

RESEARCH ARTICLE

Under calcium spraying nutrients accumulation in the initial stages of fruits development is critical in “Rocha” pears

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ABSTRACT

Balanced supply of essential nutrients in fruit trees, such as “Rocha” pears, is an important tool to achieve maximum yield and fruit quality, yet under fertilization the interactions among nutrients accumulation may be positive (synergistic) or negative (antagonistic), being also possible to have no interactions. Through foliar spraying of “Rocha” pear trees with Ca, this study aimed to assess the interactions with other micro and macronutrients in the leaves, the related implications on the synthesis of photoassimilates and the subsequent kinetics of accumulation in the fruits at harvest. Between fruit set and 30 mm diameter, two times spraying, with fifteen days range, were carried out with $\text{Ca}(\text{NO}_3)_2$ or CaCl_2 (0.1, 0.3 and 0.6 kg ha^{-1} or 0.4, 0.8 and 1.6 kg ha^{-1} , respectively). Thereafter, with similar interval periods, for all treatments, trees were sprayed with CaCl_2 (firstly at a concentration of 4 kg ha^{-1} – second phase, followed by four with 8 kg ha^{-1} – third phase). During fruit development, through the three spraying phases with Ca, the accumulation of Ca, K, P, S, Mg and Zn showed substantial variations in leaf tissues, which at fruit maturity determined some special and quantitative variations in tissues accumulation of C, H, O, K, Mg, Cl, Cu, Zn, Mn, Fe. Nevertheless, the functioning of the photosynthetic apparatus and the morphological and colorimetric parameters were not impaired. Considering that during fruits development, although Ca translocation prevails in the xylem, being its redistribution through the phloem strongly limited, it was concluded that accumulation depends of the type of Ca fertilizer and concentration, prevailing an oscillating balance among Ca K, P, S, Mg and Zn deposition in the leaves and its slow removal from the mesophyll cells to the fruits until maturity. Besides, as at fruit maturity xylem functionality can be minimal, the major accumulation of Ca in the peripheral layers of the fruits, resulted from Ca spraying, whereas translocation through the xylem favored its accumulation in the core in the early stages of fruit development. Following this behavior, C, H, O, K, Mg, Cl, Cu, Zn, Mn and Fe further showed complex accumulation interactions in the fruits.

Keywords: Chlorophyll *a* fluorescence parameters; Foliar spray with calcium; Calcium accumulation in fruits; Kinetics of calcium accumulation in leaves; Nutrients interactions; *Pyrus communis* L.

INTRODUCTION

Balanced supply of Ca is, among other nutrients, a key factor for plant growth and development, providing

stability and integrity to the cell wall (Wei et al., 2017), playing a central role in stress responses (Hocking et al., 2016), and being a cofactor of several enzymes involved in the catabolism of ATP and phospholipids (Taiz and

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Zeiger, 2002). Calcium accumulation also interferes with the equilibrium of some minerals contents in the fruits, which is a prerequisite for extending its shelf life, delaying softening and improving internal quality (Poovaiah, 1993; Raese, 1994; Zocchi and Mignani, 1995).

Calcium is normally acquired from the soil solution in the form of Ca^{2+} and transported toward the root apex, and/or regions of lateral root initiation, to the shoot through the apoplasm (which is relatively non-selective between divalent ions), in regions where Casparian bands are absent, or via the cytoplasm of unsuberised endodermal cells where Casparian bands are present (Moore et al., 2002; White, 2001). Once in the apoplasm cell water space, Ca^{2+} binds to negatively charged residues and are taken up by cells down the electrochemical gradient for Ca^{2+} , or pass through the water-free space of the cell wall to the xylem (White and Broadley, 2003). Thereafter, the translocation of Ca^{2+} (or complexed with the organic acids transport pathway) prevails in the xylem via water mass flow with the transpiration stream (Barthakur et al., 2001; Welch and Shuman, 1995), while the symplasmic movement of Ca^{2+} (*i.e.*, through the cytoplasm of cells linked by plasmodesmata) develops at a low rate. Thus, factors that diminish transpiration, like stomatal closure, can be linked to Ca transport inhibition from roots to shoots and fruits. Moreover, once uploaded from the xylem, and in particular to points downstream of transpirational flow, Ca cannot be readily remobilised (Dayod et al., 2010). In this context, although several authors pointed that Ca is immobile in the phloem and does not redistribute in plants (Ho and White, 2005; Welch, 1999; White and Broadley, 2003; Ziegler, 1975), a low kinetics rate of Ca was also reported by others (Bonomelli et al., 2019; Coelho et al., 2021; Davies and Millard, 1985; Nelson et al., 1990; Oparka and Davies, 1988).

To potentiate maximum yields, temperate fruit trees should be fertilized, namely with Ca, whenever the soil cannot provide sufficient amounts of nutrients to feed the plant. Yet, excessive nutrients availability, relatively to plant requirements, convey negative effects, namely on plant vigour, yield and fruit skin colour, abortion of flowers and even fungal diseases on leaves and fruits (Huber and Jones, 2013; Huber and Thompson, 2007; Martin et al., 2004). Besides, often an increased fruit yield does not occur upon soil fertilization because nutrients availability are already within the adequate range, but above upper threshold, toxic symptoms can develop (namely when Ca excess triggers leaf necrosis, which limits photoassimilates mobilization). Additionally, nutrients excess may further affect fruit quality at morphological, physical, chemical and organoleptic levels, which also determines that the fertilization management should be adjusted to the final destination. Besides,

fertilization techniques must be carried out with caution, as nutrients interactions can form chemical bonds between ions (thus, synthesizing precipitates or complexes at the root surface or within the plant organs) or compete for the site of adsorption, absorption, transport, and function on plant root surfaces or within all plant tissues. In this context, cation-cation and anion-anion interactions occur mostly at the membrane level and are primarily of a competitive nature, whereas cation-anion interactions occur at both the membrane and in cellular processes. Accordingly, nutrients unbalance can trigger antagonism or synergism interactions due to the similarity of size, geometry of coordination and electronic configuration. For instance, in rice, barley, corn, cucumber and tomato, Ca content is higher when ($\text{NO}_3\text{-N}$) is applied as a nitrogen source, whereas the supply of $\text{NH}_4\text{-N}$ inhibits Ca uptake (Kawasaki, 1995). In rice, Fageria (1983) also found that at higher concentration K concentrations also has an antagonistic effect (*i.e.*, quadratic decrease) on Ca^{2+} uptake, which depends on plant species and environmental conditions. Besides, it has long been reported (Lundergardh, 1934; Wall, 1940) that higher absorption of P and Ca linked to a lower concentrations of K seems to be due to the high mobility of K, which when present in higher concentrations will tend to depress the absorption of other ions. It has been further reported (Fageria and Baligar, 1999; Ishizuka and Tanaka, 1960) that P, K and Mg uptake by rice and beans is depressed with increasing Ca concentration, eventually due to a decrease in the permeability of cells (Fageria, 1983). In soybean and wheat plants increasing contents of Ca further inhibits the accumulation of Mg, Zn, Mn, and Fe (Fageria and Baligar, 1999), but high levels of Mg also decrease K and Ca in rice, eventually as a result of competition for metabolically produced binding compounds (Fageria, 1983). Acidifying effect of S applications to a highly calcareous soil mobilizes Fe, Mn, Zn, and P in crops (Soliman et al., 1992). Fageria and Baligar (1999) further reported that, Ca uptake by alfalfa, wheat, rice and red clover is depress with increasing Fe levels and Wang et al. (2018) and Bonomelli et al. (2019) reported synergistic and antagonic interactions among Ca^{2+} , Mg^{2+} , K^+ , and Na in avocado and pear trees.

Although soil fertilization might alleviate minerals deficits in plants (de Valença et al., 2017; Jha and Warkentin, 2020), to achieve maximum yields, foliar spraying can bypass the use of soil as an intermediary for plant nutrition, avoiding the negative interactions among soil characteristics and its minerals equilibria (de Valença et al., 2017). Additionally, foliar spraying might induce faster effects and its application can increase target-mineral contents in the edible parts of plants (D'Imperio et al., 2016; Garg et al., 2018). Moreover, it was found that foliar sprays with Ca in "Conference" pears affects N, Mg and K contents in the fruits (Gastol and Domagala-Świątkiewicz, 2006; Gastol and Domagala-

Świątkiewicz, 2009). Additionally, independently of the Ca fertilization type, Ca^{2+} is relatively immobile in cells and remobilized at a low rate from mature to active growing plant tissues (Dayod et al., 2010), which can ultimately result in local tissue deficits despite abundance in supply (Dayod et al., 2010). Indeed, Ca deficits prevails in younger leaves (Taiz and Zeiger, 2002) and, due to its structural functions, changes in membranes and cell walls, namely rigidity, can develop (Hocking et al., 2016). This nutrient also differs from other nutrients because it is transferred to fleshy fruit in amounts much smaller than to leaves (Saure, 2005), and only during the first part of growth or linearly until harvest, but with very low vascular mobility (Faust, 1989; Zavalloni et al., 2001).

Rocha pears are a typical Portuguese variety with a PDO (Protected Designation of Origin - “Pera Rocha do Oeste”) designation attributed in 2003 (ANP, 2021; Codimaco, 2021). This pear variety is of great important to Portugal contributing to its horticultural sector, since up to 60% of its average annual production can be exported (ANP, 2021). It is largely produced in the West region, in 29 municipalities (ANP, 2021), occupying over 12 500 ha in 2018, and reaching a production of 161 350 tonnes for the same year (INE, 2019). The fruits characteristics are closely related to its geographic production area, being influenced by soils, closeness to sea, and the micro-climatic differentiation of the region (ANP, 2021). The production of this fruit can thus influence this regions hydric and soil resources, by the use of practices such as drop-to-drop irrigation or due to the fact of being a permanent crop (rotation with other cultures is not possible). Thus, correct nutrients, fertilizing and disease management is crucial to minimize contaminations while assuring the orchards health.

This variety’s resistance to transport and handling allows a marketability to consumers during almost all year with minor wastes (ANP, 2021). Furthermore, by being a climacteric fruit (Pedro et al., 2020) perishable due to an elevated water content and active physiological activities, under correct handling during harvest and post-harvest phases, it can be stored up to 8 months (ANP, 2021).

Consumers awareness to food quality and sustainability of food chain increased in recent years. Ultimately, this study could increase Ca in fruits leading to a product with added value, providing the agro-industrial sector the possibility to attain products with functional properties (promoting health benefits beyond basic nutrition). This mineral is especially important for the prevention of pathologies such as rickets (bone deformities) or osteoporosis linked to Ca deficits, that eventually result in fractures or decrease in mobility (EFSA, 2015).

Considering the low kinetics mobility of Ca in the phloem and the consequent limited redistribution in plants, using as a test system *Pyrus communis* L. variety “Rocha” submitted to foliar fertilization with increasing concentrations of CaCl_2 and $\text{Ca}(\text{NO}_3)_2$ during the first stage of the fruit development, followed by higher levels of CaCl_2 , the concurrent kinetics interactions among nutrients, in the leaves and at tissues levels in the fruits were assessed. The related implications on the synthesis of photoassimilates and on fruits developments were further characterized.

MATERIALS AND METHODS

Experimental fields

“Rocha” pears, a typical variety of the West region of Portugal (INE, 2019), with about an annual production of 200.000 tons (ANP, 2021), were used as testing system. Seven foliar sprays with Ca were applied in an orchard (GPS coordinates 39° 23’ 28.997”N; 9° 4’ 52.483”W) of *Pyrus communis* L. variety “Rocha” pear trees, from 12th May until harvest (4 September) of 2018. During this experimental period, maximum and minimum average temperatures were 23 °C and 15 °C (with maximum and minimum values of 41 °C and 6 °C, respectively), with a mean rainfall of 0.41 mm, a daily maximum of 18.03 mm and an accumulation of 60.4 mm. Trees were sprayed, at different concentrations with $\text{Ca}(\text{NO}_3)_2$ (because neutralizes acids to detoxify the plant) or alternatively with CaCl_2 (as provides calcium immediately available to plants). Spraying was carried out in three different rows (each separated by another tree row to avoid contamination) of the orchard. Three distinct pulverization phases were carried out between fruit set and 30 mm diameter. The first phase consisted of two times spraying, with fifteen days range, with $\text{Ca}(\text{NO}_3)_2$ (0.1, 0.3 and 0.6 kg ha⁻¹, for each treatment four trees; In parallel, two other initial leaves pulverization were carried out with CaCl_2 (0.4, 0.8 and 1.6 kg ha⁻¹, for each treatment four trees. At this phase, for each treatment four trees of the same row were sprayed, but keeping three trees without spraying between each concentration to avoid contaminations. The control trees were monitored in a separate row from the treated ones. Thereafter, with fifteen days interval, for all treatments, all the 24 trees were sprayed with CaCl_2 (firstly at a concentration of 4 kg ha⁻¹ – second phase; followed by four with 8 kg ha⁻¹ – third phase). Control trees (8 trees) were not sprayed at any time with $\text{Ca}(\text{NO}_3)_2$ or CaCl_2 .

Analysis of nutrients contents in the leaves and fruits

In the leaves of *Pyrus communis* L. “Rocha” pear trees, each treatment were randomly collected at 8th and 15th of June and 10th and 20th of July), thus after the 2nd, 3rd, 4th and 5th foliar spray, respectively. Leaf samples were then washed

with deionized water, dried at 60 °C (until constant weight) and grounded. Calcium, K, P, S, Mg and Zn contents were determined by X-ray spectrophotometry (four readings per treatment), using a XRF analyzer (model XL3t 950 He GOLDD+) under He atmosphere, according to Reboredo et al. (2018).

At harvest, the fruits from all treatments, after washing with deionized water, were cut at the equatorial region and the slices (with 4 mm) were dried at 60 °C until constant weight. Four measurements per treatment were carried out with a μ -EDXRF system (M4 Tornado™, Bruker, Germany), as described in Marques et al. (2021). Additionally, five regions were defined between the peel and the core and three readings per treatment were performed. The X-ray generator was operated at 50 kV and 100 μ A without the use of filters, to enhance the ionization of low-Z elements. The following elements were measured: Ca, C, H, O, K, Mg, Cl, Cu, Zn, Mn, and Fe. To allow a better quantification of Ca, a set of filters between the X-ray tube and the sample, composed of three foils of Al/Ti/Cu (with a thickness of 100/50/25 μ m, respectively), was used. All the measurements with filters were performed with 600 μ A voltage. Detection of fluorescence radiation was performed by an energy-dispersive silicon drift detector, XFlash™, with 30 mm² sensitive area and energy resolution of 142 eV for Mn K α . Measurements were carried out under 20 mbar vacuum conditions. These point spectra were acquired during 200 s.

Chlorophyll a fluorescence parameters

Chlorophyll *a* fluorescence parameters were determined in the leaves of *Pyrus communis* L. variety “Rocha”, using a fluorimeter PAM 2000 (H. Walz, Effeltrich, Germany), as described in Rodrigues et al. (2016), with minor modifications. Briefly, minimal fluorescence from the antennae (F_o) and maximal photochemical efficiency of photosystem (PS) II (F_v/F_m) were determined in overnight dark adapted leaves, using a low irradiance red light ($< 0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$), to obtain F_o , and an actinic saturating light flash of ca. $7500 \mu\text{mol m}^{-2} \text{s}^{-1}$ to obtain maximum fluorescence from the antennae (F_m), with maximal photochemical efficiency of PSII (F_v/F_m) being calculated as $[(F_m - F_o)/F_m]$. Another set of parameters was evaluated under photosynthetic steady-state conditions under natural irradiance (ca. $1000 - 1300 \mu\text{mol m}^{-2} \text{s}^{-1}$), with superimposed saturating light flashes, and included the photochemical quenching, based on the concept of interconnected PSII antennae (q_L), the non-photochemical quenching (q_N), the actual PSII photochemical efficiency (F_v'/F_m'), as well as the estimates of the quantum yield of photosynthetic non-cyclic electron transport ($Y_{(II)}$), of the quantum yield of regulated energy dissipation of PSII ($Y_{(NPQ)}$), and of non-regulated energy (heat and fluorescence) dissipation

of PSII ($Y_{(NO)}$) (with $Y_{(II)} + Y_{(NPQ)} + Y_{(NO)} = 1$). All parameters and their meaning were obtained based in the formulas presented previously presented (Huang et al., 2011; Klughammer and Schreiber, 2008; Kramer et al., 2004; Krause and Jahns, 2004; Schreiber, 2004).

Measurements under dark and light adapted conditions were performed in the same leaves (two leaves from each of the five trees), in three different dates (19th June, 20th July and 11th September 2018, *i.e.*, after the 3rd, and 5th foliar spraying, and at harvest date, respectively).

Morphometric and colorimetric parameters of fruits at harvest

At harvest, the height and diameter of four fruits of “Rocha” pears and of each treatment were randomly selected and measured.

In three of these fruits, colorimetric parameters of the pulp and peel were also determined, using a scanning spectrophotometric colorimeter (Agrosta, European Union) equipped with a sensor with 6 phototransistors for specific transmittance regions of the spectrum (red – 670 nm; orange – 600 nm; yellow – 570 nm; green – 500 nm; blue – 450 nm and violet – 380 nm).

Statistical analysis

Data were statistically analyzed using a One-Way or Two-Way ANOVA ($P \leq 0.05$), to assess differences between treatments and experimental periods and, based on the results, a Tukey's for mean comparison was performed, considering a 95 % confidence level. For the Two-Way ANOVA, letters (A, B, C or a, b, c) correspond to significant differences between treatments for the same analytical date, or between analytical dates for each treatment, respectively.

RESULTS

Nutrients accumulation in the leaves

After the 2nd foliar spray (8th of June), relatively to the control, Ca contents in the leaves of 1.6CCa showed a significantly higher value, whereas NCa0.3, NCa0.6 and 0.4CCa revealed the lowest values (although non-significantly different from the control) (Fig. 1). By 15th June (after the 3rd foliar spraying), Ca levels of NCa0.3, NCa0.6 and 0.4CCa kept the same pattern relatively to the control, but 1.6CCa showed minimum values (Fig. 1). After the 4th spraying (10th July), the control showed the highest content of Ca in the leaves (with NCa0.3 maintaining the lowest value) and with the 5th spraying this pattern persisted (Fig. 1).

The contents of K in the leaves, after the 2nd foliar spray (8th of June), showed the highest values with

1.6CCa, whereas minimum values occurred with NCa0.6, 0.4CCa and 0.8CCa (Fig. 2). After the 3rd foliar spray the significantly highest values were found with NCa0.6, whereas NCa0.3 and 0.8CCa revealed significantly lower values and 1.6CCa had the lowest one (Fig. 2). By 10th July (after the 4th spraying), the amount of K did not vary significantly among treatments, yet with the 5th foliar spraying maximum values were obtained with NCa0.1 and the lowest with 1.6CCa.

The contents of P in the leaves, after the 2nd spraying (8th June), showed the highest value in 1.6CCa, with

minimum significant values occurring with all the treatments with $\text{Ca}(\text{NO}_3)_2$ and 0.4CCa (Fig. 2). After the 3rd and 4th foliar spraying the contents did not varied significantly among treatments, whereas after the 5th spraying (20th July) the highest value was found in 0.4CCa (Fig. 2).

In the leaves, after the 2nd foliar spraying the contents of S revealed, relatively to the lowest value (0.4CCa), the significantly highest values in 0.8CCa and 1.6CCa (Fig. 2). After the 3rd spraying (15th June) NCa0.1 and NCa0.3 showed the significantly highest values relatively

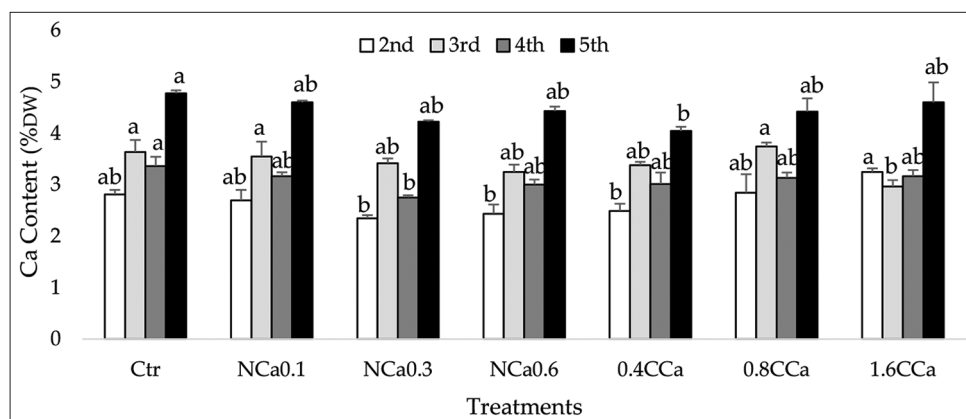


Fig 1. Average $\pm$ SE ($n = 4$) Ca contents of leaves from *Pyrus communis* L, variety "Rocha" pear, after the 2nd (at 8th June 2018, with 0.1, 0.3 and 0.6 kg $\text{Ca}(\text{NO}_3)_2 \text{ ha}^{-1}$, or 0.4, 0.8 and 1.6 kg $\text{CaCl}_2 \text{ ha}^{-1}$), 3rd (at 15th June 2018, all the previous treatments with 4 kg $\text{CaCl}_2 \text{ ha}^{-1}$), 4th and 5th leaf spray (at 10th and 20th July 2018 respectively, all the previous treatments with 8 kg $\text{CaCl}_2 \text{ ha}^{-1}$). Letters a, b represent significant differences, between treatments for each date of analysis (statistical analysis using the single factor ANOVA test, $P \leq 0.05$). Ctr = control; NCa0.1, NCa0.3, NCa0.6 correspond, respectively, to the initial foliar spray of 0.1 kg ha^{-1} , 0.3 kg ha^{-1} and 0.6 kg ha^{-1} $\text{Ca}(\text{NO}_3)_2$; 0.4CCa, 0.8CCa, and 1.6CCa, correspond respectively to the initial foliar spray of CaCl_2 0.4 kg ha^{-1} , 0.8 kg ha^{-1} and 1.6 kg ha^{-1} . After 4 kg ha^{-1} and 8 kg ha^{-1} of CaCl_2 sprays were applied to all treatments except to control.

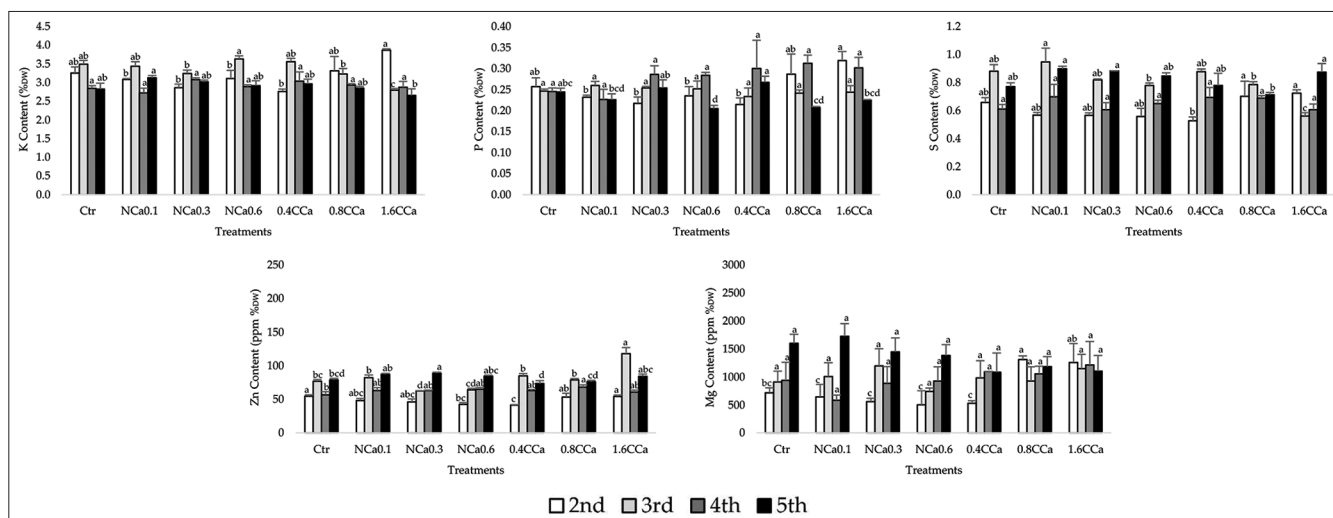


Fig 2. Average $\pm$ SE ($n = 4$) mineral content of leaves from *Pyrus communis* L, variety "Rocha" pear, after the 2nd (at 8th June 2018, with 0.1, 0.3 and 0.6 kg $\text{Ca}(\text{NO}_3)_2 \text{ ha}^{-1}$, or 0.4, 0.8 and 1.6 kg $\text{CaCl}_2 \text{ ha}^{-1}$), 3rd (at 15th June 2018, all the previous treatments with 4 kg $\text{CaCl}_2 \text{ ha}^{-1}$), 4th and 5th leaf spray (at 10th and 20th July 2018 respectively, all the previous treatments with 8 kg $\text{CaCl}_2 \text{ ha}^{-1}$). Letters a, b, c, d represent significant differences, between treatments for each date of analysis (statistical analysis using the single factor ANOVA test, $P \leq 0.05$). Ctr = control; NCa0.1, NCa0.3, NCa0.6 correspond, respectively, to the initial foliar spray of 0.1 kg ha^{-1} , 0.3 kg ha^{-1} and 0.6 kg ha^{-1} $\text{Ca}(\text{NO}_3)_2$; 0.4CCa, 0.8CCa, and 1.6CCa, correspond respectively to the initial foliar spray of CaCl_2 0.4 kg ha^{-1} , 0.8 kg ha^{-1} and 1.6 kg ha^{-1} . After 4 kg ha^{-1} and 8 kg ha^{-1} of CaCl_2 sprays were applied to all treatments except to control.

to the lower contents of S found in NCa0.6, 0.8CCa and 1.6CCa, yet after the 4th foliar spraying significant differences could not be found among treatments (Fig. 2). Following the 5th spraying, the significantly lowest value was found with 0.8CCa (relatively to NCa0.1, NCa0.3 and 1.6CCa).

After the 2nd spraying, the levels of Mg showed the highest values in 0.8CCa, yet thereafter its contents did not vary among treatments after each foliar spraying (Fig. 2).

In the leaves, Zn contents showed (Fig. 2) significantly higher values in the control and 1.6CCa (relatively to lowest occurring in 0.4CCa, after the 2nd spraying), in 1.6CCa (relatively to the lowest found in NCa0.3, after the 3rd foliar spraying), in 0.8CCa (relatively to the lowest detected in the control, after the 4th spraying) and NCa0.3 (relatively to the lowest revealed in 0.4CCa, after the 5th foliar spraying).

Between the 2nd and the 5th foliar spraying, within each treatment the average ratios of K/Ca, P/Ca and S/Ca decreased to 48.75 – 65.77%, 50.00 – 77.78% and 64.00 – 95.24%, whereas Zn/Ca and Mg/Ca varied between 84.21 – 111.77% and 58.70 – 154.17%, respectively (Fig. 2). Besides, between the 2nd and the 5th spraying, the trendlines of the average rates between K/Ca remained linear (except with 0.4CCa that showed a 2nd order polynomial equation), whereas P/Ca and S/Ca of all treatments was best translated by 3rd and 2nd order polynomials, respectively (Table 1). The average ratios Zn/Ca further revealed 3rd order polynomial trendlines (except for NCa0.6), whereas with Mg/Ca 2nd order polynomials prevailed (except for NCa0.1 and 0.8CCa) (Table 1). Accordingly, K/Ca, S/Ca and P/Ca displayed interactive linear, biphasic and triphasic concentration patterns in the leaves tissues.

Light reactions of the photosynthetic apparatus

Within the scope of the functioning of the light reactions of the photosynthetic apparatus it was found that F_v/F_m did not reveal a significant impact (or any consistent variation) in any analytical period (excepting at 20th July, among the experimental periods, with 0.4CCa), regarding both products and different concentrations used (Table 2).

Regarding $Y_{(II)}$, among the different experimental periods (Table 2), significantly lower values were only found for NCa0.3 and 0.4CCa, at 19th June and 20th July, respectively. Yet, after the 5th and 7th spraying, a slight decreasing tendency (non-significant) was observed in higher doses of both products (NCa0.6 and 1.6CCa). Additionally, at 20th July, among treatments, a significant decrease was found with 0.8CCa. Concerning to $Y_{(NPQ)}$, among the three experimental periods, significantly higher values were found at 11th September for all treatments,

Table 1: Trendlines of average rates between Ca and K, P, S, Zn and Mg, in leaves of *Pyrus communis* L, variety "Rocha" pear, between the 2nd (at 8th June 2018, with 0.1, 0.3 and 0.6 kg Ca(NO₃)₂ ha⁻¹, or 0.4, 0.8 and 1.6 kg CaCl₂ ha⁻¹), 3rd (at 15th June 2018, all the previous treatments with 4 kg CaCl₂ ha⁻¹), 4th and 5th foliar spray (at 10th and 20th July 2018, respectively), all the previous treatments with 8 kg CaCl₂ ha⁻¹). Ctr, NCa0.1, NCa0.3, NCa0.6 correspond, respectively, to the control and initial foliar spray of 0.1 kg ha⁻¹, 0.3 kg ha⁻¹ and 0.6 kg ha⁻¹ Ca(NO₃)₂, whereas 0.4CCa, 0.8CCa, and 1.6CCa, correspond respectively to the initial foliar spray of CaCl₂ 0.4 kg ha⁻¹, 0.8 kg ha⁻¹ and 1.6 kg ha⁻¹

Treatments	K/Ca			P/Ca			S/Ca			Zn/Ca			Mg/Ca		
	Trendline	R ²		Trendline	R ²		Trendline	R ²		Trendline	R ²		Trendline	R ²	
Ctr	$y = -0.182x + 1.527$	0.9777		$y = -0.0067x^3 + 0.07x^2 - 0.2433x + 0.35$	1		$y = -0.0075x^2 + 0.0255x + 0.2145$	1		$y = 0.0002x^3 - 0.0017x^2 + 0.0056x - 0.0039$	1		$y = 0.0013x^2 - 0.0061x + 0.0321$	0.9988	
NCa0.1	$y = -0.149x + 1.285$	0.9922		$y = -0.0067x^3 + 0.07x^2 - 0.2433x + 0.35$	1		$y = 0.0233x^3 - 0.265x^2 + 0.9417x - 0.8$	1		$y = 0.0002x^3 - 0.0019x^2 + 0.0068x - 0.0056$	1		$y = 0.0072x^3 - 0.0715x^2 + 0.2253x - 0.198$	1	
NCa0.3	$y = -0.133x + 1.335$	0.6156		$y = -0.02x^3 + 0.205x^2 - 0.665x + 0.76$	1		$y = -0.0025x^2 + 0.0065x + 0.2385$	0.9333		$y = -0.0002x^3 + 0.0024x^2 - 0.008x + 0.0101$	1		$y = -0.0022x^2 + 0.0184x - 0.0029$	0.7585	
NCa0.6	$y = -0.202x + 1.712$	0.972		$y = -0.0133x^3 + 0.135x^2 - 0.4417x + 0.55$	1		$y = -0.01x^2 + 0.056x + 0.159$	0.9857		$y = -0.0001x^2 + 0.0011x + 2E-05$	0.9385		$y = -0.0005x^2 + 0.0073x + 0.0077$	0.8819	
0.4CCa	$y = -0.055x^2 + 0.267x + 0.783$	0.9603		$y = -0.0183x^3 + 0.19x^2 - 0.6217x + 0.72$	1		$y = -0.0225x^2 + 0.1485x + 0.0065$	0.9084		$y = 0.0002x^3 - 0.0026x^2 + 0.0094x - 0.0087$	1		$y = -0.0042x^2 + 0.0322x - 0.0273$	0.902	
0.8CCa	$y = 0.005x^2 - 0.183x + 1.478$	0.7915		$y = -0.0283x^3 + 0.295x^2 - 0.9767x + 1.1$	1		$y = -0.005x^2 + 0.009x + 0.246$	0.8286		$y = -8E-05x^3 + 0.0007x^2 - 0.0017x + 0.0032$	1		$y = -0.0077x^3 + 0.084x^2 - 0.2953x + 0.362$	1	
1.6CCa	$y = -0.186x + 1.556$	0.9196		$y = -0.0183x^3 + 0.185x^2 - 0.5967x + 0.7$	1		$y = -0.0075x^2 - 0.0615x + 0.3115$	0.9333		$y = 0.0011x^3 - 0.0118x^2 + 0.041x - 0.0417$	1		$y = -0.0035x^2 + 0.0199x + 0.0126$	0.9556	

Table 2: Average $\pm$ SE of chlorophyll *a* fluorescence parameters, maximal photochemical efficiency of PSII (F_v/F_m), estimate of the quantum yield of photosynthetic noncyclic electron transport ($Y_{(II)}$), estimate of the quantum yield of regulated energy dissipation ($Y_{(NPQ)}$), and nonregulated energy (heat and fluorescence) dissipation ($Y_{(NO)}$) of PSII, coefficient of non-photochemical (q_N) and photochemical (q_L) fluorescence quenching, as well as actual PSII efficiency of energy conversion under light (F_v'/F_m') in leaves of *Pyrus communis* L., variety “Rocha” pear, submitted to Ca biofortification, at 19th June 2018, 20th July 2018 and 11th September 2018 (after the 3rd, 5th foliar sprays and at harvest, respectively). For each parameter, the mean values $\pm$ SE ($n = 7$) followed by different letters express significant differences among analytical dates for each treatment (a, b, c), or among treatments for the same analytical date (A, B, C) (statistical analysis using the single factor ANOVA test, $P \leq 0.05$). Ctr = control; NCa0.1, NCa0.3, NCa0.6 correspond, respectively, to the initial foliar spray of 0.1 kg ha⁻¹, 0.3 kg ha⁻¹ and 0.6 kg ha⁻¹ Ca(NO₃)₂; 0.4CCa, 0.8CCa, and 1.6CCa, correspond respectively to the initial foliar spray of CaCl₂ 0.4 kg ha⁻¹, 0.8 kg ha⁻¹ and 1.6 kg ha⁻¹. After 4 kg ha⁻¹ and 8 kg ha⁻¹ of CaCl₂ sprays were applied to all treatments except to control

Analysis Dates	19 th June 2018				20 th July 2018				11 th September 2018			
Treatments	F_v/F_m				F_v/F_m				F_v/F_m			
Ctr	0.818	$\pm$	0.006	aA	0.804	$\pm$	0.006	aAB	0.819	$\pm$	0.005	aA
NCa0.1	0.815	$\pm$	0.005	aA	0.810	$\pm$	0.010	aAB	0.828	$\pm$	0.004	aA
NCa0.3	0.817	$\pm$	0.005	aA	0.805	$\pm$	0.011	aAB	0.810	$\pm$	0.003	aA
NCa0.6	0.806	$\pm$	0.006	aA	0.816	$\pm$	0.011	aAB	0.822	$\pm$	0.007	aA
0.4CCa	0.799	$\pm$	0.006	bA	0.786	$\pm$	0.011	bB	0.833	$\pm$	0.004	aA
0.8CCa	0.808	$\pm$	0.008	aA	0.819	$\pm$	0.007	aA	0.814	$\pm$	0.003	aA
1.6CCa	0.811	$\pm$	0.006	aA	0.807	$\pm$	0.010	aAB	0.821	$\pm$	0.004	aA
$Y_{(II)}$												
Ctr	0.409	$\pm$	0.015	aA	0.423	$\pm$	0.019	aA	0.392	$\pm$	0.034	aA
NCa0.1	0.411	$\pm$	0.017	aA	0.371	$\pm$	0.036	aAB	0.403	$\pm$	0.014	aA
NCa0.3	0.377	$\pm$	0.021	bA	0.460	$\pm$	0.024	aA	0.388	$\pm$	0.016	aA
NCa0.6	0.415	$\pm$	0.014	aA	0.378	$\pm$	0.020	aAB	0.376	$\pm$	0.015	aA
0.4CCa	0.399	$\pm$	0.015	aA	0.382	$\pm$	0.016	bAB	0.401	$\pm$	0.016	aA
0.8CCa	0.413	$\pm$	0.017	aA	0.309	$\pm$	0.016	aB	0.411	$\pm$	0.012	aA
1.6CCa	0.405	$\pm$	0.018	aA	0.393	$\pm$	0.014	aA	0.364	$\pm$	0.016	aA
$Y_{(NPQ)}$												
Ctr	0.338	$\pm$	0.021	bA	0.325	$\pm$	0.026	bBC	0.464	$\pm$	0.035	aA
NCa0.1	0.344	$\pm$	0.023	bA	0.388	$\pm$	0.034	abC	0.447	$\pm$	0.016	aA
NCa0.3	0.364	$\pm$	0.025	bA	0.288	$\pm$	0.031	bAB	0.455	$\pm$	0.017	aA
NCa0.6	0.320	$\pm$	0.021	bA	0.344	$\pm$	0.026	bAB	0.475	$\pm$	0.015	aA
0.4CCa	0.312	$\pm$	0.016	bA	0.306	$\pm$	0.014	bBC	0.451	$\pm$	0.018	aA
0.8CCa	0.375	$\pm$	0.017	aA	0.413	$\pm$	0.013	aA	0.416	$\pm$	0.015	aA
1.6CCa	0.383	$\pm$	0.016	bA	0.294	$\pm$	0.040	cC	0.478	$\pm$	0.013	aA
$Y_{(NO)}$												
Ctr	0.253	$\pm$	0.017	aAB	0.252	$\pm$	0.010	aB	0.145	$\pm$	0.004	bA
NCa0.1	0.245	$\pm$	0.013	aAB	0.241	$\pm$	0.013	aB	0.151	$\pm$	0.005	bA
NCa0.3	0.259	$\pm$	0.019	aAB	0.252	$\pm$	0.012	aB	0.157	$\pm$	0.005	bA
NCa0.6	0.266	$\pm$	0.014	aA	0.278	$\pm$	0.022	aAB	0.150	$\pm$	0.006	bA
0.4CCa	0.289	$\pm$	0.009	aA	0.312	$\pm$	0.009	aA	0.148	$\pm$	0.004	bA
0.8CCa	0.212	$\pm$	0.007	bB	0.278	$\pm$	0.010	aAB	0.174	$\pm$	0.006	bA
1.6CCa	0.212	$\pm$	0.009	bB	0.314	$\pm$	0.027	aA	0.158	$\pm$	0.006	cA
q_N												
Ctr	0.667	$\pm$	0.033	bAB	0.617	$\pm$	0.032	bAB	0.840	$\pm$	0.021	aA
NCa0.1	0.685	$\pm$	0.029	bAB	0.695	$\pm$	0.022	bAB	0.835	$\pm$	0.014	aA
NCa0.3	0.698	$\pm$	0.030	bAB	0.615	$\pm$	0.042	bB	0.826	$\pm$	0.013	aA
NCa0.6	0.662	$\pm$	0.031	bAB	0.676	$\pm$	0.036	bAB	0.850	$\pm$	0.010	aA
0.4CCa	0.638	$\pm$	0.020	bB	0.606	$\pm$	0.016	bA	0.835	$\pm$	0.011	aA
0.8CCa	0.746	$\pm$	0.015	aA	0.718	$\pm$	0.013	aA	0.798	$\pm$	0.013	aA
1.6CCa	0.749	$\pm$	0.014	aA	0.582	$\pm$	0.068	bB	0.842	$\pm$	0.006	aA
q_L												
Ctr	0.413	$\pm$	0.030	bB	0.328	$\pm$	0.016	bB	0.585	$\pm$	0.037	aA
NCa0.1	0.456	$\pm$	0.032	bAB	0.349	$\pm$	0.043	bAB	0.589	$\pm$	0.021	aA
NCa0.3	0.437	$\pm$	0.037	aAB	0.464	$\pm$	0.029	aA	0.539	$\pm$	0.031	aA
NCa0.6	0.490	$\pm$	0.030	aAB	0.448	$\pm$	0.046	aAB	0.580	$\pm$	0.035	aA
0.4CCa	0.448	$\pm$	0.030	abAB	0.392	$\pm$	0.021	bAB	0.555	$\pm$	0.020	aA
0.8CCa	0.541	$\pm$	0.018	aA	0.341	$\pm$	0.036	bAB	0.565	$\pm$	0.027	aA
1.6CCa	0.515	$\pm$	0.030	aAB	0.384	$\pm$	0.033	bAB	0.526	$\pm$	0.038	aA
F_v'/F_m'												
Ctr	0.629	$\pm$	0.016	aA	0.691	$\pm$	0.013	aA	0.524	$\pm$	0.024	bA
NCa0.1	0.607	$\pm$	0.020	aAB	0.635	$\pm$	0.016	aAB	0.534	$\pm$	0.016	bA
NCa0.3	0.584	$\pm$	0.017	aAB	0.648	$\pm$	0.019	abA	0.542	$\pm$	0.017	bA
NCa0.6	0.593	$\pm$	0.017	aAB	0.582	$\pm$	0.022	abAB	0.511	$\pm$	0.017	bA
0.4CCa	0.599	$\pm$	0.011	aAB	0.613	$\pm$	0.012	aB	0.546	$\pm$	0.015	aA
0.8CCa	0.565	$\pm$	0.012	aB	0.574	$\pm$	0.012	aAB	0.554	$\pm$	0.015	aA
1.6CCa	0.571	$\pm$	0.012	abAB	0.628	$\pm$	0.033	aAB	0.525	$\pm$	0.007	bA

excepting NCa0.1 and 0.8CCa at 20th July and also 0.8CCa at 19th June, whereas among treatments of each period significant differences could not be found at 19th June and 11th September (Table 2). Relatively to $Y_{(NO)}$, all treatments of 20th July showed significantly higher values among the experimental periods (excepting with 0.8CCa and 1.6CCa at 19th June), but relatively to all treatments of each period, at 11th September significant differences could not be found, whereas significantly higher values were found with 0.8CCa and 1.6CCa (at 19th June) and control, NCa0.1 and NCa0.3 (at 20th July). During this trial, the variations of $Y_{(H)}$, $Y_{(NPQ)}$ and $Y_{(NO)}$ eventually were mostly associated with environmental conditions prevailing in each measurement date, than to the applied products or concentrations used.

The proportion of energy dissipated as heat by photoprotection mechanism (q_N) at 11th September, among the experimental periods showed the significantly higher values for all treatments, excepting for 0.8CCa (at 19th June and 20th July) and 1.6CCa (at 19th June) (Table 2). Among treatments of each period, q_N did not revealed significant variations at 11th September, but significantly higher values were found for 0.8CCa (at 19th June and 20th July) and 1.6CCa (at 19th July).

Considering q_L (Table 2), among the experimental periods and for all treatments, the significantly higher values were found at 11th September (excepting for NCa0.3 at 19th June and 20th July, as well as NCa0.6, 0.4CCa, 0.8CCa and 1.6CCa for 19th June). Within each experimental period significant differences could not be found among treatments at 11th September, whereas at the highest and lowest values were found with NCa0.3 and control (at 20th July) and 0.8CCa and control (at 19th June).

Concerning to F_v'/F_m' (Table 2), among the experimental periods and for all treatments, the lower values were found

at 11th September (excepting with 0.8CCa). Within each period significantly different values could not be found at 11th September, whereas the significantly higher and lower values were found in the control and 0.4CCa (at 20th July) and control 0.8CCa (at 19th June).

Mineral contents in pear fruit

At harvest, total Ca of “Rocha” pear fruits of all treatments showed, relatively to the control, significant increases (Fig. 3). Calcium increases for treatments with $Ca(NO_3)_2$ and $CaCl_2$ ranged between 1.6 – 2.9 fold and 1.9 – 6.4 fold, respectively. In the fruit tissues, the amount of Ca revealed a heterogeneous distribution (Fig. 3) but prevailed in the peel and core (*i.e.*, regions 1 and 5 in the equatorial zone).

Concerning to the total levels of C, H and O in “Rocha” pear fruits (Table 3), significant deviations could not be found among treatments, varying the ranges (in %) between 35.9 – 40.8, 5.01 – 5.70 and 39.8 – 45.2, respectively. Among treatments, significant differences were found (Table 3) only for NCa0.6 and 0.4CCa (for total contents of K) and control relatively to 0.8CCa and 1.6CCa or the remaining treatments (for total amounts of Mg). Concerning to Cl, all treatments were significantly different and P also revealed a similar pattern for all treatments (except 0.4CCa relatively to 0.8CCa) (Table 3). Total contents of Mn, Fe, Zn, and Cu showed an heterogeneous variation among treatments ranging (in ppm) between 4.14 – 37.9, 15.5 – 59.6, 9.55 – 27.9 and 6.36 – 21.4, respectively.

Concerning to the tissues of the five regions in the equatorial region of the fruit, a clear tendency for distribution of C, H, O, K, Mg and Cl could not be detected, with values varying (in %) between 22.5 – 43.9, 3.15 – 6.14, 25.0 – 48.7, 1.13 – 5.0, 0.0 – 14.3, 0.000 – 4.380 and 0.000 – 0.630, respectively (Table 3). Additionally, relatively to the tissues of the 5 regions of the equatorial region of “Rocha” pears, Mn, Fe,

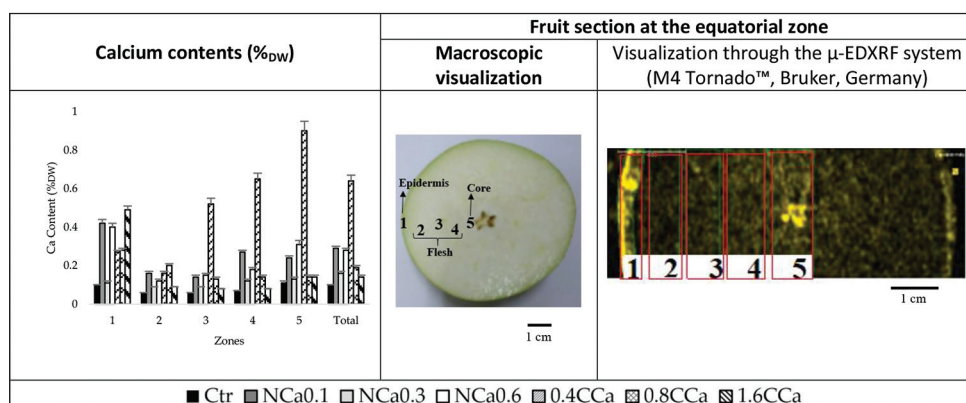


Fig 3. Average $\pm$ SE ($n = 3$) of Ca contents expressed in %_{DW}, in 5 zones (1, 2, 3, 4, 5, where 1 corresponds to the skin and 5 to core respectively), of fruits from *Pyrus communis* L, variety “Rocha” pear, at harvest (4th and 9th of September 2018 respectively). Ctr = control; NCa0.1, NCa0.3, NCa0.6 correspond, respectively, to the initial foliar spray of 0.1 kg ha⁻¹, 0.3 kg ha⁻¹ and 0.6 kg ha⁻¹ $Ca(NO_3)_2$, whereas 0.4CCa, 0.8CCa, and 1.6CCa, correspond respectively to the initial foliar spray of $CaCl_2$ 0.4 kg ha⁻¹, 0.8 kg ha⁻¹ and 1.6 kg ha⁻¹. After 4 kg ha⁻¹ and 8 kg ha⁻¹ of $CaCl_2$ sprays were applied to all treatments except to control.

Table 3: Average of different elements (C, H, O, K, Mg, Cl, Cu, Zn, Mn and Fe) $\pm$ SE (n = 3) and rates between each chemical element and Ca, considering 5 zones (1, 2, 3, 4, 5, where 1 corresponds to the peel and 5 to core respectively), of the equatorial region of the fruits from *Pyrus communis* L, variety "Rocha" pear, at harvest (4th September 2018). Ctr = control; NCa0.3, NCa0.6 correspond, respectively, to the initial foliar spray of 0.1 kg ha⁻¹, 0.3 kg ha⁻¹ and 0.6 kg ha⁻¹ Ca (NO₃)₂, whereas 0.4CCa, 0.8CCa, and 1.6CCa, correspond respectively to the initial foliar spray of CaCl₂ 0.4 kg ha⁻¹, 0.8 kg ha⁻¹ and 1.6 kg ha⁻¹. Thereafter 4 kg ha⁻¹ of CaCl₂ sprays were applied to all treatments except to control. For each nutrient significant differences for total amounts are indicated with letters from a to g

Treatments	Zones	C			H			O			K			Mg			Cl			Cu			Zn			Mn			Fe		
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Ctr	1	43.7	±2.18	6.11	±0.31	48.5	±2.42	1.62	±0.08	n.d.	n.d.	n.d.	n.d.	0.001	±0.000	4.27	±0.21	7.06	±0.35	5.23	±0.26	10.2	±0.51								
	2	43.6	±2.18	6.10	±0.31	48.4	±2.42	1.72	±0.09	n.d.	n.d.	n.d.	n.d.	0.001	±0.000	3.99	±0.20	7.08	±0.35	1.61	±0.08	11.5	±0.57								
	3	41.9	±2.09	5.86	±0.29	46.5	±2.32	1.95	±0.10	3.73	±0.19	0.030	±0.000	4.37	±0.22	7.37	±0.22	7.37	±0.37	1.13	±0.06	13.7	±0.68								
	4	43.6	±2.18	6.10	±0.30	48.4	±2.42	1.81	±0.09	n.d.	n.d.	n.d.	n.d.	0.030	±0.000	4.50	±0.22	6.60	±0.33	2.47	±0.12	10.5	±0.53								
	5	42.0	±2.10	5.87	±0.29	46.6	±2.33	2.25	±0.11	3.17	±0.16	0.050	±0.001	7.06	±0.35	9.26	±0.35	9.26	±0.46	3.27	±0.16	14.7	±0.74								
Total		40.8a	±2.04	5.70a	±0.29	45.2a	±2.26	2.23b	±0.11	5.96c	±0.30	0.020g	±0.001	6.36d	±0.32	9.55c	±0.32	9.55c	±0.48	4.13e	±0.21	15.5d	±0.77								
NCa0.1	1	39.3	±1.96	5.50	±0.27	43.6	±2.18	2.38	±0.12	8.22	±0.41	0.030	±0.000	17.5	±0.88	22.7	±1.14	144	±7.21	386	±19.3										
	2	42.3	±2.11	5.91	±0.30	46.9	±2.35	2.40	±0.12	2.23	±0.11	0.080	±0.001	9.08	±0.45	13.5	±0.67	11.6	±0.58	17.9	±0.89										
	3	42.9	±2.15	6.01	±0.30	47.7	±2.38	1.72	±0.09	1.45	±0.07	0.050	±0.000	8.72	±0.44	10.9	±0.54	5.36	±0.27	14.6	±0.73										
	4	40.9	±2.05	5.72	±0.29	45.4	±2.27	1.99	±0.10	5.69	±0.28	0.010	±0.000	11.4	±0.57	14.4	±0.72	5.01	±0.25	16.4	±0.82										
	5	40.5	±2.02	5.66	±0.28	45.0	±2.25	1.86	±0.09	6.69	±0.33	0.080	±0.000	10.8	±0.54	15.8	±0.79	3.19	±0.16	22.0	±1.10										
Total		36.5a	±1.83	5.11a	±0.26	40.6a	±2.03	2.55b	±0.13	14.8a	±0.74	0.090d	±0.001	15.3b	±0.46	21.5b	±1.08	21.8b	±1.09	59.6a	±2.98										
Nca0.3	1	43.9	±2.19	6.14	±0.31	48.7	±2.43	1.13	±0.06	n.d.	n.d.	n.d.	n.d.	0.050	±0.003	6.70	±0.34	13.7	±0.68	3.07	±0.15	14.7	±0.73								
	2	42.0	±2.10	5.87	±0.29	46.6	±2.33	1.49	±0.07	3.70	±0.18	0.050	±0.004	7.47	±0.37	16.7	±0.84	0.32	±0.02	10.1	±0.51										
	3	43.6	±2.18	6.10	±0.31	48.4	±2.42	1.61	±0.08	n.d.	n.d.	n.d.	n.d.	0.060	±0.004	5.86	±0.29	7.50	±0.37	2.16	±0.11	9.84	±0.49								
	4	43.3	±2.16	6.05	±0.30	48.1	±2.40	1.89	±0.09	0.47	±0.02	0.020	±0.001	6.81	±0.34	9.58	±0.48	2.79	±0.14	10.3	±0.52										
	5	43.5	±2.18	6.09	±0.30	48.3	±2.42	1.84	±0.09	n.d.	n.d.	n.d.	n.d.	0.080	±0.003	7.25	±0.36	11.4	±0.57	5.85	±0.29	16.3	±0.81								
Total		38.5a	±1.92	5.38a	±0.27	42.7a	±2.13	2.29b	±0.11	10.9a	±0.54	0.070f	±0.004	10.7c	±0.54	18.1b	±0.90	6.54d	±0.33	19.3d	±0.97										
NCa0.6	1	43.0	±2.15	6.02	±0.30	47.8	±2.39	2.46	±0.12	n.d.	n.d.	n.d.	n.d.	0.130	±0.005	15.5	±0.77	21.8	±1.09	12.1	±0.60	53.1	±2.66								
	2	43.3	±2.16	6.06	±0.30	48.1	±2.40	2.33	±0.12	n.d.	n.d.	n.d.	n.d.	0.070	±0.004	8.98	±0.45	15.5	±0.77	1.56	±0.08	48.4	±2.42								
	3	41.8	±2.09	5.84	±0.29	46.4	±2.32	3.24	±0.16	2.31	±0.12	0.080	±0.003	10.9	±0.54	14.1	±0.70	6.86	±0.34	23.6	±1.18										
	4	40.7	±2.03	5.69	±0.28	45.2	±2.26	4.56	±0.23	3.16	±0.16	0.130	±0.004	15.1	±0.76	21.8	±1.09	6.67	±0.33	31.5	±1.57										
	5	39.6	±1.98	5.54	±0.28	44.0	±2.20	4.99	±0.25	4.64	±0.23	0.170	±0.004	21.4	±1.07	25.4	±1.27	16.1	±0.81	39.5	±1.98										
Total		37.1a	±1.86	5.19a	±0.26	41.2a	±2.06	4.51a	±0.23	11.0a	±0.55	0.120b	±0.004	19.9a	±1.00	27.2a	±1.36	13.6c	±0.68	47.1b	±2.36										
0.4CCa	1	42.7	±2.14	5.98	±0.30	47.5	±2.37	3.19	±0.16	n.d.	n.d.	n.d.	n.d.	0.120	±0.003	15.2	±0.76	19.4	±0.97	24.4	±1.22	33.8	±1.69								
	2	36.5	±1.82	5.10	±0.26	40.5	±2.02	3.79	±0.19	13.8	±0.69	0.130	±0.002	16.1	±0.81	18.7	±0.94	8.35	±0.42	25.0	±1.25										
	3	35.5	±1.77	4.96	±0.25	39.4	±1.97	4.66	±0.23	14.3	±0.71	0.070	±0.003	23.8	±1.19	27.4	±1.37	50.3	±2.51	44.4	±2.22										
	4	40.6	±2.03	5.68	±0.28	45.1	±2.25	3.76	±0.19	3.52	±0.18	0.120	±0.004	16.8	±0.84	21.7	±1.08	36.6	±1.83	35.9	±1.79										
	5	36.6	±1.83	5.12	±0.26	40.6	±2.03	4.91	±0.25	10.9	±0.55	0.110	±0.003	20.5	±1.03	29.2	±1.46	41.8	±2.09	55.8	±2.79										
Total		35.9a	±1.79	5.01a	±0.25	39.8a	±1.99	4.63a	±0.23	13.3a	±0.66	0.110c	±0.003	21.4a	±1.07	27.9a	±1.39	37.9a	±1.90	45.8b	±2.29										
0.8CCa	1	39.4	±1.97	5.51	±0.28	43.8	±2.19	2.26	±0.11	8.76	±0.44	0.001	±0.000	64.1	±3.21	32.5	±1.63	3.85	±0.19	42.1	±2.10										
	2	38.5	±1.92	5.38	±0.27	42.7	±2.13	2.31	±0.12	10.9	±0.55	0.030	±0.002	12.6	±0.63	19.8	±0.99	11.1	±0.55	33.4	±1.67										
	3	42.8	±2.14	5.99	±0.30	47.5	±2.38	1.89	±0.09	1.56	±0.08	0.040	±0.003	6.30	±0.31	12.2	±0.61	2.28	±0.11	17.9	±0.90										
	4	43.4	±2.17	6.07	±0.30	48.2	±2.41	1.99	±0.10	n.d.	n.d.	0.140	±0.002	4.89	±0.24	10.7	±0.53	3.89	±0.19	16.3	±0.82										
	5	41.5	±2.08	5.81	±0.29	46.1	±2.31	1.92	±0.10	4.24	±0.21	0.100	±0.005	6.04	±0.30	12.3	±0.61	5.32	±0.27	17.6	±0.88										
Total		38.8a	±1.94	5.43a	±0.27	43.1a	±2.16	2.37b	±0.12	9.76b	±0.49	0.080e	±0.003	13.0c	±0.11	18.4b	±0.92	6.48d	±0.32	27.5c	±1.37										
1.6CCa	1	22.5	±1.13	3.15	±0.16	25.0	±1.25	2.00	±0.25	n.d.	n.d.	n.d.	n.d.	4.380	±0.007	9.23	±0.46	41.3	±2.06	0.67	±0.03	111	±5.53								
	2	43.2	±2.16	6.05	±0.30	48.0	±2.40	1.26	±0.06	0.85	±0.04	5.200	±0.008	3.83	±0.19	6.96	±0.35	2.12	±0.11	10.4	±0.52										
	3	43.0	±2.15	6.02	±0.30	47.7	±2.39	1.19	±0.06	n.d.	n.d.	n.d.	n.d.	1.900	±0.003	2.79	±0.14	5.24	±0.26	3.51	±0.18	13.1	±0.66								
	4	42.1	±2.10	5.88	±0.29	46.7	±2.33	1.45	±0.07	2.53	±0.13	1.230	±0.002	3.29	±0.16	6.97	±0.35	3.10	±0.15	18.7	±0.94										
	5	39.3	±1.97	5.50	±0.28	43.7	±2.18	2.19	±0.11	6.55	±0.33	2.600	±0.001	7.51	±0.38	11.4	±0.57	3.17	±0.16	19.9	±0.99										
Total		38.4a	±1.92	5.37a	±0.27	42.6a	±2.13	2.02b	±0.10	7.48b	±0.37	4.010a	±0.002	7.37d	±0.37	12.0c	±0.60	4.41e	±0.22	19.9d	±1.00										

n.d. = not detected

Zn and Cu also did not show a clear tendency (Table 3), but values varied (in ppm) between 0 – 2760, 0.32 – 144, 9.84 – 386, 5.24 – 41.3, 0.00 – 1.94 and 2.79 – 64.1, respectively.

Fruit characteristics at harvest

At harvest, fruits height (Fig. 4) did not reveal significant differences between the control and the remaining treatments. The values ranged (in mm) from 72–84.

Regarding fruits diameter (Fig. 4), values varied (in mm) between 61–70 and 61–71, being 0.8CCa significantly lower relatively to the highest treatments (0.8CCa and 1.6CCa).

The colorimetric profile of “Rocha” pears in the peel and pulps (Fig. 5) did not revealed significant differences among treatments, showing the highest values between 550 - 650 nm, and the lowest between 450 - 500 nm. Thus, Ca spraying with calcium nitrate or calcium chloride did not affect the transmittance colour of the “Rocha” pears.

DISCUSSION

Calcium is acquired from the soil solution, by the root tip and regions in which lateral roots are being initiated, and translocated to the shoot via the xylem. However, the Ca pathway across the root confers potential limitations. Through the symplastic pathway of Ca movement the rate of selectivity is controlled, yet the apoplastic pathway is relatively non-selective between divalent cations allowing the accumulation of toxic solutes in the shoot (White, 2001) and, once in the phloem, its redistribution has a low kinetic rate (Bonomelli et al., 2019; Coelho et al., 2021). Thus, to surpass the low mobilization rate of Ca (*i.e.*, potential deficiencies) from roots and to active growing tissues, namely young leaves (Dayod et al., 2010), and during fruits development (Faust, 1989; Zavalloni et al., 2001), by spraying with Ca, uptake can take place through the fruit cuticle or stomata (Saure, 2005). In this context, analysing the accumulation of Ca in the leaves of “Rocha” pear trees, during the three pulverization phases (Fig. 1) distinct physiological behavior was found. In the first pulverization phase, the significantly higher accumulation of Ca in 1.6CCa after 2nd foliar spray (*i.e.*, immediately after the end of spraying with increasing concentrations of CaCl₂), suggested the development of Ca²⁺ compartmentation in different subcellular organelles, eventually in the vacuoles (Conn and Gilliam, 2010; MacRobbie, 2006). Indeed, compartmentation of Ca²⁺ in the tonoplast of leaf cells, away from the cytosol, through the expression of particular Ca²⁺-transporters, will have avoided precipitation reactions between Ca²⁺ with inorganic phosphorous species (Pi), ATP and other inorganic phosphates (Conn and Gilliam,

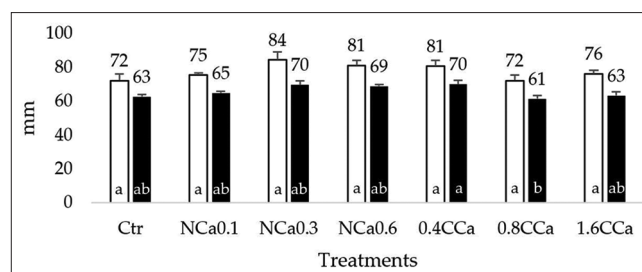


Fig 4. Average $\pm$ SE ($n = 4$) height (white) and diameter (black) of fruits from *Pyrus communis* L. variety Rocha pear, at harvesting (4th of September 2018). Letters a, b indicate significant differences, for each parameter, between the treatments (statistical analysis using the single factor ANOVA test, $P \leq 0.05$). Ctr = control; NCa0.1, NCa0.3, NCa0.6 correspond, respectively, to the initial foliar spray of 0.1 kg ha⁻¹, 0.3 kg ha⁻¹ and 0.6 kg ha⁻¹ Ca(NO₃)₂, whereas 0.4CCa, 0.8CCa, and 1.6CCa, correspond respectively to the initial foliar spray of CaCl₂ 0.4 kg ha⁻¹, 0.8 kg ha⁻¹ and 1.6 kg ha⁻¹. After 4 kg ha⁻¹ and 8 kg ha⁻¹ of CaCl₂ sprays were applied to all treatments except to control.

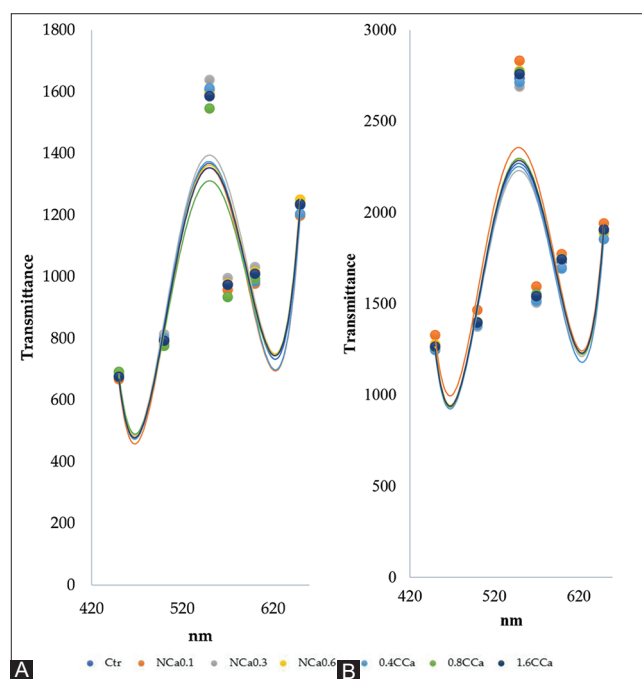


Fig 5. Visible spectra showing the average of transmittance ($n = 3$) in the peel (A) and pulp (B) of fruits from *Pyrus communis* L. variety “Rocha” pear, at harvest (4th September 2018 respectively). Ctr = control; NCa0.1, NCa0.3, NCa0.6 correspond, respectively, to the initial foliar spray of 0.1 kg.ha⁻¹, 0.3 kg ha⁻¹ and 0.6 kg ha⁻¹ Ca(NO₃)₂, whereas 0.4CCa, 0.8CCa, and 1.6CCa, correspond respectively to the initial foliar spray of CaCl₂ 0.4 kg.ha⁻¹, 0.8 kg.ha⁻¹ and 1.6 kg.ha⁻¹. After 4 kg ha⁻¹ and 8 kg ha⁻¹ of CaCl₂ sprays were applied to all treatments except to control.

2010). This accumulation Ca phase is also consistent with the hypothesis that explicit high contents of Ca in the cytosol produce defined physiological responses to specific developmental cues, through activation of specific genes (White and Broadley, 2003). With the 2nd pulverization phase (*i.e.*, after the 3rd foliar spraying, in which all the initial treatments were then treated with 4 kg ha⁻¹ CaCl₂), the

higher Ca concentration applied to the leaves showed similar values between the control and the remaining treatments, suggesting the development of Ca homeostasis in parallel with its mobilization from roots. Thus, at this stage of the productive cycle of the trees, this Ca physiotype, indicated similar contributions of symplastic and apoplastic pathways to the xylem (White, 2001) normalizing the different amounts of Ca assimilated through the 1st and 2nd foliar spraying. The final pulverization phase (*i.e.*, after the 4th and 5th foliar spraying in which all the initial treatments were then treated with 8 kg ha⁻¹ CaCl₂), which matched the natural development of “Rocha” pear fruits, in general kept a similar trend to that previously identified in the second phase. So, the higher concentration of CaCl₂ (8 kg ha⁻¹) did not triggered a substantial increase of Ca after the 4th foliar spraying, yet its nutrient accumulation after the following spraying became relevant, indicating a genotype specificity (as values remained similar to the control). Besides, as additional accumulation triggered by foliar spraying did not occur in Ca treated trees, the application 4 and 8 kg ha⁻¹ determined the higher mobilization and accumulation of Ca to the fruits (Fig. 3). Accordingly, this related Ca kinetics suggests an oscillating balance between this nutrient deposition by foliar spraying and the slow removal of Ca²⁺ from the mesophyll cells in the leaves to the fruits through the phloem (Bonomelli et al., 2019; Coelho et al. 2021; Davies and Millard, 1985; Nelson et al., 1990; Oparka and Davies, 1988).

In the leaves, although between the 2nd and the 5th foliar spraying with both chemical forms of Ca the levels of K varied among treatments between 2.66 and 3.87 % (Fig. 2), the decreased ratio K/Ca between these spraying periods (Table 1) clearly showed the prevalence of an antagonistic interaction. This trend is closely linked with the general increase of Ca between the 2nd and the 5th foliar spraying that was not followed by the kinetics of K accumulation (Fig. 2; Table 1). This inhibiting effect of higher Ca contents on K accumulation suggested a decreasing permeability of cells (Fageria, 1983). Indeed, Fageria (1983) studying the interactions between these nutrients in rice grown in nutrient solution also found an antagonistic effect, and Fageria and Baligar (1999) further found this effect on dry bean grown on an Inceptisol. Interesting was to note that the different trendlines displayed by the lower Ca treatments of both chemical forms (NCa0.3 and 0.4CCa) until the 4th foliar spraying (Table 1), revealing an increasing ratio K/Ca, which also accomplished with the findings of Ishizuka and Tanaka (1960) about synergistic interactions of both nutrients at low concentrations of Ca.

The trendline between the 2nd and the 5th foliar spraying for the ratio P/Ca, although showing similar patterns, revealed three distinct steps that corresponded to the

different phases of pulverization (Fig. 2; Table 1). In the first spraying phase (*i.e.*, after two Ca sprays, with Ca(NO₃)₂ or CaCl₂) a decrease was found, whereas in the second phase (3rd foliar spraying with 4 kg ha⁻¹ CaCl₂) the ratio P/Ca increased. Moreover, a new decrease occurred in the third phase (4th and 5th foliar spraying with 8 kg ha⁻¹ CaCl₂). Accordingly, our data showed an antagonistic interaction in the first and third foliar spraying phases, but a synergistic one during the second phase. In the first phase, that corresponded to the beginning of fruits development, the decreasing ratio P/Ca suggested a differential physiological translocation of Pi from roots, through the xylem, to the young fruits and leaves, as well as retranslocation from older leaves, as P, through the phloem (Mimura et al., 1996; Jeschke et al., 1997). Moreover, the higher foliar spraying of Ca in the second phase, might affect P homeostasis, changing its membrane transport and exchange among the intracellular (cytoplasm, vacuole, apoplast, and nucleus) pools (Schachtman et al., 1988) through precipitation. In the third phase, the higher accumulation of Ca, due to its low mobility through the phloem (Bonomelli et al., 2019; Coelho et al. 2021) determined the decrease of the relative proportion of P in the leaves (Table 1).

It has long been known that the uptake of sulfate into roots is mainly a metabolic process subject to negative feedback control by the intracellular sulfate concentration and by reduction products of sulfur metabolism (Rennenberg, 1984). Besides, the subsequent sulfate transport into isolated leaves is an active carrier-mediated process yet, whereas the rate-limiting steps of its accumulation is mainly achieved through influx into the vacuoles, its assimilation requires influx into the chloroplast that only is effectively controlled if the cytoplasmic sulfate concentration is carefully controlled (Rennenberg, 1984). In this context, the trendlines of S/Ca ratio revealed by NCa0.1, NCa0.6 and 0.4CCa, between the 2nd and 5th foliar spraying (Fig. 2; Table 1), showed a synergistic interaction with Ca until the second phase, followed by an antagonistic behavior, while in the remaining treatments prevailed a progressive antagonistic pattern. Accordingly, the synergistic interaction between Ca accumulation and S (Fig. 2; Table 1), suggested a metabolic controlled accumulation of S in the vacuoles. Moreover, the subsequent antagonism might be related with the removal by translocation of the sulfur taken up into the leaves, or the translocation of its metabolic products, or still by release into the environment (*i.e.*, into the soil or into the atmosphere after conversion into a volatile compound, namely through hydrogen sulfide emission). Concerning to the progressive antagonism interaction between Ca and S, between the 2nd and the 5th foliar spraying, for the remaining treatments, also the surplus of sulfate translocated in an acropetal direction from roots may have been retranslocated in the phloem in

a basipetal direction. Nevertheless, it must be pointed that, besides accumulation of sulfate, soluble organic sulfur also occurs (*i.e.*, enhancement of glutathione synthesis, coupled to its degradation and translocation through the phloem, and that function in cells as a reservoir of reduced sulphur) (Rennenberg, 1984).

The synergistic interaction revealed by the ratio Zn/Ca between the 2nd and 3rd foliar spraying, as well as the subsequent antagonistic behavior until the 5th foliar application (Fig. 2; Table 1), also suggested a close link between Ca for Zn redistribution from roots to the shoot, implicating xylem transport and retranslocation through the phloem (Wu *et al.*, 2016). Indeed, as Ca plays an important role in cell permeability and stabilization of plasma membrane in a physiological process implicating Zn (Prasad *et al.*, 2016), our data suggested a synergistic interaction when Ca levels remained low, thereafter followed by an antagonistic pattern with increasing foliar spraying (Prasad *et al.*, 2016; Wu *et al.*, 2016). In this context it must be pointed that the linkage of both kinetics must account for the variation of Zn accumulation during the different growth stages, leaf age and position on the stem, shoot biomass, leaf number and leaf area, which interferes with Zn distribution regulating its concentration in the leaves (Gupta *et al.*, 2016).

Trendlines displayed by the ratio Mg/Ca, between the 2nd and 5th foliar spraying, differed substantially among the three phases of foliar spraying (Fig. 2; Table 1), suggesting the absence of a direct interaction. Nevertheless, between the 2nd and the last foliar spraying, the highest values found in the most elevated treatments with CaCl₂ 0.8CCa and 1.6CCa) seems to point a long term synergistic effect, while the opposite (*i.e.*, antagonism outcome) prevails in the remaining treatments (Fig. 2; Table 1).

Although excess Ca is unlikely to cause toxicity in and of itself, changed the accumulation of other nutrients (mostly cations), and therefore potentially could affect the synthesis of photoassimilates, limiting fruits development. Indeed, the constant influx of Ca²⁺ from the roots to the shoots in the transpiration stream, linked to the three phases of foliar spraying, led to significant deviations on its contents and on some other nutrients as well (Fig. 1; Fig. 2; Table 1), which once in the cytosol of the guard cells could determine stomata closure (Dayod *et al.*, 2010). Thereafter this stomata limitation further limited leaf internal CO₂ concentrations and consequently the photosynthetic rates and productivity (Dayod *et al.*, 2010). Nevertheless following the increasing concentrations of Ca sprayed (between 4 and 8 kg ha⁻¹), the rise in Y_(NPQ) and decrease of Y_(NO) (Table 2) suggested that photosynthetic energy fluxes kept regulated. In this context, as after CaCl₂ (4 kg ha⁻¹)

spraying, only 0.8CCa significantly differed in parameters q_L and F_v'/F_m', whereas a similar tendency occurred after the 5th spraying (with only some parameters showing deviations in NCa0.3, 0.4CCa, 0.8CCa and 1.6CCa), no major effects in photosynthetic activity occurred. This trend clearly reenforced the development of Ca²⁺ compartmentation in different subcellular organelles, namely in the vacuoles (Conn and Gilliam, 2010; MacRobbie, 2006), limiting Ca augmentation in the cytosol and the consequent effect on stomata regulation (Wang *et al.*, 2019). Nevertheless, the decrease in F_v'/F_m' (Table 2) revealed a slight negative impact of PSII photochemical efficiency after application of CaCl₂ concentrations ranging between 4 and 8 kg ha⁻¹. Thus, although at harvest chlorophyll *a* parameters did not revealed major discrepancies relatively to the control, the tendency of F_v'/F_m' pointed that foliar spraying with higher concentrations of CaCl₂ could surpass the threshold of toxicity (Wójcik *et al.*, 2014).

During fruit development, the water mass flux coupled to the transpiration stream (Barthakur *et al.*, 2001; Welch and Shuman, 1995) is not constant, as at maturity xylem functionality can be minimal (which might be indirectly associated to the increased flux rates of solutes in the phloem (Marschner, 1974). Yet, as independently of the flux rate in the phloem, Ca flow to the fruits is constantly low in this tissue (Bonomelli *et al.*, 2019; Dayod *et al.*, 2010; Greenspan *et al.*, 1994), the majority of this mineral is uploaded from the xylem, and in particular to points downstream of transpirational flow, in the early phases of fruit development (Dayod *et al.*, 2010; Rogiers *et al.*, 2006). Still, independently of the foliar spraying with Ca, the photosynthetic functioning only revealed minor variations (Table 2), clearly implicating stomata opening in the maintenance of the water mass flux. Accordingly, the major accumulation of Ca in the peripheral layers of the fruits, suggests that its main source was Ca spraying between fruit set and 30 mm diameter, whereas this mineral translocation through the xylem favored the accumulation in the core (Fig. 3), particularly in the early stages of fruit development. Nevertheless, before maturity the withdrawing water through transpiration in the large surface of the leaves might became detrimental for Ca accumulation in the core of the fruit. Besides, considering that the cell walls bind, on average, about 70% of the total plant tissue content (White and Broadley, 2003), the implications on tissues homeostasis determined the heterogeneous Ca diffusion through all the flesh of the fruit (*i.e.*, higher compartmentation in the peripheral layers and core).

Within fruits of each treatment, C, H and O do not vary significantly among tissues (Table 3), as they are building blocks for plant growth, contributing to the synthesis of

essential biological compounds (namely, carbohydrates, proteins, starches, lignins and celulos). Besides, among treatments, total accumulation of these elements in all the regions of the fruits also did not varied significantly, as Ca did not substantially impaired the photosynthetic machinery (Table 2; Table 3).

Changes of K contents in the leaves and fruits (Fig. 2; Table 3), that also takes part in the conversion metabolism to sugars and further controls stomata functioning (thus keeping a check on the respiration rate, turgor pressure and ionic balance) also did not injured the photosynthetic machinery. Nevertheless, it was reported that in trees submitted to Ca pulverization, “Conference” pears and apple fruits accumulated higher and lower levels of K in the peripheral layers (Gaštol and Domagala-Świątkiewicz, 2009; Grimm-Wetzel and Schonherr, 2007) but, in general, the contents of K among tissues of “Rocha” pears from each treatment remained quite similar (Table 3). It seems therefore that different foliar spray types have an antagonism effect on the accumulation of K in “Rocha” pears, since significantly higher total accumulations prevailed in the highest treatment with $\text{Ca}(\text{NO}_3)_2$ (N $\text{Ca}0.6$) and in the lowest treatment with CaCl_2 (0.4CCa).

Although the contents of Mg, like Ca, is low in phloem-fed tissues such as fruits (Karley and White, 2009), the highest contents of Mg found in Ca treated “Rocha” pears (Table 3) clearly pointed that, as at the onset of cell expansion in fruits xylem dysfunction often begins, Mg was affected to a lesser extent (Drazeta et al., 2004). Nevertheless, this combined interaction seemed to be intraspecific for “Rocha” pears, since foliar spraying with increasing concentrations of Ca (namely with CaCl_2 and $\text{Ca}(\text{NO}_3)_2$) decreases Mg contents in fruits (Fageria, 2001; Madani et al., 2015).

During plants growth, Cl is translocated from the root to the shoot through the xylem and is redistributed via the phloem, yet tissues that are fed predominantly through the phloem tend to have the lowest Cl content (White and Broadley, 2001). Accordingly, as xylem dysfunction starts with fruit development (Drazeta et al., 2004), as found in “Rocha” pears (Table 3), Cl contents in the fruits is generally low (Levy and Shalhevet, 1990; Xu et al., 2000). Nevertheless, the increasing levels of total Cl in Ca treated “Rocha” pears (Table 3), which is involved in the transport of Mg and K (Prajapati, 2019), reinforces the trend displayed by these elements, but additionally improved the regulation of water balance parameters during fruits development (Franco-Navarro et al., 2016), contributing to the final characteristics at harvest (Fig. 4). In this context, the general highest contents of Cl found, among

treatments, in the heart of the fruit, suggested that this accumulation might result from the breakdown functioning of xylem in the “Rocha” pears during development.

The accumulation of Cu, Zn, Mn and Fe showed a synergistic interaction with Ca spraying (Table 3), which points an opposite trend relatively to other studies (Fageria and Baligar, 1999; Fageria, 2001). This means, whether these micronutrients are varying, it is necessary to examine not only the critical levels of the absolute concentrations of the elements contained in the soil, but also the interactions with nutrients foliar spraying. Indeed, with high calcium carbonate prevailing in the soils, the availability of micronutrients declines due to the raised soil pH, where OH^- reacts with Fe^{3+} , Zn^{2+} , Mn^{2+} and decreases its solubility forming ion complexes, and therefore limiting its uptake. Moreover, as translocation of Ca prevails in the xylem (whose dysfunction starts with fruit development (Drazeta et al., 2004)), subsisting a low mobility in the phloem (Barthakur et al., 2001; Bonomelli et al., 2019; Coelho et al. 2021; Davies and Millard, 1985; Nelson et al., 1990; Oparka and Davies, 1988; Welch and Shuman, 1995), our data (Fig. 3; Table 3) suggested that a gradient electrostatic pressure of the sieve cells of the phloem triggered the higher micronutrients accumulation upon Ca foliar spraying. This pressure gradient results from sucrose loading at the source end of the phloem and sucrose unloading at the sink end (*i.e.*, in mature leaves and fruits, respectively), triggering a localized drop in pressure and leading to a mass flow analogous to that of the xylem but without being drive by a negative gradient pressure.

Overall, pre-harvest foliar sprays of Ca did not affect significantly both morphological parameters. As according to ANP (2021) this variety’s caliber varies in average between 55–65 mm, while presenting oval or piriform shapes. Diameter values (Fig. 4) not only fit within this range, but also slightly surpass it. Spraying “Rocha” pears with Ca also did not triggered significant differences (Fig. 5) in the peel or pulp of the fruits, which could indicate physiological disorders that might happen in cases where Ca might vary (Wójcik et al., 2014).

CONCLUSION

In “Rocha” pears, Ca accumulation depends of the type of Ca fertilizer and concentration, prevailing an oscillating balance between Ca K, P, S, Mg and Zn deposition in the leaves and its slow removal from the mesophyll cells to the fruits until maturity. Besides, nutrients accumulation in the initial stages of fruits development is critical, since Ca translocation prevails in the xylem, being its redistribution

through the phloem strongly limited, while at maturity xylem functionality is minimal. Thus, interactions with micro and macronutrients became differentially affected, whereas the major accumulation of Ca in the peripheral layers of the fruits, results from Ca spraying, whereas translocation through the xylem favored its accumulation in the core in the early stages of fruit development. Nevertheless, the supply of essential nutrients in fruit trees did not become unbalanced, which allowed the maintenance of the functioning of the photosynthetic apparatus and the morphological and colorimetric characteristics of the fruits.

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Author contributions

All authors contributed equally to the research and discussion of the obtained data of the manuscript. Additionally, all authors further contributed to the writing of the different sections of the paper.

Abbreviations

F_o = Minimal fluorescence from the antennae;
 F_m = Maximum fluorescence from the antennae; F_v/F_m = Maximal photochemical efficiency of PSII; F_v'/F_m' = Actual PSII efficiency of energy conversion under light;
 Q_A = Quinone A in oxidized state; q_L = Photochemical quenching, based on the concept of interconnected PSII antennae; q_N = Nonphotochemical quenching; $Y_{(II)}$ ($= \phi_p$) = Estimate of the quantum yield of photosynthetic noncyclic electron transport; $Y_{(NO)}$ = Estimate of the quantum yield of nonregulated energy (heat and fluorescence) dissipation of PSII; $Y_{(NPQ)}$ = Estimate of the quantum yield of regulated energy dissipation of PSII; PSII = Photosystem II.

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