissipation of light energy (reviewed in Powles, 1984; Krause, 1988; Krause and Weis, 1991; Demmig-Adams and Adams, 1992; Long et al., 1994).

In this paper, we use whole leaf gas exchange and chlorophyll fluorescence to investigate how water stress and *X. fastidiosa*-infection affect photosynthetic carbon gain and the susceptibility of the photosynthetic apparatus to photoinhibition (Daley, 1995). To date little research has been carried out assessing gas exchange and chlorophyll fluorescence characteristics in the *X. fastidiosa* pathosystem. Our previous work has demonstrated additive interactions between water stress and *X. fastidiosa*-infection on plant water relations, stem hydraulic conductivity, and disease expression (McElrone et al., 2003). Understanding the plants' gas exchange responses to water stress and bacterial infection will help to elucidate the physiological mechanisms behind disease expression and its interaction with water stress. This information can also be used to examine the effects of these combined stresses on plant carbon gain and growth.

We hypothesized that the combination of water stress and bacterial infection would act additively to reduce photosynthetic capacity and photosynthetic rates in symptomatic leaves to a greater degree than either stress alone. We further hypothesized that water stress effects would be seen throughout the plant, while disease effects would be restricted to those basal leaf positions that expressed visual disease symptoms. Finally, we hypothesized that later disease stages and prolonged water stress would interact additively to make the photosynthetic apparatus more susceptible to photoinhibition under high light and temperature conditions. To test these hypotheses, we set up a 2 × 2 factorial experiment made up of a high water (HW)-non-infected (NI) treatment, a HW-infected (I) treatment, a low water (LW)-NI treatment and a LW- I treatment.

Materials and Methods

Plant species description

Parthenocissus quinquefolia (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 (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 locally 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

We used a 2 × 2 complete factorial experiment, with two pathogen treatments (non-infected, NI and infected, I) and two soil moisture treatments (HW and LW) to determine the response of *P. quinquefolia* plants to *X. fastidiosa*-infection and concurrent water stress. This greenhouse experiment was initially performed during the summer of 1999 and was repeated in the summer of 2000. Equal age *P. quinquefolia* shoots, produced from cuttings of nursery-grown plants, were planted in potting soil (Peters Professional Potting Soil, Scotts, Inc., Baltimore, MD, USA), watered regularly, and maintained in large pots (30 cm diameter and 45 cm depth) under ambient greenhouse conditions prior to use in the experiments. Large pots were used to minimize the effects of root zone restriction. At the beginning of the experiment, plants were randomly assigned to each of the four treatments and distributed at random on benches in the same greenhouse facility, where they remained for the duration of the experiment. Plants were fertilized using standard liquid N-P-K fertilizer (20-10-20; Peters Inc.) recommended for container grown woody ornamentals.

Xylella fastidiosa inoculations and treatment confirmation

Parthenocissus quinquefolia plants were inoculated with *X. fastidiosa* using a scalpel incision at the base of the

stem just above the soil (Sherald, 1993). Plants used in the summer 1999 experiment were inoculated once only in mid-July 1998, and plants used in the summer 2000 experiment were inoculated once only in mid-July 1999. No plants were used for more than 1 year. A Pierce's disease strain of *X. fastidiosa* (strain no. 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. Inoculum was standardized in PBCM at an optical density (OD) of 0.07–0.10 at 560 nm (corresponding to 10^7 – 10^8 cells/ml) with a Bausch & Lomb Spectronic 710 spectrophotometer (Rochester, NY, USA) (Sherald, 1993). Control plants also received scalpel incision inoculations but with sterilized PBCM solution without bacteria. Plant samples were subsequently tested for the presence of *X. fastidiosa* using an immunomagnetic separation and nested-polymerase chain reaction (PCR) technique (Pooler et al., 1997; McElrone et al., 1999) to verify successful inoculations.

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 HW or 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 1/2 field capacity (determined gravimetrically) daily, except on cloudy days, when reduced transpiration alleviated the need for watering. Water treatments were initiated on June 24 in the summer of 1999 and on July 11 in the summer of 2000, and were sustained for 52 and 76 days, respectively.

Differences between watering treatments were verified using leaf water potential measurements (Ψ_L). Leaf water potentials were measured on five replicate plants from each treatment using a PMS pressure chamber (PMS Instruments Inc. Corvallis, OR, USA) at both an apical and basal leaf location (leaf position designation to be explained below).

Environmental conditions

During the days that plant physiological responses were measured, photosynthetically active radiation (PAR), air temperature, and the leaf–air vapour pressure deficit (VPD) were collected and stored in a datalogger (CR-10X, Campbell Scientific, Logan, UT, USA). PAR, air temperature, and VPD were recorded using quantum sensors, thermocouples, and humidity sensors, respectively. Sensors were scanned every 60 s, and 30-min averages of these readings were stored in datalogger memory.

Gas exchange measurements

Since scorch symptoms in *P. quinquefolia* progress from the plant base towards the apex (McElrone et al., 2001), the 1999 measurements were focused on quantifying the effect of leaf position on photosynthetic

responses. Gas exchange and fluorescence measurements during this experiment were taken near the end of the experiment (11 August 1999 and 13 August 1999, respectively), after development of disease symptoms and 48 days of water treatment. The 5-leaf positions along the stem were chosen for measurements, ranging from the most recent, fully expanded leaf (most apical, leaf position 0) to a leaf located 20 nodes basal from leaf position 0 (leaf position 20). Moving towards the plant base, every fifth leaf was designated as a measurement position. In the year 2000 experiment, major objectives were to document the time course of disease and water stress effects. Accordingly, physiological measurements were made four different times. Once, prior to the imposition of water treatments, and three times during the course of the experiment, each separated by approximately 30 days (mid-June, mid-July, mid-August and mid-September). Diurnal cycles of net photosynthesis (A), stomatal conductance (g_s), and the ratio of intercellular CO_2 to ambient CO_2 (C_i/C_a) were measured with an open gas exchange system (LI 6400, LiCor, Inc., Lincoln, NE, USA) at 2-leaf positions along the stem segments (leaf positions 0 and 20). The response of net photosynthesis to intercellular CO_2 (C_i) at saturating irradiance (AC_i response curves) was measured on two leaves each on five different plants, one leaf at position 0 and one leaf at position 20 using the same photosynthetic system. Saturating irradiance for AC_i response curves was maintained at approximately $1950 \mu\text{mol}/\text{m}^2/\text{s}$ with a LED Light Source (LI 6400-02B; LiCor, Inc.). Maximum photosynthetic rates (A_{max}) at saturating CO_2 , CE, and CO_2 compensation point were determined from these curves using a non-linear, mixed-model statistical approach (Peek et al., 2002).

Chlorophyll fluorescence

Chlorophyll fluorescence from photosystem II (PS II) was recorded with a portable pulse amplitude fluorometer (PAM 2000, Walz, Effeltrich, Germany). The maximal photochemical efficiency of PS II was determined from the ratio of variable to maximal fluorescence, i.e. $F_v/F_m = (F_m - F_o)/F_m$, where F_o and F_m are initial and maximal fluorescence of dark-adapted leaves. F_o was determined with a modulated measuring light from a light emitting diode ($< 0.5 \mu\text{mol}/\text{m}^2/\text{s}$). F_m was obtained with a brief saturation light pulse (0.8 s , $> 5000 \mu\text{mol}/\text{m}^2/\text{s}$). In order to measure responses from the portion of the leaf receiving the highest amounts of ambient irradiance, fluorescence was measured on the adaxial side of leaves. For diurnal courses of F_v/F_m , five replicate leaves for each treatment at leaf positions 0 and 20 were dark-adapted for 15–20 min, in order to open all reaction centres, prior to determination of F_v/F_m .

Leaf chemistry

Leaf nitrogen (N) content was determined in the year 2000 experiment for five replicate leaves each at positions 0 and 20. Leaves were harvested after the four

gas exchange measurement periods and dried in an oven at 60°C for at least 1 week. Dried leaves were then finely ground using liquid N and a mortar and pestle. Samples were combusted in an elemental analyzer (Perkin-Elmer, CHN Elemental Analyzer, Boston, MA, USA) to determine leaf N content.

Statistical analysis

The textscanova were computed using SAS system version 8.0 (1999 SAS Institute Inc., Cary, NC, USA). Data transformations were performed as needed to meet ANOVA assumptions. Differences in mean values were analysed using LS Means analysis (SAS Institute, Inc.). All four possible treatment combinations were used with five replicates of each treatment in the 2 × 2 complete factorial design. For Ψ_L , A , g_s , C_i/C_a , and F_v/F_m data, we used repeated measures of ANOVA because multiple measurements (multiple leaf positions per vine or multiple times per day) were made on the same experimental unit for each sampling date.

Photosynthetic response to intercellular CO_2 was analysed using a non-linear mixed model approach to estimate three parameters; A_{max} , CE, and CO_2 compensation point (Peek et al., 2002). The following equation was fit using this model:

$$A = A_{max} * (1 - e^{-CE*(C_i - CO_2 \text{ compensation point})})$$

Results

Leaf water potentials

The effects of reduced soil water availability and infection by *X. fastidiosa* on leaf water potentials in *P. quinquefolia* have been reported elsewhere (McElrone et al., 2003), so we present only a brief summary here. In 1999, both midday and predawn Ψ_L sampled at the end of the experiment were lower in the LW-treated plants than in the HW plants ($P < 0.0001$). There were also significant infection effects, with I plants having significantly lower predawn ($P < 0.0001$) and midday Ψ_L ($P < 0.0001$) than NI plants from leaf position 10 through 20 (non-significant water × infection, $P > 0.05$) (McElrone et al., 2003). No significant differences in Ψ_L were found between treatments at any time of the day on the first sampling date of the year 2000 experiment (June 12), prior to the initiation of water treatments ($P > 0.05$) (McElrone et al., 2003). By July 31, 2000, LW plants had significantly lower midday Ψ_L than HW plants at both leaf nodes 0 and 20. Leaf 20 also showed an infection effect at midday. On the August and September sampling dates, significantly lower leaf water potentials were measured in the LW plants compared with the HW plants at all hours of the day (McElrone et al., 2003). *Xylella fastidiosa*-infected plants also had significantly lower Ψ_L in both August and September for basally located leaves throughout the day, with the lowest water potentials being recorded in the LW, I treatment.

Gas exchange responses

Midday photosynthetic rates under ambient light and temperature conditions in August, 1999 showed some variability along the stem, but were always higher for HW than LW plants ($P < 0.0001$) (Fig. 1a). Infection also reduced photosynthetic rates, but only at basal leaf positions ($P < 0.0001$) (non-significant water × infection, $P > 0.05$) (Fig. 1a). Stomatal conductance had a similar pattern, with reductions in the LW compared with the HW treatments at all leaf positions, and infection induced reductions for basal leaf positions ($P < 0.0001$) (non-significant water × infection, $P > 0.05$) (Fig. 1b). Most of the reduction in photosynthesis in the LW treatments was explained by stomatal closure. This can be seen by the decreased C_i/C_a ratio in LW plants (Fig. 1c). However, at the most basal leaf location an infection by water interaction ($P = 0.04$) increases C_i/C_a in LW plants, while decreasing C_i/C_a in the HW, I treatment.

Diurnal courses for net photosynthetic rate measured on four sampling dates throughout the summer of 2000 show the development of water stress due to low soil water conditions, and the progression of the

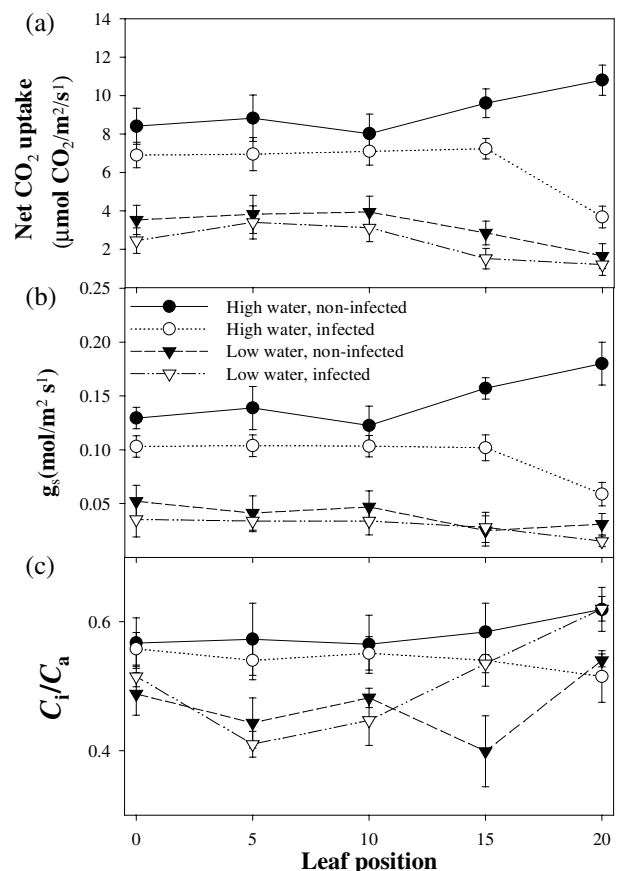


Fig. 1 Midday photosynthesis (a), stomatal conductance (g_s) (b), and the ratio of intercellular CO_2 to ambient CO_2 (C_i/C_a) measured at 5-leaf positions along the stem on 14 August 1999. Data are the mean \pm SE. HW, high water; LW, low water; NI, non-infected; I, infected (these abbreviations are the same for all other figures)

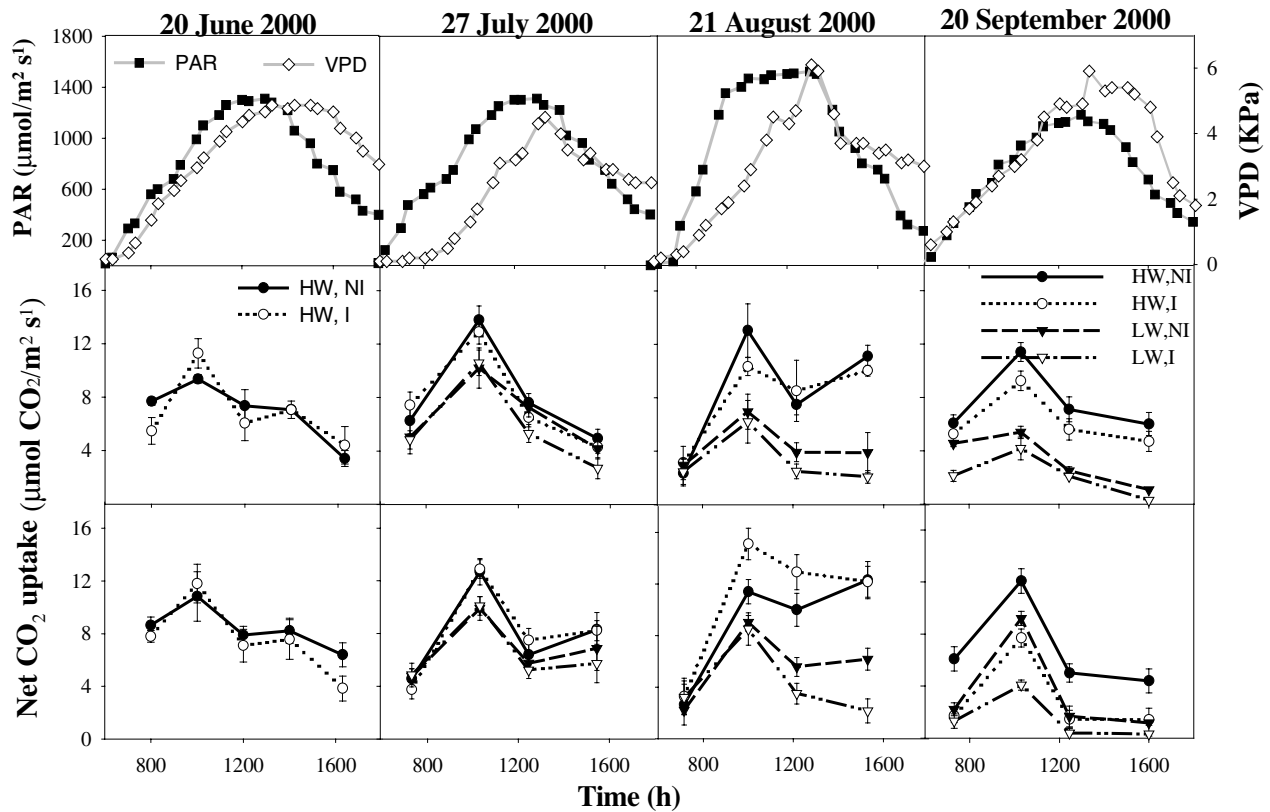


Fig. 2 Photosynthetically active radiation (PAR), vapour pressure deficit (VPD) (both in top row), and photosynthetic rates (leaf 0, middle row; leaf 20, bottom row) measured at four sampling dates (columns) throughout the 2000 experiment. Data are the mean \pm SE

disease (Fig. 2). Microclimatic conditions were similar on all four-sample dates, which were clear and warm (top panels, Fig. 2). Midday PAR exceeded $1300 \mu\text{mol}/\text{m}^2/\text{s}$ each day, and ambient air temperature ranges were $19.8\text{--}38.5$, $16.7\text{--}39.1$, $20.5\text{--}42.7$ and $20.0\text{--}42.2^\circ\text{C}$ for each of the four successive dates, respectively. High VPD levels in the afternoon resulted in partial stomatal closure and reduced photosynthesis (Figs 2 and 3). All treatments showed a displacement of peak photosynthetic and stomatal conductance rates from peak PAR values, with highest gas exchange rates becoming progressively restricted to earlier morning hours from June to September (Figs 2 and 3). Throughout the season, significant water by time and infection by time interactions were found at both leaf positions with net photosynthetic rates and stomatal conductance increasing in the morning and decreasing in the afternoon ($P < 0.01$) (Figs 2 and 3). Prior to the imposition of reduced soil water (June), net photosynthetic rates, g_s , and C_i/C_a were not significantly different among treatments ($P > 0.05$) (Figs 2–4). By July, net photosynthetic rates were significantly reduced at midday in LW plants compared with HW plants at leaf positions 0 and 20 ($P = 0.04$) (Fig. 2). The reduction in photosynthetic rates in LW plants corresponded with reduced midday g_s ($P = 0.009$) (Fig. 3) causing a decrease in C_i/C_a ($P = 0.03$) (Fig. 4). In August and September, net photosynthetic rates

and g_s were reduced by LW compared with HW plants at both leaf positions throughout the day ($P < 0.003$), and were reduced by I at leaf position 20 ($P < 0.009$) (Figs 2 and 3). No significant infection effect was found at leaf position 0 ($P > 0.05$) (non-significant water \times infection, $P > 0.05$) (Fig. 2). Reduced photosynthetic rates of LW plants were caused by decreased g_s at both leaf positions in August and at leaf 0 in September as seen in reduced C_i/C_a ratios ($P = 0.01$) (Fig. 4). Additionally, C_i/C_a was reduced by I at leaf 20 in August ($P = 0.03$). However, in September the pattern of C_i/C_a as seen throughout the season varied with LW and I treatments, with increasing C_i/C_a in late day measurements at leaf position 20, suggesting that non-stomatal limitation of photosynthesis was occurring (water \times infection \times time interaction, $P = 0.03$) (Fig. 4).

The response of net photosynthesis to intercellular CO_2 (C_i) at saturating irradiance (AC_i response curves) was measured on leaf positions 0 and 20 at four sampling dates throughout the 2000 season. No significant treatment effects were found for the three AC_i parameters (A_{max} , CE and CO_2 compensation point) in the June (predrought), July, and August sampling dates at leaf positions 0 and 20 ($P > 0.05$) (data not shown). On the September sampling date, A_{max} was reduced by LW and I treatments at leaf position 20 ($P = 0.024$ and $P = 0.019$, respectively) (Fig. 5). This reduction in

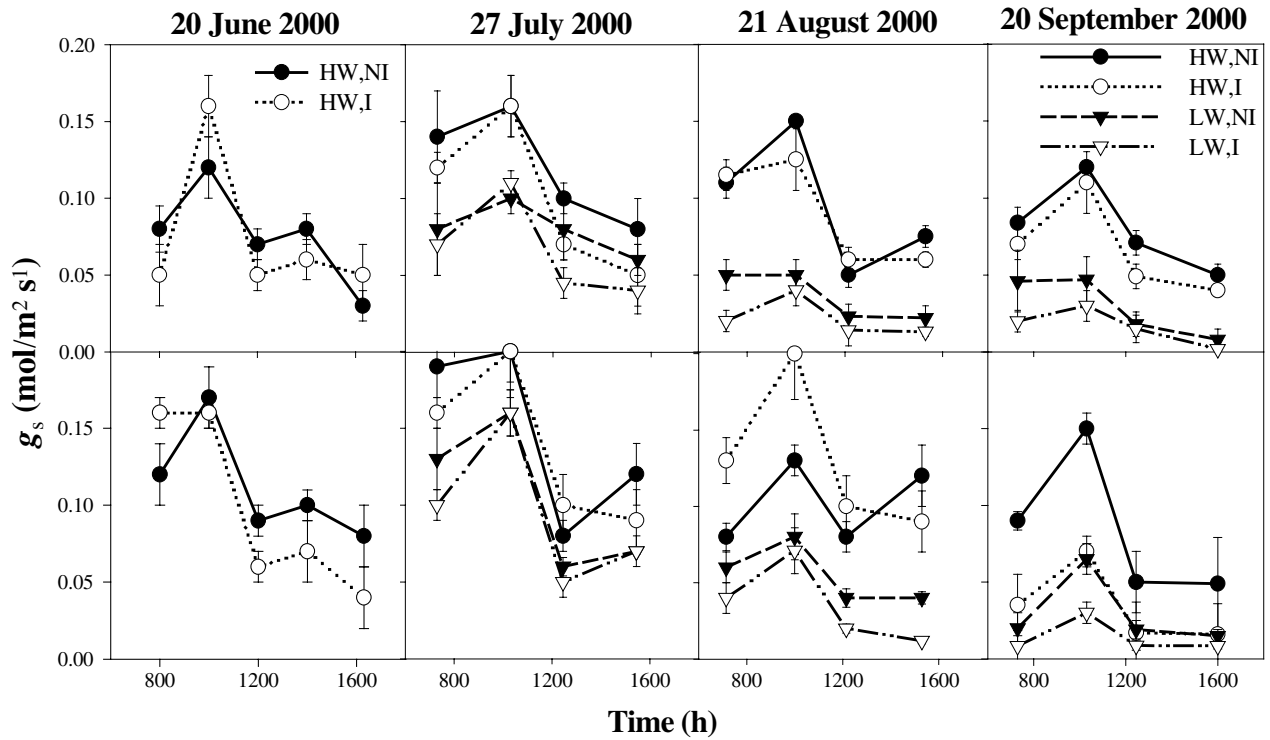


Fig. 3 Stomatal conductance (g_s) measured at four sampling dates (columns) and at 2-leaf positions (leaf 0, top row; leaf 20, bottom row) in 2000. Data are the mean \pm SE

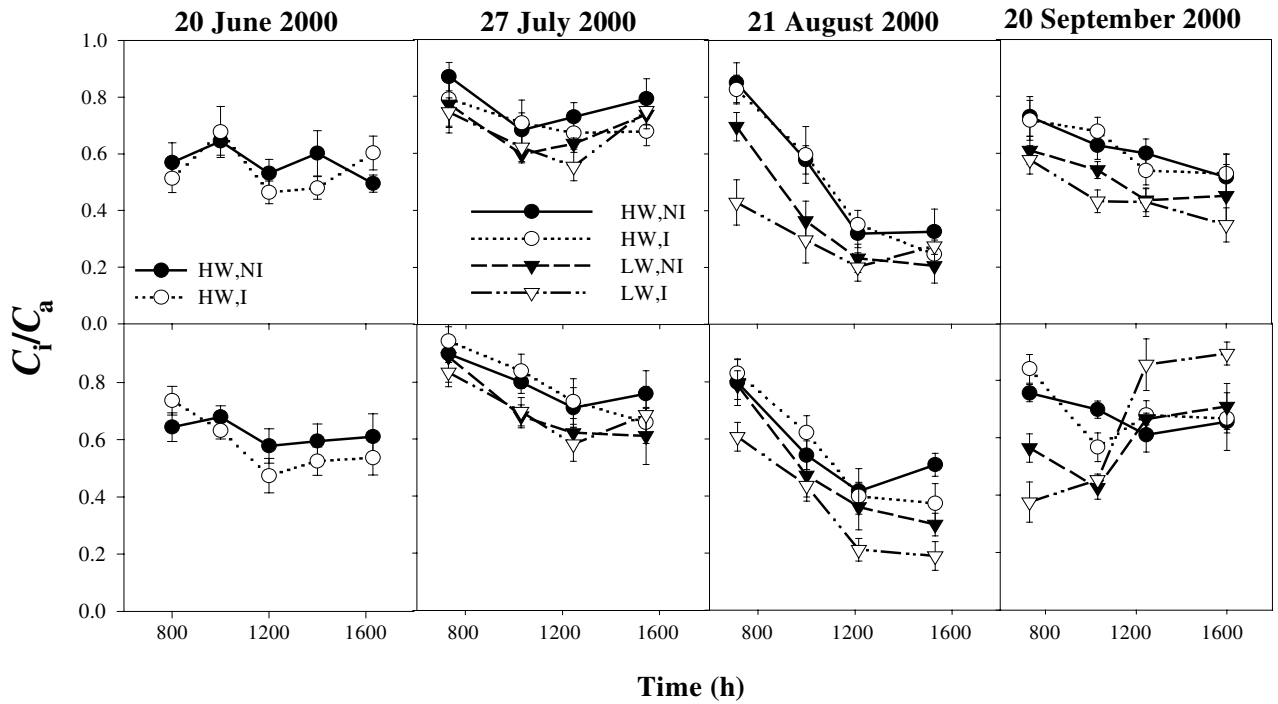


Fig. 4 The ratio of intercellular CO_2 to ambient CO_2 (C_i/C_a) (leaf 0, top row; leaf 20, bottom row) measured at four sampling dates (columns) in 2000 experiment. Data are the mean \pm SE

A_{max} by the LW and I treatments is consistent with the C_i/C_a data that indicated non-stomatal factors were limiting photosynthesis in basal leaves late in the season.

Chlorophyll fluorescence

The photochemical efficiency of PS II was measured using chlorophyll fluorescence at predawn and midday in August 1999 (Fig. 6) and tracked throughout the

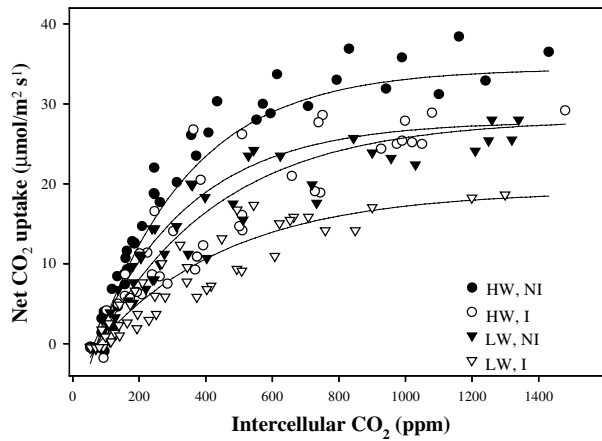


Fig. 5 The response of net photosynthesis to intercellular CO₂ (C_i) at saturating irradiance (A_{C_i} curve) measured at leaf position 20 in mid-September 2000

day at the four sampling dates throughout 2000 (Fig. 7). In August 1999, the LW treatment significantly reduced F_v/F_m at all leaf positions at both

predawn and midday samplings (Fig. 6). A water by leaf position interaction was found for predawn with F_v/F_m decreasing towards the basal leaves in the LW treatment only (P = 0.02) (Fig. 6). The lower photochemical efficiency in LW-treated plants at nodes 10, 15 and 20 at predawn indicates that non-reversible damage was occurring to PS II. At midday, the combination of LW and I treatment reduced F_v/F_m for basal leaves (P = 0.009)(leaves 10, 15 and 20) (Fig. 6), but this inhibition recovered to LW, NI levels by the following morning, indicating that most effects were reversible. Midday F_v/F_m in the HW, I treatments was significantly reduced relative to the HW, NI treatment only at leaf node 20. This midday reduction was reversible, as indicated by predawn measurements.

No significant differences were found between NI and I plants at the predrought 30 June, 2000 sampling date, nor between treatments on 28 July (Fig. 7). On 27 August 2000, midday F_v/F_m was reduced by LW at all leaf positions and by I at leaf 20 (P < 0.05) (Fig. 7). F_v/F_m recovered from photoinhibition at predawn with no significant differences between

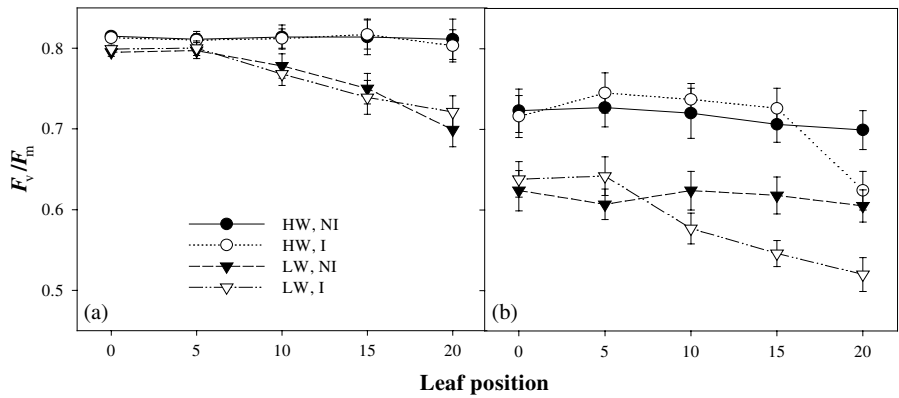


Fig. 6 Photochemical efficiency of photosystem II (PS II) (F_v/F_m) measured at predawn (a) and midday (b) on 13 August 1999 at five different leaf positions along the stem. Data are the mean ± SE

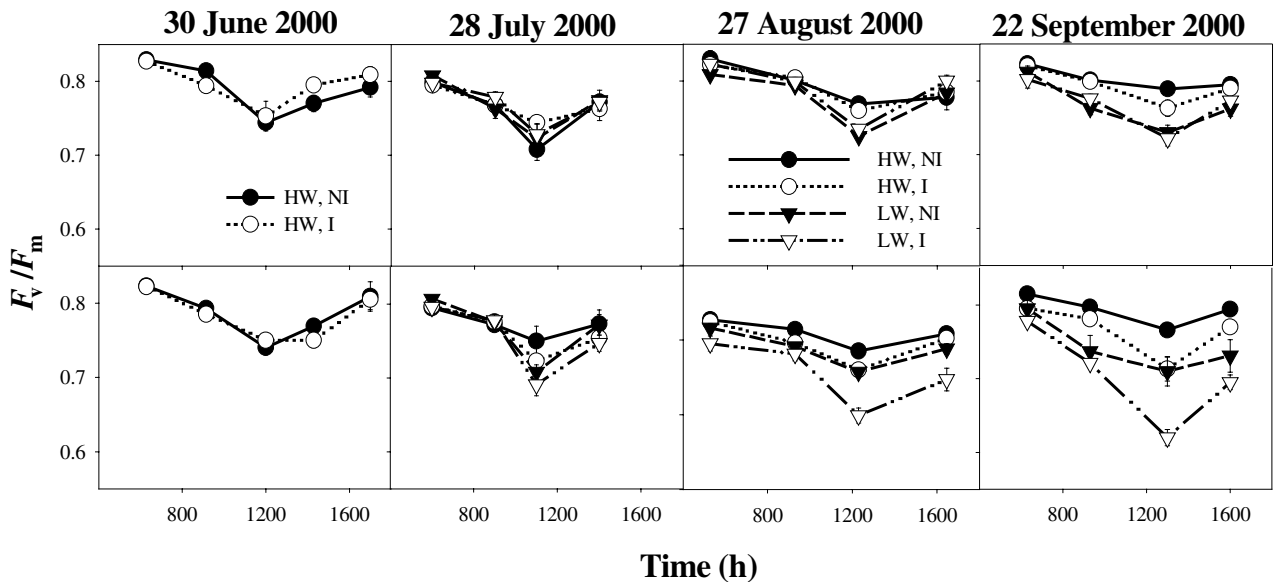


Fig. 7 Photochemical efficiency of photosystem II (PS II) (F_v/F_m) (leaf 0, top row; leaf 20, bottom row) measured at four sampling dates (columns) in 2000 experiment. Data are the mean ± SE

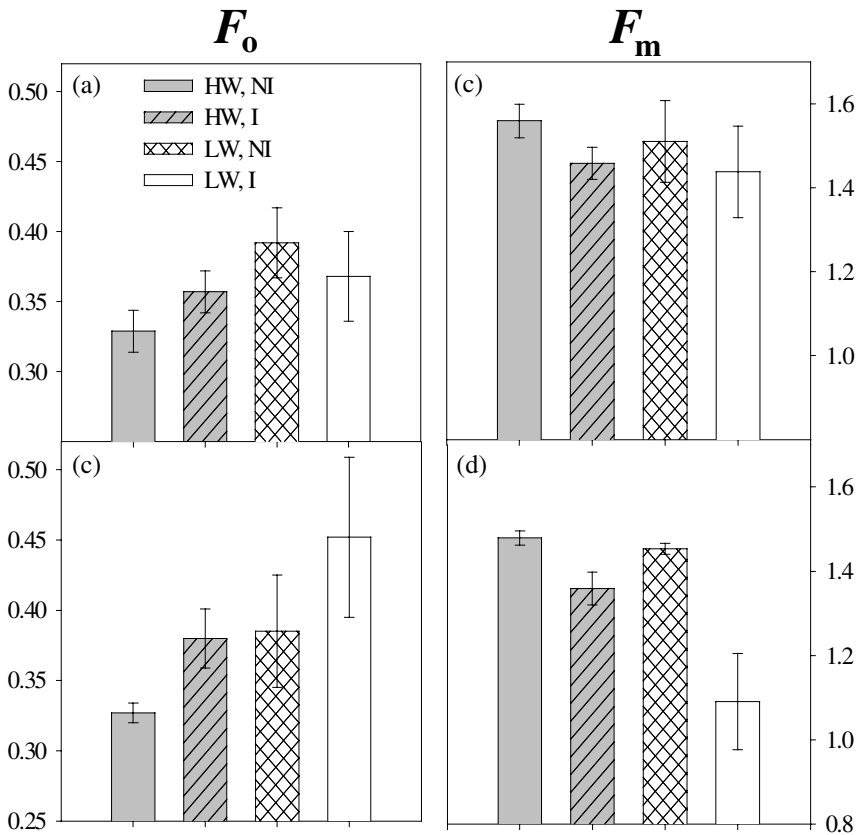


Fig. 8 Midday F_o (left) and F_m (right) (leaf 0, top row; leaf 20, bottom row) measurements on 22 September 2000. Data are the mean \pm SE

treatments ($P > 0.05$) (Fig 7). On 22 September, differences in F_v/F_m between the treatments were more pronounced. F_v/F_m was reduced by LW at all leaf positions throughout the entire day ($P < 0.05$) (Fig. 7), and was also reduced by I throughout the entire day at leaf 20 ($P = 0.04$). In order to distinguish irreversible photodamage from reversible photoprotection mechanisms, we analysed the F_o and F_m parameters at all 3-leaf positions for the 22 September measurements. F_o increased in LW plants ($P = 0.05$, combined effect of all 3-leaf positions) (Fig. 8a and c) throughout the entire day (predawn data not shown), but was not affected by *X. fastidiosa*-infection. Midday F_m was lower for I plants at leaf position 20 only ($P = 0.004$; $P > 0.05$ for leaves 0 and 15) (Fig. 8D), but was not significantly decreased by the LW treatment at any leaf position ($P > 0.05$) (Fig. 8B and D).

Leaf chemistry

Leaf percentage N was not significantly different between treatments at leaf 0 for the September sampling (Fig. 9a). However, both the I ($P = 0.030$) and LW ($P = 0.041$) treatments reduced leaf percentage N for leaf 20 (non-significant water \times infection, $P > 0.05$) (Fig. 9b).

Discussion

Our results support the hypotheses postulated in the introduction. Gas exchange parameters were affected by both *X. fastidiosa*-infection and water stress, and

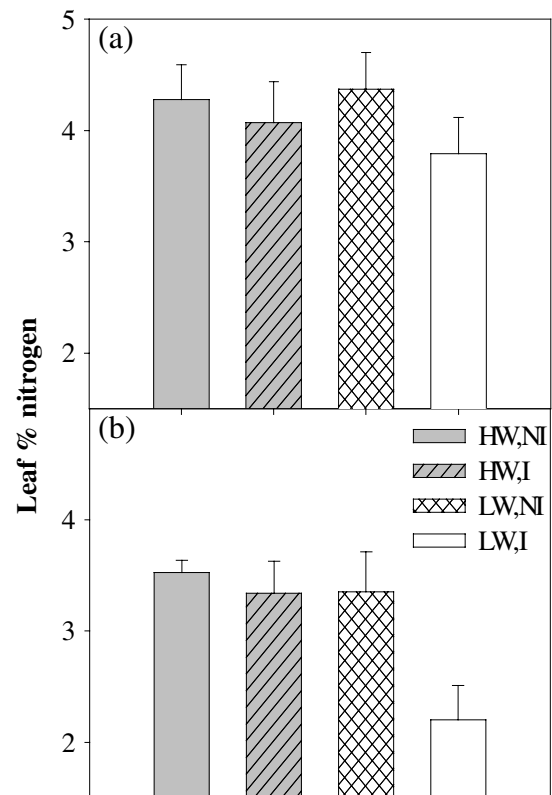


Fig. 9 Leaf percentage nitrogen for leaves from leaf position 0 (a) and leaf position 20 (b) collected on 24 September 2000. Data are the mean \pm SE

these factors acted additively in reducing gas exchange rates in basal, symptomatic leaves. Apical leaves were affected primarily by soil moisture treatments, and not by *X. fastidiosa*-infection.

Boyer (1995) proposed two mechanisms to explain how water stress increases the susceptibility of plants to attack by pathogens: (1) reduced photosynthate production induced by drought eliminates the plants' ability to defend against pathogens and/or (2) plant growth is reduced without reducing the pathogen's ability to reproduce, thus allowing further progression and increased symptom severity in the host (Boyer, 1995). Our results provide support for the operation of both of these mechanisms. Our previous work showed that water stress enhances symptom severity and progression along *X. fastidiosa*-infected *P. quinquefolia* shoots (McElrone et al., 2001). The results presented here show that water stress and bacterial infection both reduce photosynthate production, and when combined with reduced total shoot leaf area (McElrone et al., 2001), plants infected by *X. fastidiosa* and concurrently experiencing water stress produce less photosynthate for growth and defense.

As expected, our prolonged drought treatment significantly reduced leaf water potentials throughout the shoot of *P. quinquefolia*. In addition, infection by *X. fastidiosa* reduced Ψ_L of basally located leaves. Basal leaves on grapevines infected with a Pierce's disease strain of *X. fastidiosa* also had lower Ψ_L compared with healthy control plants at the same leaf positions (Goodwin et al., 1988a). Infection by *X. fastidiosa* typically induces an internal water stress by clogging xylem vessels and increasing hydraulic resistance in the xylem (Hopkins, 1989, 1995; McElrone et al., 2003). On severely scorched leaves from grapevines infected by Pierce's disease, nearly all of the vessels within petioles contained bacterial occlusions (Mircetich et al., 1976).

Photosynthetic rates in most terrestrial plants are sensitive to moderate drops in Ψ_L (Hsiao, 1973). In both years, net photosynthetic rates in *P. quinquefolia* were significantly reduced by water stress at all leaf positions and by *X. fastidiosa*-infection at basal leaf positions. Generally, initial reductions in photosynthesis induced by water stress are caused by stomatal closure (Hsiao, 1973; Chaves, 1991; Cornic, 2000). In our year 2000 results prior to September, g_s and C_i/C_a were significantly reduced by water stress at both apical and basal leaf positions and by *X. fastidiosa*-infection at the basal leaf position. Work on grapevines infected by Pierce's disease also found increased stomatal resistance to gas exchange and a reduction in net photosynthetic rates in basal leaves (Goodwin and Meredith, 1987; Goodwin et al., 1988b). Similarly, Bowden et al. (1990) were able to show that the initial decrease in photosynthesis of potato leaves infected by *Verticillium dahliae* (a vascular wilt fungus) was caused exclusively by stomatal closure. In contrast, potato infected by the combination of *Pratylenchus penetrans* (root lesion nematode) and *V. dahliae* exhib-

ited significant reductions in both CE and A_{max} , which indicated that non-stomatal effects were also involved in the reduction in photosynthetic rate (Saeed et al., 1999).

The response of *P. quinquefolia* to water stress was characterized by partial stomatal closure that maintained relatively high leaf water potentials until severe levels of water stress. This response was accompanied by little irreversible damage to the photosynthetic machinery until vascular disease symptoms and water stress were pronounced. *Parthenocissus quinquefolia* stomata are sensitive to changes in Ψ_L and VPD (Bell et al., 1988) and reduce g_s in order to prevent tissue desiccation. However, over a longer period of time, partial stomatal closure substantially reduces the photosynthate available for growth, reproduction and defense. Thus, this conservative water use pattern is subject to tradeoffs resulting in reduced growth rate and reproductive output during periods of drought (Bell et al., 1988).

Stomatal limitation of photosynthesis during midday periods of high temperature and high light also reduces the ability of leaves to dissipate incident solar radiation through photosynthesis (Long et al., 1994). Decreased utilization of incident radiation due to reduced photochemistry can lead to photoinhibition. Photoinhibition is the reduction of photosynthesis caused by excess light (Krause and Weis, 1991; Demmig-Adams and Adams, 1992; Balachandran and Osmond, 1994; Long et al., 1994; Thiele et al., 1996). Two main components of photoinhibition have been identified: (1) irreversible photodamage and (2) reversible photoprotection (Krause, 1988; Balachandran and Osmond, 1994; Thiele et al., 1996). F_v/F_m of dark-adapted leaves is commonly used as a measure of the photochemical efficiency of PS II, and changes in F_v/F_m are used to indicate photoinhibition (Balachandran and Osmond, 1994; Demmig-Adams and Adams, 1992; Krause and Weis, 1991). At all sampling dates in 2000, we found a diurnal repression in F_v/F_m , indicating inhibition of the photochemical efficiency of PS II (Damesin and Rambal, 1995). However, midday reductions in F_v/F_m recovered by predawn of the next day for most of the season. This suggests that *P. quinquefolia*, like many other plant species, utilizes photoprotective mechanisms to efficiently dissipate excess light as thermal radiation. However, under chronic, long-term stress, the photoprotective mechanisms became less effective, indicated by an increase in the magnitude of the diurnal depression in F_v/F_m as the season progressed, particularly at the basal leaf position. Thus, by the end of the season, photoinhibition was accentuated by prolonged drought and *X. fastidiosa*-infection. Damesin and Rambal (1995) found similar results in two *Quercus* species subjected to an extended drought, where the diurnal depression of F_v/F_m was larger as the season progressed.

When stomata close in response to high evaporative demands or low soil water supply, photochemical utilization of light energy is decreased by the decreased

supply of intercellular CO₂. This reduction in photochemistry lowers the amount of light needed to fix available CO₂, thus creating excess light energy under similar radiation levels (Krause, 1988; Krause and Weis, 1991; Demmig-Adams and Adams, 1992; Long et al., 1994). Plants have evolved several mechanisms to protect against excess light energy (Külheim et al., 2002). One documented mechanism is a feedback de-excitation that switches the photosynthetic antennae into a thermal dissipation mode, instead of an efficient solar energy absorption mode (Horton et al., 1996; Külheim et al., 2002). One part of this feedback de-excitation involves the xanthophyll cycle (Demmig-Adams and Adams, 1992; Demmig-Adams et al., 1995; Thiele et al., 1996). This cycle involves the conversion of violaxanthin to zeaxanthin, followed by the formation of a Chl-Zea complex and a conformational change in the thylakoid membrane that converts PS II into an energy dissipation mode (Müller et al., 2001). Increased zeaxanthin formation in leaves is concomitant with increased quenching of fluorescence parameters (specifically decreases in F_v/F_m) (Demmig-Adams and Adams, 1992; Demmig-Adams et al., 1995). The fluorescence patterns we measured in *P. quinquefolia* suggest that this species has a significant ability to photoprotect leaf photosynthetic machinery during prolonged stress. This protection only broke down late in the season under severe water stress in early senescing leaves. *Parthenocissus quinquefolia* has been shown to rapidly upgrade xanthophyll cycling to dissipate excess energy when grown in high light compared with plants grown in low light (Demmig-Adams et al., 1995), and we suspect that increased xanthophyll cycling acts as a photoprotection mechanism during extended water stress induced by drought and *X. fastidiosa*-infection.

More mature, basal leaves have been shown to have lower xanthophyll cycle activity than younger leaves (Thiele et al., 1996). This reduction could explain the more pronounced photodamage exhibited towards the base of *P. quinquefolia* plants. Thiele et al. (1996) found that irreversible photodamage in mature leaves was associated with inactivation of the D1 protein of PS II as photoprotection declined due to decreasing xanthophyll cycle activity. The irreversible photodamage component of photoinhibition has been characterized by increased F_o with decreased F_m and F_v/F_m (Krause, 1988; Balachandran and Osmond, 1994). This is the pattern of fluorescence parameters that we measured in basal leaves on the September 2000 sampling date, suggesting irreversible photodamage in these older leaves.

The fluorescence data on basal leaves is consistent with the AC_i response curve analysis for September, where A_{max} was reduced by both water stress and *X. fastidiosa*-infection, indicating a non-stomatal inhibition of photosynthesis. Non-stomatal inhibition of photosynthesis becomes more pronounced under severe water stress (Chaves, 1991; Tezara et al., 1999). Our AC_i response curve analyses detected little non-

stomatal inhibition of photosynthesis in apical leaves (leaf position 0) throughout the season (Bowden et al., 1990; Pennypacker et al., 1990; Saeed et al., 1999). However, A_{max} , a parameter measured at high CO₂ concentrations that eliminates stomatal limitations, was reduced by both water stress and *X. fastidiosa*-infection for the most basal leaf (leaf position 20) at the last sampling date in September 2000. A proposed mechanism for the reduction in A_{max} is that there was a disruption in the metabolic pathways of photosynthesis, such as a reduction in the biochemical capacity to regenerate RuBP (Farquhar and Sharkey, 1982; Saeed et al., 1999; Tezara et al., 1999).

We believe that the reduction in A_{max} in September was due to a combination of irreversible photoinhibition and the early onset of leaf senescence. Both water stress and *X. fastidiosa*-infection have been found to cause premature leaf senescence and leaf abscission (Hsiao, 1973). Treatments may have caused changes in maturity of leaves located at a similar position along the shoot (treatment-induced changes in phenology). With the onset of senescence, deciduous plants typically breakdown leaf components and export valuable nutrients that would otherwise be lost when leaves are abscised. This idea is supported by the reduction in leaf nitrogen content that we measured for basal leaves in September. Accelerated leaf senescence due to the combined effects of *X. fastidiosa*-infection and water stress may compromise the ability of the plant to avoid high light damage under partial stomatal closure. As photoprotective mechanisms break down, irreversible photodamage may be accelerated in these leaves, leading to even greater rates of leaf senescence.

In conclusion, water stress increases the severity of symptoms and the rate of spread of the pathogen *X. fastidiosa* in *P. quinquefolia*. Water stress compromises the carbon gain capacity of the plant, reducing its ability to outgrow the pathogen and produce effective defenses. In the high light, high temperature conditions of the summer growing season, initial stages of water stress are characterized by stomatal limitations to midday photosynthesis, accompanied by effective photoprotection mechanisms that prevent irreversible photodamage to the photosynthetic apparatus. However, long-term and/or severe water stress eventually outstrip photoprotective mechanisms leading to greater disease expression and accelerated leaf senescence, further compromising the ability of the plant to gain photosynthetic carbon for growth and reproduction.

Acknowledgements

The authors thank D. Hopkins (University of Florida, Leesburg) for supplying us with *X. fastidiosa* cultures; M. Pooler (U.S. National Arboretum) for providing access to her laboratory for PCR analyses of plant samples; J. Bentz (USDA-ARS, Beltsville, MD) for providing laboratory and greenhouse space in early stages of the research; I. Castellanos and P. Baldauf for technical assistance; the Behavior, Ecology, Evolution and Systematics summer fellowship program at University of Maryland for summer funding

for 2000 and 2001; and P. Barbosa, A. Grybauskas, J. Sherald, and J. Sullivan for critical reviews and helpful comments to improve this manuscript.

References

- Balachandran, S., B. Osmond (1994): Susceptibility of tobacco leaves to photoinhibition following infection with two strains of Tobacco Mosaic Virus under different light and nitrogen nutrition regimes. *Plant. Physiol.* **104**, 1051–1057.
- Bell, D. J., I. N. Forseth, A. H. Teramura (1988): Field water relations of three temperate vines. *Oecologia* **74**, 537–545.
- Bhattacharyya, A., S. Stilwagen, N. Ivanova, M. D'Souza, A. Bernal, A. Lykidis, V. Kapatral, L. Anderson, N. Larsen, T. Los, G. Reznik, E. Selkov, T. L. Walunas, H. Feil, W. S. Feil, A. Purcell, J. L. Lassez, T. L. Hawkins, R. Haselkorn, R. Overbeek, P. F. Predki, N. C. Kyrpides (2002): Whole-genome comparative analysis three phytopathogenic *Xylella fastidiosa* strains. *Proc Natl Acad. Sci. U S A* **99**, 12403–12408.
- Blanchard, R. O., T. A. Tattar (1987): *Field and Laboratory Guide to Tree Pathology*. Academic Press, New York, NY.
- Bowden, R. L., D. I. Rouse, T. D. Sharkey (1990): Mechanism of photosynthesis decrease by *Verticillium dahliae* in potato. *Plant. Physiol.* **94**, 1048–1055.
- Boyer, J. S. (1995): Biochemical and biophysical aspects of water deficits and the predisposition to disease. *Ann. Rev. Phytopathol.* **33**, 251–274.
- Chaves, M. M. (1991): Effects of water deficit on carbon assimilation. *J. Exp. Botany* **42**, 1–16.
- Cornic, G. (2000): Drought stress inhibits photosynthesis by decreasing stomatal aperture – not by affecting ATP synthesis. *Trends Plant Sci.* **5**, 187–188.
- Daley, P. F. (1995): Chlorophyll fluorescence analysis and imaging in plant stress and disease. *Can. J. Plant Pathol.* **17**, 167–173.
- Damesin, C., S. Rambal (1995): Field study of photosynthetic performance by a Mediterranean deciduous oak tree during a severe summer drought. *New Phytologist* **131**, 159–167.
- Demmig-Adams, B., W. W. Adams (1992): Photoprotection and other responses of plants to high light stress. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **43**, 599–626.
- Demmig-Adams, B., W. W. Adams, B. A. Logan, A. S. Verhoeven (1995): Xanthophyll cycle dependent energy dissipation and flexible photosystem II efficiency in plants acclimated to light stress. *Aust. Journal Plant Physiol.* **22**, 249–260.
- Farquhar, G. D., T. D. Sharkey (1982) Stomatal conductance and photosynthesis. *Ann. Rev. Plant Physiol.* **33**, 317–345.
- Gleason, H. A., A. Cronquist (1991): *Manual of Vascular Plants of Northeastern United States and Adjacent Canada*. The New York Botanical Garden, Bronx, NY.
- Goodwin, P. H., C. P. Meredith (1987) New clues in understanding Pierce's disease. *California Agricultural Exp. Station* **42**, 6–7.
- Goodwin, P. H., J. E. DeVay, C.P. Meredith (1988a): Roles of water stress and phytotoxins in the development of Pierce's disease of the grapevine. *Physiol. Mol. Plant Pathol.* **32**, 1–15.
- Goodwin, P. H., J. E. DeVay, C. P. Meredith (1988b): Physiological responses of *Vitis vinifera* cv. 'Chardonnay' to infection by the Pierce's disease bacterium. *Physiol. Mol. Plant Pathol.* **32**, 17–32.
- Hearon, S. S., J. L. Sherald, S. J. Kostka (1980): Association of xylem-limited bacteria with elm, sycamore, and oak leaf scorch. *Can. J. Botany* **58**, 1986–1993.
- Hopkins, D. L. (1989): *Xylella fastidiosa*: xylem-limited bacterial pathogens of plants. *Ann. Rev. Phytopathol.* **27**, 271–290.
- Hopkins, D. L. (1995): *Xylella fastidiosa*. In: Singh, U. S., R. P. Singh and K. Kohmoto (eds), *Pathogenesis and Host Specificity in Plant Diseases: Histopathological, Biochemical, Genetic and Molecular Bases*. Vol. 1: Prokaryotes. Elsevier Science, Inc., New York.
- Hopkins, D. L., W. C. Adlerz (1988): Natural hosts of *Xylella fastidiosa* in Florida. *Plant Dis.* **72**, 429–431.
- Horton, P., A. V. Ruban, R. G. Walters (1996): Regulation of light harvesting in green plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 655–684.
- Hsiao, T. C. (1973): Plant responses to water stress. *Ann. Rev. Plant Physiol.* **24**, 519–570.
- Kapatral, L. Anderson, N. Larsen, T. Los, G. Reznik, E. Selkov, T. L. Walunas, H. Feil, W. S. Feil, A. Purcell, J. L. Lassez, T. L. Hawkins, R. Haselkorn, R. Overbeek, P. F. Predki, N. C. Kyrpides. (2002): Whole-genome comparative analysis of three phytopathogenic *Xylella fastidiosa* strains. *Proc. Nat. Acad. Sci.* **99**(19), 12403–12408.
- Krause, G. H. (1988): Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. *Physiologia Plantarum* **74**, 566–574.
- Krause, G. H., E. Weis (1991): Chlorophyll fluorescence and photosynthesis: the basics. *Ann. Rev. Plant Physiol. Mol. Biol.* **42**, 313–349.
- Külheim, C., J. Ågren, S. Jansson (2002): Rapid regulation of light harvesting and plant fitness in the field. *Science* **297**, 91–93.
- Long, S. P., S. Humphries, P. G. Falkowski (1994): Photoinhibition of photosynthesis in nature. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **45**, 633–662.
- McElrone, A. J., J. L. Sherald, M. R. Pooler (1999): Identification of alternative hosts of *Xylella fastidiosa* in the Washington, DC area using a nested polymerase chain reaction (PCR). *J. Arboriculture* **25**, 258–263.
- McElrone, A. J., J. L. Sherald, I. N. Forseth (2001): Effects of water stress on symptomatology and growth of *Parthenocissus quinquefolia* infected by *Xylella fastidiosa*. *Plant Dis.* **85**, 1160–1164.
- McElrone, A. J., J. L. Sherald, I. N. Forseth (2003): Interactive effects of water stress and xylem-limited bacterial infection on the water relations of a host vine. *J. Exp. Botany* **54**, 419–430.
- Mircetich, S. M., S. K. Lowe, W. J. Moller, G. Nyland (1976): Etiology of the almond leaf scorch disease and transmission of the causal agent. *Phytopathology* **66**, 17–24.
- Müller, P., X.-P. Li, K. K. Niyogi (2001): Non-photochemical quenching. A response to excess light energy. *Plant. Physiol.* **125**, 1558–1566.
- Peek, M. S., E. Russek-Cohen, D. A. Wait, I. N. Forseth (2002): Physiological response curve analysis using nonlinear mixed models. *Oecologia* **132**, 175–180.
- Pennypacker, B. W., D. P. Knievel, K. T. Leath, E. J. Pell, R. R. Hill Jr (1990): Analysis of photosynthesis in resistant and susceptible alfalfa clones infected with *Verticillium albo-atrum*. *Phytopathology* **80**, 1300–1306.
- Pooler, M. R., I. S. Myung, J. Bentz, J. Sherald, J. S. Hartung (1997): Detection of *Xylella fastidiosa* in potential insect vectors by immunomagnetic separation and nested polymerase chain reaction. *Lett. Appl. Microbiol.* **25**, 123–126.
- Powles, S. B. (1984): Photoinhibition of photosynthesis induced by visible light. *Ann. Rev. Plant Physiol.* **35**, 15–44.
- Raju, B. C., J. M. Wells (1986): Diseases caused by fastidious xylem-limited bacteria and strategies for management. *Plant Dis.* **70**, 182–186.
- Saeed, I. A. M., A. E. MacGuidwin, D. I. Rouse, T. D. Sharkey (1999): Limitation to photosynthesis in *Pratylenchus penetrans* and *Verticillium dahliae*-infected potato. *Crop Sci.* **39**, 1340–1346.
- Sherald, J. L. 1993. Pathogenicity of *Xylella fastidiosa* in American elm and failure of reciprocal transmission between strains from elm and sycamore. *Plant Dis.* **77**, 190–193.
- Sherald, J. L., S. J. Kostka (1992): Bacterial leaf scorch of landscape trees caused by *Xylella fastidiosa*. *J. Arboriculture* **18**, 57–63.
- Sherald, J. L., S. S. Hearon, S. J. Kostka, D. L. Morgan (1983): Sycamore leaf scorch: culture and pathogenicity of fastidious xylem-limited bacteria from scorch-affected trees. *Plant Dis.* **67**, 849–852.
- da Silva, F. R., A. L. Vettore, E. L. Kemper, A. Leite, P. Arruda (2001): Fastidious gum: the *Xylella fastidiosa* exopolysaccharide possibly involved in bacterial pathogenicity. *FEMS Microbiol. Lett.* **203**, 165–171.
- Simpson, A. J., F. C. Reinach, P. Arruda et al. (2000): The genome sequence of the plant pathogen *Xylella fastidiosa*. *Nature* **406**, 151–157.

- Tezara, W., V. J. Mitchell, S. D. Driscoll, D. W. Lawlor (1999): Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature* **401**, 914–917.
- Thiele, A., K. Schirwitz, K. Winter, G. H. Krause (1996): Increased xanthophyll cycle activity and reduced D1 protein inactivation related to photoinhibition in two plant systems acclimated to excess light. *Plant Sci.* **115**, 237–250.
- Wells, J. M., B. C. Raju, H. Y. Hung, W. G. Weisburg, L. Mandelco-Paul, D. J. Brenner (1987): *Xylella fastidiosa*: gram-negative, xylem-limited, fastidious plant bacteria related to *Xanthomonas*. *Int. J. Syst. Bacteriol.* **37**, 136–143.