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RESEARCH PAPERS - 10TH SPECIAL ISSUE ON GRAPEVINE TRUNK DISEASES

Pathogenicity of ten *Phaeoacremonium* species associated with esca and Petri disease of grapevine

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Summary. Nineteen species of *Phaeoacremonium* have been associated with grapevines in South Africa, of which only six species have been confirmed as pathogens through pathogenicity tests conducted on field-grown grapevines. This study determined the pathogenic status of ten *Phaeoacremonium* spp. recently found for the first time on South African grapevines. These were: *Pm. australiense*, *Pm. austroafricanum, Pm. fraxinopennsylvanicum, Pm. griseo-olivaceum*, *Pm. griseorubrum, Pm. iranianum, Pm. italicum, Pm. prunicolum, Pm. scolyti* and *Pm. sicilianum*. In the pathogenicity tests, *Ph. parasiticum* was used as the positive control, and sterile water as the negative control. Up to three isolates were used per species, depending on isolate availability. Freshly cut pruning wounds in a 9-year-old Cabernet Sauvignon vineyard in Stellenbosch, South Africa, were inoculated with 200 conidia of each fungus per wound. Inoculated pruning wounds were removed after 18 months, cut longitudinally and lesion lengths were measured. Re-isolation proportions were determined by conducting isolations from inoculated spurs. All the inoculated isolates successfully colonized pruning wounds, and caused lesions that were significantly different from the negative control. All isolates were re-isolated at proportions varying from 28.6 to 85.7%. *Phaeoacremonium griseo-olivaceum* STE-U 7859 produced the longest lesions (mean = 79.5 mm) and *Pm. iranianum* STE-U 6998 the shortest (62.0 mm). No statistically significant differences in mean lesion lengths were observed between the inoculated species. There were also no significant differences between isolates of the same species, except in *Pm. prunicolum* where isolate STE-U 5968 produced longer lesions (mean = 77.3 mm) than STE-U 7857 (62.3 mm). This study confirmed the capabilities of all the tested *Phaeoacremonium* spp. to infect grapevine pruning wounds and cause lesions. The study also confirmed the importance of pruning wounds as ports of entry by these pathogens into host plants.

Keywords: *Vitis* spp., grapevine trunk diseases, pruning wound infections.

Introduction

Of the 61 *Phaeoacremonium* W. Gams, Crous & M.J. Wingf. species known, 33 have been isolated from grapevines (Gramaje *et al*., 2015; Spies *et al*., 2018). Species of *Phaeoacremonium* are known inhabitors of many woody hosts and have also been isolated from humans (Ajello *et al*., 1974; Crous *et al*., 1996; Mostert *et al*., 2005; Damm *et al*., 2008; Gramaje *et al*., 2012; Marin-Felix *et al*., 2019). Some of the species associated with grapevine have also been found from fruit and nut trees, including economic important pome and stone fruit species, olives, kiwifruit, guava, fig, pomegranate, loquat, persimmon, mulberry, date palm and medlar, as well as numerous ornamental trees and shrubs, some occurring in forests, plantations, gardens or parks (Gramaje *et al*., 2015; Spies *et al*., 2018).

Phaeomoniella (Pa.) chlamydospora (W. Gams, Crous, M.J. Wingf. & Mugnai) Crous & W. Gams, together with *Phaeoacremonium (Pm.)* spp., cause Petri disease (Scheck *et al*., 1998; Mugnai *et al*., 1999), which is commonly found in young grapevines of approx. 1-5 years age (Halleen and Groenewald, 2005). Species of *Phaeoacremonium* are well adapted endophytes, capable of becoming pathogenic when vines are sub-

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jected to stress (Scheck *et al*., 1998; Ferreira *et al*., 1999). External host decline symptoms include leaf necrosis, shoot dieback, shortened internodes or (in extreme cases) the vine death. Internal symptoms include brown streaks in xylem tissues, seen in a longitudinal cuts infected stems, or the black/brown gummoses exuded from transversally cut wounds (Scheck *et al*., 1998; Ferreira *et al*., 1999; Mugnai *et al*., 1999). *Phaeoacremonium* spp. are also associated with esca of grapevines. Esca is caused by the Petri disease pathogens together with wood rotting fungi of the Hymenochaetales (Mugnai *et al*., 1999; White *et al*., 2011a; Cloete *et al*., 2015a, b). The brown streaking found in Petri diseased and esca-affected grapevines is due to the accumulation of phenolic compounds and tyloses in infected tissues, which block the xylem vessels and impair translocation of water and nutrients (Ferreira *et al*., 1999; Edwards *et al*., 2007).

Petri disease pathogens infect grapevines primarily through susceptible pruning wounds (Larignon and Dubos, 2000; Eskalen *et al*., 2007; Van Niekerk *et al*., 2011). Sucker wounds have also been shown to be susceptible, and could act as additional infection sites during spring and early summer (Makatini, 2014). Fruiting bodies of the pathogens formed within vineyards release spores mostly during and after rainfall periods, although spore release can occur without rainfall (Edwards and Pascoe, 2001; Eskalen *et al*., 2005a; b; Rooney-Latham *et al*., 2005). Spore trapping studies conducted in Western Cape (South Africa) vineyards and rootstock mother plant nurseries have shown the presence of air-borne spores within these vineyards (Baloyi, 2016). Spore release also coincided with winter and spring pruning periods, as well as the period when rootstock canes were harvested in nurseries. Studies in France, California and Italy have also showed the availability of air-borne spores during pruning periods (Larignon and Dubos, 2000; Eskalen and Gubler, 2001; Quaglia *et al*., 2009). Winter pruning activities in the Western Cape occur between June and August, whereas de-suckering activities (winter pruning), the removal of unwanted shoots from vine trunks, cordons and spurs, occurs in spring and early summer (September to November). Rootstock canes are harvested between April and July. Multiple wounds made on each vine during these periods increase the risk of vines becomming infected. Other modes of pathogen spread have recently been reported, including arthropods which vector Petri disease pathogens to pruning wounds in vineyards (Moyo *et al*., 2014), infected pruning shears under greenhouse conditions (Agustí-Brisach *et al*., 2015), and the use of infected planting material (Fourie and Halleen, 2002; Halleen *et al*., 2003; Fourie and Halleen, 2004).

In California, pruning wound susceptibility was shown to last for up to 16 weeks, which would enable even slow-growing pathogens to colonize and infect the wounds before they heal (Eskalen *et al*., 2007). In France, wounds remained susceptible to *Pm. minimum* (Tul. & C. Tul.) Gramaje, L. Mostert & Crous for 7-9 weeks in the early dormant season, and for only 2 weeks in late winter pruning periods when bleeding occurred (Larignon and Dubos, 2000). Furthermore, Munkvold and Marois (1995) found that vines pruned towards the end of dormancy release exudates that contained carbohydrates, amino acids and organic acids, which promoted rapid growth of microflora that compete with pathogens. Other factors such as vine training method, as for bilateral cordons, result in multiple wounds and more pathogen entry sites, which may allow multiple infections (Gu *et al*., 2005). Pruning spurs to two buds has been shown to promote rapid infection of cordons, as lesions extended quicker into the cordons and trunks from the inoculated pruning wounds (Eskalen *et al*., 2007; Halleen *et al*., 2007). To date, no grapevine cultivar has been shown to be resistant to *Phaeoacremonium* spp., or to any of the trunk disease pathogens. Pruning wound protection is, therefore, advocated as a disease management strategy (Halleen *et al*., 2010; Kotze *et al*., 2011; Mutawila *et al*., 2016). However, the increasing number of fungal pathogens from unrelated fungal genera means that single products or modes of action may not be effective for control of the complex of grapevine trunk disease pathogens.

At commencement of this project, 17 *Phaeoacremonium* spp. were isolated from grapevines in South Africa. These were: *Pm. Minimum*; *Pm. italicum* Carlucci & M.L. Raimondo (reported as *Pm. alvesii*); *Pm. austroafricanum* L. Mostert, W. Gams & Crous; *Pm. iranianum* L. Mostert, Gräfenhan, W. Gams & Crous; *Pm. krajdenii* L. Mostert, Summerb. & Crous; *Pm. fraxinopennsylvanicum* (T.E. Hinds) D. Gramaje, L. Mostert & Crous; *Pm. parasiticum* (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M.J. Wingf.; *Pm. scolyti* L. Mostert, Summerb. & Crous; *Pm. sicilianum* Essakhi, Mugnai, Surico & Crous; *Pm. subulatum* L. Mostert, Summerb. & Crous; *Pm. venezuelense* L. Mostert, Summerb. & Crous; *Pm. viticola* J. Dupont.; *Pm. australiense* L. Mostert, Summerb. & Crous; *Pm. griseorubrum* L.

Mostert, Summerb. & Crous; *Pm griseo-olivaceum* (Damm, L. Mostert & Crous) Gramaje, L. Mostert & Crous; *Pm. inflatipes* W. Gams, Crous & M.J. Wingf.; and *Pm. prunicolum* L. Mostert, Damm & Crous (Mostert *et al*., 2005, 2006b; White *et al*., 2011b; Spies *et al*., 2018). Of these, only *Pm. minimum*, *Pm. krajdenii*, *Pm. parasiticum*, *Pm. subulatum*, *Pm. venezuelense* and *Pm. viticola* have been subjected to pathogenicity studies on grapevines in South Africa (Halleen *et al*., 2007). The present study was therefore conducted to assess the pathogenic status of ten of these newly found *Phaeoacremonium* species, on grapevine pruning wounds under field conditions.

Materials and methods

Isolate selection and inoculum preparation

The *Phaeoacremonium* isolates used in this study were previously recovered and identified in spore trapping studies (Baloyi, 2016) and disease incidence surveys conducted in South African vineyards (Mostert *et al*., 2005, 2006b; White *et al*., 2011b) (Table 1). The species tested included *Pm. australiense*, *Pm. austroafricanum, Pm. italicum* (previously reported as *Pm. alvesii*), *Pm. fraxinopennsylvanicum, Pm. griseo-olivaceum, Pm. griseorubrum, Pm. iranianum, Pm. prunicolum, Pm. scolyti* and *Pm. sicilianum. Phaeoacremonium parasiticum* was used as the positive control (Halleen *et al*., 2007) in the pathogenicity tests, and sterile water was used as the negative control. Up to three strains per isolate were used, depending on availability. Isolates were plated onto Potato Dextrose Agar with chloramphenicol (PDA-C), and grown at 25°C for 2 weeks. The cultures were then each flooded with 20 mL of sterile distilled double autoclaved water. Conidia were dislodged from the mycelia using a sterile glass rod, and the conidia suspensions were filtered with doubled cheese cloth. The conidia concentration was adjusted to 10^4 mL^{-1} after enumeration using a haemocytometer.

Pruning wound inoculations

The inoculation trial was conducted from August 2013 and March 2015, in a 9-year-old Cabernet Sauvignon vineyard at the Nietvoorbij Campus of ARC Infruitec-Nietvoorbij in Stellenbosch, Western Cape Province, South Africa. The trial was set up in a completely randomized block design, with 27 treatments each replicated 15 times. Pruning wounds were the experimental unit. The vines were cordon trained and spur pruned to two buds. Five pruning wounds were made per vine and individual vines received one treatment. Due to sap flow, pruning wounds were not inoculated immediately, but within 24 h of pruning they were each inoculated with 20 µL of conidium suspension (200 conidia per wound).

Trial evaluation

The trial was evaluated after 18 months from the date of inoculation. Stubs with inoculated pruning wounds were removed, individually placed in plastic bags and immediately taken to the laboratory for assessment and to conduct isolations. The stubs were each cut longitudinally to measure lesion length with a caliper. The stubs were then surface sterilized by immersing into 70% ethanol for 30 s, followed by 1 min in 3.5% sodium hypochlorite solution and again for 30 s in 70% ethanol. Pieces of small tissue sections (1 × 1 × 2 mm) were each asceptically dissected with a sterile scalpel from just below the wound scar interface, as well as along the length of the lesion, and plated onto PDA-C plates. The inoculated Petri dishes were maintained at 25°C for 4 weeks and closely monitored for any *Phaeoacremonium* spp. growth. Re-isolated pathogens were identified based on morphological characteristics and then verified by randomly selecting two representative isolates from each treatment for sequencing. DNA was extracted with CTAB buffer according to Damm *et al*. (2008). The partial beta-tubulin gene regions were amplified with PCR using primers T1 and Bt2b (Glass and Donaldson, 1995; O' Donnel and Cigelink, 1997), as decribed by Mostert *et al*. (2006b). PCR products were cleaned using an MSB Spin PCRapase kit (Invitek), and the cleaned products were sequenced with the same primers using ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (PE Biosystems). The products were then analyzed on an ABI Prism 3130XL DNA sequencer (Perkin-Elmer). Sequences were compared to reference sequences of each species in the megablast function of the NCBI's GenBank nucleotide database (www.ncbi.nlm.nih.gov), to confirm identity. Mean lesion lengths were calculated, and subjected to one way analyses of variance (ANOVA) using SAS. Student's t-test for least significant difference (LSD) was calculated (at $P \leq 0.05$) to separate means.

Table 1. *Phaeoacremonium* spp. and isolates used in the pathogenicity study.

Phaeoacremonium spp.	Accession number	Host	Location of origin
Pm. australiense	STE-U 7863	Spore trap[a]	Slanghoek, Western Cape
	STE-U 7862	Spore trap[a]	Slanghoek
	STE-U 7861	Spore trap[a]	Slanghoek
Pm. austroafricanum	LM 733	*Vitis vinifera*[b]	Wellington, Western Cape
Pm. italicum	STE-U 6988	*Vitis vinifera*[c]	Klawer, Western Cape
	STE-U 6989	*Vitis vinifera*[c]	Klawer
	STE-U 7000	*Vitis vinifera*[c]	De Rust, Western Cape
Pm. fraxinopennsylvanicum	STE-U 6987	*Vitis vinifera*[c]	Hermanus, Western Cape
Pm. griseorubrum	STE-U 7881	*Vitis* sp.	Wellington
	STE-U 7882	*Vitis* sp.	Wellington
	STE-U 7856	*Vitis* sp.	Wellington
Pm. griseo-olivaceum	STE-U 7860	Spore trap[a]	Durbanville, Western Cape
	STE-U 7859	Spore trap[a]	Durbanville
	STE-U 7858	Spore trap[a]	Durbanville
Pm. iranianum	STE-U 6998	*Vitis vinifera*[c]	Calitzdorp, Western Cape
	STE-U 6999	*Vitis vinifera*[c]	Calitzdorp
Pm. prunicolum	STE-U 5968	*Prunus salicina*[d]	Mookgopong, Limpopo
	STE-U 7857	Spore trap[a]	Stellenbosch, Western Cape
Pm. scolyti	STE-U 7854	Spore trap[a]	Slanghoek
	STE-U 7855	Spore trap[a]	Rawsonville, Western Cape
	STE-U 7876	Spore trap[a]	Rawsonville
Pm. sicilianum	STE-U 7879	Spore trap[a]	Rawsonville
	STE-U 7880	Spore trap[a]	Rawsonville
	STE-U 7877	Spore trap[a]	Rawsonville
Pm. parasiticum	STE-U 7875	Spore trap[a]	Paarl, Western Cape
	STE-U 7878	Spore trap[a]	Stellenbosch

[a] Isolates were obtained as aerial spore inoculum in vineyards (Baloyi, 2016).
[b] Mostert *et al*. (2006b).
[c] White *et al*. (2011b).
[d] Damm *et al*. (2008).

Results

Mean lesion lengths of vascular discolouration caused by the 27 treatments, and mean re-isolation percentages, are presented in Table 2. All the isolates caused black to brown discolourations, as vascular streaking inside the inoculated spurs. All isolates were considered pathogenic, as they produced lesion lengths significantly different to the negative experimental control ($P < 0.001$), and some lesions extended beyond the inoculated pruning wounds into the cordons. There was no significant variation in mean lesion lengths produced by each species. *Phaeoacr-*

Table 2. Mean lesion lengths and re-isolation proportions for *Phaeoacremonium* spp. inoculated on Cabernet Sauvignon pruning wounds.

Phaeoacremonium spp.	Mean lesion length (mm)	Mean re-isolation (%)	Accession number
Pm. griseo-olivaceum	79.53 a	78.57	STE-U 7859
Pm. prunicolum	77.27 ab	64.29	STE-U 5968
Pm. griseo-olivaceum	75.54 abc	80.00	STE-U 7858
Pm. parasiticum (positive control)	74.53 abc	78.57	STE-U 7875
Pm. sicilianum	74.44 abc	76.92	STE-U 7880
Pm. australiense	74.30 abc	73.33	STE-U 7863
Pm. griseorubrum	73.27 abc	69.23	STE-U 7881
Pm. australiense	73.06 abc	42.86	STE-U 7861
Pm. griseorubrum	72.95 abc	69.23	STE-U 7882
Pm. sicilianum	72.04 abc	76.92	STE-U 7877
Pm. scolyti	71.59 abc	85.71	STE-U 7854
Pm. scolyti	71.51 abc	84.62	STE-U 7855
Pm. sicilianum	71.51 abc	80.00	STE-U 7879
Pm. italicum	70.91 abc	35.71	STE-U 7000
Pm. iranianum	70.05 abc	71.43	STE-U 6999
Pm. austroafricanum	67.97 abc	69.23	LM 733
Pm. italicum	67.54 abc	28.57	STE-U 6988
Pm. griseo-olivaceum	67.33 abc	84.62	STE-U 7860
Pm. scolyti	65.77 abc	66.67	STE-U 7876
Pm. fraxinopennsylvanicum	65.65 abc	78.57	STE-U 6987
Pm. parasiticum (positive control)	65.21 abc	57.14	STE-U 7878
Pm. italicum	64.50 bc	53.85	STE-U 6989
Pm. australiense	64.39 bc	57.14	STE-U 7862
Pm. griseorubrum	64.17 bc	73.33	STE-U 7856
Pm. prunicolum	62.26 c	57.14	STE-U 7857
Pm. iranianum	62.00 c	60.00	STE-U 6998
Negative control (sterile water)	14.46 d	0	
LSD ($P = 0.05$)	14.93		

[a] Different letters indicate significant differences to the negative control ($P<0.001$).

emonium griseo-olivaceum strain STE-U 7859 produced the longest lesions (mean = 79.5 mm. The shortest lesions (mean = 62.0 mm) were produced by *Pm. iranianum* strain STE-U 6998. Positive control wounds inoculated with two isolates of *Pm. parasiticum* produced mean lesion lengths of, respectively, 74.5 and 65.2 mm. All the inoculated isolates produced lesion lengths similar to *Pm. parasiticum*, a known pathogen, and therefore all the isolates are confirmed as pathogenic to grapevine. There were no significant differ-

ences between isolates of the same species, except for *Pm. prunicolum*, where STE-U 5968 produced significantly longer lesions (mean = 77.3 mm) than STE-U 7857 (62.3 mm).

All isolates were re-isolated from inoculated pruning wounds after 18 months. The isolates with the greatest proportions of re-isolation were STE-U 7854 and STE-U 7855 (*Pm. scolyti*), STE-U 7860 and STE-U 7858 (*Pm. griseo-olivaceum*) and STE-U 7879 (*Pm. sicilianum*), with re-isolation proportions of 89.0 to 84.6%. Two of the three *Pm. italicum* isolates were re-isolated at the least proportions of, respectively, 28.6 and 35.7% (Table 2).

Discussion

This study showed that all 11 inoculated *Phaeoacremonium* species were pathogenic and capable of causing vascular discolouration when inoculated onto grapevine pruning wounds. The ten *Phaeoacremonium* species with unknown pathogenicity to grapevines all formed lesions that were not significantly different in mean lengths from *Pm. parasiticum*, a known pathogen of grapevines. Of the species tested, *Pm. fraxinopennsylvanicum*, *Pm. iranianum*, *Pm. scolyti* and *Pm. sicilianum* have been tested on grapevines in other grapevine-producing countries (Gramaje *et al*., 2007; Aroca and Raposo, 2009; Gramaje *et al*., 2009; Gramaje *et al*., 2010; Mohammadi and Banihashemi, 2012; Özben *et al*., 2012; Úrbez-Torrez *et al*., 2014; Mohammadi and Hashemi, 2015). All these species caused vascular discolourations inside inoculated grapevine shoots, which is consistent with the findings of the present study. Furthermore, reduced root weight, chlorotic leaves, severe defoliation and wilting symptoms have been observed from grapevine shoots inoculated with *Pm. fraxinopennsylvanicum* (Gramaje *et al*., 2007; Aroca and Raposo, 2009; Úrbez-Torrez *et al*., 2014). Low mean shoot weights were also reported in grapevine shoots inoculated with *Pm. iranianum* and *Pm. sicilianum* (Gramaje *et al*., 2009).

All *Phaeoacremonium* spp. from South African grapevine, previously described as *Pm. alvesii* (White *et al*., 2011b), were re-identified by Spies *et al*. (2018) as *Pm. italicum* based on the recent finding of this new species from Italian vineyards (Raimondo *et al*., 2014). Although these isolations were made from declining and esca-affected vines in both countries, pathogenicity studies have not been previously conducted. The present report is, therefore, the first confirmation of *Pm. italicum* as grapevine pathogen. *Phaeoacremonium austroafricanum* has only been reported from grapevines in South Africa (Mostert *et al*., 2006b), but this is also the first confirmation of the pathogenic status of this species. Until the recent finding in South Africa (Spies *et al*., 2018), *Phaeoacremonium griseo-olivaceum* and *Pm. prunicolum* have not been previously reported on grapevines. *Phaeoacremonium australiense* has previously only been isolated from grapevines in Australia and Uruguay (Mostert *et al*., 2005; Abreo *et al*., 2011), and *Pm. griseorubrum* from Italian vineyards (Essakhi *et al*., 2008), but pathogenicity studies have not been conducted for these species. Pathogenicity has only been tested on *Prunus armeniaca* L. and *P. salicina* Lindl. in South Africa (Damm *et al*., 2008). This is, therefore, the first confirmation of *Phaeoacremonium griseo-olivaceum*, *Pm. prunicolum, Pm. australiense* and *Pm. griseorubrum* as grapevine pathogens. These species were also trapped as airborne spores in Western Cape vineyards during the 2012 and 2013 seasons (Baloyi, 2016). The presence of these species as aerial inoculum, and confirmation of their capability to cause symptoms in grapevines and stone fruit, highlights the risks of establishing stone fruit orchards in close proximity to vineyards. This is also true for *Pm. iranianum* and *Pm. scolyti* (Damm *et al*., 2008; Baloyi, 2016). This conclusion agrees with a study that showed *Pm. minimum* isolated from apple trees could infect grapevine shoots and develop lesions (Cloete *et al*., 2011). Furthermore, *Phaeoacremonium minimum* isolates from apple, apricot and grapevine were pathogenic to apple, inducing wood discolouration on apple stems (Arzanlou *et al*., 2014). Aroca and Raposo (2009) also used isolates of *Pm. inflatipes* from *Quercus* and *Pm. fraxinopennsylvanicum* from *Fraxinus* to show the capability of these species to infect and cause discolouration in grapevines. *Phaeoacremonium* spp. have wide host ranges, which favours the survival and spread of these pathogens, as several woody hosts can serve as pathogen reservoirs (Spies *et al*., 2018). Cross dispersal between orchards and / or alternative hosts and vineyards is therefore of great concern, due to the increase in species diversity, and to the increasing risk of mating types being introduced that could result in production of sexual structures on vines.

All species of *Phaeoacremonium* tested on pruning wounds in this study were successfully re-isolated, although this was at varying proportions between species and isolates. The least re-isolation proportions were obtained with two of the three *Pm. italicum* iso-

lates. This species has previously only been isolated from grapevines in South Africa and Italy (White *et al*., 2011b; Spies *et al*., 2018). Spies *et al*. (2018) recently isolated *Pm. italicum* from eight additional hosts in South Africa, and Carlucci *et al*. (2015) also isolated this fungus from diseased olive trees in Italy. Infrequent release and low spore numbers in vineyards (Baloyi, 2016), isolation only from a limited number of grapevines (White *et al*., 2011b), and presence in several other hosts may indicate that this species has been introduced more recently, or that grapevine is not its primary host.

There were statistically significant difference in virulence between the *Phaeoacremonium* isolates tested in this study, except for *Pm. prunicolum* where differences were observed between the two isolates. The most virulent isolate of *Pm. prunicolum* (STE-U 5968) was previously isolated from *Prunus salicina* in the Limpopo Province (Damm *et al.,* 2008), and was significantly different from the isolate found as airborne inoculum during spore trapping studies conducted in the Western Cape Province (Baloyi, 2016). The two isolates may be genetically different, resulting in different levels of virulence. Another explanation for the apparent lack of virulence differences in most of the *Phaeoacremonium* spp. may be that several lesions extended into the inoculated cordons, which could not be measured using the methods adopted for this study.

Isolates used in the present study were obtained from different geographic locations, and were only tested in one vineyard in Stellenbosch, which is, in some instances, environmentally different from the other regions from which isolates were collected. The capability of isolates from all these regions to infect grapevine pruning wounds, irrespective of their origins, and in the case of *Pm. prunicolum* also from another host, highlights the highly adaptive character of these pathogens. This shows the importance of movement of plant material between regions, which can introduce new pathogenic species into areas where they have not been previously reported. This is particularly the case for species such as *Pm. sicilianum* and *Pm. australiense*, which have not been reported from Stellenbosch vineyards (White *et al*., 2011b; Baloyi, 2016) but have been shown to cause infections of grapevine pruning wounds in this region.

As was reported by Halleen *et al*. (2007), in the present study, many lesions extended from the inoculated two bud spurs into the cordons. This demonstrates the risk associated with this pruning method, and how rapidly pruning wound infections can spread into the cordons. Other pruning practices, such as double pruning, have been previously been reported as a strategy to manage infection by *Eutypa lata* (Pers.) Tul. & C. Tul. (Weber *et al*., 2007), but this practice has been widely used in South Africa for many years without any apparent reduction in the incidence of trunk diseases.

The pathogenicity trial in this study was not repeated. Pathogenicity of *Pm. fraxinopennsylvanicum*, *Pm. iranianum*, *Pm. scolyti* and *Pm. sicilianum* have been tested on grapevines in other countries, and the present study confirmed that South African isolates are pathogenic. Results obtained with the remaining six species, which are here confirmed as grapevine pathogens for the first time, were similar to previous pathogenicity studies conducted on grapevine pruning wounds in Stellenbosch (Halleen *et al*., 2007).

This study confirms pruning wounds as entry sites for infection by *Pm. australiense*, *Pm. austroafricanum*, *Pm. fraxinopennsylvanicum*, *Pm. griseorubrum*, *Pm. griseo-olivaceum*, *Pm. iranianum*, *Pm. italicum*, *Pm. prunicolum*, *Pm. scolyti* and *Pm. sicilianum*, irrespective of the geographic origins of the isolates. The importance of pruning wound protection in vineyards and rootstock mother fields is thus emphasized, and this should form the foundation of an integrated management strategy to combat esca and Petri diseases of grapevine.

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