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A case study about the use of precision agriculture technology applied to a Zn biofortification workflow for grapevine *Vitis Vinifera* cv Moscatel †

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Abstract: As human population is growing worldwide, the food demand is sharply increasing. Following this assumption, strategies to enhance the food production are being explored, namely smart farming, for monitoring crops during the production cycle. In this study, a vineyard of *Vitis Vinifera* cv. Moscatel located in Palmela (N 38º 35′ 47.113′′ O 8º 40′ 46.651) was submitted to a Zn biofortification workflow, through foliar application of zinc oxide (ZnO) or zinc sulfate (ZnSO4) (respectively, at a concentration of 60% and 90% - 900g.ha-1 and 1350g.ha-1). The field morphology and vigor of the vineyard was performed through Unmanned Aerial Vehicles (UAV's) images (assessed with altimetric measurement sensors), synchronized by GPS. Drainage capacity and slopes showed 1/3 of the field with reduced surface drainage and a maximum variation of 0.80 m between the extremes (almost flat) respectively. The NDVI (Normalized Difference Vegetation Index) values reflected a greater vigor in treated grapes with treatment SZn90 showing a higher value. These data were interpolated with mineral content, monitored with atomic absorption analysis (showing a 1.3 fold increase for the biofortification index). It was concluded that the used technologies furnishes specific target information in real time about the crops production.

Keywords: Grapes; NDVI; Precision Agriculture; UAV's; *Vitis Vinifera*; Zn Biofortification

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1. Introduction

By 2050, due to the increase of the world population, to avoid hunger, food production must significantly increase [1]. Besides, to ensure safety, food also must have high quality, namely at a prophylactic level, providing the necessary nutrients, since it is expected that their deficiency might affect the health of more than two billion people worldwide [2, 3]. In this context, some alternatives are being suggested, namely agronomic biofortification to increase target nutrients in edible plant tissues. This alternative can be accomplished through soil and foliar application, yet this last seems to allow plants to assimilate micronutrients with high efficiency, as it does not depend upon root-to-shoot translocation [4, 5]. Beyond the main aims of agronomic biofortification, some evidence showed that yield and nutritional quality increases with this practice [6].

Zinc deficiency continues to affect around three billion people worldwide, leading to the appearance, among others, of neurological disorders, autoimmune, degenerative diseases related to age, Wilson's disease, cardiovascular problems, and diabetes mellitus [7].

To address this increase in food demand, other factors also may be considered like climate change, the limited availability of arable lands, as well as the growing necessity for freshwater, making it indispensable to resort to new technologies such as Unmanned Aerial Vehicle (UAVs). This technology can carry different types of cameras such as multispectral that allows users to obtain vegetation indices translated by Normalized Difference Vegetation Index (NDVI), providing us with information about biomass levels and stress conditions like crop diseases, water stress, pest infestations, nutrient deficiencies and other factors that affect crop productivity [8]. Regarding other advantages of UAVs, acquirement of field data its arried out more easy, fast and cos-effective way [9]. Following this assumption, the present study used multispectral images from UAVs to monitor Zn biofortified vineyards, once this fruit plays a predominant role in the development of the world and, according to the Food and Agriculture Organization (FAO), covers 75.866 square kilometers worldwide, additionally helping in some health problems [10].

2. Materials and Methods

2.1. Experimental Field

Biofortification with Zn was performed in a *Vitis vinifera* L. variety Moscatel field located in Lau Novo, Palmela, Portugal (38º 35′ 47.113′′ O 8º 40′ 46.651′′ W), under irrigation conditions. Foliar application with zinc sulfate ($ZnSO_4$) and zinc oxide (ZnO), at concentrations of 0%, 60% and 90% (0, 900 and 1350 g ha^{-1}) was performed between 29th of June and 19th July, with harvest being carried out at 10th September of 2019.

2.2. Field Morphology and vigor of the Vine

Flight planning and execution was performed to obtain images with a high resolution RGB (20Mp) and Parrot Sequoia Plus installed multispectral cameras in an unmanned aerial vehicle (UAV), model DJI Phantom 4 Pro +. The multispectral camera had four band sensors: Green (550 BP 40), Red (660 BP 40), Red Edge (735 BP 10) and Near Infrared (790 BP 40). After acquisition of images, an orthophotomap was processed and, using the altimetry data, the digital model of the terrain (MDT) was obtained, as well as the surface drainage model (using the ARCGIS and Agisoft Photoscan software), being created the vegetation index maps that reflect the vigor of the plants (NDVI) [11].

2.3. Quantification of Zn in grapes

At harvest, grapes were cut, dried (until constant weight, at 60ºC) and subjected to an acid digestion procedure with a mixture of HNO_3-HCL (4:1), according to [12]. Then the samples were filtrated, and Zn contents were measured using an atomic absorption spectrophotometer model Perkin Elmer AAnalyst 200, fitted with a deuterium background corrector, and the AA WinLab software program.

2.4. Statistical Analyses

Data were statistically analyzed using a One-Way ANOVA ($p \leq 0, 05$) to access differences, followed by a Tukey's test for mean comparison (95% confidence level).

3. Results

The slopes of the experimental field were determined, being found a moderate surface drainage prevailing, with 63.86% of infiltration capacity (Table 1).

Table 1. Slope characterization before foliar application of Lau Novo field.

Slope classes (%)	**Surface Drainage**	**Area (m²)**	**% Area**
[0 – 5 %]	Reduced	589.9	34.87
]5 – 20]	Moderate	1080.5	63.86
> 20 %	Elevated	21.4	1.27
Total		1691.8	100

Zn contents in treated grapes showed, relatively to the control, 1.2 - 1.3 fold increases in the higher concentrations of treatments, OZn90 and SZn90 respectively (Table 2).

Table 2. Average content ± S.E. (n = 3) of Zn in fruits at harvest of *Vitis vinifera* L. variety Moscatel. Letter a indicate the absence of significant differences among treatments (P≤ 0,05). Treatments OZn60, OZn90, SZn60 e SZn90 indicate the following concentrations for zinc oxide (ZnO) or Zinc sulfate ($ZnSO_4$): 0%, 60%, 90%. (i.e., 0, 900 e 1350 g ha^{-1}).

Moscatel variety	Zn (ppm)	
	Mean	SE
Control	6.04a	± 0.67
OZn60	6.44a	± 0.50
OZn90	7.91a	± 0.28
SZn60	6.58a	± 0.65
SZn90	7.49a	± 0.75

After the 4th treatment, Moscatel treated grapes revealed a positive response, showing higher NDVI values than the control, with treatments SZn displaying highest foliage densities (Table 3; Figure 1).

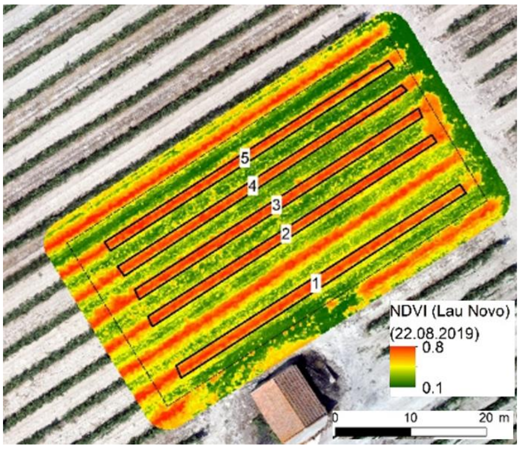

Figure 1. NDVI index of Lau Novo field after the 4th application (1-Control; 2- Treatment $ZnSO_4$ 60 %; 3- Treatment $ZnSO_4$ 90%; 4- Treatment ZnO 60%; 5- Treatment ZnO 90%).

Table 3. Average vigor ± S.E. (n = 3) in fruits of *Vitis vinifera* L. variety Moscatel after the 4th application. Letter a indicate the absence of significant differences among treatments (P≤ 0,05). Treatments OZn60, OZn90, SZn60 e SZn90 indicate the following concentrations for zinc oxide (ZnO) or Zinc sulfate ($ZnSO_4$): 0%, 60%, 90%. (i.e., 0, 900 e 1350 g ha^{-1}).

Treatment	**Mean**	**SE**
Control	0.58	0.18
OZn60	0.61	0.16
OZn90	0.61	0.18
SZn60	0.64	0.15
SZn90	0.64	0.14

4. Discussion

Climate changes are a concern among winemakers, as grapes are one of the fruit crops most sensitive to severe drought conditions and water shortage [13]. Water shortage is considered one of the most stressful promoters in grapes, leading to yield and quality losses. Under these conditions, decreases in relative water content (RWC), leaf dry matter, chlorophyll (Chl) content, net photosynthetic rate (PN), ribulose-1,5-bisphosphate carboxylase (RuBPC) and nitrate reductase (NR) activities of Riesling grapevines can develop [14].

Portuguese climate is becoming more dryer, being indispensable to develop adaptation strategies to face water scarcity. In this framework, a smart irrigation, optimizing grape composition and providing a balanced solution between environment and plant requirements can become a relevant option. Indeed, with higher dryness, and without irrigation, yield reductions were already found in Portugal (i.e., Alentejo, Lisboa, Minho and Terras-da-Beira) [15]. Moreover, an efficient irrigation showed a diminishing volume of water applied to crops fields by 30–70% and an increase crop yields by 20–90% [16]. According to our data, the experimental field with Moscatel grapes, showed a moderate capacity of surface drainage in 63.86% of the area (Table 1), which determines a moderate infiltration capacity, contributing to the groundwater recharge (i.e., water available for plant growth) [17]. Additionally, as this field is being irrigated, the potential hydric stress derived by rain scarcity, observed in the last decades, is being mitigated.

In Turkey, fertilization with Zn was carried out by leaves spraying, which determined increases in productivity of about 25% in cereals, with the concurrent augmentation of Zn contents in the edible parts of the plants [18]. Our study also showed an increase of Zn contents in grapes sprayed with ZnO and $ZnSO_4$ (Table 2). Besides, following [19, 20], Zn biofortification through foliar application, also affected yield parameters. Indeed, through vegetation indices, namely NDVI, is possible to access health conditions, providing information of photosynthetic capacity, which can be correlated with plant vigor and vegetation abundance, health and growth [21, 22]. This index has values normalized between +1 and -1, with higher values indicating a denser vegetation [22]. In fact, our data showed higher values for NDVI in the vines fertilized with $ZnSO_4$ and ZnO, relatively to the control, but treatments with $ZnSO_4$ triggered a higher vigor (Table 3).

5. Conclusions

Through images obtained with cameras attached to UAV's, it's possible to get information about morphology of the field and potential limiting conditions for vines development. Using the Moscatel field as a test system, important characteristics, namely moderate infiltration capacity and the use of irrigation, enabling vines to have more resistance to hydric stress were optimized. The obtained images further gave information about the crops state, being detected a positive response to Zn fertilization with an increase in the Zn contents and vigor of vines subjected to ZnO and $ZnSO_4$.

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