

Temporal susceptibility of grapevine pruning wounds to trunk pathogen infection in South African grapevines

JAN M. VAN NIEKERK¹, FRANCOIS HALLEEN^{1,2} and PAUL H. FOURIE^{1,3}

¹Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa

²Plant Protection Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa

³Citrus Research International, P.O. Box 2201, Stellenbosch 7602, South Africa

Summary. *Eutypa lata* and *Phaeoconiella chlamydospora*, as well as several species in Botryosphaeriaceae, *Phomopsis* and *Phaeoacremonium* are known trunk pathogens of grapevines, which use pruning wounds as infection portals. Duration of pruning wound susceptibility to some of these pathogens was largely unknown. To address this question, plants of the cv. Chenin Blanc in a vineyard in the Stellenbosch area of South Africa were pruned at two stages, and then spray-inoculated with spore suspensions of *E. lata*, *Pa. chlamydospora*, *Neofusicoccum australe* and *Phomopsis viticola* directly after pruning, and 1, 2, 3, 7, 10, 14, 17 and 21 days after pruning. Eight months after inoculation, pathogen incidence in the treated wounds was determined by means of isolation from the pith and xylem tissue of treated plants. Lesions observed in these tissues were also measured and recorded. Results indicated that, irrespective of pathogen inoculated, pathogen incidence in the inoculated pruning wounds of both mid- and late winter declined with increasing wound age. The rate of decline was much slower in 2004 compared to 2005; however, wounds remained susceptible for 3 or more weeks after pruning in both years. Late winter wounds were more susceptible to infection than wounds made earlier in the season, while xylem tissue of pruning wounds generally proved more susceptible to all pathogens compared to exposed pith tissue.

Key words: *Eutypa*, *Phaeoconiella*, Botryosphaeriaceae, *Phomopsis*, *Phaeoacremonium*.

Introduction

Several species within the Botryosphaeriaceae (van Niekerk *et al.*, 2004, 2010b; Crous *et al.*, 2006), *Phomopsis* (van Niekerk *et al.*, 2005) and *Phaeoacremonium* (Mostert *et al.*, 2006), as well as *Phaeoconiella chlamydospora* (W. Gams, Crous & M.J. Wingf. & L. Mugnai) Crous & W. Gams (Mugnai *et al.*, 1999) and *Eutypa lata* Tul. & C. Tul. (Munkvold *et al.*, 1994) have been shown to be part of a pathogen complex causing trunk diseases of grapevines (van Niekerk, 2008). Pruning wounds are known infection portals for all of these pathogens (Lehoczky, 1974, 1988; Ferreira *et al.*,

1989; Adalat *et al.*, 2000; van Niekerk *et al.*, 2005; Serra *et al.*, 2008; Rolshausen *et al.*, 2010). Several studies have indicated that air-borne inoculum of the respective pathogens is present in vineyards for long periods of time, especially when weather conditions are favourable for spore release and dispersal and inoculum is therefore available for infection of susceptible wounds (Moller *et al.*, 1977; Merrin *et al.*, 1995; Larignon and Dubos, 2000; Eskalen and Gubler, 2001; van Niekerk *et al.*, 2010a).

Due to the lack of curative control measures for trunk diseases and the importance of pruning wounds as infection portals, pruning wound protection has been the subject of numerous studies (Moller and Kasimatis, 1980; Ferreira *et al.*, 1991; Munkvold and Marois, 1993, John *et al.*, 2001; Halleen and Fourie, 2005; Sosnowski *et al.*, 2005;

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

Bester *et al.*, 2007). However, for the development of a successful pruning wound protection strategy, knowledge about the duration of pruning wound susceptibility to infection is important; especially to elucidate aspects such as the timing of treatment to prevent infection and duration of protection required. Pruning wound susceptibility has been studied for *E. lata*, *Diplodia seriata* De Not., *Pa. chlamydospora* and some *Phaeoacremonium* spp. associated with grapevine trunk diseases (Munkvold and Marois, 1995; Larignon and Dubos, 2000; Serra *et al.*, 2008). These studies indicated that pruning wounds made on grapevines early in the dormant season are more susceptible to *E. lata* infection and remain susceptible for a longer time in comparison with wounds made later in the dormant season, with wood age at time of pruning having no significant effect on wound susceptibility (Munkvold and Marois, 1995; Chapuis *et al.*, 1998).

Inoculation studies conducted by Adalat *et al.* (2000) and Larignon and Dubos (2000) revealed that both *Pa. chlamydospora* and *Phaeoacremonium aleophilum* W. Gams, Crous, M.J. Wingf. & Mugnai have the ability to infect pruning wounds. Similar to *E. lata*, pruning wound infection was more severe and susceptibility of a longer duration, when pruning was done early in the dormant season. In France, the period of susceptibility declined from 7–9 weeks with mid-winter pruning, to only 1–2 weeks with late-winter pruning (Larignon and Dubos, 2000). In California, these results were very similar to findings of Gubler *et al.* (2001), who found that pruning wounds remained susceptible to infection by *Pm. inflatipes*, *Pm. aleophilum* and *Pa. chlamydospora* for up to 4 months after pruning and that the shoot growth on infected spurs were also reduced. These findings were supported by those of Serra *et al.* (2008) that also found that pruning wounds remained susceptible for at least 16 weeks after pruning.

The aim of the present study was therefore to determine and compare the duration of pruning wound susceptibility to natural and induced infection by all major pathogens associated with grapevine trunk diseases under climatic conditions different from previous studies that had been conducted in northern hemisphere countries. Moreover, special attention was given to the first 3 weeks following pruning. This knowledge would

aid in the development of pruning wound protection strategies against all trunk disease pathogens in South African vineyards.

Materials and methods

Pruning and inoculation

Grapevines in a non-irrigated 18-year-old vineyard, cv. Chenin Blanc, in the Stellenbosch region of the Western Cape province of South Africa were spur-pruned to three buds per cane at two stages (mid- [July] and late-winter [August]) during the dormant seasons of 2004 and 2005. All pruning cuts were made at a distance of 1 cm above the third bud. At each stage, a total of 180 grapevines were pruned. Individual pruning wounds were spray-inoculated with 1 mL of a 1×10^4 spores mL⁻¹ spore suspension of either *E. lata*, *Pa. chlamydospora*, *Neofusicoccum australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips or *Phomopsis viticola* (Sacc.) Sacc. directly after pruning, and 1, 2, 3, 7, 10, 14, 17 and 21 days after pruning. At the different times of inoculation, pruning wounds were sprayed with 1 mL sterile water, or painted with a commercial non-fungicidal pruning wound sealant (ABE Tree Seal, Pruning Grade, ABE Construction Chemicals [Pty] Ltd, Cape Town, South Africa) as non-inoculated control treatments. At each wound age, a total of 20 (five single wound replicates on five different grapevines in four experimental blocks) pruning wounds were inoculated per pathogen, water-sprayed or painted with the wound sealant so that the different treatments were all repeated on a grapevine. *Phomopsis viticola* and *Pa. chlamydospora* inoculum was produced by growing the fungi on Petri dishes containing potato dextrose agar (PDA, Biolab, Wadeville, South Africa) and incubating for 2–4 weeks under constant near-UV light at 25°C. Inoculum of *N. australe*, which was shown to be a virulent species in the Botryosphaeriaceae occurring on grapevines (van Niekerk *et al.*, 2004), was produced *in vitro* according to the protocol described by van Niekerk *et al.* (2004). The *N. australe* isolate was plated out on water agar (WA, Biolab, Wadeville, South Africa) amended with 3 cm pieces of double-autoclaved pine-needles. The plates were incubated at 25°C under near-UV light in a 12 h light-dark regime for 2–4 weeks to induce pycnidia formation on the

pine needles. *Eutypa lata* inoculum was obtained from perithecia taken from stroma on diseased grapevine wood. Pieces of wood bearing stroma were soaked in water for 15 min after which the stromatic tissue covering the perithecia was removed with a scalpel to expose single perithecia. Again using a scalpel, the contents of single exposed perithecia were removed and placed in a glass bottle containing 10 mL autoclaved water. These bottles were subsequently shaken to release ascospores from the asci. A spore count was done of the 10 mL solution and the concentration adjusted to prepare the final spore suspensions used to spray-inoculate the pruning wounds.

Trial evaluation

Eight months after treatment, the distal internodes containing the treated pruning wounds were removed and taken to the laboratory for pathogen isolation. Pruning wound stubs were surface sterilised by immersion in 70% ethanol for 30 s, 1 min in 3.5% NaOCl and again for 30 s in 70% ethanol, before being split longitudinally. The occurrence of the inoculated pathogens in the xylem and pith tissues (isolation zones) directly beneath the pruning wound scar was subsequently determined. This was done by aseptically removing four tissue sections, 0.5×1.0 mm in size, from the xylem tissue on either side of the pith and also four from the pith tissue, and plating them out onto 90 mm Petri dishes containing PDA amended with 0.04 g L⁻¹ streptomycin sulphate to inhibit bacterial growth. Petri dishes were incubated at 25°C in an 8 h fluorescent light, 16 h dark regime for 2–4 weeks before morphological identification of the isolated fungi to species level in the case of *E. lata*, *Pa. chlamydospora* and *P. viticola* and to genus level in the case of Botryosphaeriaceae spp. inoculated as *N. australe*. The incidence of the inoculated pathogens were subsequently calculated for the xylem (percentage out of eight tissue sections) and pith tissues (percentage out of four tissue sections) and the whole pruning wound stub (percentage out of 12 tissue sections). Lesions observed as vascular browning in the pith and xylem tissue of the split pruning wound stubs were also measured and recorded.

Trial layout and statistical analysis

Trial was of complete randomised block design

with four block replications. Treatments were arranged as a split-plot design with the main plot a 2×4×3×9 factorial. The factors were two pruning times (July and August), four pathogens (*E. lata*, *Pa. chlamydospora*, *N. australe* and *P. viticola*), three treatments (paint, water and inoculated) and nine wound ages at treatment (0, 1, 2, 3, 7, 10, 14, 17 and 21 days after pruning). An experimental unit consisted of a single pruning wound and each treatment combination was replicated five times per block. The sub-plot treatments were two isolation zones (xylem and pith tissues) from which isolations were made onto PDA. The pathogen incidence data and lesion lengths were subsequently subjected to analyses of variance (ANOVA), Student's t-test for least significant difference, as well as linear regression analysis of pathogen incidence means over wound age at treatment using SAS (SAS Institute Inc., NC, USA).

Weather data

In the trial vineyard block, a Vantage Pro® weather station (Davis Instruments, Hayward, CA) was installed. The weather station recorded hourly measurement of rainfall, temperature, relative humidity and wind speed during the trial periods of 2004 and 2005.

Results

Pathogen incidence in treated pruning wounds

The analysis of variance of the pathogen incidence data showed a significant ($P=0.0286$) year × pruning time × wound age at treatment × treatment interaction, a significant ($P=0.0455$) pathogen × pruning time × isolation zone × treatment interaction and also a significant ($P=0.0250$) year × pathogen × wound age at treatment interaction.

The linear regression analysis of mean pathogen incidence over wound age at treatment for the year × pruning time × wound age at treatment × treatment interaction resulted in very low R^2 -values of between 0 and 0.0524 for the lines fitted to the mean pathogen incidence of the different treatments applied in July and August 2004 (Figure 1; Table 1). Wounds inoculated directly after pruning ($x=0$) in July, yielded significantly lower mean pathogen incidences compared

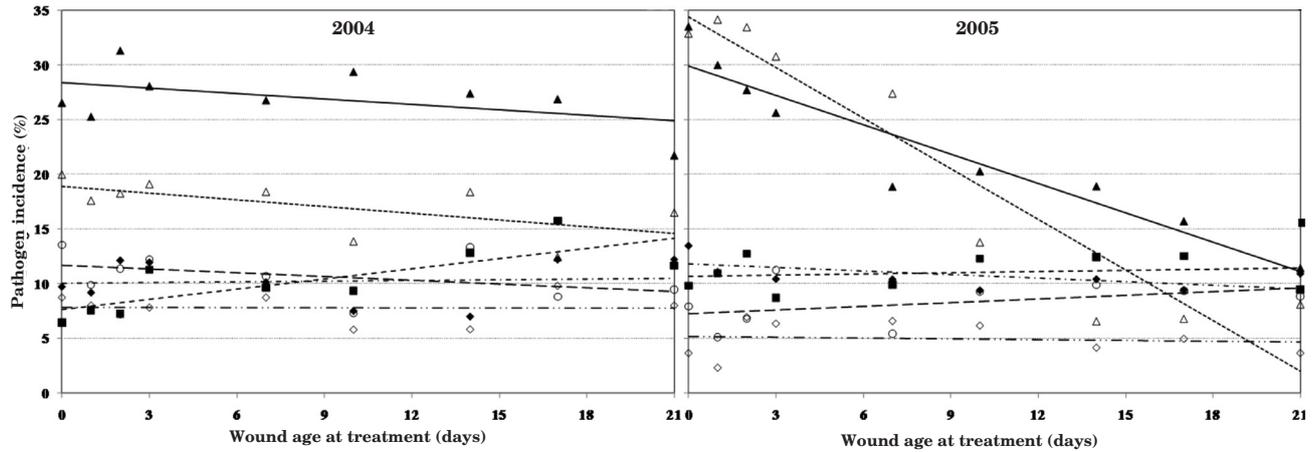


Figure 1. Linear regression lines (August inoculated [—]; July inoculated [- - -]; August painted [— —]; July painted [— -]; August water [- - -]; July water [- - -]) fitted to the mean pathogen incidence (August inoculated [▲]; July inoculated [△]; August painted [■]; July painted [○]; August water [◆]; July water [◇]) over wound age at treatment for the significant season × pruning time × wound age at treatment × treatment interaction observed in the 2004 and 2005 pruning seasons.

Table 1. Coefficients of equations ($y = a + bx$) following linear regression analysis of mean pathogen incidence in pruning wounds, which were made during July and August 2004 and 2005 and either painted, water treated or inoculated with *Eutypa lata*, *Phaeomoniella chlamydospora*, *Neofusicoccum australe* and *Phomopsis viticola* directly after pruning, 1, 2, 3, 7, 10, 14, 17 and 21 days after pruning, over wound age at treatment for the significant year × pruning time × wound age at treatment × treatment interaction.

Treatment	Pruning time	R ² -value	Intercept A ± SE ^a	Slope B ± SE
2004				
Inoculated	July	0.0524	18.86±1.63	-0.20±0.15
Inoculated	August	0.0335	28.40±1.69	-0.17±0.15
Paint	July	0.0079	11.67±2.41	-0.11±0.22
Paint	August	0.0488	7.61±2.58	0.31±0.23
Water	July	0.0000	7.80±2.22	0.00±0.20
Water	August	0.0003	10.04±2.37	0.02±0.22
2005				
Inoculated	July	0.7237	34.40±1.80	-1.54±0.16
Inoculated	August	0.2837	29.78±2.66	-0.89±0.24
Paint	July	0.0074	7.28±2.40	0.11±0.22
Paint	August	0.0004	10.68±3.63	0.04±0.33
Water	July	0.0011	5.16±1.36	-0.02±0.12
Water	August	0.0033	11.69±3.25	-0.10±0.30

^aStandard error

with wounds made in August. No significant differences in the slopes (B) of these lines were observed, with both indicating a slow decline in pathogen incidence with increasing wound age at treatment. Wounds that were treated with paint or water generally yielded similar pathogen incidences and slopes, but these were significantly lower than the inoculated treatments (Figure 1; Table 1).

In 2005, wounds inoculated directly after pruning ($x = 0$) yielded similar pathogen incidences in wounds made in July compared to August (Figure 1; Table 1). R^2 -values for these regression lines were 0.7237 for July and 0.2837 for August, and the slopes indicated a more rapid decline in pathogen incidence with increasing wound age at treatment compared to 2004. The decline was also significantly more pronounced in wounds made in July 2005, compared with August 2005. As was the case in 2004, mean pathogen incidences from the control treatments (water and paint) were significantly lower than the inoculated treatments. For both paint- and water-treated wounds, significantly higher pathogen incidences were recorded in August 2005, than in July 2005. As was the case in 2004, slopes for these regression lines indicated no change in the pathogen incidence with increasing wound age at treatment (Figure 1; Table 1).

Linear trend lines fitted to the mean pathogen incidence over wound age at treatment for the year \times pathogen \times wound age at treatment interaction indicated that the slopes of the pathogen trend lines for 2004 were between 0.070 and -0.010 (Figure 2; Table 2). In the case of wounds inoculated with *Phomopsis* spp. the mean pathogen incidence in inoculated pruning wounds increased slightly with increasing wound age at treatment. The mean pathogen incidence in wounds inoculated with *Pa. chlamydospora* remained almost constant with increasing wound age at treatment. Only in the case of pruning wounds inoculated with Botryosphaeriaceae spp. and *E. lata* did the mean pathogen incidence show a decline with increasing wound age at treatment (Figure 2; Table 2). However, in contrast to 2004, in 2005 all pathogen trend lines had negative slopes between -0.14 and -0.58 indicating a decline in the mean pathogen incidence in inoculated pruning wounds for all pathogens (Figure 2; Table 2). The mean pathogen incidence in *Phomopsis* spp. inoculated pruning wounds declined at the slowest rate with increasing wound age at treatment while the rate of decline in pruning wounds inoculated with *Pa. chlamydospora*, Botryosphaeriaceae spp. and *E. lata* were much more rapid (Figure 2; Table 2). The pathogen \times pruning time \times isolation zone \times treatment inter-

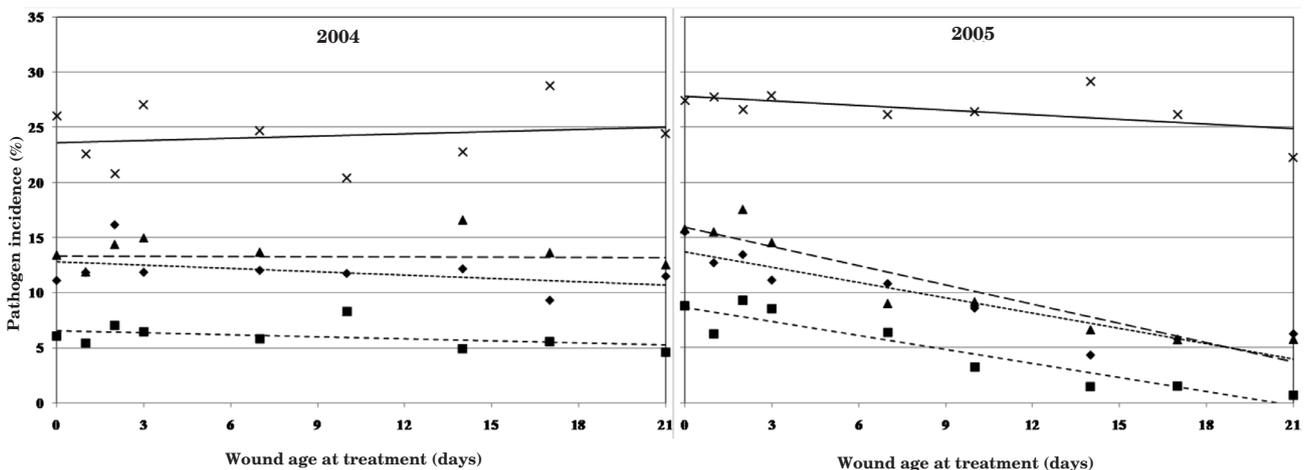


Figure 2. Linear regression lines (Botryosphaeriaceae spp. [---]; *E. lata* [---]; *Pa. chlamydospora* [—]; *Phomopsis* spp. [—]) fitted to the mean pathogen incidence (Botryosphaeriaceae spp. [◆], *E. lata* [■], *Pa. chlamydospora* [▲] and *P. viticola* [×]) over wound age at treatment for the significant year \times pruning time \times wound age at treatment interaction observed in the 2004 and 2005 pruning seasons.

Table 2. Equations of linear trend lines fitted to the mean pathogen incidence in pruning wounds, which were made during July and August 2004 and 2005 and inoculated with *Eutypa lata*, *Phaeoconiella chlamydospora*, *Neofusicoccum australe* and *Phomopsis viticola* directly after pruning, 1, 2, 3, 7, 10, 14, 17 and 21 days after pruning, over wound age at treatment for the significant year × pruning time × wound age at treatment interaction.

Pathogen	Line equation	R ² -values
2004 season		
<i>Phomopsis</i> spp.	$y = 0.07x + 23.62$	0.03
Botryosphaeriaceae spp.	$y = -0.01x + 12.81$	0.18
<i>E. lata</i>	$y = -0.06x + 6.53$	0.17
<i>Pa. chlamydospora</i>	$y = -0.01x + 13.31$	0.00
2005 season		
<i>Phomopsis</i> spp.	$y = -0.14x + 27.83$	0.32
Botryosphaeriaceae spp.	$y = -0.46x + 13.68$	0.84
<i>E. lata</i>	$y = -0.42x + 8.65$	0.87
<i>Pa. chlamydospora</i>	$y = -0.58x + 15.92$	0.87

action showed that the incidences of pathogens in the xylem and pith tissues of inoculated pruning wounds were also significantly greater than the incidence of pathogens in the paint and water treated wounds (Table 3). In the xylem and pith

tissues of pruning wounds inoculated in July, the pathogen incidences were significantly less compared to the wounds inoculated in August, with the only exceptions being *Pa. chlamydospora* in the xylem tissue and *E. lata* and species in Bot-

Table 3. Mean incidence of *Eutypa lata*, *Phaeoconiella chlamydospora*, Botryosphaeriaceae and *Phomopsis* spp. in the xylem and pith tissues of pruning wounds that were made during July and August 2004 and 2005 and either painted, water treated or inoculated with *Eutypa lata*, *Phaeoconiella chlamydospora*, *Neofusicoccum australe* and *Phomopsis viticola* directly after pruning, 1, 2, 3, 7, 10, 14, 17 and 21 days after pruning.

Treatment	Pruning time					
	July			August		
	Inoculated ^a	Paint	Water	Inoculated	Paint	Water
Xylem						
Botryosphaeriaceae spp.	25.70d	7.79l-p	0.55pqr	33.29bc	7.17m-q	6.09pqr
<i>E. lata</i>	14.91hi	2.35rs	1.04s	20.34fg	0.41s	0.08s
<i>Pa. chlamydospora</i>	21.60ef	10.47j-o	3.86qrs	20.39fg	8.59k-p	7.89l-p
<i>Phomopsis</i> spp.	31.57bc	30.06bc	20.59fg	42.25a	35.63b	33.24bc
Pith						
Botryosphaeriaceae spp.	10.71j-n	1.15s	1.04s	10.51j-n	1.08s	0.56s
<i>E. lata</i>	10.72j-n	0.30s	0.00s	10.93j-m	0.07s	0.00s
<i>Pa. chlamydospora</i>	11.89ijk	7.03n-q	2.45rs	24.95de	14.20hij	12.73ij
<i>Phomopsis</i> spp.	11.37i-l	7.97l-p	6.63opq	17.47gh	13.88ij	12.88ij
LSD ($P < 0.05$)	3.877					

^aMeans followed by the same letter are not significantly different ($P < 0.05$).

ryosphaeriaceae in the pith tissues (Table 3). In general, xylem tissues also yielded significantly greater pathogen incidences compared to the pith tissues (Table 3).

Lesions in treated pruning wounds

Analysis of variance of the xylem lesions measured in the treated pruning wounds indicated significant wound age at treatment \times treatment ($P=0.0042$) and year \times pruning time ($P=0.0003$) interactions. Quadratic regression lines were fitted to the mean lesion lengths of the wound age at treatment \times treatment interaction with R^2 values ranging from 0.14 to 0.73. Lesions in the xylem tissue of non-inoculated wounds (water treated; R^2 value of 0.73) and wounds inoculated with *Pa. chlamydospora* (R^2

value of 0.70) declined with increasing wound age at treatment, while the lesion lengths in the painted wounds (R^2 value of 0.56) showed a slight increase with increasing wound age at treatment (Figure 3). No clear trend in lesion length with increasing wound age at treatment was observed for any of the other treatments with quadratic trend lines fitting poorly to means (R^2 value of 0.14 to 0.37; Figure 3). The year \times pruning time interaction indicated that xylem lesions on pruning wounds in July (3.27 mm) and August (13.30 mm) 2004 were significantly smaller than the xylem lesions in the July (11.58 mm) and August (17.14 mm) 2005. In both years, the lesions on pruning wounds made and inoculated in August were significantly larger than the lesions from July.

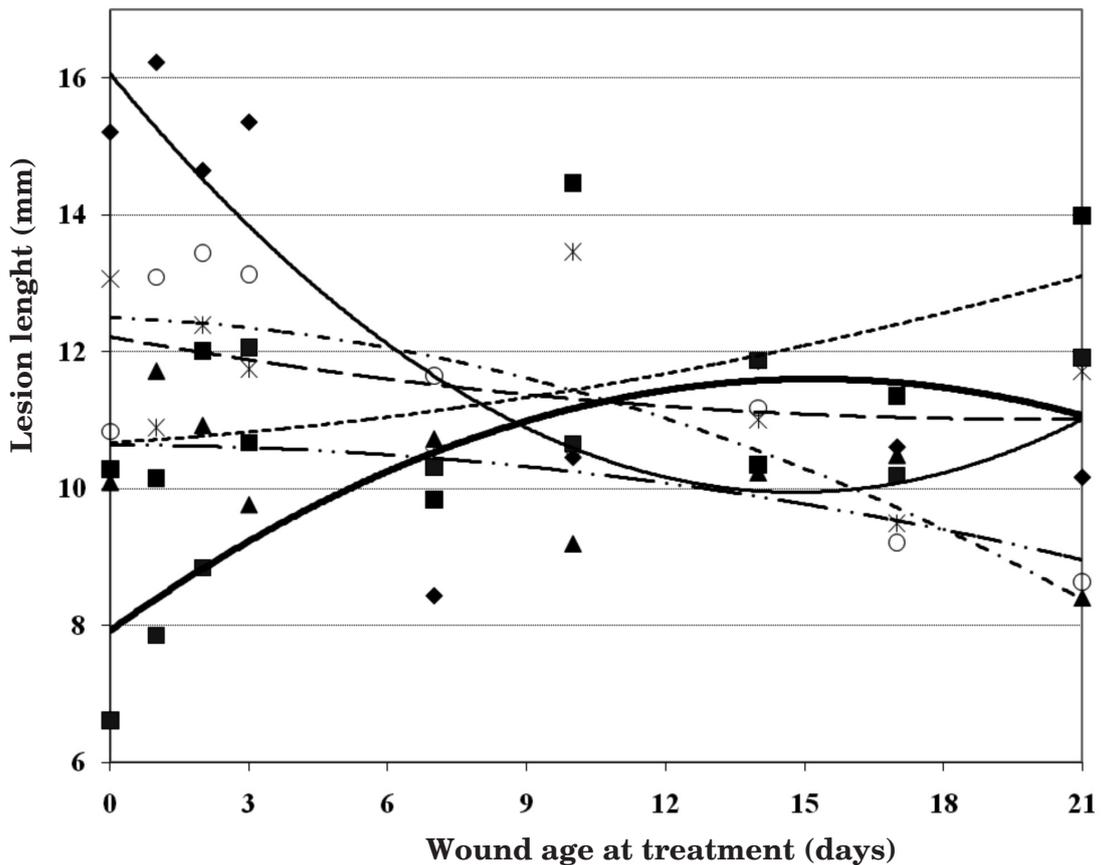


Figure 3. Quadratic regression lines (*E. lata* [---], *Phomopsis* spp. [-.-.-], *Pa. chlamydospora* [—], Botryosphaeriaceae spp. [- - -], water [- - -], paint [—]) fitted to the mean lesion lengths (*E. lata* [▲], *Phomopsis* spp. [■], *Pa. chlamydospora* [◆], Botryosphaeriaceae spp. [*], water [○], paint [■]) over wound age at treatment data for the significant wound age at treatment \times treatment interaction.

Weather data

Average temperature for the 21 days after pruning in July 2004 was 13.1°C and for the same period after pruning in August 2004 was 13.5°C. This is in comparison with 14.8°C for the period after the July 2005 pruning and 14.6°C for the August 2005 pruning time. Rainfall recorded for the 21 days after the respective pruning times was 2.6 mm for July 2004, 1.4 mm for August 2004, 3.3 mm for July 2005 and 3.3 mm for August 2005.

Discussion

Previous studies on the susceptibility of grapevine pruning wounds to pathogen infection have included some of the major trunk disease pathogens (Moller and Kasimatis, 1980; Trese *et al.*, 1980; Munkvold and Marois, 1995; Chapuis *et al.*, 1998; Larignon and Dubos, 2000; Gubler *et al.*, 2001; Serra *et al.*, 2008). These studies reported that wounds made early in the dormant season were more susceptible, and remained susceptible for longer, to infection by *E. lata*, *Pa. chlamydospora* and *Phaeoacremonium aleophilum* W. Gams, Crous, M.J. Wingf. & Mugnai as opposed to pruning wounds made later in the dormant season. All of these studies also found that wound susceptibility declined with increasing wound age.

To the knowledge of the authors, the present study is the only one evaluating and comparing pruning wound susceptibility at various wound ages and different pruning times during the pruning season to all trunk disease pathogens. Results support the previous studies, as pruning wound susceptibility declined with increasing wound age irrespective of the time of year pruning took place. However, differences in the rate of decline in wound susceptibility were noted between 2004 and 2005, similar to the variation between years reported by Chapuis *et al.* (1998) and Serra *et al.* (2008) in studies conducted over 3 years in French and Italian vineyards. The variation observed could be explained by the mechanisms involved in wound repair.

Sun *et al.* (2006) reported that 1 day after pruning grapevines, tyloses start to develop in the exposed xylem vessels of the pruned canes, continuing until almost all xylem vessels are

totally or partially occluded with tyloses after approximately 7 days. This is similar to other woody plants, where tyloses were also reported to form in response to wounding, subsequently developing lignified and/or suberised secondary walls, ultimately forming impenetrable layers in the previously open xylem vessels (Biggs, 1987). This process has been found to be dependent on temperature. Biggs (1990) showed that at higher average temperatures, the rate of suberised tissue formation in wounded bark is greater than at lower temperatures. Munkvold and Marois (1995) made similar observations in grapevines, where they reported a strong positive correlation between higher mean temperature after pruning and rate of suberin accumulation in the pruning wounds as well as the rate of colonisation of pruning wounds by naturally occurring epiphytes. Some evidence was found which indicate that this natural colonisation of pruning wounds by non-pathogenic micro-organisms contributed to reduced infection of wounds by *E. lata* (Munkvold and Marois, 1993). In the present study, weather data were recorded. This data showed that the average temperature and total rainfall for the 21 days after pruning in July and August 2005 was greater compared to the same periods for the July and August 2004 pruning times. Therefore, based on abovementioned studies, the pruning wound repair processes might have been slower in 2004 compared to 2005 due to the lower average temperature and total rainfall during the trial months of July and August. These higher temperatures and rainfall in 2005 could also have contributed to a faster colonisation of pruning wounds by naturally occurring epiphytes, thereby reducing pruning wound susceptibility.

Despite the declining trend in wound susceptibility observed in 2004 and 2005, as well as the variation between the 2 years, wounds remained susceptible to infection by all inoculated pathogens for at least 3 weeks. Previous pruning wound inoculation studies with *Pa. chlamydospora* and *Phaeoacremonium* spp. support these findings. Adalat *et al.* (2000), Larignon and Dubos (2000) and Serra *et al.* (2008) found that, depending on the time of pruning, wounds remained susceptible to *Pa. chlamydospora* and *Pm. aleophilum* infection for between 1 and 16 weeks. Similar to this, Gubler *et al.* (2001) found that wounds could

remain susceptible to infection by *Pa. chlamydospora* and *Pm. aleophilum* for up to 4 months after pruning in Californian vineyards.

Results of the current study revealed that pruning wounds that were made and inoculated during August in both years, generally had greater incidence of infection and longer xylem lesions compared to the pruning wounds inoculated in July, which suggests that wounds made later in the dormant season are more susceptible to infection than wounds made earlier. This could also indicate that it is not necessarily the time of year that pruning is done that determines the period of wound susceptibility but rather the climatic conditions experienced after pruning. This could also explain the conflict between earlier studies in California, France and Italy. These found that wounds made in late winter were less susceptible to infection than wounds made during early winter (Moller and Kasimatis, 1980; Munkvold and Marois, 1995; Chapuis *et al.*, 1998; Larignon and Dubos, 2000; Serra *et al.*, 2008), compared to the results reported in Michigan by Trese *et al.* (1980) who observed that pruning wounds made in late winter were more susceptible to infection by *E. lata* compared to early winter pruning wounds.

Based on the infection recorded in the water treated pruning wounds, it was evident that natural infection could also have contributed to the infection observed in the inoculated pruning wounds, as was evident from the relatively high pathogen incidences in painted and water treated pruning wounds. The high level of pathogen incidence in the painted pruning wounds was especially surprising as the wounds were treated with a commercial pruning wound sealant in an attempt to prevent natural infection. These results therefore clearly illustrate that treating pruning wounds with non-fungicidal paints or pruning wound sealants is not effective in preventing infection by trunk pathogens.

Several previous studies have shown that spore release by grapevine trunk pathogens is promoted by the occurrence of rainfall (Lehoczy, 1974, 1988; Trese *et al.*, 1980; Hewitt and Pearson, 1988; Larignon and Dubos, 2000). In the present study, weather data indicated rainfall did occur in the 21 day period after pruning in 2004 and 2005, which could have contributed to greater amounts of airborne inoculum of the

various pathogens that potentially led to the high levels of natural infection in the painted and water treated pruning wounds.

Apart from infection by air-borne inoculum, the pathogen incidence observed in pruning wounds could be attributed to infections already present in the grapevines prior to pruning and treatment (Feliciano and Gubler, 2001; Rooney *et al.*, 2001; Fourie and Halleen, 2002; van Niekerk, 2008). Although it is difficult to determine the level of infections already present in the grapevines prior to inoculation, the possible contribution of these infections to the pathogen incidence in the inoculated wounds cannot be disregarded. During 2004 and 2005, profuse bleeding of the freshly made pruning wounds was observed during the August pruning time, but not for the July pruning time. *Phaeoconiella chlamydospora* has been shown to grow systemically in the vascular tissue of infected grapevines, forming conidiophores that produce conidia (Feliciano and Gubler, 2001). Conidia of this pathogen were later also found to be present in wound sap of freshly pruned grapevines (Rooney *et al.*, 2001). Fourie and Halleen (2002) studied the occurrence of *Pa. chlamydospora* in rootstock canes and reported isolating species in Botryosphaeriaceae and *Phomopsis* from the basal internodes of 1-year old rootstock canes. These results were subsequently attributed to mycelium colonisation originating from rootstock mother plants that were infected by these fungi. This observation was further supported by recent findings showing species in Botryosphaeriaceae and *Phomopsis* to be present in the basal ends of pruned shoots of mature grapevines, further indicating that these pathogens might be present in the vascular tissue of shoots prior to pruning (F. Halleen, ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, personal communication). However, the statistical significance of the present results are unlikely to be compromised by this unknown variable, as the randomised block design of the field experiments provided sufficient degrees of freedom to adequately address such natural variation.

Another possible explanation for the higher susceptibility of August pruning wounds compared to July pruning wounds can be found in differences during these months in the nutritional status of grapevine shoots. In an earlier study,

Ferreira (1999) noted that carbohydrate and nitrogen concentrations were the greatest in grapevine shoots in the winter pruning period of June to August, with greatest concentrations occurring in August, and that *E. lata* had the highest growth rate in the extracts obtained from shoots in August. It is therefore also possible that the greater susceptibility of the late winter (August) pruning wounds can be attributed to high carbohydrate and nitrogen concentrations in the pruned canes during late winter, making these more favourable environments for the establishment of pruning wound infection by the various trunk pathogens.

Another interesting observation was the higher incidence of the various pathogens in the xylem tissue compared to the pith tissue. The only exception to this observation was *Pa. chlamydospora* that had a greater incidence in the pith tissue of pruning wounds inoculated in August compared to the incidence in the xylem tissue of the same wounds. In a study using tissue cultured plants, Feliciano and Gubler (2001) observed that, after inoculation, this pathogen initially invades the pith tissue from where it spreads quickly to invade the rest of the vascular tissue. However, the generally higher pathogen incidence observed in the xylem tissue versus the pith tissue could probably be explained by differences in nutrient status of the two tissue types. Xylem tissue is made up of a network of dead vessels through which water, minerals, amino acids, organic acids and sugars are transported (Mauseth, 1995; Buhtz *et al.*, 2004), while the pith tissue is made up of parenchyma cells only (Mauseth, 1995). Xylem tissue would therefore represent a source of easily accessible nutrients to an invading pathogen as opposed to the pith tissue, where cell wall breakdown must first be achieved before the invading pathogen can access the nutrients in the pith cells. Moreover, after pruning, xylem vessels are open to invasion by the different trunk pathogens offering an easy pathway to pathogen infection.

From the pathogen incidence results obtained it was evident that the different pathogens colonise the xylem and pith tissues of pruning wounds to varying levels. All the pathogens displayed greater incidences in the xylem tissue compared to the pith tissues. In this study, the susceptibility of pruning wounds to infection by *N. australe*

and *P. viticola* was examined for the first time, and although the pathogen incidences in the different pruning wound tissues varied, results indicated that the reaction of pruning wounds towards infection by these pathogens are similar to *E. lata* and *Pa. chlamydospora*: wounds made in late-winter (August) are generally more susceptible than wounds made in mid-winter (July), with susceptibility declining with increasing wound age.

Similar to previous studies, our results clearly illustrated that, despite the differences observed in the pruning wound susceptibility between pruning times and the differences between years in the rate of decline in susceptibility, pruning wounds remained susceptible to infection to all major trunk disease pathogens for an extended period after pruning. It is therefore of utmost importance that grapevine pruning wounds should be protected for prolonged periods after pruning against infection by all pathogens implicated in the trunk disease complex by means of chemical and/or biological agents.

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