Phytopathol. Mediterr. (2006) 45, S68–S86

Older and more recent observations on esca: a critical overview

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Summary. The main research findings of studies in esca carried out over the last few years in a number of laboratories are discussed. The topics include: the disease model; the relationship between black dead arm and esca; the role of toxins and xylem cavitation in the development of leaf symptoms; the artificial reproduction of leaf symptoms and wood rot; the inoculum sources of the fungi *Phaeomoniella chlamydospora, Phaeoacremonium aleophilum* and *Fomitiporia mediterranea*, and the influence of climate on the incidence of esca. The purpose was to sum up the current state of esca research and where possible to present new suggestions and sometimes alternatives to the suggestions of other researchers. The ultimate aim was to draw attention to those questions in esca research that are open to differing and even opposing explanations because the hard data are still lacking.

Key words: *Vitis vinifera,* trunk diseases, disease models, pathogens and climate, epidemiology.

Introduction

Extensive information on esca is given in some older manuals of plant pathology such as those of Brizi (1919), Arnaud and Arnaud (1931), Ciferri (1955), Goidànich (1964) and Galet (1977). In these manuals one can read that 'esca or apoplexy (the two terms were taken at that time to be synonymous) is a disease in which both symptoms and the sequence of symptom appearance are highly variable. The symptoms appear on all the green and lignified organs of the vine, including the berries but not the roots, from June to September. Apoplexy on the other hand occurs only in the hotter part of the summer and is more common on grafted vines. When an excess of water in the soil is combined with hot weather, and particularly when a hot wind blows, a dramatic imbalance between transpiration and absorption is produced that is sufficient to bring about the death of affected vines almost instantly' (Brizi, 1919). The disease occurs in almost all areas where vines are grown and is most common in older plants, especially those more than 25 years old. A number of fungi are mentioned as causal agents, all in the basidiomycetes: *Stereum hirsutum* (=*S. necator*), *S. rugosum, S. spadiceum, Fomes* (*Phellinus*) *igniarius,* and others.

At the time when the manuals mentioned above were written it was also known that 'diseased plants occur either isolated or, less frequently, grouped in small clusters. Both trunk rot and the 'mal dello spacco' related to it are sometimes preceded by extensive foliar reddening. A dark-brown stripe about 1–2 cm wide starts from an affected vine-shoot from which it advances downwards sometimes as far as the large roots; it is often vis-

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ible in the wood when the bark is removed. The slow form of esca progresses acropetally on the plant, while apoplexy migrates basipetally. The disease occurs preferentially on European rather than on American grapevines. When diseased, vines do not present symptoms regularly every year'.

A number of years after these manuals were published, many of the statements made in them are still valid, but it is clear that our understanding of esca has not only made advances, which was only to be expected, but that progress has also opened altogether new and rather complex pathological scenarios, even though a consensus about all the new findings has not yet been reached. This paper reports on some of the most important new advances in esca research over the last few years and attempts to illustrate the overall trend of these advances.

Esca today

At the first International Workshop on Grapevine Trunk Diseases - Esca and Grapevine Declines, held at Siena (Italy) in 1999, reports were made summarizing 7–8 years of research into the disease carried out in various countries, and discussing what esca was thought to be in the past and what it is thought to be now (Graniti *et al.*, 1999, 2000, 2001). It was suggested there that:

i) esca was a 'complex' disease and the syndrome of esca as a whole was caused by a number of factors interacting with each other and involving more than one causal micro-organism;
ii) it was a complex of at least two distinct diseases: l. white rot caused by *Fomitiporia mediterranea* (Fmed) or other wood-rotting basidiomycetes, and 2. a tracheomycosis characterised by dark wood streaking and gummosis, and caused by *Phaeomoniella chlamydospora* (Pch) and/or *Phaeoacremonium aleophilum* (Pal) or some other species of *Phaeoacremonium*.

Also, depending on where the infection started (in the nursery or in the field); on the fungus that is predominant or on the order in which the fungi infect the vine or become active in it, it has been possible to describe at least five syndromes, corresponding to as many diseases (Mugnai *et al.*, 1999):

1. dark wood streaking of the rooted cuttings;
2. Petri disease (also known as black goo, slow dieback, Phaeoacremonium grapevine decline, etc.), affecting very young vines;
3. young esca (tracheomycosis), characterised by dark wood streaking, occasionally with small areas of black and hard necrosis, and the typical leaf symptoms of esca, but without wood white rot;
4. white rot, occurring when a Fmed infection (or infection by other wood-rotting basidiomycetes, especially in non-European countries: see Fischer, 2005) is not accompanied by the co-presence of *Phaeoacremonium*/*Phaeomoniella* and hence by leaf symptoms;
5. esca proper, which denotes both white rot and dark wood streaking (tracheomycosis): the rot occurs on the stems and eventually spreads to the main branches of adult or older vines. This syndrome is thought to be caused by Pch and Pal, acting together or in succession, plus Fmed.

In this context, apoplexy is a peculiar event that occurs mainly, if not exclusively, in vines suffering from esca proper (see below).

As for the succession of events that lead to esca proper (and apoplexy), it has been postulated that the disease starts (or may start) in the nursery (Tables 1 and 2 show some data on the occurrence of the fungi of esca in the vine shoots of mother plants, and also in rooted vine cuttings ready for

Table 1. Incidence (%) of *P. chlamydospora* in grapevine propagating material.

Plant material	Isolation (%)	Country	Reference
Rooted cutting	0–80	Italy	Bertelli *et al.*, 1998 Surico *et al.*, 1998
Rootstock	7.5–38.4	Portugal	Rego *et al.*, 2000
Rooted cutting	20–60	Italy	Zanzotto *et al.*, 2001
Rooted cutting	12	Italy	Sidoti *et al.*, 2001
Rooted cutting and rootstock	0–37	California	Stump, 2001
Self-rooted cutting	30	Australia	Laukart *et al.*, 2001

Table 2. Incidence (%) of *P. chlamydospora* (Pch) and/or *P. aleophilum* (Pal) in the shoots of mother vines.

Plant material	Pch infection (%)	Pal infection (%)	Country	Reference
One-year-old shoot	0.5	0	France	Larignon and Dubos, 2001
One-year-old shoot	2–9	0–6	Australia	Edwards *et al*., 2003
Spur	2.5–9.7	0	South Africa	Fourie and Hallen, 2002

outplanting) and continues in the field with Petri disease or young esca, and eventually with esca proper (Surico, 2001).

In the years following 2000, after the findings presented at the Workshop had been published, various authors have reported one or other of these five syndromes in the nursery or in the field: dark wood streaks colonised by Pch/Pal in grapevine cuttings were reported, after Bertelli *et al*. (1998), by several researchers (Rego *et al*., 2000; Laukart *et al*., 2001; Stamp, 2001; Zanzotto *et al*., 2001; Rumbos and Rumbou, 2001); Petri disease was reported, following Ferreira *et al*., 1994 in South Africa, and Morton (1997) and Scheck *et al*. 1998, in the USA, also in Portugal (Chicau *et al*., 2000; Rego *et al*., 2000), Italy (Sidoti *et al*., 2000), Greece (Rumbous and Rumbou, 2001) and Spain (Armengol *et al*., 2002); young esca was described in Italy (Mugnai *et al*., 1999), Australia (Edwards *et al*., 2001), USA (Gubler *et al*., 2004a), and is commonly found in all countries where esca symptoms are present.

Study of the internal symptoms of many mature grapevines with esca leaf symptoms in various European countries has confirmed that white rot and dark wood streaking (esca proper) effectively exist side by side. These two types of symptoms may both occupy the same site on a vine or each may have a separate site.

Of the five syndromes postulated, only white rot seems to be more a hypothetical construct (supposed to be caused by Fmed acting as a primary pathogen) than a disease effectively occurring in nature, inasmuch as it has hitherto been found only in two cases, once in Italy and once in Hungary (Mugnai, unpublished data).

Reproduction of esca symptoms

In various laboratories attempts have been made to reproduce, completely or in part, the symptoms of esca, or at least to verify the pathogenicity of the fungi associated with the disease. Sparapano *et al*. (2001) inoculated cv. Matilde and Italia vines (9 and 5 years old respectively) in the field with Pch, Pal and Fmed (at that time classified as *Fomitiporia punctata*) separately and in various combinations. The results of these trials are summarized in Table 3. They show that the three fungi, alone or in combination, reproduce not only the internal symptoms of esca, but also, though not invariably, the symptoms on the leaves, and sometimes even those on the berries. This is so far the only trial in which inoculation with Pch, Pal and Fop (Fmed) successfully reproduced the full syndrome of esca, which is now termed esca proper.

Numerous other pathogenicity tests have indicated that Fmed invariably causes rot symptoms (Chiarappa, 1997; Mugnai *et al*., 1999; Sparapano *et al*., 2000) and that Pch and Pal (and other species of *Phaeoacremonium*) cause dark streaking of the vine wood (Larignon and Dubos, 1997; Mugnai *et al*., 1997; Khan *et al*., 2000; Eskalen *et al*., 2001; Sparapano *et al*., 2001; Feliciano *et al*., 2004; Wallace *et al*., 2004; Conceiçao *et al*., 2005) and in young vines signs of Petri decline as well (Scheck *et al*., 1998; Eskalen *et al*., 2001; Halleen *et al*., 2005). Direct inoculation of the berries with Pch and Pal has reproduced the symptoms of black measles (Gubler *et al*., 2004b). Another finding was that these last two fungi inhibited the formation of callus at the graft union (Wallace *et al*., 2004). Finally, Feliciano *et al*. (2004) reproduced young esca on 15-year-old grapevines cv. Thompson Seedless.

In this scenario, it should be noted that, in spite of several attempts, only two groups of researchers have so far succeeded in reproducing young esca or esca proper. This would seem to mean that, if the fungi being tested are effectively those causing esca, there must be other as yet unidentified factors whose co-involvement is necessary for such tests to succeed. But then again, positive results with inoculation tests on fairly young and mature

Table 3. Outcome of inoculations of two grapevine cultivars (Matilde and Italia, 9- and 5-year-old respectively) with *P. chlamydospora* (Pch), *P. aleophilum* (Pal) and *F. punctata* (=*F. mediterranea*) (Fop), alone and in all possible combinations (from Sparapano *et al.*, 2001).

Fungus/fungi inoculated	Symptoms detected 1, 2 and 3 years after inoculation											
	Dark streaking			White rot			Leaf symptoms			Black measles		
	1	2	3	1	2	3	1	2	3	1	2	3
Pch	+	++	+++[a]	-	-	-	-[b]	+	+	-	-	+
Pal	+	++	+++	-	-	-	-	+	+	-	-	-[c]
Fmed	±	±	±	+	++	+++	-	+	+	-	-	-
Pch+Pal	+	++	+++	-	-	-	-	+	+	-	-	-
Pch+Fop	+	++	+++	-	-	+	-	-	+	-	-	-
Pal+Fop[d]	+	++	+++	-	-	+	-	-	+	-	-	-
Pch+Pal+Fop[d]	+	++	+++	-	-	-	-	+	+	-	-	-

[a] The number of plus-signs indicates the degree of severity.
[b] Occurs occasionally on a few plants.
[c] Only in the 3rd year and only in the cv. Matilde.
[d] Antagonistic/competitive effect of Pal against Fop.

vines are not easy to assess because it is difficult to know the health status of the vines being tested. Lastly, it will also be interesting to ascertain whether any leaf symptoms that may be artificially reproduced also exhibit discontinuity from year to year, as do esca leaf symptoms in nature. Such discontinuity if it occurred would be further evidence that the artificial inoculations had successfully reproduced the disease.

In conclusion, it seems that we can say that:

1. the pathogenicity trials currently adopted in laboratories do not succeed in reproducing the syndromes of young esca and esca proper in their entirety with the fungi used; and
2. the leaf symptoms are in all likelihood a consequence of the concurrence of multiple biotic and abiotic factors that are difficult to reproduce in inoculation trials (see also the paragraphs which follow).

Black dead arm by *Botryosphaeria* spp. and esca

In 2001 Larignon and Dubos (2001a, b) reported on a new type of decline from various areas in France (Bordolais, Armagnac, Champagne, Provence). This decline had first been detected in France in 1999, and had then been attributed to esca, but Larignon and Dubos (2001a, b) stated that it was due to black dead arm (BDA). This particular form of decline had already been reported from Hungary by Lehoczky (1974) and from Italy by Cristinzio (1978) and Rovesti and Montermini (1987). In Hungary the disease had been attributed to *Botryosphaeria stevensii* and in Italy to *B. obtusa*. Larignon and Dubos in their study isolated mainly *B. obtusa* and *B. dothidea*.

In France symptoms began on the leaves of dark-berried grapevines as small wine-red spots along the leaf margins and/or on the blade. These spots then expanded and became confluent, leaving only a stripe that still remained green along the main veins of the leaves. The deteriorated areas then wilted and took on the colour of a dead leaf, with the necrotic areas separated from the remaining green area by a thin wine-red line. The overall leaf appearance was not always the same, however; sometimes the spots were small and covered the entire leaf blade (giving it an 'aspect tacheté') or the necrotic areas expanded from the leaf margins inward to the median in numerous small, light-brown tongues that were more or less regularly edged with a yellowish translucent line; in yet other cases the necrotic leaf tissue in those areas that were contiguous to the wine-red dividing line turned a reddish pink. At a later stage the leaf areas that remained green and the veins sometimes turned yellow. In the most serious cas-

es the leaves wilted completely, curled and fell, and only some leaves were left at the tip of the shoots, which could also become partially or completely withered.

On the leaves of white grapes the spots that formed were an orangy-yellow colour. As with the dark-berried vines, symptoms varied: either the spots expanded and often coalesced, while retaining their original orangy colour, and remaining surrounded by normal green tissue; or the diseased leaves retained their spotted appearance; or they formed chlorotic areas along the coloured necrotic areas. Complete wilting of the leaves and shoots was frequent, and at a more advanced stage of the disease the remaining green leaf tissue and the veins sometimes turned yellow. On both green and dark-berried vines the inflorescences and the berries often wilted.

Another symptom characteristic of the black dead arm (BDA) described by Larignon and Dubos was the formation of a dark stripe on the wood surface just below the bark. This stripe was a few centimeters wide, and in some cases extended from the symptomatic branch all the way to the graft junction. In cross-section the dark streak on the wood tissue was edged with an orangy-yellow border that was often a few mm wide - in this area the vessels were blocked. This orangy-yellow border might become necrotic, forming a sectorial, dark-grey or black canker.

Larignon and Dubos stated that the new disease in its early stages was easy to distinguish from esca. BDA leaf symptoms appeared in early June, those of esca not until late June. Vine leaves with BDA never had yellowish leaf spots, as were found with esca; instead vines with BDA showed a dark streak on the wood immediately below the bark, which is not found with esca. Lastly, in the case of dark-berried grapevines leaf necrosis with BDA was a darker red than it was with esca.

BDA has more recently also been studied in France by Lecomte *et al*. (2005). These researchers raised doubts about the claim by Larignon and Dubos (2001a, b) that the BDA they had found was a different disease from esca. In opposition to Larignon and Dubos (2001a, b) they found that it was very often difficult to distinguish between the leaf symptoms of BDA and those of esca and that many diseased vines showed the typical leaf symptoms of both BDA and esca, either simultaneously or one after the other - sometimes even on one and the same leaf. BDA symptoms appeared towards the middle of June and very often turned into the symptoms of esca and from vine wood degraded by the disease species of *Botryosphaeria* were isolated, a group of fungi considered by the authors as normal weakness parasites that often colonise the bark and wood of grapevines.

As a result of this sometimes contradictory description of BDA symptoms offered by these two studies, the question arises whether these symptoms really define a new form of vine decline, different from BDA as first described by Lehoczky (1974) and therefore to be given a different name. In reality both Lehoczky (1974) and Cristinzio (1978) in their studies found that the symptoms of BDA in the vines they studied were somewhat different from those reported by Larignon and Dubos (2001a, b) for the same disease. Lehoczky (1974) reported BDA symptoms as: slightly diffuse chlorosis on the leaves; wilting of the leaves when water flow became insufficient during the growing season; the occurrence of black stripes, initially 3–5 mm wide, in the xylem of the shoots and trunks (rarely in current-year shoots); death of the bark where it covered blackened wood; sectorial blackening, visible in cross-section, that sometimes included the pith; infected vines did not resume growth at all in spring or suddenly began to wither at some time during the growing season; the rootstock generally remained unaffected. Foliar symptoms as described by Larignon and Dubos were never recorded in Hungary nor are they recorded there now (Mugnai, personal communication).

The findings of Cristinzio (1978) on the other hand were as follows: reddening and curling of the leaf blades with loss of turgor; darkening of the xylem and parenchyma tissue of the stems and shoots, sometimes extending for the entire length of the organ. All plant growth over the darkened areas comes to a halt and the vine often dies in its entirety in the space of two years.

Rovesti and Montermini (1987) were the only researchers whose findings resembled those of Larignon and Dubos (2001a, b). They reported that BDA-infected vines had leaves with wine-red areas distributed uniformly over the leaf blade or located between the leaf veins; in other cases the leaves suddenly turned yellow and often wilted

though they remained attached to the shoots; and the grape berries gradually shrivelled. All these symptoms affected the vines in part or in their entirety, and woody organs of two years or older exhibited darkening of the wood cylinder, diffuse or limited to sectors of the xylem.

On the basis of the studies mentioned, then, there are four descriptions for the disease supposed to be BDA.

Despite what has been said, the present authors can state on the strength of experience extending over many years, that esca manifests itself in vines with a range of possible symptoms, of which the tiger-stripe design is the most typical (Fig. 1). The question that therefore presents itself is whether the BDA that was found in France was not simply one of the many possible manifestations of esca. Otherwise the assumption would have to be that vines certainly infected with esca can show esca symptoms one year, and BDA symptoms the next, or alternatively that the symptoms of one disease, BDA, can develop and turn into the typical symptoms of another, namely esca (as suggested by Lecomte *et al*. [2005] examining individual leaves in a season). It seems therefore that specific factors which we hope will soon be identified determine not only the onset of the leaf symptoms of esca, but also the particular form that those symptoms will take. Perhaps the symptoms seen on the vines are the expression either of different fungi (*P. chlamydospora*, different species of *Phaeoacremonium* and of *Botryosphaeria*) or of these fungi associated in different combinations in the vine wood. The vine may respond to different stimuli in the same or almost the same way. (It is a fact for example that the leaf symptoms of Pierce disease are reminiscent of those of esca.) It is also possible that the leaf and other symptoms conventionally attributed to esca are in reality not caused by wood-colonising parasites/pathogens and may have altogether different causes, acting singly or in combination (examples would be drought or mineral deficiencies).

Larignon and Dubos (2001) and Lecomte *et al*. (2005) attributed the symptoms they found on diseased vines to species of *Botryosphaeria,* and it is true that these species are often isolated, together with Pch and Pal, from degraded wood of esca diseased vines. (Larignon and Dubos and Lecomte and colleagues did not mention isolating Pch and/or Pal from the vines they examined.) However, in all the inoculation tests performed thus far the symptoms caused by *Botryosphaeria* always affect the wood, and never the leaves (Phillips, 2002; Larignon, 2004) (Table 4). It therefore seems that what was already found with the esca fungi is being repeated with the *Botryosphaeria* spp.: the wood symptoms are easily reproduced by artificial means but the presumed leaf symptoms are quite difficult. Lastly, as regards the dark stripe under the bark, a symptom that has mostly been ignored till now, and that was thought to be typical of BDA, although Lecomte *et al*. (2005) also reported it on vines with esca, it was already described in the plant pathology manual of Arnaud and Arnaud (1931) as occurring on vines infected with esca, and this was subsequently confirmed by Ciferri (1955) and Galet (1977). Moreover, symptoms resembling the dark stripe under the bark were reproduced after inoculation of Pch or Pal on grapevine spurs (Sparapano *et al*., 2001).

Table 4. Pathogenic effect of *Botryosphaeria* spp. on grapevine (from Phillips, 2002).

Symptom	*Botryosphaeria* species				
	B. dothidea	*B. parva*	*B. lutea*	*B. obtusa*	*B. stevensii*
Trunk dieback	-[a]	+[b]	-	-	-
Wood streaking	-	+++	-	+	+
Bud necrosis	-	+	-	-	+
Graft failure	+	++	-	+	-
Cane bleaching	+	+++++	+++++	+	-

[a] No symptoms.
[b] The number of plus-signs indicates the degree of severity.

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Fig. 1. Examples of symptom variability in the leaves of grapevine plants that have been classified as esca diseased for many years (average vine age: 25 years). 1–14, symptoms at the initial stage of development. 15–30, foliar symptoms at the final stage of esca, before leaf fall. Some of the symptoms may be typical of diseases other than esca. For example, leaf 25 exhibit a red or purplish color, a symptom that recalls leaf-roll virus disease. (However, with this latter disease the symptom is usually accompanied by a downward

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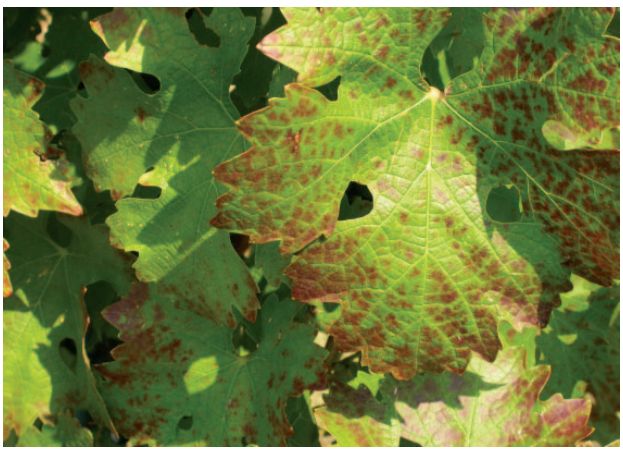

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rolling of the leaf margins and appears in late summer rather than in early summer.) Similarly leaves 4 and 17 may be confused with vines infected with verticilliosis. Should all these symptoms then be assigned to esca? Or could they have been caused by different pathogens on older vines, or by an altered physiological status of the vines? It is to be noted that all these plants also showed or had shown the typical tiger-stripe symptoms.

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The role of toxins and cavitation in the development of leaf symptoms

The reasons why leaf symptoms appear in esca-infected vines and why these symptoms sometimes do not appear for one or more growing seasons in succession are still largely unclear. Mugnai *et al.* (1999) suggested that leaf symptoms could be caused by substances that formed in degraded wood and were translocated to the leaves by sap flow. These substances could be compounds produced in the wood reacting to invasion, or they could be toxic metabolites secreted by the esca fungi (Sparapano *et al*., 1998; Evidente *et al*., 2000; Tabacchi *et al*., 2000). And, in fact, leaf symptoms similar to those of esca were reproduced when vine shoots, or detached vine leaves were allowed to absorb culture filtrates of esca fungi, or various toxins extracted from such filtrates: pullulans, isosclerone, scytalone and other naphthalenones (Sparapano *et al*., 1998, 2000; Abou-Mansour *et al*., 2004). However, these findings on toxins are only a good starting point for investigating what causes leaf symptoms, since the mere presence of biological active compounds in the culture filtrates of Pch and/or Pal, or the phytotoxic effect that they cause on vine leaves, or even their isolation from vine tissues (vivotoxin) does not necessarily mean that they also participate to the process of symptom formation.

To explain the formation of leaf symptoms other hypotheses have been formulated. For example, Lecomte *et al.* (2005) postulated that the external symptoms of esca could be due at least in part to processes of cavitation, in which the xylem conduits become filled with air, or cavitated, and hence non-functional. Cavitation was assumed to occur as a result of water stress, or at precise points of the plant where a weakness occurred: where the vine branch was bent and wounded in training; where sap flow was hampered by the scarring areas formed by pruning, etc. It is quite likely that interruptions in the water flow trigger leaf wilt (and thus apoplexy), but could cavitation and its consequences alone cause the particular foliar symptoms of esca? It is of course always possible in exceptional circumstances such as apoplexy, that the entire hydraulic system of the vine may collapse, but it seems equally likely that water stress of varying degrees, if and when it occurs, is merely one of the concurring factors causing leaf symptoms in esca diseased plants (see also next paragraph). It has been shown that the appearance of leaf symptoms is favoured by rainfall events (Surico *et al*., 2000; Marchi *et al*., this issue; see also below), especially at particular developmental stages of the vine plant, but exactly how rainfall acts on vines is still unclear. Finally, it should be added that some researchers have advanced the idea, for which there is as yet no experimental evidence, that a vine must become re-infected every year anew to show leaf symptoms. This rather unlikely, although stimulating hypothesis is now being tested in some laboratories.

Vineyard age and symptom expression

An old dictum about esca is that it becomes widespread only in old vineyards. This statement is still true: the incidence of esca increases with vineyard age and eventually levels of manifest esca (that in diseased vines whenever they show foliar symptoms) plus hidden esca (that in diseased vines whenever the vines remain asymptomatic in a year) (see Marchi *et al*., this issue) may exceed 70–80%. In the past it was thought that high levels of esca occurred only in vineyards of 25–35 years old; but such high levels are now already found in much younger vineyards. The disease therefore begins sooner in the vineyard now, and the higher incidence is reached earlier. As for example (Fig. 2) cumulated esca incidence values (calculated as the percentage of plants that each year, on the basis of data collected also in previous years, are known to be affected by esca in a given vineyard) estimated for 3 Italian vineyards, CBSI4 (province of Siena), TB (province of Ravenna) and ISO-A (province of Florence), 18, 13 and 17 years old in 2005, were 50, 30 and 51% , respectively. In the case of vineyard ISO-A and, to a lesser extent, in that of CBSI4, linear regression analysis indicates that the trend of the increase of esca incidence over time may be represented by a fitted straight line, although, in certain years, there could be a departure from linearity (Time 3 and Time 8 for vineyards ISO-A and CBSI4, respectively). The earlier onset of esca has been, or can be, attributed to the production of already infected rooted cuttings in the nursery, and/or to the occurrence of greater amounts of inoculum in nature which increases the likelihood of infection both initially in the nursery, and during the early years of vineyard life.

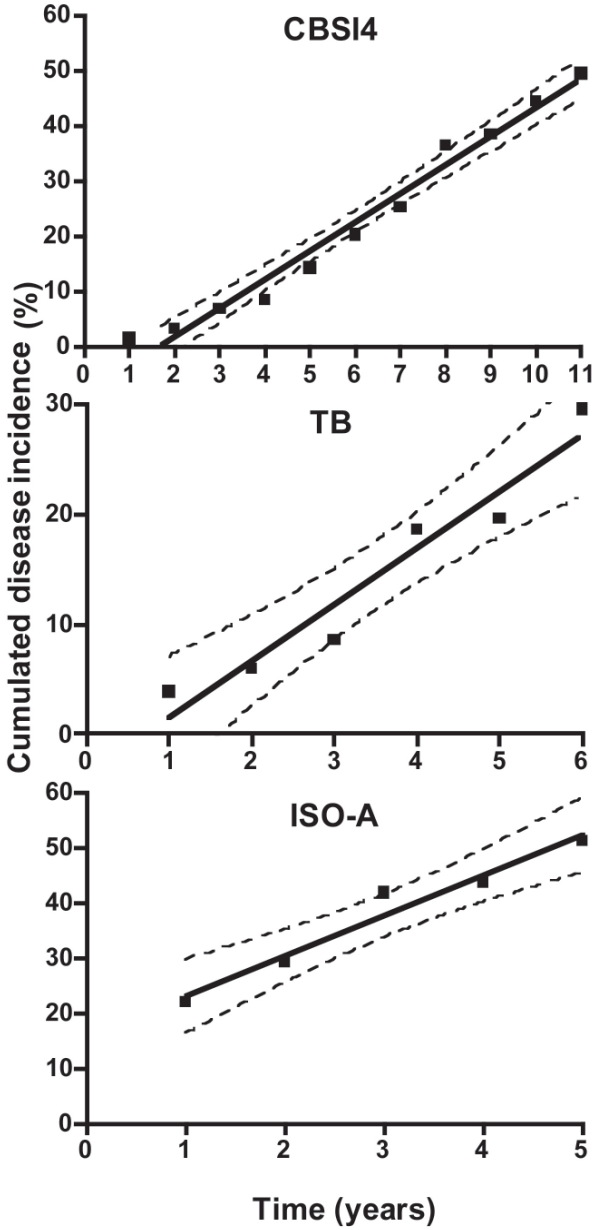

Fig. 2. Increase in cumulated esca incidence (see text for description) over time, estimated in 3 vineyards at Siena (CBSI4; 8-year-old at the beginning of the survey; surveyed for 11 consecutive years), Ravenna (TB; surveyed for 6 consecutive years from the year of plantation) and Florence (ISO-A; 13-year-old at the beginning of the survey; surveyed for 5 consecutive years). The fitted lines (straight line) and relative 95% confidence intervals (dotted lines) were obtained by linear least square regression analysis (R^2=0.97, F=343, P<0.0001; R^2=0.93, F=60.3, P<0.002; R^2=0.96, F=70.2, P<0.004 for data collected in vineyards CBSI4, TB and ISO-A, respectively).

While the correlation between esca and vineyard age has become less clear-cut, the same cannot be said of apoplexy, a sudden and rapid wilting, which is still mainly restricted to older vines, in which it is quite common, particularly in seasons that are hot and dry, but is altogether an exception in younger vines (Table 5). (When apoplexy strikes younger vines it is usually for reasons that have little or nothing to do with esca.) This suggests that the cause of leaf symptoms in esca is, or may be, different from the cause of apoplexy: the latter being more closely linked to water stress. In old vines that have already shown leaf symptoms for a number of years, white rot and dark necrosis often affect large portions of the vine including the ring of vessels that are of most recent formation and therefore most active in axial sap transport (Fig. 3). In those cases it is reasonable to suppose that the plant hydraulic system is already seriously damaged and therefore more sensitive to any water imbalances that may arise during the growing season. But when, for example, a young 3–4 year old vine shows leaf symptoms the signs of wood deterioration are often very slight, and most likely not serious enough to impair the overall functionality of the sap flow system of the vine. On the other hand it is well known that the vine, a plant with diffuse porous wood, has the capacity to repair in some way the negative effects of water imbalance because of defence systems consisting of a high root-pressure in spring; a fairly high proportion of vessels with a diameter less than 40 μm, and therefore less susceptible to cavitation, and with a more effective control of gas exchange in the vessels, etc (Tibbets and Ewers, 2000; Stevenson *et al*., 2004). Thus, isolated cases of cavitation might not have a serious impact on vine functionality and the formation of leaf symptoms. In conclusion, what can be said is that esca-diseased vines may suffer negative effects because of:

1. the production of phytotoxins that accumulate in the leaves;
2. the reduction in water conductivity as a result of:
   a. the growth of tracheomycotic fungi in the vessels, leading to their obstruction, with the concomitant production of pectinolytic enzymes;
   b. the formation of tyloses and gums produced by the vine;

Table 5. Frequency of apoplectic strokes ([No. of apoplectic plants /No. of symptomatic plants]×100) in vineyards of varying age surveyed in Tuscany in 2005.

Vineyard	Location	Age	Cultivar	Apopletic strokes (%)
CAL3	Calenzano (PO)	4	C. Sauvignon	0.0
MON19	San Casciano (FI)	11	C. Sauvignon	0.4
CAL2	Calenzano (PO)	15	C. Sauvignon-Syrah	0.0
CBSI4	Siena	18	C. Sauvignon	1.0
LAT1	Laterina (AR)	20	C. Sauvignon	4.8
CAL1	Calenzano (PO)	35	Sangiovese-Merlot	3.8
CAR1	Carmignano (PO)	>35	Sangiovese	6.8

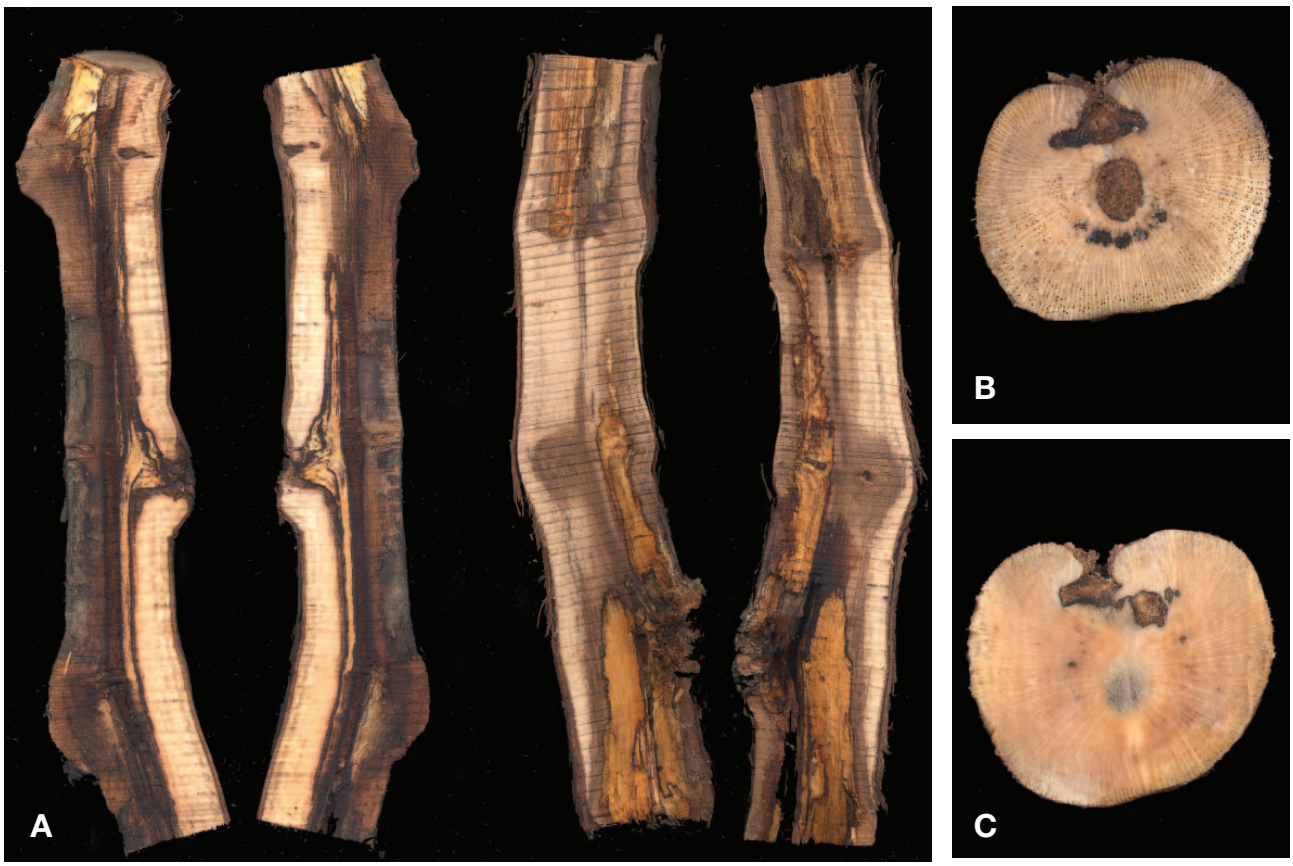

Fig. 3. A. Longitudinal sections of portions of trunk from grapevines which were symptomatic in the year they were uprooted. The figures show large portions of the wood with white decay and dark necrosis. In these vines the upward movement of the sap is through a very small portion of still functioning wood. B and C. cross sections of a trunk from grapevines which were symptomatic in the year they were uprooted. The figures show small areas of necrotic and rotted tissues near injuries caused by sucker removal.

c. cavitation of the vessels.

3. other stress factors such as drought and mineral deficiencies, or the occurrence of other disease agents.

Which of these factors are more directly involved in the formation of foliar symptoms?

Esca and climate change

The current epidemic spread of esca can be traced back roughly to the early 1980s. At that time the national and regional plant-health balance sheets in Italy and France began to show increases in the incidence of esca after it had been stable for years. Specifically an alarm was sounded in Tuscany in 1986 when an incidence of 10–15% of withered vines was recorded. At the time these high losses due to esca were attributed to the hard frost that had occurred in 1985, but upon a closer examination of the phenomenon it was generally concluded that the increase could also have had other causes as well such as: the lack of specific esca-control practices since 1977, when the use of sodium arsenite was banned from Italian agriculture; the increasing use of selective fungicides in viticulture; new techniques of nursery material production; the introduction of new grapevine cultivars and rootstocks, and new cultural practices; and the increasing use made in the '70s of low-quality nursery material (Surico *et al.*, 2004). Changes in climate may also have exacerbated the esca problem. In particular, variation in precipitation: not only the number and intensity of rain events, but also, and perhaps more importantly, the distribution of rainfall throughout the year. The incidence of manifest esca in a given year seems to be correlated with the amount of precipitation in the spring and summer period of that year (see Marchi *et al.*, this issue). (This suggests, if it is true, that the reduction in water conductivity - see above - may not be associated with the appearance of foliar symptoms.) On the other hand the 1980s were an unusually dry period in Italy — with an estimated shortfall of 340 thousand billion litres of rainwater in that time span — and it was precisely in that period that the incidence of esca increased, without its being clear whether this increase in esca concerned the foliar symptoms or apoplexy. Here it is important to stress that to avoid basing conclusions on inappropriate data, the meteorology equipment used should always be located right in the vineyards being surveyed.

Sources of inoculum, inoculum spread and infection

Trials in California, France, Australia and South Africa have established that propagules of Pch and of various species of *Phaeoacremonium* are easy to find on vine trunks, shoots and berries as well as on old tendrils that have remained attached to the training wires (Eskalen *et al.,* 2004). A Californian trial found that Pch spores were dispersed mainly in October/November and in April, and occasionally in other months, if these months were marked by a rain event(s) and at a wide range of daily temperatures, varying from as low as 5°C in winter to 20–25°C (Gubler *et al*., 2004a). In France, on the other hand, Pch spores were captured all the year round, even in winter, and the optimal conditions for spores to be dispersed by air and to infect pruning wounds were when average temperatures ranged from 7 to 15°C with a maximum of 12–18°C when accompanied by rain events (Larignon and Dubos, 2000). Pal, on the other hand, whose infectiveness has been studied only in France, did not seem to infect pruning wounds. Pal spores have never been captured in winter, only in periods that in some years ranged from early March to the first week of April, but more often from mid-May to mid-June (Larignon and Dubos, 2000).

Pruning wounds are more susceptible to fungal infection, and remain susceptible over longer periods of time when pruning is done early in winter (December, January). The infective period for Pch and Pal is not known in Italy and that for Fmed it is not known anywhere in the world. The fruiting bodies of Fmed (from which the basidiospores derive) form almost exclusively on very old vines, but Fmed carpophores have been found on many other plants besides grapevine, including some of the most characteristic plants of the Mediterranean area such as *Olea europaea, Acer negundo, Lagerstroemia indica, Actinidia chinensis, Corylus avellana, Laurus nobilis, Ligustrum vulgare, Quercus ilex, Cornus mas, Robinia pseudoacacia, Rhamnus cathartica, Salix caprea, Syringa vulgaris, Sorbus aucuparia,* and probably others (Fischer, 2002; Fischer, Mela and Mugnai, unpublished data). It

seems reasonable to assume that Fmed inoculum enters the vineyard from external sources such as very old vineyards located nearby or carpophores that have developed on other host plants (although it is not yet clear whether strains of Fmed from other plants are also pathogenic on grapevine). Within the vineyard it has been established that the disease is not transmitted between vines with tools used for pruning, although in theory this would be possible (Surico *et al.,* 2000).

Fungi are thought to invade the vine primarily through pruning wounds. However, a careful study of vines less than 10 years old that had esca symptoms showed that these vines often had dark streaks that started from those sites on the vine from which suckers had been removed (Fig. 3 B), an operation that is usually carried out in early summer. Vines of this age sometimes showed small rotted areas at the graft union starting from wounds also caused by sucker removal (Fig. 3 C). The dark streaks in these vines suggest that Pch and Pal spores are spread by air in late spring and at temperatures higher than 15–18°C.

Conclusions

When Larignon published his PhD thesis on esca of grapevine in 1991 he probably did not imagine that his thorough research study, which he carried out under the guidance of Bernadette Dubos, would mark the beginning of a new round of investigations into the disease, after a first round carried out primarily in France in the 1920s and 30s. The new studies were also a response to an urgent situation that had arisen, and still exists, in vineyards all over the world. In his thesis Larignon restated the old view of esca: that it was basically a disease that could be identified with the wood rot caused by *Phellinus igniarius,* or, more rarely, *Stereum hirsutum*. To this model of esca Larignon applied the CODIT model of rot development in forest trees. Later studies taking their clue from the work of Larignon and Dubos (1987, 1997) have determined with greater accuracy the taxonomic position of the fungi involved in the disease (in the process establishing two new genera, *Phaeoacremonium* and *Phaeomoniella*, and redefining the taxonomic position of the most known and widespread wood rotting basidiomycetes, now named *Fomitiporia mediterranea*), and have postulated that the course of the disease could be divided into a number of distinct stages, depending on: 1. the age of the vine; 2. whether the three main esca fungi (*P. chlamydospora, P. aleophilum, F. mediterranea*) acted alone, together, or in succession; 3. other factors, internal and external to the plant and still largely unknown.

In this way a total of five syndromes linked to esca have been identified: 1. dark streaking of rooted vine cuttings; 2. Petri disease; 3. young esca; 4. white rot; 5. esca proper. Here we thus propose anew the terms young esca and esca proper. Esca proper is closer to the old idea of esca, identified with white rot and apoplexy, and could indeed be called simply 'esca'. Young esca on the other hand is basically a name assigned to vines that have tiger stripes but without wood rot, and has had a more doubtful reception from researchers. However, these vines are like vines with esca proper in that they have leaf symptoms and dark wood streaks and are colonised by Pch/Pal. These points of similarity seem to warrant giving these two diseases a name that points up their relatedness. Alternatively, young esca could be called 'Phaeotracheomycosis of grapevine', a name derived from the two main causal fungi, *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum,* and from the fact that both are tracheomycotic fungi.

The disease model thus postulated is a dynamic one, in which the disease gradually moves towards full development as the vine matures, and as the various fungi involved in it become operative (or active by abandoning their endophytic stage). These fungi (which may include some species of *Botryosphaeria*) perhaps already invade the vine when it is still only a shoot on the mother plant. However, it would be rash to think that the vast quantity of experimental data on esca pathology collected in recent years in Australia, France, Germany, Italy, New Zealand, Portugal, South Africa, Spain, the United States and elsewhere, have all been assessed and interrelated correctly. Much of the information about esca, including what has been reported here, remains unclear and open to different or subsidiary interpretations. What is certain is that esca is a complex disease and that it will continue to be a focus of research activity for many years to come, as it is also suggested by the incompleteness of the information that we have on some phytopathological phenomena related to

the esca disease *sensu latu* and by the fact that several aspects of esca (in particular the expression of leaf symptoms) can often be considered from different points of view and lead to conflicting explanations.

This article has not dealt with attempts to control esca in the laboratory and in the field. The time has not yet come to offer effective strategies and means of control to viticulturists and nursery operators (hot water treatment may be one solution of interest to the latter [Waite and May, 2005]). However, some research studies are now in progress and it is not too much to expect that this aspect too will soon see interesting new developments.

Acknowledgements

Research study commissioned from ARSIA-Toscana (Regional Agency for Development and Innovation in Agriculture and Forest) by fourteen administrative Regions and one autonomous province, and financed with funds provided by the Ministero per le Politiche Agricole e Forestali (Ministry for Agriculture and Forestry Policy) to implement the inter-Regional Project 'Grapevine esca: research and experiment in the nursery and in the field for prevention and cure.'

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Accepted for publication: December 22, 2005