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Aerial inoculum patterns of Petri disease pathogens in South African vineyards and rootstock mother blocks

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Summary. Petri disease is caused by the xylem inhabiting fungi *Phaeomoniella* (*Pa.*) *chlamydospora* and several *Phaeoacremonium* (*Pm.)* species. Pruning wounds are known host ports of entry for aerial spores of these pathogens. However, knowledge is lacking on occurrence of these pathogens as aerial inoculum within South African vineyards. This study determined when spores of Petri disease pathogens are released in Western Cape Province vineyards and how these spore release events coincided with pruning activities when infections could occur. The research was conducted for two seasons from mid-May to early December 2012 and from mid-March to early December 2013. Microscope slide spore traps were affixed to arms of infected vines in six vineyards and mother vines in two rootstock mother vine nurseries. The slides were replaced weekly and fungal spores were retrieved from them, cultured, counted and identified. Colonies resembling those of *Pa. chlamydospora* and *Phaeoacremonium* spp. were subcultured for further molecular identification. Species of *Phaeoacremonium* were identified by amplification of the partial beta-tubulin gene. Taqman probes and primers were developed to facilitate fast detection of the most frequently occurring species (*Pm. minimum*, *Pm. parasiticum* and *Pm. sicilianum*), using real-time PCR. Petri disease pathogens occurred throughout the periods investigated. *Phaeomoniella chlamydospora* and *Pm. minimum* were trapped in all vineyards. A total of 14 *Phaeoacremonium* species were identified, with the greatest diversity ever recorded in vineyards, including *Pm. australiense, Pm. griseo-olivaceum, Pm. griseorubrum, Pm. inflatipes*, *Pm. iranianum, Pm. italicum, Pm. minimum, Pm. parasiticum, Pm. prunicola, Pm. scolyti, Pm. sicilianum, Pm. subulatum, Pm. venezuelense* and *Pm. viticola*. Of these, only *Pm. minimum* and *Pm. inflatipes* have been reported as aerial inoculum within vineyards. Spore release coincided with winter and spring pruning activities. The occurrence of six *Phaeoacremonium* species in rootstock mother vine nurseries highlights the high risk of pathogen spread through infected nursery material. This is the greatest *Phaeoacremonium* species diversity ever recorded in vineyards and the first detection of *Phaeoacremonium* species aerial inoculum in grapevine rootstock mother vine nurseries. The high species diversity and frequency of spore release in vineyards and rootstock mother vine nurseries coinciding with traditional pruning practices emphasizes the need to develop effective wound protection strategies to avoid infection of unprotected grapevine pruning wounds.

Keywords. *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., *Vitis* spp., epidemiology, spore release.

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INTRODUCTION

Petri disease is a serious problem in grapevines affecting grape production worldwide including South Africa, where it has been a major problem since the early 1990's (Ferreira *et al*., 1994). Petri disease is caused by *Phaeomoniella* (*Pa*.) *chlamydospora* and *Phaeoacremonium* (*Pm*.) species (Scheck *et al*., 1998a; Mugnai *et al*., 1999; Mostert *et al*., 2006a), and is usually associated with 1- to 5-year-old vines in newly established vineyards (Mugnai *et al*., 1999; Halleen and Groenewald, 2005). Seventeen species of *Phaeoacremonium* have been isolated from symptomatic vines in South Africa, including *Pm. australiense, Pm. austroafricanum*, *Pm. fraxinopennsylvanicum, Pm. griseo-olivaceum, Pm. griseorubrum, Pm. inflatipes*, *Pm. iranianum*, *Pm. italicum* (reported as *Pm. alvesii*), *Pm. krajdenii*, *Pm. minimum*, *Pm. parasiticum*, *Pm. prunicola*, *Pm. scolyti*, *Pm. sicilianum*, *Pm. subulatum*, *Pm. venezuelense* and *Pm. viticola* (Crous *et al*., 1996; Groenewald *et al*., 2001; Mostert *et al*., 2006b; White *et al*., 2011a; Spies *et al*., 2018). Infected vines show stunted growth, shortened internodes and dieback. Internal symptoms include black to brown spots and brown streaking in the xylem tissues (Ferreira, 1994; Scheck *et al*., 1998a). The black discolouration is a result of host responses to infection, which include the formation of tyloses and phenolic compounds in the xylem tissues (Mugnai *et al*., 1999; Lorena *et al*., 2001; Del Río *et al*., 2004). These responses prohibit host water uptake (Edwards *et al*., 2007), and decreases the lifespan of infected vineyards. Replanting of young vineyards is subsequently required (Scheck *et al*., 1998b; Ferreira *et al*., 1999). The pathogens associated with Petri disease are well-adapted endophytes that cause disease during stress conditions (Ferreira *et al*., 1999).

Petri disease pathogens spread within vineyards as aerial inoculum from reproductive structures (Larignon and Dubos, 2000; Eskalen and Gubler, 2001). The asexual stage of *Pa. chlamydospora* (pycnidia) has been reported as the source of aerial inoculum, but a sexual stage has not been reported (Crous and Gams, 2000; Edwards *et al*., 2001; Eskalen *et al*., 2002; Edwards and Pascoe, 2004; Baloyi *et al*., 2016). Since Mostert *et al.* (2003) confirmed the sexual stage of *Phaeoacremonium* spp. by *in vitro* pairings, five *Phaeoacremonium* sexual morph connections from isolates originating from grapevines were made, including *Pm. austroafricanum, Pm. krajdenii, Pm. viticola, Pm. parasiticum* and *Pm. minimum*. However, only three *Phaeoacremonium* sexual morphs have been found on grapevines in nature, including *Pm. minimum* (Rooney-Latham *et al*., 2005a; Baloyi *et al*., 2013), *Pm. viticola* and *Pm. fraxinopennsylvanicum* (Eskalen *et al*., 2005a; b). *Phaeoacremonium viticola* and *Pm. fraxinopennsylvanicum* perithecia have also been found on ash trees near vineyards in California, emphasizing the potential role of alternative hosts as inoculum sources (Eskalen *et al*., 2005a; b). Furthermore, the known host range of *Phaeoacremonium* species has greatly increased to include a broad range of fruit trees, ornamental plants and natural vegetation, most of which can be found in close proximity to vineyards (Spies *et al*., 2018).

Spore trapping studies have shown the presence of aerial inoculum of Petri disease pathogens in vineyards of France, California and Italy, where large spore counts coincided with rainfall events, although spores were also detected in periods of no rainfall (Larignon and Dubos, 2000; Eskalen and Gubler, 2001; Roony-Latham *et al*., 2005b; Quaglia *et al*., 2009). The spore release mechanisms of *Phaeoacremonium* perithecia might explain this phenomenon. In the absence of rainfall, but sufficient relative humidity, perithecia ooze ascospores which result in small release events, but under sufficient wetting periods, i.e. during rainfall, ascospore release takes place through forcible discharge (Rooney-Latham *et al.* 2005b).

Petri disease pathogens infect grapevines through susceptible pruning wounds (Larignon and Dubos, 2000; Eskalen *et al*., 2007; Van Niekerk *et al*., 2011a). Wound susceptibility is influenced by several factors including cultivar susceptibility, wound age, pruning date, and climate, but pruning wounds remain susceptible for at least 3 to 4 weeks (Van Niekerk *et al*., 2011a), although they could remain susceptible for as long as 3 to 4 months (Larignon and Dubos, 2000; Eskalen *et al*., 2007; Elena and Luque, 2016). Due to the long period of wound susceptibility, it is crucial that winter and spring pruning practices do not coincide with prolific spore release, as this will lead to increased probability of new wound infections.

For South African vineyards, the frequency and patterns of spore release of Petri disease pathogens are unknown. A previous attempt to quantify and correlate spore release of Petri disease causing-pathogens with rainfall using a volumetric spore trap was unsuccessful (Van Niekerk *et al*., 2010). The aim of the present study was, therefore, to determine if spores of Petri disease pathogens were present in South African vineyards, when these spores were released, and the risks these pose as inoculum during traditional pruning periods. Whiteman (2004) developed a species-specific PCR-based detection method to detect *Pa. chlamydospora* aerial inoculum in samples collected from a grapevine rootstock block. To our knowledge, the present study is the first to investigate *Phaeoacremonium* spp. aerial inoculum in grapevine rootstock mother vine nurseries.

MATERIALS AND METHODS

Site selection

Six vineyards within the Western Cape Province, South Africa, known to be infected with Petri disease pathogens, were selected for this study. These were two vineyards each in Paarl and Stellenbosch, and one vineyard in Durbanville and one in Rawsonville (Van Niekerk *et al*., 2011b; White *et al*., 2011b; Baloyi *et al*., 2013; 2016; Moyo *et al*., 2014). Two rootstocks mother vine nurseries were also included, one in Slanghoek and the other in Wellington (Table 1). Within each vineyard, five vines or five rootstock mother plants were selected and marked. The Western Cape has a Mediterranean climate with rainy winters and dry, warm to hot summers.

Spore trapping

The protocol was adopted from Eskalen and Gubler (2001). Spore traps consisted of microscopic slides coated with Vaseline® petroleum jelly on both sides, and were affixed with wire and binder clips to the vine cordon on each of the five plants. The slides were arranged directly above old pruning wounds or cracks.

The study was conducted over two seasons, from late May to the first week of December in 2012 and from mid-March to the first week of December in 2013. The spore trap slides were replaced weekly. The slides were placed individually in sterile Falcon tubes, and immediately taken to a laboratory for processing. Each slide was washed in 5 mL of sterile distilled water (dH_2O) to suspend the spores, and the resulting spore suspension was then filtered through 5 µm and 0.45 µm microfilters. This separated large from small spores. The filters were then backwashed with 1 mL dH_20 of which 200 µL was then plated onto each of three PDA-chloramphenicol (PDA-C) agar plates. The plates were sealed with Parafilm® and incubated at 25°C for 4–8 weeks. The number of *Pa. chlamydospora* and *Phaeoacremonium* spp. colonies were monitored and recorded as a total from the three plates. *Phaeomoniella chlamydospora* colonies were morphologically identified according to Crous and Gams (2000). *Phaeoacremonium* species were subcultured for further identification. In cases where more than one colony of the same species occurred on the three dishes from one grapevine, a representative sample was subcultured.

DNA extraction, PCR and sequencing

DNA was extracted from mycelia harvested from 2-week-old *Phaeoacremonium*cultures using a CTAB protocol as described by Damm *et al*. (2008). The partial beta-tubulin gene were amplified with PCR using primers T1 and Bt2b (Glass and Donaldson, 1995; O'Donnel and Cigelink, 1997). The reactions were each performed using 0.65 units of Biotaq polymerase, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 0.25 pmol of each primers, 1 mg mL^{-1} of bovine serum albumin (BSA) and 5 µL of DNA solution. The amplifications were performed on a GeneAmp PCR System 2700 (Applied system Biosystems, Foster City California). The cycling conditions were 5 min at 96°C, followed by 40 cycles of 30 s at 94°C, 30 s at 58°C, 1 min and 30 s at 72°C and a 7 min extension step at 72°C to complete the reaction. PCR products were visualised on a 1% agarose gel. PCR products were purified with Nucleospin MSB PCRapace kit (Invitek) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (PE Biosystems). Sequencing products were analysed on an ABI PRISM 3130XL DNA sequencer (Perkin-Elmer) at the Central Analytical Facility of Stellenbosch University. Sequences were trimmed in Geneious 3.5.6 (Kearse *et al*. 2012). Species identification was done using the

Table 1. Vineyards and rootstock mother vine nurseries in which spore trapping studies were conducted in 2012 and 2013.

Vineyard	Cultivar	Age of vineyard	Pruning dates	
			2012	2013
Paarl A	Red Muscadel	30	26/06/2012	27/08/2013
Paarl Z	Hanepoot	40	17/07/2012	08/07/2013
Stellenbosch B3	Mixed table grape cultivar	27	24/07/2012	27/07/2013
Stellenbosch P2	Pinotage	35	10/07/2012	27/07/2013
Durbanville	Sauvignon blanc	29	31/07/2012	18/06/2013
Rawsonville	Chenin blanc	24	24/07/2012	27/05/2013
Slanghoek	Ramsey	19	29/05/2012	06/05/2013
Wellington	Ramsey	17	04/06/2012	04/06/2013

Table 2. Primers and hydrolysis probes used in this study. Oligonucleotide modifications and template specificity are indicated.

Name	Sequence	Modifications	Specificity	Reference
(Primers)				
F_ibt_Paleo	GCTTCGACGTCCTCGA	none	*Pm. minimum*	This study
F_ibt_Ppara	GCTTCGACGACCTCGA	none	*Pm. parasiticum*	This study
F_ibt_Psicil	AGCTTCGAACCATCTCGA	none	*Pm. sicilianum*	This study
R_ibt_uni	GCATTGGCCGGTCTG	none	universal	Martin *et al.* (2012)
(Hydrolysis probes)				
Paleo	CAGAATCTACCCCAGATCATCGACCAGC	5'-FAM™, 3'-QSY®	*Pm. minimum*	This study
Ppara	CGACTCTGACCCCAAAAGCATCGAC	5'-VIC®, 3'-QSY®	*Pm. parasiticum*	This study
Psicil	CCTCGATATCGTCCTCAAAATGTCTCTCAGAC	5'-JUN®, 3'-QSY®	*Pm. sicilianum*	This study

megablast function of the NCBI's GenBank nucleotide database (www.ncbi.nlm.nih.gov).

Primer design and qPCR analysis of Phaeoacremonium minimum*,* Pm. parasiticum *and* Pm. sicilianum isolates

Primers (IDT) and hydrolysis probes (Life Technologies) were designed to bind within the beta-tubulin gene region and are listed in Table 2. Three different species-specific primer/probe sets were designed for the respective detection of *Pm. minimum*, *Pm. parasiticum* and *Pm. sicilianum*, following a similar design strategy to that used for *Pm. minimum* detection by Martin *et al.* (2012). Although this meant that the *Pm. minimum* detection system for the present study was very similar to that of Martin *et al.* (2012), there were significant sequence differences in the assay primers and probe, to provide sufficient discrimination between these *Phaeoacremonium* species. Species-specific forward primers were designed, including F_ibt_Paleo for *Pm. minimum*, F_ibt_Ppara for *Pm. parasiticum* and P_ibt_Psicil for *Pm. sicilianum*, intended for use with the universal reverse primer R_IBT_uni that recognises all *Phaeoacremonium* spp. templates. Species-specific hydrolysis probes (Paleo for *Pm. minimum*, Ppara *Pm. parasiticum* and Psicil for *Pm. sicilianum*) were 5' labelled with unique fluorophores (FAM, VIC and JUN) allowing for a multiplexed experimental design. Furthermore, all hydrolysis probes carried a 3' QSY quencher (Table 2). *Phaeoacremonium minimum* detection assays were run individually, and those for *Pm. parasiticum* and *Pm. sicilianum* were in in duplex. Reactions for *Pm. minimum* detection were each run in a 20 µL final volume containing 2× KAPA PROBE FAST qPCR Master Mix (Kapa Biosystems), 0.2 µM each of F_ibt_Paleo, R_ibt_uni and Paleo, and 0.5 µL of template (genomic DNA 10× diluted in PCR grade water). Reactions were run in a CFX96 Touch™ cycler (Bio-Rad Laboratories Inc.) using the following programme: 3 min at 95°C followed by 40 cycles of 3 s at 95°C and 30 s at 67°C. Reactions for *Pm. parasiticum* and *Pm. sicilianum* detection were each run in duplex, in a 20 µL final volume containing 2× KAPA PROBE FAST qPCR Master Mix (Kapa Biosystems), 0.1 µM each of F_ibt_Ppara and F_ibt_Psicil, 0.2 µM each of R_ibt_uni, Ppara and Psicil, and 0.5 µL of template (genomic DNA 10× diluted in PCR grade water). Alternatively, reactions were run using the following programme: 3 min at 95°C followed by 40 cycles of 3 s at 95°C and 30 s at 65°C also using the CFX96 Touch™ cycler. All assays included non-template controls as well as controls for *Pm. minimum*, *Pm. parasiticum* and *Pm. sicilianum*. All isolates not positively identified as either *Pm. minimum*, *Pm. parasiticum* or *Pm. sicilianum* using the above assays were identified through Sanger sequencing of the beta-tubulin gene region (Central Analytical Facility, Stellenbosch University).

RESULTS

Phaeoacremonium species identification

A total of 1532 representative *Phaeoacremonium* spp. isolates were recorded from the 2012 and 2013 spore trapping seasons. Of these, 919 isolates were amplified with the *Pm. minimum* Taqman probe, 157 with the *Pm. sicilianum* probe and 24 with the *Pm. parasiticum* probe. A total of 432 isolates which did not amplify with the Taqman probes were sequenced as described above. The results for the representative sequence results are presented in Table 3.

Species diversity in vineyards

Petri disease pathogens, *Pa. chlamydospora* and a number of *Phaeoacremonium* species were found in both

Table 3. *Phaeoacremonium* species identification with the partial beta-tubulin gene using the megablast search of GenBank.

Species	GenBank accession	Identities	Gaps
Pm. italicum	EU883990.1	609/612 (99%)	0/612 (0%)
Pm. australiense	EU128073.1	683/683 (100%)	0/683 (0%)
Pm. griseorubrum	EU128075.1	747/750 (99%)	0/750(0%)
Pm. griseo-olivaceum	EU128097.1	671/676 (99%)	0/676 (0%)
Pm. inflatipes	GQ903719.1	575/582 (99%)	0/582 (0%)
Pm. iranianum	KJ200941.1	504/506 (99%)	0/506 (0%)
Pm. minimum	HQ605018.1	618/620 (99%)	0/620 (0%)
Pm. parasiticum	KF870482.1	694/695 (99%)	0/695 (0%)
Pm. prunicola	EU128095.1	551/556 (99%)	0/556 (0%)
Pm. sicilianum	KM016927.1	600/603 (99%)	0/603 (0%)
Pm. subulatum	EU128092.1	616/618 (99%)	0/618 (0%)
Pm. scolyti	EU128090.1	744/751 (99%)	0/751(0%)
Pm. venezuelense	AY579318.1	441/442 (99%)	0/442 (0%)
Pm. viticola	EU128093.1	587/590 (99%)	0/590 (0%)

Table 4. Diversity of Petri disease pathogens collected from spore traps placed in six vineyards and two rootstock mother vine nurseries in the Western Cape Province during 2012 and 2013. (X) Denotes during which year and in which vineyard a particular species was trapped.

Pathogen	Vineyard												Nursery			
	PaarlA		PaarlZ		Stellenbosch B3		StellenboschP2		Durbanville		Rawsonville		Slanghoek		Wellington	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013[a]	2012	2013
Pa. chlamydospora	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pm. minimum	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pm. alvesii								X								
Pm. australiense												X	X			
Pm. griseorubrum								X								
Pm. griseo-olivaceum									X							
Pm. parasiticum	X	X	X	X		X	X	X		X	X	X				
Pm. prunicola					X			X								
Pm. inflatipes				X				X		X						
Pm. iranianum		X		X				X								
Pm. sicilianum											X	X	X			
Pm. scolyti											X	X	X			
Pm. subulatum											X	X	X			
Pm. venezuelense												X				
Pm. viticola		X			X	X										
Total	3	5	3	5	4	4	3	8	3	4	6	8	6	2	2	2

[a] Rootstock mother block was removed 13/05/2013.

seasons of spore trapping, nine *Phaeoacremonium* species were trapped in 2012 and 14 were trapped in 2013 (Table 4). Species diversity differed between vineyards, ranging from two to six Petri disease pathogens within a vineyard in 2012, and two to eight species in 2013. The least number of species within a vineyard were found in the Wellington rootstock mother block, with only two species trapped in both seasons. The greatest diversity during 2012 was recorded in Slanghoek and Rawsonville, with six species in each vineyard, whereas Stellenbosch P2 and Rawsonville vineyards recorded the greatest diversity (eight species in each vineyard) during 2013. *Phaeomoniella chlamydospora* and *Pm. minimum* were the only species trapped in all vineyards in both seasons. *Phaeoacremonium parasiticum* was the second most frequently found *Phaeoacremonium* species in all vine-

yards at least in one of the sesons, except in Slanghoek and Wellington. Pathogens found in both seasons, but not necessarly in the same vineyard, were *Pa. chlamydospora*, *Pm. minimum*, *Pm. australiense*, *Pm. parasiticum*, *Pm. prunicola*, *Pm. sicilianum*, *Pm. scolyti*, *Pm. subulatum* and *Pm. viticola*. Pathogens only reported in 2013 were *Pm. italicum*, *Pm. griseorubrum*, *Pm. inflatipes*, *Pm. iranianum* and *Pm. venezuelense*. All the species found in the 2012 trapping period except *Pm. griseo-olivaceum* were trapped again in the 2013 season. *Phaeoacremonium sicilianum*, *Pm. scolyti* and *Pm. subulatum* were only found in Rawsonville and Slanghoek. *Phaeoacremonium italicum* and *Pm. griseorubrum* were only found in Stellenbosch P2, and *Pm. griseo-olivaceum* was only found in Durbanville. *Phaeoacremonium prunicola* was found in Stellenbosch B3 during 2012 and Stellenbosch P2 during 2013.

Spore release events

Spore trapping was carried out in the six vineyards for 29–30 weeks in 2012 and 38 weeks in 2013 (Table 5). Spore release events were observed throughout the trapping periods in all the vineyards, with only a few weeks in each where no spore events were recorded. The number of weeks with spore release events varied between vineyards, ranging from 63–100% of the total number of weeks in 2012, and 87–100% in 2013. The number of weeks with spore release events during winter (73-100 % of this 11 week period in 2012, and 82–100% in 2013) and spring pruning periods (50-100 % in 2012, and 86–100% in 2013) was of particular interest, because these are the known periods for trunk pathogen infections.

Spore trapping in the two rootstock mother vine nurseries were carried out for 27–29 weeks during 2012, and 8-38 weeks during 2013 (Table 5). The Slanghoek mother block was unexpectedly removed in 2013 so only 8 weeks of data could be collected for this nursery. In the Western Cape Province it is recommended that Ramsey rootstock canes are harvested between April and July (Hunter *et al*., 2004). Within this period 78-89% of the weeks recorded spore trapping events.

Spore release in individual vineyards and rootstock mother blocks

Paarl A

Three pathogen species were trapped in 2012, including *Pa. chlamydospora*, *Pm. minimum* and *Pm. parasiticum* (Figure 1). Spore release occurred throughout the trapping period from early May to the end of November. The greatest number of colony forming units (cfu) were detected for *Pm. minimum* (525 cfu), with release detected during 45% of the trapping weeks. However, the majority (95%) of the cfu were detected between mid July and the end of November. The greatest release event occurred at the end of November (168 cfu). *Phaeomoniella chlamydospora* spores were trapped during 66% of the weeks, but these were at very low numbers of cfu (1 to 84 cfu per week), with most of these events occurring between mid August (also the highest peak) and late November. This accounted for 91% of all cfu detected. *Phaeoacremonium parasiticum* was only recorded once, late in November (21 cfu in total). The same pathogens were trapped in 2013, together with *Pm. iranianum* and *Pm. viticola* (Figure 2). *Phaeomoniella chlamydospora* was the dominant species, with 1922 cfu detected, and was recorded during 71% of the weeks. Most of the release events and cfu counts occurred between early

Table 5. Number of weeks with spore release events in the 2012 and 2013 trapping seasons.

Year	Pruning	Vineyard						Nursery	
		Paarl A	Paarl Z	Stellenbosch B3	Stellenbosch P2	Durbanville	Rawsonville	Slanghoek[c]	Wellington[c]
2012	Winter pruning[a]	8 of 11	9 of 11	10 of 11	11 of 11	11 of 11	9 of 11	8 of 11	8 of 9
	Spring pruning[b]	10 of 13	7 of 14	12 of 14	14 of 14	13 of 14	13 of 14	N/A	N/A
	Total[e]	23 of 30	19 of 30	26 of 29	29 of 29	28 of 30	25 of 29	25 of 29	25 of 27
2013	Winter pruning[a]	11 of 11	9 of 11	11 of 11	10 of 11	11 of 11	11 of 11	6 of 6[d]	10 of 18
	Spring pruning[b]	12 of 14	14 of 14	13 of 14	14 of 14	13 of 14	13 of 14	N/A	N/A
	Total[e]	35 of 38	38 of 38	33 of 38	37 of 38	37 of 38	37 of 38	7 of 8	37 of 38

[a] Winter pruning refers to pruning from mid-June to end-August.
[b] Spring pruning refers to pruning occurring from beginning of September to end-November.
[c] The optimum time for collecting Ramsy rootststock cuttings in the Western Cape is between April and June (Hunter *et al*., 2004).
[d] Rootstock mother block was pulled out during the week of 13/05/2013 and therefore data for only 6 weeks of the optimum collection time are available.
[e] Total number of spore trapping weeks for each vineyard and the total number of weeks with spore release events.

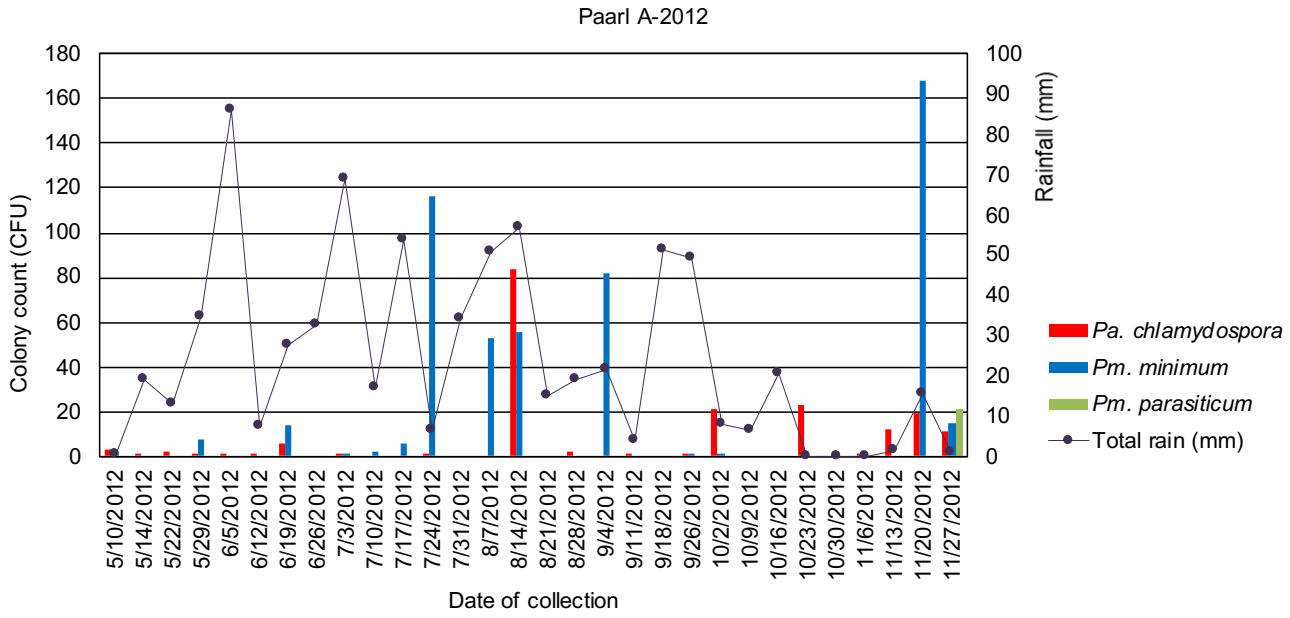

Figure 1. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to the cordons on each of five vines in vineyard Paarl A, Western Cape province in 2012. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes.

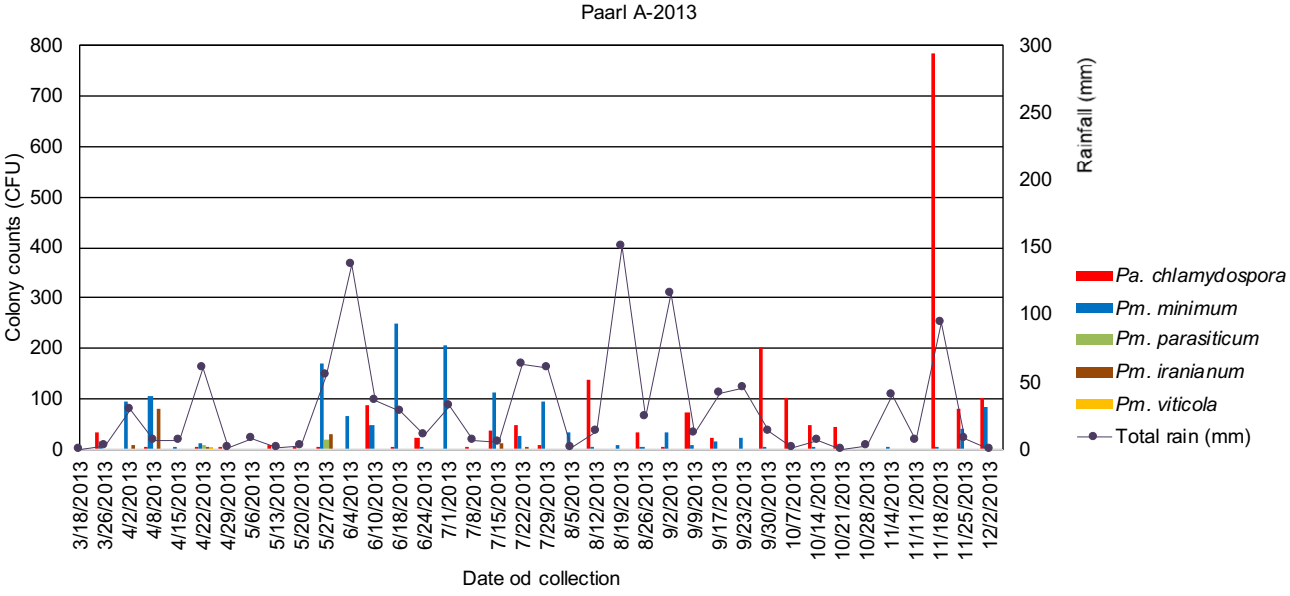

Figure 2. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to the cordons on each of five vines in vineyard Paarl A, Western Cape province in 2013. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes. Not clear on the graph, *Pm. viticola* detection date is 22/04.

June and early December, accounting for 91% of all cfu detected, with the highest spore release peak in mid November (785 cfu). *Phaeoacremonium minimum* spores were released throughout the trapping period during 76% of the weeks (1472 cfu in total). The most cfu were detected between early April and late July, account-

ing for 81% of all cfu detected, with the highest peak of 250 cfu in mid June. *Phaeoacremonium iranianum* cfu were only detected during 16% of the weeks, and at low counts (140 cfu in total), between early April and mid July, with the highest peak of 80 cfu in early April. Only two small *Pm. parasiticum* release events occurred (27 cfu in total), one in mid April and one in early May, and there was only one release for *Pm. viticola* in mid April (1 cfu).

Paarl Z

Three pathogens, *Pa. chlamydospora*, *Pm. minimum* and *Pm. parasiticum*, were recorded in 2012 (Figure 3). *Phaeoacremonium minimum* was the predominant pathogen, based on the total number of cfu detected (1820) and the number of spore release events. CFU were detected in 47% of the weeks between mid May and mid November, but the most cfu were detected between early June and mid August, accounting for 95% of all the cfu detected of which 73% were in June. *Phaeomoniella chlamydospora* was recorded in 40% of the weeks between mid May and early November (453 cfu in total), with the most cfu detected in August, accounting for 69% of all cfu. *Phaeoacremonium parasiticum* was recorded only during 3 weeks (18 cfu in total), in mid May, late July and late November. The same three pathogens together with *Pm. inflatipes* and *Pm. iranianum* were trapped in 2013 (Figure 4). *Phaeoacremonium minimum* was the predominant pathogen based on the total number of cfu detected (6613), and the number of spore release events. Spores of *Pm. minimum* were released in 84% of the weeks between mid March and late November. The highest peaks of cfu occurred between early April and early September, accounting for 97% of all cfu, with the highest peak (1340 cfu) in early April. *Phaeomoniella chlamydospora* spore release was recorded in 53% of the weeks between mid March and early December (422 cfu in total), but at low numbers of 1 to 99 cfu per week, of which 62% were detected between September and early December. The highest peak of 87 cfu was recorded in mid October. *Phaeoacremonium parasiticum* was recorded in 18% of the weeks between mid March and early November (691 cfu in total), although 95% of the total cfu were detected in April and May, with the highest peak was in early April (600 cfu). *Phaeoacremonium iranianum* was detected, mostly at low counts (217 cfu in total), during only 13% of the weeks between early April and mid July, with April accounting for 84% of the total cfu. Only one spore release event of 110 cfu was recorded for *Phaeoacremonium inflatipes* in mid June.

Stellenbosch B3

Four Petri disease pathogens were trapped during 2012, including *Pa. chlamydospora*, *Pm. minimum*, *Pm. prunicola* and *Pm. viticola* (Figure 5). *Phaeoacremonium minimum* was the predominant pathogen based on the total number of cfu detected (1208) and the number of large spore release events. Spore release occurred in 59% of the weeks between mid May and late November, although 91% of the total number of cfu were detected between late May and early September, with the highest cfu peak (615) in mid July. *Phaeomoniella chlamydospora* spore release occurred, at low counts (320 cfu in total), in 76% of the weeks between mid May to late November, with 85% of the cfu detected between June and early October. The highest peak occurred in early August (74 cfu). Only two low spore release events of *Phaeoacremonium prunicola* were recorded, one in late May (3 cfu) and one in early July (2 cfu), and one release event was detected for *Pm. viticola* in late May (1 cfu). Four pathogens were recorded in 2013, including *Pa. chlamydospora*, *Pm. minimum*, *Pm. parasiticum* and *Pm. viticola* (Figure 6). *Phaeoacremonium minimum* was the predominant pathogen based on the total numbers of cfu (2564) and spore release events. Spore release was recorded in 74% of the weeks from late March to late November. The majority of cfu (92%) were detected between early April and early September, with the highest peak in late May (998 cfu). *Phaeomoniella chlamydospora* was recorded, at low numbers (404 cfu in total), in 63% of the weeks between late March to late November, with 81% of the total number of cfu detected between early May and late August. The highest spore release peak (129 cfu) occurred in mid August. The only *Pm. parasiticum* spore release event was recorded in early March (4 cfu). *Phaeoacremonium viticola* was detected in early March (3 cfu) and mid October (1 cfu).

Stellenbosch P2

During 2012, *Pa. chlamydospora*, *Pm. minimum* and *Pm. parasiticum* twere trapped (Figure 7). *Phaeomoniella chlamydospora* was the predominant pathogen based on the total number of cfu (1798), with cfu detected during 90% of the weeks between mid May and late November. Consistent release events occurred between mid June and late October during which 94% of the total number of cfu were detected, with the highest peak observed in mid June (751 cfu). *Phaeoacremonium minimum* spore releases were recorded during 90% of the weeks between mid May and late November (1145 cfu in total), with three cfu peak periods, in late

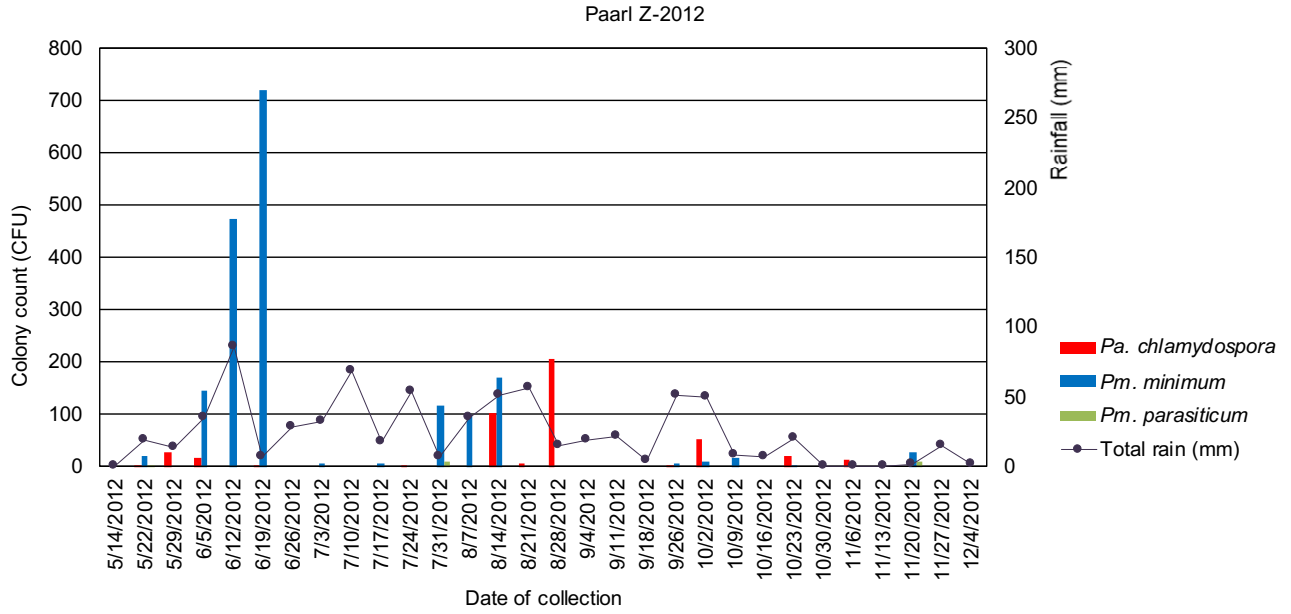

Figure 3. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to the cordons on each of five vines in vineyard Paarl Z, Western Cape province in 2012. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes. Not clear on the graph, *Pm. parasiticum* detection dates are 22/05, 31/07 and 20/11.

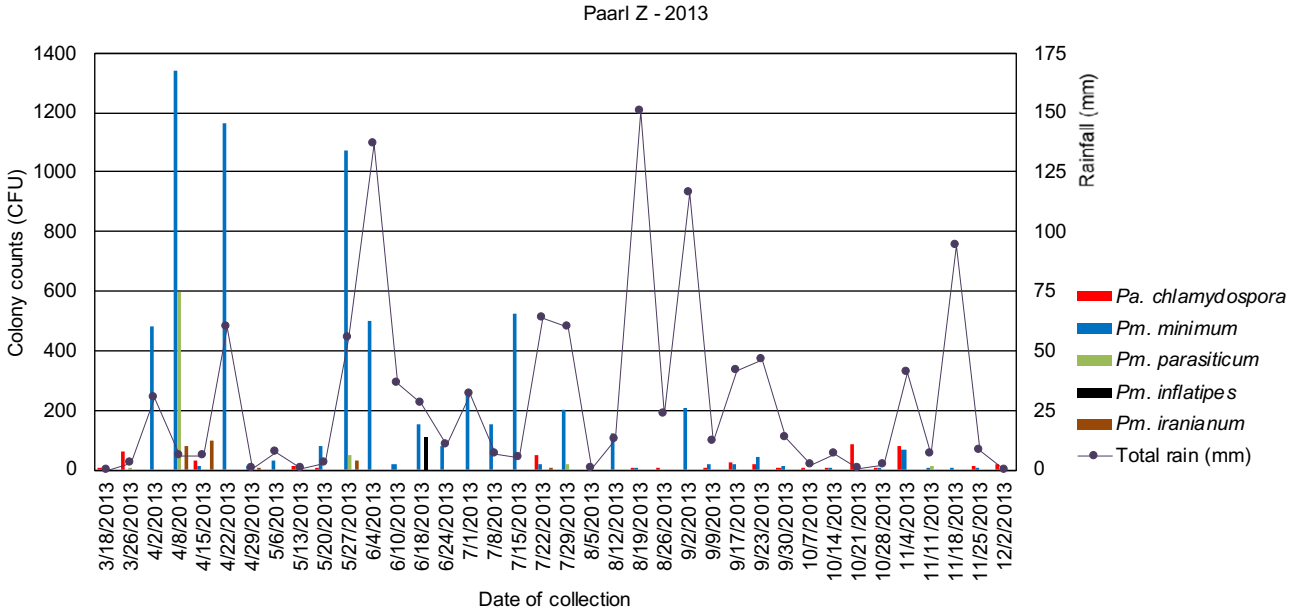

Figure 4. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to the cordons on each of five vines in vineyard Paarl Z, Western Cape province in 2013. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes.

June/early July (highest peak of 279 cfu), early October (111 cfu) and mid/late November (294 cfu). These accounte for, respectively, 39, 10 and 26% of the total number of cfu detected. *Phaeoacremonium parasiticum* spore release was recorded in 34% of the weeks between mid May and early December, at very low numbers (47

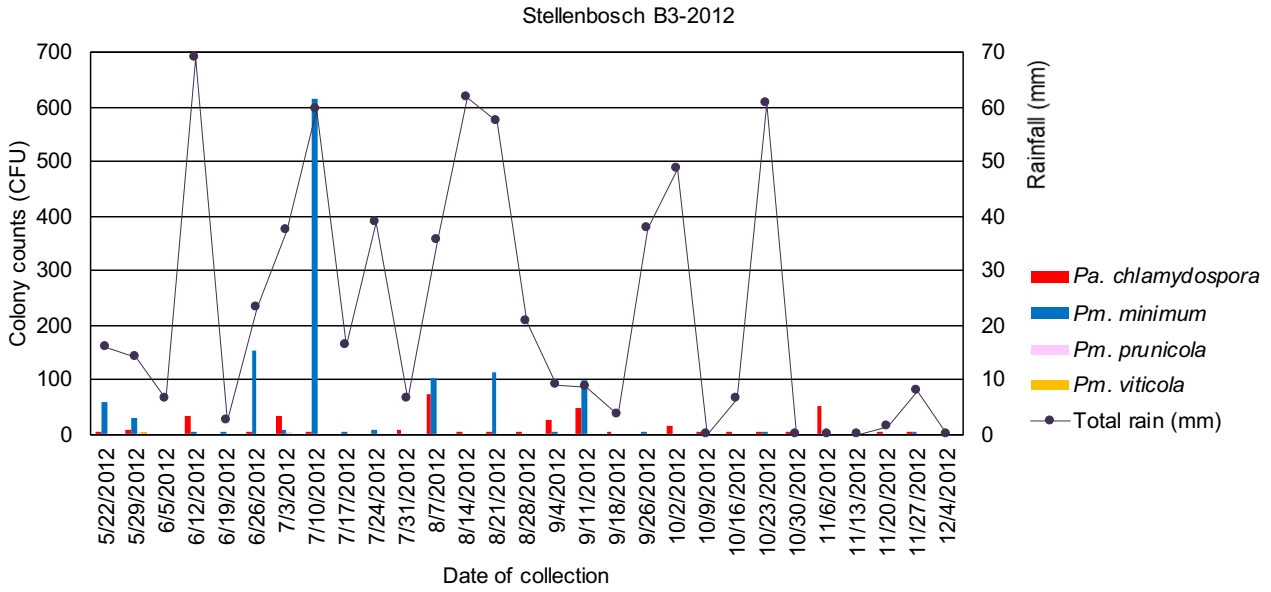

Figure 5. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to the cordons on each of five vines in vineyard Stellenbosch B3, Western Cape province in 2012. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes. Not clear on the graph, *Pm. prunicola* detection dates are 29/05 and 03/07 and *Pm. viticola* is 29/05.

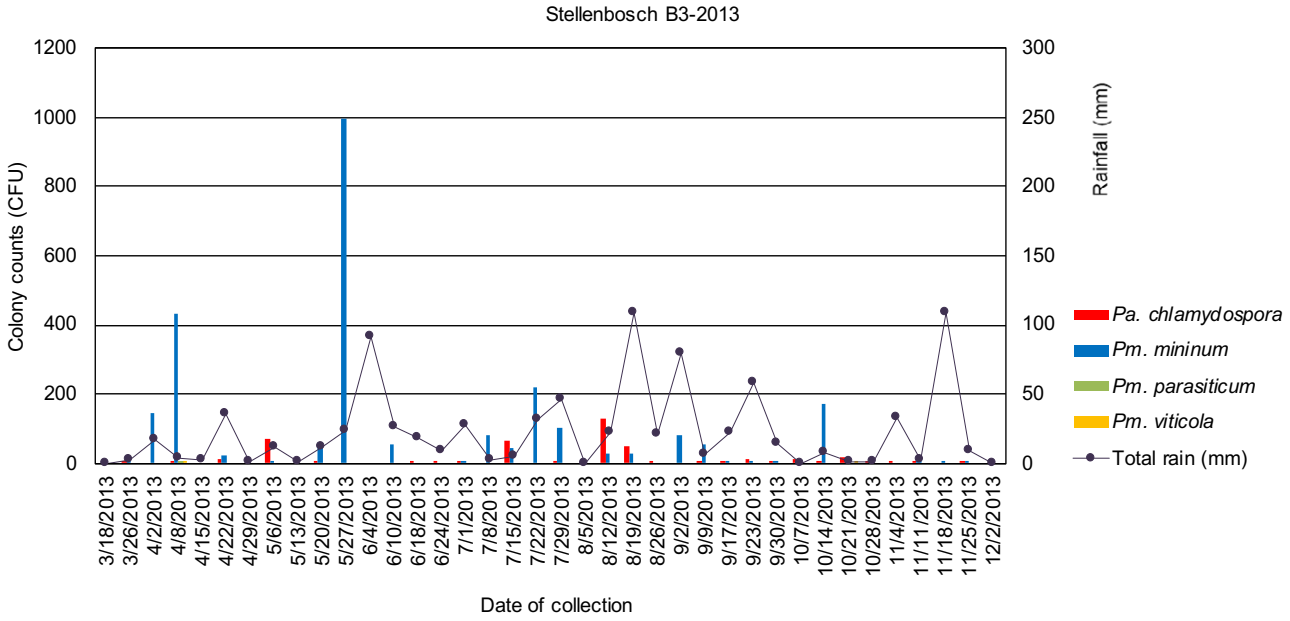

Figure 6. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to the cordons on each of five vines in vineyard Stellenbosch B3, Western Cape province in 2013. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes. Not clear on the graph, detection dates for *Pm. viticola* are 08/04 and 21/10 and for *Pm. parasiticum* is 08/04.

cfu in total), with the greatest count (11 cfu) in late May. During 2013, eight Petri disease pathogens were recorded, including *Pa. chlamydospora, Pm. minimum*, *Pm. parasiticum*, *Pm. italicum*, *Pm. prunicola*, *Pm. iranianum*, *Pm. inflatipes* and *Pm. griseorubrum* (Figure 8). *Phaeomoniella chlamydospora* was the predominant pathogen based on the total number of cfu detected (2677) and number of large spore release events. Cfu of

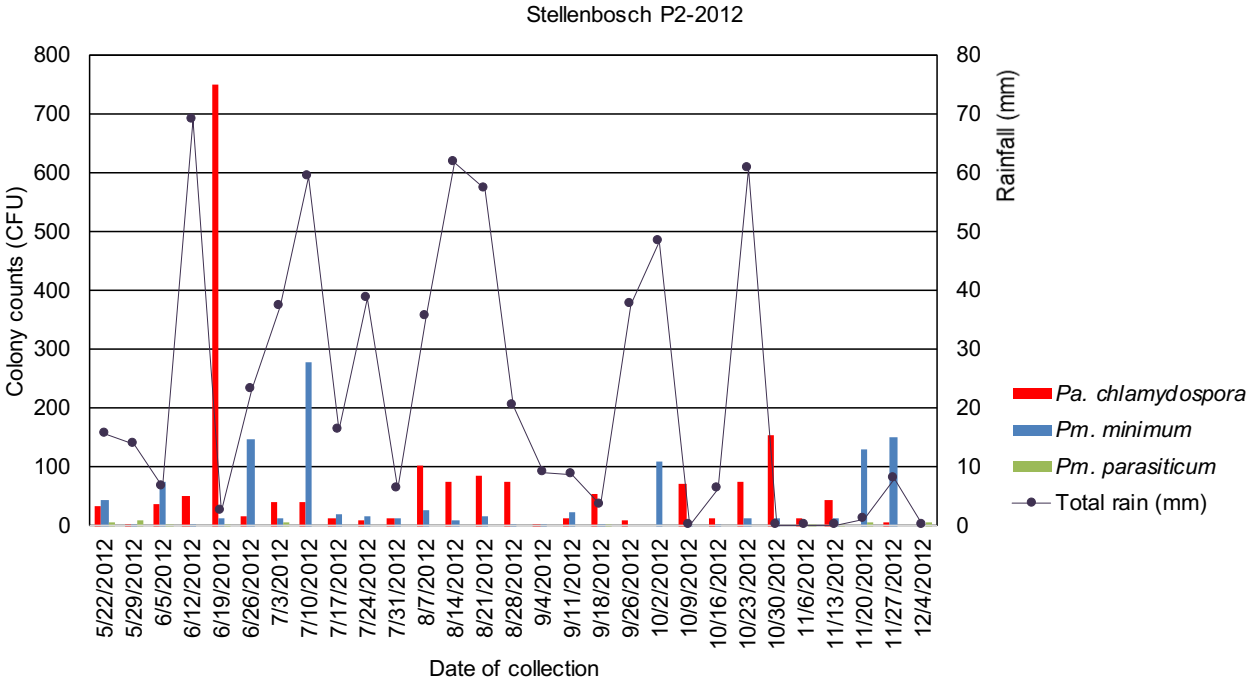

Figure 7. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to the cordons on each of five vines in vineyard Stellenbosch P2, Western Cape province in 2012. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes.

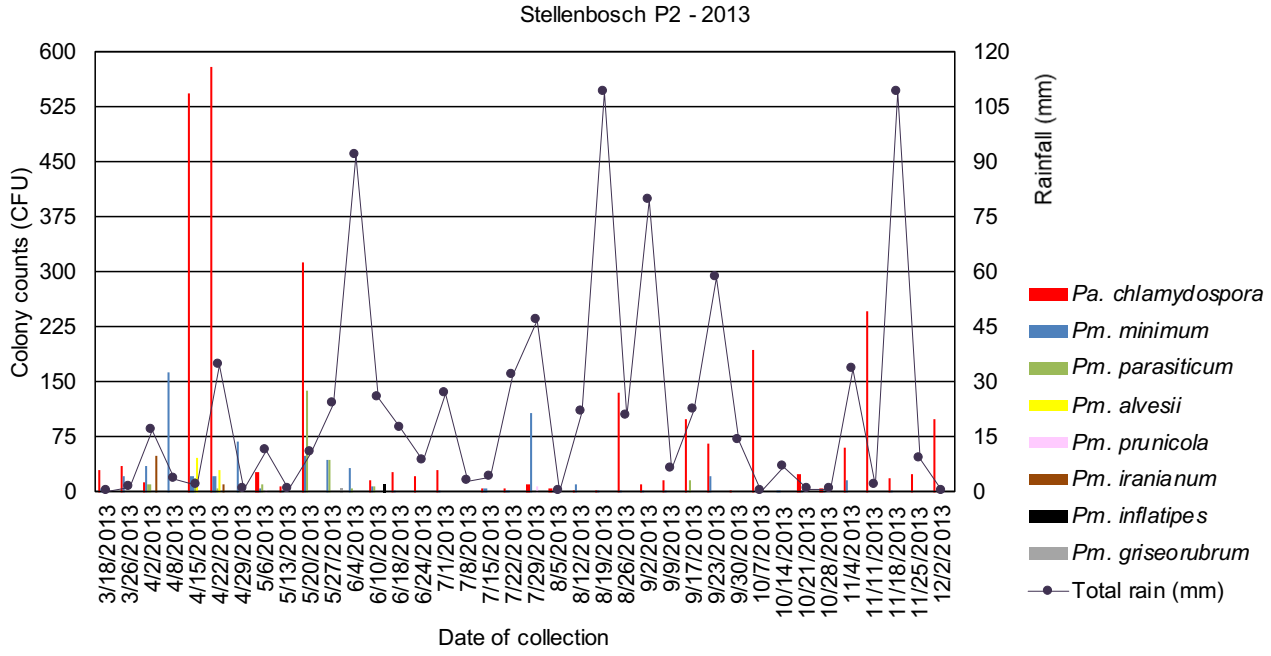

Figure 8. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to the cordons on each of five vines in vineyard Stellenbosch P2, Western Cape province in 2013. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes. Not clear on the graph, detection dates for *Pm. prunicola* are 06/05 and 29/07 and for *Pm. griseorubrum* is 27/05.

Pa. chlamydospora were recorded in 84% of the weeks between mid March to early December, with the highest cfu detection peaks observed in mid April (greatest number 580 cfu) to mid May, accounting for 55% of the total number of cfu detected, and then again during a period between late August and early December, representing 38% of the total cfu. *Phaeoacremonium minimum* cfu were detected during 79% of the weeks between mid March to late November (666 cfu in total). However, the greatest proportion (87%) of the cfu were detected between early April and late July, with the highest cfu peak recorded in early April (164 cfu). Spore release of *Pm. parasiticum* was recorded in 32% of the weeks between mid March and early December (267 cfu in total). The majority of cfu (94%) were detected between mid March and early June, with the highest cfu detection peak (139 cfu) in mid May. *Phaeoacremonium griseorubrum* and *Pm. inflatipes* were detected once in late May (5 cfu) and early June (10 cfu). *Phaeoacremonium italicum* was detected in mid March (total cfu 79), *Pm. iranianum* in early and mid March (respectively, 50 and 10 cfu), and *Pm. prunicol*a in early May (1 cfu) and late July (7 cfu).

Durbanville

Three Petri disease pathogens, *Pa. chlamydospora*, *Pm. minimum* and *Pm. griseo-olivaceum*, were trapped in 2012 (Figure 9). *Phaeomoniella chlamydospora* (626 cfu) and *Pm. minimum* (601 cfu) had very similar total cfu, and similar numbers of spore release events. *Phaeomoniella chlamydospora* spore release was recorded in 83% of the weeks between mid May to early December, although at low counts, with the highest peak (123 cfu) in late August. Spore release of *Pm. minimum* was recorded during 80% of the weeks, also at low numbers, from late May to early December, with the highest peak (122 cfu) recorded during mid August. *Phaeoacremonium griseo-olivaceum* was recorded only once (37 cfu) in early December. During 2013, four Petri disease pathogens were recorded, including *Pa. chlamydospora*, *Pm. minimum*, *Pm. parasiticum* and *Pm. inflatipes* (Figure 10). *Phaeoacremonium minimum* was the predominant pathogen, with the greatest total number of cfu detected (4016), as well as the most spore release events. Spore release was recorded during 82% of the weeks between mid March to late November, with the majority (95%) of the cfu detected between early April and late July. Although the highest cfu peak occurred at the end of June (750 cfu), the greatest total number of cfu detected per month was in April (1877 cfu). *Phaeomoniella chlamydospora* was recorded during 74% of the weeks between mid March and early December, although at low numbers (484 cfu in total). Two distinct spore release periods occurred, the first was between mid April to mid June, with the highest peak (90 cfu) occurring in late March, accounting for 59% of all the cfu. The second peak occurred between early August and early December, accounting for 38% of the toal number of cfu. *Phaeoacremonium parasiticum* was recorded during 11% of the weeks, at low numbers (26 cfu in total) between mid March and late May. *Phaeoacremonium inflatipes* was recorded in early June (50 cfu) and early July (4 cfu).

Rawsonville

During 2012, six pathogens were recorded, including *Pa. chlamydospora*, *Pm. minimum*, *Pm. parasiticum*, *Pm. scolyti*, *Pm. sicilianum* and *Pm. subulatum* (Figure 11). *Phaeoacremonium sicilianum* was the predominant pathogen, based on the total number of cfu detected (5605) and number of spore release events. *Phaeoacremonium sicilianum* was released during 62% of the weeks between mid May and early December, although the majority of cfu (90%) were detected between mid September and early December, with the highest cfu detection peak (1838 cfu) in mid November. *Phaeoacremonium scolyti* was released during 24% of the weeks between mid May and mid October (2194 cfu in total), with the highest cfu peaks in mid July (700 cfu) and mid October (1100 cfu). *Phaeoacremonium minimum* was recorded during 62% of the weeks, but at low counts (740 cfu in total), between early June and late December, with the highest cfu peak in mid October (313 cfu). *Phaeomoniella chlamydospora* spores were released during 55% of the weeks, also at low counts (560 cfu in total), between mid May and mid November, with the highest cfu peak (210) in mid September. However, the majority (89%) of these cfu were detected between mid July and mid November. *Phaeoacremonium parasiticum* was recorded at low counts during 10% of the weeks (46 cfu in total) in early June, early November and early December, and early December was when the highest cfu peak of 25 cfu was recorded. *Phaeoacremonium subulatum* was only trapped once in late June (16 cfu).

During 2013, eight pathogen species were trapped, including *Pa. chlamydospora*, *Pm. minimum*, *Pm. parasiticum*, *Pm. sicilianum*, *Pm. subulatum*, *Pm. australiense*, *Pm. scolyti* and *Pm. venezuelense* (Figure 12). *Phaeoacremonium sicilianum* was the predominant pathogen, with the greatest total number of cfu detected (5748) and the greatest number of spore release events, with cfu detected during 74% of the weeks between mid March and early December. How-

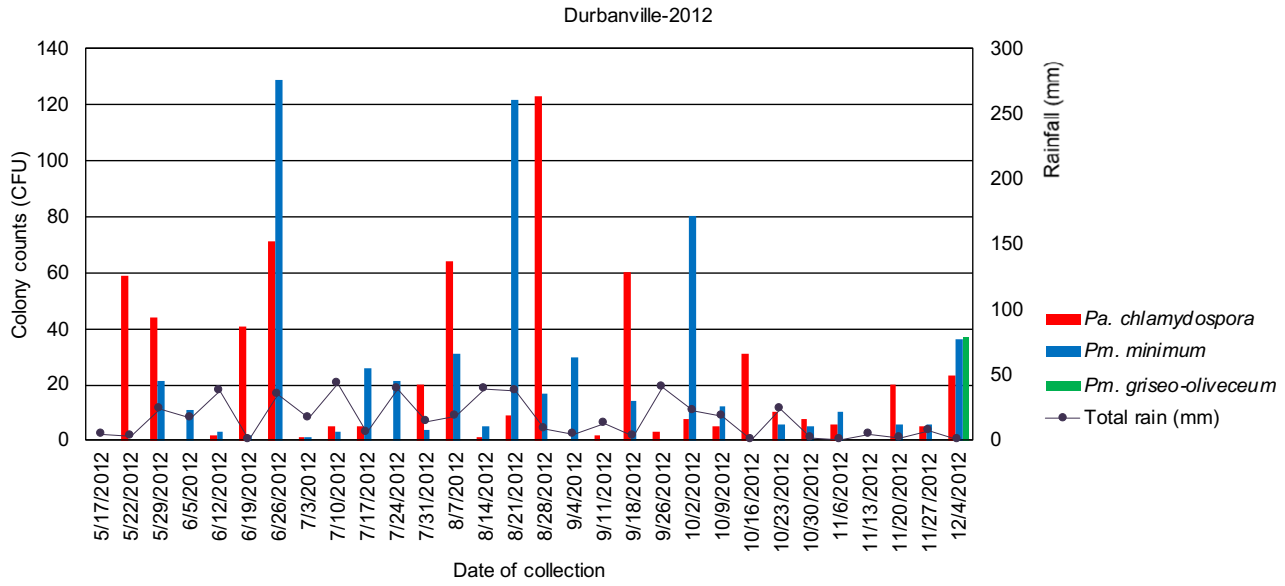

Figure 9. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to the cordons on each of five vines in a vineyard in Durbanville, Western Cape province in 2012. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes.

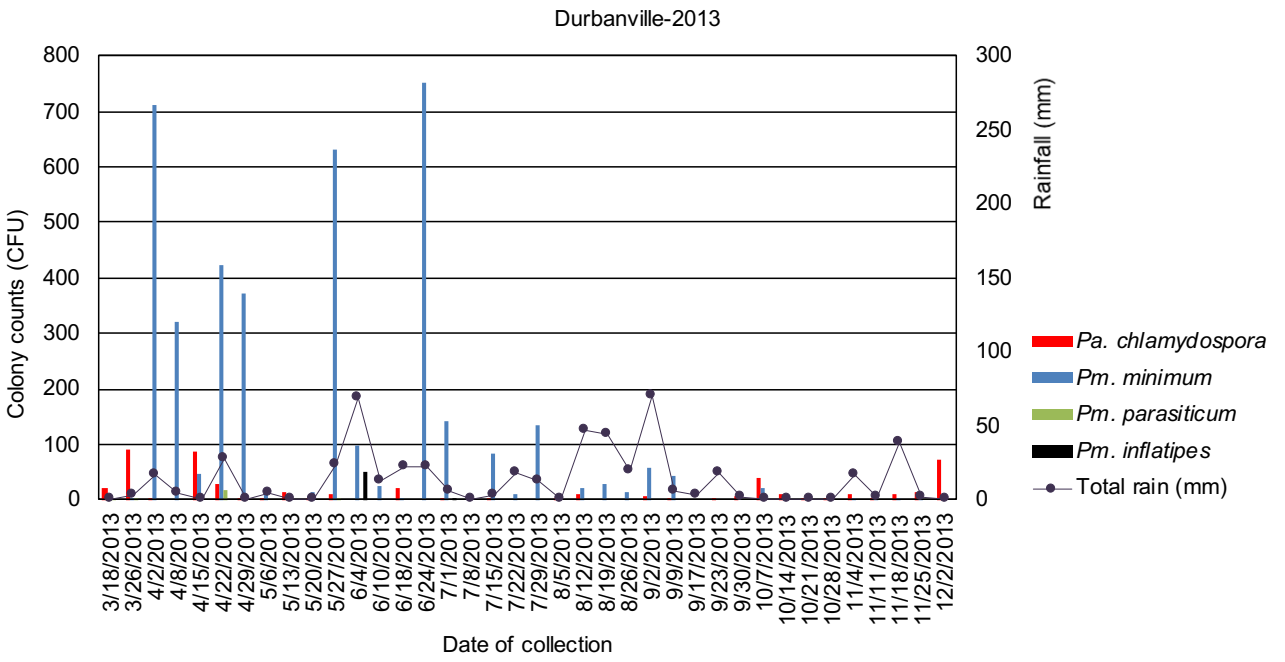

Figure 10. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to the cordons on each of five vines in a vineyard in Durbanville, Western Cape province in 2013. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes.

ever, the majority of the cfu were detected between mid March and late July, accounting for 74% of the total cfu detected, with the highest cfu detection peaks in mid April (665 cfu) and mid May (608 cfu). A high peak also occurred in early December (663 cfu), accounting for 12% of the total cfu. *Phaeoacremonium minimum* was trapped during 55% of the weeks between late March and late November (1506 cfu in total). The

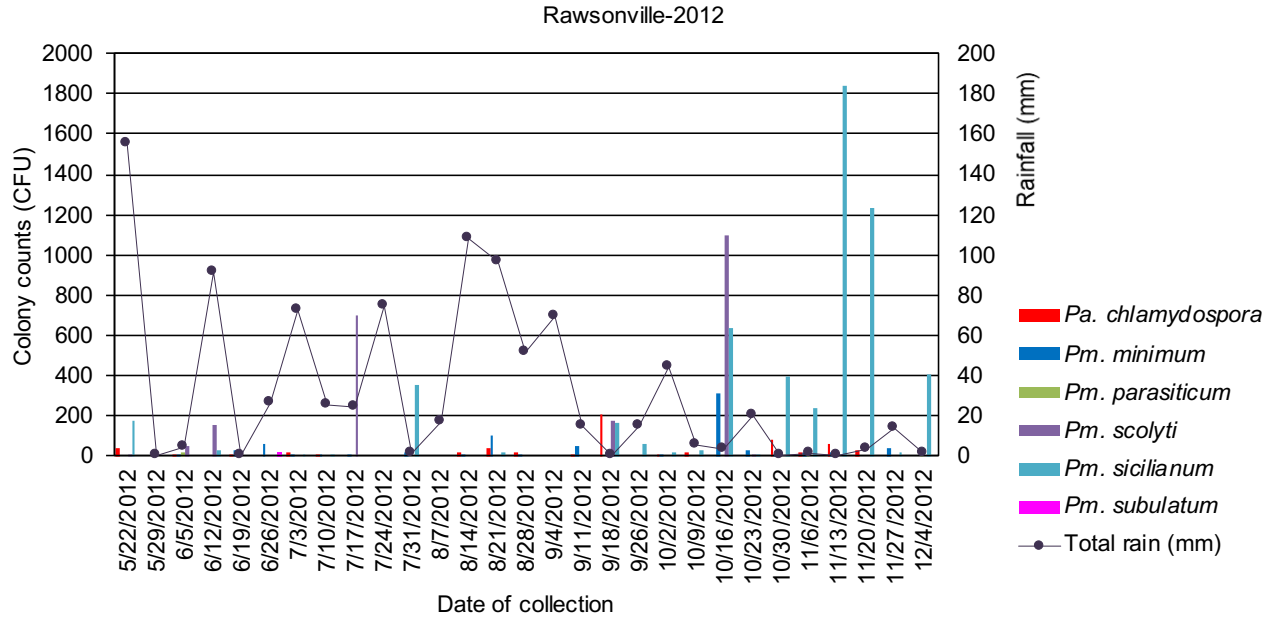

Figure 11. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to the cordons on each of five vines in a vineyard in Rawsonville, Western Cape province in 2012. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes. Not clear on the graph, detection dates for *Pm. parasiticum* are 05/06, 06/11 and 04/12 and for *Pm. subulatum* is 26/06.

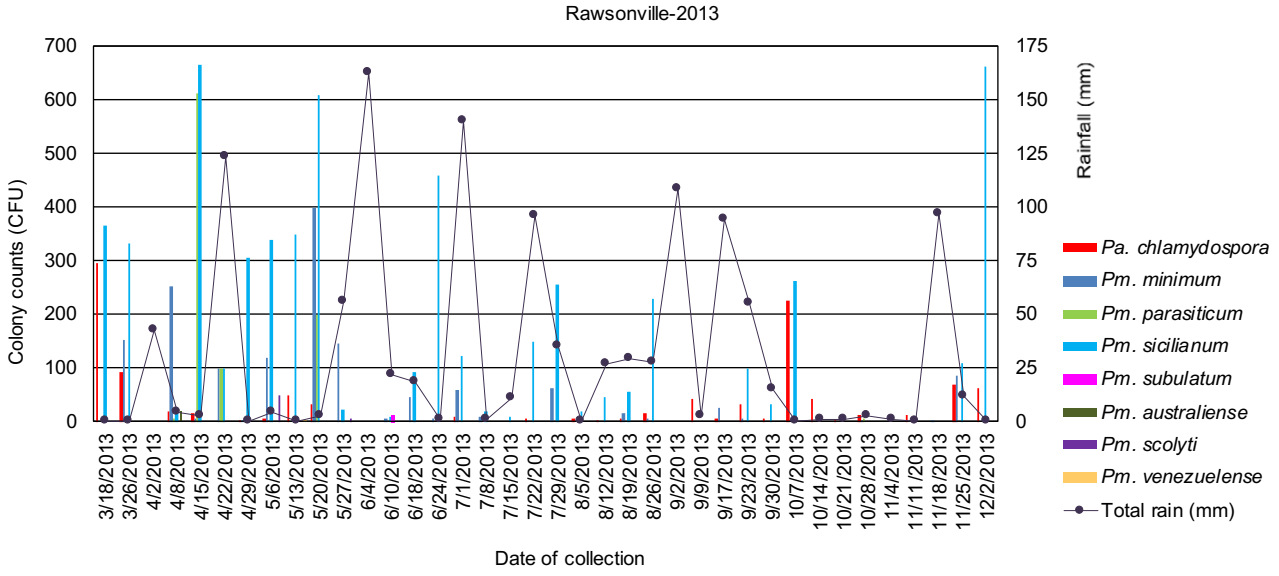

Figure 12. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to the cordons on each of five vines in a vineyard in Rawsonville, Western Cape province in 2013. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes. Not clear on the graph, the detection date for *Pm. australiense* is 08/04 and for *Pm. venezuelense* is 13/05.

majority of these cfu were detected between early April and late July, accounting for 80% of all cfu detected, with the highest peak of 400 cfu recorded in mid May. *Phaeoacremonium parasiticum* was trapped during 13% of the weeks of spore trapping (924 cfu in total). The majority of these cfu (99%) were detected between early April and late May with, the highest peak in mid April (614 cfu). *Phaeomoniella chlamydospora* spores were

released during 66% of the weeks between mid March to early December (1059 cfu). The greatest number of cfu were detected during two distinct periods. The first was between mid March and late May (49% of all cfu detected), with the highest peak (295 cfu) in mid March. The second period was between early September and early December, representing 47% of the total number of cfu, with the highest peak (225 cfu) in early October. *Phaeoacremonium scolyti* spores were trapped in early May (50 cfu) and in late May (5 cfu). *Phaeoacremonium australiense* was trapped in early April (20 cfu), *Pm. venezuelense* in early May (1 cfu) and *Pm. subulatum* in early June (13 cfu).

Slanghoek

Six Petri disease pathogens were reported in 2012, including *Pa. chlamydospora*, *Pm. minimum*, *Pm. australiense*, *Pm. scolyti*, *Pm. subulatum* and *Pm. sicilianum* (Figure 13). *Phaeomoniella chlamydospora* had the greatest total cfu count (947), and spores were released during 66% of the weeks between early June and late November. The greatest cfu numbers were detected between early July and late August, accounting for 85% of all cfu, with the highest peak in early July (587 cfu). *Phaeoacremonium minimum* was trapped during 76% of the weeks between mid June and early December (827 cfu in total), although the majority (76%) of the cfu were detected between mid June and late August, with the highest peak in late June (239 cfu). *Phaeoacremonium scolyti* was recorded during 34% of the weeks between mid June and early November (595 cfu in total), with the greatest number of cfu detected between mid June and mid August, and the highest peak (195 cfu) occurred in late June. *Phaeoacremonium australiense* was only recorded between early August, with the highest peak of 100 cfu, and early October (108 cfu in total). *Phaeoacremonium sicilianum* was only trapped once in early December (7 cfu). *Phaeoacremonium subulatum* was trapped twice, in early September (1 cfu) and mid October (3 cfu). Due to the unforeseen removal of grapevine plants, only 8 weeks of spore trapping were completed in 2013, between mid March and early May. During this period, *Pa. chlamydospora* and *Pm. minimum* were recorded (Figure 14). *Phaeoacremonium minimum* was the predominant pathogen trapped within this period, with 2693 cfu in total. Six high spore release events of 301 to 810 cfu per week were recorded, with the greatest release occurring in early April. *Phaeomoniella chlamydospora* was only detected during mid March (15 cfu in total).

Wellington

Only two pathogens, *Pa. chlamydospora* and *Pm. minimum*, were trapped during 2012 (Figure 15). *Phaeomoniella chlamydospora* was the predominant pathogen (total of 1281 cfu detected) and the number of spore release events, with spores released during 85% of the weeks between early June and early December, although the majority (82%) of the cfu were detected between early June and end of August. The highest peak of 578 cfu occurred during early June with a second peak of 188 cfu in late August. *Phaeoacremonium minimum* spore release was recorded during 74% of the weeks between early June and early December (575 cfu in total), although the majority (91%) of cfu were detected between early June and early September, with the highest peak (103 cfu) in early July. During 2013, only *Pa. chlamydospora* and *Pm. minimum* were trapped (Figure 16). *Phaeoacremonium minimum* was the predominant pathogen based on the total number of cfu (4061) and number of high spore release events, with spores trapped during 68% of the weeks between late March and early December. The majority (81%) of the spores were trapped between mid April and mid September, with two high peaks in late May (800 cfu) and mid July (800 cfu). *Phaeomoniella chlamydospora* was recorded during 42% of the weeks between mid April and early December. The majority (82%) of the cfu were detected between the early July and mid October, with the highest peak (465 cfu) detected in late July.

Spore release events during and after pruning and rootstock cane harvesting

The pruning and rootstock harvesting dates are listed in Table 1. A conservative arbitrary period of 4 weeks has been selected for illustration purposes, as the period when grapevines are likely to be most susceptible to infections after pruning, although it is known that pruning wounds remain susceptible to infection for 4 to 16 weeks (Eskalen *et al*., 2007; Elena and Luque, 2016). *Phaeomoniella. chlamydospora* and/or *Pm. minimum* spore release events occurred during the week of pruning or within 4 weeks after pruning, and rootstock cane harvesting in all the vineyards and rootstock mother blocks, although only a few *Pa. chlamydospora* and *Pm. minimum* cfu were detected during this period in the Paarl A vineyard in 2012. Cfu of *Pm. sicilianum* were also detected after pruning in Rawsonville in 2012 and 2013, while *Pm. subulatum* and *Pm. scolyti* were also trapped in 2013. *Phaeoacremonium inflatipes* spores were detected, although at very low numbers, 3 weeks after

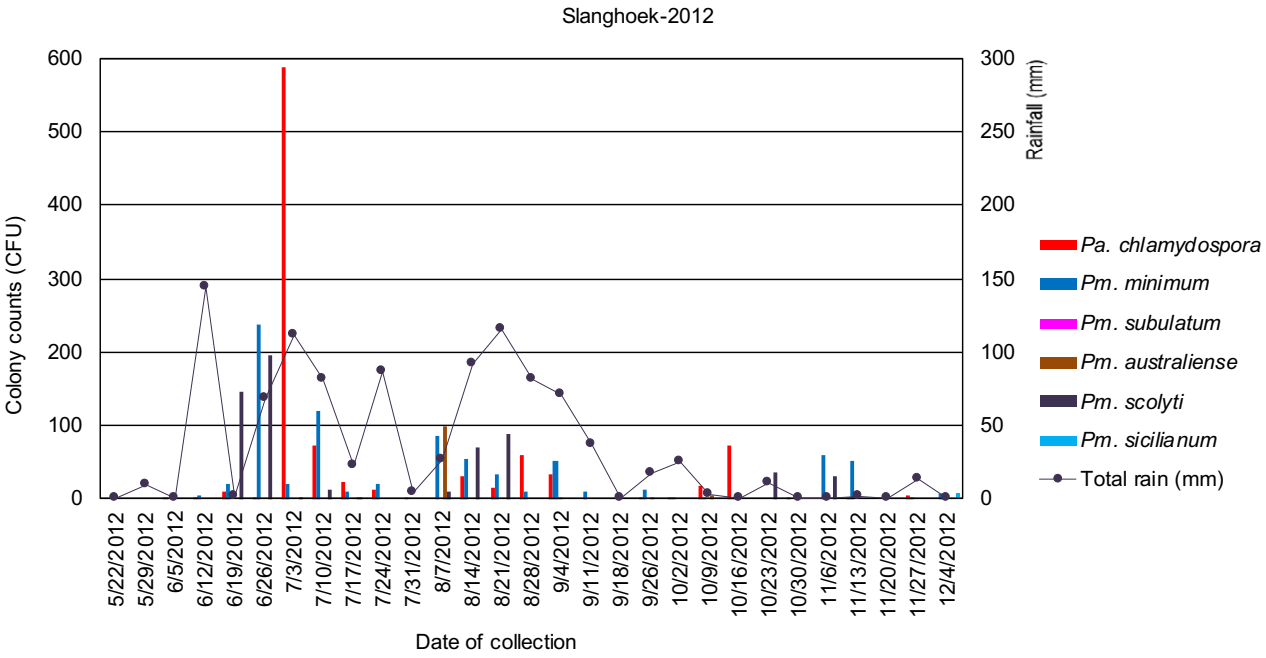

Figure 13. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to each of five rootstock mother vines in a rootstock mother block in Slanghoek, Western Cape province in 2012. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes. Not clear on the graph, detection dates for *Pm. subulatum* are 04/09 and 16/10.

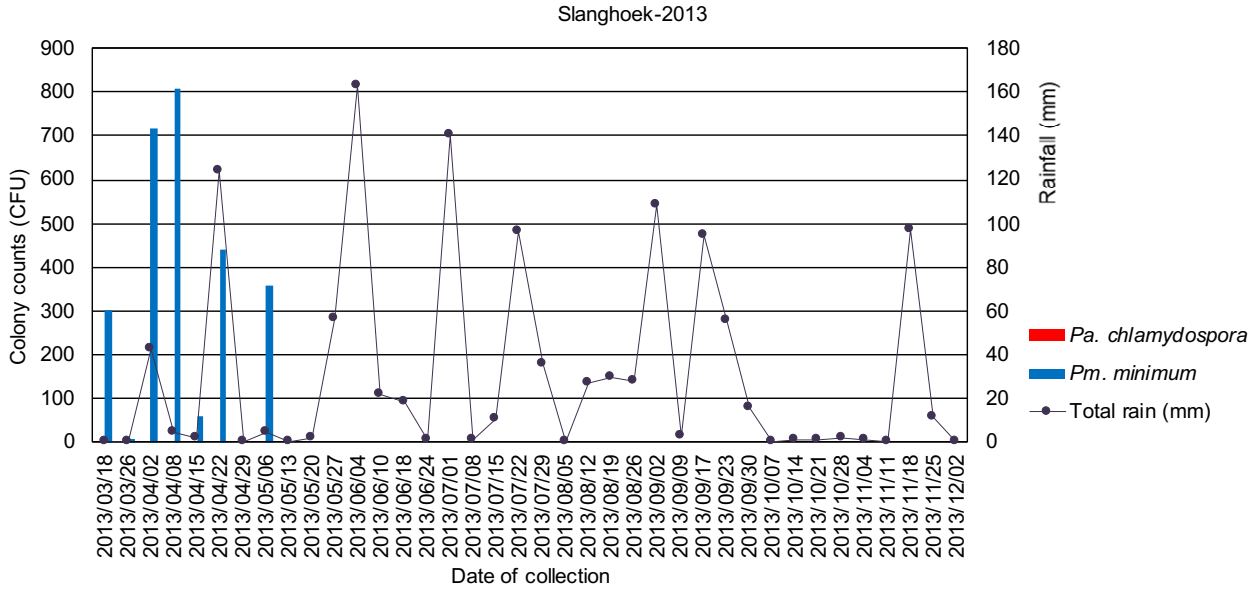

Figure 14. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to each of five rootstock mother vines in a rootstock mother block in Slanghoek, Western Cape province in 2013. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes. Not clear on the graph, the detection date for Pa. chlamydospora is 18/03. The rootstock mother block was removed 13/05/2013 and therefore no sampling occurred beyond this date.

pruning in Durbanville in 2013, and *Pm. prunicola* in Stellenbosch P2 in 2013. *Phaeoacremonium iranianum* and *Pm. parasiticum* were trapped within 3 and 4 four weeks of pruning in Paarl Z in 2013, while *Pm. parasiticum* was trapped within 3 weeks in 2012. No cfu were detected during the first 2 weeks of harvesting rootstock

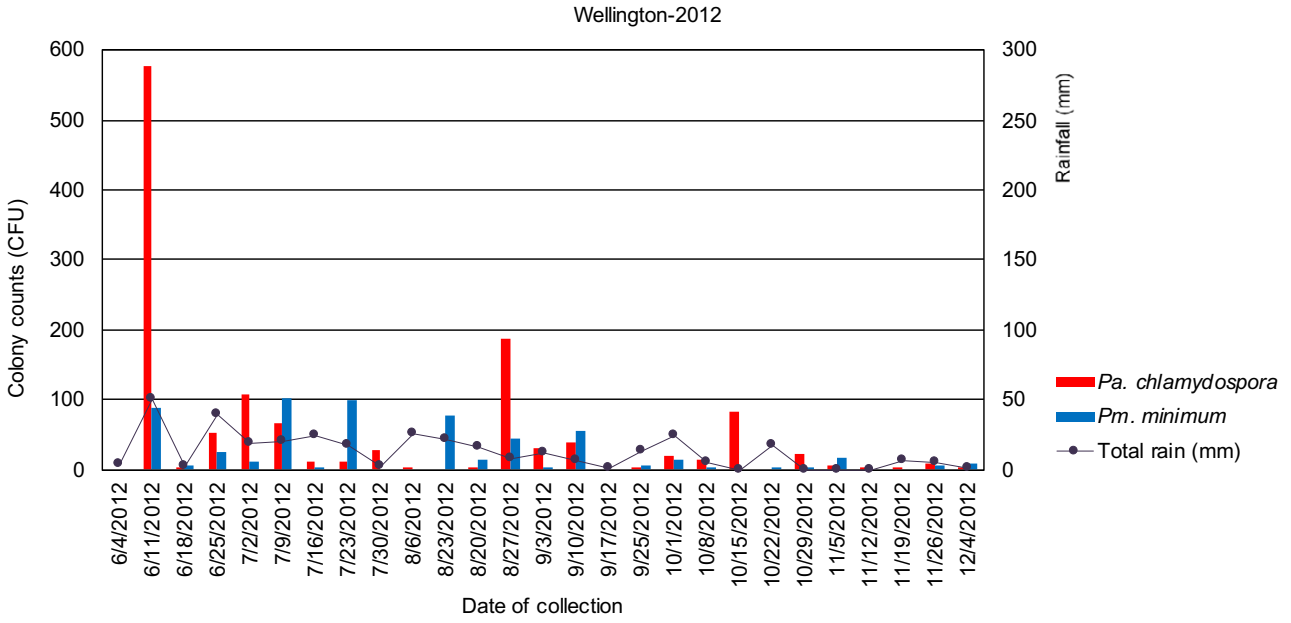

Figure 15. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to each of five rootstock mother vines in a rootstock mother block in Wellington, Western Cape province in 2012. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes.

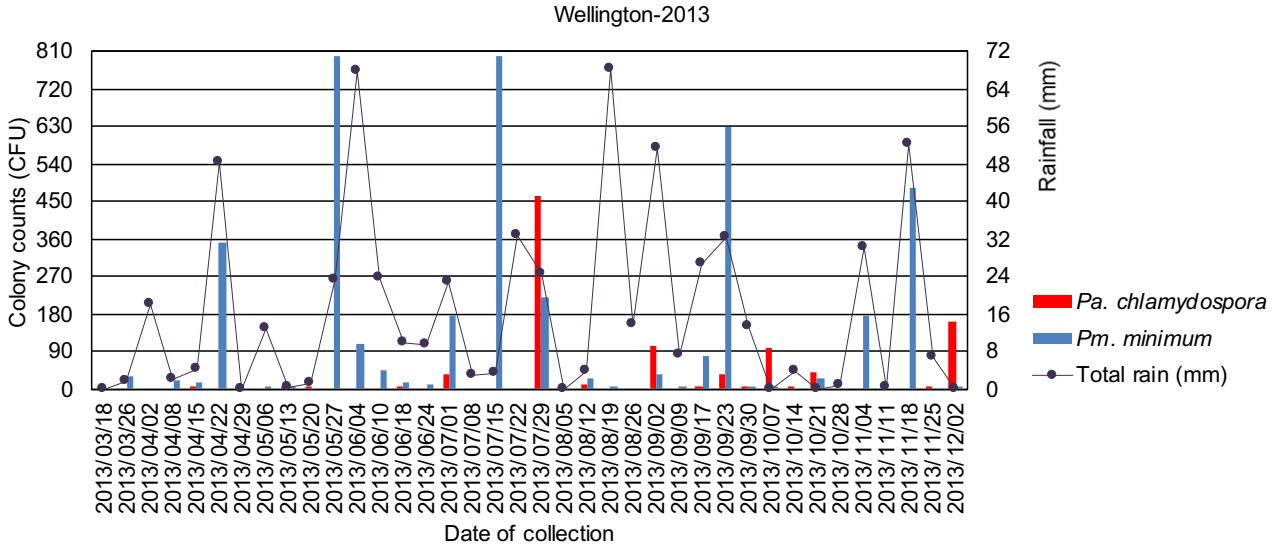

Figure 16. *Phaeomoniella* and *Phaeoacremonium* spp. cfu cultured from five vaseline coated microscope slides affixed to each of five rootstock mother vines in a rootstock mother block in Wellington, Western Cape province in 2013. The slides were replaced weekly. Each bar represents the total number of cfu cultured on three Petri dishes.

canes in Slanghoek, although a few *Pa. chlamydospora* and *Pm. minimum* cfu were detected 3 and 4 weeks after harvesting, with significant cfu counts of *Pm. scolyti* during weeks 4 and 5.

DISCUSSION

The present study conducted during the 2012 and 2013 seasons, trapped aerial spores of *Pa. chlamydospo-*

ra and *Phaeoacremonium* species from six vineyards and two rootstock mother vine nurseries located in six grape-growing areas of the Western Cape Province. A total of 14 *Phaeoacremonium* species were detected, including *Pm. australiense*, *Pm. griseo-olivaceum*, *Pm. griseorubrum, Pm. inflatipes, Pm. iranianum, Pm. italicum, Pm. minimum*, *Pm. parasiticum*, *Pm. prunicola, Pm. scolyti*, *Pm. sicilianum*, *Pm. subulatum, Pm. venezuelense* and *Pm. viticola*. Compared to similar spore trapping studies conducted in other countries, this is the greatest species diversity recorded in vineyards. Only two Petri disease pathogens were detected in vineyards in France (*Pa. chlamydospora* and *Pm. minimum*) (Larignon and Dubos, 2000) and three pathogens in California (*Pa. chlamydospora*, *Pm. minimum* and *Pm. inflatipes*) (Eskalen and Gubler, 2001). Of the 17 *Phaeoacremonium* species previously isolated from diseased grapevines in South African vineyards (Crous *et al*., 1996; Mostert *et al*., 2005; Mostert *et al*., 2006a; White *et al*., 2011a: Spies *et al*., 2018), only *Pm. krajdenii*, *Pm. austroafricanum* and *Pm. fraxinopennsylvanicum* could not be cultured from spore traps during the present study. This study is the first to report aerial inoculum of *Pm. australiense, Pm. griseo-olivaceum, Pm. griseorubrum, Pm. iranianum, Pm. italicum, Pm. parasiticum, Pm. prunicola, Pm. scolyti, Pm. sicilianum, Pm. subulatum, Pm. venezuelense and Pm. viticola* in vineyards.

Two pathogen species, *Pa. chlamydospora* and *Pm. minimum*, were trapped in all the vineyards and rootstock mother vine nurseries during both seasons of the study. The reason for their abundance as aerial inoculum was probably linked to the occurrence of their fruiting bodies in Western Cape vineyards (Baloyi *et al*., 2013; 2016), and possibly for *Pm. minimum,* the presence on *Proteaceae* twig litter (Marincowitz *et al*., 2008). One of these two pathogens was the predominant species detected in all vineyards, except in Rawsonville where *Pm sicilianum* was the predominant species. *Phaeomoniella chlamydospora* and *Pm. minimum* are widely distributed throughout most grape growing regions of the world (Mostert *et al*., 2006a; Gramaje *et al*., 2015), which is testimony to their adaptive characters and abilities to grow in a wide range of biotic conditions (Whiting *et al*., 2001; Mostert *et al*., 2006a). *Phaeoacremonium sicilianum* was previously isolated from vineyards in Calitzdorp (White *et al*., 2011a) and was also found in Italian (Essakhi *et al*., 2008) and Spanish vineyards (Gramaje *et al*., 2009). However, fruiting structures of this species have not been found in nature or through *in vitro* mating studies, and the origin of this inoculum is still unclear. Similar to previous isolation studies, after *Pm minimum*, *Pm. parasiticum* appears to be the most common *Phaeoacremonium* species associated with grapevine (Mostert *et al*., 2006a), as this species was detected in four and six vineyards, respectively, during 2012 and 2013, with significant numbers of spores released in at least two vineyards during 2013. Perithecia of *Pm. parasiticum* have been found on *Proteaceae* twig litter in the Western Cape (Marincowitz *et al*., 2008), but not from grapevines. Altough *Pm. scolyti* only occurred in Rawsonville (in 2012 and 2013) and Slanghoek (only 2012), significant spore releases occurred especially during the 2012 season in Rawsonville. Together with *Pm minimum*, *Pm. scolyti* was also the most dominant species isolated from *Prunus* spp. (Damm *et al*., 2008).

The total number and composition of *Phaeoacremonium* species varied between vineyards and seasons. Vineyards of Stellenbosch P2 and Rawsonville had the greatest species diversity (eight species each), compared to the two species found in the Wellington rootstock mother vine nursery. The reason for this is unknown, but the age of the vineyards, proximity to other older vineyards, proximity to other woody hosts and sanitation practices may play roles. The Wellington rootstock mother vine nursery was more isolated from other fruit orhards and woody hosts, and although there are other rootstocks and vineyards within the immediate vicinity, the sampled nursery was surounded by wheat fields. Some of the *Phaeoacremonium* species that were detected less frequently occurred within the same or nearby geographical areas. For example *Pm. prunicola* only occurred in the two Stellenbosch vineyards, *Pm. australiense*, *Pm. subulatum*, *Pm. scolyti* and *Pm. sicilianum* were trapped in the Rawsonville and Slanghoek areas, while *Pm. iranianum* was trapped in the two Paarl vineyards. These results suggests that aerial spores were dispersed between vineyards that were in close proximity to each other. This can also be the case for vineyards established in close proximity to fruit orchards, ornamental trees or numerous other woody hosts. All the vineyards were in close proximity to other vineyards, fruit orchards or other woody hosts. Stellenbosch P2 was located directly next to a plum orchard and various other *Prunus* spp. were just a few metres further away. The extent of the host ranges associated with *Phaeoacremonium* species in South Africa was recently confirmed by Spies *et al.* (2018), who linked *Pm. scolyti* with 20 woody hosts, *Pm. minimum* with 19, *Pm. parasiticum* with 17, *Pm. inflatipes* with 11, *Pm. italicum* with ten, *Pm. subulatum* with ten, *Pm. prunicola* with ten, *Pm. viticola* with eight, *Pm. iranianum* with seven, and *Pm. sicilianum* with four woody hosts.

Eight *Pm.* species associated with *Prunus* spp. in South Africa (Damm *et al*., 2008) were trapped as aeri-

al inoculum in the present study, including *Pm. scolyti, Pm. minimum, Pm. australiense, Pm. griseo-olivaceum, Pm. griseorubrum, Pm. iranianum, Pm. prunicola* and *Pm. parasiticum*. The possibility that *Phaeoacremonium* sexual morphs may form on alternative hosts in close proximity to vineyards further emphasizes the importance of these hosts as inoculum sources. Eskalen *et al.* (2005a; b) found *Pm. fraxinopennsylvanicum* and *Pm. viticola* perithecia on ash trees growing in close proximity to vineyards in California.

Petri disease pathogens were detected throughout the trapping periods in all the vineyards, with only occasional weeks of no spore release. However, cfu numbers differed between the vineyards. The reasons for this are unknown, but these could be related to differences in inoculum type (sexual or asexual), inoculum abundance and/or favourable microclimatic conditions. Spore release of the two most predominant species, *Pa. chlamydospora* and *Pm. minimum,* occurred in a large proportion of the vineyards during most of the sampling months, although sometimes in very low numbers. However, if the total numbers of cfu detected in all the vineyards are taken into consideration, the greatest spore release events for *Pa. chlamydospora* differed between the two seasons. During 2012, the peak spore release months occurred in winter declining towards spring, while during the 2013 season spore release increased in spring and early summer with another high peak in autumn. The greatest spore release events for *Pm. minimum* were in winter 2012 and in autumn/winter 2013. *Phaeoacremonium sicilianum* possibly favoured the warm humid months of spring/early summer (2012) and autumn (2013), while *Pm. parasiticum* spore numbers peaked in autumn and those of *Pm. scolyti* peaked in winter/spring. For the other species, it is difficult to determine specific patterns, or to link them to specific seasons, because of the low detection numbers, species distribution amongst the vineyards and seasonal differences. In Californian vineyards, *Pa. chlamydospora*, *Pm. minimum* and *Pm. inflatipes* were also trapped throughout the year, but *Pa. chlamydospora* and *Pm. inflatipes* spore release coincided with rainfall events mostly in late winter and early spring. Trapping of *Pm. minimum* coincided less with rainfall events and even during periods of no rain during early to mid summer (Eskalen and Gubler, 2001). However, Rooney-Latham *et al.* (2005b) contradicted this when they reported large numbers of *Pm. minimum* spores that were trapped following rain in Californian vineyards, a phenomenon linked to the ability of *Pm. minimum* asci to forcibly discharge ascospores after thorough wetting. Trapping of *Pm. minimum* spores in dry summer months was attributed to overhead irrigation. In French vineyards *Pa. chlamydospora* was also trapped throughout the year, although pruning wound infections correlated with winter rainfall events, whereas *Pm. minimum* were mostly trapped during the grapevine vegetative period (Larignon and Dubos, 2000).

Spore release occurring during winter (June-August) and spring (September-November) coincides with traditional winter and spring grapevine pruning periods in the Western Cape Province. Winter and spring wounds remain susceptible for long periods (Eskalen *et al*., 2007; Van Niekerk *et al*., 2011a; Makatini, 2014). In the present study, spore release events were observed during the 4-week period after winter pruning when wounds are at their most susceptible to infections. During this period, aerial inoculum of *Pa. chlamydospora*, *Pm. minimum*, *Pm. parasiticum*, *Pm. inflatipes*, *Pm. prunicola*, *Pm. iranianum*, *Pm. sicilianum*, *Pm. scolyti* and *Pm. subulatum* were recorded. The capability of *Pa. chlamydospora* to infect spring wounds under field conditions has recently been shown (Makatini, 2014). Spring wounds also stayed susceptible to infection for 4 weeks, although susceptibility declined after the first week. The large number of spore release events, large aerial inoculum numbers and large diversity of Petri disease pathogens emphasize that pruning activities in South African vineyards occur during periods of high risk of infections. This shows that wound protectants that provide prolonged protection against Petri disease pathogens and those that cause other grapevine trunk diseases are very important.

The occurrence of aerial spore inoculum of *Pa. chlamydospora* and *Phaeoacremonium* species in rootstock mother vine nurseries is of concern, especially since these pathogens were trapped within the week in which cuttings were harvested as well as subsequent weeks when the wounds were still susceptible. The high *Phaeoacremonium* species diversity (six) within the Slanghoek mother vine nursery should be of major concern for South African vine improvement efforts. There are currently no guidelines on the productive lifespans of rootstock mother vines. From the present results it is clear that inoculum may build up in particular blocks, most likely increasing as the vines age. There are also no guidelines, or any regulations, regarding pruning wound protection or management of grapevine trunk diseases in general. The risks of mother vines becoming infected and supplying propagation material with compromised phytosanitary status could seriously impact spread of trunk diseases, as infected asymptomatic shoots may be used for grafting or planting into newly established vineyards with severe disease transmission results (Bertelli *et*

al., 1998; Fourie and Halleen, 2002; Halleen *et al*., 2003; Fourie and Halleen, 2004).

Establishing new vineyards in close proximity to older vineyards is a common practice in South Africa. In this study, spore traps were also placed in newly established vineyards adjacent to two of the spore trapping vineyards, one in Rawsonville and one in Paarl (data not shown). Pathogens trapped in the older vineyards were also trapped in the young vineyards, including *Pa. chlamydospora*, *Pm. minimum*, *Pm. sicilianum* and other trunk disease pathogens. Petri disease pathogens can also be dispersed from one vineyard to another by arthropods which act as vectors (Moyo *et al*., 2014), and on pruning shears (Augustí-Brisach *et al*., 2015). This emphasizes the need to adopt integrated disease approaches throughout vineyards, from as early as the establishment phase by sanitation practices to reduce inoculum sources and wound protection strategies to prevent infections. Gramaje and Armengol (2011) and Gramaje *et al*. (2018) have provided a comprehensive international overview of grapevine trunk disease management during the propagation process as well as in established vineyards.

The aim of this study was to provide new insight into the inoculum ecology of Petri disease pathogens in South African vineyards and rootstock mother vine nurseries. In total, 15 Petri disease pathogens, including *Pa. chlamydospora* and 14 *Phaeoacremonium* species were trapped as aerial spore inoculum. The spore release events coincided with periods of pruning and rootstock harvesting. Spores were available even during spring pruning and the late summer periods, which some viticulturist believe to be free of spores and consequently correct times to conduct clean pruning practices where large wounds are created. Wound protectants should be applied whenever vine wounding occurs, irrespective of the time of the year. New wound protectant formulations should consider the period of wound susceptibility, as well as the constant availability of aerial spore inoculum of many pathogens. However, to determine the best timing for such preventive management options, further research into the development of epidemiological models accounting for the specific environmental conditions required for spore dispersal and infection should be undertaken. A recent first step was achieved with a model to evaluate disease risk for *Pa. chlamydospora* in Spain, where hydro-thermal time was shown to be the best descriptor for predicting dispersal of this pathogen (González-Domínguez *et al*., 2020). Further studies on sources of inoculum within vineyards and mother vine nurseries are also highly recommended in order to reduce aerial pathogen inoculum.

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