Phytopathol. Mediterr. (2001) 40, Supplement, S303–S310

Young esca in Australia

JACQUELINE EDWARDS[1,2], GUIDO MARCHI[3] and IAN G. PASCOE[1,2]

[1]Cooperative Research Centre for Viticulture, P.O. Box 154, Glen Osmond, South Australia, Australia 5064
[2]Agriculture Victoria - Knoxfield, Private Bag 15, Scoresby Business Centre, Victoria, Australia 3176
[3]Dipartimento di Biotecnologie Agrarie-Sezione di Patologia vegetale, P.le delle Cascine 28, 50144, Firenze, Italy

Summary. Young esca was monitored on 3 to 7-year-old grapevines in two vineyards in the Riverland region of Australia over two seasons (1999–'00 and 2000–'01). The affected cultivars were own-rooted Shiraz, own-rooted Cabernet Sauvignon and Merlot grafted onto Kober 5BB. Some of the Cabernet Sauvignon vines began to show symptoms as young as two-years-old. *Phaeomoniella chlamydospora* was the only esca-associated fungus consistently isolated from symptomatic vines, suggesting that *P. chlamydospora* alone is responsible for esca symptoms. Spatial analysis was unable to reveal any consistent pattern of symptom distribution.

Key words: *Phaeomoniella, Phaeoacremonium*, grapevine disease.

Introduction

Esca is a grapevine trunk disease generally associated with mature vines more than ten years old. External symptoms present as interveinal chlorosis and necrosis of the leaves, shrivelling of berries and occasional rapid collapse of the whole vine (apoplexy). When the trunk is cut open, the internal symptom is a soft white heart rot bordered by a black line. The longevity of vines is severely reduced. In Europe, the incidence of esca has dramatically increased over the past 15 years, particularly in southern Europe (Graniti *et al.*, 2000). In Australia, however, the disease is rare and its presence has only recently been confirmed (Pascoe, 1999).

Corresponding author: J. Edwards
Fax: +61 3 98003521
E-mail: Jacky.edwards@nre.vic.gov.au

Although esca usually affects mature vines, sometimes vines less than ten years old express classic external symptoms but no white rot in the trunk and this syndrome has been called young esca (Mugnai *et al.,* 1999). Chicau *et al*. (2000) reported esca-like foliar symptoms associated with black wood-streaking in 3 to 11-year-old *Vinho Verde* grapevines grown in northwest Portugal. In Italy, Serra *et al*. (2000) examined thirty one 5 to 6-year-old grapevines, eight of which had classic esca foliar symptoms and three of which had suffered apoplexy. However, they were unable to decide what pathogen was responsible for the symptoms.

The aetiology of esca has been the focus of research for the past century but remains unresolved as Koch's postulates have never been fully satisfied (Chiarappa, 2000). The external symptoms are erratic and may not show every year, and the factors controlling symptom expression are unknown. Although the internal wood symptoms have been

successfully reproduced, the foliar and fruit symptoms have not. Fungi which have been isolated from esca-affected vines and implicated as causal organisms include *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingf. & L. Mugnai) Crous & W. Gams, *Fomitiporia punctata* (P. Karst.) Murrill, *Stereum hirsutum* (Willd.) S.F. Gray, *Phaeoacremonium aleophilum* W. Gams, Crous, M.J. Wingf. & L. Mugnai and *Eutypa lata* (Pers. Fr.) Tul. (Larignon and Dubos, 1997). This has led to debate as to which organism or organisms are necessary for the expression of esca (Mugnai *et al.,* 1999; Graniti *et al.,* 2000).

In Australia, young esca first came to our notice in 1999 when it affected 3 to 7-year-old vines growing in the Riverland-Sunraysia region of Australia. This paper reports on a study to determine the cause of young esca affecting three grapevine cultivars in two Australian vineyards.

Materials and methods

Sampling sites

Two vineyards from the Riverland-Sunraysia grape-growing region of Australia, site 1 at Waikerie in South Australia and site 2 at Wentworth in New South Wales, reported esca foliar symptoms on their young vines, which was often associated with shrivelling of the fruit. In this region, the growing season is from September to early March, with periods of very high temperatures (>40°C) over summer (December to February). Vines are grown under irrigation on sandy, alkaline, sodic soils and are vigorous and high-yielding. At both sites, esca foliar symptoms were observed during three seasons, 1998–'99, 1999–'00 and 2000–'01, first becoming noticeable in late spring (November).

At site 1, the affected vines were own-rooted Shiraz planted in 1994. They had been grown under a restricted-deficit-irrigation (RDI) schedule until February 1998 when severe wilting and fruit shrivelling occurred. RDI was abandoned and irrigation increased, but esca symptoms were noticed the following season (1998–'99).

At site 2, esca symptoms were also noticed during 1998–'99, on own-rooted Cabernet Sauvignon planted in 1997. The following season, Merlot grafted onto Kober 5BB, also planted in 1997, showed symptoms.

Spatial analysis

Symptomatic vines at each site were tagged during 1999–'00 and 2000–'01 in order to follow disease progress. In 2001, the tagged vines were mapped and spatial analysis (ordinary runs analysis), as described by Surico *et al.* (2000), was performed on the data to determine whether the symptomatic vines were randomly spread throughout the agronomic rows or clustered together.

Preliminary examination of vines

During 1999–'00, several vines were removed from each site for examination. At site 1, three symptomatic Shiraz vines were uprooted during autumn (March 2000), their trunks cut into pieces (both transversely and longitudinally) and isolations made from any areas with wood discolouration, which was mainly black streaking. For this purpose, small slivers (5×50 mm) of internal wood were surface sterilised in 0.5% NaOCl and plated onto potato dextrose agar amended with 50 ppm Achromycin® (a.i. tetracycline hydrochloride; American Cyanamid Company, USA). After incubation at room temperature for 2 to 3 weeks, the plates were examined for fungal growth.

At site 2, a symptomatic Cabernet Sauvignon and a symptomatic Merlot on Kober 5BB vine were removed in autumn (March 2000) for examination as described above, and in the following spring (November 2000) five more symptomatic Merlot on Kober 5BB vines were removed and examined.

Systematic examination of vines

When symptoms were observed at both sites for a third season (2000–'01), a more systematic approach to vine examination was taken. In late summer (February and March 2001), from site 1 we chose one asymptomatic vine, one vine which showed symptoms for the first time in 2000–'01 and one vine which had been symptomatic during both 1999–'00 and 2000–'01. From site 2, seven Merlot on Kober 5BB vines were selected: two asymptomatic vines, one that had expressed symptoms only during 1999–'00, three showing symptoms for the first time in 2000–'01 and one which had been symptomatic during 1999–'00 and then died in 2000–'01. Four Cabernet Sauvignon vines were also selected: one asymptomatic vine, one which expressed symptoms only during 1999–'00, one with symptoms for the first time in 2000–'01

and one which had been symptomatic both seasons.

The harvested trunks were cut into 10 cm segments and then surface flame-sterilised. Internal symptoms were described. Small pieces of internal wood were taken from areas with black and/or brown streaking and plated onto potato dextrose agar amended with lactic acid (2 drops lactic acid / 100 ml medium). All remaining trunk pieces were moist incubated and examined at ×40 for the presence of esca-associated fungi after 4 to 8 weeks.

Results

Spatial analysis

One dimensional spatial analysis of the symptomatic vines showed that they were randomly distributed at site 1 (Figure 1) but clustered at site 2 (Figure 2) along the agronomic rows.

Preliminary examination of vines

Internal examination of the trunks showed that all had some black wood-streaking, particularly near the base, which in combination with the foliar symptoms is consistent with young esca. Subsequent isolation and moist incubation confirmed that all of the sampled vines were infected with *P. chlamydospora*. Other fungi isolated were species of ubiquitous genera such as *Penicillium, Alternaria, Cladosporium* and *Gliocladium*.

Systematic examination of vines

Site 1: Shiraz

All the own-rooted Shiraz vines had black wood-streaking and *Phaeomoniella chlamydospora* present in the trunk (Table 1). Vine 1, symptomatic during both seasons, had black wood-streaking along the entire length of the trunk and *P. chlamydospora* present in the bottom 20 cm. Vine 2, symptomatic in 2000–'01, had both black wood-streaking and *P. chlamydospora* in the lower third of the trunk and again in the top third associated with a deep crack downwards from the cordons. *P. chlamydospora* was found sporulating abundantly in this crack, which was very similar to the sites of *in situ P. chlamydospora* sporulation found by Edwards *et al*. (2001). Vine 3, asymptomatic, had very little black wood-streaking and some *P. chlamydospora* scattered throughout the trunk. The only other esca-associated fungus found was *Phaeoacremonium aleophilum* in a single portion of vine 2.

Site 2: Merlot grafted onto Kober 5BB

All vines had black wood-streaking and *P. chlamydospora* in the rootstock just below the graft union (Table 2). In the asymptomatic vines (6 and 7) and vine 3 (symptomatic in 2000–'01), black wood-streaking and *P. chlamydospora* were limited to the rootstock, but in the other vines these were also evident in the scion wood. *P. aleophilum* was found in vines 1, 3 and 6.

Site 2: Cabernet Sauvignon

Black wood-streaking and *P. chlamydospora* were present in all the symptomatic vines (vines 1–3) but not in the asymptomatic vine (vine 4) (Table 3). *P. aleophilum* was present in two vines (1 and 4), and an unidentified basidiomycete associated with white heart rot was present in the below-ground portion of vine 2.

Once again, the only other fungi isolated from the trunks were species of ubiquitous genera such as *Penicillium, Alternaria, Cladosporium* and *Gliocladium*.

Discussion

In all cases of young esca examined in the present study, the common factor was the presence of *Phaeomoniella chlamydospora* associated with black wood-streaking in the affected trunks. Other esca-associated fungi were rare. *Phaeoacremonium aleophilum* was observed in six of the 19 vines examined (4 symptomatic and 2 asymptomatic), and an unidentified basidiomycete associated with white heart rot was present in only one vine (symptomatic). *P. chlamydospora* was also isolated from all except one of the young esca vines examined in Italy (Serra *et al*., 2000) and Portugal (Chicau *et al*., 2000). Sparapano was able to reproduce foliar symptoms resembling esca such as interveinal chlorosis and necrosis in 6-year-old vines by injecting liquid cultures of *P. chlamydospora* into current-season shoots (Sparapano *et al*., 2000a). He then demonstrated that similar symptoms could be produced by injecting metabolites extracted from *P. chlamydospora* culture filtrates and from discoloured wood infected with *P. chlamydospora* (Sparapano *et al*., 2000b). In 1999, Mugnai posed the question as to whether esca is (a) a disease complex requiring the interaction of two or more organisms to produce the overall syndrome, (b) a complex of

Table 1. Internal symptoms and esca-associated fungi (*Phaeomoniella chlamydospora, Pch; Phaeoacremonium aleophilum, Pal*) found in the trunks of 7-year-old own-rooted Shiraz vines from site 1, Waikerie, South Australia, Australia.

Trunk portion[a]	Vine 1: esca 1999–00 and 2000–01		Vine 2: esca 2000–01		Vine 3: asymptomatic	
	Symptoms[b]	Fungus	Symptoms	Fungus	Symptoms	Fungus
0	+	*Pch*	+	-	-	-
1	+	*Pch*	+	*Pch*	+	-
2	+	-	+	*Pch+Pal*	-	*Pch*
3	+	-	+	*Pch*	-	-
4	+	-	-	-	-	-
5	+	-	-	*Pch*	-	-
6	+	-	-	-	-	-
7	+	-	-	-	-	*Pch*
8	+	-	-	*Pch*	+	*Pch*
9	+	-	-	-	-	-
10			-	-	-	-
11			-	-	-	-
12			+	*Pch*	-	*Pch*
13			-	*Pch*	-	*Pch*
14			+	*Pch*	-	-
15			-	*Pch*	-	-
16			+[c]	*Pch*		
17			+[c]	*Pch*		
18			+[c]	*Pch*		

[a] Trunks were cut up into portions approx. 10 cm length. Portion 0 was below ground, portions 1–18 were sequential beginning at ground level.
[b] Internal wood symptoms: +, wood discolouration, usually black wood streaking; -, no internal symptom observed.
[c] Symptoms in these portions included a large split down the trunk from the top where the cordons differentiated. *Pch* was found sporulating in abundance inside this split.

two distinct diseases: white heart rot caused by *Fomitiporia punctata* and black wood-streaking caused by *P. chlamydospora,* or (c) a disease induced by *P. chlamydospora* alone (Mugnai *et al*., 1999). The results outlined above suggest that esca is a disease induced by *P. chlamydospora* alone.

However, there are still unanswered questions. *P. chlamydospora* has been identified as the cause of stunted growth and decline of young grapevines, a disease now known as Petri disease (Ferreira *et al*., 1994; Crous *et al*., 1996; Scheck *et al*., 1998; Pascoe and Cottral, 2000). Why, in this case, did the vines succumb to young esca and not Petri disease.

Why are symptoms erratic from year to year? In the present study, 3 of the 4 vines without symptoms had both black wood-streaking and *P. chlamydospora* in the trunks, although to much lesser extent than the symptomatic vines.

What factors control the expression of esca symptoms? In the case of Merlot on Kober 5BB, all of the vines we examined had black wood-streaking and *P. chlamydospora* in the rootstock, but only the symptomatic vines had these in the scion. This suggests that the disease was not being expressed until the infection had spread from the rootstock past the graft union into the scion. Spatial analysis of the distribution of symptomatic vines in both vineyards was inconclusive as site 1 symptoms were randomly distributed, yet site 2 symptoms were clustered. We need to explore whether these patterns can be correlated with soil or microclimatic factors. In 2000–01, the owner of site 2 observed that symptoms appeared two weeks earlier on Merlot than on Cabernet Sauvignon. At this site, Cabernet Sauvignon reaches harvest two weeks later than Merlot, so in fact the symptoms appeared

Table 2. Internal symptoms and esca-associated fungi (*Phaeomoniella chlamydospora, Pch; Phaeoacremonium aleophilum, Pal*) found in the trunks of 4-year-old Merlot vines on Kober 5BB rootstock, from site 2, Wentworth, New South Wales, Australia.

Trunk portion[a]	Vine 1: esca 1999–'00		Vine 2: esca 1999–'00 dead 2000–'01		Vine 3: esca 2000–'01		Vine 4: esca 2000–'01		Vine 5: esca 2000–'01		Vine 6: asymptomatic		Vine 7: asymptomatic	
	Symptoms[b]	Fungus	Symptoms	Fungus	Symptoms	Fungus	Symptoms	Fungus	Symptoms	Fungus	Symptoms	Fungus	Symptoms	Fungus
0	-	-	+	*Pch*	+	*Pal*	+	*Pch*	+	*Pch*	+	*Pch*	-	-
1	-	-	+	-	(G) +	*Pch*+*Pal*	+	*Pch*	+	*Pch*	(G) +	*Pch*	-	-
2	(G)[c] +	*Pch*	(G) +	*Pch*	-	-	(G) +	*Pch*	(G) +	*Pch*	-	-	+	-
3	+	*Pch*			-	-	+	*Pch*	+	*Pch*	-	-	(G) +	*Pch*
4	+	-			-	-	+	-	-	-	-	-	-	-
5	+	-			-	-	+	-	-	-	-	-	-	-
6	+	*Pal*			-	-	+	*Pch*	-	-	-	*Pal*	-	-
7	+	-			-	-	-	-	-	-	-	-	-	-
8	-	-			-	-	-	-	-	-	-	-	-	-
9	-	-			-	-	-	-	-	-	-	-	-	-
10	+	-			-	-	-	-	-	-	-	-	-	-
11	-	-			-	-	-	-	-	-	-	-	-	-
12					-	-			-	-			-	-
13					-	-								

[a] Trunks were cut up into portions approx. 10 cm length. Portion 0 was below ground, portions 1–13 were sequential beginning at ground level.
[b] Internal wood symptoms: +, wood discolouration, usually black wood streaking; -, no internal symptom observed.
[c] (G), graft union.

Table 3. Internal symptoms and esca-associated fungi (*Phaeomoniella chlamydospora, Pch*; *Phaeoacremonium aleophilum, Pal*; white heart rot fungus, basidio) found in the trunks of 4-year-old own-rooted Cabernet Sauvignon vines from site 2, Wentworth, New South Wales, Australia.

Trunk portion[a]	Vine 1: esca 1999–'00		Vine 2: esca 1999–'00 and 2000–'01		Vine 3: esca 2000–'01		Vine 4: asymptomatic	
	Symptoms[b]	Fungus	Symptoms	Fungus	Symptoms	Fungus	Symptoms	Fungus
0	+	*Pch* + *Pal*	+[c]	Basidio	-	-	-	*Pal*
1	+	*Pch*	+	*Pch* + basidio	-	-	-	-
2	+	*Pch*	+	*Pch*	+	-	-	-
3	-	-	+	-	+	-	-	-
4	+	*Pch*	+	-	+	-	-	-
5	-	-	+	-	+	-	-	-
6	-	*Pal*	+	-	+	-	-	-
7	-	-	+	-	+	-	-	-
8	-	*Pal*	+	-	+	*Pch*	-	-
9	-	-	+	-	+	-	-	-
10	-	*Pch*	+	-	+	-	-	-
11	-	-	+	-	+	-	-	-
12				-	-	-	-	-

[a] Trunks were cut up into portions approx. 10 cm length. Portion 0 was below ground, portions 1–12 were sequential beginning at ground level.
[b] Internal wood symptoms: +, wood discolouration, usually black wood streaking; -, no internal symptom observed.
[c] Symptoms in this portion were white heart rot and black wood streaking.

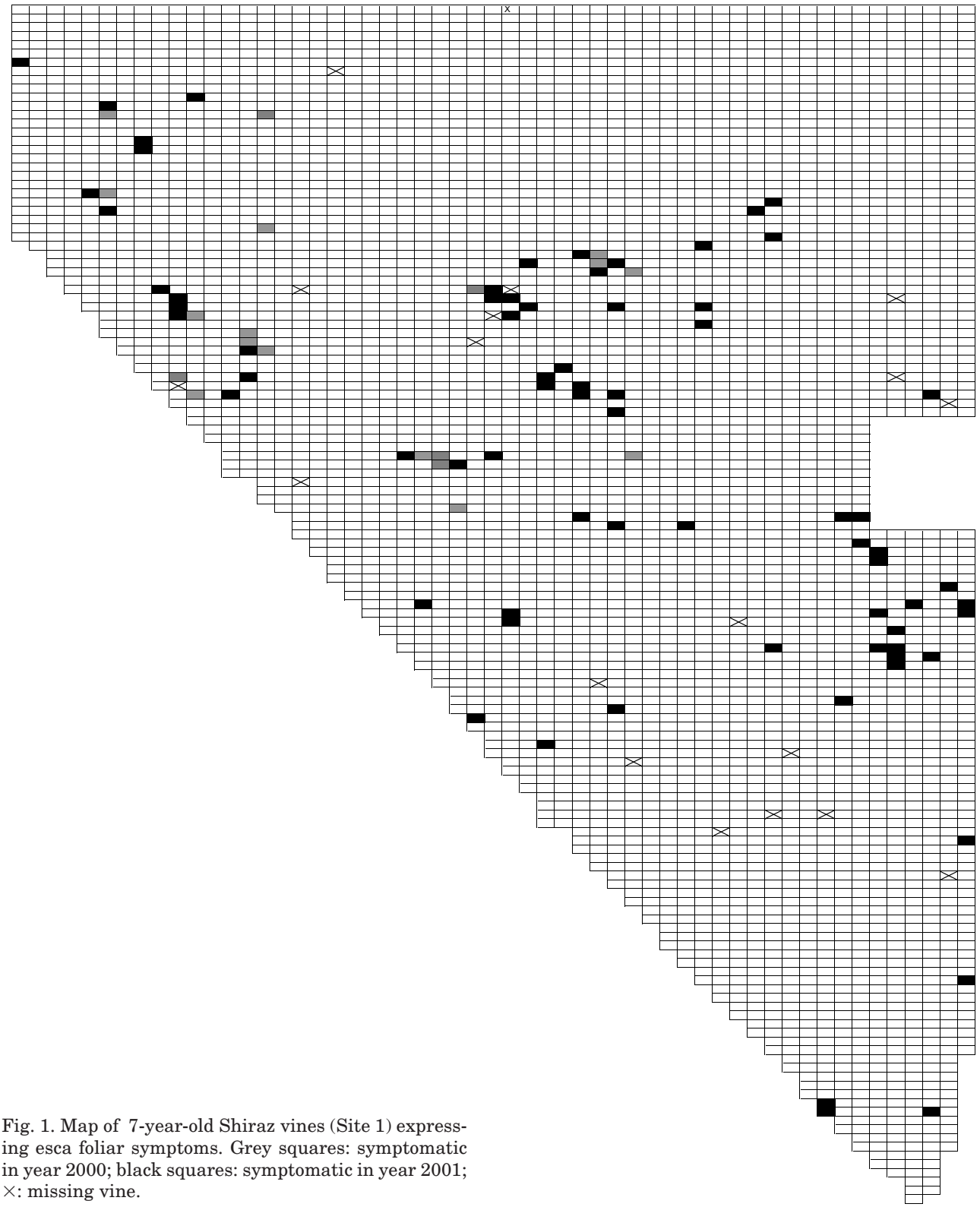
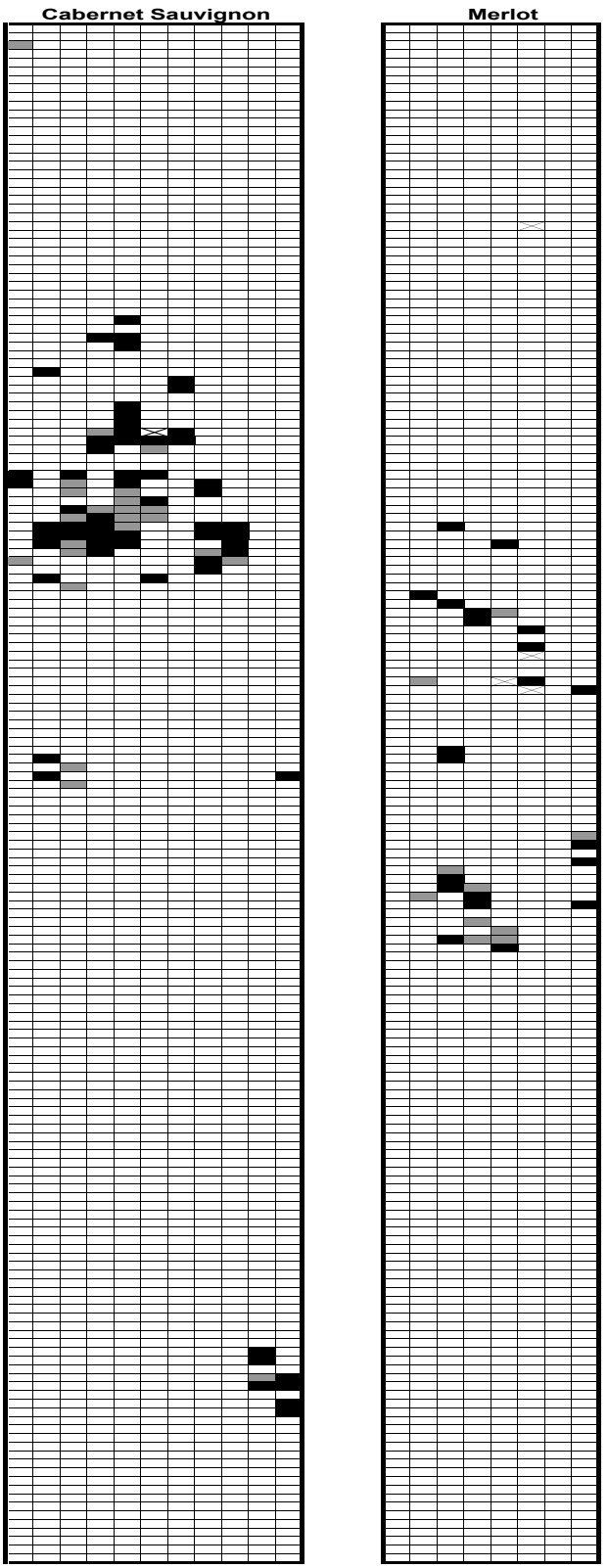


Fig. 1. Map of 7-year-old Shiraz vines (Site 1) expressing esca foliar symptoms. Grey squares: symptomatic in year 2000; black squares: symptomatic in year 2001; ×: missing vine.



when the two cultivars had reached the same developmental stage sometime between flowering and veraison. Are the symptoms caused by a fungal toxin induced by some hormonal trigger in the host? Continued monitoring and further data collection may provide some answers in the future.

Acknowledgements

Funding was provided by the Fondazione Carlo e Giulio Marchi, Italy, and the Australian Government's Commonwealth Cooperative Research Centres Program through the CRC for Viticulture. We thank the vineyard owners for their cooperation, and Mrs. Natalie Laukart for technical assistance.

Literature cited

Chiarappa L., 2000. Esca (black measles) of grapevine. An overview. *Phytopathologia Mediterranea* 39, 11–15.

Chicau G., M. Aboim-Inglez, S. Cabral and J.P.S. Cabral, 2000. *Phaeoacremonium chlamydosporum* and *Phaeoacremonium angustius* associated with esca and grapevine decline in *Vinho Verde* grapevines in northwest Portugal. *Phytopathologia Mediterranea* 39, 80–86.

Crous P.W., W. Gams, M.J. Wingfield and P.S. van Wyk, 1996. *Phaeoacremonium* gen. nov., associated with wilt and decline diseases of woody hosts and human infections. *Mycologia* 88, 786–796.

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

Ferreira J.H.S., P.S. van Wyk and E. Venter, 1994. Slow dieback of grapevine: association of *Phialophora parasitica* with slow dieback of grapevines. *South African Journal of Enology and Viticulture* 15(1), 9–11.

Graniti A., G. Surico and L. Mugnai, 2000. Esca of grapevine: a disease complex or a complex of diseases? *Phytopathologia Mediterranea* 39, 16–20.

Larignon P. and B. Dubos, 1997. Fungi associated with esca disease in grapevine. *European Journal of Plant Pathology* 103, 147–157.

Mugnai L., A. Graniti and G. Surico, 1999. Esca (black measles) and brown wood-streaking: two old and elusive diseases of grapevines. *Plant Disease* 83, 404–417.

Fig. 2. Map of 4-year-old Cabernet Sauvignon vines (left) and Merlot vines on Kober 5BB rootstock (right) (Site 2) expressing esca foliar symptoms. Grey squares: symptomatic in year 2000; black squares: symptomatic in year 2001; ×: missing vine.

Pascoe I.G., 1999. Grapevine trunk diseases – black goo decline, esca, Eutypa dieback and others. *The Australian Grape Grower and Winemaker* 414, 135–136.

Pascoe I.G. and E. Cottral, 2000. Development of grapevine trunk diseases research in Australia. *Phytopathologia Mediterranea* 39, 68–75.

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

Scheck H.J., S.J. Vasquez, W.D. Gubler and D. Fogle, 1998. Three *Phaeoacremonium* spp. cause young grapevine decline in California. *Plant Disease* 82, 590 (abstract).

Sparapano L., G. Bruno, C. Ciccarone and A. Graniti, 2000a. Infection of grapevines by some fungi associated with esca. II. Interaction among *Phaeoacremonium chlamydosporum*, *P. aleophilum* and *Fomitiporia punctata*. *Phytopathologia Mediterranea* 39, 53–58.

Sparapano L., G. Bruno and A. Graniti, 2000b. Effects on plants of metabolites produced in culture by *Phaeoacremonium chlamydosporum*, *P. aleophilum* and *Fomitiporia punctata*. *Phytopathologia Mediterranea* 39, 169–177.

Surico G., G.A. Marchi, F.J. Ferrandino, P. Braccini and L. Mugnai, 2000. Analysis of the spatial spread of esca in some Tuscan vineyards (Italy). *Phytopathologia Mediterranea* 39, 211–224.

Accepted for publication: December 6, 2001