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Monitoring a Calcium Biofortification Workflow in an Orchard of *Pyrus communis* var. Rocha Applying Precision Agriculture Technology†

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Abstract: Smart farming techniques can be used to maximize food production. This can be achieved by rapid detection of variations in crops and clever use of resources such as water and fertilizers, which might minimize crop stress through direct target practices. In an orchard located in the West region of Portugal (GPS coordinates 39°23′28.997′′N; 9°4′52.483′′W), a Ca biofortification workflow with 7 foliar sprays of $CaCl_2$ (4 $kg.ha^{-1}$ and 8 $kg.ha^{-1}$) was used to increase Ca contents in "Rocha" pear trees. During the biofortification process, an Unmanned Aerial Vehicle synchronized by GPS, was used to characterize the orchard regarding it`s morphology (slope) and to monitor trees (NDVI - Normalized Difference Vegetation Index). These data were correlated with Ca content (assessed by X-Ray fluorescence analysis) and photoassimilates synthesis (assessed by leaf gas exchange measurements). The orchard showed no major slopes and after 4 sprays with $CaCl_2$, NDVI values revealed no major differences between the control and sprayed trees. Accordingly, leaf gas exchange parameters did not reveal negative impacts in the photoassimilates synthesis of the sprayed trees, although in the leaves Ca content significantly increased. The use of precision agriculture techniques in correlation to other analysis to assess plant stress is discussed.

Keywords: Biofortification; Calcium; Leaf gas exchange; NDVI; Pears; Precision Agriculture; X-Ray fluorescence analysis

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1. Introduction

The agro-industrial sector will face challenges to feed an exponential growing population, set to reach 9.1 billion by 2050 [1]. Besides, consumers needs must avoid the development of pathologies related to nutrition deficits, in face of scarcity of resources [2]. Since calcium (Ca) largely performs structural functions in the human body, its deficits are related to the development of pathologies such as rickets, osteopenia or osteoporosis, which can interfere with bone`s formation (deformities) and structure (leading to fractures) [3].

To address this issue, adaptation and optimization of agricultural practices are bound to happen. In this context, precision agriculture focuses on the optimization and sustainable production of crops, using both sensors and software to retrieve data (namely edaphoclimateric conditions) and acquire high-resolution images (from satellites or air-borne platforms) for further processing [4]. Very recently UAVs (Unmanned Aerial Vehicles) technology has evolved to allow its use for assessment of agricultural plots, in contrast to the NDVI (Normalized Difference Vegetation Index) techniques more commonly used, being its acquisition based in aerial images that can be an alternative to proximal (on soil) sensors [5]. The efficient use of both techniques can contribute to financial savings in productivity factors of crops and these practices can also be implemented in small farms, with the economic gains surpassing the costs of these technology`s implementation [5]. On the other hand, recent studies using agronomic biofortification techniques have been performed in different cultures such as rice, wheat, grapes, potatoes, and apples to increase mineral content of Se, Zn and Ca respectively, providing the opportunity to attain functional products [6-11]. Agronomic biofortification workflows with foliar sprays can thus be used to increase the content of a target mineral in the edible part of plants, however, it is also important to avoid negative impacts on the agronomic performance of plants [12,13].

Rocha pears are a typical Portuguese variety, produced mainly in the West region (11 000 ha) of Portugal, with an average annual total production of 173000 tons, where at least 60 % is exported [14]. This study will merge the use of precision agriculture (UAVs), *in situ* and laboratory analysis, to characterize and monitor an orchard of *Pyrus communis* L. var Rocha during an Ca biofortification workflow.

2. Materials and Methods

2.1. Biofortification Itinerary

In an orchard (coordinates 39° 23′ 28.997′′N; 9° 4′ 52.483′′W) alocated in the West region of Portugal, a total of seven foliar sprays, spacing 15 days between each, were performed between 20th April to 3rd August 2019. Three rows with 12 trees each were monitored (one row was kept between sprayed rows to avoid contaminations). One was kept as the control (*i.e.*, no Ca sprays were performed), and for the remaining two rows, $CaCl_2$ (4 $kg.ha^{-1}$) was applied three times (thus in a total of 24 trees). Then for the last four sprays, the concentration in one of the rows was doubled to 8 $kg.ha^{-1}$. Fruits were harvested on 3rd of September 2019. During the experimental trial (April to September), air temperatures varied between 8 - 33 °C and total precipitation reached 11.68 mm.

2.2 Orchard Characterization and NDVI

The orchard was flow over twice with Unmanned Aerial Vehicle (equipped with altimetric measurement sensors) synchronized by GPS, like described in other studies [9]. The first flight was performed before applying the biofortification itinerary for morphological characterization (namely slopes), while the later meant to characterize vegetation indexes (monitor differences in vigor between the control and sprayed trees after four sprays) and further interpolation with levels of mineral content.

2.3. Leaf Gas Exchange

Leaf gas exchange parameters were determined using 4-6 randomized leaves per treatment, on 12th June, 26th July and 12th September, as described in other studies [15]. Under photosynthetic steady-state conditions after *ca*. 2 h of illumination (in the middle morning), leaf rates of net photosynthesis (P_n), stomatal conductance to water vapor (g_s) and transpiration (E) were attained. Through P_n-to-E ratio, leaf instantaneous water-use efficiency (iWUE) was calculated. The measurements were obtained with a portable open-system infrared gas analyzer (Li-Cor 6400, LiCor, Lincoln, NE, USA) under environmental conditions, with external CO_2 (*ca.* 400 ppm) and PPFD varying between 1200 - 1400 $\mu mol\ m^{-2}\ s^{-1}$.

2.5. Calcium Content in Leaves

In randomized leaves, Ca contents were determined four times during trees productive cycle (on the 21st May, 12th June, 11th and 25th of July 2019) using a XRF analyzer (model XL3t 950 He GOLDD+) under He atmosphere [7]. The leaves were cut, dried (at 60 °C, until constant weight), and then grounded and processed into pellets.

2.6. Statistical Analysis

Statistical analysis was carried out using a Two-Way ANOVA ($P \leq 0.05$), to assess differences between treatments (a, b) and between different moments of analyses (r, s, t), and then a Tukey's for mean comparison was performed, considering a 95 % confidence level.

3. Results

Nearly 80% of the total area of the orchard presents a low drainage surface (Figure 1), while the remaining area is classified as moderate, indicating the absence of high drainage sections. Regarding NDVI values (Table 1), the mean value of control trees was slightly higher than sprayed trees, and minimum and maximum values varied between 0.420 - 0.440 and 0.906 - 0.914, respectively.

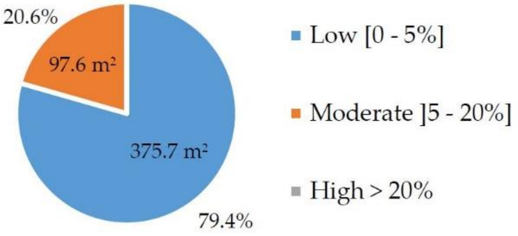

Figure 1. Slope / Surface Drainage characterization calculated from images of UAVs of an orchard from *Pyrus communis* L., variety Rocha, before leaves spray.

Table 1. Minimum, maximum, and mean values of normalized vegetation index (NDVI) ± SD calculated from images of UAVs (before foliar sprays) in trees (n = 12) from *Pyrus communis* L., variety Rocha, after the 4th (25-6-2019) leaf spraying. Ctr = Control; 4% correspond to the exclusive pulverization of 4 kg.ha^{-1}; 8% correspond to three pulverization of 4 kg.ha^{-1}, and four pulverizations with 8 kg.ha^{-1}.

Treatments	Minimum	Maximum	Mean ± SD
Ctr	0.440	0.914	0.800 ± 0.093
4%	0.440	0.906	0.781 ± 0.111
8%	0.420	0.914	0.797 ± 0.110

Considering leaf gas exchanges (Table 2), for the last two dates of analyses, trees sprayed with 8% revealed a slight tendency for higher P_n, g_s and E values and minor iWUE in comparison to the control.

Table 2. Average (n = 4) ± SE of leaf gas exchange parameters, P_n, g_s, E and iWUE in leaves of *Pyrus communis* L., variety Rocha pear, submitted to Ca biofortification, at 12th June, 26th July and 12th September 2019 (after the 3rd , 6th and 7th sprays, respectively). Letters a, b, c indicate significant differences between the treatments, and r, s, t between different moments of analyses (statistical analysis using the single factor ANOVA test, $P \leq 0.05$). Ctr = Control; 4% correspond to the exclusive pulverization of 4 kg.ha^{-1}; 8% correspond to three pulverization of 4 kg.ha^{-1}, and four pulverizations with 8 kg.ha^{-1}.

Treatments	12th June	26th July	12th September
	P_n (μmol CO$_2$ m^{-2}s^{-1})		
Ctr	17.69±0.24a,r	16.09±0.55as	7.77±0.25at
4%	17.70±0.13a,r	15.49±0.23as	7.47±0.39at
8%	17.85±0.19a,r	16.49±0.32as	8.37±0.33at

g_s **(mmol H_2O $m^{-2}s^{-1}$)**			
0%	255.6±6.8ar	199.0±10.9bs	68.5±3.8at
4%	252.4±5.1ar	221.1±4.9bs	65.5±5.1at
8%	245.2±3.9ar	269.0±8.8ar	76.6±5.5as
E **(mmol H_2O $m^{-2}s^{-1}$)**			
0%	3.27±0.06ar	2.21±0.07bs	2.03±0.09bs
4%	3.38±0.04ar	2.40±0.05bs	2.17±0.13bs
8%	3.24±0.03ar	3.29±0.09ar	2.53±0.15as
iWUE **(mmol CO_2 mol^{-1} H_2O)**			
0%	5.43±0,06as	7.29±0.17ar	3.95±0.15at
4%	5.26±0.05as	6.53±0.17br	3.51±0.07abt
8%	5.51±0.06ar	5.08±0.12cr	3.41±0.09bs

Regarding Ca contents in the leaves (Figure 2), for the same analytical experimental period, the control was significantly lower than the treatment 8% from the 3rd spray, and from treatment 4% from the 5th foliar spray onwards. For all treatments, the last two analytical dates (after 5th and 6th foliar sprays), revealed values significantly higher than the second and the first moment of analyses (after 3rd and 2nd foliar sprays respectively).

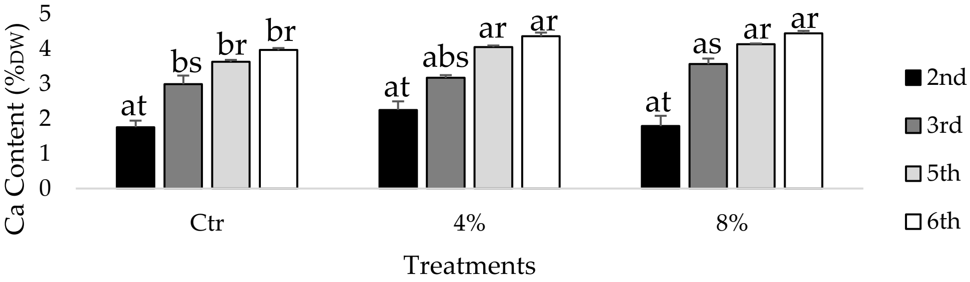

Figure 2. Mean values of Ca contents ± SE (n = 3) in leaves from *Pyrus communis* L., variety Rocha, after the 2nd (21-5-2019), 3rd (12-6-2019), 5th and 6th leaves spray (at 11-7-2019 and 25-7-2019 respectively). Letters a, b indicate significant differences between the treatments, and r, s, t between different moments of analyses (statistical analysis using the single factor ANOVA test, $P \leq 0.05$). Ctr = Control; 4% correspond to the exclusive pulverization of 4 kg.ha^{-1}; 8% correspond to three pulverization of 4 kg.ha^{-1}, and four pulverizations with 8 kg.ha^{-1}.

4. Discussion

Water is vital for nutrient plants uptake, and the morphologic aspect of agricultural fields can consequently have a high impact in crops health, since slopes influence water drainage and infiltration [9]. The correct functioning of photosynthetic mechanisms also depends on water for photoassimilates production and translocation [16].

In regard to our study, the orchard presents mostly a smooth slope morphology, indicating an even accumulation of water (and subsequent infiltration into the ground) for all sprayed trees. Artificial irrigation was also performed in the orchard, thus, water was not a conditional factor for nutrient absorption from soils. Leaf gas exchange parameters also supported this conclusion, with minor decreases of P_n and g_s for the later period of analysis, being probably related to the end of the production cycle of Rocha pear trees, with the harvest phase typically beginning late August to September [14].

This study also showed an increase of Ca content in leaves after foliar sprays, and the slightly higher values of treatment 8% relates to the increase of $CaCl_2$ concentration to double after the 3rd spray. Furthermore, in the perspective of fruits biofortification, an increase of Ca in fruits from this variety, using the same workflow, has been reported [17]. Ca content can be linked to photosynthesis since its deficits affect mainly new leaves growth and photoassimilates production and mobilization [18]. Leaf gas exchanges and NDVI indexes support the absence of toxicity signs throughout the workflow, with sprayed trees revealing similar values to the control. NDVI values can vary between 1 and -1, and thus, higher values indicate healthy vegetation [4]. Since all trees presented values higher than 0.75, at this point of the workflow, no signs of disrupted vegetation were detected [5]. Additionally, the results are in accordance with this mineral`s role in the preservation of photosynthetic capacity and high stomatal opening [19] linked to the stabilization of chlorophyll complexes and the maintenance of high photochemical efficiency of PSII [18].

5. Conclusions

Foliar applications of $CaCl_2$ in concentrations of 4 and 8 kg.ha^{-1}, increased Ca contents in leaves of *Pyrus communis* L., variety Rocha. However, vigor and respective photosynthesis mechanism of sprayed trees were not affected by the applied workflow. These interactive factors can also be due to the terrain morphology, which promotes identical water supply to plants. In this context, the use of precision agriculture techniques (namely UAVs), was successfully used with other analyses, not only to characterize the terrain, namely to monitor plants during their productive cycle.

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