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Inner necrosis in grapevine rootstock mother plants in the Cognac area (Charentes, France)

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Summary. The incidence and quantification of decline-associated inner necrosis in grapevine rootstock mother plants have rarely been studied. In an experimental vineyard planted in 1991 at Saintes (Charentes), susceptibility to esca was evaluated in eleven common rootstock varieties. Fifty vines per rootstock variety were used as mother plants producing long canes which were severely pruned every year. No foliar symptoms, typical of grapevine wood diseases, were seen in field inspections conducted in the summer of 1996, 2002, 2003 and 2006. In 2007, nine trunks per variety were randomly selected and were cross-sectioned at the point of greatest diameter. All sections revealed typical esca necrosis, central and/or sector-shaped, indicating that such necrosis is very common. Every section was photographed and the percentage of necrotic area was calculated by either visual assessment or image-analysis. No significant difference was detected between these two calculating methods. Based on the mean percent necrotic area, rootstock varieties were ranked in order of susceptibility from the least susceptible, '1103 Paulsen' (33%), to the most susceptible, '101-14 MGT' (71%). The percent of necrotic area was correlated significantly with i) the incidence of mortality and ii) the percentage of vine sections showing white rot, a type of necrosis indicating an advanced stage of wood deterioration. This study confirmed that necrosis in grapevine wood is not always associated with foliar symptoms, but that it is related positively with grapevine mortality. Furthermore, wood necrosis in mother-plants poses a risk of disseminating associated fungi through propagation material.

Key words: *Vitis vinifera,* trunk diseases.

Introduction

Grapevine (*Vitis vinifera* L.) is a perennial crop widely cultivated throughout the world for raisin, table grape and wine grape production. Most varieties are grafted on rootstock cultivars, predominantly to counter phylloxera, but also to prevent soil problems such as chlorosis (Delas, 1992). Among fungal diseases affecting yield, trunk diseases can severely damage crops in most vine-growing areas (Mugnai *et al.*, 1999). Esca, Eutypa dieback and Botryosphaeria cankers are the main wood diseases of grapevine and they are common in adult vineyards. These disorders commonly cause a gradual decline in production as the vines age. Cultivar susceptibility is well-known to be a factor in determining the severity of the foliar expression of Esca or Eutypa dieback, or in the development of wood deterioration and

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necrosis (Carter, 1991; Péros and Berger, 1994; Mugnai *et al*., 1999; Marchi, 2001; Feliciano *et al*., 2004; Sosnowski *et al*. 2007). In France, information on cultivar susceptibility comes mainly from several surveys carried out in the last two decades (Péros, 1995; Dubos, 2002). Some varieties, such as 'Cabernet-Sauvignon' and 'Sauvignon', are particularly susceptible (Kobès *et al*., 2006; Fussler *et al*., 2008).

Although Mugnai *et al*. (1999) reported that the external and internal symptoms of esca have been observed on American mother vines, studies on foliar symptoms and decline-related necrosis in rootstock mother vines are still rare, after the very first report by Petri (1912). Fourie and Halleen (2002, 2003), in South Africa were probably the first to employ isolations to quantify latent infections of trunk disease pathogens in rootstock mother vines. From wounds of 2-yr-old stubs (Fourie and Halleen, 2003), *Phaeomoniella chlamydospora* was the pathogen the most frequently isolated and *Phaeocremonium* spp., *Cylindrocarpon* spp., *Botryosphaeria* and *Phomopsis* species were also detected. Similar results were also reported from a survey in Spain (Aroca *et al*., 2008). Furthermore, two other studies showed differences in susceptibility among rootstocks following artificial inoculations with the main fungi associated with grapevine decline (Eskalen *et al*., 2001; Luque *et al*., 2007).

The Cognac region in France is severely affected by grapevine trunk diseases, in particular Eutypa dieback on Ugni Blanc (Dumot *et al*., 2004; Kobès *et al*., 2006). The objective of the present study was to assess the behaviour and mortality of different rootstock varieties during a 16-yr field trial and to compare the susceptibility of these varieties to trunk diseases under natural conditions. An experimental collection of rootstock mother vines planted in 1991 in this area by the "Bureau National Interprofessionnel du Cognac" (BNIC) was used for the tests. In 2007, just before uprooting, the opportunity was taken to examine the trunk internal tissues for inner wood rot.

Materials and methods

Rootstocks and experimental design

Eleven rootstock varieties, commonly used in the Cognac area and in other regions of France and elsewhere, were used in the experiment. The following varieties were planted in 1991 at Saintes in Charentes (France): '41B MGT' ('Millardet et de Grasset'), '101-14 MGT' 'Millardet et de Grasset', '110 Richter', '140 Ruggeri', '161-49 Couderc', '333 EM' ('Ecole de Montpellier'), '1103 Paulsen', 'Fercal', 'RSB1' ('Rességuier Sélection Birolleau No.1'), 'Rupestris du Lot', 'SO4' 'Sélection Oppenheim de Téléki No.4'. The genetic origin and vigour of these varieties are summarized in Table 1.

Five replications of 10 vines per variety were arranged in a completely randomized design on a sandy clay soil. Vines were treated as conventional mother-plants producing long canes and pruned severely every year at 10–30 cm length (Fig. 1 A and B).

During the 16 yr trial, the percentage of dead vines per cultivar was monitored every year and the final mortality rate was determined at the beginning of 2007. The exact cause of the mortality was not investigated. Moreover, summer foliar symptoms typical of wood diseases (either Eutypa dieback or esca) were rated in 1996, 2002, 2003 and 2006.

Before uprooting in April 2007, 1 or 2 randomly selected trunks per replicate were cut transversally with a saw at the point of greatest diameter, as shown in Fig. 1C, D, E, F and 2. An attempt was made to measure the length of the wood lesions, but because the plants were too short, this proved less convenient to assess the severity of internal necrosis, which was mainly located in the upper part of the trunks (Fig. 1A and 2). A total of 9 trunks per rootstock cultivar were tested.

Description and assessment of internal necrosis

All internal lesions were recorded, noting for each lesion its location (central and/or sector-shaped) and the colour and texture of the deteriorated wood (brown or discoloured wood with a hard consistency or a wood with a soft spongy white/yellow rot) according to previous descriptions from similar areas (Larignon and Dubos, 1997; Larignon, 2004).

Every section was photographed. The severity of each necrotic area was assessed from the photographs using two methods, image-analysis and visual assessment. Two image-analysis softwares were used: 1. Adobe Photoshop 7.0 (Adobe: "www.adobe.com") to select the wood surface to be analyzed and, 2. Image

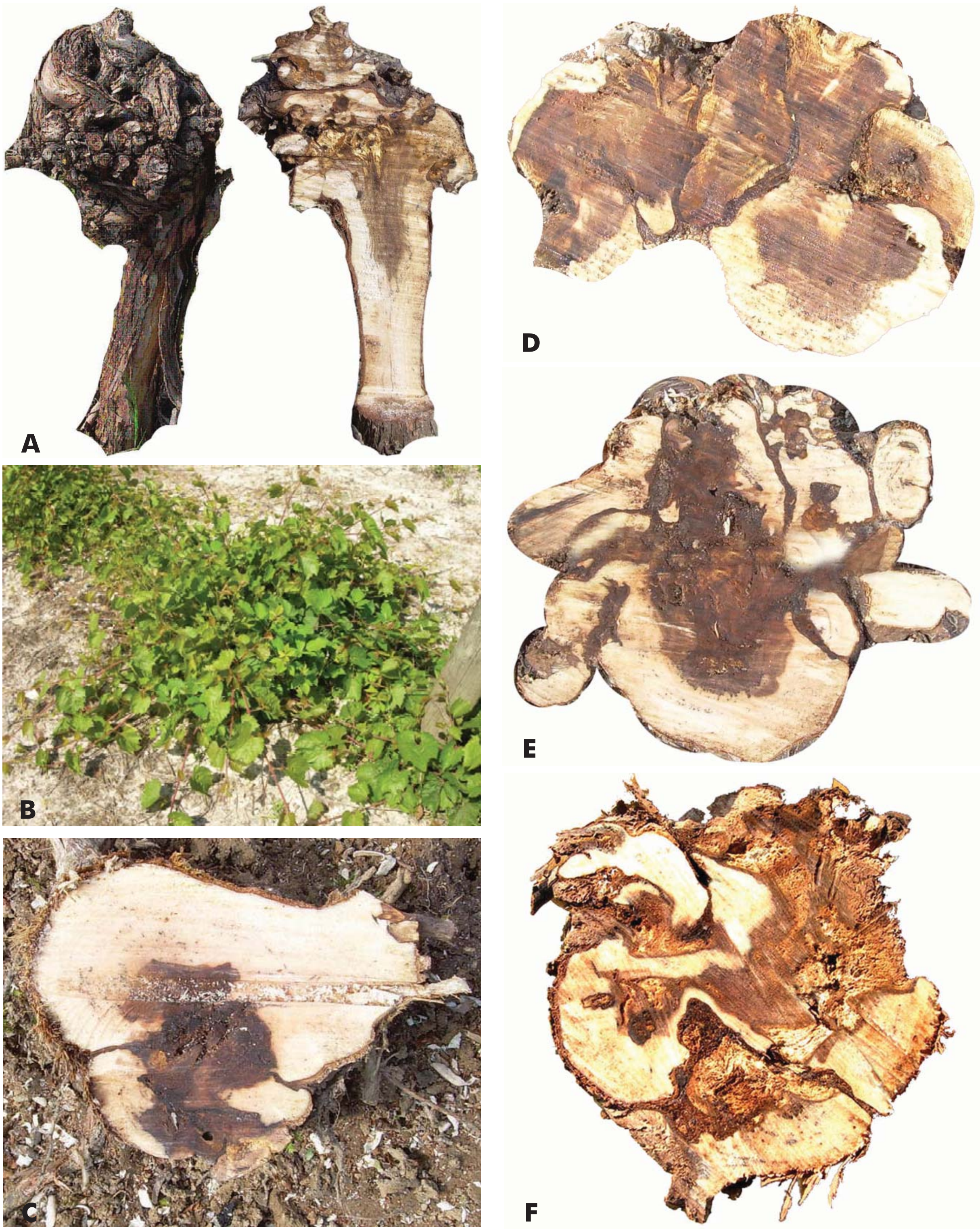

Fig. 1. Examples of rootstock mother plants (A, B) and necrosis observed in cross sections after saw-sectioning (C, D, E, F)

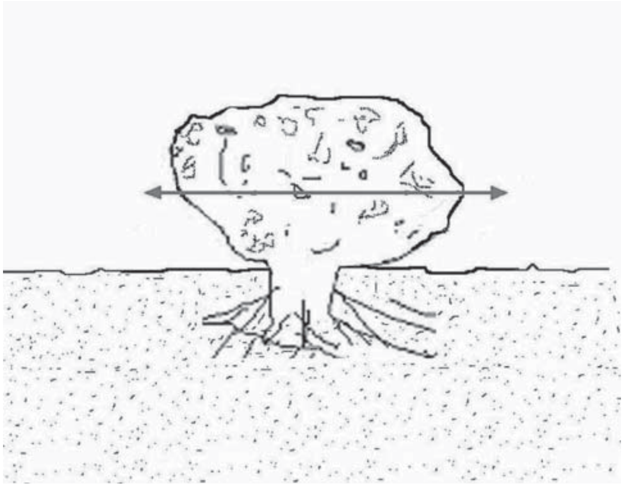

Fig. 2. Schematic illustration of a rootstock mother vine before saw-sectioning at the greatest diameter (line with arrows).

J ("www.rsbweb.nih.gov/ij") to determine the extent of wood necrosis as a proportion of brownish and darkened wood tissues (*vs* whitish healthy wood). Visual assessment was done with two observers (1 and 2), following a preliminary standardization based on a set of 12 random sections where visual assessments were compared with data from the image-analysis software. Visual assessments were also expressed as the percentage of wood that was discoloured.

Variance analysis and Pearson's correlation tests were conducted using Statbox Pro 5.0 (Grimmersoft, Issy Les Moulineaux, France). In variance analysis, data from the two different procedures (3 blocks corresponding to image analysis, visual assessments done by thw two observers 1 and 2) were compared, as well as the rootstock varieties (one main factor). In the correlation analyses, the mean necrotic area (%) was calculated from the three assessment methods (image analysis, observer 1 and 2).

Table 1. Characteristics of the 11 grapevine rootstock varieties (genetic origin and vigour), mortality percentage and main decay features from 9 trunk cross sections per rootstock and ranking according to the mean percentage of necrotic area

Rootstock variety	Genetic origin[a]	Vigour[b]	Mortality (%) (Confidence interval)[c]	Main decay features: Location[d]			Main decay features: Sight, consistency[e]			Mean necrotic area (%)[f]	Homogeneous group[g]
				I	II	III	I	II	III		
1103 Paulsen	*V. rupestris* × *V. berlandieri*	L	6 (1–17)	5	2	2	6	0	3	33.18	A
SO4	*V. riparia* × *V. berlandieri*	H	2 (0–11)	6	1	2	7	0	2	36.66	B
333 EM	*V. berlandieri* × *V. vinifera*	M	0 (0–7)	3	2	4	3	2	4	38.74	B
41B	*V. berlandieri* × *V. vinifera*	H	5 (1–16)	4	2	3	4	0	5	43.74	C
161-49C	*V. riparia.* × *V. berlandieri*	M	11 (4–23)	4	0	5	5	1	3	46.63	D
RSB 1	*V. riparia* × *V. berlandieri*	H	4 (0–14)	4	1	4	5	0	4	49.07	D
Rupestris du Lot	*V. rupestris*	M	2 (0–11)	4	2	3	3	2	4	52.96	E
140 Ruggeri	*V. rupestris* × *V. berlandieri*	L	7 (2–18)	1	2	6	3	1	5	57.18	F
110 Richter	*V. rupestris* × *V. berlandieri*	L	24 (13–38)	3	4	2	3	0	6	58.92	F
Fercal	*V. berlandieri* × *V. vinifera*	M-H	18 (9–31)	2	0	7	3	0	6	62.51	G
101-14	*V. riparia* × *V. rupestris*	M-H	20 (10–34)	2	1	6	2	0	7	70.92	H
	Total			38	17	44	44	6	49		

[a] According to Reynier (2003).
[b] Intrinsic vigor: L, low; M, moderate; H, high, according to Cordeau (2002), Reynier (2003) and L. Bordenave and J-P. Soyer (personal communications).
[c] Dead vines (%) recorded before uprooting in 2007. Confidence interval at P=0.05 according to the binomial distribution.
[d] Necrosis location: I, mostly central; II, mostly sector-shaped; III, both locations observed.
[e] I, discolorations, brown wood, hard consistency; II, soft, spongy and yellow (white rot); III, both types of necrosis.
[f] Mean of three assessments (visual and image analysis) based on 9 vine sections per rootstock
[g] According to Newman-Keuls's test (P=0.05) after variance analysis.

Results

Field summer inspections in 1996, 2002, 2003 and 2006, did not detect any foliar symptoms typical of grapevine wood diseases. As regards vine mortality, the cumulative incidence (%) of dead vines measured in winter 2007 on each rootstock variety is shown in Table 1. It varied strongly from 0% ('333 EM') to 24% ('110 Richter'). Three varieties showed a high mortality percentage greater than 18%: 'Fercal', '101-14' and '110 Richter'.

In cross-sections, all mother vines revealed typical esca necrosis (Fig. 1C, D, E and F). As detailed in Table 1, 38% of vines exhibited mainly central necrosis, and 44% of the vines had both central and sector-shaped necrosis, as illustrated in Fig. 1. Consequently, central necrosis was seen in approximately 83% of the vine sections examined. Only 17% of vines showed mostly sector-shaped necrosis. Although the vines were young, white rot was frequently (50%) associated with brown and/or discoloured necrosis (Fig. 1A, D and F). This last type of necrosis was observed in nearly 94% of all vine sections.

As shown in Table 2, variance analysis detected no significant differences (P=0.05) between the two assessment procedures (i.e. the two visual assessments and image analysis). According to image analysis, the mean necrosis area was 52% (Table 2). Although there were variations from vine to vine, the average percentage of surface necrosis (Table 1) differed significantly between rootstock varieties (F=151.6; P=0). The varieties were then ordered according to the Newman-Keuls's test from least necrotic ('1103 Paulsen', 33%) to most necrotic ('101-14 MGT', 71%).

Figure 3 compares, for each rootstock, the mean percent of necrotic area with the cumulated percentage of dead vines in the vineyard. A significant and positive correlation was found (Pearson's R=0.743; P=0.004) and a linear regression was fitted (y=0.507x -16.354; R^2=0.552). However, there were two outliers: i) 'Rupestis du Lot', which exhibited a low percent mortality associated with a large necrotic area and ii) '110 Richter', which had the highest percent mortality but not the greatest mean necrotic area. Figure 4 compares the mean percentage of necrotic area with that of vine sections showing white rot (in Table 1, sight, consistency, columns II and III). A significant and positive correlation (Pearson's R=0.772; P=0.003) was found and a linear regression was fitted (y=1.133x–1.180; R^2=0.596).

Lastly, no correlation was found (Pearson's R=-0.033; P=0.461) between the mean area of the deteriorated wood and the intrinsic vigour of each rootstock, as established in French conditions (Table 1). In the same way, no clear information in relation to the genetic origin could be noticed: '1103 Paulsen', which originated from a cross between *Vitis rupestris* and *Vitis berlandieri* appeared rather tolerant while '140 Ruggeri' and '110 Richter', both from the same origin, appeared much more susceptible. Similarly, '333 EM' and '41B' can be compared with 'Fercal', which all originated from a cross between *V. berlandieri* and *Vitis vinifera*.

Table 2. Variance analysis of the 3 assessment procedures to evaluate the necrotic area

Mean	Visual assessment (%)		Image analysis (%)
	Observer 1	Observer 2	
F: 1.22 P=0.29	50.2 (14.8[a])	48.2 (14.6[a])	51.7 (14.9[a])

[a] Residue standard deviation

Discussion

The mother vines surveyed in this study were uprooted when the plants were 16 year-old. This period of cultivation was sufficient to cause vine mortality of up to 24%. In this vineyard, the mortality rates were positively correlated with the mean necrotic area in the trunk cross-sections. Although the exact cause of the mortality was not investigated, this result indicates that grapevine trunk diseases were at least in part, a cause of this mortality. In the case of 'Rupestris du Lot', the low percentage of dead vines indicated that this variety may be tolerant to necrosis inside the trunk. Interestingly, '333 EM', with no dead vines at the end of the trial, still had significant mean necrosis area of 39% in the trunks. This may be due to the plant metabolism of vines from this rootstock cultivar and to the effectiveness of the plant defence (Estiarte and Penuelas, 1999; Coviella *et al.,* 2002).

The detailed description and quantification of necrosis from the trunk cross sections showed clearly that necrosis occurred frequently with a transverse extent that differed depending on the variety of the rootstock mother plants. An attempt was made to cut the trunks in order to determine the length of the necrosis, but this was found to be impracticable because the trunks were too close to the soil, and the longitudinal sections were not always more informative.

Necrotic wood i.e. wood showing either discolorations and hard brown parts and typical soft rot or both, was chosen as an indicator of varietal susceptibility to grapevine trunk diseases, in contrast to healthy zones without necrosis. It was noticeable that the necrosis in the rootstock wood were mostly central, and visually quite similar to necrosis in the wood of different grapevine varieties grown in Europe and affected with esca (Mugnai *et al*., 1999; Fisher and Kassemeyer, 2003; Péros *et al*., 2008). As a confirmation, there was a positive correlation between mean necrotic area and the incidence of white rot, a type of necrosis often stated to be an indicator of an advanced stage of wood deterioration (Larignon and Dubos, 1997; Mugnai *et al*., 1999; Dubos, 2002; Dumot, 2007; Lecomte *et al*., 2008a, 2008b). Although we did not perform any fungal isolation, the most common pathogenic fungi generally involved in such grapevine wood rot are well-known (Botryosphaeriaceous species, *Eutypa lata, Fomitiporia mediterranea, Phaeoacremonium* spp., *P. chlamydospora*), in particular in Europe and the Mediterranean countries (Larignon and Dubos, 1997; Mugnai *et al*., 1999; Pollastro *et al*., 2000; Armengol *et al*., 2001; Rumbos and Rumbou, 2001; Fisher and Kassemeyer, 2003; Berraf and Péros, 2005; Choueiri *et al*., 2006; Calzarano and Di Marco, 2007; Martin and Cobos, 2007; Péros *et al*., 2008). Nevertheless, further studies should be carried out to determine precisely the associated fungi and any differences in wood colonization due to the genetic background of the rootstock variety.

To determine the size of the necrotic area, the two methods were compared. Visual assessment was not significantly different from image analysis and can thus be considered a reliable indicator. Consequently, visual assessment is a rapid procedure for similar experiments valid under a range of conditions. Statistical analysis also detected significant differences in the mean percentage of the necrotic area between the rootstocks tested. The lowest average necrotic area was 33%. Therefore, as Eskalen *et al*. (2001) also reported, none of the rootstocks were truly resistant. The necrotic area that was found in all varieties may have been favoured by other factors such as the training system or the severe pruning of all canes every year. Internal necrosis and fungal invasion may have been enhanced by numerous associated wounds and a concentration of deteriorated wood areas (unhealed drying areas) that arose as a result of such pruning regime (Lecomte *et al*., 2008a, 2008b).

The rootstock varieties with the most extensive necrosis in our experiment were '140 Ruggeri', '110 Richter', 'Fercal' and '101-14 MGT'. '1103 Paulsen' had the smallest necrotic areas. This finding was in agreement with Eskalen *et al*. (2001) who inoculated *Phaeoacremonium aleophilum* and *P. chlamydospora* on this and other rootstocks. Significant differences between studies exist which are possibly related to the experimental approach: field observations or mechanical inoculation and assessment of necrosis or assessment of vascular streaking. Thus, the ranking of rootstocks reported here provides additional information on rootstock behaviour but further surveys under other cultural conditions are required to ascertain the true susceptibility of rootstocks to trunk diseases. In the same way, further inoculation tests with fungi, alone or combined, should be carried out to compare the susceptibility of the rootstocks in standardized or controlled conditions. Determination of the most resistant rootstock variety would be useful for breeding programmes.

Our study demonstrated that wood necrosis can be very common and the necrotic area in the trunk is important in most grapevine rootstock mother plants. Foliar symptoms were not investigated every year and only four inspections were made during the experiment. Nevertheless, inspected vines were all 5 years old or more. No leaf symptoms were seen even in the varieties most affected. This condition of adult vines with frequent internal necrosis but no leaf symptom, is not unusual for esca and has already been reported in the same region or in the same country (Dubos, 2002; Dumot, 2007; Lecomte *et al*., 2008a, 2008b). The occurrence of internal necrosis was not related to either the intrinsic vigour or the rootstocks or the genetic origin of the rootstock varieties.

Among the fungi that can presumably spread from the infected mother plants to rootstock cuttings through the vessels, *P. chlamydospora* is disseminated via its propagules or spores (Crous *et al.,* 1996; Fourie and Halleen, 2002, 2003; Edwards *et al*. 2004; Larignon *et al*., 2004). This shows that the means of fungal dissemination in plant propagation material can play a significant role in the early epidemiology of grapevine trunk diseases. It is now also clear that grapevine trunk disease pathogens occur in plant propagation material before, during or after the nursery process (Larignon *et al.*, 2004, 2008; Zanzotto *et al.*, 2007; Aroca *et al.*, 2008; Pichierri *et al.*, 2008; Pollastro *et al.*, 2008; Serra *et al.*, 2008; Vigues *et al.*, 2008). However, the occurrence of a fungus or fungi in a vine does not mean that that vine will necessarily become diseased. This also depends to a large extent on environmental predisposing factors in the nursery or during and after planting in the vineyard (Ferreira *et al.*, 1999; Stamp, 2001; Lecomte *et al.*, 2008a, 2008b; Serra *et al.*, 2008).

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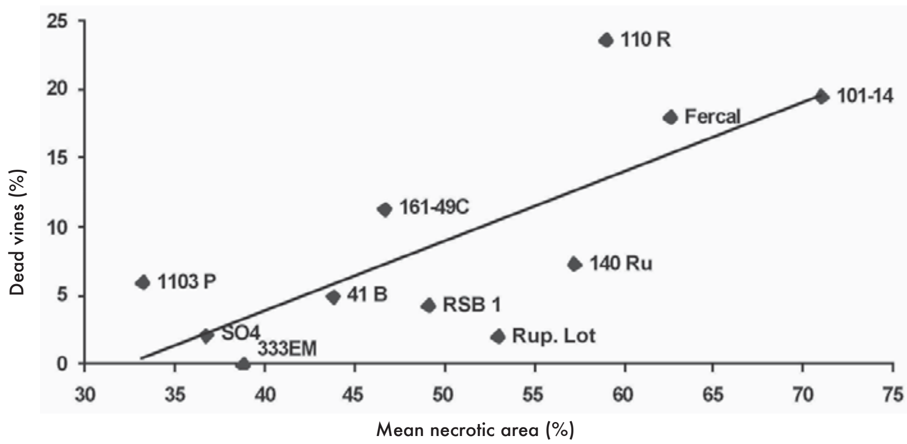

Fig. 3. Linear regression and correlation between the mean necrotic surface and the percentage of dead vines recorded at the end of the experiment (y = 0.507x -10.354; R2 = 0.552).

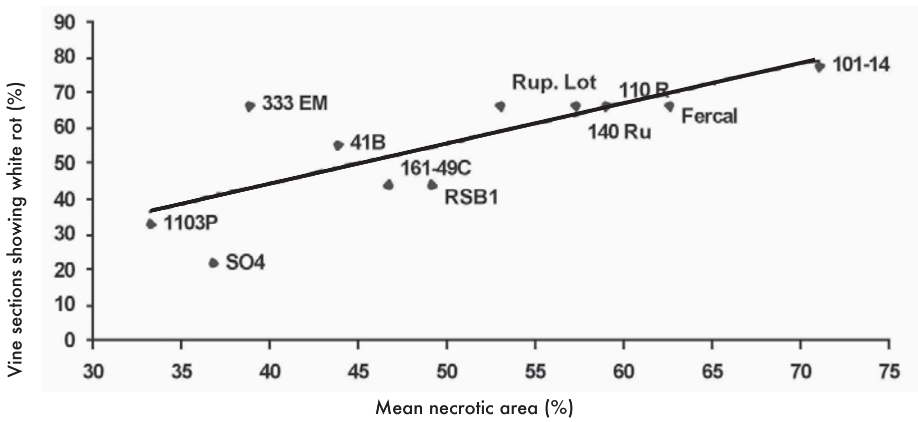

Fig. 4 Linear regression and correlation between the mean necrotic surface and the percentage of vine sections showing white rot (y = 1.133x – 1.180; R^2 = 0.5956).

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