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Proceeding Paper

Physiological Assessment of Rocha Pear Trees to Agronomic Enrichment with $CaCl_2$ and $Ca(NO_3)_2$ †

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Abstract: The exponential increase of the world's population is a major concern for the food sector because the quantity and quality of food products need to be ensured for consumers. Thus, in an orchard of pears located in Portugal, a total of seven foliar sprays, using $CaCl_2$ and $Ca(NO_3)_2$, were performed; the first two sprays with three different concentrations each ($CaCl_2$—0.4, 0.8, and 1.6 $kg \cdot ha^{-1}$; $Ca(NO_3)_2$—0.1, 0.3, and 0.6 $kg \cdot ha^{-1}$), the third with $CaCl_2$ 4 $kg \cdot ha^{-1}$, and the remaining four with $CaCl_2$ 8 $kg \cdot ha^{-1}$. During the workflow, a normalized difference vegetation index (NDVI) was attained with an unmanned aerial vehicle (UAV) and was later correlated with photoassimilates synthesis (assessed by a portable open-system infrared gas analyzer) and Ca content in leaves and fruits (assessed by X-Ray fluorescence analysis). Regarding NDVI values, the exclusive use of $CaCl_2$ presented slightly inferior values; however, no major signs of disrupted vegetation were detected. For leaf gas exchange, only minor changes occurred (namely E and iWUE parameters), while calcium content in leaves during the workflow and fruits at harvest increased. In conclusion, smart farming techniques can be correlated with in situ analysis to monitor Rocha pear trees, and the concentrations used in this study increased Ca content in fruits without reaching toxicity levels.

Keywords: calcium; foliar sprays in pears; leaf gas exchange; NDVI; X-ray fluorescence analysis

1. Introduction

Rocha pears are a variety of *Pyrus communis* largely produced in Portugal, with orchards prevailing in the country's western region, occupying up to 11,000 ha. and providing an average annual production that reaches over 170,000 tons [1,2]. Thus, it contributes to the country's fruit sector because up to 60% of the total production is exported to other countries, such as Brazil, Morocco, the United Kingdom, France, and Germany, among others [2]. When considering the exponential growth of the world's population [3], the agroindustry will face challenges related to the production of food, having not only to deliver enough quantity to meet the population demands but also to maintain its quality to assure the nutritional needs of the human body [4]. In this regard, calcium (Ca) is a macronutrient performing both structural and signaling functions in the body, and its deficits are associated with pathologies such as osteopenia and osteoporosis, increasing the risk of fractures [5].

Agronomic enrichment of plants with minerals focuses on the use of soil or spray fertilizers in order to increase a certain mineral in its comestible parts [6]. It is already practiced on staple crops and also in tubers and fruits (such as vineyards or orchards) to increase the mineral levels of micronutrients and macronutrients such as Ca [7–13], giving the option to attain food products with added value. Pre-harvest Ca enrichment using sprays was already performed on apples and pears, and increases of this mineral in fruits were reported [11,12,14]; however, in pears, damages to foliage were observed [14]. Precision agriculture is gradually being implemented in the food industry to optimize agricultural practices by assisting in the monitorization of crops and allowing early detection of stress signals in cultures, namely in the context of resources scarcity [15]. The normalized difference vegetation index (NDVI) is emphasized by the same authors as a component of remote sensing (using platforms such as UAVs—unmanned aerial vehicles) and is used to provide information regarding the vegetation dynamics and health monitoring of crops [15].

This study thus aimed to apply agronomic enrichment practices to increase Ca content in pears and simultaneously monitor its effects on the orchard with UAVs and in situ analysis.

2. Materials and Methods

2.1. Calcium Workflow Applyed in Orchard

In an orchard located in Caldas da Rainha (Portugal), seven foliar sprays (spaced 15 days apart each), were performed between 12 May to 25 August 2018. Three rows with 12 trees (of *Pyrus communis* from cultivar Rocha) each, were monitored (one row was kept between sprayed rows to avoid contaminations). One row was kept as the control (no Ca sprays were performed), and for the remaining two rows, different concentrations (four trees per concentration) of $CaCl_2$ (0.4, 0.8, and 1.6 $kg \cdot ha^{-1}$) and $Ca(NO_3)_2$ (0.1, 0.3, and 0.6 $kg \cdot ha^{-1}$) were applied on the first two sprays. For the third spray, $CaCl_2$ (4 $kg \cdot ha^{-1}$) was applied on all 24 trees (except the control). Then, for the last four sprays, the concentration of $CaCl_2$ was doubled to 8 $kg \cdot ha^{-1}$. Fruits were harvested at the beginning of September (4th) 2018. Between May and September (i.e., during the experimental trial), total precipitation reached 60.4 mm and air temperatures was in the range of 6–41 °C.

2.2. Normalized Difference Vegetation Index Attained from UAVs

One flight after the 3rd leaf spray (on 19 June 2018) was performed using an Unmanned Aerial Vehicle (equipped with altimetric measurement sensors, RGB, and multispectral cameras) synchronized by GPS, as described in other studies [9]. Data was then processed with ArcGIS PRO to produce orthophotomaps and consequent determination of the normalized difference vegetation index (NDVI). The flight aimed to characterize vegetation indexes (mainly to monitor differences in vigor between the control and sprayed trees) and further interpolation with levels of mineral content.

2.3. Leaf Gas Parameters with Portable Open-System Infrared Gas Analyzer

Leaf gas exchange parameters were determined using up to 6 randomized leaves per treatment, on 19 and 11 of June and September 2018, respectively, as depicted in other studies [16].

2.4. Calcium Content in Leaves and Fruits by X-ray Fluorescence Analysis

For leaves, Ca content was determined three times during the workflow (on the 8 June, 15 June, and 20 July 2018) and at harvest of fruits (4 September 2018), using an XRF analyzer (model XL3t 950 He GOLDD+) under He atmosphere, as described in other studies [7].

2.5. Statistic

Statistical analysis was performed using a one-way ANOVA ($p \leq 0.05$) to assess the differences between treatments (a to e), and a Tukey test for mean comparison was carried out, considering a 95% confidence level.

3. Results

Normalized vegetation index values (Table 1) after the 3rd spraying varied between 0.88 and 0.94, with treatments with the exclusive use of $CaCl_2$ presenting slightly lower values. In general, minimum values for these treatments were inferior to the control, while treatments with $Ca(NO_3)_2$ were superior.

Table 1. Mean ± SD, maximum and minimum values of normalized vegetation index (NDVI) in trees (n = 4) from *Pyrus communis* L., variety Rocha, after the 3rd leaf spraying.

Treatments	Mean ± SD	Maximum	Minimum
Control	0.94 ± 0.02	0.97	0.79
0.1 kg·ha^{-1} $Ca(NO_3)_2$	0.94 ± 0.02	0.97	0.84
0.3 kg·ha^{-1} $Ca(NO_3)_2$	0.94 ± 0.02	0.97	0.81
0.6 kg·ha^{-1} $Ca(NO_3)_2$	0.94 ± 0.03	0.97	0.77
0.4 kg·ha^{-1} $CaCl_2$	0.91 ± 0.06	0.96	0.63
0.8 kg·ha^{-1} $CaCl_2$	0.88 ± 0.08	0.96	0.56
1.6 kg·ha^{-1} $CaCl_2$	0.89 ± 0.09	0.96	0.53
Field mean value	0.92 ± 0.05	0.91	0.70

Leaf gas exchange values (Figure 1), after the 3rd and 7th sprays, showed an increase of g_s and E values over time, while iWUE values decreased. Leaf rates of net photosynthesis (P_n) values from treatments with $Ca(NO_3)_2$ and treatment 0.8 kg·ha^{-1} $CaCl_2$ presented a slight decrease, while the remaining treatments showed a slight increased. For the first date of analysis, on P_n and g_s, only one treatment (0.1 kg·ha^{-1} $Ca(NO_3)_2$) was significantly higher than the control. Furthermore, treatments with $Ca(NO_3)_2$ were all superior to the control, while the ones with exclusive use of $CaCl_2$ were all inferior. For parameters E and iWUE, the control values corresponded to the lowest and highest, respectively, and only treatments 0.4 kg·ha^{-1} $CaCl_2$ or 0.1 kg·ha^{-1} $Ca(NO_3)_2$ were not significantly different from this. For the last moment of analysis for parameters P_n, g_s, and iWUE, all treatments were inferior to the control, except for 0.4 kg·ha^{-1} $CaCl_2$ on g_s, while in parameter E, the control was inferior to the remaining treatments. Thus, for E and iWUE, all treatments were significantly different from the control, while for P_n and g_s, only two treatments (0.6 kg·ha^{-1} $Ca(NO_3)_2$ and 0.8 kg·ha^{-1} $CaCl_2$) were significantly different.

Regarding Ca content in leaves (Figure 2), after the 2nd spray, no significant differences between the control and the remaining treatments were observed; however, two treatments with $Ca(NO_3)_2$ (0.3 and 0.6 kg·ha^{-1}) presented lower values, while for $CaCl_2$, only the lowest concentration presented a lower value. For the 3rd spray (with exclusive use of 4 kg·ha^{-1} $CaCl_2$), only treatment 1.6 kg·ha^{-1} $CaCl_2$ was significantly inferior to the control. After 5 sprays (two with 8 kg·ha^{-1} $CaCl_2$) only treatment 0.4 kg·ha^{-1} $CaCl_2$ was significantly inferior to the control. At harvest (Figure 2), all sprayed fruits presented values significantly higher than the control.

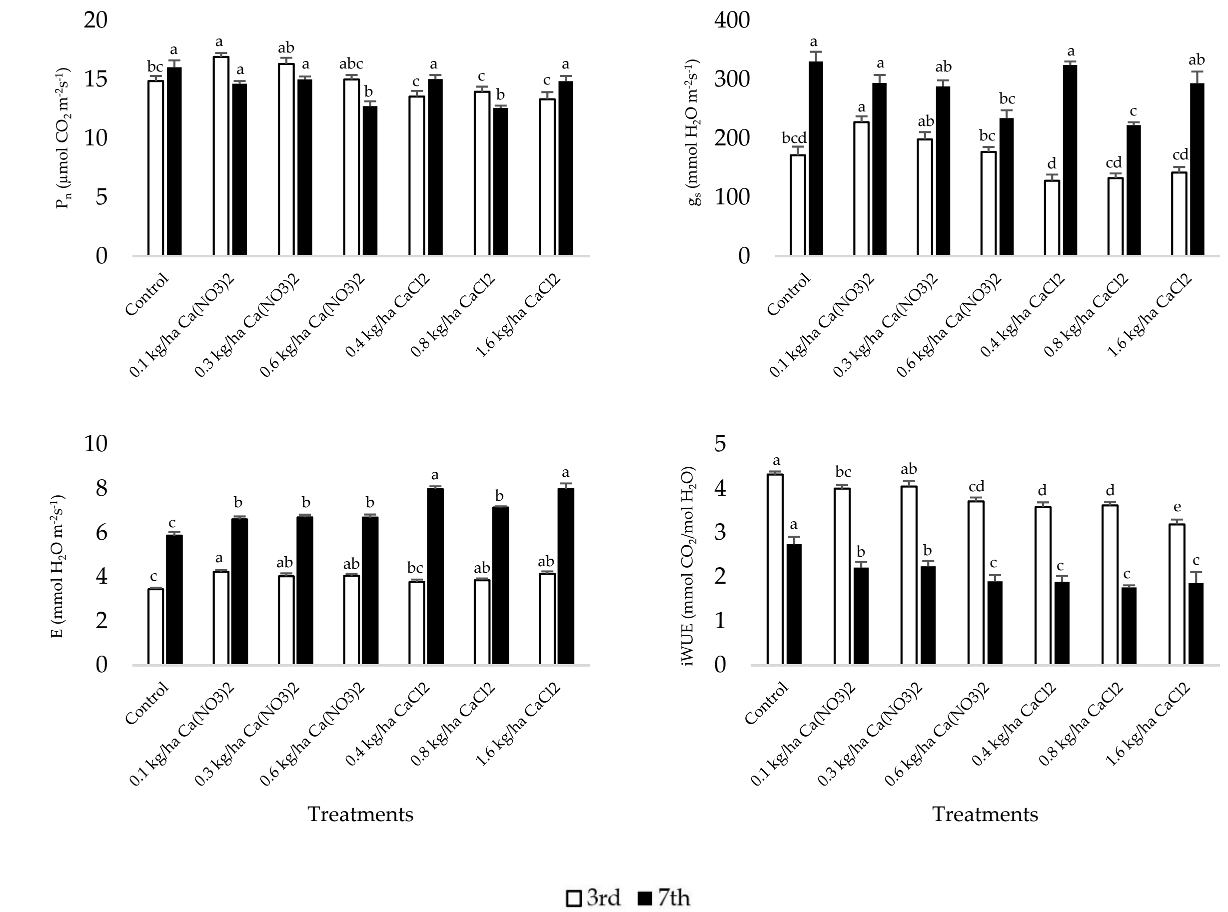

Figure 1. Mean (n = 4) ± SE of leaf gas exchange parameters (P_n, g_s, E, and iWUE) in leaves of *Pyrus communis* L., variety Rocha, submitted to a Ca workflow in June and September (after the 3rd and 7th sprays, respectively). Letters a to e indicate significant differences between the treatments (statistical analysis using the single factor ANOVA test, $p \leq 0.05$).

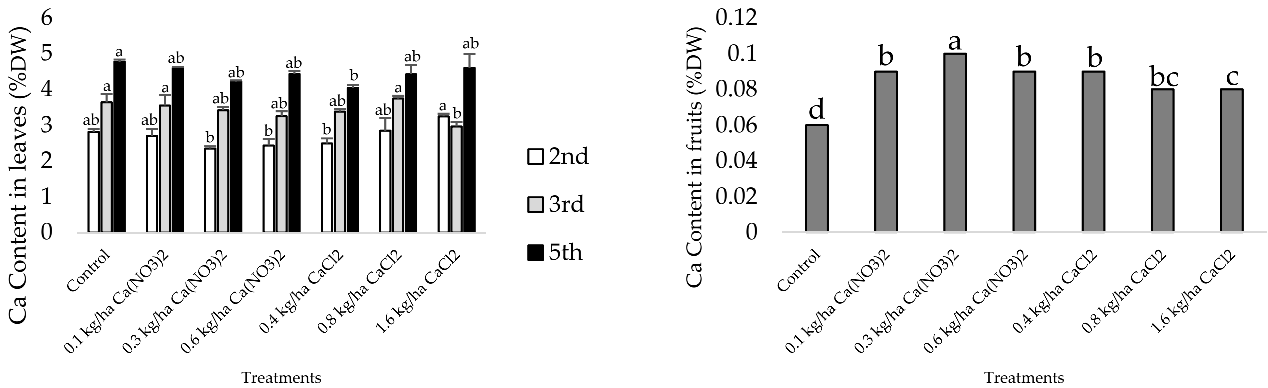

Figure 2. Mean values of Ca contents (n = 3) ± SE in leaves and fruits (n = 4) from *Pyrus communis* L., variety Rocha, after the 2nd (8 June), 3rd (15 June), and 5th (20 July) leaf sprays, or at harvest (4 September). Letters a to d indicate significant differences between the treatments (statistical analysis using the single factor ANOVA test, $p \leq 0.05$).

4. Discussion

The NDVI values can vary between 1 and −1, where higher values correspond to a higher density of green leaves, and thus lower values correspond to less chlorophyll or leaves [15]. All mean values attained in this study were higher than 0.85, being in accordance with the absence of toxicity signals, indicating a healthy orchard [15,17]. This analysis correlates with leaf gas exchange, and both suggest the absence of toxicity signals.

Calcium is a nutrient that is involved with tolerance to environmental stresses, but also other physiological processes related to the growth and development of plants, and thus photosynthetic activity [9,18]. The external application of Ca may have led to minor differences in leaf gas exchanges parameters, due to its role in stomatal closure, non-photochemical quenching, and photosystems function [19,20]; however, accumulation of Ca in fruits was not affected. Because there was no major impairment on photoassimilates synthesis, toxicity levels were not reached. Additionally, for the concentrations applied in this field trial, no toxicity signs such as leaf injuries occurred, but a study using foliar sprays with concentrations varying between 10 and 25 kg ha^{-1} $CaCl_2$ reported damages such as leaf burn or defoliation [14].

Regarding Ca, the absence of significantly higher levels of this mineral in leaves after sprays indicates a translocation to other plant tissues, such as fruits. Other studies also reported a Ca increase in fruits after foliar sprays with $CaCl_2$ and $Ca(NO_3)_2$ [11,12,14]. For apples, the exclusive use of $CaCl_2$ was better for Ca increases in Jonagold apple fruits, while a combination of $CaCl_2$ and $Ca(NO_3)_2$ was better for Golden apples [12]. Furthermore, although Ca has more mobility on the xylem [21], Ca increases with foliar sprays of $CaCl_2$ and $Ca(NO_3)_2$ have been reported on tubers, rising between 5–40%, suggesting a complementary redistribution through the phloem [9].

5. Conclusions

The implemented workflow increased Ca content in fruits at harvest, and concentrations up to 8 $kg \cdot ha^{-1}$ $CaCl_2$ did not have negative impacts on pear trees. Additionally, in situ and precision agriculture techniques can be used in combination to assess orchards health during the different phases of production.

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