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Proceeding Paper

Interactions between Zn Enrichment of Grapes *cv.* Syrah Fertilized with ZnO and Photoassimilates Mobilization †

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Abstract: Micronutrients have an important role in crops, namely Zn, being responsible for several physiological pathways, improving crops quality and growth. Zn role is related to enzymes activity, carbohydrate metabolism, photosynthesis, protein metabolism and maintenance of the integrity of biological membranes. Considering his importance and the deficiency observed worldwide, an itinerary of foliar spraying with zinc oxide (ZnO) in vines of *cv.* Syrah during the production cycle, was outlined at Biscaia field in Palmela, Portugal (38° 35′23.629″ N; 8° 51′ 46.208″ W). The treatment applied have concentrations of 10% and 30% (150 and 450 g ha^{-1}). At harvest, Zn concentration in grapes, reached a maximum increase of 55% with the highest treatment concentration, face to control. Also, leaf gas exchange after foliar spraying, didn't present toxic signs in both concentrations, even being observed a positive impact in Pn and iWUE, thus contributing to biomass levels. Moreover, remote detection through Unmanned Aerial Vehicles (UAV's), allowed to obtain the morphology of the field, being observed a superficial drainage capacity of 65% with water lines in the direction of NW-SE and SE sense, along the lines of the vines, also contributing to quality of the crops. This strategy of Zn enrichment, demonstrated to have potential benefits for crops, being additionally advantageous for consumption once this micronutrient has several important functions.

Keywords: leaf gas exchange; Syrah; Zn; ZnO

1. Introduction

Grapes are one of the fruits with an important role in the total global production, particularly in Europe [1], since this continent presents the main wine production and vineyard area in the world [2]. There are 60 species of grapes of the genus Vitis worldwide, with *Vitis vinifera* being the European grape, therefore, the most common [3]. Among the advantages of vine cultivation, its composition has shown to have potential benefits for human well-being, related to the antioxidant components that are mainly responsible, namely anthocyanins, resveratrol and flavonoids, which, in addition to their antioxidant capacity, have cardioprotective, anticancer, anti-inflammatory, anti-aging and antimicrobial characteristics [4].

In order to provide a good quality of grapes, Zinc (Zn) is an essential micronutrient involved in crop growth and reproduction, also contributing to human health [5]. Considering the different cultures, grapes are in the group that are more vulnerable to Zn deficits [6], with the concentration of this micronutrient varying according to the different parts of the vine, with the roots showing higher concentrations compared to the fruit [7]. Zinc is related to several physiological functions in crops: membrane structure, photosynthesis and sugar formation, phytohormones activity, lipid and nucleic acid metabolism, gene expression and regulation, protein synthesis and also defense against drought and disease. Moreover, his role is also related to its activity as a cofactor for many hormones (*i.e,* auxin), which will affect plant growth and development. [6]. Regarding the photosynthesis, Zn's role influences the repair of processes of PSII, the conversion of carbon dioxide to reactive bicarbonate species required for the fixation to carbohydrates and stomata opening [8], which in the case of deficiency can lead to a loss of quality in crops growth, once 90% of biomass results from photosynthesis [9]. Therefore, it is important an adequate supply of nutrients, being mineral fertilizers, an option used to potentiate a positive response on food production [10]. Additionally, the nutrient content is influenced by water status of the soil, being an important factor to guarantee a proper nutrition of crops [11], and the normal function of photosynthesis, because soil water content affects leaf phenology and photosynthetic rate [12].

2. Materials and Methods

2.1. Experimental Field

The itinerary of ZnO (Zinc oxide) foliar spraying in grapes of cv. Syrah, was implemented at Biscaia, an irrigated terrain, in Palmela, Portugal (38° 35′23.629″ N; 8° 51′ 46.208″ W). Pulverizations during the production cycle with treatment ZnO occurred between 16th of June and 25 September, with concentrations of 10 and 30%, 150 and 450 g ha^{-1} respectively. Harvest was performed on 11 October of 2018.

2.2. Quantification of Zn in Grapes

At harvest, Zn content in grapes was measured using an atomic absorption spectrophotometer model Perkin Elmer AAnalyst 200, fitted with a deuterium background corrector, and the AA WinLab software program. Previously, grapes were cut, dried (until constant weight, at 60 °C) and subjected to an acid digestion procedure with a mixture of HNO^{3-}HCL (4:1), and then the sample is filtrated [13]

2.3. Leaf Gas Exchange

A portable open-system infrared gas analyzer (Li-Cor 6400, LiCor, Lincoln, NE, USA) was operated under environmental conditions, with external CO_2 (ca. 400 ppm) and PPFD ranging between 1200–1400 μmol m^{-2} s^{-1}. Following Rodrigues et al. (2016), leaf gas exchange parameters were obtain using 4-6 randomized leaves per treatment of Biscaia field, in 13 September, after three foliar applications. Leaf rates of net photosynthesis (Pn), stomatal conductance to water vapor (gs) and transpiration (E) were determined under photosynthetic steady-state conditions after ca. 2 h of illumination (in the middle morning). Leaf instantaneous water-use efficiency (iWUE) was calculated as the Pn-to-E ratio, representing the units of assimilated CO_2 per unit of water lost through transpiration [14].

2.4. Morphology of the Field

High resolution RGB (20Mp) and Parrot Sequoia Plus multispectral cameras installed in an unmanned aerial vehicle (UAV), model DJI Phantom 4 Pro +, were used for obtaining images of the field. The multispectral camera has four band sensors: Green (550 BP 40), Red (660 BP 40), Red Edge (735 BP 10) and Near Infrared (790 BP 40). Data acquired, was processed in to an orthophotomap and using the altimetry data, a digital model of the terrain (MDT) was obtained, as well as the surface drainage model (using the ARCGIS and Agisoft Photoscan software) [15]. Analyses considered Direção Geral de Agricultura Desenvolvimento Rural (1972), were the highest class and lowest, corresponded to the land that enhances the surface runoff of the water and does not promote infiltration and to flat surfaces, that can potentiate accumulation of surface water respectively.

2.5. Statistical Analyses

Applying the One-Way ANOVA ($p \leq 0, 05$), statistics of data were determined to access differences. After, a Tukey's test was performed for mean comparison with 95% of confidence level.

3. Results

3.1. Quantification of Zn in Grapes

Zn content in grapes presented values between 6.7–12.3 ppm, being found a higher value with concentration of 30%, achieving an increase of 55% and demonstrating significative differences compared to control (Figure 1).

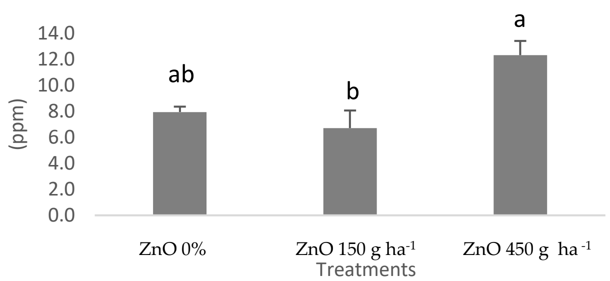

Figure 1. Average content + S.E. (n = 3) of Zn in fruits at harvest of *Vitis vinifera* L. variety Syrah. Different letters (a, b) indicate significant differences among treatments ($p < 0.05$).

3.2. Leaf Gas Exchange

After the 3rd application of ZnO in Syrah grapes, significant differences were found in relation to the control, in the parameters E and iWUE, with a lower and higher value being observed, respectively (Table 1). Since iWUE is the ratio between Pn and E, although it is not significant, the grapes fertilized with ZnO had a higher value for Pn, thus justifying the increase of iWUE (Table 1). Regarding gs parameter, control grapes present a higher value, which is also according to the value of E parameter, being higher for control (Table 1).

Table 1. Average ± SE of leaf gas exchange parameters-net photosynthesis (Pn), stomatal conductance to water vapor (gs), transpiration (E) rates, and as well as variation in the instantaneous water use efficiency (iWUE = Pn/E) after 3rd leaf application. Different letters (a, b) indicate significant differences between treatments ($p < 0.05$).

Treatment	Photosynthetic Parameters
	Pn (μmol CO_2 m^{-2} s^{-1})
0%	13.6 ± 0.4 a
ZnO 30%	13.7 ± 0.1 a
	gs (mmol H_2O m^{-2} s^{-1})
0%	201.3 ± 5.8 a
ZnO 30%	197.8 ± 4.4 a

	E (mmol H_2O m^{-2} s^{-1})
0%	5.4 ± 0.1 a
ZnO 30%	4.6 ± 4.4 b
	iWUE (mmol CO_2 mol^{-1} H_2O)
0%	2.5 ± 0.1 b
ZnO 30%	3.0 ± 4.4 a

3.3. Morphology of the Field

Biscaia field showed a surface drainage network along the lines of the vines, with NW-SE direction, flowing to SE (Figure 2). According to the quota of the field, most of the area presents a high inclination, being found only 35% with morphological characteristics prone to surface water accumulation (or infiltration, depending on the permeability characteristics of the land) (Figure 2).

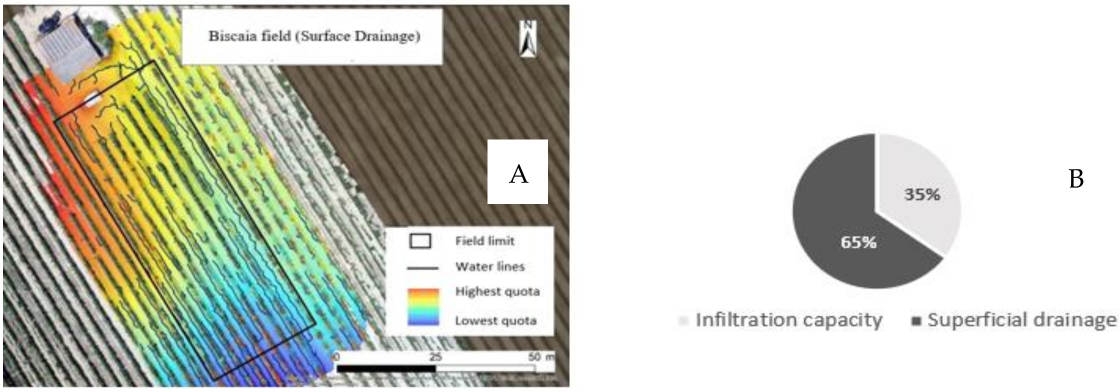

Figure 2. Orthophotomaps of grapes *Vitis vinifera* cv. Syrah. (**A**) Digital elevation model of grapevines and water lines. (**B**) Water accumulation capacity according to slope classes [15].

4. Discussion

Water scarcity is a universal problem with increasing climate change [16], with vine being a crop that is often subject to low soil water availability [17]. Despite the resistance of this crop to water stress due to a developed root system [17], depending on the severity of the induced stress, a negative impact on several vital processes such as photosynthesis and consequently growth (i.e., reaching losses of 50% on yields) and crop productivity may be observed. This condition of water deficit leads crops to suffer through changes in the water relationship, biochemical and physiological processes, membrane structure and ultrastructure of subcellular organelles [18], as an adaptation mechanism for survival [19]. Therefore, it is essential to optimize the WUE (water use efficiency) of crops, this is to lose less water and have more carbon assimilation, through stomatal control. Being even used in several studies deficit irrigation strategies, proving to be efficient in improving WUE and consequently in the quality of the fruits, without having a great impact on yields [20].

In fact, the grapes in this study even being subjected to irrigation are more susceptible to water deficits since they have only 35% of the water storage capacity, namely in the SE region, implying less water available in a dry climate that has been observed in recent years. Without water, crops cannot adequately absorb nutrients [19], which poses another important concern related to nutrient supply, placing an additional stress on the grapes. Following this assumption, many vine soils are Zn deficient, accentuating the water deficit this problem. Furthermore, previous studies have shown Zn with a protective function in plants against abiotic and biotic stresses [16], which can be related to his involvement in redox reactions and protection of plasma membranes from oxidative damage, even being observed in a study those effects with the application of a low amount of Zn [21].

It has been reported [22] that grapes Zn fertilizers improve the photochemical reactions occurring in thylakoid membrane (ensuring membrane integrity), electron transport through PSII and increases photosynthetic rate and chlorophyll content.

With this study, a positive trend was also found, since with increasing Zn content in grapes (55% higher amount of Zn was found in the highest concentration of ZnO-30%, 450 g ha^{-1}, relatively to grapes without fertilization) an increase in Pn and WUE was found.

5. Conclusions

Fertilization with ZnO (30%, 450 g ha^{-1}) was efficient in increasing Zn amount in Syrah grapes, being also observed a positive effect in photosynthesis parameter iWUE. Therefore, this fertilization can decrease Zn deficits in grapes and at the same time, potentiate a positive response in vineyards growth.

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