Symptoms and fungi associated with esca in South African vineyards

Chana-Lee WHITE[1], Francois HALLEEN[1,2] and Lizel MOSTERT[1]

[1]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa
[2]Plant Protection Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa

Summary. In the past, only a few incidences of esca diseased grapevines were reported from the Slanghoek and Rawsonville areas of South Africa. The damage was believed to be of little importance and therefore the disease has not been studied in South Africa. In the present study, vines with internal or external symptoms of the esca disease complex were sampled from table, raisin and wine grape cultivars from 37 production areas in the Western Cape, Northern Cape and Limpopo provinces of that country. Most vines were greater than 10 years old, but younger vines (3 and 5 years old) were also found to be infected. External symptoms, including dieback, tiger striped leaves, berry symptoms (shrivelling, insufficient colouring) and apoplexy, resembled those found on grapevines in Europe and the USA, although the typical tiger stripe symptom was observed less frequently. The internal stem and trunk symptoms were similar to European symptoms, and included white rot, black and brown wood streaking, brown necrosis within white rot, sectorial brown necrosis and brown/red/margins next to decay, which often included black lines delimiting white decay. The fungi isolated mostly from the white rot were basidiomycetes species (30.4 %). Black and brown wood streaking was primarily caused by *Phaeomoniella chlamydospora* (45.4%). Brown necrosis within the white rot was linked to colonization by basidiomycetes (20.4%), *Phaeoacremonium aleophilum* (15.9%) and *Pa. chlamydospora* (13.6%). *Phaeomoniella chlamydospora* (20.8%) and Botryosphaeriaceae species (10.7%) were isolated the most from the sectorial brown necrosis and *Pa. chlamydospora* (29.1%) from the brown/red margins and black lines next to decay. Given the wide distribution of esca complex wood and foliar symptoms in the grape growing regions investigated, this disease should be considered as an important limiting factor in the productive lifespan of vineyards and the quality of produce from grapevine in South Africa.

Key words: Basidiomycetes, *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp.

Esca is the name given to one of the most destructive diseases of grapevine (Mugnai *et al.*, 1999). The disease affects the trunks and arms of grapevines and is widespread in older vineyards in Europe (Mugnai *et al.*, 1999; Surico *et al.*, 2006).

In the last 20 years esca was interpreted as a disease caused by a succession of a complex of fungi (Larignon and Dubos, 1987), but also as a complex of different diseases, a vascular disease (or tracheomycosis) and a wood decay disease (Mugnai *et al.*, 1999; Graniti *et al.*, 2000; Tabacchi *et al.*, 2000; Surico, 2001; Surico *et al.*, 2006). Research in several countries on the cause of the disease revealed that several pathogens could be isolated from esca-affected vines. These include: *Fomitiporia mediterranea* M. Fischer (which is the most common basidiomycete species in Europe); *F. polymorpha* M. Fischer (North America); *F. australiensis* M. Fisch., J. Edwards, Cunnington & Pascoe (Australia); *Phellinus* sp. (Europe and North America); *Stereum hirsutum* (Willd.) Pers.; *Phaeomoniella chlamydospora* (W. Gams, Crous & M.J. Wingf. & L. Mugnai) Crous & W. Gams; *Phaeoacremonium aleophilum* W. Gams, Crous, MJ Wingf. & L. Mugnai (Crous *et al.*, 1996); other *Phaeoacremonium* spp.; *Eutypa lata* Tul. & C. Tul.; Botryosphaeriaceae and *Phomopsis* spp. (Mugnai *et al.*, 1996; Larignon and Dubos, 1997; Armengol *et al.*, 2001; Rumbos and Rumbou, 2001; Fischer, 2002; Fischer and Kassemeyer, 2003; Fis-

Corresponding author: F. Halleen
Fax +27 21 8093002
E-mail: halleenf@arc.agric.za

cher and Binder, 2004; Fischer, 2006; Martin and Cobos, 2007). The view of esca as a complex of different diseases lead to the proposal of the term "esca" which is to be restricted to grapevine wood rot (or white rot) caused by basidiomycete fungi (i.e. its original meaning), and that the term "grapevine leaf stripe disease" be used for the tracheomycosis caused by *Pa*. *chlamydospora* and *Phaeoacremonium* spp. (mainly *Pm*. *aleophilum*) which is associated with the typical tiger stripe symptoms (Surico, 2009). "Esca proper" was proposed to designate the co-occurrence of esca and grapevine leaf stripe disease (Surico, 2009). Species such as *Eutypa lata* (Larignon and Dubos, 1997; Mugnai *et al*., 1999), members of the Botryosphaeriaceae (Mugnai *et al*., 1999) such as *Diplodia seriata* De Not. (Armengol *et al*., 2001; Crous *et al*., 2006; Calzarano and Di Marco, 2007) and *Phomopsis* species, particularly *Phomopsis viticola* (Sacc.) Sacc. (van Niekerk *et al*., 2005), are all involved in their own symptomology. However, the role of these fungi in wood degradation, as well as the interactions with the vascular and decay fungi in the esca complex, still needs to be determined (Péros *et al*., 2008).

In recent years the incidence of "esca" (here used as a general term including occurrence of internal and/or external symptoms) has increased markedly in Europe. There are great economic losses associated with replacing esca-infected vineyards (Rumbos and Rumbou, 2001). In Italy the disease is found in all the grape growing regions and in some areas affects up to 100% of vineyards over 15 years old. Throughout Europe, average incidence varies from 1 to 50% or more, and this increases in vineyards older than 10 years (Mugnai *et al*., 1999; Reisenzein *et al*., 2000; Surico, 2001; Péros *et al*., 2008; Romanazzi *et al*., 2009). However, younger vines (4 to 6 years old) are also found with typical tiger stripe leaf symptoms (Serra *et al*., 2000; Edwards *et al*., 2001).

Foliar symptoms include the typical 'tiger stripe' symptom which can appear red in some cultivars (Viala, 1926; Mugnai *et al*., 1999; Bruno and Sparapano, 2006). These tiger stripes appear as light green/chlorotic rounded/irregular spots which occur between the veins and margins of the leaves (Mugnai *et al*., 1999; Sparapano *et al*., 2001; Bruno and Sparapano, 2006). The tiger stripes eventually coalesce and become necrotic, leaving green areas along the leaf veins of unaffected areas (Surico *et al*., 2006). Foliar symptoms become visible in summer and autumn (Mugnai *et al*., 1999). It is very difficult to correlate foliar with internal symptoms, as foliar symptom expression is influenced by various metabolites produced by the different fungi involved (Serra *et al*., 2000; Calzarano and Di Marco, 2007) and are also influenced by various environmental factors (Petit *et al*., 2006). Additionally, blocked vessels within the affected plants could change physiological processes and contribute to foliar symptoms (Péros *et al*., 2008).

Berry symptoms consist of diminutive spots which are dark brown, violet or purple and are known as 'black measles'. These symptoms are common in California (Chiarappa, 1959; Gubler *et al*., 2004) and southern Italy (Mugnai *et al*., 1999; Bruno *et al*., 2007) and have also been reported in France (Mugnai *et al*., 1999) and Austria (Reisenzein *et al*., 2000). Infected berries not showing spots tend to have a violet colour and show reduced turgor, causing them to shrivel (Reisenzein *et al*., 2000). These symptoms may, or may not, be associated with foliar symptoms (Mugnai *et al*., 1999).

Apoplexy, the sudden wilt and collapse of vines, is the symptom originally described as the acute syndrome of esca (Viala, 1926). This was reported to usually occur during hot summers, especially when rainfall is followed by hot, dry weather (Galet, 1977), and more precisely shown to occur under conditions of water stress and high temperatures (Surico *et al*., 2000a; Bruno *et al*., 2007).

The internal stem and trunk symptoms associated with the esca complex in Europe have been described by various authors as combinations of different symptom types. Up to seven different symptom types have been described (Serra *et al*., 2000). Although different symptom categories have been developed, commonly found internal symptoms include brown or black wood streaking, brown necrosis that can be sectorial, pinkish-brown or brown-red central necrosis, white rot which can be bordered by black lines and brown margins of decayed wood (Larignon and Dubos, 1997; Mugnai *et al*., 1999; Pollastro *et al*., 2000; Serra *et al*., 2000; Sofia *et al*., 2006; Calzarano and Di Marco, 2007; Péros *et al*., 2008).

Esca has been studied in various grapevine growing countries including Australia, France, Germany, Greece, Italy, Portugal, Spain and the United States of America (Chiarappa, 1959; Larignon and Dubos, 1997; Mugnai *et al*., 1999; Pascoe and Cottral, 2000; Armengol *et al*., 2001;

Rumbos and Rumbou, 2001; Fischer and Kassemeyer, 2003; Gubler *et al*., 2004; Sofia *et al*., 2006). There is little information available on the occurrence of esca in South Africa. Few incidences were reported prior to 1981 in the Rawsonville and Slanghoek areas of the Western Cape Province and it was thought to be of no economic concern. *Stereum hirsutum* and *Phellinus igniarius* (L.) Quél. were believed to be the causal organisms, but no study confirmed this (Marais, 1981). Du Plessis (1947) acknowledged the fact that "esca" and apoplexy were observed in local vineyards, but mentioned that typical esca leaf symptoms were not common. At the time there was also confusion in distinguising Eutypa dieback from esca, since some of the symptoms overlap. Perold (1926) regarded apoplexy as a physiological disorder which was the result of high plant transpiration and lack of sufficient water uptake from the roots. Apoplexy was frequently observed, but never in large numbers.

The aims of the present study were to determine the presence and extent of esca diseased vines in the different grapevine production areas of South Africa, to describe the external and internal symptoms associated with diseased vines and to identify the fungi isolated from specific symptom types found in these diseased vines, with specific reference to the basidiomycete fungi.

Materials and methods

Sampling of esca diseased vines

Vineyards showing foliar symptoms of esca, esca proper or general decline were identified in all the major wine, raisin and table grape production areas of South Africa between 2001 and 2008. The presence of external symptoms was not always recorded, since several vines were collected during winter when farmers traditionally remove old or unproductive vines. Vines showing typical tiger stripe foliar symptoms and also declining vines were cut open and those showing the whole range of internal symptoms (white rot and different types of discolourations) were removed and immediately taken to the laboratory where fungal isolations were made.

Fungal isolations from diseased vines

Cross and longitudinal sections were made at various places in the cordons and trunk of each plant to investigate internal necrosis. For fungal isolations, wood sections with internal necrosis were selected and cut into two smaller sections adjacent to each other, in order to obtain two mirror images of the same symptom type. This was also done to facilitate the use of two sterilisation techniques to ensure fungal isolation from soft, spongy material. A photograph of each wood section showing the various symptom types was taken. One section was flame sterilized by holding the wood with sterile forceps, lightly spraying it with 70% ethanol and passing it through a flame. The other piece was triple sterilized as follows: 30 s in 70% ethanol, 2 min in 3.5% NaOCl and 30 s in 70% ethanol. Twelve small sections of wood (1×1×2 mm) from each of the different symptom types were then aseptically removed with a scalpel and placed onto Potato Dextrose Agar (PDA, Biolab, South Africa) plates containing 250 mg chloramphenicol (four pieces per plate). Plates were incubated at 24(±1) ºC for approximately 4 weeks. Fungal growth from tissue pieces was monitored daily.

Selection and storage of cultures

Fungi isolated from the various symptom types and suspected of being involved in the complex of fungi associated with esca were recorded, identified (where possible) and subcultured (hyphal tip or single spore) for later identification. Isolates were also selected to represent the various geographical regions. Pure cultures were stored in sterile distilled water in 14 ml McCartney bottles kept at 4°C. Representative isolates are stored in a fungal culture collection at ARC Infruitec-Nietvoorbij, Stellenbosch and the STE-U culture collection of the Department of Plant Pathology, Stellenbosch University, South Africa.

Identification of fungi

Fungal isolates obtained from the various symptom types were identified to genus level with phenotypic characteristics, including cultural growth and micromorphology. For a selected number of isolates, species identification was completed with PCR and sequence comparisons (White *et al*., 2011).

Internal wood symptoms

Photographs of the wood section were used to identify and compare the different internal symptom types. Fungi that were isolated were linked to the specific symptom type and recorded on the different photographs. From this, the total number of fungi isolated from each symptom type from each section of wood was calculated.

Results

Distribution of esca

Diseased vines representative of the search criteria were found in 31, five and one production areas of the Western Cape, Northern Cape and Limpopo Provinces, respectively (Table 1). In total, 212 vines were collected from vineyards covering an area of 207 ha. Of these vines, fungi were isolated from 181 vines, representing 18 cultivars.

The majority of diseased vines were found in older vineyards. A total of 3% of the vines sampled were between 3 and 10 years of age, 39% were between 11 and 20 years, 56% were older than 21

Table 1. The geographic distribution of esca diseased vines in South Africa, the cultivars sampled and their age.

Province	District [a]	Town	Affected cultivars [b]
Western Cape	Botriver	Botrivier	Chenin blanc (20, 23)
	Calitzdorp	Calitzdorp, De Rust, Ladismith, Montagu, Oudtshoorn, Prins Albert	Chenin blanc (28, 38), Colombar (27), Fransdruif (33), Hanepoot (37), Merlot (3), Pinotage (29), Red Muscadel (31)
	Cape Point	Constantia	Sauvignon blanc (18, 25)
	Darling	Darling	Chenin blanc (21, 23)
	Lutzville Valley	Klawer, Lutzville	Chenin blanc (41), Colombar (20), Fransdruif (35)
	Overberg	Grabouw	Chardonnay (15), Sauvignon blanc (15)
	Paarl	Franschoek, Paarl, Wellington	Cabernet sauvignon (13, 14), Chenin blanc (18, 20, 25, 40) Hanepoot (22)
	Robertson	Ashton, Bonnievale, Klaas Voogds	Red Globe (10)[c], Sauvignon blanc (20), Shiraz (30)
	Stellenbosch	Somerset West, Stellenbosch	Cabernet sauvignon (15, 19, 31, 32), Chenin blanc (26), Hanepoot (12), Malbec (12), Pinotage (28), Ruby cabernet (22), Tinta barocca (28), Sauvignon blanc (16, 23, 25)
	Swartland	Piketberg, Porterville, Malmesbury, Riebeek Kasteel, Riebeeck Wes	Dan Ben Hannah (19)[c], Chenin blanc (19, 20, 36), Colombar (15), Pinotage (36)
	Tulbagh	Tulbagh	Chenin blanc (24, 28)
	Tygerberg	Durbanville	Chenin blanc (26, 34), Sauvignon blanc (23), Shiraz (21)
	Walker Bay	Hermanus	Chardonnay (21)
	Worcester	De Doorns, Rawsonville, Slanghoek	Chenin blanc (11, 20), Hanepoot (40), Sultana (18)[c], Red Globe (9)
Northern Cape		Keboes, Keimoes, Kanon Eiland, Marchand, Prieska	Chenin blanc (18), Colomino (18), Sultana (26, 40)[c,d]
Limpopo		Marken	Prime seedless (5)[c]

[a] Districts as described by South African Wine Industry Information and Systems (SAWIS) (Anonymous, 2010).
[b] Cultivars affected by esca with the age of the sampled vines indicated in brackets.
[c] Table grape cultivars.
[d] Raisin cultivars; those which are not indicated are wine grapes.

years and the age of the remaining 3% of vines sampled was unknown.

Esca foliar symptoms observed in the field

Although extensive surveys of the same number of vineyards of the same age in each of the regions could not be conducted, symptoms typical to those described for esca were observed in several vineyards. External symptoms included foliar and berry symptoms, as well as apoplexy.

The first symptom observed in the beginning of each growing season was typical dieback, consisting of vine arms not producing any green shoots. Apoplexy, i.e. the sudden die-back and wilt in the hot summer months, was observed occasionally in vineyards 13 years of age and older.

Foliar symptoms, when present, appeared mainly in a short window period between the end of January and the end of March each year. Foliar symptoms (Figure 1) observed in some vineyards included; 1) typical tiger stripe symptoms, as found in European vineyards, 2) general scorching on the leaves where eventual necrosis occurred, 3) and general vine decline symptoms which included smaller leaves, shoots with shortened internodes and stunted shoots. The tiger stripe symptoms included scorching on the edges of the leaf laminae and chlorosis along the leaf veins, with red grape cultivars showing reddish scorching along the edges of the leaves. White grape cultivars did not have this red tinge and leaf scorching appeared whitish in colour.

Examples of berry symptoms were also observed. Symptoms on red berries appeared as a pale discoloration and some shriveled berries. Symptoms on white berries appeared as black spots on the berries. This was only observed on one occasion on berries of a Hanepoot vine located in Stellenbosch.

Internal wood symptoms

Five predominant symptom types were identified, and these included white rot, black and brown wood streaking, brown necrosis within the white rot, sectorial brown necrosis, and a brown/red margin next to decay often including black lines at the borders of decayed wood (Figure 2). The white rot included areas which appeared white, yellow or yellow-orange, and ranged from spongy to hard in texture. Black and brown wood streaking took the

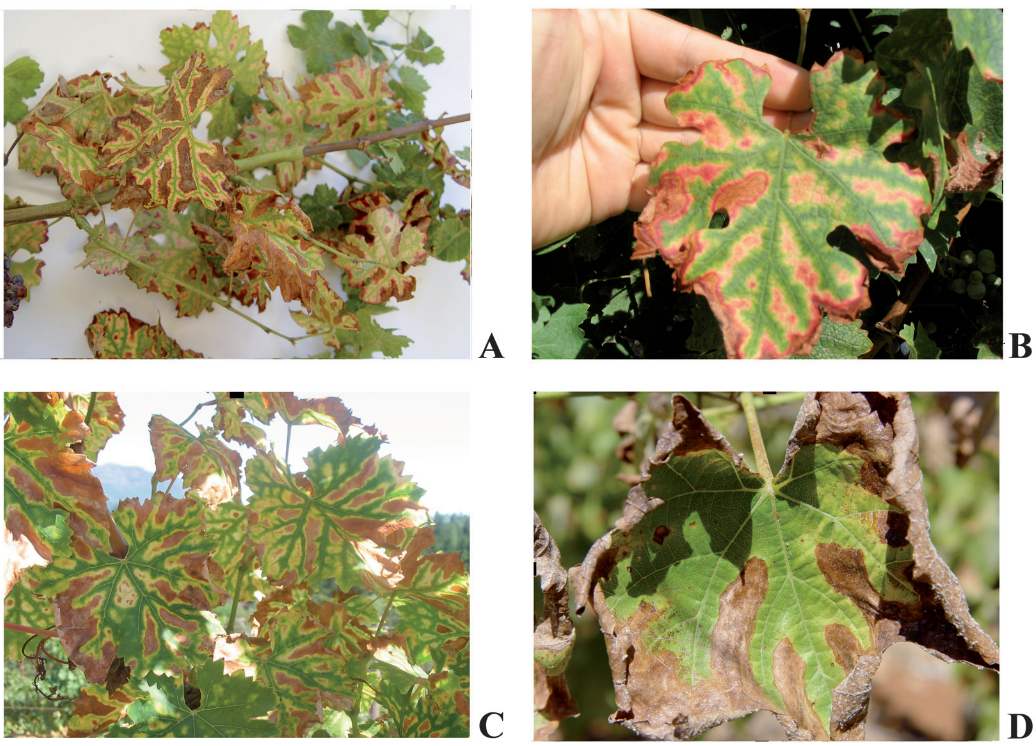


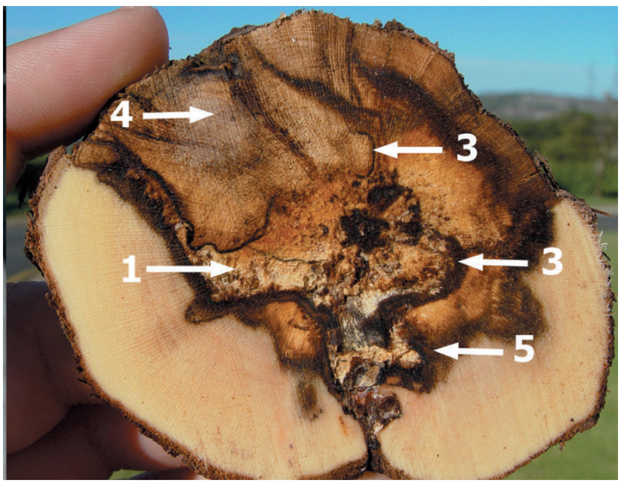
Figure 1. Tiger stripe foliar symptoms as observed in South Africa on red grapevine cultivars (A and B), a white grape cultivar (C) and leaf scorching (D).

form of brown to black dots in transverse sections that were either separate or clustered. These were assumed to be the same wood symptoms as recorded in Petri disease, the decline form described in new vineyards. The brown necrosis within the white rot was identified as brown to dark brown necrotic wood of a hard consistency where the necrotic areas were surrounded by white rot on all sides. The sectorial brown necrosis started from below the bark and then progressed inwards sometimes taking on a V-shape. This sectorial necrosis appeared brown to dark brown. The brown, red or black margins next to decay appeared darker in colour than neighbouring symptoms (especially when surrounding the sectorial necrosis).

Fungi associated with specific symptom types

A trunk or cordon section representative of all wood symptoms detected in each sampled vine was selected. A total of 24,715 isolations were made and 13,669 fungal isolates were obtained from the five symptom types associated with esca diseased vines in South Africa (Table 2). Basidiomycetes (4,695 isolates), *Pa. chlamydospora* (4,595) and *Pm. aleophilum* (2,386) were by far the most frequently isolated pathogens associated with these symptoms and represent 34.3, 33.6 and 17.5% of the total isolates obtained, respectively. Ten unknown basidiomycete species were identified (White *et al.*, 2011), four within the *Inocutis* group, four within *Inonotus*, one *Phellinus* and one new *Fomitiporia* sp. closely related to *F. mediterranea*. Other pathogens included Botryosphaeriaceae, other *Phaeoacremonium* spp., *Eutypa lata*, *Phomopsis viticola*, other *Phomopsis* spp. as well as *Pleurostomophora richardsiae* (Nannf.) L. Mostert, W. Gams & Crous. Most of the isolates were retrieved from the central brown/red margins of stem decay with or without black lines (5,257 isolates), white rot (4,554) and sectorial brown necrosis (2,405), followed by black and brown wood streaking (914) and brown necrosis within white rot (539). The number of isolates obtained for each taxon per symptom type is presented in Table 2.

The basidiomycetes, as expected, were the primary cause of the white rot (30.4%; Table 2). *Phaeoacremonium aleophilum*, *Pa. chlamydospora* and *Phaeoacremonium* spp. were also found in the white rot, but at much lower incidences (7.4%, 4.8% and 2.1%, respectively). It appeared that the first stage of the white rot was often orange in colour



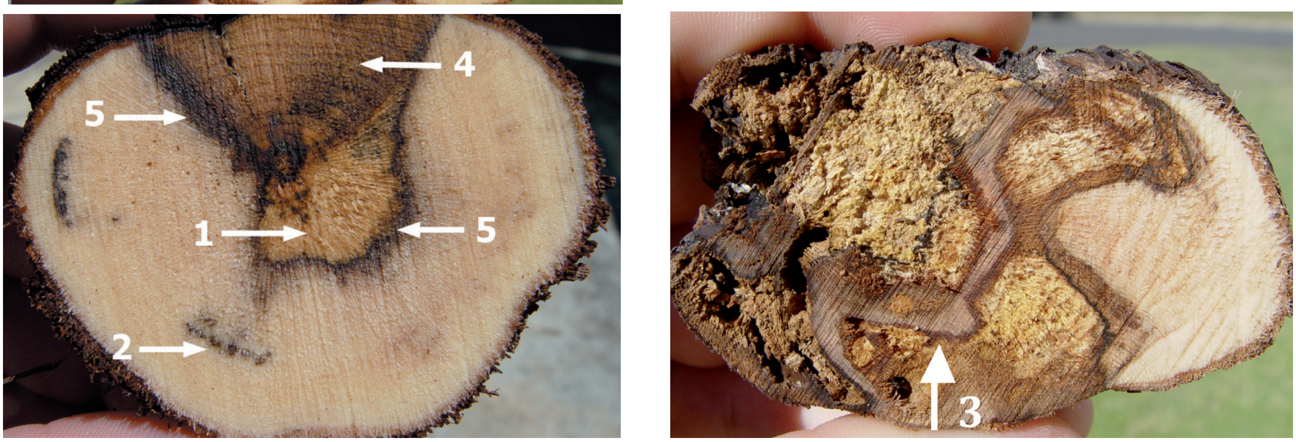


Figure 2. Cross sections showing the internal symptoms and symptom types found in diseased grapevines: 1. White rot, usually central. 2. Black and brown wood streaking. 3. Brown necrosis within white rot. 4. Sectorial brown necrosis. 5. Brown-red margin next to decay and black line surrounding the decayed wood.

Table 2. The total number of isolations and fungi isolated from each of the five symptom types associated with esca diseased vines collected in South Africa.

Symptom type	Isolated fungi [a]									Total No. of isolates[b]	Total No. of isolations
	Basidiomycetes species	*Phaeoacremonium aleophilum*	*Phaeoacremonium* spp.	*Phaeomoniella chlamydospora*	*Phomopsis viticola*	*Phomopsis* spp.	Botryosphaeriaceae	*Eutypa lata*	*Pleurostomophora richardsiae*		
White rot	30.4	7.4	2.1	4.8	0.1	0	0.4	0.4	0.1	4554 (33.3)	9944
Brown, red margin and black line next to decay	15.1	13.6	2.5	29.1	1.5	0	2.1	1.1	0.01	5257 (38.5)	8072
Brown necrosis within white rot	20.4	15.9	1.4	13.6	0	0	1.5	0.4	1.1	539 (3.9)	992
Sectorial brown necrosis	5.5	9.4	1.2	20.8	6.3	0.1	10.7	7.5	0.03	2405 (17.6)	3904
Black and brown wood streaking	1.8	1.4	1.1	45.4	0	0	1.1	0	0	914 (6.7)	1803
No. of isolates[b]	4695 (19)	2386 (9.7)	491 (2)	4595 (18.6)	380 (1.5)	3 (0.1)	662 (2.7)	430 (1.7)	27 (0.1)	13669	24715

[a] The numbers represent the percentage of isolates of a specific fungus from the total number of isolations that was made from that symptom type.
[b] The number in brackets represents the percentage of isolates retrieved from that symptom type.

and hard in texture, which then progressed to become whiter and semi-spongy to spongy and soft. The development of symptom types could be seen in the differences between trunks of younger vines compared to older vines and sometimes within the same vine. In younger vines, the areas of the brown discoloured wood were often larger than the area of white rot, indicating that there could be a succession of infection and symptom development in the wood. White rot dominated over the other symptom types as wood degradation advanced.

Black and brown wood streaking was primarily caused by *Pa. chlamydospora* (45.4%). No *Phomopsis* species, *Pl. richardsiae* or *Eutypa lata* were found in this symptom and the other species occurred at a relatively low frequency (1.1% to 1.8 %).

From the brown necrosis within white rot, six taxa were isolated in the following frequencies: basidiomycetes (20.4%), *Pm. aleophilum* (15.9%), *Pa. chlamydospora* (13.6%), Botryosphaeriaceae (1.5%), *Pl. richardsiae* (1.1%) and *E. lata* (0.4%). *Phomopsis* spp. were not isolated from this symptom type.

The most predominant species isolated from the sectorial brown necrosis were *Pa. chlamydospora* (20.8%). Botryosphaeriaceae (10.7%), *Pm. aleophilum* (9.4%), *E. lata* (7.5%), basidiomycetes spp. (5.5%), *Phomopsis viticola* (6.3%). *Phaeoacremonium* spp. (1.2%), *Phomopsis* spp. (0.1%) and *Pleuros-*

tomophora richardsiae (0.026%) comprised the rest of the species isolated from this type of symptom.

Phaeomoniella chlamydospora (29.1%) was the primary fungus that was isolated from the brown/red margins of decay with or without black lines next to decay, which appeared as the remains of brown discoloured necrotic wood observed in younger vines. The basidiomycetes (15.1%), *Pm. aleophilum* (13.6%), *Phaeoacremonium* spp. (2.5%), Botryosphaeriaceae (2.1%), *Phomopsis viticola* (1.5%), *E. lata* (1.1%) and *Pleurostomophora richardsiae* (0.01%) comprised the rest of the species isolated from this symptom.

Discussion

Esca, as a complex of wood diseases that may also show typical foliar symptoms (Surico, 2009), occurs over a wide range of grapevine producing areas in South Africa. Esca was found in 31 production areas in the Western Cape, five production areas in the Northern Cape and in one production area in the Limpopo province on a range of cultivars of wine, table and raisin grapes. This illustrates the prominence of the disease in the country. Esca proper, where wood rot coexists with grapevine leaf stripe disease (Surico, 2009), was only observed in a small number of vineyards.

In the present study, 95% of the esca diseased vines were from vineyards older than 10 years. This correlates with other studies, which have shown that higher incidences of disease were common in vines aged 10 years and older, very often in the more complex form of "esca proper", where decay coexists with the vascular disease (Mugnai *et al.*, 1999; Reisenzein *et al.*, 2000; Surico *et al.*, 2006; Péros *et al.*, 2008; Romanazzi *et al.*, 2009).

External symptoms observed in a few South African vineyards were very similar to those found in Europe and other countries, including foliar, berry and apoplexy symptoms traditionally associated with the esca complex. Symptoms associated with "hoja de malvón", where the leaves are smaller than normal, chlorotic and with the edges rolled downwards (Gatica *et al.*, 2000) were not observed. The recent observations made in South African vineyards were also consistent with external symptoms reported by Marais (1981) resembling tiger-stripes, apoplexy, dieback and decline of the vines. Apoplexy was rarely found in the present study, while general decline symptoms were much more prevalent. Measle-like berry symptoms were only found on one occasion. This observation differs with results from Europe, where tiger stripe symptoms are common (Mugnai *et al.*, 1999), but is similar to the situation in Australian vineyards, where foliar symptoms are also not common (Pascoe and Cottral, 2000). The typical tiger stripe symptoms, which as stated above were rarely found, were observed mainly in the same period as in the Northern Hemisphere, during full summer. These symptoms can sometimes be missed because of natural deterioration of the leaves due to unfavourable weather conditions like strong winds and high temperatures which are common during that particular time of the year. However, given the nature of this study where all the vineyards were not visited during the same time period, conclusions regarding the occurrence of various symptoms cannot be made.

The combination of fungal species in affected grapevine wood, their contribution to wood degradation, their interactions amongst each other and the toxins produced by the different species are all thought to play roles in symptom expression. This interaction, however, is not fully understood (Mugnai *et al.*, 1999; Stefanini *et al.*, 2000; Surico *et al.*, 2000a, 2000b; Surico *et al.*, 2006; Calzarano and Di Marco, 2007). External symptoms can also be influenced by vine age, farming practices, soil type, the slope of the land and the cultivar type (Surico *et al.*, 2000a), as well as environmental and seasonal factors (Mugnai *et al.*, 1999). Expression of external symptoms have been found to be erratic or inconsistent since vines showing symptoms in one season might not show them the following season (Surico *et al.*, 2000b; Redondo *et al.*, 2001; Sofia *et al.*, 2006).

Species of the basidiomycetes, Botryosphaeriaceae, *E. lata*, *Pm. aleophilum,* other *Phaeoacremonium* spp., *Pa. chlamydospora*, *Phomopsis viticola*, other *Phomopsis* spp. as well as *Pl. richardsiae* were found in this study. Five symptom types were found including; white rot, black and brown wood streaking, brown necrosis within the white rot, sectorial brown necrosis, and brown/red margins with or without black lines next to decay. Internal symptoms observed, and the fungi isolated from each type, were similar to those observed in Europe and Argentina (Larignon and Dubos, 1997; Mugnai *et al.*, 1999; Gatica *et al.*, 2000; Pollastro *et al.*, 2000; Sofia *et al.*, 2006; Calzarano and Di Marco, 2007; Péros *et al*., 2008; Sánchez-Torres *et al*., 2008). Marais (1981) only reported internal symptoms of yellow rot surrounded by a black zone.

From the white rot symptom, basidiomycete species were, as expected, the most predominant species with low numbers of *Pm. aleophilum* and *Pa. chlamydospora* isolated in this study. Many other studies have shown that *F. mediterranea* was the main casual agent in the white rot of grapevine in Europe (Larignon and Dubos, 1997; Mugnai *et al*., 1999; Pollastro *et al*., 2000; Sofia *et al*., 2006; Calzarano and Di Marco, 2007; Péros *et al*., 2008; Sánchez-Torres *et al*., 2008), while other basidyomycetes species are found in different grape growing areas (Fischer, 2006). Another basidiomycete species, *Inocutis jamaicensis*, was the most dominant species found in the soft decay associated with "hoja de malvón"-affected vines (Gatica *et al*., 2000; Lupo *et al*., 2006).

The role of *Stereum hirsutum* in esca is still unclear (Reisenzein *et al*., 2000). This fungus was isolated from only 5% of the esca-affected vines investigated in France (Larignon and Dubos, 1997). The pathogen was believed to be the casual agent in South African esca diseased vines (Marais, 1981), probably following suggestions from early French literature reports. *Stereum hirsutum* was not isolated in the present study and therefore cannot be considered as a main esca-associated pathogen in South Africa.

The black and brown wood streaking observed was primarily caused by *Pa. chlamydospora*. This is consistent with other studies (Larignon and Dubos, 1997; Mugnai *et al*., 1999; Pollastro *et al*., 2000; Sofia *et al*., 2006; Calzarano and Di Marco, 2007; Péros *et al*., 2008; Sánchez-Torres *et al*., 2008). In the present study, basidiomycetes, *Pm. aleophilum* and species of Botryosphaeriaceae were also isolated from black and brown wood streaking, but below 2% for each species found. Gatica *et al*. (2000) mostly found Botryosphaeriaceae, followed by *Pa. chlamydospora* and *Phaeoacremonium* spp.

From the brown necrosis within the white rot, basidiomycetes, *Pm. aleophilum* and *Pa. chlamydospora* were isolated. The greatest number of fungal isolates from this symptom were the basidiomycetes (20%). Serra *et al*. (2000) isolated mostly sterile mycelium and *Fomitiporia* sp. from this symptom type. Even though basidiomycetes are more commonly associated with white rot, pathogenicity studies showed that basidiomycetes can also cause brown discolouration of grapevine wood during the first stages of colonisation (Sparapano *et al*., 2001).

Phaeomoniella chlamydospora was the primary fungus isolated from the brown/red margins next to wood decay. Other studies have also found *Phaeoacremonium* spp., *Pa. chlamydospora* and the Botryosphaeriaceae in the black lines and within the brown discoloured wood (Larignon and Dubos, 1997; Mugnai *et al*., 1999; Pollastro *et al*., 2000; Serra *et al*., 2000; Sofia *et al*., 2006; Calzarano and Di Marco, 2007; Péros *et al*., 2008; Sánchez-Torres *et al*., 2008).

Phaeomoniella chlamydospora and species of Botryosphaeriaceae were the most numerous fungi isolated from the sectorial brown necrosis symptoms. This symptom type is likely to be the first to develop. Gatica *et al*. (2000) also found *Pa. chlamydospora*/*Phaeoacremonium* spp. and Botryosphaeriaceae, as well as *I. jamaicensis* from the corresponding zones in "hoja de malvón"-affected vines. Serra *et al*. (2000) isolated sterile mycelium, *Sphaeropsis* sp. (Botryosphaeriaceae), *Phomopsis* sp. and *E. lata* from the brown necrosis. In the present study, *E. lata* was rarely isolated (only 1.7 % of the total isolates) and predominantly from the sectorial brown necrosis. *Eutypa lata* was reported as a pioneer fungus pre-disposing the wood for colonization by the decay agent *F. mediterranea* (Larignon and Dubos, 1997). However, *E. lata* is likely to be the agent causing Eutypa dieback, a disease which has its own symptomology (Mugnai *et al*., 1999). It can simply be coincidental that this fungus is present in vines infected by esca pathogens. This fungus may simply be coincidental in vines infected by esca pathogens.

Members of the Botryosphaeriaceae are known to be associated with esca diseased vines (Mugnai *et al*., 1999), but their role is unclear (Calzarano and Di Marco, 2007). In general, these fungi can be isolated from most of the symptom types associated with the esca complex (Larignon and Dubos, 1997; Mugnai *et al*., 1999), although they are more frequently associated with sectorial necrosis (Van Niekerk *et al*., 2006). As in the case of *E. lata*, they may be coincidentally present in affected vines, even if, when present, they could have an influence on foliar symptoms.

Phomopsis species, particularly *Phomopsis viticola*, have also been associated with grapevine trunk diseases (van Niekerk *et al*., 2005). However, their role needs to be determined in esca diseased vines (Péros *et al*., 2008). The majority of the *Phomopsis* isolates were isolated from sectorial brown necrosis, but they appeared to play a minor role due to low frequencies found in comparison with the other fungi. The *Phomopsis* spp. were only found in the sectorial brown necrosis. The *Phomopsis* spp. and *Phomopsis viticola* were not isolated in the black and brown wood streaking or in the brown wood streaking within white rot.

Pleurostomophora richardsiae was rarely found in the present study. This is a lesser known grapevine trunk disease pathogen that has been associated with vascular streaking (Halleen *et al*., 2007), although its involvement in esca is unknown. The fungus has frequently been isolated from young diseased vines, specifically from around the pith areas in graft unions (Halleen and Groenewald, 2005).

Esca is more prominent and widespread in South Africa than previously recognized, and occurs in the three provinces where grapevine production dominates. External symptoms associated with the esca complex were observed in the field, and five internal symptom types in grapevine wood were identified. The external symptoms and the predominant fungi isolated were similar to those observed in Europe and the USA. The isolated fungi included basidiomycete species, *Pa. chlamydospora* and *Phaeoacremonium* spp. (mostly *Pm. aleophilum*), confirming these taxa as the main causal organisms of esca diseased vines. Additionally, *Phomopsis* species, Botryosphaeriaceae and *E. lata* were also found, but to a lesser extent. It must be emphasized that this study was carried out with the aim of completing a wide survey of fungal pathogens, selecting vines showing the full range of internal symptoms (white rot and different types of discolouration), and therefore did not record the presence of symptomatic vines showing only the vascular disease. This aspect will be investigated in future studies.

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