# The impact of *Phaeomoniella chlamydospora* infection on the grapevine's physiological response to water stress Part 2: Cabernet Sauvignon and Chardonnay

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**Summary.** *Phaeomoniella chlamydospora* is a vascular pathogen that colonises the xylem tissues of the grapevine. It is associated with the diseases, esca and Petri disease, often considered to be 'stress-related' diseases. In glasshouse experiments using Cabernet Sauvignon and Chardonnay, stomatal conductance was higher in infected plants, implying that infection interferes with stomatal control. In Cabernet Sauvignon, leaf water potentials were lower in infected plants subjected to water stress, indicating that infection made it more difficult for the vine to get water to the leaf. This was less apparent in Chardonnay. Clearly, infection alters the grapevine response to water stress and some cultivars are affected more than others.

**Key words:** *Vitis vinifera,* water stress, grapevine trunk disease, stomatal regulation, leaf water potential.

## Introduction

The grapevine trunk diseases, Petri disease and esca, are caused by the xylem-inhabiting fungal pathogen, *Phaeomoniella chlamydospora*, although other fungi have been implicated (Crous and Gams, 2000). Grapevines can be infected with *P. chlamydospora* yet show no external symptoms of disease (Bertelli *et al.,* 1998; Edwards *et al.,* 2001; Halleen *et al.*, 2003; Edwards and Pascoe, 2004). Infected grapevines are more vulnerable to stress, however, and this can trigger disease expression (Ferreira *et al.,* 1999; Fourie and Halleen, 2004).

In 2004 and 2005, we demonstrated in glasshouse trials that infected Zinfandel grapevines responded differently to uninfected Zinfandel subjected to the same water stress (Edwards *et al.,* 2007). In 2005 and 2006, we conducted similar experiments using Cabernet Sauvignon and Chardonnay grapevines, the results of which are reported here.

## Materials and methods

Sets of four year old Cabernet Sauvignon and Chardonnay were inoculated near the base of the trunk while dormant (mid July 2005), with either 200 $\mu$l sterile distilled water or 200 $\mu$l spore suspension made up to deliver 50 spores of *P. chlamydospora*. They were then grown in the glasshouse and, at the time of the experiments, the most uniform plants were chosen to provide 18 infected and 18 uninfected plants of each cultivar.

The factorial set of 6 treatments consisted of

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Table 1. Measurements of water stress (WS) in experimental Cabernet Sauvignon and Chardonnay grapevines.

| WS parameter | Time of measurement | Measures per vine |
|---|---|---|
| Daily stomatal conductance ($g_L$) | 3–4 pm, $\times$3/week Mon/Wed/Fri | 3 leaves, mature sunlit, midshoot |
| Daily leaf water potential ($\psi_L$) | 3–4 pm, $\times$3/week Mon/Wed/Fri | 1 leaf, mature sunlit, midshoot |
| Diurnal stomatal conductance ($g_L$) | 6 am, 3 pm, 6 pm; days 9, 11, 14 | 1 leaf, mature sunlit, midshoot |
| Diurnal leaf water potential ($\psi_L$) | 6 am, 3 pm, 6 pm; days 9, 11, 14 | 1 leaf, mature sunlit, midshoot |

three levels of stress, 'no stress', '50% of water requirement' and '25% of water requirement' combined with one of two levels of infection, either 'infected' or 'not infected'. There were 6 replicates of the six treatments and the pots were laid out on 6 benches with each bench a replicate. The Cabernet Sauvignon trial was conducted from 14 November–23 December 2005 and the Chardonnay trial from 20 February–31 March 2006.

The methodology was the same as reported in Edwards *et al.* (2007). Water was completely withheld from the Cabernet Sauvignon stress treatments for 4 days from day 23–26, and from the Chardonnay stress treatments for 3 days from day 27–29, after which the treatments were reapplied. This was to determine whether infection interfered with the grapevines' capacity to recover from a short severe stress. This severe stress was restricted to 3 days for Chardonnay as this cultivar did not tolerate the stress as well as the Cabernet Sauvignon. The variables measured were leaf water potential and stomatal conductance, as per Table 1. The diurnal measurements consisted of three measurements per day at 6 am, 3 pm and 6 pm, for three days in the second week (days 9, 11 and 14).

The daily measurements data were analysed using the AREPMEASURES procedure of Genstat, which uses an analysis of variance to analyse the data but allows for the correlation of measurements over time. The diurnal measurements data were analysed using a standard split plot analysis with each time of day analysed as a separate variable.

## Results

### Cabernet Sauvignon, stress imposed 14 November – 23 December 2005

For the daily measurements, the results of the analyses were similar for both stomatal conductance and leaf water potential. The overall main effects of stress, infection and date were signifi-cant ($P<0.001$) but the only significant interaction was that of stress with date indicating that the size of the stress effect changed over time.

Stomatal conductance of infected plants was higher than that of uninfected plants, regardless of stress treatment (Fig. 1) and this effect did not change over time. Leaf water potentials of infected plants became lower than the corresponding uninfected plants as the stress was prolonged, particularly in the more severe treatment of 25% water (Fig. 2).

The possibility of a difference between treatments in the 'recovery' period was considered. On days 23, 24, 25 and 26, the pots receiving the stress treatments received no water at all while the remaining unstressed treatments received the normal allocation. On day 27, the normal watering recommenced. At 3 pm, the usual measurements were taken and although leaf water potential showed an immediate recovery (Fig. 2), stomatal conductance (Fig. 1) did not show recovery until day 31 and the 25% stress treatment barely recovered at all. Leaf water potential measures also took longer to recover in infected vines versus uninfected vines, particularly in the 25% stress treatment.

The diurnal measurements showed that for both stomatal conductance (Fig. 3) and leaf water potential (Fig. 4), infected plants responded differently to water stress than uninfected plants. This difference was most apparent in the 50% water treatment using stomatal conductance and the 25% water treatment using leaf water potential.

With regards to stomatal conductance, at 6 am, there was a significant overall stress main effect but no main effect of infection. There was no evidence of stress by infection interaction at 6 am across the 3 days. At 3 pm, the overall effects of both stress and infection were significant with weak evidence of an interaction between these two factors ($P=0.091$). At 6 pm, the overall main effects of infection and stress were significant, as was their
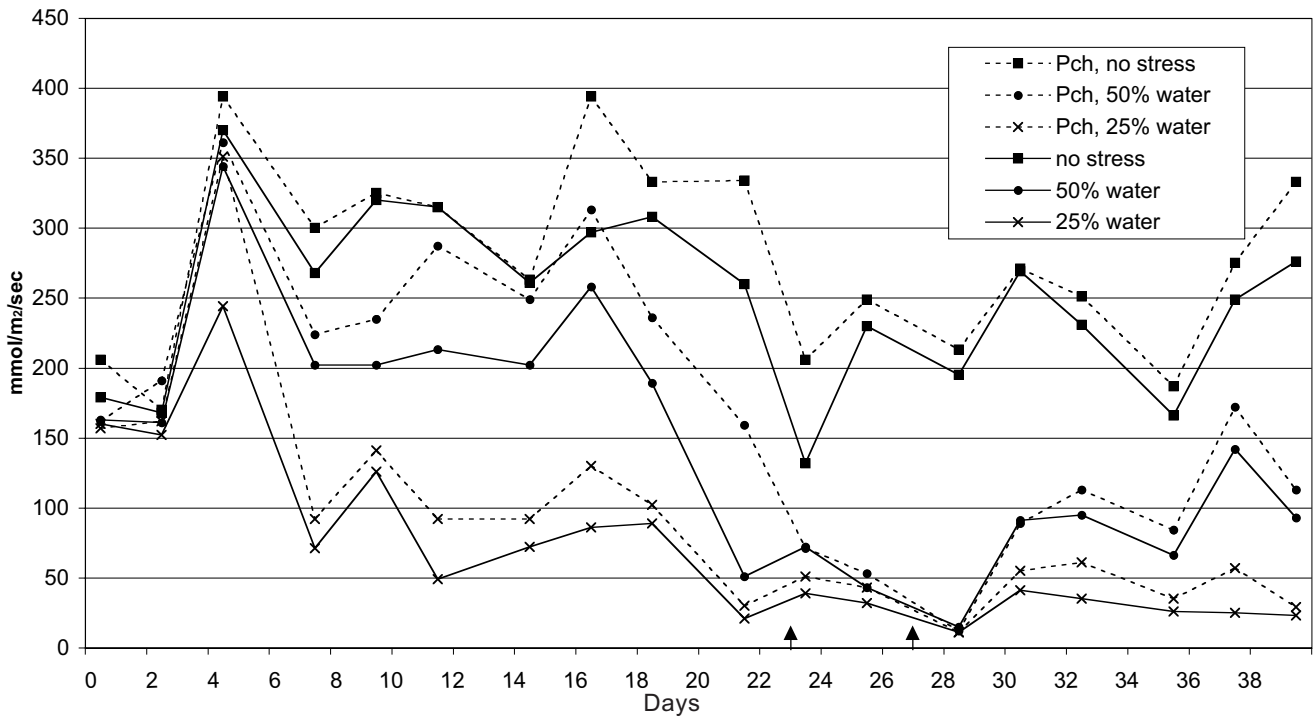
Fig. 1. Effect of water stress on stomatal conductance of infected and uninfected Cabernet Sauvignon, November 2005. The arrows indicate the period when water was completely withheld from the stress treatments.
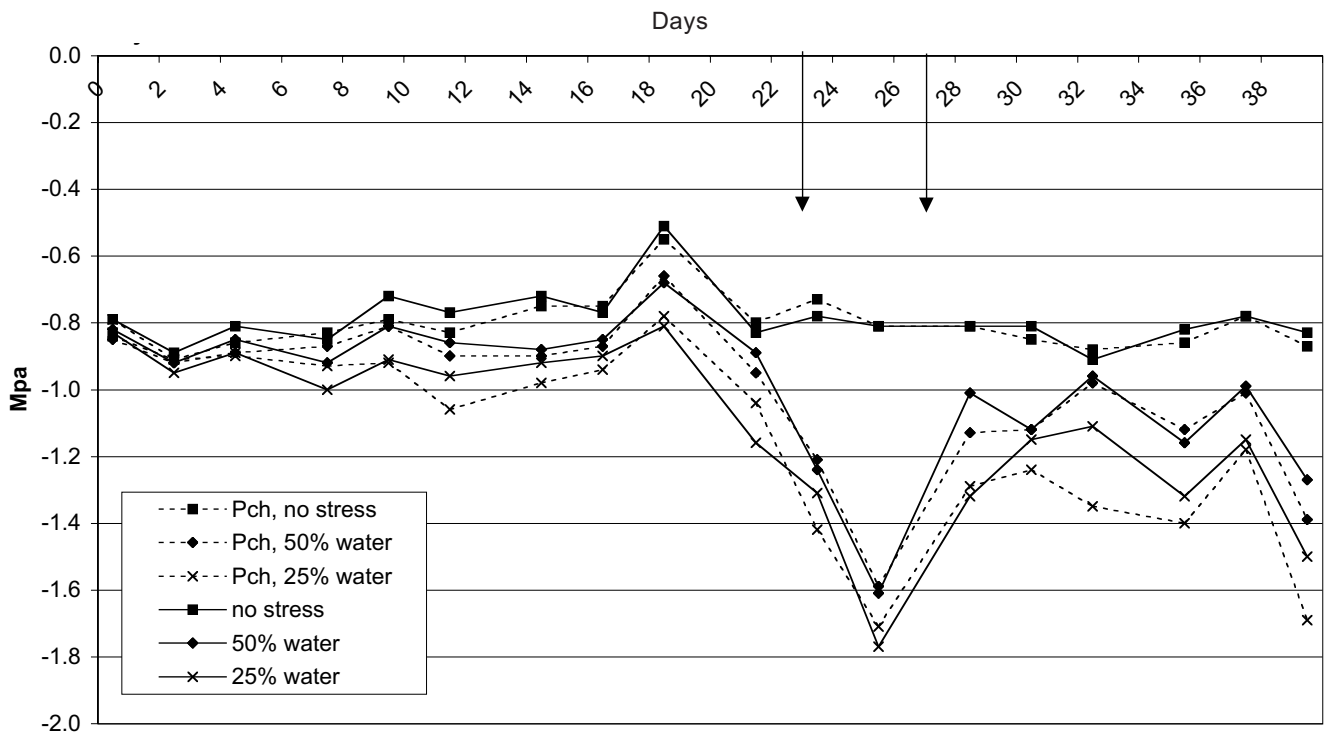


Fig. 2. Effect of water stress on leaf water potential of infected and uninfected Cabernet Sauvignon, November 2005. The arrows indicate the period when water was completely withheld from the stress treatments.
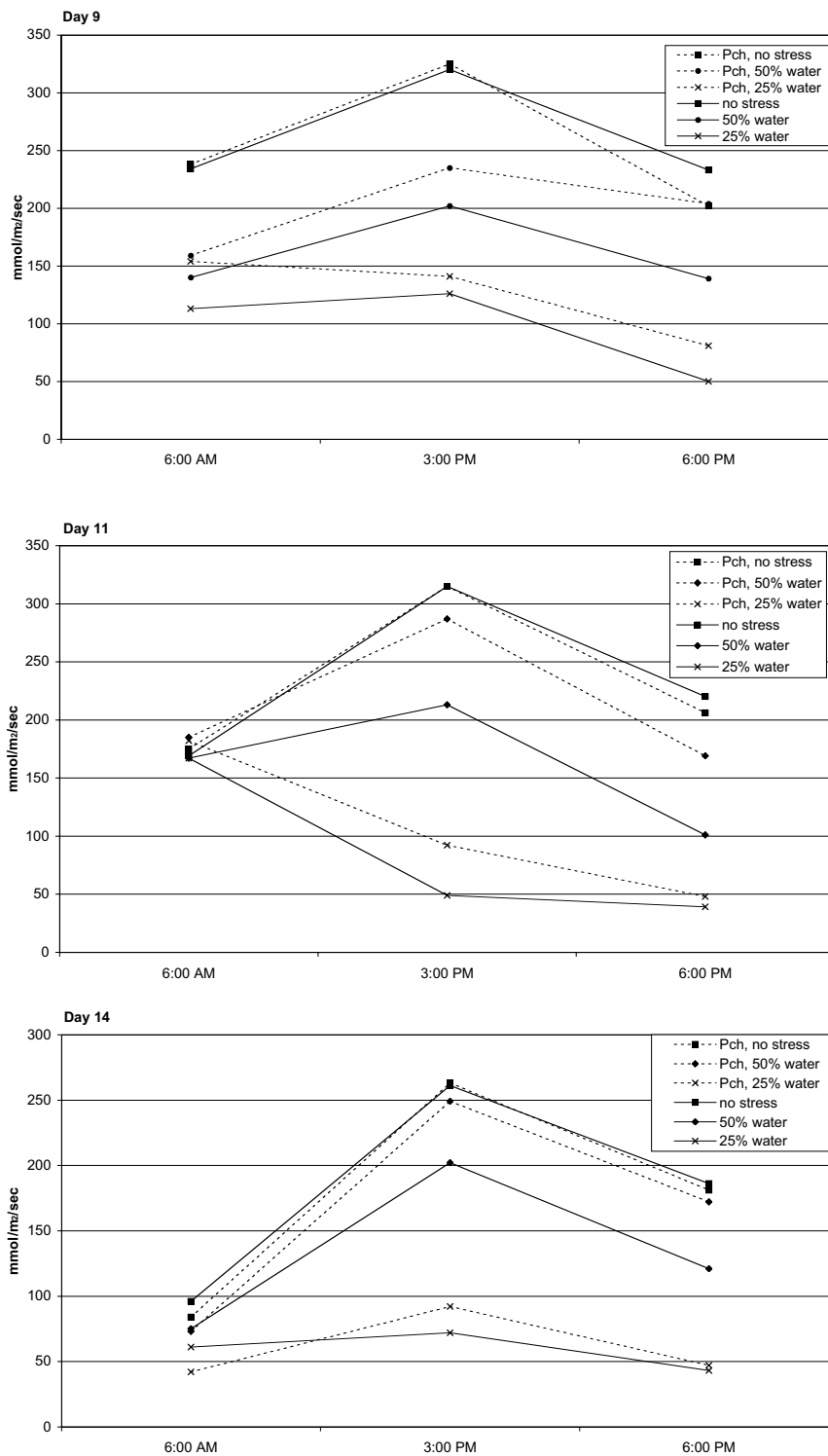
Fig. 3. Effect of 9, 11 and 14 days water stress on stomatal conductance of infected and uninfected Cabernet Sauvignon, November 2005.
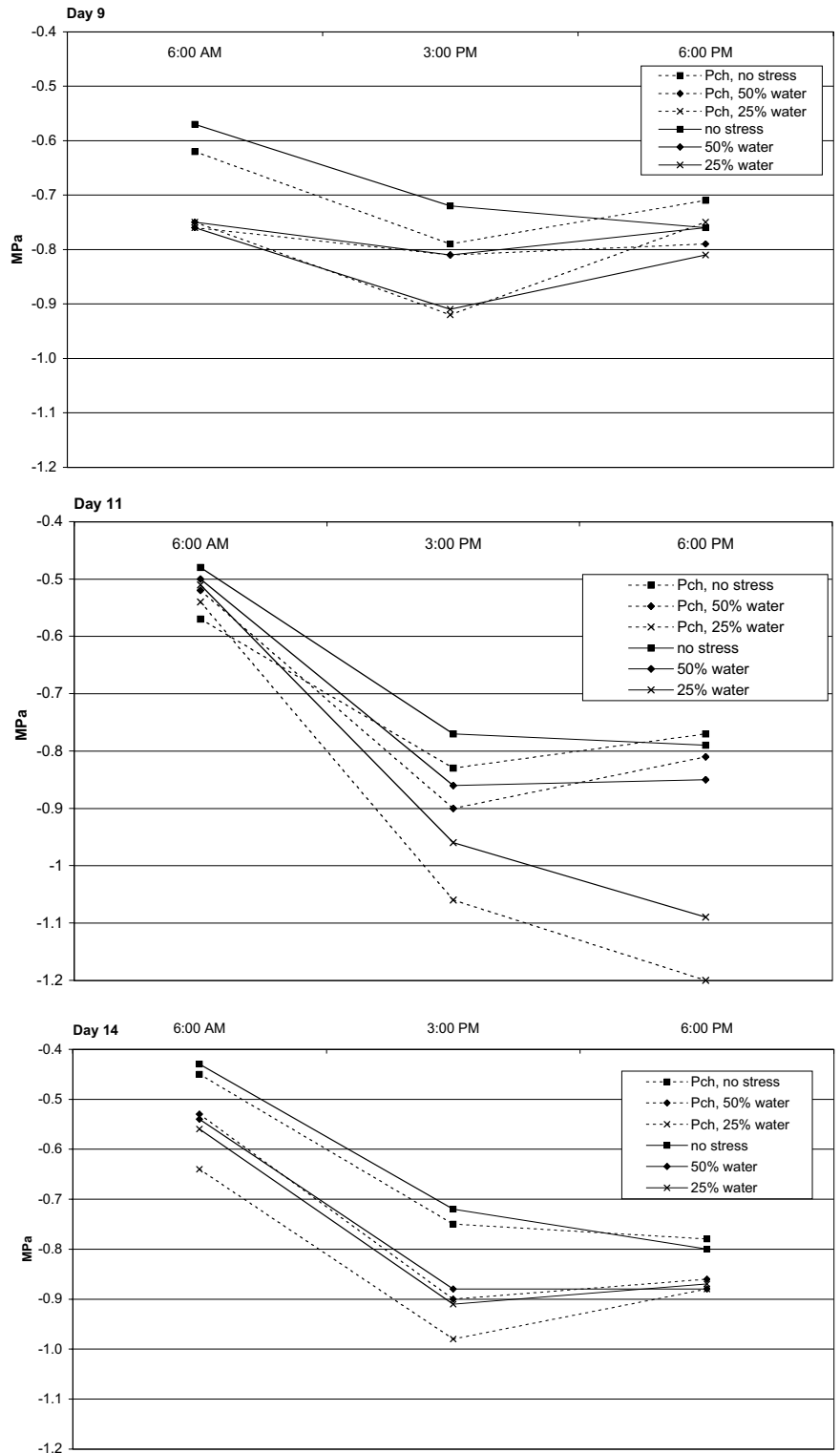
Fig. 4. Effect of 9, 11 and 14 days water stress on leaf water potential of infected and uninfected Cabernet Sauvignon, November 2005.
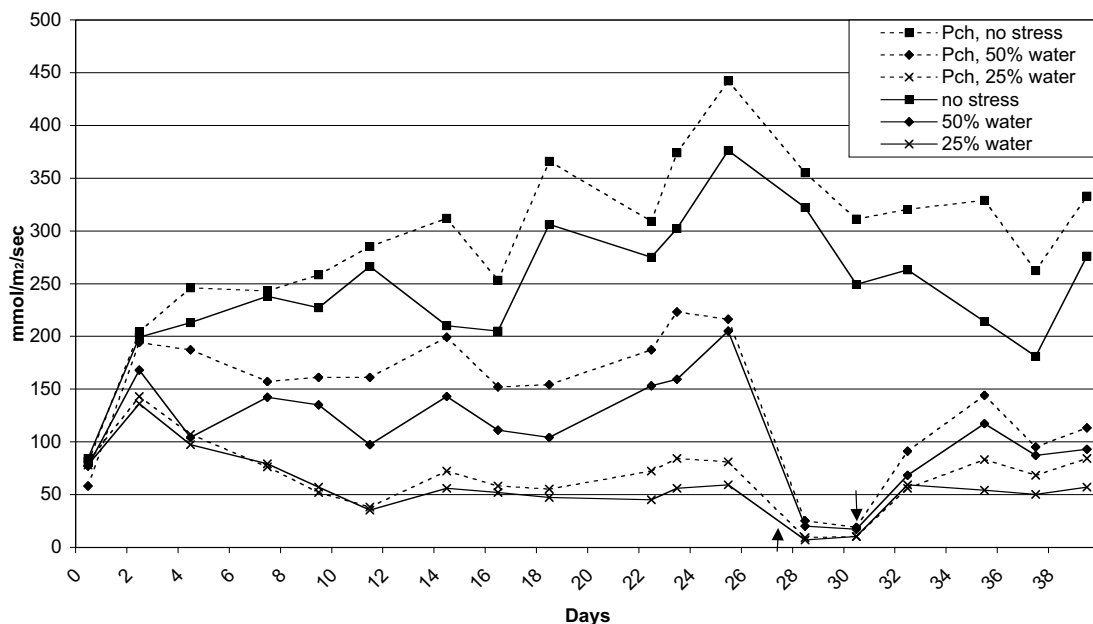
Fig. 5. Effect of water stress on stomatal conductance of infected and uninfected Chardonnay, March 2006.

interaction. This was consistent over the three dates of measurement.

Using leaf water potential as a measure, at 6 am there was a significant overall stress main effect with only weak evidence of an overall infection effect at this time of day ($P$=0.078). The interaction of stress with infection was not significant. At 3 pm, the overall main effects of both stress and infection were significant, but there was no significant interaction effect. At 6 pm, there was an overall significant stress effect, which changed significantly across the three days, but infection and the interactions were not significant.

**Chardonnay, stress imposed 20 February – 31 March 2006**

Again, stomatal conductance of infected plants was consistently higher than that of uninfected plants (Fig. 5). However, whereas leaf water potential was lower in the infected, stressed treatments using Cabernet Sauvignon, the opposite occurred in Chardonnay (Fig. 6).

The overall results of the analyses of daily measurements were similar for both stomatal conductance and leaf water potential. The main effects of infection, stress and date were significant, as were the two-way interactions of infection and stress, infection and date and stress and date. The three-

way interaction of time x infection x stress was also very slightly significant ($P$=0.071 for stomatal conductance and 0.083 for leaf water potential), probably due to the catastrophic effect of giving no water to the four stress treatments on days 27–29.

The possibility of a difference between treatments in the 'recovery' period was again considered. On days 27, 28 and 29, the pots receiving the stress treatments received no water at all while the remaining unstressed treatments received the normal allocation. As expected the measurements taken on day 28 showed extreme values for both leaf water potential and stomatal conductance. On day 30, the normal watering recommenced. At 3 pm, the usual measurements were taken and although leaf water potential showed an immediate recovery, stomatal conductance did not show recovery until day 32. There was a significant difference in the recovery of infected versus uninfected vines, but unexpectedly, the infected vines recovered more rapidly than the uninfected vines.

The diurnal measurements again showed that infected plants responded differently to water stress than uninfected plants at both 3 pm and 6 pm but particularly at 3 pm (Fig. 7 and 8). As mentioned above, the Chardonnay grapevines behaved differently to the Cabernet Sauvignon grapevines in that the leaf water potentials of the stressed
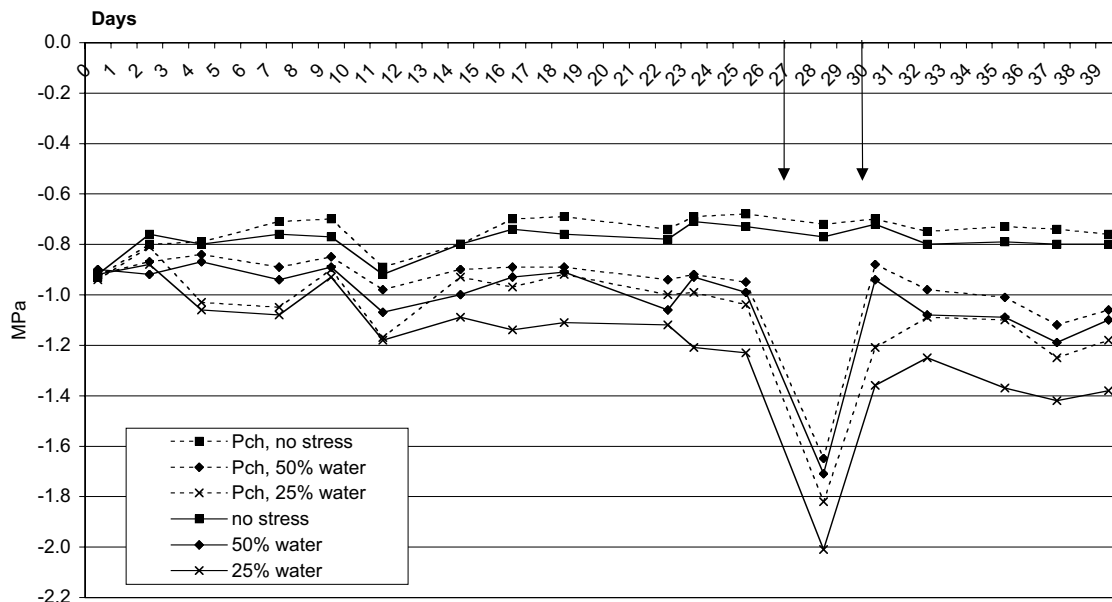
Fig. 6. Effect of water stress on leaf water potential of infected and uninfected Chardonnay, March 2006.

uninfected vines were lower than those of the stressed infected vines, particularly for the 3 pm and 6 pm measurements. This implied that the uninfected vines were less tolerant of the stress than the infected vines.

For stomatal conductance measures, at 6 am, there were overall main effects of stress and infection but no interaction between them. The size of the main effects was different on the three days; this difference was significant for infection and stress but not for the interaction. At 3 pm, the differences were greatest, as this is the hottest time of day. The infection and stress interaction was not only significant overall but was significantly different between days. However, this may be due to temperature differences between the days. The data for 6 pm required a log transformation for the analysis. Overall, the main effects of infection and stress were significant. There was weak evidence of an overall interaction between stress and infection ($P$=0.087).

For leaf water potential measures, at 6 am, there were significant main effects of stress and infection but no interaction between stress and infection. At 3 pm and 6 pm, there were significant overall main effects of stress and infection but the interaction of these two factors was not significant.

## Discussion

The results presented here clearly showed that *P. chlamydospora* infection interfered with the water relations of the grapevine, particularly under conditions of water deficit. In glasshouse experiments, the physiological responses to water stress of infected and uninfected Zinfandel (Edwards *et al.,* 2007), Cabernet Sauvignon and Chardonnay were measured. Stomatal conductance was higher in infected plants, implying that infection interferes with stomatal regulation. In Zinfandel (Edwards *et al.,* 2007) and Cabernet Sauvignon, leaf water potentials were lower in infected plants subjected to water stress, indicating that infection made it more difficult for the vine to get water to the leaves. Cultivar differences were noticed, with infected Chardonnay responding differently to the other two cultivars.

In the glasshouse experiments described here, two cultivars were trialled: Cabernet Sauvignon and Chardonnay. Plants were subjected to a single steady stress and to the imposition of a short severe stress. Under both circumstances, grapevines infected with *P. chlamydospora* responded differently to comparable uninfected grapevines.

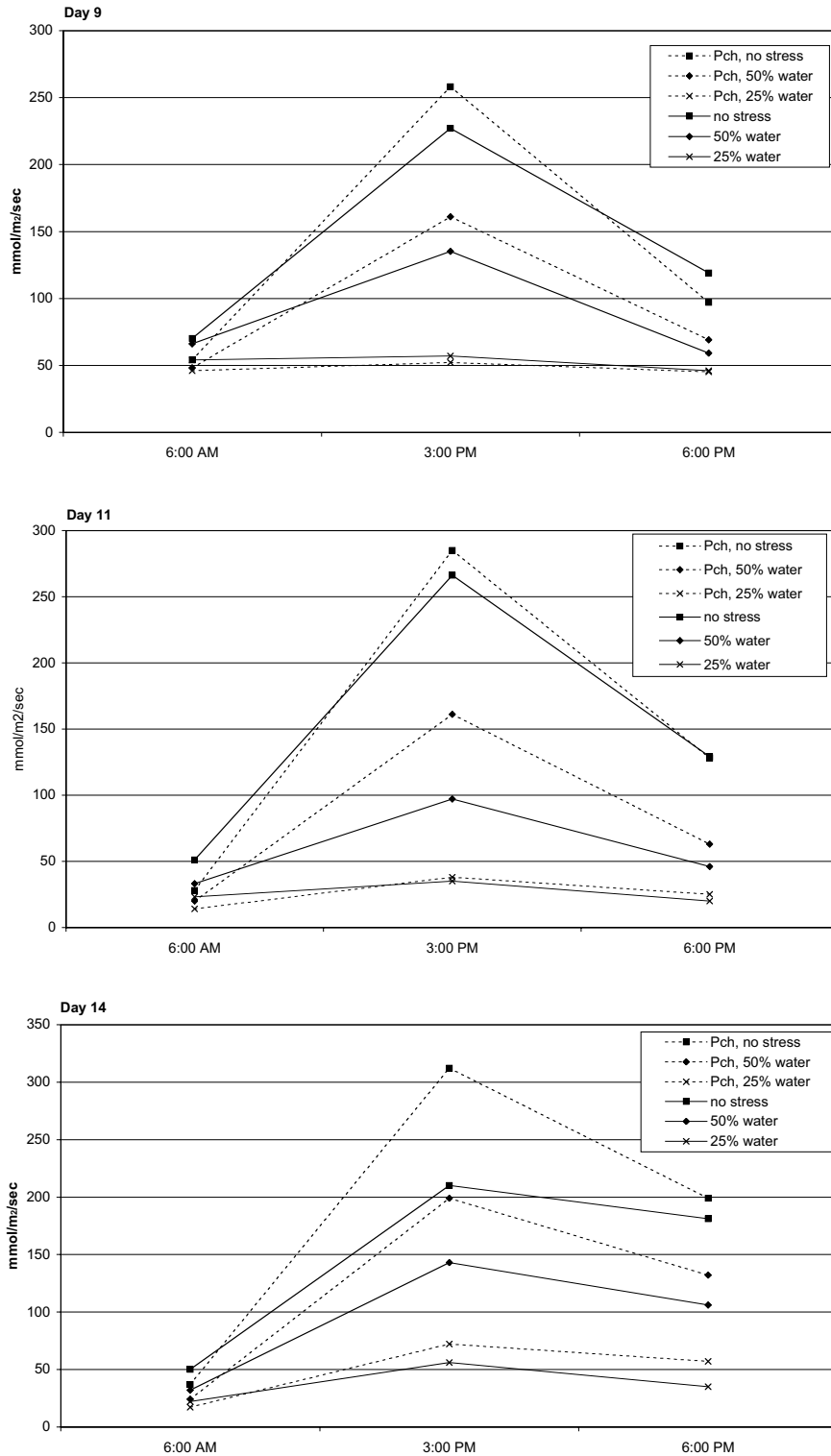Stomatal conductance was higher in infected

Fig. 7. Effect of 9, 11 and 14 days water stress on stomatal conductance of infected and uninfected Chardonnay, March 2006.
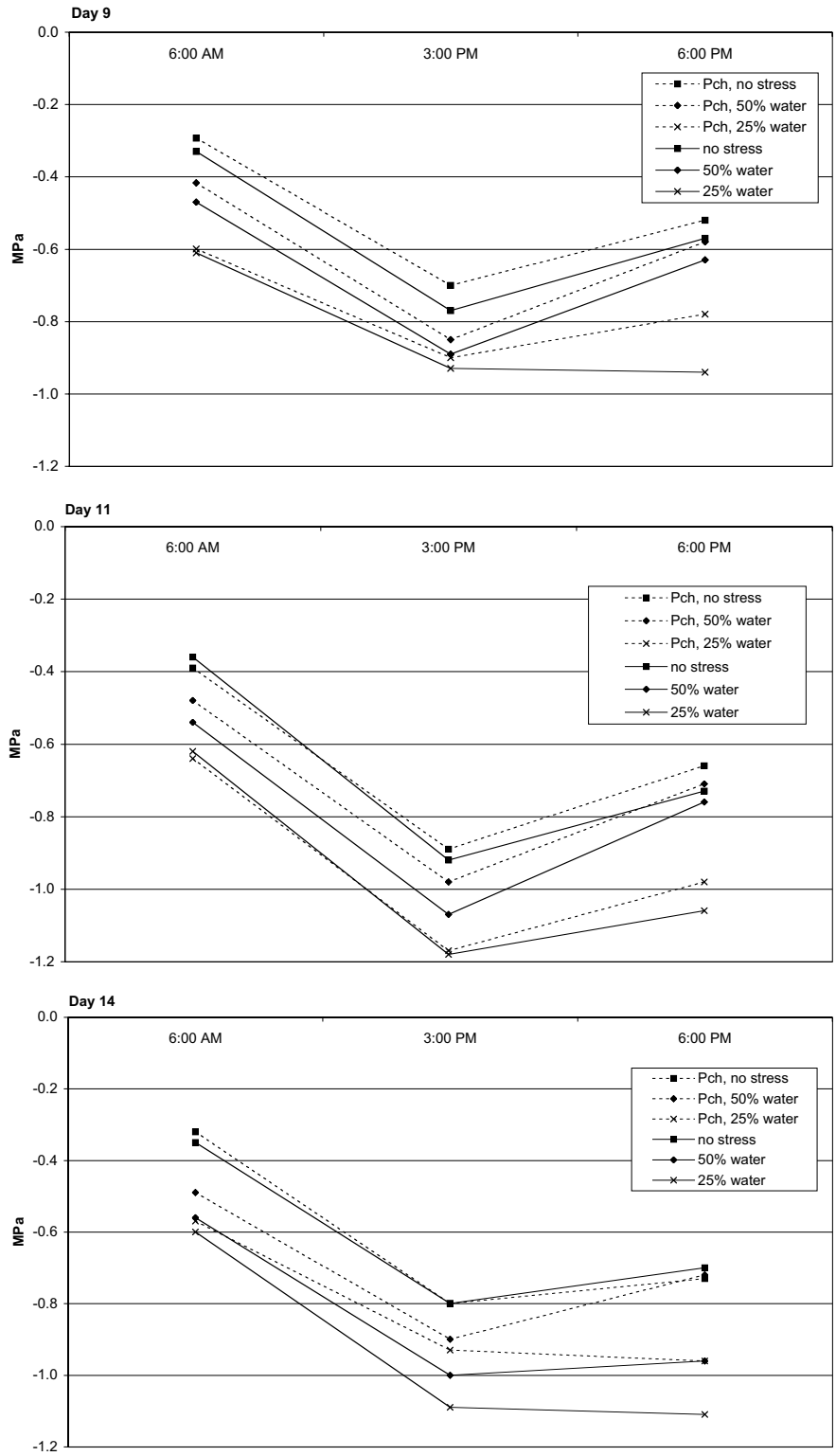
Fig. 8. Effect of 9, 11 and 14 days water stress on leaf water potential of infected and uninfected Chardonnay, March 2006.

plants than uninfected plants, indicating that infection interferes with stomatal regulation. The cultivars appeared to differ slightly in reaction, with the most noticeable effect on Chardonnay, followed by Zinfandel (Edwards *et al.,* 2007) and least noticeable in Cabernet Sauvignon. Under water stress, infected Zinfandel (Edwards *et al.,* 2007) and Cabernet Sauvignon had consistently lower leaf water potentials, but infected Chardonnay had higher leaf water potentials. As a general rule, the lower the leaf water potential, the more stressed the plant. This suggests that Zinfandel and Cabernet Sauvignon were more adversely affected by the combination of infection and stress than Chardonnay. Differences in cultivar response to water stress have been linked to the geographical origin of the cultivar (Winkel and Rambal, 1993). These researchers compared the physiological response to water stress of three cultivars from different geographical origins. Carignane, originating from semi-arid Aragon in Spain, physiologically adjusted to water stress using stomatal control. Shiraz, originating from the Rhone valley in France, apparently adjusted by limiting leaf area. The response by Merlot, from Bordeaux in France, was intermediate between the two. Cultivars are also known to respond differently to infection by *P. chlamydospora* (Adalat *et al.*, 2000; Feliciano *et al.*, 2004). Therefore, it is to be expected that cultivars will differ in their physiological response when these two stresses, lack of water and infection, are overlaid.

The diurnal measurements (taken throughout a single day) showed that the stress × infection interaction was more pronounced in the afternoon, when plant water demand was highest. The 6 am measurements showed that over time the infected plants were less able to recover overnight than the uninfected plants, and the 3 pm and 6 pm measurements showed that the infected plants were less able to cope with the additional burden of afternoon temperature. The level of stress was also an important factor in how well the vine was able to respond. In the Cabernet Sauvignon experiment, the impact of infection was most evident in the intermediate (i.e. 50%) water stress treatment. Under higher stress (i.e. 25%), all plants were severely stressed whether infected or not.

A short severe stress (i.e. no water) was imposed to determine if infection affected the plants' ability to recover. Cabernet Sauvignon plants (at the more severe 25% water treatment) were less able to recover than uninfected plants subjected to the same severe stress. This has also been observed in infected Zinfandell (Edwards *et al*., 2007) .The Chardonnay plants responded differently, with infected plants seemingly recovering better than uninfected plants, particularly at the 25% water treatment.

Two of the most important driving forces in Australian viticulture today are water shortages and rises in temperature due to climate change. The current trend towards growing grapes for quality and the increased awareness of water as a limited resource has meant there is considerable interest in applying irrigation scheduling which involves periods of water stress. Infections by grapevine trunk disease pathogens are perennial (i.e. a grapevine is infected for life) and symptomless, with disease expression triggered in response to environmental stress. Edwards and Pascoe (2004) showed that these diseases are 'sleepers' and symptomless infection is widespread. It is unknown how infected grapevines will respond to deficit irrigation schedules, and whether these practices will have detrimental effects on long-term vineyard health. Future research is required into defining this relationship between stress and disease expression, so that deficit irrigation schedules can be applied safely to produce high quality wine grapes without compromising vine health. It is important to determine how infected grapevines will respond to the challenges of increased water and heat stress, and how best to manage them for long-term vineyard health and productivity.

## Acknowledgements

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