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Agronomic Strategies to Manipulate Kiwifruit Calcium Content to Understand Its Role in Fruit Physiology

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Abstract: Calcium (Ca) is one of the most important nutrients involved in fruit quality and storability; therefore, its application in fruit trees is often used in pre- and post-harvest. The aims of this study were to manipulate soil Ca, K, and N availability, photosynthetic active radiation, and fruit transpiration rate to understand their implication on fruit Ca accumulation on green-flesh kiwifruit grown in calcareous soil. Our results show that Ca partitioning into the fruit is not affected by the applications of Ca, K, and N, as well as the increase of photosynthetic active radiation. However, the presence of reflective films reduced fruit firmness and increased soluble solid content at harvest and during cold storage, thus enhancing fruit quality. Fruit calcium accumulation is decreased by the reduction of fruit transpiration rate; however, it has the possibility to recover, even close to fruit harvest, when the fruit transpiration is restored. The presence of bags reduced fruit weight from 84 to 63 g even though bags were removed. Our data provide evidence of the inefficiency of calcium fertilization in kiwifruit in calcareous soils and demonstrate the extension of calcium transportation into the fruit, which seems to occur during the entire growing season.

Keywords: calcium uptake; reflective film; water potential; fruit quality; *Actinidia chinensis* var. *deliciosa*; fruit growth; fruit calcium accumulation

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1. Introduction

According to the Food and Agriculture Organization, the worldwide annual production rate of kiwifruit in 2023 was nearly 2.36 million metric tons, with China being the main world producer, followed by New Zealand, Italy, and Greece [1]. Green-fleshed kiwifruit is still dominating the global market, even though yellow- and red-fleshed fruits are gaining more importance.

Calcium (Ca) is a macronutrient essential for plants, serving both physiological and structural functions. Calcium serves as a second messenger in a variety of processes ranging from root or pollen tube growth and fertilization to responses to abiotic as well as biotic stress [2–4]. Besides acting as an intracellular messenger in the cytosol and as a counter-cation for inorganic and organic anions in the vacuole, the ion Ca^{2+} is required for structural functions in the cell wall and organization of plasma membranes [5]. Optimal Ca^{2+} concentrations in harvested fruits improve their storability and reduce economic

losses due to its role in maintaining tissue mechanical strength [6]. In particular, high Ca^{2+} concentration is also associated with reductions in the incidence of pre- and post-harvest physiological disorders in fruits such as apple [7], table grape [8], and kiwifruit [9]. Calcium is a low-mobile nutrient that is mainly accumulated in leaves, where it accounts for approximately 85% [10] to 93% [11] of total Ca^{2+} uptake. As an indirect consequence, fruit is a weaker sink compared to leaf, because of its low transpiration rate during its growth [12]. According to Montanaro at al. [10], maximum Ca^{2+} accumulation in kiwifruit occurs at the beginning of stage II (approximately 55 days after full bloom) in addition it was reported that 61–75% of Ca^{2+} fruit content at harvest is accumulated 8 weeks after fruit set [13]. During the rest of the growing season, Ca^{2+} enters the fruit at a low rate, with the last 6 weeks of fruit growth resulting in a minimal gain in Ca^{2+} [10]. On the other hand, the amount of Ca^{2+} removed yearly by fruits of *Actinidia chinensis* is limited compared to leaves [14], meaning that Ca requirements of the fruit are smaller than those of the leaves.

Calcium movement in the plant is almost exclusively via xylem, mainly driven by transpiration [15]. It was demonstrated [10] that the highest fruit water loss occurs after fruit set, and thereafter it declines towards a minimum value at the end of stage II; these values are then maintained until the end of the season [10]. Higher transpiration rates promote higher Ca^{2+} accumulation in the fruit [10], even if, at low transpiration rates, the proportionality is not respected [16]. Other factors responsible for Ca^{2+} accumulation in fruits are photosynthetic active radiation (PAR), vapor pressure deficit (VPD), and wind speed. The increase of PAR enhances total fruit Ca^{2+} content up to 80% during the first 40 days after flowering [17]. VPD seems to be the main driving force of fruit transpiration [18], thus influencing Ca^{2+} accumulation in fruits. The transpiration rate of fruits is affected by the boundary layer conductance, which is influenced by wind speed [19]. However, the proportional relationship between Ca^{2+} delivery and transpiration is weak, also when its concentration in the xylem sap is low [15], supporting the idea that transpiration is not the only factor governing the movement of Ca. High Ca concentration in kiwifruit fruit is a pre-requisite for lower incidence of diseases and high fruit quality in pre- and post-harvest.

Balanced fertilization ensures application of the appropriate amount and proportions of macro- and micronutrients. Nitrogen (N) as nitrate or ammonium affects absorption of other nutrients, such as potassium (K) and Ca [20]; consequently, increasing N application could negatively affect plant nutrition. In an experiment on apples, a decrease in Ca and K content in fruits was observed as a consequence of increasing N in soil solution [21].

The aim of the present research was to clarify aspects of Ca accumulation into green-fleshed kiwifruit fruit through three different experiments. To define the best strategy to enhance Ca content in fruit, we aimed specifically at evaluating the effect of: (1) application of Ca, K, and N (experiment 1); (2) modification of photosynthetic active radiation (experiment 2); and (3) fruit transpiration rate on Ca partitioning into the fruit (Experiment 3).

2. Materials and Methods

2.1. Ca, K, and N Application—Experiment 1

2.1.1. Orchard Description and Treatments

The trial was conducted in 2020 in Italy in a kiwifruit orchard located in the Emilia-Romagna region (44°26′65″ N–11°93′21″ E) on a Hypocalcic Haplic Calcisols soil [22]. The main soil properties are described in Table 1. The climate in the area is temperate, with an average annual precipitation of 674 mm (in the period 2015–2019) and an average temperature of 14.9 °C; in 2020 the average temperature was 14.6 °C, precipitation 562 mm [23].

Table 1. Main soil properties of the two fields where the study was conducted.

Parameter	Experiment 1	Experiments 2 and 3
pH	7.56	8.16
Total lime (% $CaCO_3$)	3	13
Active carbonate (% $CaCO_3$)	12.7	11.6
Organic matter (%)	3.32	1.97
Total N (%)	2.04	1.30
Available P (mg kg^{-1})	98	29
Exchangeable K (mg kg^{-1})	322	324
Exchangeable Ca (mg kg^{-1})	5512	4141
Exchangeable Mg (mg kg^{-1})	183	408
Salinity (mS cm^{-1})	2.75	-
CEC [1] (meq 100 g^{-1})	30.6	25.0
Sand (%)	35	10
Loam (%)	49	49
Clay (%)	16	41

[1] CEC = cation exchange capacity. Analyses were performed according to official Italian methods for soil analysis by an external laboratory. In detail, pH was determined with a potentiometric method; total lime with the De Astis calcimeter; active carbonate with the method of Drouineau; organic matter with the Walkley–Black methods; total N with the Kjeldhal method; available P with the Olsen method; exchangeable K, Ca, and Mg and CEC with extraction in DTPA. Soil texture was determined according to USDA classification.

The experiment was conducted on self-rooted vines of the cultivar Hayward (*Actinidia chinensis* var. *deliciosa*) planted in 2017, with a distance of 5 m between rows and 2 m between two plants on the row, trained to a pergola system. Water was provided through drip irrigation and sprinkler emitters, according to daily evapotranspiration.

The study compared, in a split-split-plot experimental design with 4 replications (consisting of 5 plants each), two levels of N application (0 and 200 kg ha^{-1}) as a main plot, two levels of K (0 and 200 kg ha^{-1}) as a sub-plot, and two levels of Ca (0 and 200 kg ha^{-1}) as a sub-sub-plot, for a total of 32 plots. During the growing season, starting from 5 May, the fertilizers were manually distributed on the vine row every 10 days. Nitrogen (as ammonium nitrate; N = 34.5%), was applied from flowering (5 May) until 3 August (fruit at 70% of final weight); application of K (as potassium sulphate; K = 44%) started 10 days after N (15 May) and stopped on 13 August; Ca (as calcium chloride; 27.8%) was applied from the beginning of June (4 June) until the beginning of August (3 August).

During winter, older fruiting canes were removed to leave, for each plant, 12 younger shoots growing from the base of the previous year's canes, spaced every 20–30 cm along the permanent main branch.

2.1.2. Sampling and Analysis

From May until harvest, every 21 days, six leaves and six fruits from each plot were collected: fresh and dry weight were measured, and samples were analyzed to quantify the total N, K, and Ca concentration. Leaf and fruit N concentration were determined with the Kjeldahl method [24], while K and Ca were determined by spectrometry emission (ICP-OES, Ametek Spectro, Arcos, Kleve, Germany) after mineralization [25] in an Ethos TC microwave lab station (Mile-stone, Bergamo, Italy). Fruit N, K, and Ca content was calculated by multiplying fruit nutrient concentration by fruit dry mass. At harvest (21 October), vine yield was assessed; from each plot, 15 fruits were sampled and used for quality parameter determination. Fruit firmness was measured using a penetrometer with an 8 mm diameter tip (FTA53220 Güss, Strand, South Africa). Fruits were then cut into slices; part was squeezed to obtain juice for determination of soluble solid content (SSC) with the employment of a digital refractometer PR-1 (Atago Tokyo, Japan). One slice per fruit was placed in the oven for dry matter content; another slice was frozen at −20 °C for

mineral analysis. Two more samples of 15 fruits each were stored in a cold storage room at 0–2 °C (without moisture control) and analyzed after 107 (5 February) and 154 (24 March) days of storage; on each date, firmness, SSC, and dry matter were measured, as described above. Frozen fruits were lyophilized and analyzed, as described above.

2.1.3. Physiological Measurements

Water potentials ($\Psi\omega$) of stem ($\Psi\omega_S$), leaf ($\Psi\omega_L$), and fruit ($\Psi\omega_F$) were measured at solar noon, following methods and procedures from [26], using a Scholander pressure chamber (Soilmosture Equipment Corp., Santa Barbara, CA, USA). Stem $\Psi\omega$ was taken after isolating a leaf, distant from the fruit, close to the trunk, in ad-hoc envelopes (aluminum foil on the outside and black on the inside). Leaf $\Psi\omega$ was estimated from well-illuminated leaves, distant from fruit. Fruit $\Psi\omega$ was taken from representative fruit, not exposed to light. Leaf gas exchanges were measured with a Li-COR 6400 (Li-COR 6400, LI-COR Inc., Lincoln, NE, USA), integrated with a fluorimeter chamber that allowed the setting of the actual radiation in the orchard. Well-illuminated leaves were chosen on one-year shoots, distant from both fruit and apical leaves.

2.1.4. Statistical Analysis

Data were analyzed as a split-split plot experimental design, with N rate (2 levels: 0 and 200) as the main plot; K rate (2 levels: 0 and 200) as the sub-plot; and Ca rate (2 levels: 0 and 200) as the sub-sub-plot. Physiological parameters were also analyzed with the software R (version 4.3.3) according to a discriminant canonical analysis (DCA). DCA is a dimension-reduction technique related to principal component analysis and canonical correlation, and it finds linear combinations of the quantitative variables that provide maximal separation between classes or groups.

2.2. Solar Radiation—Experiment 2

2.2.1. Orchard Description and Treatments

The trial was conducted in 2021, in Italy, in a kiwifruit orchard located in the Emilia-Romagna region (44°19′36.1″ N–11°56′02.5″ E) on a Fluvic Cambisol soil [22]. The main soil properties are described in Table 1 and the climate in the area is the same as described for experiment 1, with an average temperature of 14.3 °C and average rainfall of 473 mm in 2021. The experiment was conducted on self-rooted vines of the cultivar Hayward (*Actinidia chinensis* var. *deliciosa*) planted with a distance of 4.7 m between rows and 2 m between two plants on the row (1064 plants ha^{-1}), trained to a pergola system. Water was provided through drip irrigation and sprinkler emitters and nutrients were supplied according to the Integrated Crop Management guideline of the Emilia-Romagna region [27].

To modify orchard microclimate and PAR, on 5 May (petal fall stage) reflective white films (Extenday®, Extenday New Zealand, Auckland, New Zealand) were positioned on the soil, between two tree rows (interrow), and kept onsite until harvest (Figure 1); this treatment (FILM) was compared to a control (NO FILM) without reflecting films. The trial was conducted in a single row, divided in half; in one part, on both the left and right sides, reflective sheets were positioned in the interrow; the rest of the row was the untreated control. Fifteen border plants were left between the two treatments to avoid the influence of the reflective sheet on the control plants. In each treatment, 4 plots (replicates) of three plants each were defined, for a total of 12 plants monitored per treatment. The experiment was conducted as a complete randomized design with 4 plots (replicates) of three plants each, for a total of 12 plants per treatment.

Figure 1. Reflective white films positioned on the soil of the kiwifruit orchard.

2.2.2. Sampling, Analysis, and Physiological Measurements

Starting from 16 June, 5 fruits from each plot were periodically sampled to measure DM. On 13 July, fully expanded leaves were sampled from each plot, washed, oven-dried and analyzed for all macro- and micronutrient concentration (as described above). At harvest (25 October), vine yield was assessed; in addition, 15 fruits were sampled from each plot and used for quality parameter determination (as described above). Thirty more fruits were set aside and stored at 0–2 °C for 3 and 5 months before quality analysis. On 16 June, 13 July, 25 August, and 5 October, 8 vines were selected for measurements of Ψ_{wS}, Ψ_{wL}, and Ψ_{wF} plus leaf gas exchanges.

2.2.3. Statistical Analysis

The data were analyzed as a complete, randomized design. Physiological parameters were also analyzed with the software R (version 4.3.3) according to a discriminant canonical analysis (DCA).

2.3. Fruit Transpiration—Experiment 3

2.3.1. Experimental Conditions and Measurements

This experiment was conducted in the same orchard as experiment 2. On 11 May, three plants were randomly chosen, and on each vine, 70 fruits were enclosed in a previously perforated plastic bag (BAG) (Figure 2); plastic bags were kept on fruits until 13 July. The period of closure was defined in relation to xylem functionality [6]. Three more plants were used as un-bagged controls (Control). From 23 June, 5 fruits inside the bag and five control ones were periodically sampled and measured for DM, as described before. At harvest, after 3 and 5 months of cold storage, the main quality parameters (see above) were determined. Fruit $\Psi\omega$ was monitored during the season, in both fruit enclosed in bags and controls.

Figure 2. Bagged fruits.

2.3.2. Statistical Analysis

Data were analyzed as a complete, randomized design. Physiological parameters were also analyzed with the software R (version 4.3.3) according to a discriminant canonical analysis (DCA).

3. Results

3.1. Ca, K, and N Application—Experiment 1

The supply of Ca, N, and K did not significantly influence nutrient concentrations in leaves and contents in fruits during the vegetative season.

No interaction between N, K, and Ca application was observed; consequently, in Tables 2 and 3, only the effects of principal factors were reported. Yield was higher in plots not fertilized with Ca and was not influenced by N and K supply, while fruit weight at harvest was not influenced by N, K, and Ca supply (Table 2).

Table 2. Effect of N, K, and Ca supply on fruit size and yield—experiment 1.

Treatment	Fruit Average Weight (g)	Total Yield (kg pt^{-1})
N0	75.1	21.0
N200	74.1	19.4
Significance	*ns*	*ns*
K0	74.9	20.4
K200	74.4	20.0
Significance	*ns*	*ns*
Ca0	76.1	21.7
Ca200	73.1	18.7
Significance	*ns* [1]	*
N × K	*ns*	*ns*
N × Ca	*ns*	*ns*
K × Ca	*ns*	*ns*
N × K × Ca	*ns*	*ns*

[1] *ns*, *: effect not significant or significant at $p \leq 0.05$, respectively.

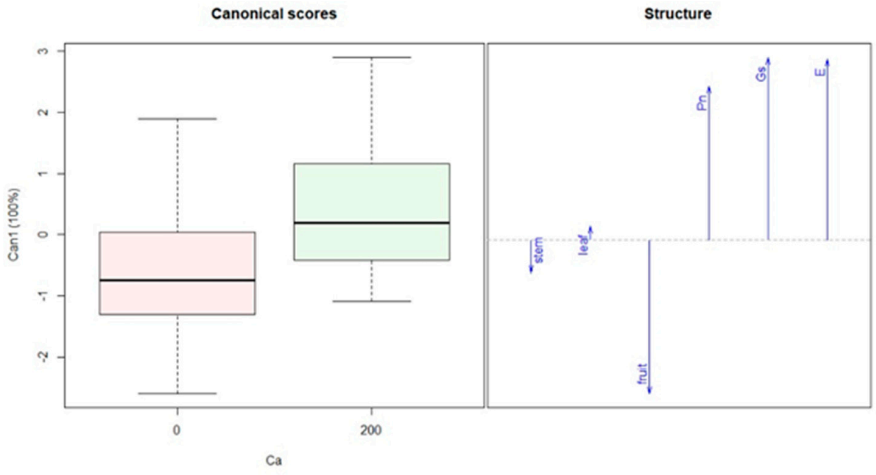
Table 3. Effect of application of N, K, and Ca on fruit dry matter soluble solid concentration (SSC) and firmness at harvest and after storage.

Treatment	Harvest			107 Days of Storage			154 Days of Storage		
	Dry Matter (%)	SSC (°Brix)	Firmness (kg)	Dry Matter (%)	SSC (°Brix)	Firmness (kg)	Dry Matter (%)	SSC (°Brix)	Firmness (kg)
N0	19.3	8.0	7.63	19.8	16.2	1.92	19.3	16.0	1.04
N200	19.2	8.1	7.53	19.7	16.9	1.67	19.2	16.2	0.99
Significance	*ns* [1]	*ns*	*ns*	*ns*	*ns*	*	*ns*	*ns*	*ns*
K0	19.3	8.1	7.54	19.6	16.2	1.73	19.0	16.1	1.04
K200	19.1	8.0	7.63	19.8	16.9	1.86	19.6	16.1	1.00
Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*	*	*ns*	*ns*
Ca0	19.1	7.9	7.72	19.6	16.6	1.86	19.1	16.0	1.05
Ca200	19.4	8.2	7.45	19.9	16.5	1.73	19.5	16.2	0.99
Significance	*ns*	**	*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
N × K	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
N × Ca	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
K × Ca	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
N × K × a	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

[1] *ns*, *, **: effect not significant or significant at $p \leq 0.05$ or $p \leq 0.01$, respectively.

Fruit firmness was lower in Ca200 compared to Ca0 at harvest, while no differences were observed after storage (Table 3). The applications of Ca induced an increase in SSC at harvest, while no significant differences were observed between the two treatments during cold storage (Table 3). Fruit dry matter was not influenced by treatments (Table 3).

The discriminant canonical analysis performed on the main physiological parameters ($\Psi\omega_S$, $\Psi\omega_L$,$\Psi\omega_F$, Pn, gs, and E) showed that 100% of the variability of this data set was represented by the first canonical variable; as a consequence, the distribution of variables was represented only for this axis (Figure 3). The box plot clearly separates Ca0 from Ca200; leaf $\Psi\omega$ was not influenced by Ca supply, while fruit and stem $\Psi\omega$ showed a positive effect of Ca application (Figure 3). Plant photosynthesis, as well as stomatal conductance and leaf transpiration, were enhanced by the supply of Ca (Figure 3) with significant differences in June and August (Table S1 in Supplementary Materials). The supply of N and K did not influence the physiological parameters.

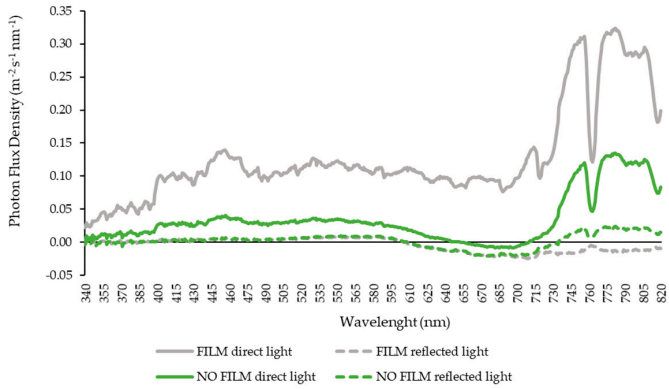


(**a**) (**b**)

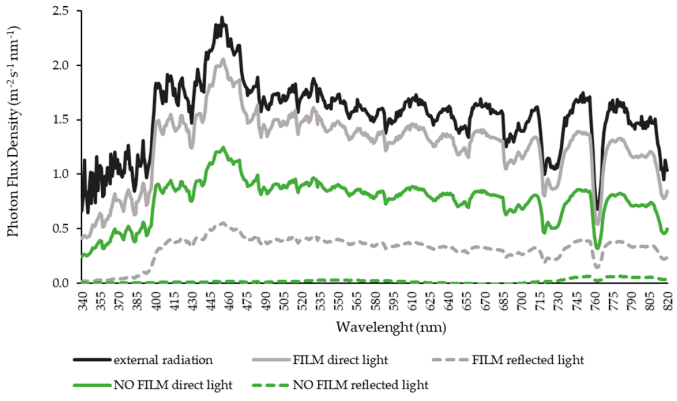
Figure 3. Discriminant canonical analysis (DCA) of the effect of Ca applications on physiological traits (**a**) and the corresponding weights (**b**) of stem Ψω (stem), leaf Ψω (leaf), and fruit Ψω (fruit), as well as on total photosynthesis (Pn), stomatal conductance (Gs), and transpiration rate (E) during the entire growth season—experiment 1.

3.2. Solar Radiation—Experiment 2

Reflective films induced an increase in photon flux density of direct light beneath the row (Figure 4a); in the interrow, both direct and reflected light was higher as a consequence of reflective film positioning, in comparison to the control (Figure 4b).



(**a**)



(**b**)

Figure 4. Effect of reflecting film on photon flux density beneath the row (**a**) and in the interrow (**b**)—experiment 2.

The use of reflective films induced an increase in fruit weight at the beginning of September, while non-significant differences were observed on other sampling days (Figure 5a). Fruit Ca content was not influenced by the treatment (Figure 5b).

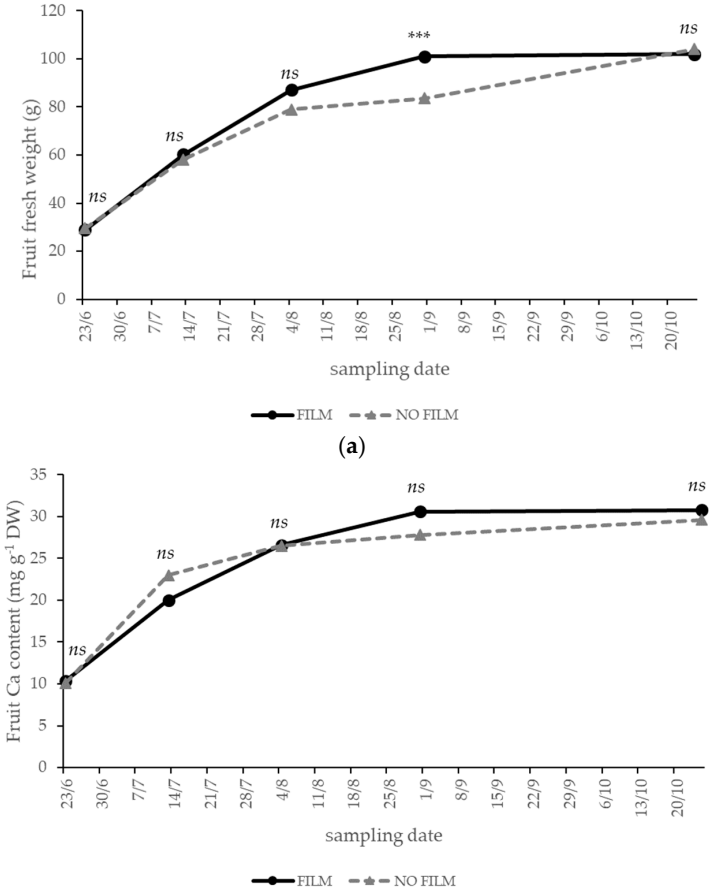


Figure 5. Effect of reflecting film on fruit fresh weight (**a**) and fruit Ca content (**b**)—experiment 2. *ns*, ***: effect not significant or significant at $p \leq 0.001$, respectively.

Fruit dry matter was enhanced by reflective film application in all sampling dates, with the exception of mid-July and harvest (Figure 6). Yield was not influenced by the treatments.

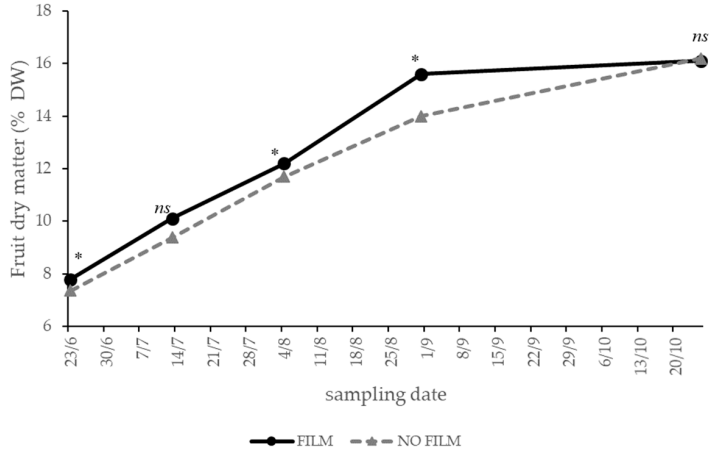


Figure 6. Effect of film application on fruit dry matter during the season—experiment 2. *ns*, *: effect not significant or significant at $p \leq 0.05$, respectively.

Film application increased fruit firmness at harvest and after 3 months of cold storage, while no differences were observed at 5 months (Table 4). Soluble solid content and DM (only after cold storage) were higher in plots with the reflective films than in the control ones (Table 4).

Table 4. Effect of reflecting film on fruit firmness, soluble solid concentration (SSC), and dry matter (DM) at harvest and after 3 and 5 months of cold storage—experiment 2.

Treatment	Firmness (kg)	SSC (%)	DM (%)
		HARVEST	
NO FILM	6.97	6.20	16.2
FILM	7.53	6.92	16.1
Significance	* [1]	***	*ns*
		3-MONTH COLD STORAGE	
NO FILM	1.76	11.8	13.5
FILM	2.36	12.8	15.7
Significance	***	***	***
		5-MONTH COLD STORAGE	
NO FILM	1.18	12.2	14.7
FILM	1.25	14.5	17.1
Significance	*ns*	***	***

[1] *ns*, *, ***: effect not significant or significant at $p \leq 0.05$, $p \leq 0.001$, respectively.

The discriminant canonical analysis performed on physiological parameters ($\Psi\omega_S$, $\Psi\omega_L$, $\Psi\omega_F$, Pn, gs, and E) showed that 100% of variability of this data set was represented by the first canonical variable; as a consequence, the distribution of variables was represented only for this axis (Figure 7). The box plot separated film from control; $\Psi\omega_L$ was influenced by the absence of the reflective film, while all other parameters, although with varying magnitude, were more influenced by the film positioning (Figure 7), even if no significant differences between treatments were observed (Tables S2 and S3).

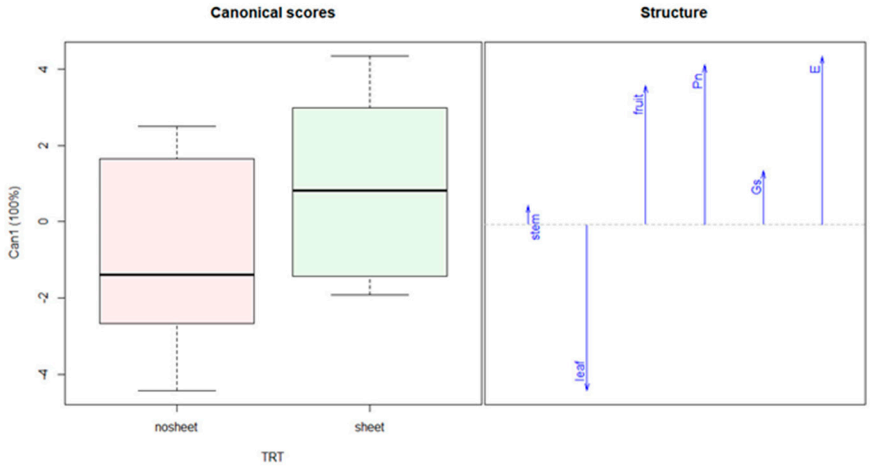


Figure 7. Discriminant canonical analysis (DCA) of the effect of soil white cover/reflective film on physiological traits on the **left** side of the figure and the corresponding weights (on the **right**) of stem $\Psi\omega$ (stem), leaf $\Psi\omega$ (leaf), and fruit $\Psi\omega$ (fruit), as well as on total photosynthesis (Pn), stomatal conductance (Gs), and transpiration (E) during the entire growth season.

3.3. Fruit Transpiration—Experiment 3

Fruit weight was higher in control fruits than in bagged ones during the entire season (Figure 8a). In addition, fruit Ca content was higher in control fruit in July and August, while no differences between treatments were observed in other sampling dates (Figure 8b). Fruit dry matter accumulation during the season was not influenced by the presence of plastic bags (Figure S1).

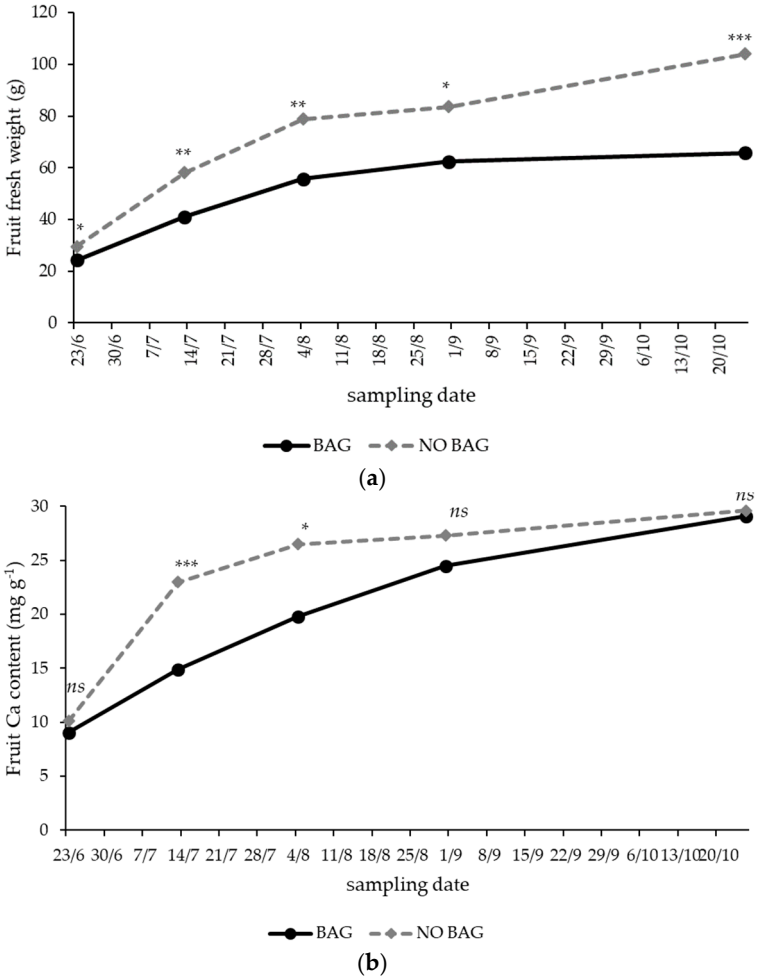


Figure 8. Effect of bag application on fruit fresh weight (**a**) and Ca content (**b**)—experiment 3. *ns*, *, **, ***: effect not significant or significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

At harvest, fruit firmness was higher in control fruits than in those closed into a plastic bag, while no effect was observed during cold storage (Table 5). Soluble solid concentration was higher in bagged fruits only after 5 months of cold storage, while no differences were observed at harvest and after 3 months (Table 5). Fruit DM was higher in bagged fruits than in control after 3 and 5 months of cold storage, while no differences were observed at harvest (Table 5). No effect of the bag was observed on Ψ_{wS}, Ψ_{wL}, and Ψ_{wF} (Table S4).

Table 5. Effect of film application on fruit firmness, soluble solid concentration (SSC), and dry matter (DM) at harvest and after 3 and 5 months of cold storage—experiment 3.

Treatment	Firmness (kg)	SSC (%)	DM (%)
		HARVEST	
NO BAG	6.97	6.20	16.2
BAG	5.82	6.03	16.2
Significance	*** [1]	*ns*	*ns*
		3-MONTH COLD STORAGE	
NO BAG	1.76	11.8	13.5
BAG	1.74	12.2	14.7
Significance	*ns*	*ns*	**
		5-MONTH COLD STORAGE	
NO BAG	1.18	12.1	14.8
BAG	1.15	13.2	18.8
Significance	ns	*	*

[1] *ns*, *, **, ***: effect not significant or significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

4. Discussion

Calcium application to kiwifruit has received considerable attention since maintaining relatively high Ca concentrations in fruit tissues is believed to promote nutrient balance, stabilize and maintain cell wall stabilization and integrity, and improve fruit quality and storability [28,29]. However, as for all the other nutrients, Ca application should also be planned according to environmental availability. Calcium excesses are detrimental to plant physiology and negative from an economic point of view. In our experimental conditions, Ca applications were probably not necessary, since soil and irrigation water are rich in Ca as a consequence of the high level of limestone in the soil. In the present experiment, soil analyses showed considerable native soil fertility that has reduced or masked the effects of Ca, K, and N fertilization on fruit weight, quality, and storability. The lack of effectiveness of soil-applied Ca solution in increasing fruit Ca concentration was also found in other investigations [30,31], and it could be due to the soil type. As a result, leaf Ca concentration was also not modified by Ca fertilization.

The application of additional Ca, in some circumstances, showed a negative effect on yield and firmness, bringing evidence to the hypothesis that the high Ca availability can become toxic for the cultivar Hayward. In broad bean, extracellular Ca^{2+} at high concentrations (i.e., >200 mM) was found to block water channels and reduce water flow into the guard cells with stomatal closure [32]. On the other side, in experiment 1, Ca was found to improve the C assimilation rate, and, at harvest, a positive effect was evidenced on soluble solid concentration. The latter result, together with the reduction in fruit firmness, could lead to the hypothesis of a possible early ripening effect, the opposite of what was expected. In fact, auxin and abscisic acid pathways involve Ca as a protein-binding secondary messenger and in membrane transport mechanisms, thus modifying turgor and solute accumulation [33]. An increase in Ca availability, by acting on hormonal pathways, has the potential of altering expression levels of enzymes that modify cell turgor pressure, apoplast solute accumulation, and cell wall modification, thus influencing fruit softening.

The application of Ca at a rate of 200 kg ha^{-1} decreased the stem $\Psi\omega$; similarly, in grape, pedicel hydraulic conductivity of xylem vessels decreased throughout ripening, potentially due to occlusion formed by pectin deposition [34]. Likewise, the formation of physical barriers linked to increased Ca^{2+} availability could be the cause of lower values of fruit water potential; in confirmation of this, a study on kiwifruit showed a general increase in xylem hydraulic resistance during the second half of fruit development [35].

The light-dependent import of Ca^{2+} into the chloroplast and dark-stimulated stromal Ca^{2+} flux, shows that Ca^{2+} is closely linked to photosynthesis. Indeed, Ca mainly acts as a structural component of photosystem II, having a regulatory function in the Calvin–Benson cycle, thus playing a pivotal role in cyclic and linear electron flow and minimizing photo-oxidative damage [36]. Consequently, the increased availability of Ca could have positively affected physiological patterns of plants.

The properties of reflective films are able to modify the orchard microclimate by reflecting solar radiation into the tree canopy [37,38]. Several experiments evidenced an increase in photosynthetically active radiation (PAR) absorption of the apple tree canopy, varying from 40% [39] to 68% [40]. Kiwifruit berries growing in light-exposed positions in the canopy were of better quality and could be stored for longer periods [41]. Similarly, it was evidenced [42] that a high level of light availability determines an increase in xylem stream, leading to higher Ca accumulation in different organs and improvement of fruit storage quality. This seems linked to the positive effect of light on vascular development of the fruit and its pedicel [43]; in our experiment, indeed, the use of reflective film increased light in the row and interrow by around 23% and 60%, respectively. The enhancement of light availability was able to increase canopy photosynthesis and assimilate supply to the

fruit, which led to higher dry matter in fruits during the season. Films also led to increased transpiration and, thus, a more negative leaf water potential; this could affect the irrigation strategy, which should probably be modified with a higher supply of water when reflective films are used. No effect of reflective film coverage was observed on fruit Ca accumulation; however, fruits from covered plots evidenced higher fruit quality, both at harvest and after cold storage.

The use of plastic bags to reduce the fruit transpiration rate had the objective of clarifying Ca accumulation in fruits. The reduction of fruit transpiration during the first phase of fruit growth was expected to impair Ca fruit partitioning. In fact, it's widely demonstrated that early fruit development is a pivotal period for Ca accumulation in kiwifruit. Predominantly, this happens since Ca is xylem-mobile but phloem-immobile [44]. In our experiment, as expected, we observed a reduction of fruit size in fruit wrapped into bags with a constant difference during the entire vegetative season, although bags were removed after 63 days. On the other hand, the trend of Ca partitioning into the fruit showed an increase after bag removal, indicating the capability of fruit to also accumulate Ca in the last part of the season. There may be 2 explanations: (1) Ca does not only move through the xylem, or (2) Ca moves with the xylem flow during the whole season according to fruit request. These hypotheses open a new perspective on the Ca nutrition of kiwifruit. In addition to fruit transpiration, its requirement for Ca and the physicochemical features of its conducting tissues (e.g., ion adsorption and desorption occurring at exchange sites across the walls of the xylem pathway) could influence the movement of Ca in plant organs [45]. In apple, for example, Ca fruit accumulation seems variety-dependent [46] and lasts for a longer period than previously suggested, reaching harvest time.

5. Conclusions

From our data, we can conclude that in limestone soils, the application of soil Ca is not useful for improving fruit quality, whereas a possible alternative could be the use of reflective films, which showed encouraging results on fruit quality. Finally, our results open questions on the timing of Ca accumulation in the fruit, which seems longer than that reported in the literature, with effective calcium partitioning into the fruit up to 60 days before harvest, laying the basis for possible modification of kiwifruit orchard management strategies, with the goal of increasing the calcium supply in the fruit.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11030237/s1, Table S1: Effect of sheet application on total photosynthesis (Pn), stomatal conductance (Gs), and transpiration (E) during the season—experiment 1; Table S2: Effect of sheet application on stem, fruit, and leaf water potential (bar) during the season—experiment 2; Table S3: Effect of film application on total photosynthesis (Pn), stomatal conductance (Gs), and transpiration (E) during the season—experiment 2; Figure S1: Effect of plastic bag on fruit dry matter accumulation during the season—experiment 3; Table S4: Effect of sheet application on stem, fruit, and leaf water potential (bar) during the season—experiment 3.

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Abbreviations

The following abbreviations are used in this manuscript:

Ca	Calcium
N	Nitrogen
K	Potassium
PAR	Photosynthetic active radiation
VPD	Vapor pressure deficit
SSC	Soluble solid content
DM	Dry matter
DCA	Discriminant canonical analysis
Ψω	Water potentials
ΨωS	Stem water potential
ΨwL	Leaf water potential
ΨwF	Fruit water potential
Pn	Total photosynthesis
Gs	Stomatal conductance
E	Transpiration

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