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Proceeding Paper

Primary Metabolites (Free Sugars, Amino, Organic and Fatty Acids) of Grape Berries as Influenced by Esca Complex Disease (Grapevine Leaf Stripe) Foliar Symptom Severity †

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Abstract: In this study, berry samples were collected from healthy (control) and symptomatic vines of the white varieties Viosinho and Malvasia-fina. Symptomatic vines showed two different degrees of severity at harvest, namely chlorotic and scorched leaves (severity level 1) and tiger stripe leaves (severity level 2). The total fatty acid content was reduced in both varieties and the total organic acid content was unchanged. The total free sugar content increased with symptom progression in Viosnho but remained unchanged in Malvasia-fina. Varietal differences were observed in the response of amino acids, whose levels increased in Malvasia-fina and decreased in Viosinho.

Keywords: grapevine leaf stripe disease; *Phaeomoniella chlamydospora*; biosynthetic pathways; disease resistance and tolerance; *Fomitiporia mediterranea*; primary metabolism; disease susceptibility; *Phaeoacremonium minimum*

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1. Introduction

Several diseases directly affect the sensory quality of grapevine berries. Infection on inflorescences, young berries and bunches by downy mildew are initially seen as oily violet-brown areas; infected berries later shrivel and look like raisins [1]. Berry symptoms of Grapevine Leaf Roll Associated Virus complex (GLRaVs) include delayed maturity and diminished yield [2]. With powdery mildew, infected berries often are misshapen or have rusty spots on the surface. The entire berry may be covered with a white powdery growth and severely affected fruit eventually crack and dry out [3].

With some other grapevine diseases, however, the effects the associated pathogens have on the sensory quality of the berries are not clear. Examples are grapevine trunk diseases (GTDs), a group of diseases that primarily affect grapes through pruning wounds, subsequently colonizing the perennial organs causing vascular infections, internal necrotic lesions, wood discolorations, brown wood streaking, white decays and cankers, and brown stripes in the outer xylem [4–8]. Diseased vines may also show external foliar symptoms, with their expression often discontinuous from one year to the next [9]. Associated with foliar symptoms, berry symptom may appear due to environmental and climatic changes. With the GTD Botryosphaeria dieback, berry discolorations can be visually identified after infections with *Phomopsis viticola* [6] and *Neofusicoccum parvum* [7].

In the case of the GTD esca complex disease, the berries of infected vines usually appear similar to those of healthy vines, although spotting of berry skins (dark-brown or purple measles) and shriveling/withering of grape bunches have been reported [10]. Moreover, some berries might not fill properly and will fail to reach maturity, indicating that esca complex could cause delayed ripening [11]. In most cases, however, diseased vines do not present any fruit symptoms at all for several growing seasons in succession, making it difficult to assess the impact of GTDs on grape quality.

The effect of esca complex on berry quality has been the subject of some investigations during the last decades [5,9,10–13], looking at sensory and chemical attributes. It was found that berries from symptomatic vines were firmer, and less elastic compared to berries from healthy vines, indicating reduced ripening and a watery structure of the berries [5]; these berries also had high levels of nitrogen [11]. Esca complex moderately affected the phenolic composition of berries by decreasing the skin concentrations of catechin and epicatechin [12]. The authors found that a '25% of Esca-affected grapes' threshold was necessary before there was any adverse effects on chemical and phenolic composition before maceration [12]. Thirteen proteins were shown to be influenced by esca complex during the ripening process [13]. Gubler et al. [10] however, did not find a consistent relationship esca disease severity (lesion size) and total soluble solids in berries.

The lack of visual berry symptoms on esca-affected vines highlights the relevance of further studies on how esca complex affects grape quality. The chemical analyses conducted in this study aimed to identify amino acids, free sugars, organic acids, and fatty acids in grape berries whose levels could be affected by the severity of foliar symptoms of esca complex disease. Hence, relationships between severity of the disease and reductions in grape quality were explored.

2. Materials and Methods

2.1. Collection of Berries

Grapes were collected from two white vine varieties grown in different plots in a vineyard [14,15]: Viosinho and Malvasia-fina. The distance between the two plots was ca., 100 m. Esca severity in the vineyard was monitored by visual inspection and three levels of foliar symptom severity were delineated as healthy, moderate, and severe. Healthy vines were those with no symptoms (S0; severity level 0). Vines with moderately affected leaves were labelled S1 (severity level 1) and comprised of vines with mostly chlorotic, spotted, and scorched leaves. Vines with mostly tiger stripe-like foliar symptoms were considered severely affected and labelled S2. For each group of vines, 100 visually healthy berries were harvested from the wings, tips, and centers of several bunches of three vines located in different parts of the plots and used as replicates. The berries were immediately frozen at −80 °C until lyophilized and pulverized into a powder for chemical analyses.

2.2. Chemical Analyses

Extraction and analysis of fatty acids was in accordance with the method described by Goufo et al. [4] for grapevine leaves. The resulting FAMEs were analyzed with a gas chromatograph equipped with a flame ionization detector and the amounts of unsaturated (UFA), polyunsaturated (PUFA), monounsaturated (MUFA), saturated (SFA) and total fatty acids (TFA) calculated and expressed in g mL^{-1}. For the determination of total (TAA), essential (EAA) and non-essential amino acids (NEAA), analytical conditions were adapted from Goufo et al. [16]. Sugar analysis was based on a previously described method [16]; the samples were analyzed with a High-Performance Liquid Chromatography system connected to photodiode array detector, and total sugars contents (TSS) calculated. Organic acids of the berry powders were extracted according to a slight modification of previously described method [11].

2.3. Statistical Analyses

All measurements were performed in triplicate. One-way analysis of variance and Tukey's honestly significant difference tests were performed to detect significant differences among treatments regardless of variety. The confidence levels of all analyses were set at 95% and values with $p \leq 0.05$ were considered significant. Statistical analyses were conducted using SPSS 16.0 and results expressed as means (bars in the figures) and standard deviations (error bars in the figures).

3. Results and Discussion

In this study, a global chemical analysis of grape berries was conducted in order to discriminate berries from healthy vines and berries from esca-affected vines with two degrees of leaf necrosis (chloroses/scorches and tiger stripes).

A progressive decrease of TFA with increasing leaf symptom severity was observed for both Vioshinho and Malvasia-fina, which was mainly due to SFA (Figure 1). Indeed, a slight SFA decrease was observed between healthy and moderately affected grapes, but there was a sharp decrease between healthy and severely affected vines. Fatty acid data from berries were not in line with leaf data previously obtained using Malvasia-fina; the previous study found that levels of SFA (expressed in % FAME) increased in diseased leaves of esca-affected vine [4], which is the opposite of data obtained in this study for berries. However, the data of the present study were expressed g mL−1 grape sample. For both cultivars, fairly similar results were obtained for UFA, with a strong increase in berries from severely affected vines (Figure 1).

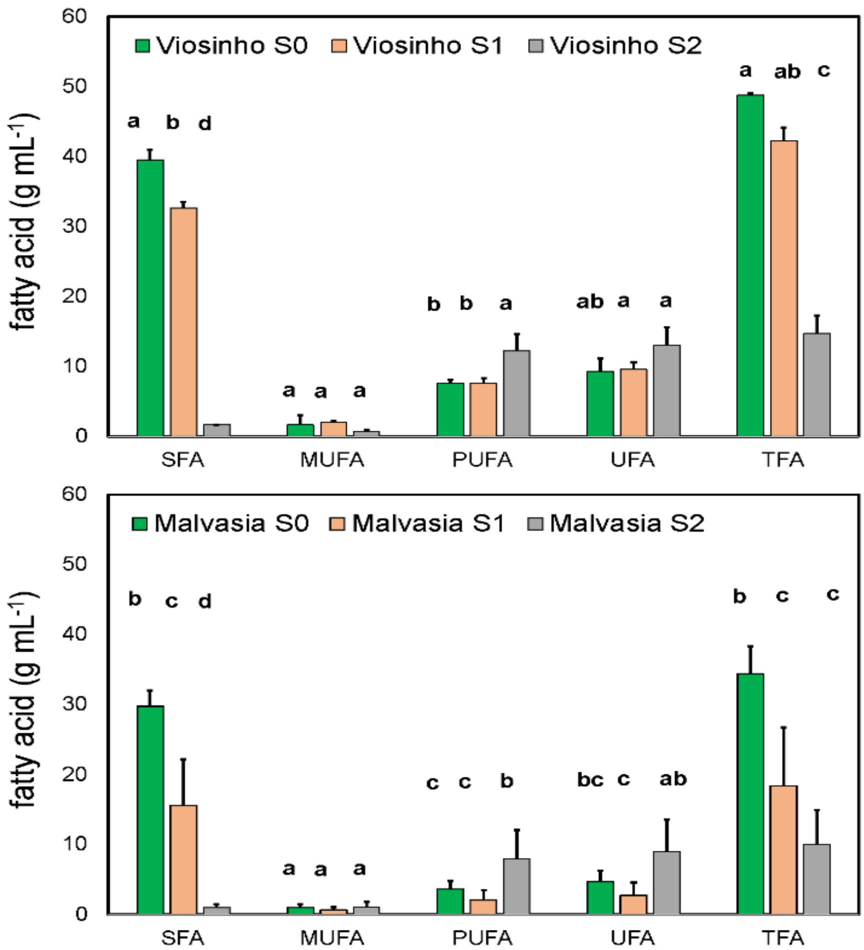

Figure 1. Contents of fatty acids in berries of Viosinho and Malvasia-fina grapevine varieties healthy (S0; severity level 0), moderately (S1; severity level 1) and severely (S2; severity level 2) affected by

esca complex disease. Error bars represent standard deviation over three replications. Comparing the two varieties, value bars with different letters for each parameter are different (Turkey's test, $p \leq 0.05$). SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, UFA = unsaturated fatty acids, TFA = total fatty acids.

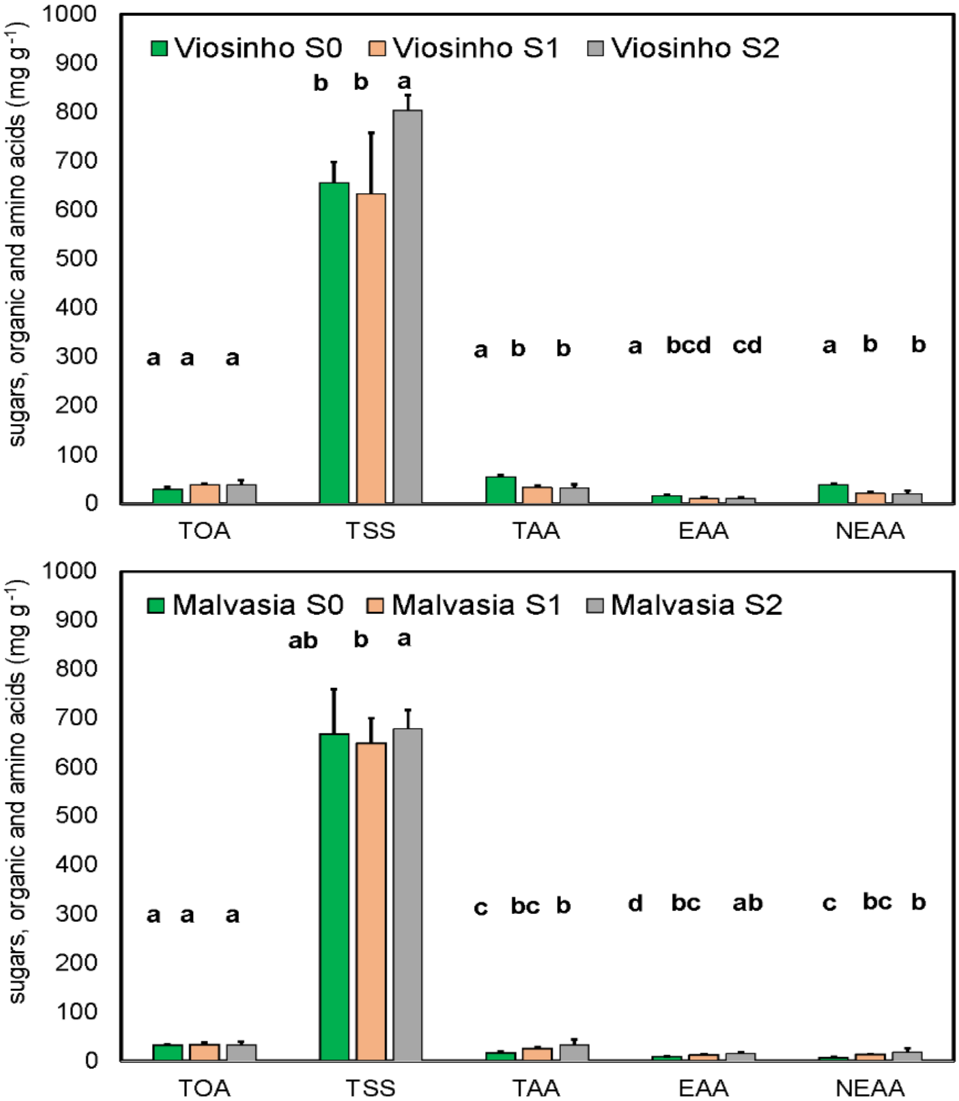

Figure 1. Contents of total organic acids (TOA), total sugars (TSS), total amino acids (TAA), essential amino acids (EAA), and non-essential amino acids (NEAA) in berries of Viosinho and Malvasia-fina grapevine varieties healthy (S0; severity level 0), moderately (S1; severity level 1) and severely (S2; severity level 2) affected by esca complex disease. Error bars represent standard deviation over three replications. Comparing the two varieties, value bars with different letters for each parameter are different (Turkey's test, $p \leq 0.05$).

With regard to organic acids, no significant differences were observed between the TOA in berries from healthy and esca-affected vines (Figure 2). The must of symptomatic vines has been reported to have higher levels of malic and tartaric acids than the must of healthy and asymptomatic vines [9]. In the same study [9], a strong reduction in sugar levels of the must from symptomatic vines was found. In the present study, TSS levels remained unchanged in the berries of Viosinho vines moderately affected but increased in berries of vines severely affected by esca complex. For Malvasia-fina vines, no effect was observed in relation to healthy vines, but berries from severely affected vines had higher TSS levels than berries from moderately affected vines (Figure 2). A cultivar effect was observed for the TAA parameter. Berries from Viosinho-affected vines had a 39.76–

41.67% lower TAA content than berries from healthy vines. In the case of Malvasia-fina, the opposite was found, with a 33.04–123.34% increase. For both varieties, variations in EAA and NEAA levels in the berries were correlated with esca symptom severity (Figure 2).

4. Conclusions

A quality wine is dependent on grapes with optimum sensory and nutritional characteristics. The data from the present study show a correlation between esca complex leaf symptom severity and several chemical parameters of grape berries. Notably, amino acids progressively increased in Malvasia-fina with leaf deterioration and decreased in Viosinho. A decrease in saturated fatty acids was observed, concomitantly with an increase in unsaturated fatty acids. Changes in all fatty acids' parameters were consistent and similar between the grapevine varieties Viosinho and Malvasia-fina. There was no correlation between the severity of the leaf symptoms and organic acids and sugars. TOA remained unaffected in both varieties; TSS increased in Viosinho and remained unaffected in Malvasia-fina. Strategies directed towards the reduction of foliar symptom expression in vineyards might provide winegrowers with the tools to adapt to the constraints of esca complex disease and produce table grapes and wines of high quality.

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Data Availability Statement: The data that support the findings of this study are available within the article.

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