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## Chapter

# Overview of the Esca Complex as an Increasing Threat in Vineyards Worldwide: Climate Change, Control Approaches and Impact on Grape and Wine Quality

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## Abstract

Esca is an increasing threat to global viticulture causing significant losses by reducing yields, declining or wilting vines, and shortening the productive life of vineyards. Recent findings indicate that the disease may also affect the quality of grapes and the chemical composition of musts and wines. However, more research in this field is needed. Esca seems to affect the ripening process of grapes resulting in lower sugar content, higher acidity, and increased nitrogen concentrations. Regarding polyphenolic compounds, reduction on the concentrations of (+)-catechin, (-)-epicatechin, anthocyanins, and tannins has been observed due to the alteration of flavonoid metabolism. Esca is a complex-chronic disease, where several fungal pathogens act simultaneously or successively, to cause necrosis to the vascular tissues of grapevines by blocking the xylem vessels and by producing enzymes and phytotoxic metabolites. As genotype affects stress response, specific *Vitis vinifera* cultivars present higher levels of resistance to the disease than others. There is evidence that varieties such as Merlot, Grenache Rouge, and Roussanne are relatively resistant, compared with more susceptible Cabernet Sauvignon, Mourvèdre, Sauvignon Blanc, and Semillon. Another main objective of the current work was to investigate the possible effects of climate change on Esca development and propose appropriate control strategies.

**Keywords:** Esca, *Vitis vinifera*, plant stress, fungal pathogens, grapevine, wine quality, must composition, climate change

## 1. Introduction

Grapevine trunk diseases (GTDs) are an increasing threat to global viticulture [1, 2]. The causal agents of GTDs belong to diverse groups of both ascomycetes and

basidiomycetes that colonise mainly the vascular tissues of grapevines, interfering with plant physiology, microbial ecology and activating plant response mechanisms [3, 4]. The Esca complex is considered as one of the most common and catastrophic wood diseases of the European grapevine "*Vitis vinifera* L." [5]. Losses and damages caused by the disease are currently in an increasing rate, especially in warm viticultural regions. Therefore, it might be assumed that climate change influences the development of Esca and the responses of the host towards the pathogens [6]. Mature vines (over 15 years old) present greater possibility to be affected by Esca and other GTDs [7]. It is generally known that old vines are responsible for the production of some of the world's finest wines, expressing high quality and typicity according to their region [8]. Therefore, Esca is considered as a serious threat, especially in regions where the production of wine from old—usually ungrafted—vines is economically important. Other forms of the disease occur in young grapevines and are usually associated with contaminated propagating material [9, 10]. Esca is considered as a complex disease, as it is caused by the action of several fungal pathogens infecting the vines in various manners. However, it has been proposed that Esca can also be described as a complex of different diseases that coexist on the trunk of mature grapevines [11, 12]. Some issues regarding aetiology, epidemiology, the role of pathogens and the exact factors that influence the development of the disease remain unclear.

Typical symptoms of Esca include inner wood necrosis, either as brown-black streaks caused by ascomycetes (although teleomorphs are not known for all these fungi), or as white rot (a yellowish-white discoloration of the wood, which is soft, spongy and brittle) caused by basidiomycetes, while in the leaves, peripheral and interveinal chlorosis, which results in necrosis (tiger stripes), is often observed in the summer due to insufficient water supply and the presence of phytotoxic metabolites [13, 14]. However, the latter symptom is often described as a separate disease within the Esca complex and is called "grapevine leaf stripe disease" (GLSD). In severe cases, gradual necrosis of arms may be observed or even sudden wilt and death of the entire vine (apoplexy). Esca is found in most viticultural regions worldwide, and the disease is believed to occur for as long as grape growing exists [11].

Climatic and soil parameters significantly affect the frequency of Esca-related symptoms and implications [15–17]. Drought and temperature are the most crucial abiotic factors for the enhancement of the pathogens affecting growth rates, propagule germination and the rates of inoculum production [18]. Intense heat and drought increase the respiration rates of plants, causing carbon losses and even plant death [19, 20]. In general, water availability induces modifications of vessel diameter in various plant species [21, 22]. The worsening of the symptoms observed in Esca-infected plants may be partially due to the size of the vessels that have been formed by altering water supply [23]. Research on the possible pathological nature of Esca began in France in 1898 [24]. However, great progress and additional findings regarding aetiology, pathogenesis and diagnosis have been revealed only recently. Abiotic factors such as climate, soil type and topography as well as biotic factors such as age of the vines and cultivar (even clone) appear to affect disease incidence [2, 7]. Further research is needed to thoroughly investigate all the aspects of aetiology, ecology and conditions that favour the development of the disease, as this knowledge can contribute to designing control strategies, while it is known that conventional chemical control is not easily applicable in the case of Esca [17, 25, 26]. Moreover, the impact of climate change needs to be addressed, as Esca is an increasing threat in vineyards worldwide and water stress seems to play a role [27].

## 2. Pathogens - Syndromes

A complex of several fungi coexists to variable levels in inner vessels, causing certain syndromes. It is commonly agreed that two species of ascomycetes, *Phaeoacremonium minimum* (previously known as *Phaeoacremonium aleophilum*) and *Phaeoconiella chlamydospora* as well as one species of basidiomycetes, *Fomitiporia mediterranea*, are the predominant fungi involved in the development of Esca on a global scale [28, 29]. *P. minimum* and *Pl. chlamydospora* are responsible for the brown streak syndrome of the wood, while wood decay (white rot) is caused by *F. mediterranea* and other basidiomycetes. Nevertheless, it has been found that many more fungal species are involved in the Esca complex or in Esca-related symptoms and damages. In addition to *F. mediterranea*, other Basidiomycetes have been reported by Fischer and González-García [30] either as primary pathogens (*Inonotus hispidus*) or as secondary infections developing on already dead tissues of grapevine trunks, causing white rot (*Stereum hirsutum*, *Trametes hirsuta* and *Schizophyllum commune*). *F. mediterranea* is a newly found species [31], identified after molecular analysis was conducted to isolates that were previously believed to belong to *F. punctata*. Those isolates differed from *F. punctata*, and therefore, a new species *F. mediterranea* (M. Fischer) was established and believed to exist widely in grapevines across the Mediterranean basin [29, 32]. Different *Fomitiporia* species have been associated with Esca in north America (*F. polymorpha*), Australia and New Zealand (*F. australiensis*), while other basidiomycetes (*Inocutis jamaicensis* and *Fomitiporella vitis*) have been found to cause white rot in South America [33]. In South Africa, a diverse group of basidiomycetes was found in grapevine trunks, including fungi of the genera *Inocutis*, *Inonotus*, *Phellinus* and *Fomitiporia* [34, 35].

Regarding ascomycetes, a diversity of fungi, mainly within the order Diaporthales, exists in grapevine trunks. However, *P. minimum* and *Pl. chlamydospora* are considered as the most common species in both Esca and Petri disease. Many *Phaeoacremonium* species have been characterised as vascular plant pathogens, producing enzymes and phytotoxic metabolites, causing dieback and wilt to several woody hosts [36, 37]. Multiple genotypes of *P. minimum* can be found within the micro-environment of a single vineyard [38]. At least 24 species of the genus *Phaeoacremonium* have been reported to infect grapevines in a global scale [39]. In South Africa, there is extended diversity of *Phaeoacremonium* species probably due to the large flora diversity, and newly found species, include *P. album*, *P. aureum*, *P. bibendum*, *P. gamsii*, *P. geminum*, *P. junior*, *P. longicollarum*, *P. meliae*, *P. oleae*, *P. paululum*, *P. proliferatum*, *P. rosicola* and *P. spadicum* [37]. Most of those species have been found in grapevines and to lesser extent in other tree crops.

According to a classic definition [12], diseases within the Esca complex are distinguished into five typical syndromes:

- a. *Brown wood-streaking*: This form causes damages to rooted cuttings and root-stocks, usually in nurseries. It is associated with Ascomycetes of the genus *Phaeoacremonium* (or related genera), often without showing external symptoms.
- b. *Petri disease*: It is caused by the early infection of propagating material or young grapevines by species of *Phaeoacremonium* or related genera.
- c. *Young vine Esca*: Fungi of the genus *Phaeoacremonium* infect young grapevines (up to 8–10 year old) through wounds, and they cause black or brown

wood-streaking and vascular gummosis inside the trunk, with or without foliar symptoms.

- d. *White rot (Esca)*: When infection through wounds is caused exclusively or mainly by Basidiomycetes (such as *F. punctata*, *F. mediterranea*, etc.), a spongy wood decay (white rot) occurs, which may or may not be associated with leaf and fruit symptoms.
- e. *Esca proper*: White rot develops in the trunk of old vines, simultaneously or after the development of brown wood-streaking. This full-scale syndrome is caused by the combined or sequential action of both ascomycetes and basidiomycetes.

### 3. Geographical distribution of Esca

Grapevine trunk diseases, especially Esca, are increasing in incidence and severity within vineyards and nurseries worldwide [3] and threaten the wine industry globally [40]. It has become increasingly devastating during the past three decades and represents today a major concern in all wine-producing countries [41]. Over the past two decades, GTDs (especially the Esca complex) have been reported in European, American and South African vineyards [35, 42]. A survey on GTDs was carried out during 2015 and 2016 in 18 European and four Mediterranean countries on a regional and a national level. Result showed that both chronic form and apoplexy occurred in all the surveyed countries [42]. However, not all the viticultural regions are affected equally. A dramatic increase of the disease has been reported in some Mediterranean regions such as Tuscany, where more than 50% of the vineyards have a disease incidence ranging from 20% to 30% [43]. Similar observations have been made for France, Greece or Portugal [11, 44–47]. The estimated annual increase is estimated to be 4–5% [11].

In many regions, implications caused by Esca have a substantial impact in viticulture and wine production. It has been recently observed that Esca affects numerous grapevines in the volcanic island of Santorini (Cyclades—Greece) and many more Greek islands including Crete. Santorini is considered as the most important white wine region in Greece, producing distinctive and terroir driven wines from old ungrafted vines (up to 250 year old) on a phylloxera safe volcanic soil. Increasing losses and damages have been recently reported in many regions across the Mediterranean basin. Nevertheless, current research indicates that Esca is an increasing phenomenon on a global scale, especially in wine regions that depend on the production of wines from mature vines. Esca is also gaining increasing importance in Central European wine-growing countries [32].

### 4. Infection - Epidemiology

Infections are mainly caused by fungal spores produced on infected or dead vines and transmitted via wind or rainfall. Spores usually invade their host through wounds created by various causes such as pruning, severe frosts and mechanical damages to the trunk or root system [48]. However, pruning incisions are considered as the main portals of infection [15, 49]. It has been recently recorded that arthropods, such as millipedes, ants, spiders or even beneficial predatory insects, can vector pathogens related to Esca and other GTDs, via wounds of freshly pruned grapevines [50]. Regarding the forms of the disease that occur in young grapevines, the infection is

usually associated with the use of contaminated propagating material or insufficient hygiene conditions and practice in nurseries [9, 10].

*P. minimum* and *Pl. chlamydospora* are characterised by their aerial dispersion [51, 52]. The release of *Pl. chlamydospora* spores is associated with rainfall, while *P. minimum* occurs during the bud break period without the presence of rainfall [53, 54]. Sources of infection have been observed in protected areas of wood within deep cracks [55, 56]. Both *Pl. chlamydospora* and *P. minimum* can also invade their hosts via grafting or spread through contaminated plant material [9, 57]. These infections are associated with Petri disease, but it is possible that the pathogens remain in a latent form and appear later for the development of Esca proper. As *F. mediterranea* and *P. minimum* reproduce sexually, basidiocarps and perithecia, respectively, may represent sources of inoculum in the field [38, 43, 56]. Many studies indicate the existence of pathogens that do not directly manifest the disease (latent infection). Factors that affect pathogenesis on *V. vinifera*, as well as the appearance of symptoms, include age of vines, training system, size of the pruning wounds, time of pruning, climatic conditions and susceptibility of certain varieties.

Esca is considered as a catastrophic, non-selective grapevine disease, and remedies such as specialised fungicides or totally resistant varieties do not exist [17, 58]. Nevertheless, various *V. vinifera* cultivars present different levels of susceptibility or resistance to the pathogens. In a study conducted in Greece, 'Agiorgitiko' and 'Soultanina' displayed susceptibility, while 'Assyrtiko' and 'Xinomavro' showed resistance to *Pl. chlamydospora* [59]. However, in homologous experiments that took place in Spain, all the varieties (including 'Godello', 'Albarín Blanco', 'Doña Blanca', 'Pan y Carne', 'Palomino', 'Zamarrica', 'Mencía' and 'Brancellao') were susceptible to the fungus [60]. Moreover, the major Spanish red grape Tempranillo is known to be very susceptible to most types of Esca [17]. In previous bioassays conducted near Siena, Italy, several *V. vinifera* varieties were tested in terms of symptom severity. Results indicated that the 17 vine cultivars were categorised into four groups of varying susceptibility to Esca. Semillon was shown to be the most susceptible cultivar, while Roussanne was the most resistant [61]. The above study also suggested that various rootstocks can affect susceptibility to Esca. In other experiments, Esca caused reduction (about 70%) in carbon assimilation and stomatal conductance. These effects were greater in varieties such as Cabernet Sauvignon and Sangiovese compared to Trebbiano [58]. Regarding table grape varieties, a previous study indicated that *V. vinifera* cv. Matilde was more resistant compared to *V. vinifera* cv. Italia, in terms of internal symptoms' severity [62]. Thompson seedless was also evaluated as relatively susceptible [63]. Even at a clonal level, there might be a difference on the expression of the disease. A study in France confirmed a clone-dependent expression of Esca in Chardonnay [2]. Overall, important international wine-grape varieties such as Cabernet Sauvignon, Sauvignon blanc, Trebbiano, Mourvèdre and Cinsault are more susceptible to Esca compared to the relatively resistant Pinot Noir, Merlot, Grenache, Carignan and Roussanne [55, 63–65].

## 5. The effects of climate change on Esca development

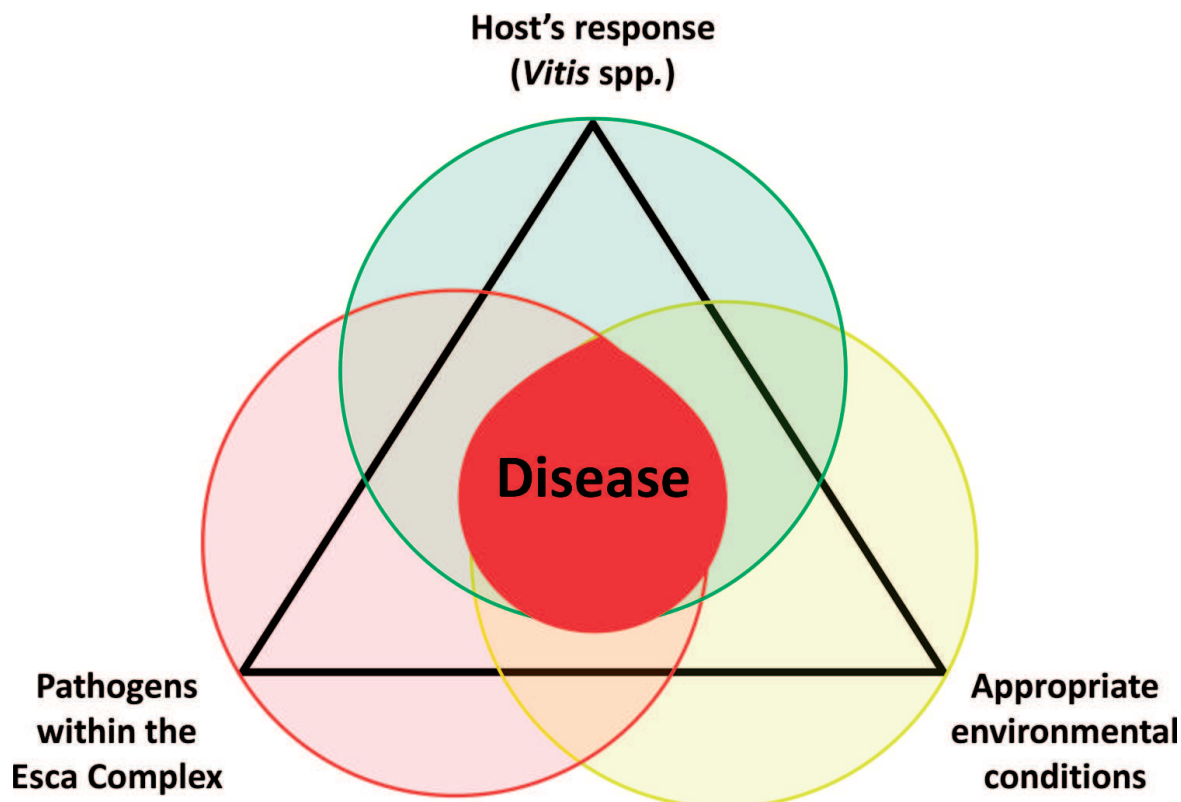
Pathogens may be established in grapevines at early stages but remain in a latent form. The impact of the disease becomes greater as the vines mature. Older plants also experience more infection cycles via pruning wounds [7, 66, 67]. Since the time

of trunk infection, and until the onset of symptoms, the disease progresses slowly. As latent pathogens, fungi involved in the development of Esca begin their life cycle inside plant vessels but without causing any symptoms. Years post infection, fungi become more invasive resulting in symptom appearance [68].

Abiotic factors, such as environmental conditions, are also recorded to significantly influence the development of the disease and the time of symptom manifestation [15, 16, 58]. Spore infections are favoured by intense humidity. Since most Esca-related fungi are anaerobic, intense oxygenation inhibits their growth, while carbon dioxide favours it. The optimal growth temperature for most of those fungi is 20–25°C. Research regarding the effects of abiotic factors on Esca was conducted at three commercial Tempranillo vineyards located in three different wine regions of Spain [17]. Those viticultural regions differ with each other in terms of average annual temperature, lowest and highest temperatures during the coldest and warmest months, respectively, climate classification and planting arrangements. The area that presented the highest vine infection (30%) is characterised by relatively high annual temperature and quite high temperatures during the hottest months, but normal lower temperatures in the coldest months. The climate of the region was described as warm, extremely humid with hot summers. The planting distance was 2 × 3 m (low planting density).

Symptomatic vines often present significantly lower leaf photosynthesis rates [69, 70]. A progressive decline in net photosynthesis was observed in asymptomatic leaves from symptomatic shoots [71]. Reduced photosynthesis at the early stages of GLSD symptoms might be a result of stomatal closure and can be magnified under water stress and high-temperature conditions. Alterations in carbohydrate physiology of grapevines may begin with various stress types such as low temperatures, drought or chemicals and can lead to considerable disorders of carbon sustenance, as a result to Esca infection. Studies indicate that highly infected grapevines may perceive some signals and react precociously by reducing photosynthesis and by triggering various defence mechanisms [71, 72].

Most Esca symptoms appear on vines during summer, where transpiration rates and demand for water increase [48, 73]. It is known that when plants are exposed to various stresses, the expression of several genes is exorted. Consecutively, enzymes and hormones with multiple biological functions are produced due to environmental stimuli. Therefore, toxicity from the action of pathogens, host defence responses, and environmental conditions compose the disease triangle [6] (**Figure 1**). Exceptionally hot and dry weather periods have been repeatedly recorded worldwide during the past two decades. Among these, the most remarkable in Europe have been the summers of 2003, 2006, 2011 and 2018. Most infected plants remain symptomless externally for years after infection. Appearance of external symptoms is not directly related to inner wood decay and may be associated with cultivation practices or environmental stresses such as high temperature, water shortage or overstock. Water stress has been proven to significantly influence characteristics such as shoot length, stem weight, expression of wood symptoms and plant survival, when young vines were artificially inoculated with conidial suspensions of *Pl. chlamydospora* [74]. Moreover, in the case of *V. vinifera*, water stress has been recorded to eventually alter the size of vessels and to affect xylem hydraulic conductivity, resulting in reduced water flow rates [22, 23, 75]. This is crucial for the outbreak of the symptoms. Foliar symptoms may also be linked to stress-related pathways of *V. vinifera*, which are stimulated during water, salt and oxidative stresses [76]. However, the effects of environmental stresses on the transcriptional responses of grapevines to the Esca complex have not been fully explored yet. Researchers



**Figure 1.**  
*Depiction of the disease triangle (combined factors that cause the disease) in the case of Esca.*

investigated the role of water stress-related genes while studying the early events that take place before apoplexy [72]. None of the tested genes was affected in visually healthy pre-apoplectic leaves, but they were down-regulated in drying leaves. Distribution and symptomatology of most GTDs are associated with the exposure of vines to different climatic conditions [15]. The influence of heat and water stress on grapevine physiology was recorded in the case of *Eutypa dieback* [77]. Back to the case of Esca, correlation between rainfall, high temperatures and the severity of GLSD symptoms was observed in recent studies conducted in Italy [16].

## 6. Detection methods

It is important to use detection methods to detect the presence of Esca pathogens before the onset of symptoms (**Table 1**). This will enable grapevine nurseries to stop or at least limit the spread of infection. Using detection methods, it was reported that infection may take place in nurseries during the propagation process [78], storage [79] or due to the use of infected mother plants [80, 81], while rootstocks used for propagation have been reported to harbour Esca pathogens [82, 83]. Tools used in nurseries such as hydration tanks, grafting tools and callus media [84–86] were also shown to spread infection. Therefore, detection methods will help to improve the sanitary measurements to avoid contamination in these facilities. Molecular detection tools can also provide markers for Esca pathogens. These molecular markers can be used as tools in taxonomy allowing the discrimination on strain level [87]. Examples for such tools are sequencing of the internal transcribed spacer region (ITS) [29, 31, 88], restriction fragment length polymorphism (RFLP) [43, 88] and sequence-characterised amplified region (SCAR) [89]. Molecular markers shed light on the genetic variation among



Detection and control of Esca		
Detection methods	Molecular	Physical
	Molecular markers: ITS, RFLP, SCAR, RAPD	Spectral: hyperspectral, multispectral
Control methods	Prevention in nurseries	Prevention in vineyards
	<ul style="list-style-type: none"> <li>• Chemical treatment of cuttings</li> <li>• Hot water treatment of cuttings</li> <li>• Antagonist treatment of cuttings</li> </ul>	<ul style="list-style-type: none"> <li>• Use of clean planting material</li> <li>• Removal of infected parts</li> <li>• Optimizing pruning method (method and period)</li> <li>• Treatment of pruning wound chemical or biologically</li> </ul>
	Curative measures in vineyards	
	<ul style="list-style-type: none"> <li>• Trunk renewal</li> <li>• Over-grafting</li> <li>• Trunk surgery</li> <li>• Trunk injection</li> </ul>	

**Table 1.**

Synopsis of detection methods and control strategies to prevent/lessen Esca.

Esca pathogens over geographical areas as well as way of dispersion. Genetic variation among isolates of pathogens was investigated with random amplified polymorphic DNA (RAPD) [89, 90]. It was suggested that variation within species may be related to the geographic location of the isolates [90, 91]. It has also been suggested that *F. mediterranea* is spread via airborne basidiospores and that outcrossing occurs [90]. Further studies on the occurrence of *Pl. chlamydospora* in different regions as well as the use of additional markers may provide more information on introduction frequencies, geographical spread and to determine the mechanisms of inoculum dispersal [38, 92]. Another molecular tool in Esca research is the whole genome sequencing. It is considered one of the most important tools in understanding the biology of organisms. Recently, the genome sequence of the two most common and important Esca pathogens, *Pl. chlamydospora* and *P. minimum*, has been released [93, 94]. These results will reveal the genetic makeup of these two pathogens, which, in turn, will help discovering new genes involved in pathogenicity as well as markers that could further differentiate between species.

Another detection method is spectral imaging using sensors to detect foliar symptoms before being visible to the human eye using ground-based hyperspectral and airborne multispectral imaging. Since chronic symptoms do not develop every year [3], an annual monitoring is a fundamental tool to estimate disease incidence in the vineyard [95]. Leaf measurements can either be recorded in the whole spectral region (hyperspectral) or at selected spectral bands only (multispectral) [96]. According to a recent study, external symptoms could be detected pre-symptomatically; however, the authors emphasised that these results need further evaluation [95]. A similar imaging approach using RGB images was used to detect Esca symptoms. The detection accuracies were 88% and 91% for white and red cultivars, respectively [97]. The reason why spectral imaging seems to be promising is the fact that net photosynthesis rate was shown to decrease gradually in asymptomatic leaves [69, 71]. However, it is not known so far how early can spectral imaging techniques detect Esca infection.

## 7. Conventional and modern control approaches

Control strategies against Esca mainly include preventive measures and cultivation practices (**Table 1**). In many wine regions across the Mediterranean Sea, sodium arsenite was extensively used as a chemical control mean. However, sodium arsenite is now banned in most countries. Other compounds, such as copper oxychloride, benodanil, fosetyl-Al, hydrogen peroxide, glutaraldehyde and furmetamide, have also been tested as inhibitors of Esca. Nevertheless, it is certain that wood decay is irreversible, and there are no remedies or conventional control methods to cure Esca [17, 25]. The use of a mixture consisting of calcium chloride, magnesium nitrate and *Fucales* seaweed extract resulted in a significant reduction of symptoms in Trebbiano d'Abruzzo and Montepulciano d'Abruzzo vineyards [70]. This application also seemed to increase the quantity and quality of grapes, without causing any phytotoxic effects on the berries. It is currently believed that research should focus on the use of incorporating solutions that could activate plants' natural defence responses, as well as studies of molecules that exhibit antimicrobial properties. The use of relatively resistant cultivars and healthy propagating material are considered as preventing means of great importance [48].

*Preventive measures in nurseries:* The use of fungicides to control Esca pathogens in nurseries is difficult. Chemical dips are usually used to control external pathogens, but they do not penetrate grapevine cuttings and, hence, are ineffective against pathogens inhabiting the vascular tissues [98]. Indeed, Chinosol (hydroxyquinoline sulfate) was reported to be the most commonly used fungicide; however, it was reported to be ineffective against *Pl. chlamydospora* and *P. minimum* in grapevine nurseries [99]. Treating propagation material with hot water is considered the most effective method to disinfect dormant canes during the propagation process [98, 100]. However, some reports showed negative side effects on grapevine growing from these cuttings such as poor shoot development and less rootstock vigour [101], delayed callusing and rooting of cuttings [98], delayed development or bud death in cuttings and grafted vines [99, 102, 103], and incomplete healing of graft unions and fermentation in cold storage [104]. This, in turn, leads other authors to suggest that cuttings taken from vines grown in warm climates may tolerate hot water treatment (HWT) better than cuttings taken from vines grown in cool climates [105]. HWT can be applied to rootstock cuttings prior to grafting [73, 106] or to young, grafted vines just prior to dispatch [106, 107]. Another method is the use of biological control agents such as *Trichoderma* in various formulations such as powder, granules/pellets and dowels [108]. Incidence of *Pl. chlamydospora* and *Phaeacremonium* spp. in rootstock cuttings was reduced by soaking the planting material in *Trichoderma* formulations [106]. Moreover, it was demonstrated that the application of *Trichoderma atroviride* at hydration, callusing and pre-planting stages in nurseries reduced infection by *Pl. chlamydospora* and *P. minimum*, hydration treatments being the most effective [109].

*Preventive measures in vineyards:* The first line of preventive measures is the use of pathogen free material obtained from healthy vines. However, there is no guarantee since asymptomatic vines may harbour Esca pathogens. For this reason, many nurseries treat cuttings with fungicides, hot water or biological agents to ensure that cuttings are pathogen free. Another preventive measure is the phytosanitation, which includes the destruction of affected parts, pruning debris or whole vines. Infected pruning residues should be immediately removed from the vineyard and destroyed since fungal fruiting bodies can become a potential source of inoculum for new infections [110]. Since pruning wounds are the major entry point for Esca pathogens [108, 111],

keeping the wounds at minimum and treating these wounds are very important for protecting the vines before spores germinate and start colonising the open xylem vessels and pith parenchyma cells at wound area [50, 112]. Another important aspect is to avoid pruning during rainy or humid warm days as spores are released under these environmental conditions [113, 114]. Late pruning (prior to budbreak) is also recommended because wound healing is rapid during this time [115]. Moreover, double pruning [116], which consists of mechanical pruning in the winter followed by hand pruning before budbreak, is a viable technique to complete pruning in large vineyard areas [117]. Wound treatment can be done chemically through chemical sealant or biologically by an antagonist. Several compounds to treat pruning wounds were tested in paste forms such as thiophanate-methyl (Topsin M<sup>®</sup>), cyproconazole + iodocarb treatment (Garrison<sup>®</sup>—commercial tree wound past), boric acid in a wound sealant paste (Biopaste<sup>®</sup>) and a pyraclostrobin formulation (Cabrio<sup>®</sup>) and showed positive results as wound protectants [73]. Others were tested as sprays such as myclobutanil that gave positive results against Esca pathogens [118]. Protecting the wound biologically can be done by *Trichoderma* sprayed on the fresh pruning wounds one to two days after pruning. Results showed a 45–65% reduction of leaf symptoms in treated vines when compared to the untreated vines [119]. Last but not least, balanced nutrition and irrigation are also important, as preventive measures, as they enable vines to fight the infection [6].

*Curative measures in vineyards:* There are several ways to manage infected vines; however, some of these measures cannot guarantee the removal of Esca pathogens completely. These methods include trunk renewal, over-grafting, trunk surgery and trunk injection. Trunk surgery is an old practice especially in fruit trees in the Mediterranean area. Surgery is a technique that relies on cutting the trunk open with a chainsaw and removing of decayed wood from the heart of the trunk allowing quick recovery of symptomatic vines by keeping their active root systems and, therefore, maintaining the quality of the product that is linked to the vine age [120]. Trunk surgery is considered more expensive and time consuming compared to trunk renewal [26] and needs well-trained personnel [120] not to compromise the main sap flow during surgery, preferring to act only on one side of the plant [121]. Another curative measure is to cut back the trunk below infected wood until healthy/clean wood is visible. One of the healthy suckers (water shoots) is then trained up to become a replacement trunk. These suckers arise from base buds at prior node positions on the vine trunk. This technique also works with other trunk diseases. It presents the advantage of saving the vine root system. Training suckers to new trunks and arms may precede trunk removal; therefore, no yield needs to be lost [122]. If suckers are not available, an over-grafting or re-grafting may be done, while after cutting the vine and reaching healthy tissues, the vine is then over-grafted or re-grafted. According to some empirical experimentation, the treated vines could reach productivity in 3 years, because of their mature root system [123].

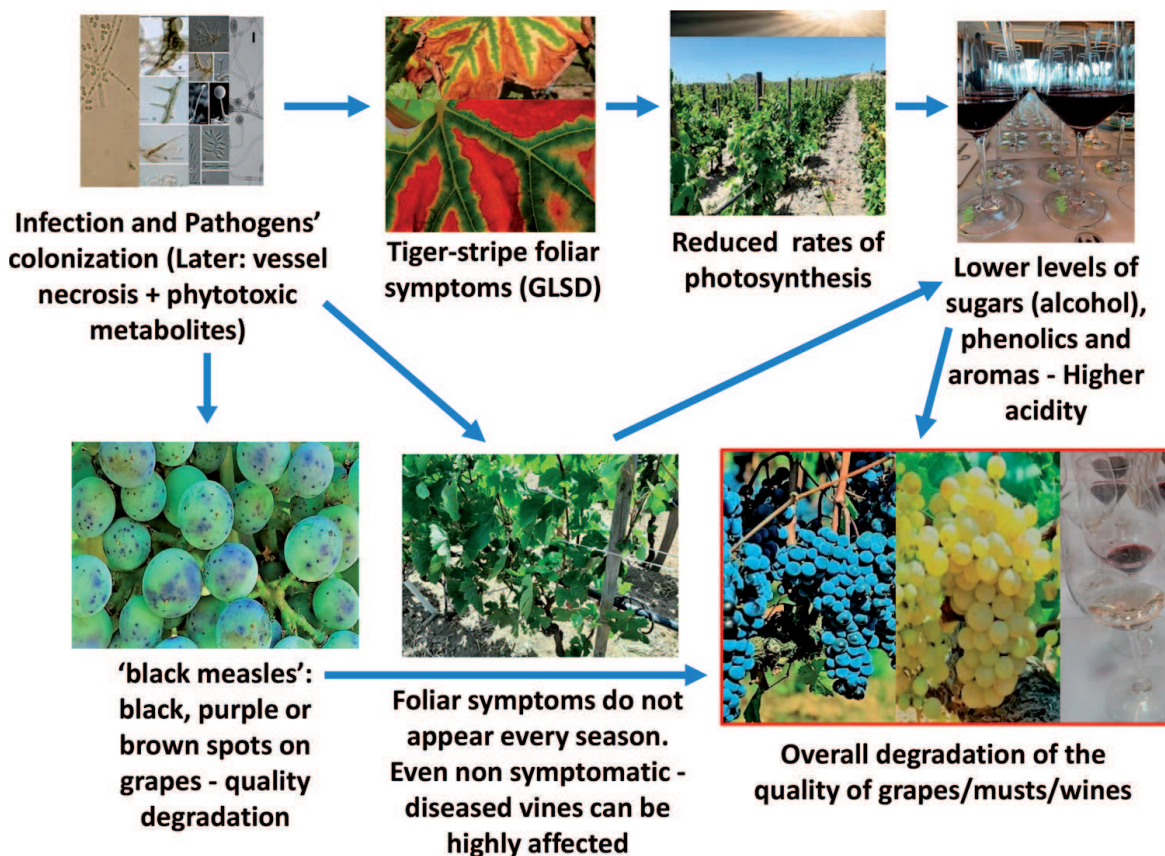
Curative chemical treatment seems difficult since Esca pathogens exist in the trunk. However, in recent years, some methods have been developed to deliver fungicides in the trunk using trunk injection, also called endotherapy. Early trunk injections on Esca-infected vines showed negative results [25, 124]. However, these studies used exclusively the presence of leaf symptoms to evaluate treatment success and did not examine changes in the presence of pathogens in the wood pre- or post-treatment [125]. A new injection technique based on encapsulated nanoparticles has shown promising results. Several fungicides have been already successfully encapsulated for being delivered in the trunk. In a recent study, fungicides were coated with lignin to

attract Esca pathogens. Indeed, lignin-coated fungicides successfully inhibited the growth of *Pl. chlamydospora* and *P. minimum* even after a period of four years, proving a drastic reduction in Esca leaf symptoms after a single injection [126]. Trunk injection encouraged some winegrowers to inject many compounds such as hydrogen peroxide into grapevine trunks; however, more testing is required before this technique can be recommended.

## 8. Impact of Esca on the quality of grapes and wines

Grapevines infected by Esca-related pathogens do not consistently demonstrate foliar and fruit symptoms every growing season [3, 61]. However, those vines tend to present decreased vigour and lower yields. The Esca complex disease causes significant losses in wine production worldwide [58]. Esca has also been reported to affect grapes' quality and, subsequently, the quality of wines produced from those grapes [108] (**Figure 2**). In many cases, quality degradation can even be observed on grapes macroscopically. As a result of chronic development of Esca, small dark-brown-to-purple spots, described as 'black measles', often appear on the berries [36]. The ripening process of grapes is also affected indirectly, as Esca-infected vines present altered rates of photosynthesis [69, 71].

In a study conducted in Bordeaux, France, levels of ripeness were significantly delayed in vines infected by Esca. In addition to reduced yield, a decline of the sugar content (~10%) as well as higher acidity (~20%) was observed. The organoleptic characteristics were also affected, as wines partially made from Esca-infected vines



**Figure 2.**  
*Development of Esca and its implications on the quality of grapes and wines.*

presented lower levels of fruity aromas and elevated levels of vegetal-herbaceous characters [127]. In another research that took place in Italy, the effects of Esca on the quality of grapes and the composition of wines were tested in two Trebbiano d'Abruzzo vineyards [128]. Three different groups were evaluated: (a) grapes from diseased and symptomatic vines, (b) grapes from vines that were asymptomatic at the time of experiments but were previously confirmed to be diseased and (c) healthy grapes from healthy vines. The results indicated notably lower levels of sugars and substantially higher nitrogen concentration in the berries from diseased-symptomatic grapevines compared to the other two groups (which did not differ significantly with each other). Regarding polyphenolic concentrations, significant differences between healthy and unhealthy vines were also observed, but results were conflicted between the two vineyards. Consequently, wines produced from diseased-symptomatic vines displayed lower alcohol content by ~1% as well as higher levels of tartaric and malic acid and higher total nitrogen. However, no significant differences were detected in the cases of pH and total acidity [128]. Subsequent experiments were performed in the same vineyard areas (Controguerra and Giulianova in Teramo, Italy). In the latter bioassays, notably higher levels of trans-resveratrol as well as iron and magnesium levels in musts from diseased-symptomatic vines were observed [25]. Symptomatic vines also produced higher concentrations of nitrogen confirming the results of 2001. This constant increase of nitrogen may be a result of protein hydrolysis that occurs in diseased leaves and generates amino acids that migrate to the grapes. Moreover, grapes from symptomatic vines presented significantly lower sugar levels and musts from symptomatic vines had significantly higher levels of total acidity, malic acid and total polyphenols compared to the musts from the other groups.

High-performance liquid chromatography was used to detect reduction in concentrations of (+)-catechin, (–)-epicatechin and anthocyanins on skins of Cabernet Sauvignon grapes, from Esca-infected vines, in the region of Bordeaux during 2009 and 2010 [65]. However, no statistical difference was detected in the case of total tannins. In wines, significantly lower alcohol content was produced from infected vines, but there was no difference in terms of total acidity. Wines were made using various percentages of berries from Esca-infected vines (0, 5, 15, 25, 50, 75 and 100%). The phenolic composition of grapes was affected, and the sensorial quality of wines was decreased, when Esca-infected fruit was used in percentages as low as 5%. It was reported that Esca affects normal maturation of berries and alters the flavonoid metabolism, which regulates the production of anthocyanins and tannins [65].

In the case of Sauvignon Blanc, symptomatic vines produced grapes of inferior quality, but when curettage was applied, it seemed to have a positive effect. Cured and asymptomatic grapevines produced wines with favourable chemical and organoleptic characteristics, maintaining high quality and typicality of Sauvignon Blanc [5]. Overall, Esca seems to reduce sugars, aromas and some phenolic compounds, resulting in the deterioration of the organoleptic quality of wines [4, 25, 65, 128].

## **9. Final remarks**

Esca is a complex disease. Several pathogens and factors act in association or in succession, to cause the syndromes, which become fully expressed only under specific environmental conditions. The appearance of symptoms as well as the severity of the

disease seems to be substantially affected by abiotic factors. Temperature increases transpiration and, therefore, is a key factor, affecting not only the time of symptom appearance but also infection, pathogenesis, fungal growth and the level of damage. Esca is currently considered as an increasing threat, becoming severe in many viticultural regions across the globe. Climate change might be a reason for the dispersal and harshness of the disease, as losses and damages are associated with plant responses to various stresses.

Control approaches include preventive measures in both vineyard and nurseries, as well as curative techniques in Vineyards. Despite the progress, direct control remains difficult. Certain varieties present some levels of resistance to Esca, compared to others, but there are no varieties with immunity to the disease. The reasons behind susceptibility or resistance are probably related to some interactions between Esca pathogens and grapevine physiology, but this phenomenon is still poorly understood. Most publications indicate resistance of specific varieties to a single pathogen only, without examining the response of various cultivars to the entire complex under field conditions.

It is known that Esca causes substantial economic losses to global viticulture by reducing yields and shortening the productive life of grapevines. Moreover, it seems to affect the quality of grapes and wines as infected vines (both symptomatic and asymptomatic) tend to produce less concentrated grapes with lower amounts of sugars and phenolic compounds. The fact that even asymptomatic vines produce grapes of inferior quality must be seriously taken into consideration. However, further research is needed to examine accurately and precisely the effects of Esca on the quality of grapes and the chemical composition of musts/wines. Overall, additional research must be conducted to investigate all the aspects of vine physiology and plant responses, in regard to ecology, pathogenesis, lifecycle, physiology and diversity of the fungal pathogens that are involved in the Esca complex.

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