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# Zn Nutrition of *Vitis vinifera* White Grapes: Characterization of Antagonistic and Synergistic Interactions by µEDXRF Tissue Analyses †

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**Abstract:** Nutritional status performs an essential role in agriculture, affecting productivity and keeping crops functioning properly. Despite being required in smaller amounts, micronutrients are also needed for adequate growth, namely Zn (zinc) with regulatory, catalytic and structural functions. Fertilization with Zn is used to ameliorate the deficits of this micronutrient in more susceptible crops, such as grapes. Yet, the management of this application must consider the antagonistic and synergistic interactions among nutrients, as it affects their uptake and translocation rates. Therefore, a workflow with three ZnO foliar applications (30% and 60%, 450 and 900 g $ha^{-1}$, respectively) in the variety *Vitis vinifera* cv. Fernão Pires, was implemented in a field located in Palmela, Portugal. The concentration of Zn in the tissues was therefore evaluated by microenergy X-ray dispersion fluorescence (µEDXRF), showing an increase of 1.82 and 2.54 times in the seed and flesh of grapes fertilized with a concentration of 60% compared to control grapes, respectively. Using the same method, a synergistic relationship was observed for macronutrients, such as Ca and K, and micronutrients, such as Fe, P and Mn. In addition, a complementary analysis of grapes' density was carried out to verify changes in quality, in which no negative impact was observed due to Zn application. This study allows us to verify that the concentration of the applied Zn fertilizer brings benefits in the amount of nutrients that are important for development and crops quality.

**Keywords:** antagonism; grapes; synergism; *Vitis vinifera*; Zn fertilizer

## 1. Introduction

Micronutrient deficiencies or hidden hunger is affecting more than 2 billion people worldwide, leading to the development of strategies to mitigate this problem [1]. In this context, agronomic biofortification has become a tool to improve the mineral content in the edible parts of food crops faster, more reliably, more sustainably and economically [2]. Through foliar fertilization, it becomes possible to mitigate crop deficiencies, selecting target minerals [3] without limiting the fixation and retention of nutrients in the soil [4].

Fertilizing fruit trees, namely the vineyard, is considered an important tool to meet nutritional needs and optimize the yield and quality of the grapes [5,6]. Zinc (Zn) deficits are common worldwide, affecting the vine [7] with visible symptoms, such as chlorosis, necrotic spots and small leaves [6]. In this regard, Zn is an important micronutrient for plant growth, with catalytic, structural and regulatory functions [8], as a co-factor in the auxin metabolism, enzymatic activation, chlorophyll and nucleotide synthesis and genes expression and regulation [9]. According to research in grapefruit, Zn agronomic biofortification has been shown to be efficient and, in addition, enhancing growth and development [10].

Likewise, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe) and manganese (Mn) are also essential, affecting crops productivity [11]. Yet, through application of one nutrient, absorption and utilization of other nutrients can be affected in a positive or negative way, in a synergistic and antagonistic relationship, respectively [12]. These interactions become visible through growth and changes in nutrient amounts [13] affecting several metabolic processes [14].

## 2. Materials and Methods

### *2.1. Experimental Field*

The experimental work was carried out in a *Vitis vinifera* cv. Fernão Pires, at a field located in Palmela, Portugal (38°35′41.467″ N O 8°50′44.535″ W). Grapes were pulverized three times, during the reproductive cycle, with ZnO at concentrations of 30% and 60% (corresponding to 450 and 900 g $ha^{-1}$). Harvest was performed on 17 September.

### *2.2. Quantification of Nutrients in Grapes and Accumulation Level in Grape Tissues*

The nutrient content in the grapes was determined in the tissues (flesh and seeds) at harvest, with a micro-energy X-ray dispersion fluorescence (μ-EDXRF) (M4 Tornado™, Bruker, Berlin, Germany) system, according to Ref [15], with an adaptation of the assessment of nutrients, such as Zn, Fe, Mn, P, Ca, K and Mg in grapes.

### *2.3. Physicochemical Parameter of Grapes*

Determination of density (kg $m^{-3}$) was performed considering three randomized grapes per treatment.

### *2.4. Statistical Analysis*

Data were statistically treated, applying one-way ANOVA ($p \leq 0.05$) to determine differences, and before, a Tukey's test for mean comparison (95% confidence level) was performed.

## 3. Results

### *3.1. Quantification of Nutrients in Grapes Flesh and Seed*

Zn seed concentration reached an increase of 1.51 and 1.82 times more than control, with ZnO 30% and 60%, respectively (both significantly different). Regarding Mn and P, the same trend was observed, with ZnO 60% treatment demonstrating the highest value, followed by ZnO 30%, both being significantly different compared to control. For Mn and P, the range of values varied between 8.22 and 35.9, and 0 and 3403 ppm, respectively. Iron amount did not show significant differences between all samples, presenting values between 91.6 and 106 ppm.

The flesh of the grapes showed a significant increase in Zn concentration of 2.32 and 2.54 times more with ZnO 30% and 60%, respectively, compared to the control. Additionally, for the nutrient Fe and P, significant differences were observed with both treatments (i.e., ZnO 30% and 60%) compared to the control, but, contrary to what was observed in the seeds (i.e., Zn, Mn and P), the lower concentration of Zn fertilizer showed the highest value for these nutrients. On the other hand, for Mn, the same tendency of the seeds was observed, where the concentration of ZnO 60% presented a higher amount, with the two treatments being significantly different in relation to the control.

Seed Ca levels were higher with the 30% treatment (2.98%), followed by the 60% treatment (1.88%), both being significantly different compared to the control. In seed, the nutrient K, with the application of Zn fertilizer, did not result in significant differences in relation to the control, showing values between 5.16 and 6.40%. The concentration of Mg in seeds was significantly different and higher for control compared to grapes treated with ZnO.

In relation to the grape flesh, the nutrients Ca and K submitted for treatment increased significantly in relation to the control, with the highest values for the ZnO 30% treatment (1.89 and 15.4% for Ca and K, respectively). Likewise, for the relationship observed in the seeds, for the pulp, Mg significantly decreased with the application of the two treatments.

### *3.2. Density*

Fertilized grapes showed a higher value, although not demonstrating significant differences compared to control. Values for density in Fernão Pires grapes showed a range between 1150 and 2185 kg $m^{-3}$.

## 4. Discussion

Nowadays, to meet the food necessities according to population growth, different techniques are being explored to increase agricultural crops yields [16]. Accordingly, mineral nutrition is among the most used agricultural techniques to obtain higher yields and quality [17], namely Zn fertilization through soil and/or foliar applications [18]. Most studies were carried out with soil fertilization, implying the influence of physicochemical and biological soil properties, such as pH, water, structure, cation exchange capacity, redox conditions and activity of microorganisms [19]. As mentioned in the literature, Zn fertilization in soil has an antagonistic response with most nutrients, such as Fe, Ca, Mg, K, Mn and P, related to similar absorption mechanisms that lead to competition (i.e., except for P, which is a specific interaction) [20].

In this context, the present study carried out foliar applications with ZnO at different concentrations (i.e., 450 and 900 g $ha^{-1}$) in vines, proving to be efficient in increasing the Zn content in grapes, reaching 2.54 times more in the flesh and 1.82 in seed than without fertilization (Table 1). Furthermore, the other nutrients accessed showed mainly an interaction of synergism in the case of micronutrients and macronutrients, such as P, Mn, Ca, Fe and K (i.e., except Mg) (Tables 1 and 2). The results obtained showed that increase in Zn concentration in the leaves enhanced the uptake of other nutrients, being in agreement with Ref [21], suggesting that micronutrients such as Zn stimulate the plant metabolism, resulting in an intensified uptake of nutrients through the roots. Additionally, Zn fertilization is related to positive effects on photosynthesis and chlorophyll synthesis, which also facilitates the absorption and accumulation of nutrients in the leaves [22]. Likewise, a study with foliar application of Zn in a fruit tree showed the same tendency of increasing the amount of Zn in the leaves to promote nutrients such as P and Ca and demonstrated to be antagonistic with Mn and Fe [22]. In the case of Fe, our results showed a different response, with Zn fertilization at concentrations applied (i.e., 450 and 900 g $ha^{-1}$), showing a relation of synergism in grapes flesh (Table 1). As for Potassium, it presented the same response as Fe, with a synergism relation only in the flesh, with the 30% (i.e., 450 g $ha^{-1}$) treatment being the one with the greatest increase in the amount of this element (Table 2). A greater increase was noticed in most results with the lowest ZnO 30% treatment, namely for the macronutrients Ca and K, and for the micronutrients Fe and P (i.e., in the flesh) (Tables 1 and 2). As for the antagonistic interactions, in this study, they only occurred in Mg, where the greatest amount of treatment with Zn significantly reduced the Mg concentration. Magnesium has a competitive relationship with other nutrients; according to Ref [23], the nutrient Ca, due to a greater affinity with the binding sites of the root plasma membrane, decreases the amount of Mg [23]. In fact, in the present study this condition is present with an increase in Ca content being observed with Zn fertilization.

**Table 1.** Average content ($n$ = 3) of Zn, Fe, Mn and P (ppm) in grape flesh and seeds (after dehydration) in Fernão Pires grapes at harvest and the respective degree of uncertainty. Letters a, b, c indicate significant differences between treatments ($p < 0.05$). Treatments ZnO with concentrations of 0%, 30%, 60%. (i.e., 0, 450 and 900 g $ha^{-1}$).

| | | **Micronutrient (Seed)** | | |
|---|---|---|---|---|
| Sample | Zn | Fe | Mn | P |
| Control | 24.6 ± 1.23 c | 91.6 ± 4.58 a | 8.22 ± 0.41 c | 0.00 ± 0.00 c |
| ZnO 30% | 37.1 ± 1.85 b | 106 ± 5.31 a | 14.8 ± 0.74 b | 1148 ± 57.4 b |
| ZnO 60% | 44.7 ± 2.24 a | 105 ± 5.24 a | 35.9 ± 1.79 a | 3403 ± 170 a |
| | | **Micronutrient (Flesh)** | | |
| Control | 23.4 ± 1.17 b | 91.2 ± 4.56 c | 8.66 ± 0.43 c | 0.00 ± 0.00 c |
| ZnO 30% | 54.4 ± 2.72 a | 278 ± 13.9 a | 27.4 ± 1.37 b | 2662 ± 133 a |
| ZnO 60% | 59.4 ± 2.97 a | 228 ± 11.4 b | 51.8 ± 2.59 a | 2203 ± 110 b |

**Table 2.** Average content ($n$ = 3) of Ca, K and Mg (%) in grape flesh and seeds (after dehydration) in Fernão Pires grapes at harvest and the respective degree of uncertainty. Letters a, b, c indicate significant differences between treatments ($p < 0.05$). Treatments ZnO with concentrations of 0%, 30%, 60%. (i.e., 0, 450 and 900 g $ha^{-1}$).

| | **Macronutrient (Seed)** | | |
|---|---|---|---|
| Sample | Ca | K | Mg |
| Control | 0.82 ± 0.04 c | 6.11 ± 0.31 ab | 12.4 ± 0.62 a |
| ZnO 30% | 2.98 ± 0.15 a | 6.40 ± 0.32 a | 0.39 ± 0.02 c |
| ZnO 60% | 1.88 ± 0.09 b | 5.16 ± 0.26 b | 2.78 ± 0.14 b |
| | **Macronutrient (Flesh)** | | |
| Control | 0.35 ± 0.02 c | 5.44 ± 0.27 c | 10.0 ± 0.50 a |
| ZnO 30% | 1.89 ± 0.09 a | 15.4 ± 0.77 a | 5.84 ± 0.29 b |
| ZnO 60% | 1.24 ± 0.06 b | 10.9 ± 0.55 b | 5.51 ± 0.28 b |

Grape density is also important, as it depends on important biophysical parameters (i.e., grape diameter, length, volume and mass) and biochemical composition [24]. According to the literature, higher densities (i.e., ≥1088 kg $m^{-3}$) demonstrated benefits, being more advantageous for health and sensorially [25]. This experimental work with Fernão Pires grapes presented greater values ranging between 1150 and 2185 kg $m^{-3}$ (Figure 1), where fertilization did not negatively affect the quality of grapes, this being important for consumer acceptance. In fact, fertilized grapes showed higher values than control, although not significantly.

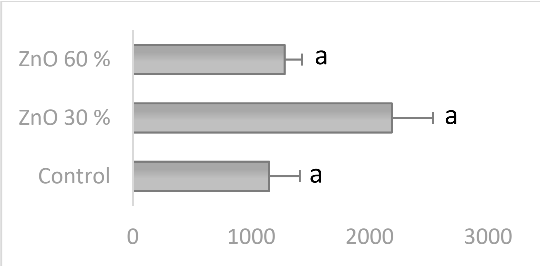


**Figure 1.** Average content ($n$ = 3) of density (kg $m^{-3}$) in Fernão Pires grapes at harvest and the respective degree of uncertainty. Letter a indicates the absence of significant differences between treatments ($p < 0.05$). Treatments ZnO with concentrations of 0%, 30%, 60%. (i.e., 0, 450 and 900 g $ha^{-1}$).

## 5. Conclusions

Zinc foliar fertilization with ZnO is efficient, increasing the amount of this micronutrient in the flesh and seeds of the grape cv. Fernão Pires, and presenting a synergistic relationship with P, Fe, Mn, Ca and K. Thus, this technique is an important tool to improve the nutritional status and quality of the grape. An antagonistic relationship with the macronutrient Mg was also observed.

Additionally, fertilization did not affect density, with Fernão Pires grapes presenting values greater than 1088 kg $m^{-3}$, this being important for health and sensory capacity.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/IOCAG2022-12317/s1.

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