



Influence of ZnO Fertilization of Grapes *cv.* Syrah on Photosynthesis [†]

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Abstract: Zinc has an important role in crops; it is responsible for several physiological pathways and improving crop quality and growth. Zinc plays an important role related to enzyme activity, carbohydrate metabolism, photosynthesis, protein metabolism, and maintenance of the integrity of biological membranes. Considering its importance and the deficiency observed worldwide, a workflow of foliar spraying with zinc oxide (ZnO) in vines of *cv.* Syrah during the production cycle was implemented at the Biscaia field in Palmela, Portugal. The treatment applied had concentrations of 150 and 450 g ha^{−1}. At harvest, Zn concentration in grapes reached a maximum increase of 55% with the treatment ZnO- 450 g ha^{−1}, face to control. Furthermore, leaf gas exchange after foliar spraying did not present toxic signs in both concentrations and was even observed as a positive impact on net photosynthesis and leaf instantaneous water-use efficiency, thus contributing to biomass levels. Moreover, remote detection through Unmanned Aerial Vehicles allowed us to obtain the morphology of the field, and we 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 the quality of the crops. This strategy of Zn fertilization is demonstrated to have potential benefits for crops and is additionally advantageous for consumption as 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 vine-

yard area in the world [2]. There are 60 species of the genus *Vitis* worldwide, with *V. vinifera* being the European grape, therefore, the most common [3]. Among the advantages of vine cultivation, its composition has been shown to have potential benefits for human well-being. Those are related to the antioxidant components that are mainly responsible, namely anthocyanins, resveratrol, and flavonoids. Additionally, they have cardioprotective, anticancer, anti-inflammatory, anti-aging, and antimicrobial characteristics [4].

In order to provide good quality grapes, Zinc (Zn) is an essential micronutrient involved in crop growth and reproduction, also contributing to human health [5]. Considering the different cultivars, the grapevine is 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, defense against drought and disease. Moreover, it plays the role of a cofactor for many hormones (i.e., auxin), which can 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 of 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, are an option used to potentiate a positive response for food production [10]. Additionally, the nutrient content is influenced by the water status of the soil, which is an important factor to guarantee the proper nutrition of crops [11,12] and the normal function of photosynthesis.

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). The pulverizations were conducted during the production cycle between 16 June and 25 September with concentrations of 0, 10, and 30%, 0, i.e., 150 and 450 g ha⁻¹, respectively (control was sprayed with water). The tests were carried out in rows of vines, where one row corresponds to the control and two distinct rows to the treatment's concentrations. Harvest was performed on 11 October 2018.

2.2. Quantification of Zn in Grapes

In sample preparation, grapes were cut, dried (until constant weight, at 60 °C), subjected to an acid digestion procedure with a mixture of HNO₃–HCL (4:1), and then the sample was filtrated [13]. At harvest, Zn content in grapes was measured using an atomic absorption spectrophotometer model Perkin Elmer AAnalyst 400 (Waltham, MA, USA), fitted with a deuterium background corrector, and the AA WinLab software program (version 32).

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 CO₂ (ca. 400 ppm) and PPFD ranging between 1200 and 1400 μmol m⁻² s⁻¹. Following Rodrigues et al. (2016), leaf gas exchange parameters were obtained using 4–6 randomized leaves per treatment of the Biscaia field, on 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 CO₂ per unit of water lost through transpiration [14].

2.4. Morphology of the Field

High resolution RGB (20 Mp) 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). Using the altimetry data, a digital model of the terrain (MDT) was obtained, as well as the surface drainage model (using the ARCGIS (version 2.7.0) and Agisoft Photoscan software (version 1.6.2) [15]. Analyses considered Direção Geral de Agricultura Desenvolvimento Rural (1972), where 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 a 95% of confidence level.

3. Results

3.1. Quantification of Zn in Grapes

Zn content in grapes presented values between 6.7 and 12.3 ppm, finding a higher value with a concentration of 450 g ha⁻¹, achieving an increase of 55%, and demonstrating significant differences compared to control (Figure 1).

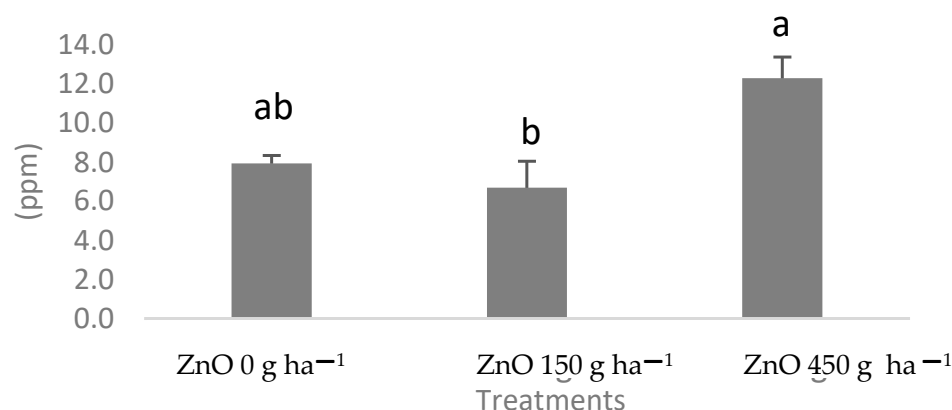


Figure 1. Average content + S.E. ($n = 3$) of Zn in fruits at harvest of *Vitis vinifera* L. variety Syrah. Different letters (a, b) indicate significant differences among treatments ($p < 0.05$).

3.2. Leaf Gas Exchange

After the third 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 in iWUE (Table 1). Regarding the gs parameter, control grapes presented a higher value, which is also according to the value of the E parameter, which is higher for the control (Table 1).

Table 1. Average \pm SE ($n = 6$) of leaf gas exchange parameters-net photosynthesis (Pn), stomatal conductance to water vapor (gs), transpiration (E) rates, and variation in the instantaneous water use efficiency (iWUE = Pn/E) after the third leaf application (i.e., control with water). Different letters (a, b) indicate significant differences between treatments ($p < 0.05$).

Treatment	Photosynthetic Parameters
	Pn ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)
0 g ha ⁻¹	13.6 \pm 0.4 a
ZnO 450 g ha ⁻¹	13.7 \pm 0.1 a
	gs ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)
0 g ha ⁻¹	201.3 \pm 5.8 a
ZnO 450 g ha ⁻¹	197.8 \pm 4.4 a
	E ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)
0 g ha ⁻¹	5.4 \pm 0.1 a
ZnO 450 g ha ⁻¹	4.6 \pm 4.4 b
	iWUE ($\text{mmol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$)
0 g ha ⁻¹	2.5 \pm 0.1 b
ZnO 450 g ha ⁻¹	3.0 \pm 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, with only 35% with morphological characteristics prone to surface water accumulation (or infiltration, depending on the permeability characteristics of the land) [15] (Figure 2).

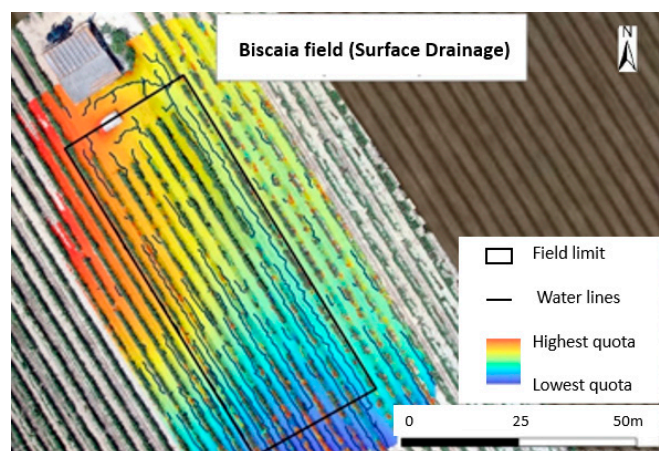


Figure 2. Orthophotomaps of grapes *Vitis vinifera* cv. Syrah. Digital elevation model of grapevines and water lines and water accumulation capacity.

4. Discussion

Grapevines have a developed root system that contributes to resistance to water stress [16]. Crops under this deficit suffer changes in biochemical and physiological processes, membrane structure, and ultrastructure of subcellular organelles [17]. Depending on the severity of the stress, a negative impact on several vital processes such as photosynthesis, growth (i.e., reaching 50% productivity losses), and crop productivity may be observed [17]. Therefore, it is essential to optimize the WUE (water use efficiency) of crops, so we lose less water and have more carbon assimilation through stomatal control. This was used in several studies on deficit irrigation strategies, proving to be efficient in improving WUE and, consequently, the quality of the fruits without having a great impact on yields [18].

In fact, the grapes in this study 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 availability in a dry climate than has been observed in recent years. Without water, crops cannot adequately absorb nutrients [19], which poses another important concern related to nutrient supply, placing additional stress on the grapes. Following this assumption, many vine soils are Zn deficient, accentuating the water deficit of this problem. Furthermore, previous studies have shown Zn with a protective function in plants against abiotic and biotic stresses [20], which can be related to its involvement in redox reactions and protection of plasma membranes from oxidative damage, even being observed in a study of 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 the thylakoid membrane (ensuring membrane integrity), the electron transport through PSII, and increase the photosynthetic rate and chlorophyll content.

In this study, a positive trend was also found. With the increasing Zn content in grapes (55% higher amount of Zn was found in the highest concentration of ZnO- 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 and was also observed as a positive effect on the photosynthesis parameter iWUE. Therefore, this fertilization can decrease Zn deficits in grapes and, at the same time, potentiate a positive response in vineyard growth.

Supplementary Materials: The poster presentation can be downloaded at <https://www.mdpi.com/article/10.3390/IECPS2021-11936/s1>.

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