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ORCID:
GDF: 0000-0002-4811-714X
FC: 0000-0002-4339-093X
RBF: 0000-0002-5027-7564

New and Opinion

Understanding the control strategies effective against the esca leaf stripe symptom: the edge hypothesis

Giovanni DEL FRARI[1,*], Francesco CALZARANO[2], Ricardo BOAVIDA FERREIRA[1]

[1] LEAF-Linking Landscape, Environment, Agriculture and Food-Research Center, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal
[2] Faculty of Bioscience and Technologies for Food, Agriculture and Environment, University of Teramo, Via Renato Balzarini, 1, 64100 Teramo, Italy
*Corresponding author. E-mail: gdelfrari@isa.ulisboa.pt

Summary. A peculiar symptom that may develop in grapevines affected by wood pathogens involved in the esca complex of diseases is the leaf stripe symptom, which also gives the name to the Grapevine Leaf Stripe Disease. Multiple studies have revealed strong links between fungal presence, wood symptomatology and expression of the leaf stripe symptom. However, numerous other factors have been shown to play roles in symptom onset, incidence, severity and yearly fluctuation of this disease. While the factors triggering the leaf stripe symptom are still under investigation, three control strategies have been proven effective for substantially reducing its expression, namely trunk surgery, and applications of sodium arsenite or a fertilizer mixture. These control strategies are examined here, including their (putative or confirmed) modes of action, and how they may influence the leaf stripe symptom development. In this article, we also propose the 'edge' hypothesis to tentatively explain symptoms onset, keeping in consideration past knowledge and recent advances in the understanding of the esca leaf stripe symptom. Ultimately, it is our intention to offer food-for-thought and stimulate debate within the phytopathological community.

Keywords. Grapevine trunk diseases, interveinal necrosis, grapevine, Fomitiporia mediterranea, trunk surgery.

INTRODUCTION

Esca is a complex of wood diseases that belongs to the Grapevine Trunk Diseases (GTD) cluster, i.e., fungal diseases that affect perennial and annual tissues of grapevine. The role of numerous fungi as causal agents of internal wood symptoms, including brown wood streaking, necrotic lesions and white rot, has been widely demonstrated (Mugnai *et al*., 1999; Bertsch *et al*., 2013; Gramaje *et al*., 2018). However, the relationship among fungal pathogens, wood symptoms and the leaf stripe symptom remain to be fully validated. This uncertainty has led to the proposal of a separate disease, namely Grapevine Leaf Stripe Disease (GLSD), characterized by permanent wood symptoms (i.e., brown wood streaking and necrotic lesions) and a discontinuous leaf stripe symptom. Nevertheless, the leaf stripe symptom is also found in esca proper-affected vines, the difference between GLSD and esca-proper being the presence of white rot in the latter (Surico, 2009).

Since the review by Mugnai *et al.* (1999), three main theories have been proposed, and partly explored, to identify the triggering factor(s) implicated in the onset of the leaf stripe symptom.

(a) The toxins hypothesis. Several studies support the involvement of toxic metabolites of fungal origin (Bruno and Sparapano, 2006; Bruno *et al.*, 2007; Andolfi *et al.*, 2011; Schilling *et al.*, 2021), a well-known mechanism in two major GTDs (i.e., Eutypa dieback and Botryosphaeria dieback; Colrat *et al.*, 1999; Masi *et al.*, 2018; Trotel-Aziz *et al.*, 2019; Cobos *et al.*, 2019; Schilling et al., 2021). Still, the evidence provided to explain the leaf stripe symptom development and yearly fluctuation in esca-affected vines remains unsatisfactory.

(b) The by-products of wood degradation hypothesis. 'By-products of wood degradation' is an umbrella term that groups all the molecules that may develop as a consequence of the plant-pathogens interaction (Mugnai *et al.*, 1999). In their recent articles, Moretti *et al.* (2021), Schilling *et al.* (2021) and Pacetti *et al.* (2022) explored a number of pathways that lead to potential triggering factors. Among them, we find (a) wood extractives, such as tannins and polyphenols, (b) lignocellulose-degrading enzymes, (c) fungal or plant derived non-enzymatic proteins, and (d) low molecular weight diffusible compounds generated during the process of cellulose and lignin degradation (e.g., DAMPs; Héloir *et al.,* 2019). It remains to verify empirically if any of them, alone or in combination, is the primary responsible for the leaf stripe symptom expression.

(c) The competition-induced metabolites hypothesis. Brought forward by Bruez *et al.* (2020), this recent hypothesis suggests that the microbe-microbe interaction among esca-associated fungi and/or other components of the endophytic microbiome (e.g., bacteria) may trigger the release of phytotoxic secondary metabolites. While certainly plausible, in light of multiple examples in the literature (Hardoim *et al.*, 2015), to date, no experimental evidence supports this hypothesis.

Despite these different hypotheses, it is commonly agreed that the translocation of fungal-derived or plant-derived molecules from wood to leaves is a key component in the leaf stripe symptom onset (Bruno *et al.*, 2007). In the following text, to simplify communication, we have grouped all possible fungal toxins, byproducts of wood degradation and competition-induced metabolites, into a single category, namely Leaf Stripe Symptoms-Inducing Molecules (LSSIM).

Since the early 1990ies, numerous studies have shown that multiple biotic and abiotic factors, as well as agronomic practices, are correlated to onset, discontinuity, incidence and severity of the leaf stripe symptom (Table 1; Fischer and Ashnaei, 2019; Claverie *et al.*, 2020; Del Frari *et al.*, 2021). Among these, meteorological conditions (e.g., rainfall, drought, temperature, soil moisture) play major roles in yearly symptom fluctuations (Surico *et al.*, 2000; Marchi *et al.*, 2006; Bruez *et al.*, 2013; Andreini *et al.*, 2014; Calzarano *et al.*, 2018;

Table 1. Biotic and abiotic factors and agronomic practices correlated to esca leaf stripe symptoms in grapevine.

Biotic factors	Abiotic factors	Agronomic practices
Plant vigour [a]	Climatic conditions [h]	Pruning and training system [m]
Plant physiology [b]	Plant protection products [i]	Trunk surgery [n]
Xylem vessels characteristics [c]	Soil [j]	Grafting type [o]
Scion and rootstock genotype [d]	Nutrients [k]	
Grapevine age [e]	Water availability [l]	
Fungal endophytes (wood pathogens and/or biocontrol agents) [f]		
Rhizosphere microbiome [g]		

[a]Lecomte *et al.*, 2011; Fischer and Ashnaei, 2019
[b]Christen *et al.*, 2007; Magnin-Robert *et al.*, 2011; Fontaine *et al.*, 2016a; Goufo and Cortez, 2020; Calvo-Garrido *et al.*, 2021
[c]Pouzoulet *et al.*, 2014
[d]Marchi, 2001; Bruez *et al.*, 2013; Andreini *et al.*, 2014; Murolo and Romanazzi, 2014; Pouzoulet *et al.*, 2014; Borgo *et al.*, 2016
[e]Mugnai *et al.*, 1999; Graniti *et al.*, 2000; Surico *et al.*, 2008
[f]Larignon and Dubos, 1997; Peros *et al.*, 2008; Bigot *et al.*, 2020; Nerva *et al.*, 2019; Del Frari *et al.*, 2021; Fotios *et al.*, 2021; Di Marco *et al.*, 2022
[g]Landi *et al.*, 2021
[h]Surico *et al.*, 2000; Marchi *et al.*, 2006; Bruez *et al.*, 2013; Calvo-Garrido *et al.*, 2021
[i]Di Marco *et al.*, 2011; Songy *et al.*, 2019; Bruez *et al.*, 2021
[j]Surico *et al.*, 2000; Guérin-Dubrana *et al.*, 2005; Fischer and Ashnaei, 2019; Nerva *et al.*, 2019
[k]Calzarano *et al.*, 2007; Calzarano *et al.*, 2014; Calzarano and Di Marco, 2018
[l]Marchi *et al.*, 2006; Andreini *et al.*, 2014; Calzarano *et al.*, 2018; Serra *et al.*, 2018; Bortolami *et al.*, 2021; Calvo-Garrido *et al.*, 2021
[m]Lecomte *et al.*, 2018
[n]Cholet *et al.*, 2021; Pacetti *et al.*, 2021
[o]Mary *et al.*, 2017

Serra *et al.*, 2018; Bortolami *et al.*, 2021; Calvo-Garrido *et al.*, 2021).

Several studies demonstrated that grapevines infected and symptomatic in the wood may remain fully asymptomatic in the leaves for several consecutive years (Surico *et al.*, 2000; Calzarano *et al.*, 2018), despite an apparently unaltered wood infection and symptomatology. As wood infections and related symptomatology tend to build up with time, leaf symptoms also become more frequent as vines age. This may be why studies have often correlated the presence of white rot with leaf symptoms manifestation (Pollastro *et al.*, 2000; Peros *et al.*, 2008; Kuntzmann *et al.*, 2010; Maher *et al.*, 2012; Bruez *et al.*, 2014; Elena *et al.*, 2018; Del Frari *et al.*, 2021). Indeed, the older the vine, the greater is the chance of it being infected by white rot agents. On the contrary, less frequent are the studies dedicated to young or adult vines showing the leaf stripe symptom in plants seemingly unaffected by white rot (Edwards and Pascoe, 2004; Calzarano and Di Marco, 2007; Romanazzi *et al.*, 2009; Hofstetter *et al.*, 2012; Raimondo *et al.*, 2019).

Interestingly, esca-associated fungi are occasionally found in asymptomatic wood, suggesting – under some circumstances – a non-pathogenic behaviour (e.g., Graniti *et al.*, 2000; Hofstetter *et al.*, 2012; Del Frari *et al.*, 2019a). This confirms that the microbiological aspect of the wood of leaf symptomatic vines is only one of the factors of a bigger equation. For example, even the grapevine rhizosphere, in particular arbuscular mycorrhizal fungi, may alter their abundance in leaf symptomatic vines (Landi *et al.*, 2021).

Regardless of this multiplicity of factors, which complicates the achievement of clear etiological and epidemiological patterns, three control strategies strongly influence the expression of the esca leaf stripe symptom. These are trunk surgery (Cholet *et al.*, 2021; Pacetti *et al.*, 2021) and sodium arsenite (Songy *et al.*, 2019; Bruez *et al.*, 2021), which decrease symptoms for multiple sequential years after a single treatment, and a fertilizer mixture, which reduces symptoms incidence and severity, when sprayed on vine canopies, in the year of application (Calzarano *et al.*, 2014; Calzarano and Di Marco, 2018). A fourth method, namely the application of *Trichoderma* products for pruning wound protection (e.g., Di Marco *et al.*, 2004), prevents fungal infection, and it was recently validated as a means of reducing the leaf stripe symptom incidence (Bigot *et al.*, 2020; Di Marco *et al.*, 2022).

In the following section, we have examined the putative or confirmed modes of action of the first three control strategies mentioned, and we elaborate a hypothesis that joins LSSIM, the grapevines health status and the leaf stripe symptom expression.

WHY DO DIFFERENT TREATMENTS REDUCE LEAF SYMPTOMS?

Trunk surgery. The earliest version of trunk surgery has ancient origins, being employed in Greece and Asia Minor since Roman times (Mugnai *et al.*, 1999). This consisted in mechanically splitting the trunk of esca leaf symptomatic vines and placing a stone between the two trunk halves (Figure 1). This technique was used sporadically over the years, and revived in North-East Italy about 30 years ago, where it was named '*Metodo Armano*', after the agronomist who spread its use. Allegedly, treated vines remained asymptomatic in the leaves for several years, regardless of the presence of wood symptoms. However, no scientific survey was conducted to assess the validity of these claims. Another similar and recently re-discovered technique is the *slupatura* (in Italian), also known as *curettage* (in French) or trunk surgery (in English), which is commonly used on olive trees in the Mediterranean region. This consists in removing all decayed tissue (*i.e.*, the white rot) from the vine trunk, using a small chainsaw (or a tool like the one called *malepeggio*, in Italy). Albeit white rot being associated with vine apoplexy since the report of Ravaz (1909), and only later with the leaf stripe symptom by Viala (1926), Fischer (2002) identified *Fomitiporia mediterranea* as the main causal agent of white rot in grapevines in the Mediterranean region – up to Iran (Moretti *et al.*, 2021). Trunk surgery has been recently validated as an effective control strategy to reduce the leaf stripe symptom relapse and to improve several physiological parameters in treated vines (Cholet *et al.*, 2021; Pacetti *et al.*, 2021).

Why is trunk surgery effective?

According to several authors (Cholet *et al.*, 2021; Pacetti *et al.*, 2021; Del Frari *et al.*, 2021), the success of this technique lies in its drastic effect on the presence of white rot and, as a consequence, on the abundance of *F. mediterranea*. In their study, Pacetti *et al.* (2021) detected significant alterations in the fungal and bacterial microbiota of vines treated with trunk surgery. With concern to fungi, the authors report a massive decrease in *F. mediterranea* abundance, while esca-associated pathogens *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp., and other GTD-associated fungi (e.g., *Eutypa* spp. and *Botryosphaeriaceae*, causal agents of Eutypa dieback and Botryosphaeria dieback) remained unaltered by the treatment. The bacterial genera most affected by trunk surgery are *Burkholderia*, *Massilia* and *Pantoea* (Pacetti *et al.*, 2021). Some of the putative driv-

Figure 1. 'Low-tech' grapevine trunk surgery (known in some Italian regions as '*Metodo Armano*'). It consists of splitting the trunk of a leaf symptomatic vine and fitting a stone in between the two trunk halves. These two treated vines were photographed in December 2016 (A) and in June 2017 (B), in two vineyards near Spessa, Friuli, Italy.

ers of these microbial alterations may be biotic factors, such as the fungus-fungus interaction resulting from variations in niches composition, or competition with wood colonizers; abiotic factors, such as the oxygenation levels in grapevine wood, or increased temperature fluctuations in the, now thin, trunk. In Pacetti *et al.* (2021), the authors speculate that the strong reduction in the leaf stripe symptom is linked to the second theory of symptoms manifestation, i.e. the byproducts of wood degradation hypothesis, despite concluding that more evidence is required to validate this assumption.

In addition to the microbiological aspect, trunk surgery may trigger strong defense responses in vines, such as release of phenolic compounds and PR proteins, which normally occur after host wounding (e.g., during annual pruning) (Blanchette and Biggs, 1992; Ferreira *et al.*, 2007; Pouzoulet *et al.*, 2013). Future research may investigate their putative role in contributing to LSSIM mitigation at leaves level.

Sodium arsenite. During the 20th century, sodium arsenite ($NaAsO_2$) was the only known chemical treatment capable of greatly reducing the leaf stripe symptom manifestation in grapevines. Its use in vineyards was discontinued in 2003, due to its highly toxic effects on humans, animals, plants and the environment in general (Carbonell-Barrachina *et al.*, 1997a; Larignon *et al.*, 2008; Songy *et al.*, 2019), even if symptoms incidence had been increasing in some European regions, France included (Fontaine *et al.*, 2016b). The grapevine endophytic microbiota is known to be affected by the use of several fungicides (Del Frari *et al.*, 2019b) and, unsurprisingly, $NaAsO_2$is among them (Larignon and Fontaine, 2018; Bruez *et al.*, 2021). In their article, Larignon and Fontaine (2018) explored a century of knowledge over the use of $NaAsO_2$ in viticulture, especially interesting for French-speaking readers.

Recently, Songy *et al.* (2019) studied the effects of $NaAsO_2$ on the physiology of young grapevines, following an application on the stem during plant dormancy, and located the presence of this chemical in all vine organs, including leaves, confirming some results obtained in earlier studies on adult vines (Carbonell-Barrachina *et al.*, 1997b; Larignon *et al.*, 2008). Songy *et al.* (2019) also detected a number of physiological alterations in response to $NaAsO_2$ application, including photosynthesis-related parameters and activation of a plant defence response (up-regulation of genes *SOD*, *GST1* and *CHV5*, in different plant organs). Sodium arsenite has *in vitro* fungicidal activity, also affecting some wood pathogens at different concentrations (Santos *et al.*, 2006; Larignon *et al.*, 2008; Bruez *et al.*, 2021). For example, *F. mediterranea* is strongly affected, even at low $NaAsO_2$ concentrations (IC_{50}= 0.4 mg L^{-1}), while tracheomycotic fungi and other endophytes assessed were relatively tolerant to the chemical (e.g., *P. chlamydospora*, IC_{50}= 6 mg L^{-1}; *Trichoderma* sp., IC_{50}= 29 mg L^{-1}; *Penicillium* sp., IC_{50}= 139 mg L^{-1}) (Bruez *et al.*, 2021). In a recent study, Bruez *et al.* (2021) examined the effects of $NaAsO_2$ on adult vines, confirming its fungicidal activity *in planta*. The most striking observation was the collapse of *F. mediterranea* abundance in white rot (-90%), a tissue known to accumulate $NaAsO_2$ following application on trunks (Larignon *et al.*, 2008). Other known wood pathogens, including *P. chlamydospora*, were not affected by the chemical, or even increased in relative abundance, depending on the woody tissue analyzed. Similarly, no major changes occurred in the endophytic bacterial microbiome, despite $NaAsO_2$ bactericidal properties (Larignon and Fontaine, 2018). Bruez *et al.* (2021) confirmed the treatment efficacy, noting that none of the treated plants manifested leaf stripe symptoms during the following growing season, while all untreated vines did.

Why is sodium arsenite effective?

Available evidence suggest that the most likely explanation to the this question lies in the drastic effect of $NaAsO_2$ on *F. mediterranea* abundance (Bruez *et al.*, 2021), in agreement with the trunk surgery technique. *F. mediterranea* is highly sensitive to this chemical, which also accumulates in *F. mediterranea* preferred niche. Consequently, important changes occur in the microbial composition within vine trunks due to $NaAsO_2$ fungicidal activity, such as the creation of pathogen-free host tissues, and newly available nutrients for other components of the endophytic microbiota. Following this reasoning, the wood affected by white rot would not be degraded by *F. mediterranea*, but – hypothetically – by other organisms (e.g., bacteria; Haidar *et al*., 2021), which do not contribute to the production of LSSIM, altogether suggesting a role of the microbiota as a whole. Before its ban, the application of sodium arsenite in vineyards used to be carried out following a 2 years on – 2 years off regime (Di Marco *et al*., 2000), and symptoms reappeared if the treatment was discontinued as the chemical gradually lost activity via plant detoxification (Larignon and Fontaine, 2018) and/or it is expelled via the root system (Carbonell-Barrachina *et al*., 1997b). The leaf stripe symptom relapse suggests that *F. mediterranea* may reclaim its original niche or infest asymptomatic wood, but more research is necessary to clarify this point.

However striking the effect on *F. mediterranea* may be, the involvement of $NaAsO_2$ at different levels should not be excluded. For example, the up-regulation of genes implicated in detoxification and stress tolerance may be partly involved in the mitigation of negative LSSIM effects. Another hypothesis worth exploring concerns the presence of $NaAsO_2$ in leaves throughout the growing season (Carbonell-Barrachina *et al*., 1997b) and its possible interference with some biochemical and/or physiological processes that mediate the vine-LSSIM interaction (Carbonell-Barrachina *et al*., 1997a; Larignon and Fontaine, 2018).

Fertilizer mixture. In multiple studies, Calzarano *et al.* reported that a mixture of nutrients and seaweed extract significantly reduced the incidence of the esca leaf stripe symptom in grapevine (Calzarano *et al*., 2014; Calzarano and Di Marco, 2018; Calzarano *et al*., 2021). This mixture, based on $CaCl_2$, $Mg(NO_3)_2$, and *Fucales* seaweed extract (Algescar®, Natural Development Group, Castelmaggiore, Bologna, Italy), was developed following observations of high calcium contents in leaves of diseased but leaf-asymptomatic grapevines, when compared to never symptomatic and leaf-symptomatic plants (Calzarano *et al*., 2009). Magnesium was added to the mixture to avoid possible imbalances following Ca applications, while the seaweed extract acts as a carrier of Ca and Mg, thereby improving their activity on the leaf stripe symptom reduction, which was confirmed by the increased druses in the cells of treated leaves (Calzarano *et al*., 2014).

After five applications of the fertilizer mixture, at fruit set and berries pea-sized growth stages, Calzarano *et al*. (2021) recorded greater Ca and Mg contents in asymptomatic diseased vine leaves, compared to leaves from untreated never symptomatic and from symptomatic diseased vines. Instead, at berries pea-sized stage, leaves of pre-symptomatic shoots had very low contents of Ca and Mg, and contents of *trans*-resveratrol and flavonoids also increased in leaves of treated vines (Calzarano *et al*., 2014). Until the pre-bunch closure stage, contents of *trans*-resveratrol, *trans*-ε-viniferin and *trans*-δ-viniferin were greater in treated asymptomatic leaves compared to those untreated asymptomatic leaves (Calzarano *et al*., 2017a). Calzarano *et al*. (2021) also recorded high values of normalized difference vegetation index (NDVI) and green normalized difference vegetation index (GNDVI) at fruit set, in treated asymptomatic leaves from diseased vines. From full flowering to fruit set, the same leaves also had greater water indices (WIs) compared to untreated leaves.

Why is the fertilizer mixture effective?

The above-mentioned findings highlight the role of Ca and Mg in reducing the leaf stripe symptom development and severity. According to some authors, these symptoms are caused by the oxidative burst generated by the plant response, rather than by a direct effect of LSSIM on leaf tissues (Petit *et al*., 2006; Andolfi *et al*., 2009; Magnin-Robert *et al*., 2011). The hypothesis that necrosis formation is comparable to a hypersensitive reaction (HR) is reinforced by the high levels of phytoalexins in symptomatic leaves. Indeed, high levels of phytoalexins are usually synthesized after the HR (Heath, 2000; Lima *et al*., 2012; Calzarano *et al*., 2016).

The calcium ion is an intracellular second messenger involved in plant defense responses. Variations in the cytosolic concentration of Ca^{2+} ($[Ca^{2+}]$cyt) are linked to the triggering of such responses. Salicylic acid, a key molecule in signal transduction, is also required for these responses, and it can cause a strong oxidative response, as it happens in the HR. Availability of Ca may reduce host oxidative responses and HR necrosis. Calcium, penetrating into the leaves, can increase the synthesis of calmodulin, which regulates salicylic acid and reduces the HR (Lecourieux *et al*., 2006; Du *et al*., 2009). High numbers of druses in treated vine leaves also suggest that Ca accumulation in extracellular spaces may increase phytoalexin synthesis and/or strengthen the cell walls (Conway *et al*., 1991; Tavernier *et al*., 1995; Lecouriex *et al*., 2006; Calzarano *et al*., 2014). The role of Mg in the control of the leaf stripe symptom could result from general improvements of host plant health, and in delay of chlorophyll degradation (Datnoff *et al*., 2007). However, Mg could also act directly, detoxifying toxic metabolites produced by esca fungi, as has been observed for *Eutypa lata* infections, where eutypin was detoxified to eutypinol by Mg^{++} and Mn^{++} (Colrat *et al*., 1999).

In untreated vines, phytoalexin synthesis was probably not involved in the reduction of the leaf stripe symptom (Calzarano *et al*., 2017b). Instead, in the leaves of asymptomatic vines, following applications of the fertilizer mixture, early and high phytoalexin synthesis does not exclude their possible contribution to the control of the leaf stripe symptom (Calzarano *et al*., 2017a). The effects of Ca and Mg may be boosted by accumulation of *trans*-resveratrol and other phytoalexins, at berries pea-sized and until pre-bunch closure. In addition, the improved physiological efficiency, demonstrated in treated vines by leaf reflectance measurements (NDVI and GNDVI), and from increased WI, in the first growth stages until fruit set, could indirectly modulate the host responses (Calzarano *et al*., 2021).

In summary, reductions in the leaf stripe symptom, achieved from fertilizer mixture applications, could depend on several complex effects that interfere with mechanisms of symptom development, which operate in the early growth stages of grapevine growing seasons, and lead to the manifestation of symptoms in later stages. The findings discussed above do not bring about evidence in support of any of the three theories for the leaf stripe symptom expression; nevertheless, it further confirms the earliest observation that metabolites brought to the leaves cause leaf reactions and symptoms development (Mugnai *et al*., 1999).

THE 'EDGE' HYPOTHESIS

Considering the different modes of action of the three techniques described above, we here propose the 'edge' hypothesis as a possible explanation for the leaf stripe symptom onset and yearly fluctuation. According to this hypothesis, all grapevines affected by esca-associated pathogens, and related internal symptoms, are constantly under the influence of LSSIM, which are transported in the host vascular system and reach the canopies. However, as long as the concentration of these molecules remains below an hypothetical edge (i.e., a multi-factor threshold), the triggering of the leaf stripe symptom does not occur. The edge concept aims at identifying the current health status of each vine, which is influenced by the biotic and abiotic factors, and agronomic practices listed in Table 1.

To clarify this hypothesis and the concept of edge, four hypothetical case scenarios could apply (Figure 2). These are:

1. A vine with a fungal infection and wood symptoms, such as those found in GLSD or esca proper, does not exhibit leaf symptoms if the abundance of LSSIM remains below the edge (Figure 2A). This represents a condition favourable to the vine, which may occur when environmental factors positively affect the plant health status, raising the edge and, therefore, the tolerance to LSSIM; or during an early fungal infection, when the fungal load, wood symptoms and LSSIM are present in relatively low abundance.

2. If one or more environmental factors negatively affect the vine, for example adverse climatic conditions and/or poor nutritional status, the edge lowers. Consequently, a similar abundance of LSSIM, as in Figure 2A, is sufficient to trigger leaf symptoms (Figure 2B). Nevertheless, since the factors that are known to be involved in this process are numerous, current predictors of leaf symptoms manifestation remain uncertain.

3. As vines age and fungal infections progress, leading to increased pathogen abundance and species richness, along with wood symptomatology extent, the plants increasingly reach a stage where LSSIM levels surpass the edge (Figure 2C). This may be due to increased LSSIM abundance, unfavourable environmental conditions, and/or protracted decrease in the edge, due to prolonged stress conditions (i.e., several years of fungal pathogens activity in the wood). At this stage, leaf symptom development, year after year, becomes more probable, when compared to plants like those in Figure 2B).

4. Treating plants with $NaAsO_2$ or trunk surgery drastically affects the abundance of LSSIM, by targeting *F. mediterranea* (Figure 2D); and may improve the vines tolerance to LSSIM, by acting on vines physiology and defense response. In these cases, the edge may or may not be lowered, based on the possible direct negative effects of the treatments on host plants. Nevertheless, the abundance of LSSIM remains below the edge for multiple years post-treatment.

The fertilizer mixture may improve host nutritional status and defenses, thereby increasing the edge, instead of lowering LSSIM abundance (e.g., Figure 2A).

Every year, numerous factors play roles in tweaking the edge, which may fluctuate throughout the growing season, based on the environment and human interventions (Table 1). This may explain yearly fluctuations as well as the differences in leaf symptoms manifestation during each growing season (e.g., plants manifesting symptoms during late spring – early summer – midsummer – late summer). Environmental changes may have a

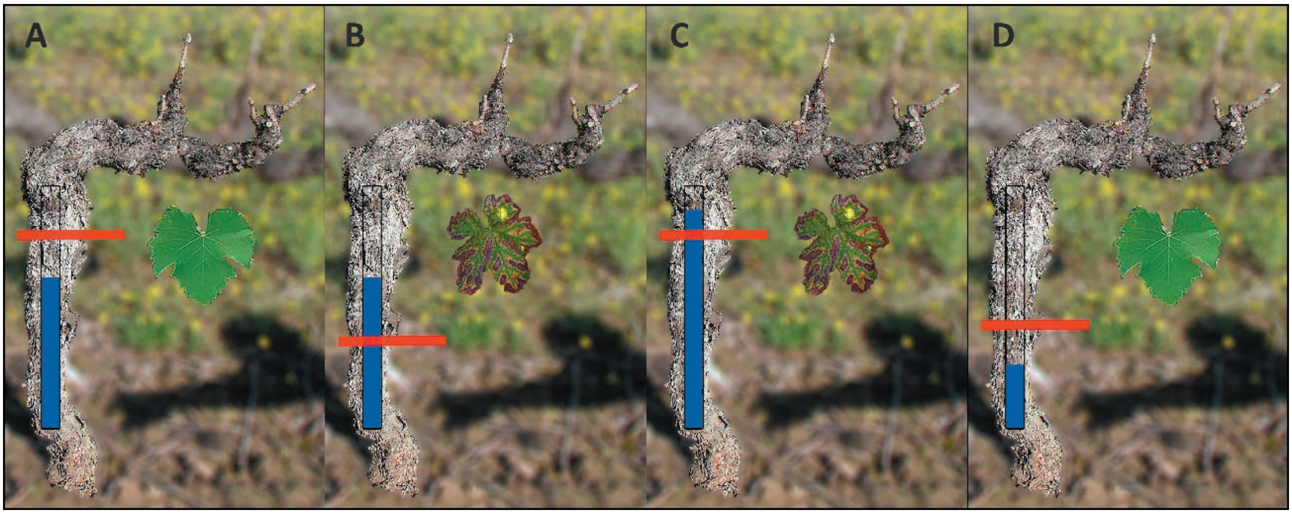

Figure 2. The results of joint effects of different factors on the edge hypothesis. Blue bars represent the hypothetical abundance of LSSIM in grapevine perennial and/or annual organs. Red bars represent the hypothetical edge, above which hosts manifest leaf symptoms. In leaf asymptomatic vines (A and D), LSSIM abundance remains below the threshold. In leaf symptomatic plants (B and C), the threshold is surpassed. Biotic and abiotic factors may act as stressors, contributing to lowering the threshold (B and D).

direct effect on the host plant and/or on the endophytic microbiome, the latter being of increasing interest in grapevine health and GTD research (Del Frari *et al.*, 2019a; Bruez *et al.*, 2020; Bettenfeld *et al.*, 2021; Fotios *et al.*, 2021; Geiger *et al.*, 2021).

While it is generally accepted, but with little experimental evidence, that the triggering of the leaf stripe symptom depends on the concentration of LSSIM and the health status of each grapevine plant, the edge hypothesis aims to consolidate this correlation by giving it a name (i.e., edge) and, possibly in the future, a numerical value to the so-called health status. The edge hypothesis may be validated by identifying, qualitatively and quantitatively, the disease triggering factors (i.e., LSSIM; blue bars in Figure 2), and by giving a numerical value to the hypothetical edge (red bars in Figure 2). While the former may be determined with current technology and targeted experimental approaches (e.g., sampling pre-symptomatic leaves and other plant organs); the latter, being still an abstract concept, may require assistance from advanced computing technology (e.g., machine learning and mathematical models), that integrates information of the multiple factors known to influence the leaf stripe symptom expression (Table 1).

CONCLUSION

In this paper, we offer an overview of the three control strategies known to strongly reduce the esca leaf stripe symptom manifestation in grapevines, acknowledging an effective fourth one, that acts on the origin of the wood infection, reducing wood colonization by protecting pruning wounds (Di Marco *et al.*, 2022). For obvious reasons, sodium arsenite was banned from vineyards and it is not a viable option anymore, while trunk surgery is an expensive technique, not suitable for all vine growers. Nevertheless, understanding their mode of action, seemingly very different – yet quite similar, may help us shed some light over some key features of the esca leaf stripe symptom.

Trunk surgery and sodium arsenite mostly affect the wood pathogen *Fomitiporia mediterranea* and/or related wood symptomatology, i.e., white rot. This suggests key roles of this fungus, or of the complex biological phenomena that precede or follow its degrading activity, towards leaf stripe development. Disease management strategies that heavily affect *F. mediterranea* have been shown to be an interesting medium-term solution, however, one question remains: can we control the leaf stripe symptom with $NaAsO_2$ or the Metodo Armano, treating vines apparently unaffected by *F. mediterranea* and white rot? If the answer was positive, we could certainly exclude *F. mediterranea* as key component of the leaf stripes symptom riddle. Instead, treatments effectiveness may be a result of the mitigation of LSSIM negative effects by acting on the vine physiological and biochemical processes, and defense response.

These different modes of action underline the relevance of an edge hypothesis, whereby a combination of

LSSIM abundance and multiple environmental factors that affect grapevine health status are responsible for the expression of the esca leaf stripe symptom. This hypothesis tentatively answers some questions relating to the causes of symptoms, such as symptoms onset and yearly fluctuations, yet, some of the most basic questions, e.g., the qualitative and quantitative influence of the triggering factors, remain unanswered. The esca leaf stripe symptom will certainly keep researchers busy in the years to come.

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