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The effects of Pierce's disease on leaf and petiole hydraulic conductance in *Vitis vinifera* cv. Chardonnay

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In this study, we test the hypothesis that the symptoms of Pierce's Disease (PD) result from the occlusion of xylem conduits by the bacteria *Xylella fastidiosa* (*Xf*). Four treatments were imposed on greenhouse-grown *Vitis vinifera* cv. Chardonnay: well-watered and deficit-irrigated plants with and without petiole inoculation with *Xf*. The hydraulic conductance of the stem-petiole junction (k_{jun}) and leaves (k_{leaf}) were measured, and *Xf* concentrations were established by quantitative polymerase chain reaction (qPCR). Leaf hydraulic conductance decreased with increasing leaf scorch symptoms in both irrigation treatments. The positive relationship between *Xf* concentration and symptom formation in deficit-irrigated plants suggests that water-stress increases susceptibility to PD. In field-grown vines, water relations of symptomatic leaves were similar to naturally senescing leaves but differed from green control leaves. Overall, these results suggest that the development of PD symptoms represents a form of accelerated senescence as part of a systemic response of the plant to *Xf* infection.

Introduction

Pierce's disease (PD) is a grapevine disease caused by the xylem limited bacteria *Xylella fastidiosa* (*Xf*). The visual symptoms of PD include marginal leaf scorch and uneven periderm maturation; for most cultivars of *Vitis vinifera*, PD is reported to cause plant death, two to three seasons after the first symptoms appear (Varela et al. 2001). The work of Goodwin et al. (1988a, 1988b) demonstrated that PD was associated with reductions in petiole hydraulic conductance, stomatal conductance, photosynthesis, and leaf water potential (Ψ_L) in symptomatic leaves relative to healthy leaves growing under the same environmental conditions. These results, combined with observations that the number of xylem vessels colonized is higher in symptomatic than non-symptomatic leaves (Hopkins 1989, Newman et al. 2003) has lead to a general consensus that the symptoms of PD result from occlusion of xylem conduits by *Xf* bacteria or associated gels and tyloses that impose water deficits on leaves distal to the blockage (Committee on Californian Agricultural Research Priorities 2004).

However, the low overall level of xylem vessel occlusion observed in the above studies, e.g. 2–4% in (Newman et al. 2003), along with the absence of a correlation between *Xf* concentrations and leaf scorch symptoms (Gambetta et al. 2007), suggests that *Xf* populations alone are insufficient to induce water-stress. Furthermore, Thorne et al. (2006) demonstrated that there are qualitative and quantitative differences between visual symptoms resulting from experimentally imposed water deficits and PD in greenhouse-grown Chardonnay. Indeed, in these experiments

Abbreviations – PCR, polymerase chain reaction; PD, Pierce's disease; qPCR, quantitative polymerase chain reaction; *Xf*, *Xylella fastidiosa*.

stomatal conductance and leaf water potential were not reduced in infected plants. These observations raise questions as to the basic mode of action by which PD kills grapevines.

Previous measurements relating to PD infection have not incorporated possible changes in the hydraulic conductance of the leaf (k_{Leaf}), which has been shown to contribute at least 30% of whole plant hydraulic resistance (Sack and Holbrook 2006) and is a site in which *Xf* can be prolific. Previous studies have also indicated that gel and tylose formation associated with *Xf* infection are initially restricted to the leaves and petioles (Stevenson et al. 2004). Therefore, evaluating the extent to which the hydraulic capacity of the leaf vasculature is impaired by PD is an important step in clarifying the role of changes in hydraulic conductance in symptom formation. In this study, the relationships between watering regime, bacterial population growth, hydraulic capacity of leaves and symptom formation (leaf scorch) were examined in *Vitis vinifera* cv. Chardonnay.

Materials and methods

Plant material and growth conditions

Own-rooted *Vitis vinifera* (L. Chardonnay) plants were grown from dormancy in 4 l pots filled with 1/3 peat, 1/3 sand, 1/3 redwood compost, with 2.4 kg m^{-2} dolomite lime in a greenhouse (30/20°C ±3°C; 40/70% ± 10% relative humidity (RH); and natural light with a daily maximum of 1200 µmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR)). The vines were pruned to two shoots, and the shoots were vertically trained to approximately 1.5 m. Pots were drip irrigated with dilute nutrient solution (90 ppm calcium, 24 ppm magnesium, 124 ppm potassium, 6 ppm nitrogen as NH_4, 96 ppm nitrogen as NO_3, 26 ppm phosphate, 16 ppm sulfate, 1.6 ppm Iron, 0.27 ppm manganese, 0.16 ppm copper, 0.12 ppm zinc, 0.26 ppm boron, and 0.016 ppm molybdenum at pH 5.5–6.0).

Grapevines were grown from dormancy under well-water conditions for 70 days and then divided into two watering treatments: well-watered (WW; 800 ml per day), and deficit-irrigated (DI; 400 ml per day). For each watering treatment, half of the vines were inoculated with *Xf* and half were dummy inoculated with media.

Experiments utilized a *Xf* (Temecula) strain engineered to express green fluorescent protein (Newman et al. 2003). *Xf* was cultured at 29°C on solid periwinkle wilt (PW) media (Davis et al. 1981) supplemented with 20 µg ml^{-1} kanamycin until colonies became visible. The *Xf* was harvested by washing the plate with PW solidified with gellan gum (PWG) media (Hill and Purcell 1995) supplemented with 20 µg ml^{-1} kanamycin, centrifuged, and the concentration adjusted to approximately 4 × 108 CFU ml^{-1} (Minsavage et al. 1994). For the inoculation of greenhouse-grown grapevines *Xf* was used immediately. Inoculations were carried out by placing a 20 µl drop of the *Xf* solution or media described above at the base of the petiole and piercing the petiole through the drop with a sterile hypodermic needle. One petiole was inoculated on each cane at midday. The 20 µl droplets were readily taken up by the plants within seconds.

Hydraulic measurements

Hydraulic measurements commenced 70 days after inoculation. For each cane, transpiration rate (E, mmol m^{-2} s^{-1}) and stomatal conductance (g_s, mmol m^{-2} s^{-1}) were measured on each leaf using a steady state porometer (Li-1600, Licor, Lincoln, NE) from 10:00 and 10:30 in the morning. The Ψ_L of three leaves on each cane was measured using a pressure chamber (Soilmoisture Equipment Corp., Santa Barbra, CA). Canes were then cut underwater at the base and transported to the laboratory with their cut end submerged in water. Once back in the laboratory, canes were cut into pieces for hydraulic measurements. For each cane, three leaves were selected for leaf hydraulic conductance (k_{leaf}, mmol m^{-2} MPa^{-1} s^{-1}) and hydraulic conductance of the stem-petiole junction (k_{jun}, mmol m^{-2} MPa^{-1} s^{-1}) measurements. Cane segments were cut underwater 5 cm below and 2 cm above the node. The cut ends of each segment were shaved with a fresh scalpel blade and the petiole was cut midway along its length with a razor blade. The cut petiole ends were wrapped with parafilm and connected to a flow meter. Measurements of k_{leaf} were made with the leaf submerged in water to prevent evaporation. The petiole was connected to flexible tubing and a graduated 1 ml glass pipette filled with filtered, degassed 10 m*M* KCl perfusing solution. The volume flow rate (F, mmol s^{-1}) into the leaf was measured by following the progress of a meniscus along the pipette after a pressure difference (ΔP, MPa) of 0.15 MPa was applied from a pressurized gas source. The flow rate usually stabilized after 10–15 min of perfusion at a constant pressure. The flow rate was then averaged over a 3 min period for calculation of k_{leaf}. After each measurement, the surface area of the leaf (A_L, m^2) was measured using a digital camera (Power Shot S2IS, Canon, Tokyo, Japan) and imageJ software (free-ware available from http://rsb.info.nih.gov/ ij/). The leaf hydraulic conductance was calculated as $k_{leaf} = F/(\Delta P A_L)$. Because these measurements were made at a pressure of 0.15 MPa it is assumed that the measured k_{leaf} represents the maximum hydraulic

conductance with reference to blockage by embolism (see section on Discussion).

The petiole-stem junction conductance was measured from the petiole cut surface through the junction and out of the stem cut end. The reverse flow path was used because perfusate leaked from the axial bud in large quantities when flow was driven in the normal direction. The cut surface of the stem above the node was sealed with cyanoacrylic glue (Loctite superbond 409). A pressure of 0.15 MPa was applied to a reservoir of perfusing solution to drive flow through the junction. The flow through the junction was measured by the rate at which solution moved onto an electronic balance (Sartorius CP225D, Goettingen, Germany); the data were collected from the balance by WinWedge software (TALtech, Philadelphia, PA) and transferred into an Excel spreadsheet where flow rate was calculated and graphed. For some junctions, a vacuum pump was used to draw solution off the balance by applying sub-atmospheric pressure at the cut petiole end such that solution moved in the normal direction of flow. The hydraulic conductance values calculated with either method were very similar, although usually slightly higher when positive pressure was used. After flow rate had stabilized, hydraulic conductance of the junction was calculated as $k_{jun} = F/(\Delta P A_L)$, with the A_L taken from the leaf distal to that junction. A total of four plants were harvested from each treatment, with two canes per plants and three leaves per cane.

Bacterial population and leaf symptoms

Leaves and petioles used in hydraulic measurements were stored in sealed plastic bags and frozen in a −20°C freezer. The population of *Xf* in each leaf was established by quantitative polymerase chain reaction (qPCR) as described by Gambetta et al. (2007). Briefly, using the primer probe pair described by Gambetta et al. (2007), the amplification was performed in a total reaction volume of 12 µl. Reactions included 4 µl of template DNA, 6 µl of TaqMan Universal PCR Master Mix (2×), 1 µ*M* forward primer *EFTu*_for-1, 1 µM reverse primer *EFTu*_rev-1, 250 n*M* *EFTu*-1 probe, and sterile molecular biology grade water to a total volume of 12 µl. All PCR reactions were performed in 96 well plates and the exact reaction cycling conditions were as follows: 95°C for 10 min, 40 cycles of 95°C for 10 s and 60°C for 1 min. Amplification and data analysis were carried out on a ABI PRISM 7700 sequence detector system (Applied Biosystems). Negative controls containing either no template DNA, or plant leaf DNA were subjected to the same procedure. Each sample was run in triplicate.

The image collected for each leaf to measure A_L was also used to assess visual symptoms of Pierce's disease. The proportion of leaf scorch relative to green area was measured using imageJ software. This was then expressed on a 'leaf state' scale from 0 to 5 with 0 indicating no scorch and 5 indicating no green area.

Impacts of PD and senescence on leaf tissue water relations in field vines

A total of 36 leaves (12 for each treatment) were collected at midday on 4 and 22 October 2007, from field-grown Chardonnay vines located on a commercial vineyard (Beringer Vineyards, Yountville, CA, USA). PD plants were identified that exhibited all of the hallmarks of *Xf* infection and were confirmed *Xf* positive by PCR. Control vines did not exhibit PD symptoms and were negative when assayed for *Xf* by PCR on multiple occasions. Leaf water (Ψ_L) and solute potentials (Ψ_S) were determined in leaf punches (d = 15 mm) by isopiestic thermocouple psychrometry (Schultz and Matthews 1993, Boyer 1995). Leaf punches were taken and immediately placed at the bottom of a psychrometer cup that was coated with melted and resolidified petrolatum, and transferred to the laboratory to measure the water status of leaf tissue. Turgor pressure (Ψ_P) was calculated by subtracting osmotic potential from water potential without correction of apoplastic water dilution. The individual leaves, punches and data are presented in Appendix S1, Supporting Information.

Results

Impact of watering regime and *Xf* inoculation

The k_{leaf} and k_{jun} of deficit-irrigated plants was 50–60% lower than in well-watered plants, but were not different between infected and non-infected plants (Fig. 1A). This was reflected in significantly lower Ψ_L in deficit-irrigated than in well-watered plants (Fig. 1B). Although there were corresponding decreases in *E* across irrigation treatments, the differences in *E* were small relative to decreases in k_{leaf} and k_{jun} (Fig. 1A).

Within infected plants, average populations of *Xf* were approximately 10^4–10^5 greater in petiole inoculated leaves than in the non-inoculated leaves on the same plant (Fig. 2). When measurements were separated between inoculated and non-inoculated leaves for each treatment, there was a 65% decrease in k_{leaf} in inoculated leaves (Fig. 3A). This difference in k_{leaf} was not present between dummy inoculated leaves and non-inoculated leaves on control plants. There was a difference in k_{jun} between inoculated and non-inoculated leaves on

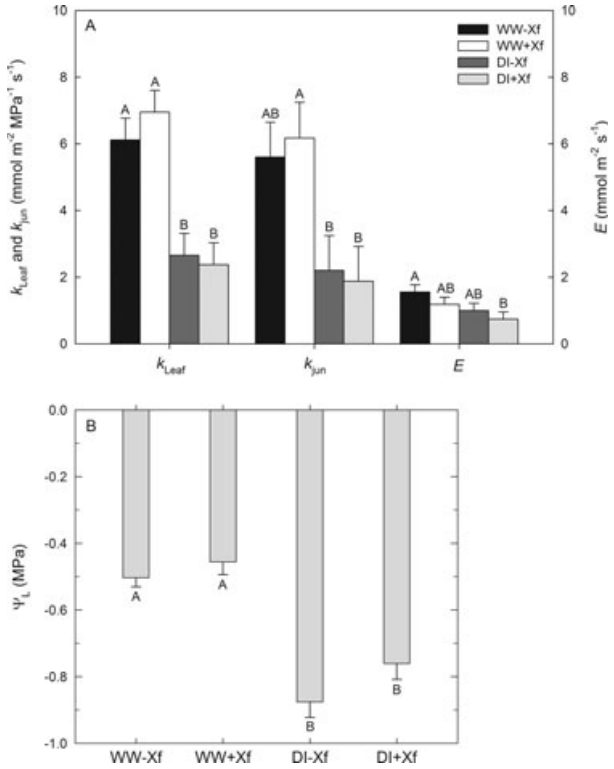

Fig. 1. (A) Leaf hydraulic conductance (k_{leaf}), stem-petiole junctions hydraulic conductance (k_{jun}), transpiration rates (E) and (B) leaf water potential (Ψ_L) for leaves of *Vitis vinifera* cv. Chardonnay exposed to four treatments. Well-watered and infected with *Xylella fastidiosa* (WW + *Xf*), well-watered controls (WW − *Xf*), deficit-irrigated and infected with *X. fastidiosa* (DI + *Xf*), deficit-irrigated controls (DI − *Xf*). Values are means with bars showing SE. For each parameter, different letters indicate a significant difference ($P < 0.05$) between means (Tukey's HSD).

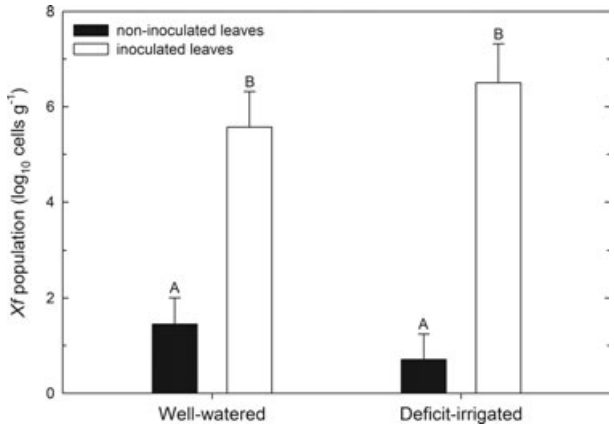

Fig. 2. Populations of *Xylella fastidiosa* in inoculated and non-inoculated leaves of *Vitis vinifera* cv. Chardonnay. All leaves are from plants on which one leaf per cane was petiole inoculated. Within each treatment, different letters indicate a significant difference ($P < 0.05$) between means (*t*-test).

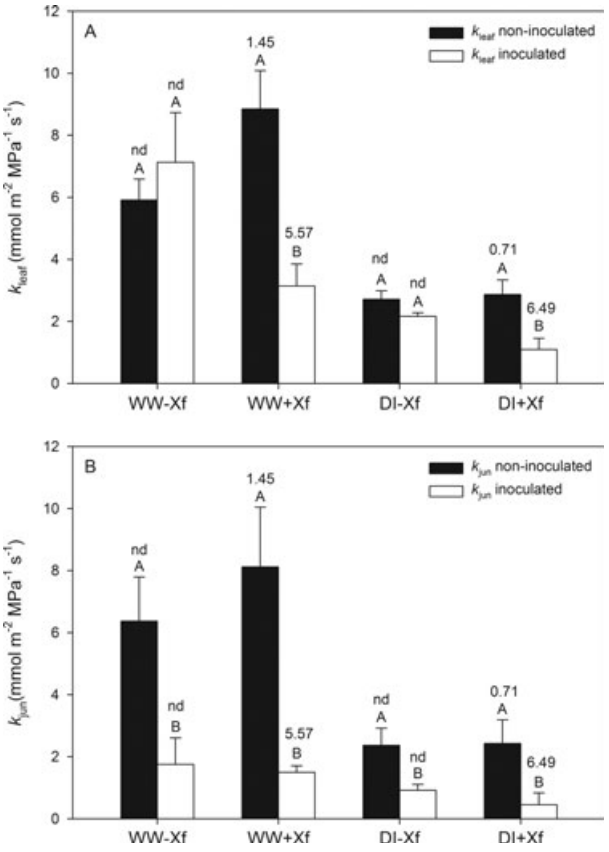

Fig. 3. (A) Leaf hydraulic conductance (k_{leaf}) and (B) stem-petiole junction hydraulic conductance (k_{jun}), for leaves of *Vitis vinifera* cv. chardonnay inoculated with *Xylella fastidiosa* and non-inoculated leaves under four treatments. Well-watered and infected (WW + *Xf*), well-watered controls (WW − *Xf*), deficit-irrigated and infected with *X. fastidiosa* (DI + *Xf*), deficit-irrigated controls (DI − *Xf*). Values are means with bars showing SE. Within each treatment, different letters indicate a significant difference ($P < 0.05$) between means (*t*-test). The numbers above each bar indicate the average population of *Xf* (log$_{10}$ cells g^{-1}) detected in leaves (nd = not detected).

infected plants; however, this difference was also present between dummy inoculated and non-inoculated leaves on control plants (Fig. 3B). This could be explained by the shoot position of inoculated leaves, which were located between node 8 and node 14 counting from the base of the cane. Because k_{jun} decreased basipetally, the population of inoculated leaves was biased toward lower k_{jun} (Fig. 4C, D). Leaf position and k_{leaf} were independent of one another (Fig. 4A, B).

Relationships between hydraulics, *Xf* population and symptom development

When linear relationships between variables were analyzed, there was a significant negative relationship between k_{leaf} and *Xf* population ($r^2 = 0.65$, $P < 0.001$)

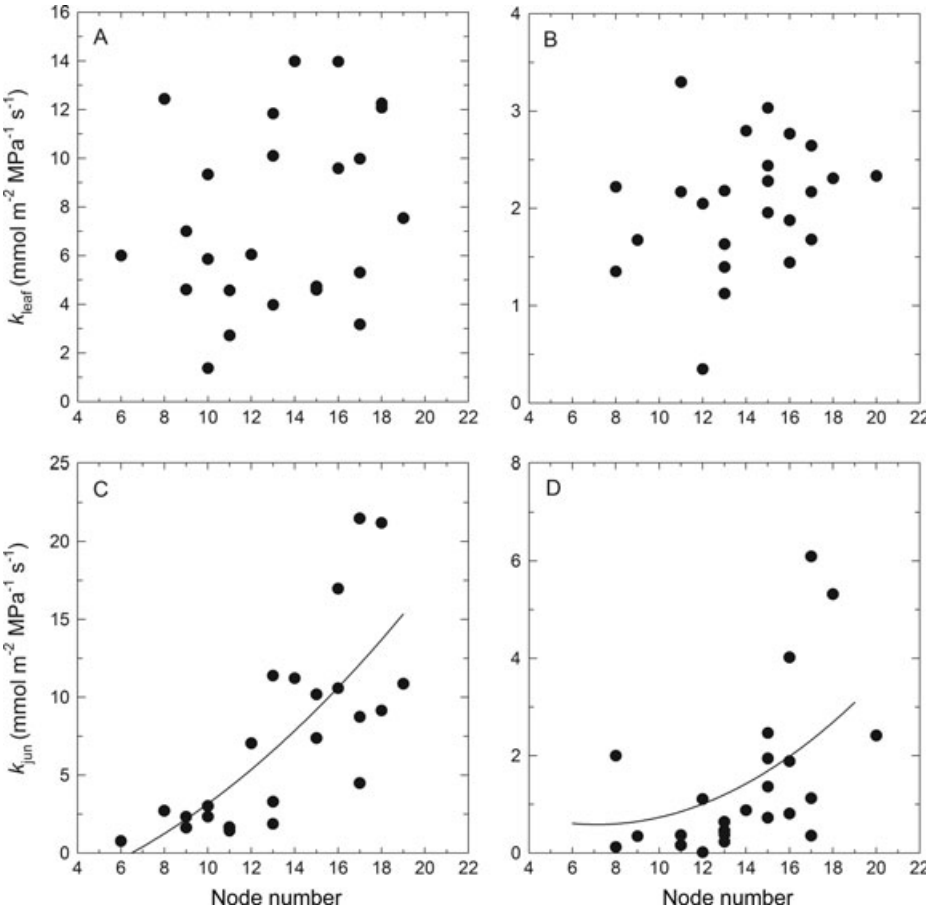

Fig. 4. Leaf hydraulic conductance (k_{leaf}) and stem-petiole junction hydraulic conductance (k_{jun}) as a function of node number counted from base to apex for leaves of *Vitis vinifera* cv. Chardonnay subjected to well-watered and deficit-irrigated treatments. (A) k_{leaf} for well-watered plants (B) k_{leaf} for deficit-irrigated plants (C) k_{jun} for well-watered plants, and (D) k_{jun} for deficit-irrigated plants.

and between k_{jun} and *Xf* population ($r^2 = 0.24$, $P = 0.046$) in deficit-irrigated plants but not in well-watered plants (Fig. 5A–D). There was a significant positive relationship between *Xf* population and lesion area ($r^2 = 0.54$, $P < 0.001$) for deficit-irrigated plants but not in well-watered plants (Fig. 5E, F). For Fig. 5E, data were re-analyzed with one data point removed in which a high lesion area was observed despite the absence of detectable *Xf* populations. With this point removed, there was a weak positive trend but no significant relationship between the two variables ($r^2 = 0.21$, $P = 0.07$). There was a significant negative relationship between k_{leaf} and lesion area for well-watered ($r^2 = 0.41$, $P = 0.005$) and deficit-irrigated ($r^2 = 46$, $P = 0.003$) treatments (Fig. 6A, B). When leaf hydraulic conductance was expressed on a green leaf area basis (k_{leafGA}), it was independent of lesion area for well-watered plants ($r^2 = 0.08$, $P = 0.26$) (Fig. 6C). In deficit-irrigated plants, there was a positive relationship between lesion area and k_{leafGA} ($r^2 = 0.60$, $P = 0.0002$) but this was largely a function of one leaf with less than 2% green leaf area (Fig. 6D). When this point was removed from the analysis the variables were independent of one another ($r^2 = 0.03$, $P = 0.49$).

Impacts of PD and senescence on leaf tissue water relations in field vines

In field-grown vines, mean psychrometrically determined Ψ_L was significantly lower, and Ψ_S was significantly higher, in PD symptomatic leaves compared to control leaves, with senescent leaves being intermediate (Table 1, see Appendix S1 in Supporting Information). There was a positive correlation between the fraction of visibly chlorotic (yellow) sampled area and both Ψ_L

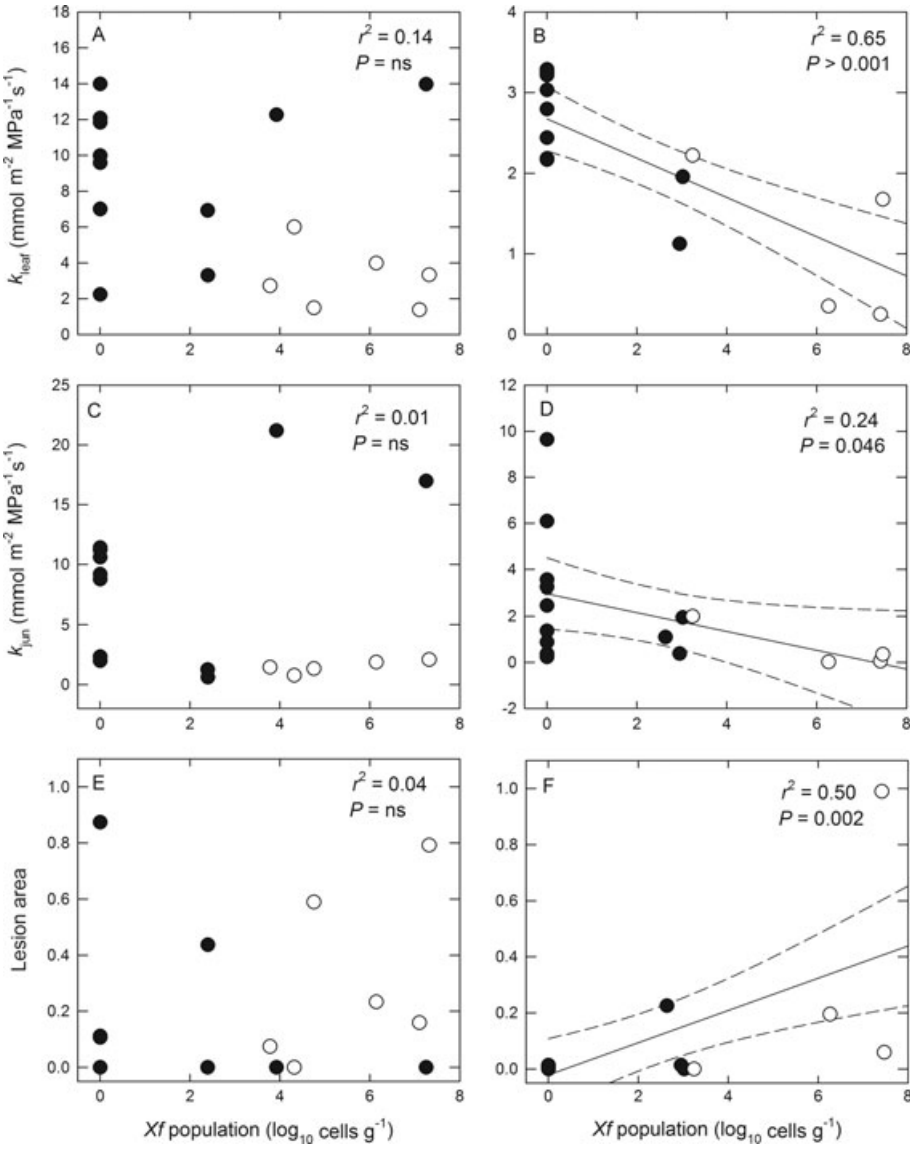

Fig. 5. Relationships between *Xylella fastidiosa* populations and leaf hydraulic conductance (k_{leaf}), stem-petiole junction hydraulic conductance (k_{jun}), and leaf lesion area in leaves of *Vitis vinifera* cv. chardonnay. (A) *Xf* population vs. k_{leaf} in well-watered plants, (B) *Xf* population vs. k_{leaf} in deficit-irrigated plants, (C) *Xf* population vs. k_{jun} in well-watered plants (D) *Xf* population vs. k_{jun} in deficit-irrigated plants (E) *Xf* population vs. lesion area in well-watered plants, and (F) *Xf* population vs. lesion area in deficit-irrigated plants. Hollow symbols represent leaves inoculated directly with *Xf* and filled symbols are non-inoculated leaves on infected plants. Lines are linear regressions with 95% confidence intervals (dotted lines).

and Ψ_S, with an analysis of covariance indicating a significant effect of chlorotic area as a covariate for Ψ_L and Ψ_S, respectively (analysis not shown). When adjusted to an average level of chlorotic area (average = 16%, range 0–100%), the adjusted (least squares) means had the same ranking as the unadjusted means, but showed no significant difference between PD symptomatic and senescent leaves for both Ψ_L and Ψ_S (Table 1). In both statistical approaches, Ψ_P was significantly lower ($P < 0.05$) in symptomatic leaves than in senescent leaves and lower in senescent leaves than in control leaves.

Discussion

To our knowledge, this is the first study in which quantitative estimates of *Xf* populations have been related to changes in the hydraulic capacity of leaves. Leaves are significant bottlenecks in the hydraulic pathway (Sack and Holbrook 2006) and the principal site of bacterial proliferation and symptom formation

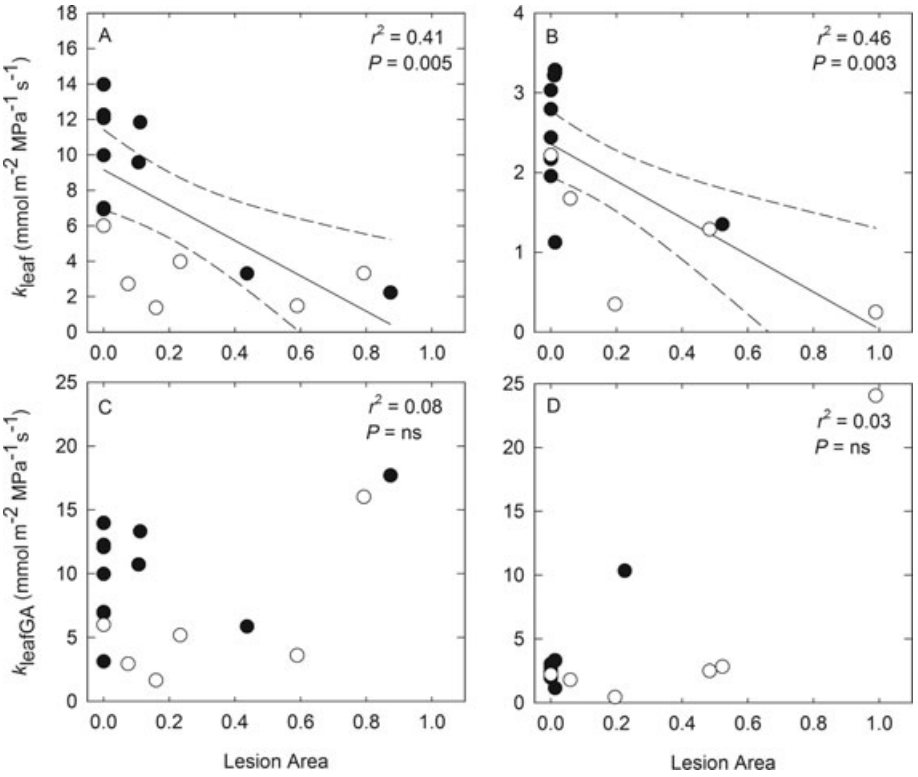

Fig. 6. Relationship between leaf lesion area and leaf hydraulic conductance (k_{leaf}) and leaf hydraulic conductance on a green area basis (k_{leafGA}) for *Vitis vinifera* cv. Chardonnay. (A) Lesion area vs. k_{leaf} in well-watered plants, (B) lesion area vs. k_{leaf} in deficit-irrigated plants, (C) lesion area vs. k_{leafGA} in well-watered plants, and (D) lesion area vs. k_{leafGA} in deficit-irrigated plants. One extreme point in panel D (circled) was not included in the correlation analysis. Hollow symbols represent leaves inoculated directly with *Xf* and filled symbols are non-inoculated leaves on infected plants. Lines are linear regressions with 95% confidence intervals (dotted lines).

Table 1. Water (Ψ_L), solute (Ψ_S) and pressure (Ψ_P) potential in control leaves, Pierce's Disease symptomatic leaves, or leaves undergoing natural senescence, for field grown *Vitis vinifera* cv. Chardonnay. Mean values followed by a different letter are significant at $P < 0.05$ of Tukeys HSD. Adjusted means (LS-means) calculated using the fraction of chlorotic area in the psychrometer are also shown, and when followed by a different letter are significant at $P < 0.05$ using a *t*-test.

	Ψ_L (MPa)		Ψ_S (MPa)		Ψ_P (MPa)	
	Mean	Adjusted mean	Mean	Adjusted mean	Mean	Adjusted mean
Control	−1.09a	−1.03a	−2.35a	−2.27a	1.26a	1.24a
Senescent	−1.26ab	−1.32b	−2.12ab	−1.96b	0.85b	0.87b
Symptomatic	−1.48b	−1.49b	−1.95b	−2.19b	0.47c	0.47c

in PD (Hopkins 1981). While leaf scorch symptoms were correlated with a decline in k_{leaf} in both well-watered and deficit-irrigated plants, we found a significant relationship between *Xf* concentrations and k_{leaf} only in inoculated leaves of deficit-irrigated plants. Therefore, our results suggest that under some environmental conditions, notably water-stress, high bacterial populations are associated with a functional occlusion of the leaf xylem conduits, either by bacteria or by the formation of gels and tyloses. However, the lack of relationship between leaf scorch symptoms and bacterial population in well-watered plants, where high populations of bacteria also occurred, raises questions as to whether high bacterial populations in leaf and petiole tissue are sufficient to cause PD symptoms.

Relationship between hydraulic parameters, bacterial population and PD symptoms

Previous studies indicate that there is a positive relationship between the number of vessels occluded by

Xf and leaf scorch symptoms (Hopkins 1981, Newman et al. 2003, Alves et al. 2004) and that petiole and stem hydraulic conductance is reduced in infected plants exhibiting leaf scorch symptoms (Goodwin et al. 1988b). In the present study, the significant relationship between *Xf* population and k_{leaf} and between *Xf* population and lesion area observed in deficit-irrigated plants but not in well-watered plants suggests that water-stressed plants are more sensitive to the presence of *Xf* bacteria. This is consistent with previous studies that have shown accelerated symptom development in water-stressed plants (McElrone et al. 2001, Thorne et al. 2006). There are a number of possible explanations for increased sensitivity of the water-stressed plants to pathogens. Hydraulic capacity was dramatically reduced in deficit-irrigated plants and any further loss caused by *Xf* may have had more immediate consequences in terms of localized leaf water deficits and marginal necrosis. It is also possible that host defense mechanisms (e.g. release of antimicrobial compounds) are suppressed by water deficits (Kruger and Manion 1994, Desprez-Loustau et al. 2006), although *Xf* populations were not higher in deficit-irrigated plants than in well-watered plants.

Very few previous studies have quantified the relationship between leaf scorch symptoms and absolute *Xf* populations. Gambetta et al. (2007) reported that, while there was an increase in the *Xf* population over time and in more symptomatic plants, this did not translate to a positive relationship between leaf scorch symptom severity and *Xf* population of individual leaves. In Gambetta et al(2007) and in the present study, some leaves with no detectable *Xf* concentrations exhibited leaf scorch symptoms (Fig. 5E), indicating that the formation of leaf scorch symptoms may be associated with factors other than high, localized concentrations of *Xf* (Krell et al. 2006, Gambetta et al. 2007). These factors may include plant derived gums and tyloses that occlude leaf and petiole vasculature as part of a systemic plant response (Stevenson et al. 2004). In support of this, Perez-Donoso et al. (2007) reported that symptomatic leaves have higher rates of ethylene production compared to control leaves with increased ethylene production being implicated in the formation of gums and tyloses in grapevine (Sun et al. 2006, Sun et al. 2007). Alternatively, recent work has re-evaluated the possibility that PD symptoms are caused by phytotoxins (Reddy et al. 2007) or by induction of programmed cell death (Gilchrist and Lincoln 2006), both of which could result in development of leaf scorch symptom in the absence of water deficits, although conclusive data to support these hypotheses are still lacking.

Reductions in hydraulic conductance under deficit-irrigation and *Xf* infection

Deficit-irrigated plants had greatly reduced k_{leaf} and k_{jun} compared to well-watered plants, but there was no significant difference between k_{leaf} and k_{jun} of infected and non-infected plants within a watering treatment when all leaves were averaged for a treatment. The decreased conductance in deficit-irrigated plants could result from drought-induced embolism, vessel occlusions (gums, tyloses) or differences in xylem structure. The extent of embolism was not established by our measurements, although the pressures used to drive flow (0.15 MPa) should have been great enough to cause the dissolution of emboli. If emboli were driven back into solution during the measurement, a slow increase in flow rate over the course of the measurements is expected. This was not observed in measurements from deficit-irrigated plants, suggesting that the decline in k_{leaf} and k_{jun} was caused by permanent occlusion of xylem conduits. Reduced k_{leaf} and k_{jun} could have been caused by altered xylem development in deficit-irrigated plants vs well-watered plants, i.e. narrower or less frequent conduits (Lovisolo and Schubert 1998). This seems unlikely because deficit-irrigation was applied after leaves were fully expanded and the majority of xylem development had been completed.

McElrone et al. (2003) showed that the whole shoot hydraulic conductance of *Parthenocissus quinquefolia* was reduced by water deficits and by *Xf* infection in an additive manner such that the well-watered control plants had the highest conductance and water-stressed infected plants had the lowest conductance. This difference in hydraulic conductance between infected and non-infected plants was not due to the formation of embolism in infected *P. quinquefolia*, leading McElrone et al. (2003) to suggest that the declining conductance in infected plants was due to clogging of xylem vessels. In contrast, Perez-Donoso et al. (2007) concluded that there was a significant increase in cavitation of stem xylem vessels in inoculated grapevines, although the MRI observations utilized in this study were unable to differentiate embolisms from tyloses. It is possible that cavitation may represent an intermediate step in permanent vessel occlusion during the progression of PD, and measurements taken at longer times after inoculation may miss the initial cavitation events (Newbanks et al. 1983).

Unexpectedly, some of the highest values of k_{leaf} and k_{jun} were observed in leaves from infected plants that harbored high populations of *Xf* but were not exhibiting visible leaf scorch symptoms. Perez-Donoso et al. (2007) observed a transient increase in hydraulic conductivity of grapevine shoots inoculated with *Xf* and suggested

that this occurred because intervessel pit membranes were digested by *Xf* bacteria during colonization. This is consistent with reports that genes encoding for polygalacturonase are required for *Xf* pathogenesis in grapevine, suggesting that digestion of pit membranes is an essential component of infection (Roper et al. 2007). Intervessel pit membranes contribute a large proportion of total xylem hydraulic resistance (Schulte and Gibson 1988, Choat et al. 2008). Although Chatelet et al. (2006) demonstrated that long, open pathways are naturally present in the primary xylem of grapevine, the majority of xylem conduits in the petiole and leaf are relatively short. Therefore, digestion of pit membranes or primary cell walls in petiole and leaf xylem may have led to the high values of k_{leaf} and k_{jun} observed for some leaves with high *Xf* populations.

PD symptoms as accelerated leaf senescence

Although the formation of PD symptoms was associated with declines in hydraulic conductance of stem-petiole junctions and leaves, the key question remains whether these declines are great enough to cause localized leaf water deficits and the initiation of cell death in the leaf margins. We observed reduced k_{leaf} with increasing lesion area on the leaf, however, it can be expected that conductance will decline with decreasing leaf area in any event. When k_{leaf} was expressed on a green leaf area basis, we found no significant relationship between lesion area and k_{leaf} (Fig. 6C, D). Therefore, the capacity of the vasculature to supply the transpiring leaf surface was not significantly reduced as green leaf area declined. Thorne et al. (2006) demonstrated that dramatically reducing water supply to half of a leaf by severing all but one secondary vein did not result in PD like symptoms. The authors concluded that leaf vasculature is 'overbuilt' such that the plant could suffer significant loss of vascular function and still maintain sufficient water transport capacity to satisfy the requirements of leaf gas exchange. It is possible that reductions in k_{leaf} play a less direct role in initiating cell death at the leaf margins. Natural leaf senescence is also associated with declines in k_{leaf}, leading some to speculate that changes in k_{leaf} act as a signal for leaf senescence (Salleo et al. 2002, Brodribb and Holbrook 2003).

In field grown plants, Ψ_L was significantly lower in symptomatic leaves than in green control leaves but was similar to naturally senescing leaves. The decrease in Ψ_L in leaves of field grown plants could be viewed as evidence of localized water deficits caused by vascular occlusion; however, the increase in Ψ_S is not consistent with this view because cellular dehydration causes concentration of solutes. The increase in Ψ_S and decrease in Ψ_P observed in symptomatic leaves is more indicative of a passive or active loss of solutes by leaf cells, as may be suggested by the increased electrolyte leakage observed in symptomatic tissue by Goodwin et al. (1988b).

The similarity of changes in the water status of symptomatic leaves and naturally senescing leaves suggests that the process of symptom formation in PD may be more akin to a program of natural senescence than simply to cellular level water deficits caused by vascular occlusions. Advances in our understanding of the molecular mechanisms involved in defense and senescence related pathways (reviewed in Lim et al. 2007) may provide the potential to confirm that leaf symptom formation during PD is programmed cell death as suggested by Gilchrist and Lincoln (2006) and to determine the possible role of physiological changes (decline in k_{leaf}) in triggering this process.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Images of individual leaves used for psychrometrically determined leaf water potential (Ψ_L), solute potential (Ψ_S) and tugor pressure (P) from field-grown *Vitis vinifera* cv. Chardonnay. Leaves were classed as control, Pierce's Disease (PD) symptomatic, and naturally senescent, with PD leaves coming from vines in which infection with *Xylella fastidiosa* had been previously confirmed using qPCR. The white circle on each leaf shows the position of leaf disc punches taken for isopiestic psychrometry. Leaves in panel A and B were collected on 4 and 22 October 2007, respectively.

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