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ORIGINAL RESEARCH

Temporal spore dispersal patterns of grapevine trunk pathogens in South Africa

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Abstract Trunk disease pathogens of grapevines, *viz. Phaeomoniella chlamydospora*, *Eutypa lata* and several species in Botryosphaeriaceae, *Phaeoacremonium* and *Phomopsis* are known to infect fresh pruning wounds by means of air-borne inoculum released after rainfall or prolonged periods of high relative humidity. Recent surveys have demonstrated that most or all of these pathogens are present in climatically diverse grape growing regions of South Africa. However, the factors controlling spore dispersal of these pathogens in vineyards were largely unknown. To address this question, spore trapping was done in a Chenin Blanc vineyard in the Stellenbosch area, South Africa, for 14 weeks during the grapevine pruning period from June to mid-September of 2004 and 2005. Hourly recordings of weather data were done by a weather station in the row adjacent to the spore trap. Spores of *E. lata* and *Phomopsis* and species in Botryosphaeriaceae were trapped throughout the trapping periods of 2004 and 2005, with higher levels of trapped spores recorded in 2005. The spores of all three pathogens were trapped during or after periods of rainfall and/or high relative humidity. In neither of the 2 years were spores of *Pa. chlamydospora* or *Phaeoacremonium* spp. trapped. Results indicated that spore event incidence, as well as the amount of spores released during a spore event of above-mentioned pathogens, were governed by rainfall, relative humidity, temperature and wind speed prior to and during the spore events.

Keywords Botryosphaeriaceae · *Phomopsis* · *Eutypa lata* · Epidemiology · Air-borne inoculum · Spore trapping

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Introduction

Pathogens associated with trunk diseases of grapevines include several species in the Botryosphaeriaceae (Crous et al. 2006; van Niekerk et al. 2004), *Phaeoacremonium* (Mostert et al. 2006) and *Phomopsis* (van Niekerk et al. 2005), as well as *Phaeomoniella chlamydospora* (W. Gams, Crous & M.J. Wingf. & L. Mugnai) Crous & W. Gams (Mugnai et al. 1999) and *Eutypa lata* (Pers.) Tul. & C. Tul. (Munkvold et al.

1994). All of these pathogens have been found to infect fresh pruning wounds (Lehoczky 1974; Ferreira et al. 1989; Larignon and Dubos 2000; van Niekerk et al. 2005), and wound infecting air-borne inoculum of many of these pathogens have been detected in vineyards (Moller et al. 1977; Hewitt and Pearson 1988; Merrin et al. 1995; Larignon and Dubos 2000; Eskalen and Gubler 2001).

Eutypa lata ascospores were shown to be the primary form of inoculum responsible for spreading of the pathogen, causing new infections where they land on susceptible pruning wounds (Moller et al. 1977). These spores are produced by perithecia formed in layers of stromatic tissue found on dead host tissue (Carter 1957; Magarey and Carter 1986). However, stromata are only known to occur in areas with an annual rainfall exceeding 350 mm or where overhead irrigation is applied, thereby creating conditions favourable for its development (Carter 1957; Magarey and Carter 1986). Mature perithecia are reported to start discharging ascospores within 3 h after the onset of rainfall, once the stromata have been exposed to at least 2 mm of rainfall at temperatures above 0°C (Pearson 1980; Magarey and Carter 1986). The amount of air-borne ascospores was furthermore found to vary between seasons. In South Australia, California and Michigan, peak air-borne ascospore concentrations in vineyards and apricot orchards were found to occur after rainfall in late autumn and spring (Moller and Carter 1965; Magarey and Carter 1986). These results are in contrast with Pearson (1980) who found that peaks in ascospore discharge in New York vineyards occur in winter or early spring after rainfall or periods of melting snow.

Epidemiological studies of *Pa. chlamydospora* and *Phaeoacremonium* spp. similarly showed that air-borne spores of these pathogens are present in vineyards. However, periods of maximum air-borne inoculum concentration were shown to be different for these two pathogens. In France, spores of *Pa. chlamydospora* were found to occur in vineyards throughout the year, while *Pm. aleophilum* W. Gams, Crous, M.J. Wingf. & Mugnai spores occurred only during the vegetative period of the vineyards (Larignon and Dubos 2000). Rainfall was, however, shown to be correlated with the peak periods of spore release of both pathogens (Larignon and Dubos 2000). Similar observations were made in California where air-borne conidia of *Pm. aleophilum* were detected in vineyards in early and mid-summer as opposed to air-borne conidia of *Pa. chlamydospora* being present during the winter (Eskalen and Gubler 2001). Sources of *Phaeoacremonium* inoculum in vineyards were found to include pycnidia formed on grapevine canes (Larignon and Dubos 2000), as well as perithecia of *Togninia minima* (Tul. & C. Tul.) Berl. and *T. fraxinopennsylvanica* (T. E. Hinds) Hausner, Eyjólfsd. & J. Reid (teleomorph species of *Phaeoacremonium*) (Eskalen et al. 2005; Rooney-Latham et al. 2005), which contribute to aerial inoculum of *Phaeoacremonium* in vineyards under favourable environmental conditions (Rooney et al. 2004).

The large number of species in the Botryosphaeriaceae reported to occur on grapevines (van Niekerk et al. 2004) has complicated epidemiological studies. It has, however, been reported that pycnidia are formed on diseased wood and pruning debris and that spores are released during wet, rainy weather (Lehoczky 1974; Hewitt and Pearson 1988). This rain-related spore release from fruiting bodies on diseased wood and pruning debris has been reported from other Botryosphaeriaceae hosts such as pistachio (Ahimera et al. 2004), and peach (Pusey 1989) where severe infections are often associated with prolonged periods of rain and high relative humidity (Michailides and Morgan 1993). Similar to species in Botryosphaeriaceae, severe incidences of Phomopsis cane and leaf spot, caused by *Phomopsis viticola* (Sacc.) Sacc., have been associated with high rainfall during late winter and early spring (Anderson and Colby 1943). This pathogen forms pycnidia on infected wood and dormant shoots and spurs from where conidia are released when exposed to prolonged periods of rain (Cucuzza and Sall 1982).

Previous studies have focussed on one or two of the pathogens in the trunk disease complex only. Studying the interaction between different pathogens in the complex is important as all of these air-borne, wound infecting pathogens have been found to be present at varying frequencies in diseased grapevines displaying different internal wood necrosis and wood rot symptoms (Serra et al. 2000; Fischer and Kassemeyer 2003). This indicates that air-borne inoculum of all or some of these pathogens could be present simultaneously in vineyards, infecting pruning or other wounds, causing the above-mentioned wood decay symptoms. No attempt was made to model the

spore dispersal patterns of the different pathogens as influenced by a variety of weather conditions.

The aims of this study were to collectively study the temporal spore dispersal patterns of the different trunk disease pathogens in an attempt to develop models to predict spore dispersal that could be used to predict peak spore dispersal periods, which in turn could be used to schedule pruning practices during periods of low inoculum availability. However, the anamorph/teleomorph associations of many of these trunk pathogens are unknown, while the spore morphology of many of the species is also unknown or overlapping as in the case of *Phomopsis* spp. (Jacobs and Rehner 1998; van Niekerk et al. 2005; Crous et al. 2006; Mostert et al. 2006). This diversity consequently made it impossible to do microscopic spore counts of the different pathogen species directly on the disc of the spore trap. Conventional spore trapping techniques could therefore not be employed, creating the necessity to develop the spore trapping protocol used in this study, which allowed the trapping and identification of morphologically diverse spores.

Materials and methods

Spore trapping

Multiple spore trapping was done for a period of 14 weeks from June to mid-September 2004 and 2005 in an 18+ year-old Chenin Blanc vineyard in the Stellenbosch area, South Africa. This period was targeted for the spore trapping study as it coincides with the pruning time of most wine grape cultivars grown in the area. A Quest volumetric spore trap (Interlock Systems, Pretoria, South Africa) was placed inside a vineyard row with the orifice 0.5 m above soil level. In the adjacent vineyard row, a Vantage Pro® weather station (Davis Instruments, Hayward, CA) was installed. The weather station made hourly recordings of rainfall, temperature, relative humidity and wind speed during the entire spore trapping period.

The spore trap disc consisted of eight 1 d segments, each of which was further divided into 24×1 h segments. Prior to placing the disc in the spore trap, it was covered with clear adhesive plastic and sprayed with petroleum jelly spray (PJS, Interlock Systems, Pretoria, South Africa). After 5 to 6 days in the vineyard, the disc was replaced with a clean disc and brought into the laboratory for further processing. When processing the disc, each day of 24 h was divided into four 6 h segments. The adhesive plastic covering each of the 6 h segments on the disc was excised and placed into small glass bottles containing 10 ml of sterile water. These bottles were then heated for 2 min at 50°C in a warm water bath to melt the PJS and put the spores into suspension. The resulting suspension was plated onto five 65 mm water agar (WA, Biolab, Wadeville, South Africa) dishes (2 ml per dish), which were placed in a laminar flow cabinet to allow the excess water to evaporate. After 36 h of incubation at 25°C, dishes were viewed at 50× magnification under a stereomicroscope with bottom illumination. All germinating spores observed were transferred to 65 mm potato dextrose agar (PDA, Biolab, Wadeville, South Africa) dishes and incubated at 25°C for 2–4 weeks before morphological identification of these single spore cultures based on colony characteristics and spore morphology. This method allowed for the identification and quantification of the variety of spores trapped on each disc, as well as correlation with the corresponding weather data for each 6 h period. The effect of the PJS and washing temperature on spore germination of *E. lata*, *Pa. chlamydospora*, *Pm. aleophilum*, *P. viticola* and species in the Botryosphaeriaceae was also tested and no negative effect was found, with spore germination in all cases being above 95% (results not shown).

Fruiting body survey

Pruning debris and other dead grapevine material found on the vineyard floor surrounding the spore trap location were collected. Samples were collected in a radius of 3 m, at 1 m intervals, from the point where the spore trap was placed in the vineyard row. After collection, samples were brought back to the laboratory where they were left to dry on the laboratory bench at 22°C prior to further examination. Forty-eight h before microscopic examination of the material, it was placed in moist chambers and incubated at 22°C to induce sporulation. Individual pieces of pruning debris were subsequently examined under 18–50× magnification using a stereomicroscope with top illumination. Any fruiting structures that were observed were squash mounted in 70% lactic acid and studied using a compound light microscope. Fungi isolated in this

manner were morphologically identified to genus level. Photographs of microscopic fungal structures observed on the squash mounts were taken using a Nikon Eclipse E600 compound microscope with a 12 megapixel Nikon DXM1200 digital camera.

Data collection

After identification of the single spore cultures, the number of cultures representing the different pathogen genera was determined. Based on these culture counts, calculations were made for total pathogen spore count for each of the 6 h periods occurring during the trapping periods of 2004 and 2005. For each of the 6 h periods, the corresponding averages of the measured weather factors were also calculated. From these data, spore events for each pathogen were identified. A spore event was defined as one or more consecutive 6 h periods during which spores were trapped, the shortest event being a single 6 h period.

Based on preliminary data analyses (results not shown), which included weather variables describing weather conditions up to 14 d prior to the spore events, and relative humidity thresholds below 75%, twelve descriptive weather variables were defined. Six variables described the weather conditions of the 48 h period preceding the spore event: the number of hours with relative humidity above 75% (T48HrsRH $\geq$75), mean relative humidity (T48MeanRH), minimum temperature (T48minTemp), maximum temperature (T48maxTemp), number of hours of rain (Hrs-rain-before) and rainfall amount (mm-Rain-before). The remaining six variables described the weather conditions prevailing during the spore event: minimum temperature (MinTemp), maximum temperature (maxTemp), minimum relative humidity (MinRH), maximum relative humidity (MaxRH), amount of rainfall (mm Rain) and mean wind speed (Mean-Wind). Day length as variable was not included due to negligible differences in day length during the months of July and August in the area where the study was done.

Statistical analysis

For each of the pathogen genera encountered during the spore trapping, the spore events, as well as 1 to 4 non-events (6 h periods when no spores were trapped) prior to the spore event, were qualitatively (event incidence) analysed by awarding a value of 1 to a spore event and a value of 0 to a non-event. The spore counts of the identified spore events were quantitatively analysed further by grouping them in five statistically calculated spore count classes, with class 1 equalling 0 spores, class 3 being the median class and class 5 comprising the highest spore counts. Spearman's Rank correlation analyses (Snedecor and Cochran 1967) were conducted to determine the correlation between the weather variables and spore event incidence, spore count or spore count class. Step-wise regression analysis (Draper and Smith 1980), using the same data, was also performed to obtain statistical models for the prediction of event incidence, spore count and spore count class. All statistical analyses were done using SAS Version 8.2 (SAS Institute Inc. NC, USA).

Results

Spore trapping

Spores of *E. lata* and species in Botryosphaeriaceae and *Phomopsis* were trapped throughout the trapping periods in 2004 and 2005. However, in both years, a short period of approximately 2 weeks was observed during which little or no spores were trapped for any of the pathogens. In both years, these periods were characterised by little or no rainfall. No spores of *Pa. chlamydospora* or *Phaeoacremonium* spp. were trapped in either of the seasons. Spores of the species in Botryosphaeriaceae were trapped in both years during or after the occurrence of as little as 0.25 mm rainfall, with higher levels of spores occurring in 2005, which had a higher rainfall (339.2 mm; 48 rain days) during the trapping period compared to 2004 (207.3 mm; 44 rain days). In both years, spores of this group of pathogens were also trapped during periods of relative humidity at or above 70% (Figs. 1 and 2).

Similar to the species in Botryosphaeriaceae, spores of *Phomopsis* spp. were trapped in both years during or after as little as 0.25 mm rainfall occurring. Higher spore levels for *Phomopsis* were also recorded in 2005. However, in both years, the levels of *Phomopsis* spores trapped were markedly lower compared to the spore levels recorded for the species in Botryosphaeriaceae (Figs. 3 and 4). *Eutypa lata* spores were also trapped throughout the trapping periods of both years. In general, the spores were also

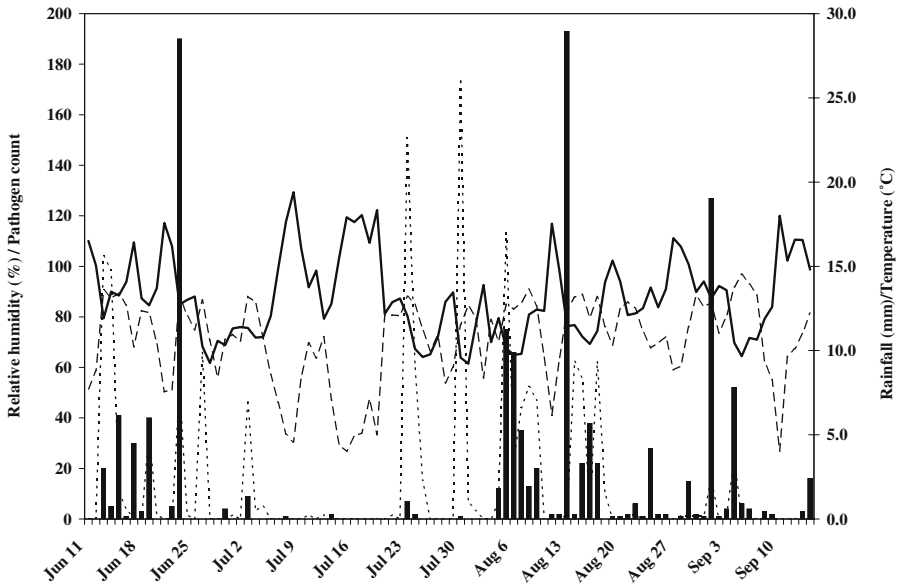

Fig. 1 Spore events and counts (■) recorded for species in Botryosphaeriaceae during 2004 plotted against daily average relative humidity (– – –), temperature (——) and daily rainfall (- - -)

trapped during or after the occurrence of rainfall of more than 1 mm. However, in some cases where sufficient rainfall did occur, no spores were trapped. Although the lowest levels of trapped spores were recorded for *E. lata*, the levels in 2005 were, similar to *Phomopsis* and species in the Botryosphaeriaceae, higher compared to 2004 (Figs. 5 and 6).

Fruiting body survey

On the pruning debris collected in a 3 m radius around the spore trap location, fruiting structures of various trunk disease pathogens were found. Abundant mature pycnidia producing conidia typical of the Botryosphaeriaceae anamorph genus, *Dothiorella* (Fig. 7a, b)

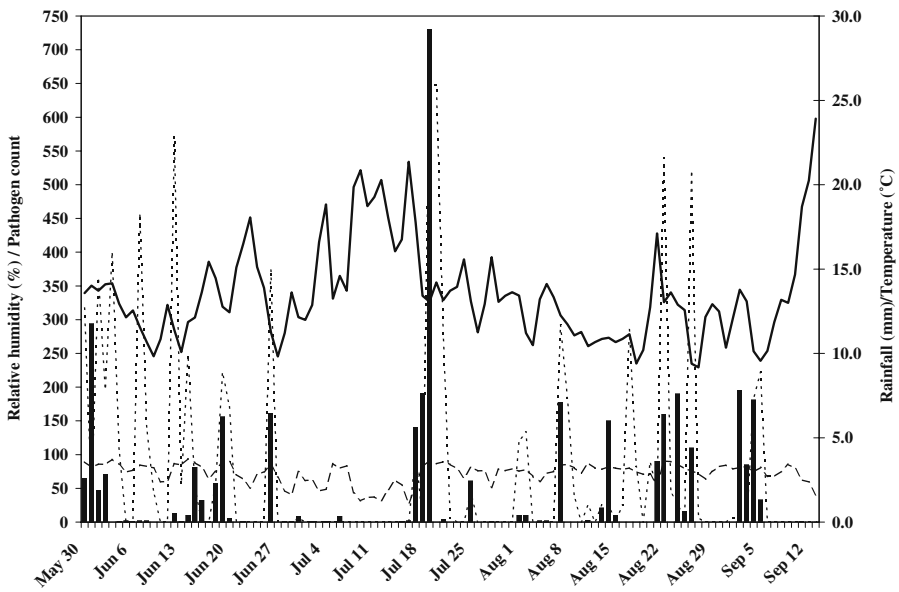

Fig. 2 Spore events and counts (■) recorded for species in Botryosphaeriaceae during 2005 plotted against daily average relative humidity (– – –), temperature (——) and daily rainfall (- - -)

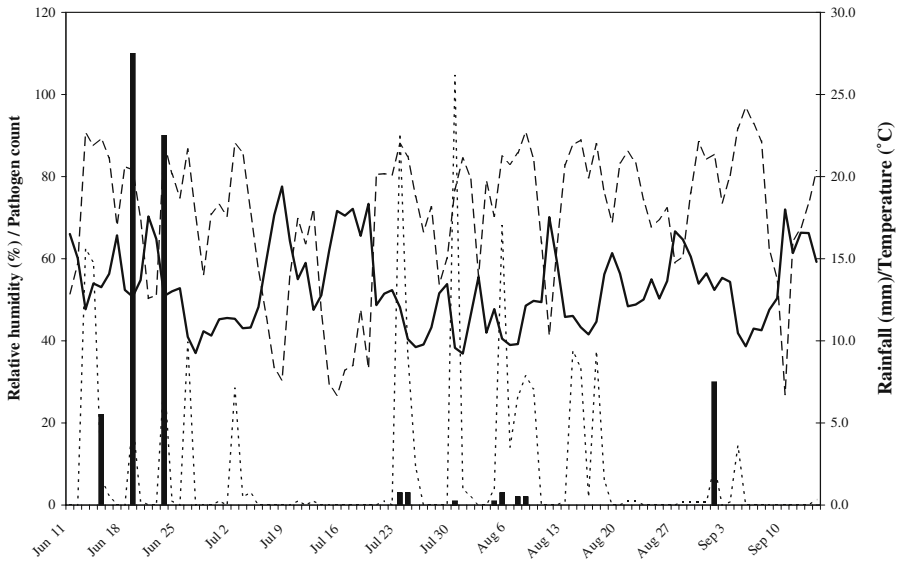

Fig. 3 Spore events and counts (■) recorded for species in *Phomopsis* during 2004 plotted against daily average relative humidity (– – –), temperature (——) and daily rainfall (- - -)

and *Diplodia* (Fig. 7c) were found on pruning debris. Within 2 m of the spore trap, pieces of pruning debris were found that bore small areas of stromatic tissue containing mature perithecia with characteristic asci and ascospores of *E. lata* (Fig. 7d, e). No *Phomopsis* fruiting structures were found in the surveyed area. No fruiting structures of either of *Pa. chlamydospora* or *Phaeoacremonium* spp. were found.

Statistical analysis

Species in the Botryosphaeriaceae Results from the Spearman's Rank correlation analysis indicated a positive and statistically significant ($P \leq 0.10$) correlation between species in the Botryosphaeriaceae spore event incidence and the weather variables mmRain, MaxRH, MinRH, Mean Wind, Hrs-Rain-before, T48HrsRH ≥75, T48MeanRH, and mm-Rain-before, while a negative correlation with MinTemp was observed (Table 1). A positive and statistically significant ($P \leq 0.10$) correlation was furthermore observed between spore count and the weather variables mmRain, Mean Wind, MaxRH, MinRH, mm-Rain-before, Hrs-Rain-before and T48MeanRH. A negative correlation with MinTemp was again observed (Table 1). Similar correlations were also

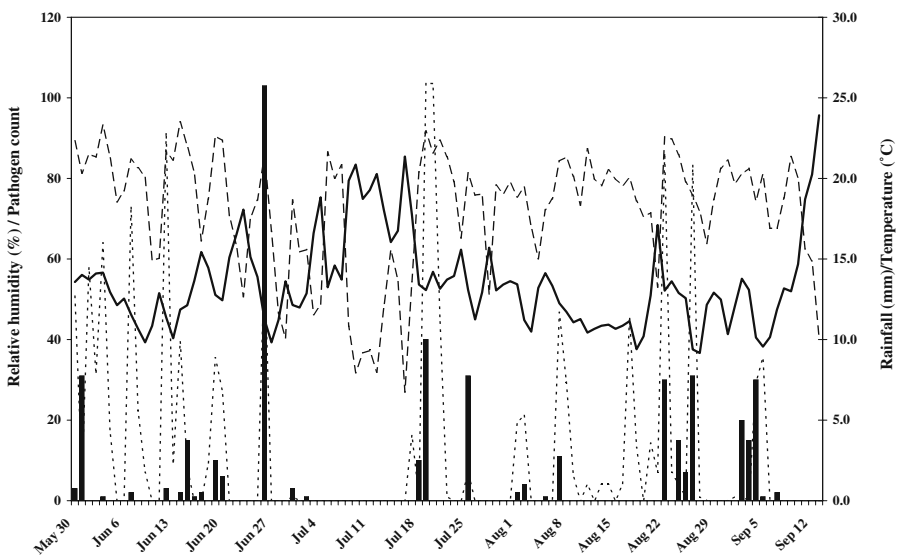

Fig. 4 Spore events and counts (■) recorded for species in *Phomopsis* during 2005 plotted against daily average relative humidity (- - -), temperature (——) and daily rainfall (- - -)

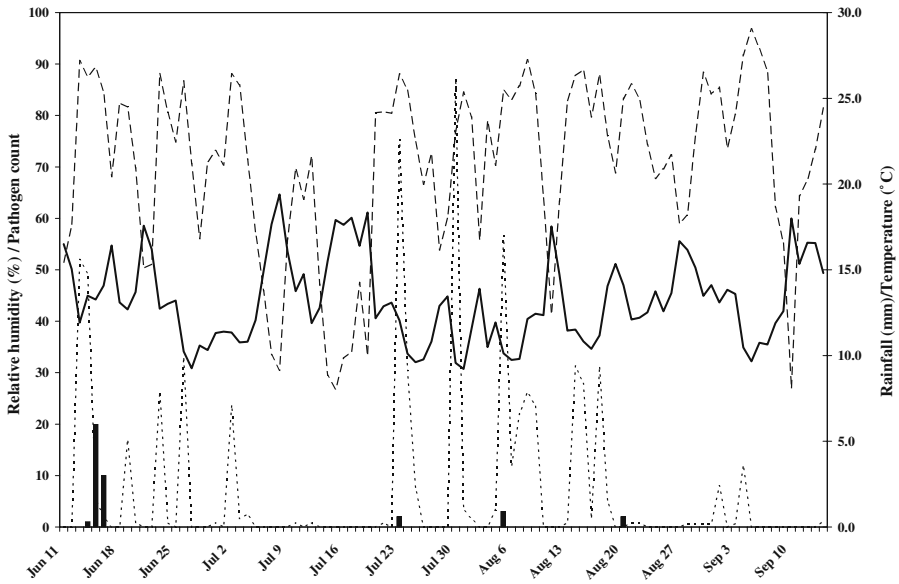

Fig. 5 Spore events and counts (■) recorded for species in *Eutypa lata* during 2004 plotted against daily average relative humidity (– – –), temperature (——) and daily rainfall (- - -)

observed in the case of spore count class. This pathogen variable also showed the best correlation with mmRain followed by MaxRH, Mean Wind, mm-Rain-before, Hrs-Rain-before, T48MeanRH, as well as a negative correlation with MinTemp.

Step-wise regression analyses yielded a model for species in the Botryosphaeriaceae spore event incidence that included the weather variables MinTemp, MaxTemp, MaxRH and mmRain. According to the partial R^2-values (pR^2) of the respective weather variables, the most important variable was mmRain (pR^2 = 0.17) followed by MinTemp (pR^2 = 0.07), MaxRH (pR^2 = 0.05) and MaxTemp (pR^2 = 0.02) (Table 2). The R^2-value of the model was subsequently calculated as 0.31 with a probability of $P<$ 0.0001 (Table 2; Equation 1: Species in Botryosphaeriaceae spore event incidence = −0.55 0.14 × MinTemp + 0.16 × MaxTemp + 0.01 × MaxRH + 0.15 × mmRain). Variables included in the spore count model were T48MaxTemp and mmRain. The

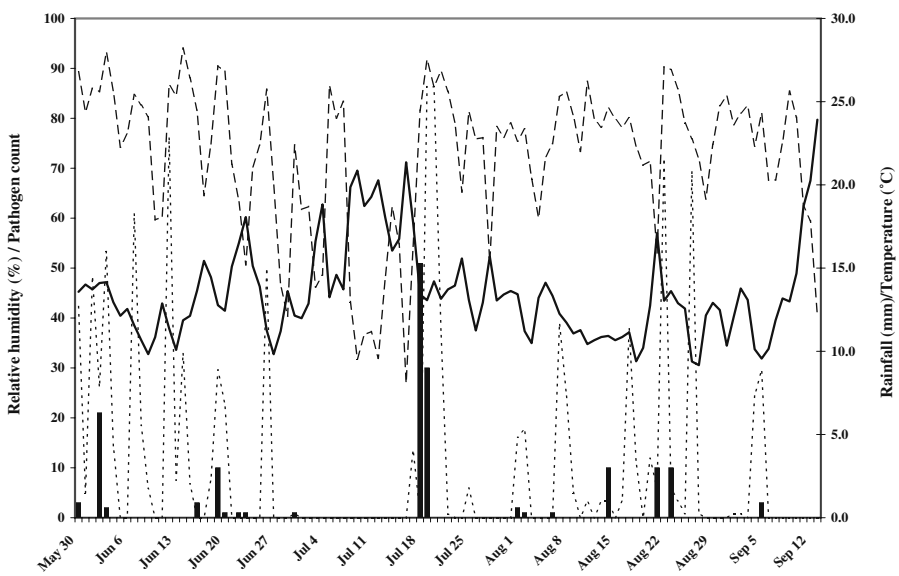

Fig. 6 Spore events and counts (■) recorded for species in *Eutypa lata* during 2005 plotted against daily average relative humidity (– – –), temperature (——) and daily rainfall (- - -)

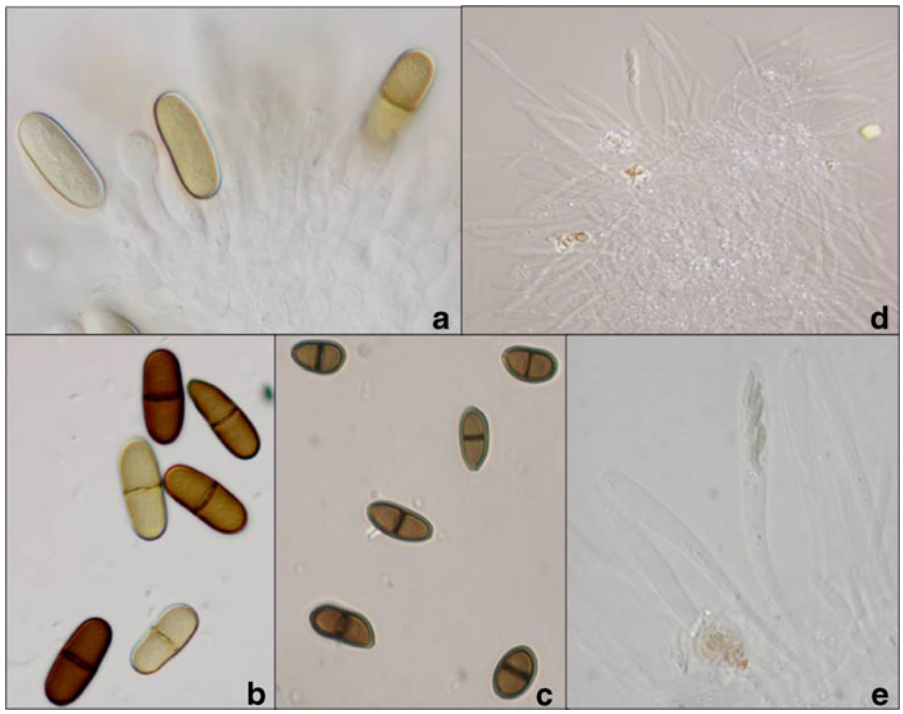

Fig. 7 Microscopic structures of grapevine trunk pathogens observed on squash mounts of fruiting structures obtained from pruning debris in vicinity of spore trap. **a.** Conidia and conidiogeneous cells of *Dothiorella* spp.(100× magnification under oil immersion), **b.** Conidia of *Dothiorella* spp., **c.** Conidia of *Diplodia* spp. (100× oil immersion), **d.** Mature and immature asci of *Eutypa lata* (100× oil immersion) and **e.** Mature *Eutypa lata* ascus with ascospores surrounded by immature asci (40× magnification)

R^2-value of this model was 0.28 with a probability of $P<0.0001$ (Table 2; Equation 2: Species in the Botryosphaeriaceae spore count = −33.42 + 2.16 × T48maxTemp + 49.82 × mmRain). In this model, mmRain were again the most important variable with a pR^2-value of 0.27, with T48maxTemp having a pR^2-value of only 0.01 (Table 2). The third model, for spore count class, included MinRH, MaxRH and mmRain and had the best R^2-value of 0.54 and a probability of $P<0.0001$ (Table 3; Equation 3: Species in Botryosphaeriaceae spore count class = 0.61–0.12 × MinRH + 0.13 × MaxRH + 0.49 × mmRain). Similar to the other two species in Botryosphaeriaceae models, mmRain were again, according to the pR^2-values, the most important weather variable included, followed by MinRH and MaxRH (Table 2).

Phomopsis spp For *Phomopsis* spore event incidence, correlation analyses showed a positive and statistically significant ($P\leq0.10$) correlation with mmRain, MaxRH, MinRH and Mean-Wind (Table 3). A negative correlation with MinTemp was observed (Table 3). *Phomopsis* spore count was positively correlated with mmRain, MaxRH, MinRH and Mean-Wind, with again a negative correlation with MinTemp. *Phomopsis* spore count class on the other hand was positively correlated only with variables describing the weather conditions during the spore event, *viz.* mmRain, MaxRH, MinRH and Mean Wind. Similar to the other Botryosphaeriaceae variables, a negative correlation with MinTemp was observed (Table 3).

For *Phomopsis* spore event incidence, only mmRain was included as weather variable in the step-wise regression model. The model had a R^2-value of 0.20 at a probability of $P<0.0001$ (Table 4; Equation 4: *Phomopsis* spore event incidence = 0.14+0.31 × mmRain). Weather variables included in the *Phomopsis* spore count model were T48maxTemp and mmRain, with a R^2-value of 0.14 (Table 4; Equation 5: *Phomopsis* spore count = −12.51 + 0.84 × T48maxTemp + 8.52 × mmRain). In this model, mmRain was also the most important weather variable with a pR^2-value of 0.11 (Table 4). *Phomopsis* spore count class yielded a model with MinRH, MaxRH and mmRain included as weather variables, and R^2 of 0.41 and probability of $P<0.0001$ (Table 4; Equation 6: *Phomopsis* spore count class = 0.50–0.22 × MinRH + 0.23 × MaxRH + 0.31 × mmRain). Here again, mmRain were, similar to the other two *Phomopsis* models, the most important weather variable included, followed by MinRH and MaxRH (Table 4).

Eutypa lata From the results illustrated in Figs. 5 and 6, *E. lata* spores were trapped on very few

Table 1 Spearman's Rank correlation coefficients of species in Botryosphaeriaceae spore event incidence, spore count and spore count class with twelve weather variables

	Weather variables											
	48 hrs before spore event						During spore event[c]					
Pathogen variables	T48 HrsRH ≥75	T48 MeanRH	T48 minTemp	T48 maxTemp	HrsRain Before	mmRain Before	Min Temp	Max Temp	MinRH	MaxRH	mmRain	Mean Wind
Event incidence	0.117[a] (0.063)[b]	0.132 (0.036)	−0.016 (0.798)	−0.024 (0.701)	0.139 (0.027)	0.154 (0.014)	−0.230 (0.0002)	0.067 (0.285)	0.136 (0.030)	0.380 (<0.0001)	0.490 (<0.0001)	0.160 (0.011)
Spore count	0.096 (0.126)	0.106 (0.091)	0.032 0.617)	−0.026 (0.675)	0.118 (0.060)	0.134 (0.032)	−0.249 (<0.0001)	0.080 (0.201)	0.108 (0.087)	0.408 (<0.0001)	0.548 (<0.0001)	0.188 (0.0026)
Spore count class	0.096 (0.125)	0.106 (0.093)	0.033 (0.604)	−0.025 (0.686)	0.120 (0.057)	0.136 (0.030)	−0.243 (<0.0001)	0.079 (0.211)	0.103 (0.1007)	0.340 (<0.0001)	0.540 (<0.0001)	0.186 (0.003)

[a] Correlation coefficients

[b] Statistical significance at $P \leq 0.10$

[c] One or more consecutive 6-h periods

occasions in 2004 and 2005. As a consequence very few spore events were identified for this pathogen and very few weather variables showed significant correlation ($P \leq 0.10$) with any of the *E. lata* variables. *Eutypa lata* spore event incidence was positively correlated with MaxRH and mmRain, while negatively correlated with T48maxTemp (Table 5). The remaining two pathogen variables were correlated with the same weather variables. Both *E. lata* spore count and spore count class was positively correlated with MaxRH, mmRain and mm Rain Before (Table 5). Similar to spore event incidence, the *E. lata* spore count and spore count class were negatively correlated with T48MaxTemp. Step-wise regression analyses yielded models for *E. lata* spore event incidence and spore count class only. In the spore event incidence model, T48maxTemp, MinRH and MaxRH were included as variables with R^2-value of 0.19 and probability of $P = 0.0173$ (Table 6; Equation 7: *E. lata* spore event incidence = 0.50–0.04 × T48max-Temp – 0.05 × MinRH + 0.06 × MaxRH). All of these variables also had similar pR^2-values and could therefore not be ranked in order of importance. The same weather variables, except T48maxTemp, were included in the *E. lata* spore count class models but the R^2-value of this model was slightly higher at 0.27 and a *P*-value of 0.0001 (Table 6; Equation 8: *E. lata* spore count class = 0.49–0.20 × MinRH + 0.21 × MaxRH). In this model, MinRH was the most important variable included with a pR^2-value of 0.21 that were almost 4 times the pR^2-value of MaxRH (Table 6).

Discussion

Numerous epidemiological studies of the various pathogens associated with grapevine trunk diseases have reported that the air-borne spores of these pathogens are abundant during or after periods of rainfall or prolonged wetness (Lehoczky 1974; Magarey and Carter 1986; Hewitt and Pearson 1988; Larignon and Dubos 2000). However, the present study is the first that attempted to model the quantitative and qualitative spore release of the different pathogen genera and species.

The protocol used in this study negated the problem of unknown anamorph/teleomorph associa-

Table 2 Statistical models for species in Botryosphaeriaceae spore event, spore count and spore count class, resulting fromstep-wise regression analysis

Dependent variable	Model weather variables (independent variables)								
	Intercept	T48 maxTemp	Min Temp	Max Temp	MinRH	MaxRH	mmRain	Model R^2	Model probability[d]
Pathogen spore event incidence	−0.55[a] (0.26)[b]	–	−0.14 (0.02) (0.07)[c]	0.16 (0.02) (0.02)	–	0.007 (0.002) (0.05)	0.15 (0.03) (0.17)	0.31	<0.0001
Pathogen spore count	−33.42 (19.47)	2.16 (1.07) (0.01)	–	–	–	–	49.82 (5.10) (0.27)	0.28	<0.0001
Pathogen spore count class	0.61 (0.28)	–	–	–	−0.12 (0.01) (0.22)	0.13 (0.01) (0.03)	0.49 (0.08) (0.28)	0.54	<0.0001

[a] Parameter estimate

[b] Standard error

[c] Partial model R^2

[d] $P \leq 0.05$

tion and unknown or overlapping spore morphology of the many species involved by allowing the single spores to develop into pure cultures that could easily be identified to genus level based on colony and morphological characteristics. An accurate count of the different pathogens trapped could thus be made based on the number of cultures obtained. Identification of the trapped pathogens was therefore not dependent on spore morphology. As is the case generally in aerobiology, one should take into account that a proportion of the spores trapped during a specific 6 h period might have been released in previous 6 h periods. However, in assuming that the majority of spores trapped were released in the corresponding 6 h period, spore trap data associated with a specific 6 h period were correlated with the weather data recorded during that specific period.

In the current study, no spore events were recorded for *Pa. chlamydospora* and/or any of the different *Phaeoacremonium* spp. associated with grapevines. This result was especially surprising since *Pa. chlamydospora* was isolated at relatively high incidences from naturally-infected pruning wound stubs in the Chenin Blanc vineyard where the spore trapping was done (unpublished results). In previous studies, aerial inoculum of these pathogens were trapped using petroleum jelly covered microscope slides mounted in close proximity to the surface of grapevine cordons (Eskalen and Gubler 2001; Eskalen et al. 2005). From these studies it was reported that spore release by these pathogens are closely related with the occurrence of rainfall (Eskalen and Gubler 2001; Eskalen et al. 2005). However, in Italy, Michelon et al. (2007) also failed to trap spores of these two pathogen genera using a volumetric spore trap.

This lack of recorded spore events for the above-mentioned pathogens could possibly be explained by the differences in spore size and growth rate of *Pa. chlamydospora* and *Phaeoacremonium* spp. relative to the other pathogen species in the trunk disease complex. Even though the spore trapping protocol developed allowed for trapping of morphologically diverse spores from different pathogens, it also allowed for the trapping of all air-borne spores by the Quest volumetric spore trap. This led to a mixed spore suspension being plated out onto the WA dishes from which the single germinating conidia were subsequently isolated. Conidia of *Pa. chlamydospora* and *Phaeoacremonium* spp. ranges between 3.0–5.0× 1.0–1.5 µm in size (Crous and Gams 2000; Mostert et al. 2005), which is much smaller than other trunk disease pathogens such as *E. lata* (Carter 1988) and the various species in *Phomopsis* (van Niekerk et al. 2004) and species in the Botryosphaeriaceae (van Niekerk et al. 2005). It is therefore quite possible that, at 50× magnification, the transfer of germinating conidia was biased towards the fungi with larger conidia. The growth and germination rates at ±25°C of *Pa. chlamydospora* (Crous and Gams 2000) and the different *Phaeoacremonium* spp. (Mostert et al. 2005) is also considerably slower in comparison with other trunk disease pathogens, and these spores might not have germinated when the single spore isolation was conducted. However, in preliminary evaluation of

Table 3 Spearman's Rank correlation coefficients of *Phomopsis* spore event incidence, spore count and spore count class with twelve weather variables

	Weather variables											
	48 hrs before spore event						During spore event[c]					
Pathogen variables	T48 HrsRH ≥75	T48 MeanRH	T48 minTemp	T48 maxTemp	HrsRain Before	mmRain Before	Min Temp	Max Temp	MinRH	MaxRH	mmRain	Mean Wind
Event incidence	0.063[a] (0.400)[b]	0.070 (0.355)	0.035 (0.642)	−0.031 (0.677)	0.071 (0.341)	0.077 (0.306)	−0.178 (0.018)	−0.046 (0.539)	0.173 (0.021)	0.308 (<0.0001)	0.469 (<0.0001)	0.175 (0.020)
Spore count	0.066 (0.381)	0.074 (0.324)	0.067 (0.372)	0.010 (0.897)	0.074 (0.323)	0.084 (0.267)	−0.169 (0.024)	−0.024 (0.748)	0.179 (0.016)	0.332 (<0.0001)	0.492 (<0.0001)	0.170 (0.024)
Spore count class	0.063 (0.405)	0.073 (0.336)	0.070 (0.350)	−0.008 (0.916)	0.072 (0.338)	0.082 (0.280)	−0.171 (0.023)	−0.024 (0.749)	0.178 (0.018)	0.331 (<0.0001)	0.492 (<0.0001)	0.172 (0.022)

[a] Correlation coefficients

[b] Statistical significance at $P \leq 0.10$

[c] One or more consecutive 6-h periods

the technique, *Pa. chlamydospora* and *Pm. aleophilum* conidia from *in vitro* cultures germinated within 36 h on WA, and were visible at 50× magnification.

In France and California, pycnidia of *Pm. aleophilum* as well as perithecia of *Togninia minima* and *T. fraxinopennsylvanica* (*Phaeoacremonium* teleomorph species) were found to occur on grapevines in the field (Eskalen et al. 2005; Rooney-Latham et al. 2005). The hyphomycete and pycnidial synanamorphs of *Pa. chlamydospora* were also observed sporulating on protected surfaces inside cracks on grapevine trunks (Edwards et al. 2001). Under favourable environmental conditions these structures are therefore regarded to contribute to the aerial inoculum present in vineyards. The survey of fruiting structures present in the vicinity of the spore trap done as part of the current study failed to detect any of the sporulating structures associated with these two genera. However, given the frequent isolation of these pathogens from the grapevines in the vicinity of the spore trap, inoculum of these pathogens had to be present to cause infections. It is a possibility that the spores produced by the pycnidial and hyphomycete synanamorphs of *Pa. chlamydospora* and *Phaeoacremonium* spp. are not necessarily wind dispersed but rather by other means such as water splash and insect vectors. Several *Phaeoacremonium* spp. associated with grapevines have been isolated from insect galleries on various woody hosts indicating that insects might play a role as vectors (Edwards et al. 2001; Mostert et al. 2005). In the case of grapevines, the possibility of insect vectors for *Phaeoacremonium* spp. spore dispersal was shown in California where *Pm. aleophilum* colonies grew after insects trapped in vineyards were plated on artificial media (A. Eskalen, University of California, Riverside, personal communication).

Unlike spores of *Pa. chlamydospora* and *Phaeoacremonium* spp., spores of *E. lata*, species in Botryosphaeriaceae and *Phomopsis* were trapped throughout the trapping periods of 2004 and 2005. The spores of the various pathogens were observed to be present during or after rainfall occurring. In the case of species in the Botryosphaeriaceae, it was also noted that air-borne spores were even present after periods of relative humidity (RH) at or above 70%. However, in both years, a short period of approximately 2 weeks was observed during which no or very few spores of these pathogens were trapped. Weather data indicated that in both years, little or no

Table 4 Statistical models for *Phomopsis* spore event, spore count and spore count class, resulting from step-wise regression analysis

	Model weather variables (independent variables)								
Dependent variable	Intercept	T48 maxTemp	Min Temp	Max Temp	MinRH	MaxRH	mmRain	Model R^2	Model probability[d]
Pathogen spore event incidence	0.14[a] (0.03)[b]	–	–	–	–	–	0.31 (0.05) (0.20)[c]	0.20	<0.0001
Pathogen spore count	−12.51 (6.00)	0.84 (0.35) (0.03)	–	–	–	–	8.52 (1.78) (0.11)	0.14	<0.0001
Pathogen spore count class	0.50 (0.35)	–	–	–	−0.22 (0.03) (0.17)	0.23 (0.03) (0.02)	0.31 (0.14) (0.23)	0.41	<0.0001

[a] Parameter estimate

[b] Standard error

[c] Partial model R^2

[d] $P \leq 0.05$

rainfall occurred during these periods, while RH levels were also very low. Given these general results it therefore seemed that spore release of *E. lata*, species in Botryosphaeriaceae and *Phomopsis* were governed primarily by rainfall and/or high RH during our study and that rainfall of as little as 0.25–1 mm can lead to spore release.

Previous studies on the spore dispersal of different species in Botryosphaeriaceae occurring on woody hosts, including grapevines, indicated that increased levels of air-borne inoculum were associated with rainfall and periods of high RH (Hewitt and Pearson 1988; Pusey 1989; Ahimera et al. 2004). Statistical analysis of the species in Botryosphaeriaceae spore trapping data and recorded weather data supported previous observations. Correlation analysis indicated significant positive correlation coefficients for the occurrence of a spore event on any given time to be governed by high RH and rainfall occurring in the previous 48 h as well as during the spore event. The negative correlation with MinTemp recorded during the event therefore indicates that if the temperature during the event increases too much, the likelihood of a species in Botryosphaeriaceae spore event occurring declines. Apart from determining the occurrence of a species in Botryosphaeriaceae spore event it was furthermore observed that rainfall and RH levels also play an important role in determining the spore count and spore count class of the spore event. Again it was seen that an increase in temperature negatively influences the amount of spores released during the spore event. This might indicate that, in the Stellenbosch area, which is a winter rainfall area, increasing temperature causes the RH to decline, as well as the likelihood of rainfall occurring. Wind was also observed to be an important weather factor for the occurrence of a spore event, as well as the amount of spores released, possibly acting as a mode of dispersal of the released spores.

The importance of temperature, RH and rainfall in determining species in Botryosphaeriaceae spore events taking place, as well as the amount of spores being released during these events were further confirmed by the statistical models derived from the step-wise regression analyses. Despite the low R^2-values obtained for all three species in Botryosphaeriaceae models, it still gives a valuable insight into the biology of this group of grapevines pathogens. As evident from the spore event incidence model, a complicated interaction exists between the four weather variables. For example, the minimum requirements for a spore event to take place were a MinTemp ≥1°C, MaxTemp ≥4.5°C, ≥75% MaxRH with at least 3 mm of rainfall. However, when no rainfall occurred, and the MinTemp and MaxTemp was above 13°C and 16°C, respectively, the maxRH value needed to be 100% for a spore event to take place. From the model it is therefore clear that following rainfall, spore events of the Botryosphaeriaceae should occur at lower temperatures and RH values, indicating the important role of rainfall in a spore event taking place.

Similar to the species in Botryosphaeriaceae event incidence model, and based on pR^2-values, mmRain was also the most important variable in the spore

Table 5 Spearman's Rank correlation coefficients of *E.lata* spore event incidence, spore count and spore count class with twelve weather variables

	Weather variables											
	48 hrs before spore event						During spore event[c]					
Pathogen variables	T48 HrsRH >75	T48 MeanRH	T48 minTemp	T48 maxTemp	HrsRain Before	mmRain Before	Min Temp	Max Temp	MinRH	MaxRH	mmRain	Mean Wind
Event incidence	0.127[a]	0.174	0.048	−0.2274	0.104	0.170	−0.074	−0.003	0.134	0.220	0.307	0.058
	(0.237)	(0.103)	(0.656)	(0.033)	(0.333)	(0.111)	(0.488)	(0.977)	(0.210)	(0.038)	(0.004)	(0.590)
Spore count	0.126	0.163	0.066	−0.205	0.120	0.182	−0.070	0.011	0.134	0.240	0.313	0.067
	(0.241)	(0.126)	(0.541)	(0.054)	(0.264)	(0.089)	(0.516)	(0.915)	(0.209)	(0.024)	(0.003)	(0.533)
Spore count class	0.126	0.165	0.061	−0.210	0.120	0.183	−0.070	0.010	0.133	0.236	0.314	0.067
	(0.238)	(0.121)	(0.568)	(0.048)	(0.262)	(0.086)	(0.512)	(0.925)	(0.214)	(0.026)	(0.003)	(0.536)

[a] Correlation coefficients

[b] Statistical significance at $P \leq 0.10$

[c] One or more consecutive 6-h periods

count class model, followed by MinRH and MaxRH. Manipulation of this model revealed that increasing rainfall (mmRain) combined with increasing MaxRH during the spore event led to a sharp increase in the spore count class. It was also observed that even though MinRH has a negative parameter estimate in the model, increasing MinRH does not have such a big negative influence on the final spore count class due to the positive intercept of this model. Similar to the other two statistical models derived for species in Botryosphaeriaceae, mmRain was also by far the most important weather variable in the spore count model with a pR^2-value of 0.27 out of a model R^2 of 0.28. These results therefore conclusively showed that, similar to previous studies on grapevines and other woody hosts (Hewitt and Pearson 1988; Pusey 1989; Ahimera et al. 2004), spore events and the number of spores released by Botryosphaeriaceae species in South African vineyards are dependent on high RH and rainfall occurring prior to and during the period of spore release, although a slight increase in temperature was required to trigger a spore event.

Apart from the previously stated similarities, it was found that the sources of inoculum and the mechanism of dispersal found in the current study were similar to previous reports. Pycnidia of *Diplodia mutila* (Fr.) Mont. and *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. have been reported to occur on pruning debris and diseased plant material in vineyards and that conidia are released after periods of rain and/or high RH, with water splash and wind being the main modes of dispersal (Lehoczky 1974; Hewitt and Pearson 1988). Results of the fruiting structure survey conducted showed that abundant pycnidia of *Diplodia* and *Dothiorella* spp. were present in the vicinity of the spore trap indicating that these structures were in all likelihood the source of propagules of Botryosphaeriaceae species trapped during the current study. As stated above, wind was positively correlated with species in Botryosphaeriaceae spore event incidence, spore count and spore count class, clearly showing that the mechanism involved in spore dispersal during the current study was probably wind dispersal, similar to the reports by Lehoczky (1974) and Hewitt and Pearson (1988). Rain splash was observed to play an important role in dispersal of *Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not. in pistachio orchards (Ahimera et al. 2004). One would therefore expect that in vineyards, rain splash also plays an important role in

Table 6 Statistical models for *E. lata* spore event, spore count and spore count class, resulting from step-wise regression analysis

Dependent variable	Model weather variables (independent variables)								
	Intercept	T48 maxTemp	Min Temp	Max Temp	MinRH	MaxRH	mmRain	Model R^2	Model probability[d]
Pathogen spore event incidence	0.50[a] (0.35)[b]	−0.04 (0.02) (0.06)[c]	–	–	−0.05 (0.02) (0.07)	0.06 (0.02) (0.06)	–	0.19	0.0173
Pathogen spore count	–	–	–	–	–	–	–	–	–
Pathogen spore count class	0.49 (0.48)	–	–	–	−0.20 (0.04) (0.21)	0.21 (0.04) (0.06)	–	0.27	<0.0001

[a] Parameter estimate

[b] Standard error

[c] Partial model R^2

[d] $P \leq 0.05$

liberating conidia from gelatinous cirrhi that exude from pycnidia into aerosols, which are subsequently wind-dispersed. However, species in Botryosphaeriaceae spore events were also recorded in the absence of rain, indicating that other mechanisms are also involved in this process. Alternatively, perithecia should be considered to play a more prominent role in contributing to aerial inoculum of species in Botryosphaeriaceae.

Similar to the various species in Botryosphaeriaceae, severe outbreaks of Phomopsis cane and leaf spot has been associated with high rainfall, which causes abundant spore release and subsequent heavy infections of new green shoots and leaves (Anderson and Colby 1943). The results obtained during the current study showed great similarities with these earlier reports. It was clearly shown that high RH combined with rainfall are the major weather variables dictating when a *Phomopsis* spp. spore event takes place, as well as the amount of spores released during a given event. Increasing temperature, probably due to its drying-out effect on fruiting structures, was also observed to have a negative effect on spore event incidence, as well as amount of spores released. However, in the case of species in Botryosphaeriaceae, rainfall and RH levels prior to spore events were also observed to be important especially for spore events to occur. While in the case of *Phomopsis*, only the rainfall, RH and temperature during the event itself were shown to be important.

These weather variables were consequently also the most important variables included in the statistical models derived for *Phomopsis* spp. These models again provided useful insight into the pathogen biology despite the low R^2-values obtained. Rainfall (mmRain) recorded during a spore event was the most important weather variable in all the *Phomopsis* models based on its pR^2-values. Manipulation of the *Phomopsis* spore event incidence model indicated that rainfall of between 2.75 and 3 mm is needed to initiate a spore event. T48maxTemp was also included in the spore count model but had a very low partial R^2-value indicating that it is of much less significance compared to rainfall in determining the spore count during a spore event. The spore count class model for *Phomopsis* spp. was, with respect to the weather variables included, exactly the same as the count class model obtained for species in Botryosphaeriaceae. In terms of importance, the variables were also ranked the same based on their pR^2-values with mmRain being the most important followed by MinRH and MaxRH. Despite the negative parameter estimate for MinRH, the combined effect of mmRain and MaxRH negated any possible negative effect that a sharp increase in MinRH during the spore event might have on the number of spores released. Rainfall and RH were therefore again the most important factors determining spore release by *Phomopsis* spp., as well as the amount of spores being released.

Very few spore events were recorded for *E. lata* in 2004 and 2005. However, if the *E. lata* spore trapping data were examined more closely, it is evident that the spore events occurred during, or shortly after periods when at least 2 mm of rainfall was recorded. This corresponds with findings of Pearson (1980) and Magarey and Carter (1986), which stated that dis-

charge of *E. lata* ascospores starts within 3 h of perithecia being exposed to at least 2 mm of rain. These results were furthermore supported by the positive correlation observed between the different pathogen variables and the amount of rainfall occurring before a spore event. The correlation analysis also indicated that the amount of spores released during an *E. lata* spore event is dependent on the RH values and amount of rainfall recorded during the spore event. However, on several occasions significant amounts of rainfall were recorded but no *E. lata* spores were trapped. This might possibly be explained by the negative correlation observed between spore event incidence and T48maxTemp. One can hypothesise that perithecium-bearing stroma dehydrate when temperatures are too high, thereby reducing the likelihood of spore production and release.

Despite all the positive correlations between weather variables and *E. lata* pathogen variables, step-wise regression analyses yielded models for *E. lata* spore event incidence and spore count class that had very low R^2-values. The negative influence of T48maxTemp was also reflected in the spore event incidence model where T48maxTemp had a negative parameter estimate indicating a negative influence on spore event incidence. With respect to the weather variables determining the amount of spores released during a spore event, *E. lata* showed similar results compared to Botryosphaeriaceae and *Phomopsis* spp. Spore count, as well as spore count class for this pathogen, was positively correlated with the MaxRH and rainfall recorded during the spore event. MinRH and MaxRH was also included in the *E. lata* spore count class model.

The most important sources of *E. lata* inoculum in apricot orchards and vineyards are perithecia formed in stromatic tissue on diseased wood (Carter 1957; Moller and Carter 1965; Moller et al. 1977; Magarey and Carter 1986). Pruning debris bearing stromatic tissue and mature *E. lata* perithecia were found within a 2 m radius of the spore trap location in the current study. This close proximity of perithecia to the spore trap therefore indicates that ascospores released by perithecia were probably, similar to above-mentioned studies, the primary source of *E. lata* propagules trapped during the current study.

Given the correct weather conditions, inoculum of *E. lata*, *Phomopsis* and/or species in Botryosphaeriaceae could be present, and available to infect susceptible pruning wounds, in vineyards throughout the South African pruning period from June to September. The level of inoculum present can, however, vary between years due to weather differences. It was furthermore found that the occurrence of spore events and the number of spores released during the spore events of these pathogens are controlled by rainfall, relative humidity and temperature conditions prior to the spore event and during the event itself. Differences between pathogens were observed, highlighting the differences in the biology of the various pathogens. The use of the statistical models obtained in this study is currently restricted to giving insight into the biology of especially species in *Phomopsis* and the Botryosphaeriaceae. For the development of models that would allow more accurate forecasting of spore events for the various pathogens, spore trapping would have to be done over a number of years, as well as over an entire year to include all seasons. It is also clear that certain aspects pertaining to the biology of *Pa. chlamydospora* and *Phaeoacremonium* spp. still needs to be investigated further, including the mechanisms involved in spore dispersal, as well as the possibility that other vectors, particularly insects, might be involved in dispersing the propagules of these two pathogen genera.

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References

Ahimera, N., Gisler, S., Morgan, D. P., & Michailides, T. J. (2004). Effects of single-drop impactions and natural and simulated rains on the dispersal of *Botryosphaeria dothidea* conidia. *Phytopathology, 94*, 1189–1197.

Anderson, H. W., & Colby, A. S. (1943). Dead arm disease of grape in Illinois. *The Plant Disease Reporter, 27*, 245–246.

Carter, M. V. (1957). *Eutypa armeniacae* Hansf. & Carter, sp. nov., an airborne vascular pathogen of *Prunus armeniaca* L. in Southern Australia. *Australian Journal of Botany, 5*, 21–35.

Carter, M. V. (1988). Eutypa dieback. In R. C. Pearson & A. C. Goheen (Eds.), *Compendium of grape diseases* (pp. 32–34). Minnesota: APS.

Crous, P. W., & Gams, W. (2000). *Phaeomoniella chlamydospora* gen. et comb. Nov., a causal organism of Petri grapevine decline and esca. *Phytopathologia Mediterranea, 39*, 112–118.

Crous, P. W., Slippers, B., Wingfield, M. J., Rheeder, J., Marasas, W. F. O., Phillips, A. J. L., et al. (2006). Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology, 55*, 235–253.

Cucuzza, J. D., & Sall, M. A. (1982). Phomopsis cane and leaf spot disease of grapevine: effects of chemical treatments on inoculum level, disease severity, and yield. *Plant Disease, 66*, 794–97.

Draper, N. R., & Smith, H. (1980). *Applied regression analysis* (2nd ed.). New York: Wiley.

Edwards, J., Laukart, N., & Pascoe, I. (2001). *In situ* sporulation of *Phaeomoniella chlamydospora* in the vineyard. *Phytopathologia Mediterranea, 40*, 61–66.

Eskalen, A., & Gubler, W. D. (2001). Association of spores of *Phaeomoniella chlamydospora*, *Phaeoacremonium inflatipes* and *Phaeoacremonium aleophilum* with grapevine cordons in California. *Phytopathologia Mediterranea, 40*, S429–S432.

Eskalen, A., Rooney-Latham, S., & Gubler, W. D. (2005). Occurrence of *Togninia fraxinopennsylvanica* on esca-diseased grapevines (*Vitis vinifera*) and declining ash trees (*Fraxinus latifolia*). *Plant Disease, 89*, 528.

Ferreira, J. H. S., Matthee, F. N., & Thomas, A. C. (1989). Fungi associated with dieback and pruning wounds of grapevines in South Africa. *South African Journal of Enology and Viticulture, 10*, 62–66.

Fischer, M., & Kassemeyer, H.-H. (2003). Fungi associated with Esca disease of grapevines in Germany. *Vitis, 42*, 109–116.

Hewitt, W. B., & Pearson, R. C. (1988). Diplodia cane dieback and bunch rot. In R. C. Pearson & A. C. Goheen (Eds.), *Compendium of grape diseases* (pp. 25–26). Minnesota: APS.

Jacobs, K. A., & Rehner, S. A. (1998). Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botryosphaeria* and related taxa. *Mycologia, 90*, 601–610.

Larignon, P., & Dubos, B. (2000). Preliminary studies on the biology of *Phaeoacremonium*. *Phytopathologia Mediterranea, 39*, 184–189.

Lehoczky, J. (1974). Black dead-arm disease of grapevines caused by *Botryosphaeria stevensii* infection. *Acta Phytopathologica Academiae Scientiarum Hungaricae, 9*, 319–327.

Magarey, P. A., & Carter, M. V. (1986). New technology facilitates control of Eutypa dieback in apricots and grapevines. *Plant Protection Quarterly, 1*, 156–159.

Merrin, S. J., Nair, N. G., & Tarran, J. (1995). Variation in *Phomopsis* recorded on grapevine in Australia and its taxonomic and biological implications. *Australasian Plant Pathology, 24*, 44–56.

Michailides, T. J., & Morgan, D. P. (1993). Spore release by *Botryosphaeria dothidea* in pistachio orchards and disease control by altering the trajectory angle sprinklers. *Phytopathology, 83*, 145–152.

Michelon, L., Pellegrini, C., & Pertot, I. (2007). First observations of esca disease in the Trentino area, northern Italy: Monitoring of spores, evolution of symptoms and evaluation of incidence. *Phytopathologia Mediterranea, 46*, 105.

Moller, W. J., Braun, A. J., Uyemoto, J. K., & Kasimatis, A. N. (1977). *Eutypa armeniacae* inoculum associated with dead arm-affected grapevines in New York and Ontario. *Plant Disease Reporter, 61*, 422–423.

Moller, W. J., & Carter, M. V. (1965). Production and dispersal of ascospores in *Eutypa armeniacae*. *Australian Journal of Biological Sciences, 18*, 67–80.

Mostert, L., Groenewald, J. Z., Summerbell, R. C., Sutton, D. A., Padhye, A. A., & Crous, P. W. (2005). Species of *Phaeoacremonium* associated with human infections and environmental reservoirs in infected woody plants. *Journal of Clinical Microbiology, 43*, 1752–1767.

Mostert, L., Halleen, F., Fourie, P. H., & Crous, P. W. (2006). A review of *Phaeoacremonium* species involved in Petri disease and esca of grapevines. *Phytopathologia Mediterranea, 45*, S12–S29.

Mugnai, L., Graniti, A., & Surico, G. (1999). Esca (black measels) and brown wood-streaking: two old and elusive diseases of grapevines. *Plant Disease, 83*, 404–416.

Munkvold, G. P., Duthie, J. A., & Marois, J. J. (1994). Reductions in yield and vegetative growth of grapevines due to Eutypa dieback. *Phytopathology, 84*, 186–192.

Pearson, R. C. (1980). Discharge of ascospores of *Eutypa armeniacae* in New York. *Plant Disease, 64*, 171–174.

Pusey, P. L. (1989). Availability and dispersal of ascospores and conidia of *Botryosphaeria* in peach orchards. *Phytopathology, 79*, 635–639.

Rooney, S., Eskalen, A., & Gubler, W. D. (2004). Ascospore discharge and occurrence of *Togninia minima* (anamorph = *Phaeoacremonium aleophilum*) in California vineyards. *Phytopathology, 94*, S57.

Rooney-Latham, S., Eskalen, A., & Gubler, W. D. (2005). First report of naturally occurring *Togninia minima* perithecia. *Phytopathologia Mediterranea, 44*, 105.

Serra, S., Borgo, M., & Zanzotto, A. (2000). Investigation into the presence of fungi associated with esca of young vines. *Phytopathologia Mediterranea, 39*, 21–25.

Snedecor, G. W., & Cochran, W. G. (1967). *Statistical methods* (6th ed.). Ames: The Iowa State University Press.

Van Niekerk, J. M., Crous, P. W., Groenewald, J. Z., Fourie, P. H., & Halleen, F. (2004). DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevines. *Mycologia, 96*, 781–798.

Van Niekerk, J. M., Groenewald, J. Z., Farr, D. F., Fourie, P. H., Halleen, F., & Crous, P. W. (2005). Reassessment of *Phomopsis* species on grapevine. *Australasian Plant Pathology, 34*, 27–39.