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Influence of water stress on Botryosphaeriaceae disease expression in grapevines

JAN M. van NIEKERK[1], ALBERT E. STREVER[4], P. GERHARD du TOIT[4], FRANCOIS HALLEEN[1, 2] and PAUL H. FOURIE[1,3]

[1]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Stellenbosch, 7602, South Africa
[2]Plant Protection Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa
[3]Citrus Research International, P.O. Box 2201, Stellenbosch 7602, South Africa
[4]Department of Viticulture and Oenology, University of Stellenbosch, Private Bag X1, Stellenbosch, 7602, South Africa

Summary. Several species in Botryosphaeriaceae have been associated with grapevine trunk diseases. To evaluate the effect of water stress on infection of grapevines by Botryosphaeriaceae spp., 1-year-old Shiraz/101-14 Mgt nursery grapevine plants were planted in plastic potting bags and placed outdoors under shade netting. Five weeks after planting, vines were pruned and the pruning wounds inoculated with spore suspensions of *Neofusicoccum australe*, *Neofusicoccum parvum*, *Lasiodiplodia theobromae* or *Diplodia seriata*. Control treatments consisted of applications of sterile water or a *Trichoderma harzianum* spore suspension. Stem inoculations were done by inserting a colonised or uncolonised agar plug into a wound made in each stem. Four different irrigation regimes were introduced 12 weeks after planting to simulate varying degrees of water stress. Measurements of stomatal conductance, photosynthetic rate and leaf spectrometry were made to monitor physiological stress. Eight months after inoculation, vines were uprooted and the root, shoot and plant mass of each vine determined. Lesions observed in the inoculated pruning wounds and stems were also measured. Vines subjected to the lowest irrigation regime were significantly smaller than optimally irrigated vines. Water stressed vines also had significantly lower photosynthetic rates and levels of stomatal conductance compared with vines receiving optimal irrigation, indicating that these plants experienced significantly higher levels of physiological stress. The mean lesion length was significantly longer in the pruning wounds and stems of plants subjected to the lowest irrigation regime, with lesion length declining linearly with increasing irrigation volume. These results clearly indicate that when a grapevine is exposed to water stress, colonisation and disease expression by Botryosphaeriaceae spp. are much more severe.

Key words: photosynthetic rates, stomatal conductance, leaf spectrometry, endophytes.

Introduction

Species in the Botryosphaeriaceae have been shown to be cosmopolitan in nature with wide host ranges (Crous *et al*., 2006). These fungi have the ability to survive endophytically in their hosts, causing disease when the host is exposed to a predisposing stress condition or when conditions are favourable for disease development (Smith *et al*., 1996; Stanosz *et al*., 2001; Desprez-Loustau *et al*., 2006; Slippers and Wingfield, 2007). Several species have been reported to occur on grapevines where they are associated with a wide range of symptoms, though they also occur in asymptomatic tissue (van Niekerk *et al*., 2004; 2006; 2010). Pruning and other mechanical wounds are known to be important infection portals for these pathogens (Lehoczky, 1974; Halleen and Fourie, 2005). However, apart from infection of mature vines in the field, propagation material taken from apparently healthy mother vines can already be latently infected by various species in the Botryosphaeriaceae prior to grafting or become infected during the different nursery processes (Halleen *et*

Corresponding author: P.H. Fourie
Fax: +27 865 717273
E-mail: phf@cri.co.za

al., 2003; Fourie and Halleen, 2004a). These early field or nursery infections remain latent until the vines are exposed to some sort of stress and/or conditions favourable for disease development.

In field-grown grapevines, it is often observed that symptom development and disease expression associated with species in the Botryosphaeriaceae are much more severe in cases where the plants have been, or still are, exposed to periods of water stress. Initial reports on the effect of environmental stresses, such as water stress, on host pathogen interactions were based on observations of a variety of hosts in the field, similar to the field observations on grapevines (Schoeneweiss, 1981). From these observations it was concluded that, infection by especially canker and dieback causing pathogens, such as species in the Botryosphaeriaceae, were more severe when the host plant was exposed to predisposing stress (Smith *et al*., 1996; Blodgett *et al*., 1997). However, no experimental evidence existed to substantiate these field observations (Schoeneweiss, 1981). Subsequently, the effect of water stress on infection of woody hosts by different species in the Botryosphaeriaceae has been studied (Schoeneweiss, 1981; Blodgett *et al*., 1997; Ma and Michailides, 2001; Stanosz *et al*., 2001; Luque *et al*., 2002). The results of these studies conclusively showed that, when the different hosts were subjected to water stress prior to, or after inoculation, the symptom development was more severe in comparison to non-stressed control plants.

This influence of water stress on Botryosphaeriaceae symptoms or disease development can be attributed to its effect on the physiology of the host plant and therefore the capacity of the plant to resist infection (Desprez-Loustau *et al*., 2006). Low moisture in the soil translate to lower water potentials in plants, which in turn negatively influences a number of plant physiological processes (Schoeneweiss, 1978). A decline in the water potential in plants was found to lead to an increase in the concentration of abscisic acid ([ABA]) in the xylem sap (Schoeneweiss, 1978; Schulze, 1991). This increase in [ABA] in the xylem is related to stomatal closure, but the relationship is not direct, since the effect of ABA on the stomata is modified by changes in the pH of the xylem sap (Schulze, 1991). Progressive soil drying also changes the amount of nitrate taken up by the roots. This raises the pH of the xylem sap, and this increases the sensitivity of the stomata to ABA. Stomatal closure is therefore the end result of a number of changes occurring in the composition of the xylem sap (Schulze, 1991; Boyer, 1995). In grapevines, the ABA produced in the leaves has also been observed to contribute to this interaction since after it is formed it remains in the leaf and further raises the leaf [ABA] that triggers stomatal closure (Soar *et al*., 2004). A consequence of reduced stomatal conductance due to stomatal closure is a hyperbolic decline in the photosynthetic rate of the vine (Boyer, 1995; Christen *et al*., 2007).

Apart from reductions in the stomatal conductance and the photosynthesis, the production of energy (ATP) in a plant is also inhibited by any stress factor (Schoeneweiss, 1978; Ayers, 1984; Boyer, 1995). This reduction in ATP production, coupled with lower photosynthate availability caused by a reduced photosynthetic rate, causes a decline in the production of enzymes and proteins that play an essential role in the pathogen defence system of a host plant. The chain of events brought about by soil water deficits therefore causes the host plant to be more susceptible to infection and to exhibit more severe symptom (Schoeneweiss, 1978; Boyer, 1995). Apart from the effects on the physiology and pathogen defence systems of the host plant, water stress also reduces cell growth and hence biomass production, causing water-stressed plants to often be smaller compared to plants with optimal water supply (Maia Souza and Cardoso, 2003). In grapevines, low plant water potentials caused by water stress were also found to similarly inhibit physiological processes in the vine (Flexas *et al*., 1999; Christen *et al*., 2007).

These observations about stress and disease severity have not been scientifically substantiated for grapevines in the field. The aim of this study was, therefore, to elucidate how water stress influences the colonisation of Botryosphaeriaceae spp. and disease expression in grapevines infected with these pathogens.

Materials and methods

Plant material and planting

Although species in Botryosphaeriaceae are common in various grapevine and rootstock varieties, physiological stress, and in particular water

stress, have mostly been studied on wine grape cultivars, with Shiraz on different rootstocks often being included in these studies (Winkel and Rambal, 1993; Dry and Loveys, 1999; Dry *et al*., 2000; Chalmers *et al*., 2004; Soar *et al*., 2006). Rootstock 101-14 Mgt was reported to have a poor tolerance of water stress (Nikolaou *et al*., 2003). Consequently, 1-year-old Shiraz/101-14 Mgt nursery vines were selected for use in the trial. Prior to planting, dormant vines were subjected to hot water treatment (30 min at 50°C) to reduce any latent pathogen infections (Fourie and Halleen, 2004b). The vines were subsequently planted during early spring 2005 (September 2005) in 7 L plastic potting bags filled with a composted potting mixture (pH 5.5) and placed outdoors under 50% shade netting. Initially, the plants were drip-irrigated to field capacity (2.5 L water/plant/day) as calculated from the physical characteristics of the potting medium. Additional fertilisation consisting of 5 g KOMPEL Chemicult® (Starke Ayres, South Africa) hydroponic nutrient powder and 1.25 g $Ca(NO_3)_2$ were applied every 14 days. Powdery and downy mildew were controlled by foliar sprays of copper ammonium carbonate [Copper Count N, SL, Hygrotech Seed (Pty) Ltd, P.O. Box 17720, Pretoria North, 0116] (500 mL 100 L^{-1} water) every 10 days.

Inoculation

Five weeks (early summer, on 4 November 2005) after planting, vines were pruned back to two shoots per plant, with the strongest shoot on each plant pruned to three buds. The fresh pruning wound on the 3-bud shoot was inoculated with 100 µL of a 1×10^5 spores mL^{-1} suspension of either *Neofusicoccum australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, *N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. or *Diplodia seriata* De Not. All the isolates used in the study were identified using the morphological and DNA sequence data of van Niekerk *et al*. (2004). Inoculum of the different species in Botryosphaeriaceae were also produced according to the protocol described by van Niekerk *et al*. (2004), which entailed plating the different species out on water agar (WA, Biolab, Wadeville, South Africa) amended with four pieces of autoclaved pine needles, and incubating it at 25°C under near-UV light for 2 weeks to induce pycnidial formation. Control treatments consisted of applications of 100 µL sterile water or, as a non-pathogen control, 100 µL of a 0.5 g L^{-1} suspension of Eco-77® [Plant Health Products (Pty) Ltd, South Africa]. Eco-77® is a *Trichoderma harzianum* Rifai based biocontrol product registered for the protection of grapevine pruning wounds against *Eutypa lata* (Pers.) Tul. & C. Tul.. On the same vines where pruning wound inoculations were made, stem inoculations were done by inserting a colonised agar plug, taken from 1-week-old cultures, or an uncolonised agar plug (control) into a wound made with a flame sterilised 5 mm cork borer through the phloem tissue 10 cm above soil level. Prior to wounding, all loose bark on the stem was removed and the exposed stem area surface sterilised by wiping with a paper towel wetted with 70% ethanol. After inoculation, wounds were covered with Parafilm®.

Irrigation and plant physiology measurements

Twelve weeks after planting (mid-summer on 21 December 2005), four different irrigation regimes were introduced that were applied until late autumn 2006 (13 May 2006). The four irrigation regimes consisted of the optimum irrigation volume (100% field capacity) and regimes having 70, 50 or 20% of field capacity at the same frequency. Soil moisture of each irrigation regime was monitored continuously (measurements every 10 min) in two potting bags using ECH_2O soil moisture probes and Em50 data loggers (Decagon Devices Inc., Pullman, WA, USA). This was done to ensure that the available soil moisture content in the potting bags of the different irrigation regimes remained at the correct levels. Scheduling of the four different irrigation regimes was adjusted according to these measurements. Rainfall at the trial site during the trial period was monitored by a weather station located 500 m from the trial site.

In order to monitor the physiological stress experienced by vines under the different irrigation regimes, stomatal conductance and photosynthetic rate were measured on three representative vines from each irrigation regime, using a CIRAS-1 infrared gas analyser (PP Systems International, Inc., Amesbury, MA, USA). After clipping the cuvette on a leaf, the CIRAS-1 was

allowed to take measurements and equilibrate for 30 s before a value was recorded. Before taking any measurements, the Ciras-1 was calibrated for ambient temperature, relative humidity (RH) and light quantity (PAR) as measured by both a hand held RH meter and a ceptometer. To stabilize the carbon dioxide (CO_2) and humidity levels within the measuring cuvette of the Ciras-1, the air supplied was scrubbed of all CO_2 and water vapour, and adjusted amounts of each were supplied and finely controlled by the instrument. CO_2 was supplied at a concentration of 380 ppm sourced from a small internal gas canister, freshly inserted before measurements started. The amount of water vapour was supplied and controlled by the semi-permeable grid of the Ciras-1. Measurements were made four times, on 20 January (mid-summer), 2 February (mid-summer), 9 March (beginning of autumn) and 20 April (mid autumn) 2006. Spectral reflectance was also measured using a Fieldspec Pro spectroradiometer (Analytical Spectral Devices, Inc., Boulder, CO, USA) and an ASD Contact Probe from the same company, with a leaf clip attachment. Apart from the light source, the leaf clip also had a built-in Spectralon (Labsphere, North Sutton, NH, USA) 100% reflectance standard, which facilitated regular white standard referencing of measurements (after each reflectance measurement). These measurements were made to investigate the reaction of leaf pigment to the water stress treatment by comparing the spectral reaction of the control vines with that of the water stressed treatment vines. The photochemical reflectance index [PRI=(R531–R570) / (R531+R570)] (Gamon *et al*., 1997) was calculated for each sample. This index was also utilised by Blanchfield *et al*. (2006) to investigate the reaction of photoprotective pigments in Phylloxera-infested grapevines. In their study, as well as that of Sims and Gamon (2002), it was found that the PRI is sensitive to the total carotenoid and chlorophyll ratio, as well as to changes in the xanthophyll cycle pigments.

Trial evaluation

The trial layout was a randomised split plot design with the irrigation regime the main plot and the inoculation treatment the sub-plot. For each irrigation regime, 24 plants were inoculated with each Botryosphaeriaceae spp. or subjected to the control inoculations of water or *T. harzianum,* a total of 144 plants per irrigation regime. During mid-winter 2006 (21–23 June), eight months after inoculation, vines were uprooted and the roots thoroughly washed to remove the potting medium. After washing, vines were left for 10 min to dry. After drying, the remaining leaves on the shoots were stripped off before determining the root, shoot and plant mass (plant mass=root + shoot mass) of each vine. Stems with inoculated pruning wounds, were taken back to the laboratory for further evaluation. In the laboratory the inoculated pruning wound stubs and the stems of 16 randomly selected vines per treatment per irrigation regime were split longitudinally to reveal any internal lesions in the pith and/or xylem tissue. Any lesions observed were measured using a digital calliper (Mitutoyo 500-196-20) and the lesion lengths recorded. The pruning wound stubs and stems of the remaining eight vines were used for back isolations. These vine sections were surface sterilised by immersion in 70% ethanol for 30 s, 1 min in 3.5% NaOCl and again 30 s in 70% ethanol. After sterilisation, pruning wound stubs and stems were split longitudinally and any internal lesions observed in the pith and/or xylem tissue were measured and the lesion lengths recorded. In order to determine whether the lesions were caused by the pathogens inoculated, five 0.5×1 mm pieces of wood were removed aseptically from the tissue at the leading edges of the lesions and plated on 90 mm Petri dishes containing potato dextrose agar (PDA, Biolab, Wadeville, South Africa) amended with 0.04 g L^{-1} streptomycin sulphate to inhibit bacterial growth. After isolation, the dishes were incubated at 23°C for 2 weeks before identifying the isolated fungi based on morphological and cultural characteristics (van Niekerk *et al*., 2004).

Statistical analysis

The recorded root, shoot and plant mass and measured lesion lengths were subjected to analysis of variance (ANOVA) and Student's t-test for the least significant difference. Linear regression analysis of the mean root, shoot and plant mass, as well as the mean lesion length for each irrigation regime, were also conducted with Student's t-test for the least significant difference used to compare the slopes and intercepts of the linear regression lines. Physiological processes measured data were also subjected to ANOVA and Student's t-test for

the least significant difference. Statistical analyses were done using SAS (SAS Institute Inc. NC, USA) and Statistica 7.0 (Statsoft Inc., Tulsa, USA).

Results

Soil moisture measurements and rainfall

Soil moisture measurements from 13 January 2006 (mid-summer) until 13 May 2006 (late autumn) are shown in Figure 1. The initial scheduling of the different irrigation regimes to commence at 8 am every day led to clear differences in the percentage volumetric water content (%VWC) in the potting bags subjected to the 100, 50 and 20% irrigation regimes, with the differences between the 100 and 70% regimes being less clear (Figure 1). However, physiological measurements taken on 20 January (mid-summer) did not show significant differences in the stomatal conductance and the photosynthetic rate between vines receiving the 50%, 70% and optimal irrigation regimes, while there were significant differences between the 20% and the optimally irrigated vines. Irrigation cycles were reduced, with the different irrigation regimes still commencing at 8 am, but now only on Mondays, Wednesdays and Fridays. However, based on the %VWC measurements, this scheduling caused the vines in the optimal irrigation regime to be sub-optimally irrigated (Figure 1). A second irrigation cycle, starting at 12 noon, was therefore introduced on 10 February 2006 (late summer) when all irrigation regimes operated on Mondays, Wednesdays and Fridays (Figure 1). This schedule corrected the suboptimal irrigation problem, but the interval in irrigation from Friday to Monday was still too long, causing the vines in the optimal irrigation regime to suffer wa-

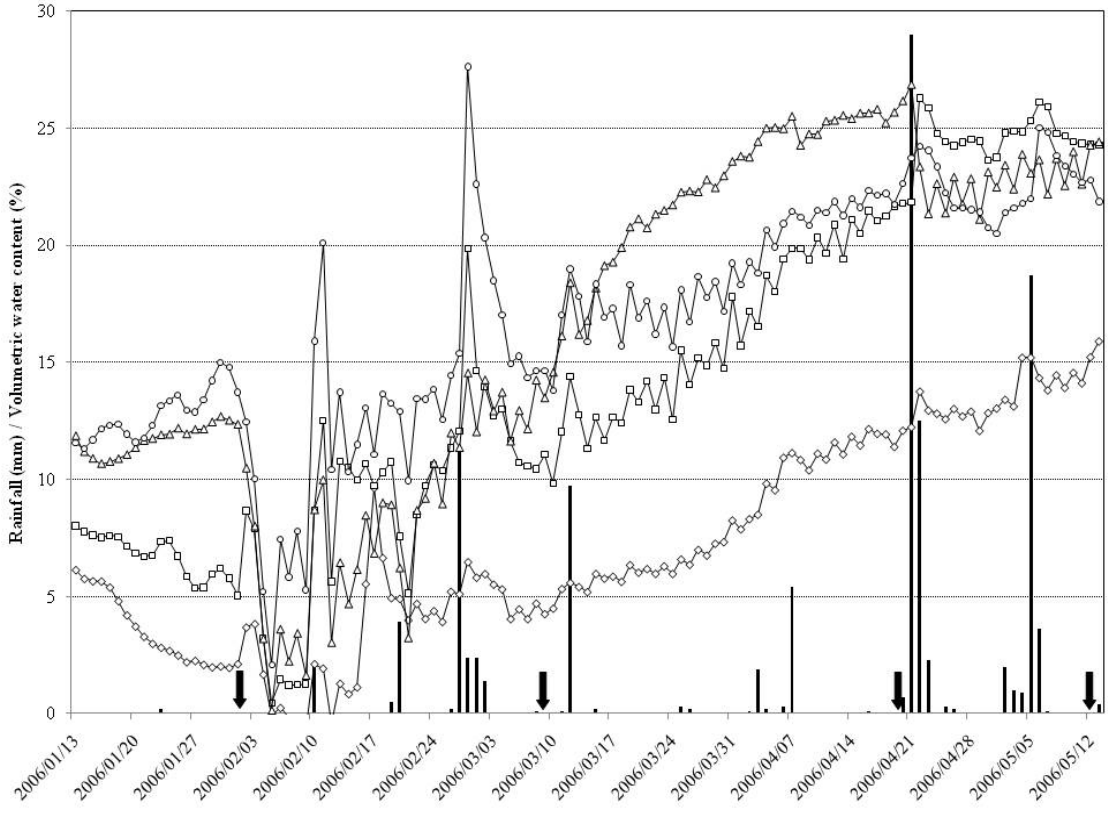

Figure 1. Daily rainfall (—) recorded at the trial site and mean daily volumetric water content (%) in the potting mixture of potted grapevines receiving 20% (◊), 50% (□), 70% (Δ) and optimal (100%) (○) irrigation as measured and recorded by ECH_2O soil moisture probes and Em50 data loggers from 13 January 2006 until 13 May 2006. Dates on which physiological measurements were taken are indicated by black arrows.

ter deficits during the weekend (Figure 1). The irrigation schedule was consequently changed again on 3 March 2006 (early autumn) to irrigation commencing every second day at 12 noon. This schedule remained unchanged until 12 May 2006 (late autumn) when the different irrigation regimes were terminated. While the irrigation schedules were in effect, the %VWC in the potting bags of the 20% irrigation regime was always much lower than the %VWC in the other three irrigation regimes (Figure 1). However, in the period between 10 March (early autumn) and 21 April 2006 (mid autumn) the %VWC in the potting bags of the 70% irrigation regime was actually higher than the %VWC in the potting bags of the 50 and 100% irrigation regimes, and these two regimes did not differ clearly in their %VWC (Figure1). From 21 April until 13 May 2006 the differences in %VWC of potting bags in the 100, 70 and 50% irrigation regimes were almost negligible, while the potting bags in the 20% irrigation regime still had a noticeably lower %VWC (Figure 1). Between 13 May 2006 and 21 June 2006, when the trial was evaluated, the only watering the plants received was a the rainfall at the trial site, which amounted to 221.4 mm.

Between January and May 2006, when the different irrigation regimes were applied, rainfall was recorded at the trial site on 34 days with a total of 114.8 mm of rainfall recorded. The daily rainfall on these days varied from 0.2 and 29.0 mm. As expected, this rainfall produced relatively similar increases in the %VWC for all the irrigation regimes as seen on 26 February–2 March, 12 March, 6, 7 April, 20–23 April and also on 2–7 May 2006 (Figure 1).

Measurements of root, shoot and plant mass

Analysis of variance of the root, shoot and plant mass measurements revealed significant ($P \leq 0.05$)

Table 1. Mean intercept (A), slope (B) and R^2-values of equations (y=ax + b) following linear regression analysis of the means of root, shoot or plant mass over the irrigation regime of potted grapevines subjected to control treatment or inoculated with *Neofusicoccum australe*, *N. parvum*, *Lasiodiplodia theobromae* or *Diplodia seriata*.

Treatment	R^2-value	Intercept[a] A	Slope[a] B
Root mass			
N. australe	0.689	84.74 ab	0.72 ab
D. seriata	0.788	93.47 a	0.64 b
N. parvum	0.429	103.15 a	0.41 b
L. theobromae	0.821	80.07 ab	0.70 b
Eco-77	0.867	95.67 a	0.64 b
Water	0.769	61.51 b	1.22 a
LSD		28.936	0.507
P-value		0.0988	0.0695
Plant mass			
N. australe	0.710	103.78 ab	0.87 ab
D. seriata	0.935	113.09 ab	0.85 ab
N. parvum	0.547	125.59 a	0.53 b
L. theobromae	0.886	98.94 ab	0.85 ab
Eco-77	0.943	117.65 a	0.80 ab
Water	0.820	82.58 b	1.38 a
LSD		31.953	0.561
P-value		0.1394	0.0974

[a]Root, and plant mass means followed by the same letter are not significantly different ($P \leq 0.05$).

irrigation regime × treatment interactions for root (df=15; F=1.99; P=0.0144) and plant mass (df=15; F=1.76; P=0.0383), but not for shoot mass (df=15; F=1.45; P=0.1211) (ANOVA not shown). The linear regression analysis of the mean root mass over irrigation regime data resulted in good fits for all treatments (R^2-values from 0.689–0.867), with only the R^2-value of the *N. parvum* line being low at 0.429 (Table 1). The intercepts of the lines fitted to the mean root mass of the *N. australe*, *L. theobromae*, *D. seriata*, *N. parvum* and Eco-77 inoculated plants did not differ significantly, but intercepts of the latter three were significantly higher than the intercept of the water treated plants (Table 1). A similar trend was seen in the slopes of the abovementioned lines, with the water treatment line having a significantly higher positive slope (Table 1). Except for *N. parvum*, the linear regression lines fitted to the mean plant mass over the irrigation regime data had very good R^2 -values between 0.710 and 0.943 (Table 1) and indicated similar trends in the intercepts and positive slopes to the trends observed for the root mass.

For shoot mass, significant effects were observed for the treatment (df=5; F=3.44; P=0.0046), as well as for the irrigation regime (df=3; F=46.26; P<0.0001) (ANOVA not shown). *N. australe* and *L. theobromae* inoculated vines had mean shoot masses significantly (LSD=2.821) lower than the mean shoot mass of the water-treated plants (27.90 g and 27.63 g respectively), while *D. seriata* and *N. parvum* (31.91 g and 29.40 g respectively) did not differ from the control treatments (water and Eco-77; 31.17 g and 31.37 g respectively). Vines receiving optimal and 70% irrigation had mean shoot masses of 34.96 g and 34.37 g, respectively, which were significantly (LSD=5.634) higher than the vines receiving the 20 and 50% irrigation regimes (26.75 g and 23.59 g respectively).

Measurements of vine physiology

Analysis of variance of the stomatal conductance and the photosynthetic rate data detected a significant (P≤0.10; ANOVA not shown) effect of irrigation regime on stomatal conductance and the photosynthetic rate on 20 January (df=3, F=4.57, P=0.0235 and df=3, F=2.86, P=0.816 respectively; ANOVA not shown) and 9 March (df=3, F=2.83, P=0.0597 and df=3, F=5.20, P=0.0066 respectively; ANOVA not shown), as well as on stomatal conductance on 2 February (df=3, F=10.29,

Table 2. Mean stomatal conductance and photosynthetic rate of potted grapevines subjected to 20, 50, 70% or optimal (100%) irrigation regimes as measured on 20 January, 2 February, 9 March and 20 April 2006.

Data surveyed/date	Irrigation regime[a, b]			
	20%	50%	70%	100%
Stomatal conductance (mmol/m^2/s)				
20 January 2006	195.8 b	436.0 a	339.0 ab	376.2 a
2 February 2006	114.3 b	142.8 b	119.5 b	222.8 a
9 March 2006	253.9 b	326.4 a	285.4 ab	320.4 a
20 April 2006	107.8 a	84.7 a	109.5 a	111.3 a
Photosynthesis (μmol/m^2/s)				
20 January 2006	7.4 b	10.5 a	10.0 ab	10.3 a
2 February 2006	6.4 a	5.8 ab	3.9 b	5.3 ab
9 March 2006	7.4 b	10.6 a	10.7 a	10.8 a
20 April 2006	5.4 a	4.8 a	5.7 a	4.6 a

[a] See Table 1.
[b] Irrigation regime calculated as the percentage of the optimal irrigation volume based on the physical properties of potting medium.

P=0.0012, ANOVA not shown). On the former dates, the stomatal conductance and the photosynthetic rate of the vines receiving optimal irrigation were significantly higher than those of the vines receiving the 20% irrigation regime (Table 2). On 2 February, the stomatal conductance of vines receiving the optimal irrigation regime was almost double that observed in vines receiving the 20, 50 and 70% irrigation regimes (Table 2). These differences were not mirrored by the photosynthetic data. The measurements taken on 20 April, the last date, did not show any significant differences in the stomatal conductance or photosynthetic rate between the plants of the different irrigation regimes (Table 2).

Results from the leaf spectrometry, collected on 9 March along with the physiological measurements, showed that the PRI values correlated weakly, although significantly (R^2=0.229; P=0.0101), with the photosynthetic rate values (Figure 2), whereas stomatal conductance was not significantly correlated with the PRI values (R^2=0.010; P=0.6000). Statistical analysis of the data collected on this sampling date also showed significantly lower PRI values with the 20% irrigation regime, compared with the other regimes (Figure 3). Spectral measurements were also conducted on 12 May, without measuring other physiological parameters, and they indicated significantly lower values of PRI for both the 20% ($P\leq0.05$) and the 100% ($P\leq0.10$) irrigation regimes (Figure 4).

Lesion measurements

Analysis of variance of the lesion measurements revealed significant ($P\leq0.05$) irrigation regime × treatment interactions for pruning wound pith lesions (df=15, F=2.71, P=0.0005) and stem xylem lesions (df=15, F=10.03, $P<0.0001$), but not for pruning wound xylem lesions (df=15, F=0.92, P=0.5409) (ANOVA not shown). However, a significant effect (df=5, F=3.98, P=0.0015) of treatment on pruning wound xylem lesions was seen (ANOVA not shown). The linear regression lines of the analysis of the mean stem xylem lesion over irrigation regime had, for all pathogen treatments, good R^2-values of between 0.898 and 0.979 (Table 3; Figure 5). The R^2-values of the Eco-77 and water treatment lines were much lower at 0.273 and 0.562 respectively. The intercepts of the pathogen treatment lines (18.2 to 21.4) were significantly higher than the intercepts of the water (4.0) and Eco-77 treatment (3.8) lines. The intercepts of the different pathogen lines were very similar, with only the intercept of the *L. theobromae* line (21.4) being significantly higher than the *D. seriata* line (18.2). The slopes of all the pathogen lines were also negative (-0.12 to -0.14) and differed significantly from the slopes of the lines for the Eco-77 and the water treatments (-0.004 and -0.009 respectively). The longest lesions therefore occurred in the stem xylem tissue of plants in the 20% irrigation regime, with the lesion length decreasing with increasing irrigation volume, the shortest lesions occurring in the vines with the optimal irrigation regime.

Linear regression analysis of the mean pruning wound pith lesions over the irrigation regime revealed regression lines for the *N. australe*, *D. seriata* and *L. theobromae* treatments that had good R^2-values of 0.819, 0.868 and 0.941 respectively, similar to the regression lines obtained for the stem xylem lesions (Table 3; Figure 6). The R^2-values for the *N. parvum*, Eco-77 and water treatment lines were much lower at 0.249, 0.015 and 0.112 respectively. The intercepts of the four pathogen treatment lines were not significantly different (7.2 to 8.6), but were significantly higher than the intercepts of the control treatment lines (2.0 to 2.9). The slopes of all the pathogen treatment lines were also negative (-0.03 to -0.05), although not as steep as the slopes of the lines fitted to the stem xylem lesion data (Table 3; Figure 5). However, the negative slopes of the treatment lines again indicated that, as with the stem xylem lesions, lesion length in the pruning wound pith tissue decreased with increasing irrigation volume. Slopes for the lines for the Eco-77 and water treatments were relatively flat (0.003 to -0.003, respectively).

Although no significant irrigation regime × treatment interaction was observed for pruning wound xylem lesions (df=15, F=0.92, P=0.5409, ANOVA not shown), mean lesion lengths also increased with decreasing irrigation volume. The mean lesion length was not significantly different between irrigation regimes. In vines growing under the 20% irrigation regime, the mean lesion length was 2.05 mm, in vines with the 50% irrigation regime, 1.93 mm, in vines with the 70% ir-

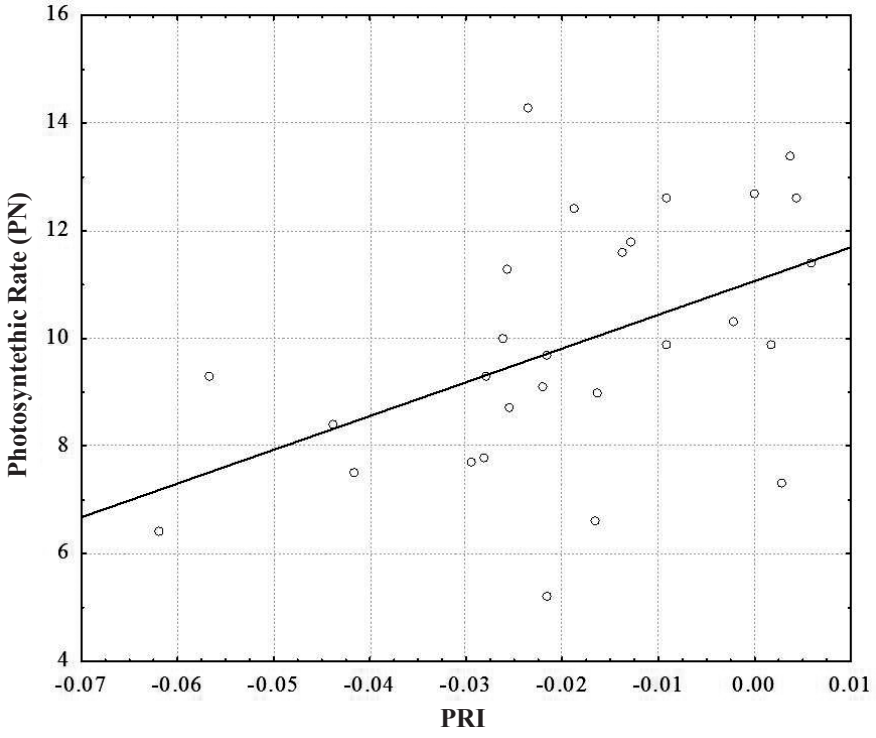

Figure 2. Significant, positive linear correlation observed on 9 March 2006 between the photosynthetic rate and the PRI values of potted grapevines receiving 20, 50, 70% and optimal (100%) irrigation (PRI:PN: R^2=0.2286; P=0.0101).

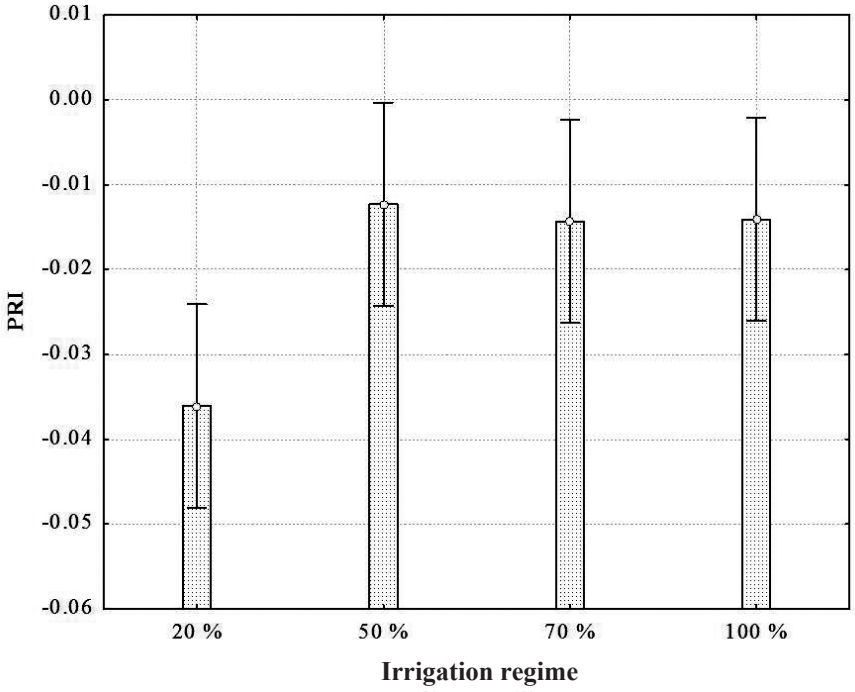

Figure 3. Mean photochemical reflective index (PRI) values as measured on 9 March 2006 for potted grapevines receiving 20, 50, 70% and optimal (100%) irrigation. Vertical bars denote confidence intervals at P=0.05.

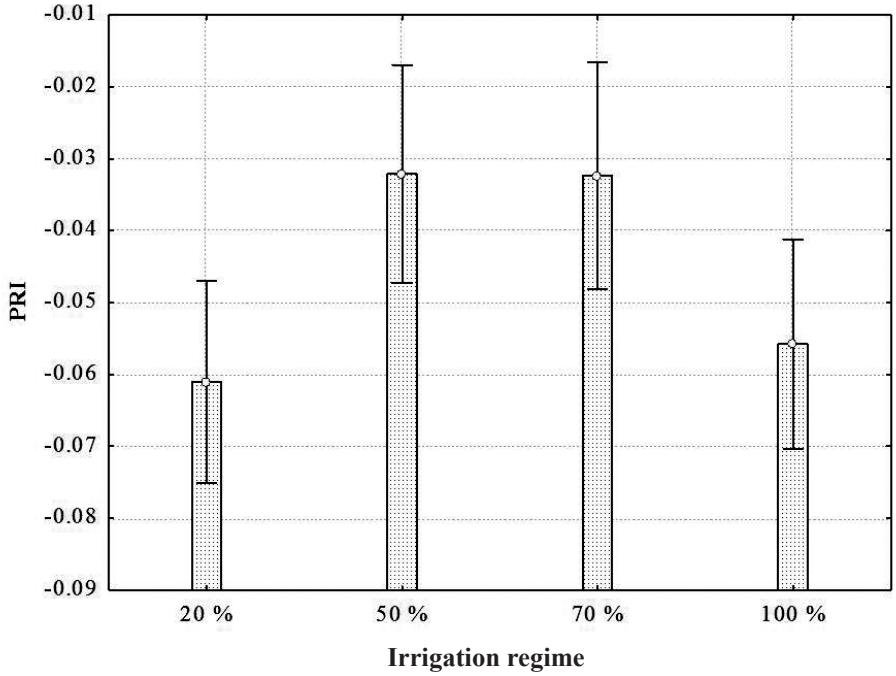

Figure 4. Mean photochemical reflective index (PRI) values as measured on 12 May 2006 for potted grapevines receiving 20%, 50%, 70% and optimal (100%) irrigation. Vertical bars denote confidence intervals at P=0.05.

Table 3. Mean intercept (A), slope (B) and R^2-values of equations (y=ax + b) following linear regression analysis of mean lesion length of the stem xylem and the pruning wound pith recorded in potted grapevines subjected to control treatment or inoculated with *Neofusicoccum australe*, *N. parvum*, *Lasiodiplodia theobromae* or *Diplodia seriata* over the 20, 50, 70% or optimal (100%) irrigation regimes .

Treatment	R^2-value	Intercept [a] A	Slope [a] B
Stem xylem lesion			
N. australe	0.956	18.71 ab	-0.14 b
D. seriata	0.976	18.24 b	-0.12 b
N. parvum	0.979	19.84 ab	-0.14 b
L. theobromae	0.898	21.38 a	-0.14 b
Eco-77	0.273	3.77 c	0.00 a
Water	0.562	4.05 c	-0.01 a
LSD		2.771	0.038
P-value		<0.0001	<0.0001
Pruning wound pith lesion			
N. australe	0.819	8.58 a	-0.05 c
D. seriata	0.868	7.19 a	-0.03 bc
N. parvum	0.249	7.20 a	-0.03 bc
L. theobromae	0.941	7.59 a	-0.03 bc
Eco-77	0.015	2.04 b	0.00 a
Water	0.112	2.85 b	0.00 ab
LSD		2.150	0.029
P-value		<0.0001	0.0139

[a]Means for stem, pruning wound pith and xylem lesions followed by the same letter are not significantly different (P≤0.05).

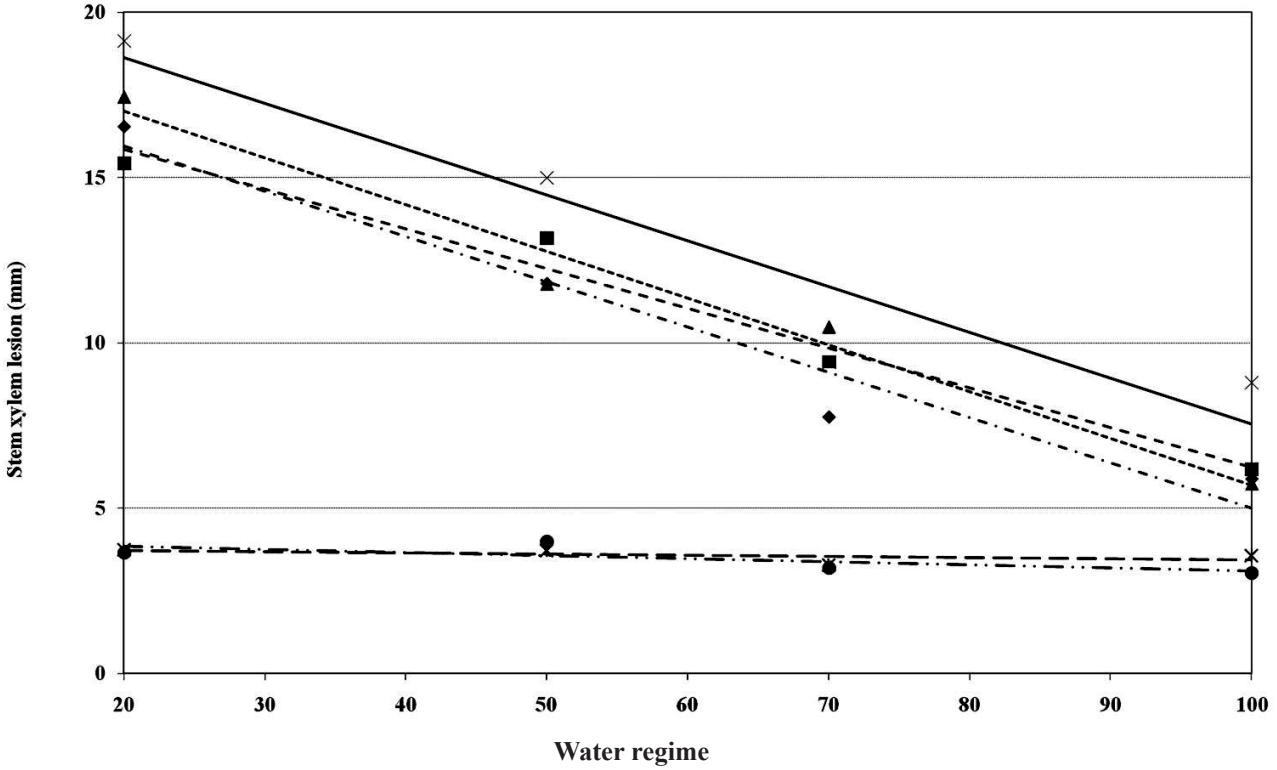

Figure 5. Linear regression lines [Eco-77 (— — —), water (— · · —), *Lasiodiplodia theobromae* (——), *Neofusicoccum australe* (— · —), *N. parvum* (- - -), *Diplodia seriata* (– – –)] fitted to the mean stem xylem lesions found in the stems of potted grapevines receiving the control treatments, Eco-77 (x) or water (●), or inoculated with *N. australe* (♦), *D. seriata* (■), *N. parvum* (▲), *L. theobromae* (x) and receiving 20, 50, 70% and optimal (100%) irrigation.

rigation regime, 1.34 mm, and in vines receiving optimal irrigation regime, 0.88 mm.

As for the mean pruning wound xylem lesions, the four species in the Botryosphaeriaceae treatments all caused lesions in the pruning wound xylem tissue of inoculated vines that were significantly longer than the lesions in the water (0.73 mm) or in Eco-77 (0.48 mm) treated vines (LSD=1.043). Although the lesion lengths were not significantly different between the four Botryosphaeriaceae species, *L. theobromae* caused the longest lesions in the pruning wound xylem (2.36 mm), followed by *D. seriata* (1.98 mm), *N. australe* (1.89 mm) and *N. parvum* (1.84 mm). In the case of the mean stem xylem lesions, the four Botryosphaeriaceae treatments again caused lesions significantly longer than the lesions in the water (3.47 mm) or in Eco-77 (3.57 mm) treated vines (LSD=1.040). *L. theobromae* caused significantly longer lesions in the stem xylem (13.09 mm) than the other three Botryosphaeriaceae spp. *D. seriata* (11.05 mm), *N. australe* (10.52 mm) and *N. parvum* (11.36 mm) caused lesions that were not significantly different, but they were smaller than the lesions produced by *L. theobromae*.

Discussion

Several previous studies have reported that when plants have been, or are being subjected to water stress, disease and symptoms caused by various Botryosphaeriaceae spp. become more severe (Schoeneweiss, 1981; Ma and Michailides, 2001; Stanosz *et al*., 2001; Luque *et al*., 2002). This effect of water stress has been attributed to the water deficit inhibiting plant growth and plant physiological processes and therefore the plant's

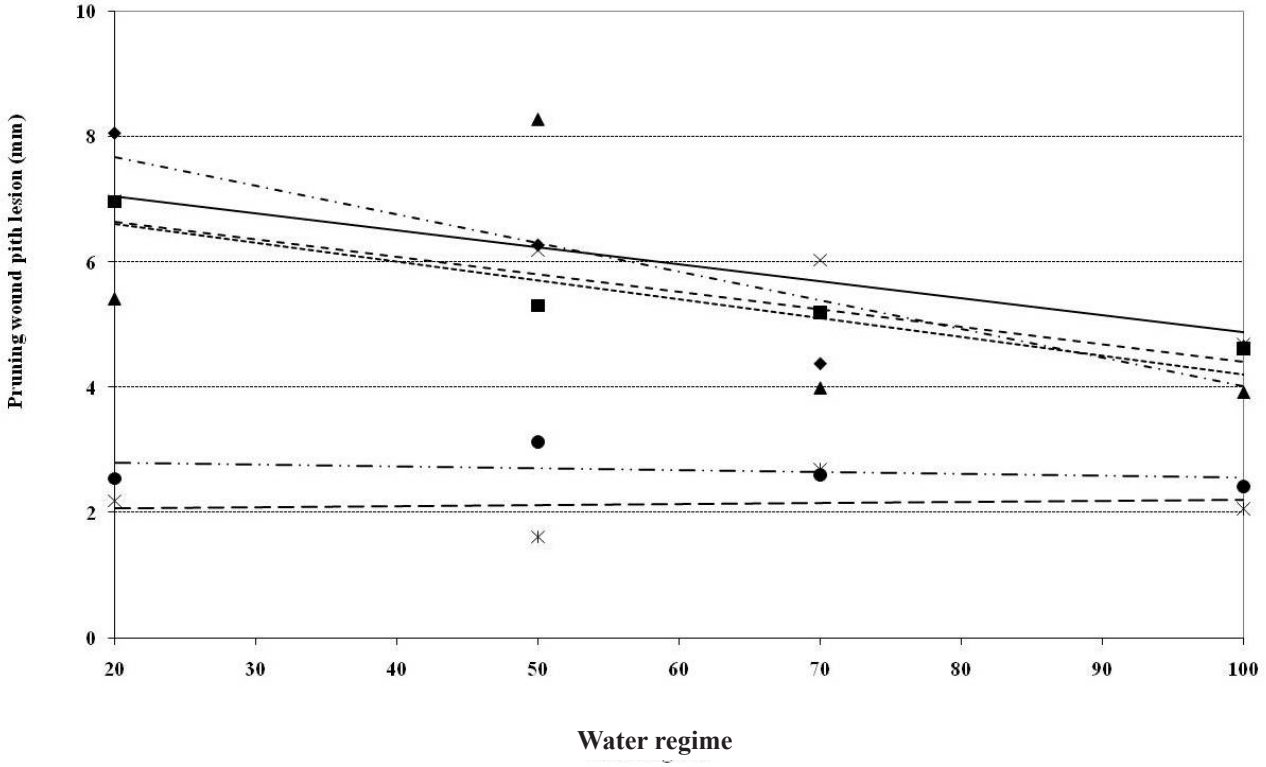

Figure 6. Linear regression lines [Eco-77 (— — —), water (— · · —), *L. theobromae* (——), *N. australe* (— · —), *N. parvum* (- - -), *D. seriata* (– – –)] fitted to the mean pruning wound pith lesions observed in the stems of potted grapevines subjected to the control treatments, Eco-77 (**x**) or water (●), or inoculated with *N. australe* (♦), *D. seriata* (■), *N. parvum* (▲), *L. theobromae* (**x**) and receiving 20, 50, 70% and optimal (100%) irrigation.

ability to defend itself against pathogen attack (Schoeneweiss, 1978; Maia Souza and Cardoso, 2003; Desprez-Loustau *et al*., 2006). In the case of grapevines, where species in the Botryosphaeriaceae are important pathogens (van Niekerk *et al*., 2004; 2006), field observations have led researchers to think that infection of vines are even more severe conditions of water stress, although this hypothesis has not been corroborated by scientific proof. The current study, is the first study to prove these field observations.

The study found that root and vine mass increased linearly with increased irrigation. Vines growing under the two highest irrigation regimes had significantly higher shoot masses than the vines growing under the two lowest irrigation regimes. These results are consisted with previous findings on other hosts, where it was found that water-stressed plants were smaller than non-stressed plants (Maia Souza and Cardoso, 2003; Desprez-Loustau *et al*., 2006). Not only did a suboptimal irrigation regime reduce shoot mass, some of the species in the Botryosphaeriaceae also did so. *N. australe* and *L. theobromae* significantly reduced shoot mass of vines, as compared with the controls.

Stressed plants are smaller and more susceptible to severe disease expression, because the stress changes the plant physiology. The stomatal conductance and photosynthetic rate of the vines varied at different dates. Levels of both these processes were similar on 20 January 2006 and 9 March 2006. The measurements taken on 2 February 2006 and 20 April 2006 were similar and higher than the previous measurements. Investigation revealed that solar irradiation levels were much higher on 2 February 2006 and on 20 April 2006 than at the other two dates (results not shown). High solar radiation depresses both stomatal conductance and photosynthetic activity (Winkel and Rambal, 1993) and this may explain

the lower levels recorded on 2 February 2006 and 20 April 2006.

Rainy events were fairly frequent at the trial site while the irrigation regimes were carried out. In previous studies on water stress in grapevines it was found that rainfall led to a temporary increase in the leaf water potential, with a consequent reduction in water stress to such an extent that the leaf water potentials of stressed and non-stressed vines became alike (Smart, 1974; De Souza *et al*., 2005). Soil moisture measurements indicated that rain events temporarily increased the %VWC in the potting bags. From the end of February 2006 onwards the %VWC in the potting bags tended to rise with all the irrigation regimes, probably because of the regular rainfall that occurred (Figure 1). Heavy rainfall in late April and early May 2006 probably caused the %VWC in the potting bags of the 50, 70 and 100% irrigation regimes to become very similar during the latter stages of the trial. Nonetheless, vines growing under the 20% irrigation regime consistently exhibited markedly lower %VWC (Figure 1).

The physiological measurements indicated that vines in the 100% irrigation regime had significantly higher rates of stomatal conductance than vines in the 50% irrigation regimes. Vines with 100% irrigation volume also had significantly higher shoot and root masses than vines in the 50% irrigation regime. This shows that the larger vines in the 100% irrigation regime were physiologically more active, so that the %VWC in the potting bags became lower despite the combined effect of rainfall plus optimal irrigation. Vines growing under the 50% irrigation regime had lower levels of physiological activity, as a result of which they took up less water from the potting medium and this, combined with the effect of irrigation and rainfall could have increased the %VWC in the potting bags. Vines in the 70% irrigation regime were similar in size and physiological activity to optimally irrigated vines. .

The effect that rainfall had on the physiological measurements can clearly be seen from the measurements taken on 20 April 2006. On this day 0.70 mm rain was recorded at the trial site, after which no differences were seen in the stomatal conductance and photosynthetic rate with any of the irrigation regimes, indicating a an absence of water stress in all vines (Smart, 1974; Poni *et al*., 1994; De Souza *et al*., 2005; Christen *et al*., 2007).

Despite the variation observed between measurement days, differences were constantly seen between vines with the lowest irrigation regime and optimally irrigated vines. Vines receiving the least water had significantly lower stomatal conductance and photosynthetic rate than optimally irrigated grapevines. The lower levels of these two physiological processes therefore translated to higher physiological stress in the 20% irrigated vines. Leaf spectrometry measurements taken on 9 March 2006 and 12 May 2006 supported the other physiological measurements. The PRI value of the 20% irrigated vines was significantly lower than the PRI of optimally irrigated vines. A low PRI values corresponded to a lower photosynthetic rate that is related to a higher carotenoid/chlorophyll ratio (Blanchfield *et al*., 2006). Several previous studies on water stress in grapevines (Flexas *et al*., 1999; Choné *et al*., 2001; Paranychianakis *et al*., 2004; Cifre *et al*., 2005; Christen *et al*., 2007) reported that these physiological results indicate that vines receiving 20% irrigation had greater physiological stress than optimally irrigated vines.

Based on the plant mass and the physiological measurements, suboptimal irrigation of vines had a stressing effect on the potted grapevines, and this may have caused vines inoculated with the various Botryosphaeriaceae species to exhibit more severe symptoms of infection. Inoculating a vine by wounding it and inserting a colonised agar plug in the wound, as was done with the stem inoculations in this study, is a very aggressive inoculation technique. This technique was also used by van Niekerk *et al*. (2004) and the stem lesions produced in the current study corresponded to those other studies in the length and nature of the lesions. The natural way for Botryosphaeriaceae spp. to infect grapevines is by airborne propagules that land on pruning wounds. In the current study, pruning wounds were also inoculated using spore suspensions in an attempt to simulate natural infection. This method produced very small lesions in the pruning wound pith and xylem. Interestingly, the significant irrigation regime × treatment interactions observed for pith lesion lengths corresponded to the longer stem lesions, but the pruning wound xylem lesions were too small to show significant effects for this interaction. This is consistent with Feliciano and Gubler (2001) who

found that *Phaeoacremonium (Pm.) inflatipes* and *Pm. aleophilum* spread more rapidly in the pith of inoculated grapevine shoots than in the xylem. The current study indicates that the pith tissue is more susceptible to the Botryosphaeriaceae spp. than the xylem, and also possibly that the pith is more susceptible to water stress.

The linear regression analysis of the lesion data over the irrigation regime clearly showed that, in the case of pruning wound lesions of both pith and stem xylem, the largest lesions occurred in inoculated vines growing under the 20% regime. The negative slopes of the regression lines fitted to this data also indicated that the mean lesion length in pruning wound pith and stem xylem tissue decreased with increasing irrigation, although the rate of decline was slower in the pith. In the xylem lesions the irrigation regime effect was not as pronounced, but the four pathogen treatments again caused significantly larger lesions than the control treatments. This study, in line with studies on other woody hosts (Schoeneweiss, 1981; Ma and Michailides, 2001; Stanosz *et al*., 2001), clearly demonstrates that in water stressed grapevines infection and symptom caused by species in the Botryosphaeriaceae were more severe.

This effect could possibly be attributed to lower host defence reaction in sub-optimal irrigated vines, brought about by lower stomatal conductance and photosynthetic rate as recorded during the trial. The study clearly indicates that in grapevines, as in other woody hosts, Botryosphaeriaceae spp. cause much more severe symptoms in stressed than in unstressed vines, and hence any stress in vineyards should be avoided.

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