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Research Article

Effect of organic calcium uptake and biostimulants during integrated nutrient management (INM) cultivation of kiwifruit cv. 'Hayward'

Thomas SOTIROPOULOS[1*], Ioannis MANTHOS[2], Theocharis CHATZISTATHIS[3], Nektarios KOUNTIS[4], Olga DICHALA[5], Georgios TSOKTOURIDIS[6,7]

[1]*Hellenic Agricultural Organization 'Demeter', Institute of Plant Breeeding and Genetic Resources, Department of Deciduous Fruit Trees, GR-59035 Naoussa, Greece; thsotiropoulos@elgo.gr (*corresponding author)*
[2]*Hellenic Agricultural Organization 'Demeter', Institute of Plant Breeding and Genetic Resources, Department of Nut Trees, GR-35100 Neo Krikello-Lamia, Greece; jmanthos@elgo.gr*
[3]*Hellenic Agricultural Organization 'Demeter', Institute of Soil and Water Resources, GR-57001 Thessaloniki (Thermi), Greece; t.chatzistathis@elgo.gr*
[4]*VITRO HELLAS S.A., GR 59300, Alexandria, Greece; kountisnektarios@outlook.com*
[5]*International Hellenic University, School of Agriculture, GR-57400 Thessaloniki (Sindos), Greece; olga.dichala@gmail.com*
[6]*Hellenic Agricultural Organization Demeter, Institute of Plant Breeding and Genetic Resources, Thessaloniki, GR-57001, Greece; gtsok@elgo.gr*
[7]*'THEOFRASTOS', Industrial Zone Korinthos, Irinis and Filias, GR-20100, Examilia Korinthias, Greece*

Abstract

Foliar application with calcium (Ca) fertilizers improves quality and flesh firmness of kiwifruit and affects the mineral composition of leaves and fruits. The impact of preharvest foliar sprays, using a commercial fertilizer with organic Ca content in combination with an integrated nutrient management (INM) fertilizers and biostimulants was assessed for the quality properties and nutritional status of the kiwi cv. 'Hayward' over a 2-year period. Fruit flesh firmness increased under all organic Ca applications. In addition, all treatments resulted in elevated dry mass content, which is a common quality index. In leaves, the concentrations of N, P, K and B were higher compared to control. In fruits, the concentrations of N, B, K and Ca increased, in contrast to Mg, which was reduced. The content of Mn, Zn, Fe and Cu was not affected either in leaves or fruits. Application of organic Ca-fertilizers led to increased flesh firmness, total soluble solids, total acidity, and dry mass percentage. Foliar application with the addition of the biostimulant THEOFAST resulted in the elongation of stigmas area in kiwi cv. 'Hayward' fruits.

Keywords: calcium application; dry mass; fertilization; flesh firmness; fruit quality; fruit mineral composition; nutrition

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Introduction

Kiwifruit is a deciduous vine, originated from southern China (Ferguson, 2011). It is recognized across the world for its distinct flavour, which includes sweet and slightly acidic flesh, high nutritional value minerals and beneficial metabolites, particularly the high ascorbic acid vitamin-C content (Jesion *et al*., 2013). Moreover, during the last years, there was a dynamic expansion and development of the cultivation, not only in the European countries, but also in China and New Zealand (Williams *et al*., 2003; Latocha *et al*., 2021). In 2015 the kiwifruit yield and harvested area in China accounted for 52.7% and 69% of the global kiwifruit yield and area (FAO, 2018). 'Hayward', is the most widely spread variety which is mainly cultivated in Greek orchards. It is rich in vitamin C (Ferguson, 2011), carotenoids, phenolics, flavonoids, chlorophyll (Jesion *et al*., 2013), and minerals (Na, K, Ca, Mg, Mn, Fe, Cu, Zn) (Latocha *et al*., 2021). Many different parameters affect the quality and storage life of kiwifruit during cultivation; the most important factor affecting fruit quality is the appropriate nutrition, which also contributes to a large extent to the fruit's storage life (Williams *et al*., 2003).

The foliar analysis is a precious tool which could be used to improve the nutrient inputs of the orchard and consequently improve the yield, conservation, and quality attributes of kiwi fruits. Fruit firmness is a valuable fruit characteristic that determines post-harvest life. Previous research showed that the concentration of mineral elements played a vital role in the firmness of kiwifruits at the time of harvest or at the end of storage (Huang *et al*., 2020).

A significant preharvest factor, aiming for the highest yield and fruit quality, is fertilization targeted to fruit postharvest characteristics (Cassano *et al*., 2006; Jesion *et al*., 2013). Proper fertilization is essential to establish fruit quality and thus increase market value. Although a balanced fertilization provides sturdy plants and high yield of high-quality fruits, the excessive or imbalanced fertilization have detrimental effects (Pacheco *et al*., 2008). The optimum combination of macro and micronutrients in a soluble form of fertilizers is the key to produce high quality kiwifruits. However, the combined use of conventional, organic fertilizers and biostimulants (the so-called integrated nutrient management; INM) represents a strategy that could sustain high yields and limit undesirable impact (Selim, 2020; Gezahegn, 2021). Research in agriculture has developed distinct groups of materials with innovative properties during the last twenty years in order to improve farming and increase crop production. It is known that diverse fertilization strategies may differentially affect not only plant growth and yield, but also product quality (Kakar *et al*., 2020). The INM fertilization treatment consisted of commercial fertilizers, which were included polysaccharides, biostimulants, organic calcium and urea as nitrogen source. Polysaccharide-based resources are enhancing fertilizer efficiency, they are non-toxic, water-soluble, increase the availability of nutrients, play an important role in plant growth and development and are suitable for agricultural applications without toxicity problems (Tripathi *et al*., 2014; Chiaregato *et al.*, 2022). Plant biostimulant is any material applied to plants, aiming to increase nutrients absorbance, abiotic stress tolerance and/or crop quality characteristics, regardless of its nutrients content (Jardin, 2015).

Calcium is an essential plant nutrient required for structural roles in the cell wall and membranes, as a counter cation for inorganic and organic anions in the vacuole, and as an intracellular messenger in the cytosol. Therefore, it is a nutrient that improves the quality of kiwifruit, and the application of Ca fertilizers may increase its use by the plant (Feng *et al*., 2006). The significant positive correlation between Ca concentration in the fruit and its flesh firmness, demonstrates the necessity to use foliar applications of calcium fertilizer during the fruit growth. The foliar applications are effectively increasing fruit quality at the post-harvest stage (Feng *et al*., 2006; Antunes *et al*., 2007; Vajari *et al*., 2018). Calcium is responsible for the strength of cell wall, the cell division, protecting the penetrability of the membrane which results in flesh firmness (Moras and Nicolas, 1987; Mass *et al*., 1994; White and Broadley, 2003; DeFreitas and Mitcham, 2012). The maturity of kiwifruits is usually indicated by the flesh firmness and is considered the major postharvest physiological property (DeFreitas and Mitcham, 2012; Madani *et al*., 2015). Recently, 51 members of PG gene family in kiwifruit were identified; in addition, the activities of PG and PE were generally correlated with the pectin content and firmness changes (Huang et al., 2020).

The aim of this research was to analyse the impact of an INM fertilization program containing an organic calcium fertilizer, on fruit quality attributes and leaf and fruit nutritional status of the 'Hayward' kiwifruit cultivar. This cultivar was chosen for experimentation, since it is the most common and economically important one existing in kiwi orchards, producing high fruit quality.

Materials and Methods

Site, plant material and fertilizer treatments

The research was conducted for three consecutive years (2018, 2019 and 2020) in a commercial kiwifruit (*Actinidia deliciosa* (A. chev.) C.F. Liang et A.R. Ferguson var. deliciosa) orchard located in Naoussa (northern Greece, long. 22^0 04' 00'' E; lat. 40^0 29' 04'' N; elevation 350 m). The vines of the cultivar 'Hayward' were 4 years old, planted at a spacing of 3.5 × 2.5 m apart and trained in a T-bar trellis planting system (Pomares *et al*., 2015). The trees received standard horticultural practices regarding pruning and irrigation. Research was conducted at industrial level with 13 pretreatments (in 2018) using several different commercial fertilizers and biostimulants in an INM fertilization programme leading to the selection of best combination treatments for the main experimentation in the next two consecutive years in 2019 and 2020. The cost of fertilizers in relation to the results obtained in the end of the cultivation period were significant for the INM fertilization scheme and the experimental design. These treatments and data are demonstrated in the Supplementary material section of the article.

Soil samples from the experimental orchard were collected from a depth of 0–60 cm and analysed (Page et al., 1982). The soil was characterized as clay loam, slightly alkaline (pH 7.7), with low electrical conductivity (1.20 mS cm^{-1}), sufficient organic matter content (2.7%) and calcium carbonate content at 8.3%. The concentrations of nutrients in the soil are shown in Table 1.

Table 1. Soil nutrient concentrations (mg kg^{-1}) of the experimental orchard

P	K	Ca	Mg	B	Mn	Zn	Fe
25	311	1612	647	0.59	21	2.12	21.34

During the second year of the experimentation (2019), the initial fertilization was on May 20, applied with root irrigation in all vines (10 L of solution per vine), with the following products: (1) THEORUN (% w/w N 17, % w/w P 0, % w/w K 1.5, % w/w organic matter 3.2, polysaccharides, C/N 0.09); (2) THEOBORO (% w/w B 8, % w/w Na 0.2, % w/w organic matter 2.9, C/N 11.2); (3) THEOZINC (% w/w Zn 10.5, % w/w organic matter 0.16, C/N 0.31); (4) THEOMASS (% w/w organic matter 5.4); ('Theofrastos' Fertilizers, Korinthos, Greece) and (5) Total soluble fertilizer 12-7-36 + 2 MgO + TE (4 kg t^{-1}) (Haifa-group fertilizers). Fertilizer doses for the initial stage of experiment are depicted in Table 2. Subsequently, five treatments [Control – C (No fertilization), A1, A2, A3, A4] which contained THEOCAL (% w/w Ca 30; % w/w organic matter 35%) in different concentrations, were initially applied at the stage of petals' drop and then were replicated at June 14, June 28, July 25 and August 20 and September 12 (about 1 month before harvest) using both foliar applications and root application (Table 3). Control plants were selected in the experimental orchard. The amount of fertilizer solution that was applied via root watering was 10L per vine. Fruits from all treatments were harvested at the same time, at commercial maturity, and were transported directly to the laboratory for analyses.

At the third year of experiment (2020), the first application of fertilization was the same for all vines and contained the same fertilizers, as well as THEOFAST (% w/w organic matter 4.4), ('Theofrastos'

Fertilizers, Korinthos, Greece). THEOFAST was applied via foliar spray in doses that are depicted in Table 4. Thereinafter, the followed treatments were based on different concentrations of THEOCAL (C, B1, B2, B3, B4 and B5) and used the same pattern of applications as the experimental year of 2019, as illustrated in Table 5. All fertilizers among treatments were applied both with foliar spraying and root irrigation except of B4 and B5 treatments that were applied only via root watering. All fertilizers mentioned above are used commercially. Regarding THEOBORO, the maximum boron limits were considered in its formulation and the dose used is safe for application of kiwifruits.

Table 2. Fertilizers doses for the initial stage of trees in 2019

Fertilizer	Dose (L t^{-1})
Theorun	3
Theoboro	1
Theozinc	1
Theomass	3

Table 3. Treatment application during the second year of experimentation (2019)

Treatment	Fertilizer	Dose (per 1000 L) for foliar application	Dose (per 1000 L) for root watering
A1	Theorun	8.0 L	3.0 L
	Theoboro	1.0 L	–
	Theocal	2.0 Kg	1.5 Kg
	Theomass		3.0 L
	Total soluble 12-7-36 + 2 MgO + TE	5.0 Kg	4.0 Kg
A2	Theorun	8.0 L	3.0 L
	Theoboro	1.0 L	
	Theocal	2.0 Kg	3.0 kg
	Theomass	–	3.0 L
	Total soluble 12-7-36 + 2 MgO + TE	5.0 Kg	4.0 Kg
A3	Theorun	8.0 L	3.0 L
	Theoboro	1.0 L	–
	Theocal	4.0 Kg	1.5 Kg
	Theomass	–	3.0 L
	Total soluble 12-7-36 + 2 MgO + TE	5.0 Kg	4.0 Kg
A4	Theorun	8.0 L	3.0 L
	Theoboro	1.0 L	–
	Theocal	4.0 Kg	3.0 Kg
	Theomass	–	3.0 L
	Total soluble 12-7-36 + 2 MgO + TE	5.0 Kg	4.0 Kg

Table 4. Fertilizer's doses during the third year of application, before blooming (2020)

Fertilizers	Dose (per 1000 L) for foliar application	Dose (per 1000 L) for root watering
Theorun	7.0 L	0.4 L
Theoboro	1.0 L	–
Theofast	5.0 L	–
Theomass	–	3.0 L
Theozinc	1.0 Kg	–
Theocal	–	1.5 Kg
Total soluble 12-7-36 + 2 MgO + TE	5.0 Kg	4.0 Kg

Table 5. Treatment application during the third year of experimentation (2020)

Treatment	Fertilizer	Dose (per 100 L) for foliar application	Dose (per 1000 L) for root watering
B1	Theorun	8.0 L	3.0 L
	Theoboro	1.0 L	–
	Theocal	2.0 Kg	3.0 Kg
	Theofast	5.0 L	–
	Theomass	–	3.0 L
	Total soluble 12-7-36 + 2 MgO + TE	5.0 Kg	4.0 Kg
B2	Theorun	8.0 L	3.0 L
	Theoboro	1.0 L	–
	Theocal	4.0 Kg	3.0 Kg
	Theofast	5.0 L	–
	Theomass	–	3.0 L
	Total soluble 12-7-36 + 2 MgO + TE	5.0 Kg	4.0Kg
B3	Theorun	8.0 L	3.0 L
	Theoboro	1.0 L	–
	Theocal	6.0 Kg	3.0 Kg
	Theofast	5.0 L	–
	Theomass	–	3.0 L
	Total soluble 12-7-36 + 2 MgO + TE	5.0 Kg	4.0 Kg
B4	Theorun	–	3.0 L
	Theoboro	–	–
	Theocal	–	3.0 Kg
	Theomass	–	3.0 L
	Total soluble 12-7-36 + 2 MgO + TE	–	4.0 Kg
B5	Theorun	–	3.0 L
	Theoboro	–	–
	Theocal	–	6.0 Kg
	Theomass	–	3.0 L
	Total soluble 12-7-36 + 2 MgO + TE	–	4.0 Kg

Fruit quality measurements

During harvest, 150 fruit samples were collected randomly from the five trees of each replication, therefore 30 fruit per tree. Fruits were sampled at commercial maturity. Yield per tree was also measured for all the experimental trees. In 50 fruits, the mean weight (g) as well as the flesh firmness (kg cm^{-2}), were measured with an Effegi penetrometer with an 8-mm tip (Effegi Model FT 327, Alfonsine, Italy). Dry matter was measured according to the method described by Schotsmans *et al.* (2007). The fresh kiwifruits were peeled and were crushed through a juice extractor. Soluble solids (%) were measured with an HI96800 Digital Refractometer for Refractive Index and Brix (HANNA Insruments Inc., Woonsocket, RI, USA) and acidity (% citric acid) was measured after titration with 0.1N NaOH (Ough and Amerine, 1988). The remaining one hundred fruits of each treatment placed into a cooling chamber (+0.5 °C) for four months and the same quality attributes determined after two and four months. Leaf samples were collected manually at midsummer August 7 for both years. Each leaf sample consisted of the third leaf past the final fruit on a fruiting lateral. All leaf samples were initially washed once with tap water and then twice with distilled water.

Mineral analysis

Leaf samples were dried in a forced draft oven at 68 °C for 72 h and ground in a mill to pass a 30-mesh screen. A portion of 0.5 g of the fine powder of each sample was dry-ashed in a muffle furnace at 515 °C, for 5 h. Then, the ash was dissolved with 3 mL of 6 N HCl, diluted with double distilled water up to 50 mL, and the concentrations of P, K, Ca, Mg, Fe, Mn, Zn, and Cu were determined by ICP (Perkin Elmer- Optical Emission Spectrometer, OPTIMA 2100 DV) (Hansen *et al*., 2013). Fruit samples for mineral analyses were collected at harvest period. Nitrogen was determined by the method of Kjeldahl (Chapman and Pratt, 1961) and B by the azomethine-H method (Wolf, 2008).

Statistics

The adopted experimental design was a randomized block with five replications of 4 treatments in 2019 and five treatments in 2020 (five vines per replication were used). Differences between means were evaluated by using Duncan's test ($P \leq 0.05$). Statistical analysis was performed using SPSS statistical package (SPSS Statistics for Windows, Version 17.0. SPSS Inc.; 2008, Chicago, IL, USA).

Results and Discussion

Quality characteristics of kiwifruits showed that for all treatments the fruit weight and eventually yield increased compared to control, in both years of experiments (Figures 1 and 2). In 2019, the highest mean fruit weight and yield per tree were measured under A4, followed by A3, A2 and A1 treatments (Figure 1), while in 2020 for all treatments the fruit weight and eventually yield increased compared to the control (Figure 2; Figure 3). This is in accordance with the results of Zhang *et al*. (2020), who found that the application of inorganic fertilizers significantly increased fruit yields, leaf number, leaf area and stem diameter; moreover, our data are partially in accordance to the results of Liu *et al*. (2020), who found that long-term organic fertilization in kiwifruit orchards improved their productivity. Elongation of kiwi fruits at stigmas after an INM foliar application with THEOFAST compared to control was observed (Figure 4).

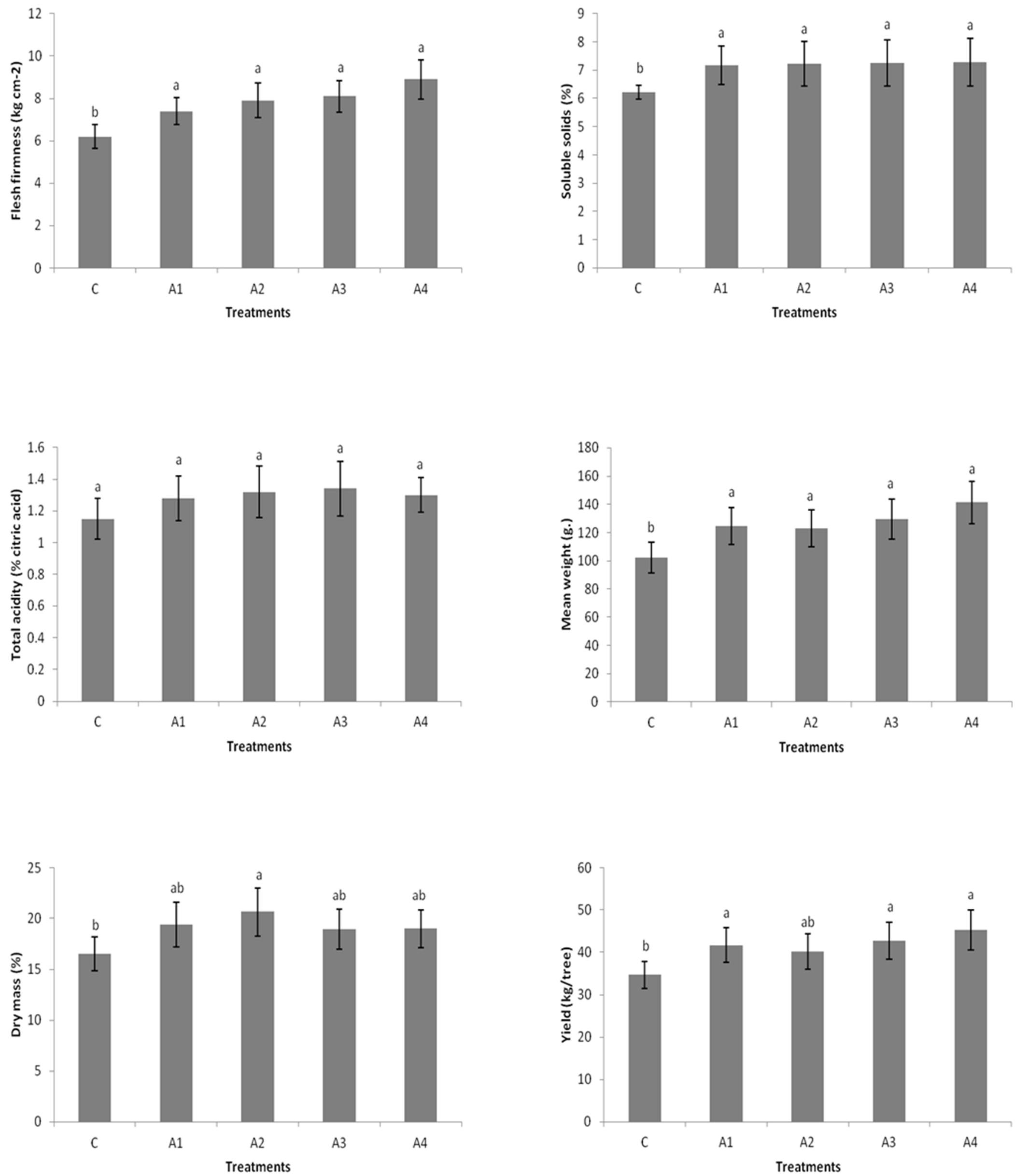

Figure 1. Effect of different organic Ca concentrations (THEOCAL) during an integrated nutrient management (INM) programme with the commercial biostimulant THEOMASS, via foliar and root application on kiwi cv. 'Hayward' cultivation. Presentation of quality characteristics of fruits in relation to fruit firmness, soluble solids, total acidity, mean weight, dry mass and yield, comparing diferent organic Ca treatments with the control for the cultivation period in 2019

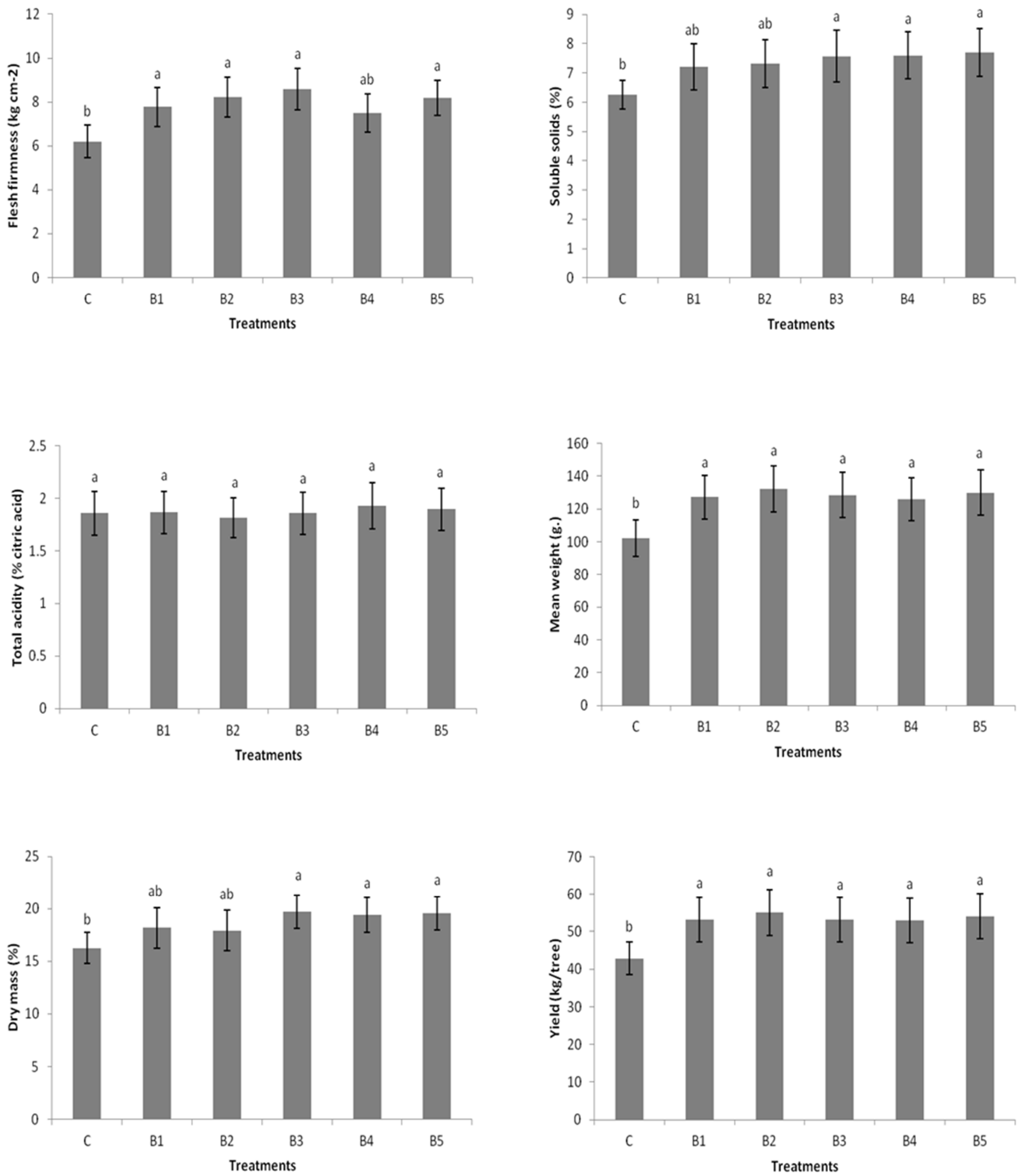

Figure 2. Effect of different organic Ca concentrations (THEOCAL) during an integrated nutrient management (INM) programme with the additional commercial biostimulants THEOMASS and THEOFAST, via foliar and root application on kiwi cv. 'Hayward' cultivation. Presentation of quality characteristics of fruits in relation to fruit firmness, soluble solids, total acidity, mean weight, dry mass and yield, comparing diferent organic Ca treatments with the control for the cultivation period in 2020

Figure 3. Effect of different organic Ca concentrations (THEOCAL) during an integrated nutrient management (INM) programme with biostimulants via foliar and root application on kiwi cv. 'Hayward' cultivation. Presentation of the best yield treatment and fruit characteristics compared to control, (A4) cultivation period in 2019 without any biostimulator and (B2) cultivation period in 2020 with the biostimulant THEOFAST

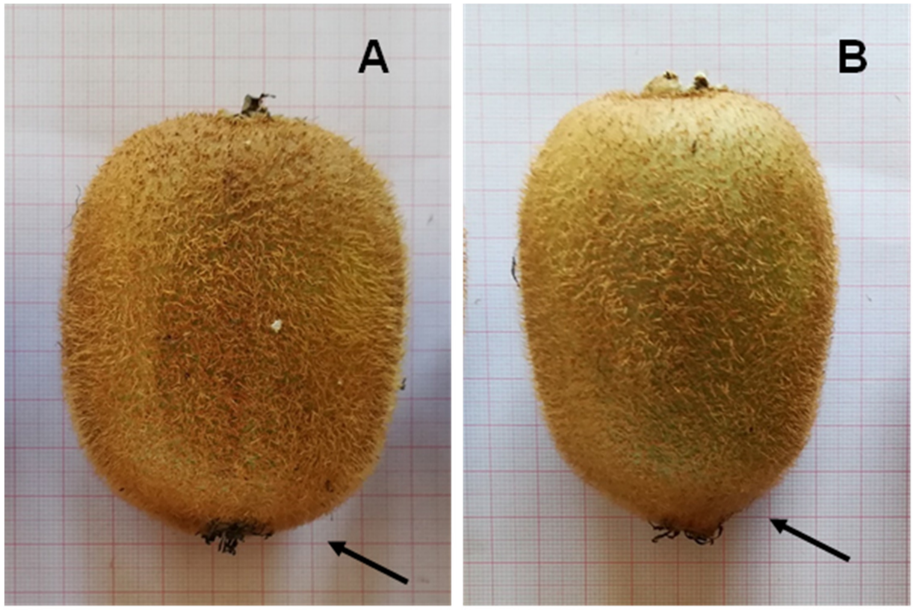

Figure 4. Fruit characteristics of kiwi cv. 'Hayward' after foliar fertilization with the commercial biostimulator THEOFAST during an integrated nutrient management (INM) programme. Elongation of kiwi fruits at stigmas (arrow) after an INM foliar application with THEOFAST (B) compared to control (A)

Soluble solids content of fruits increased, compared to control; however, no differences were found among the different treatments and followed the same pattern in both years. The flesh firmness increased compared to control and the highest values were found under A4 and A3 treatments in 2019 and B3 and B2 in 2020. The highest acidity was found in A2 and A3 treatments in 2019, while in 2020 it was found in B4 (Figures 1 and 2). Our findings are in accordance with the results of Sotiropoulos *et al.* (2021) and Hashmatt *et al.* (2019) for different varieties of kiwi vines. In addition, Zhang *et al.* (2020) found that the combinational application of inorganic and organic fertilization improved kiwifruit quality, as a result to improve fruit chemical composition, such as soluble solids and reduced sugar. In our study, fruit quality improved by Ca application at the stage of maturity as was also shown for peach by Sotiropoulos *et al.* (2010). On the other hand, Ca applications did not positively affect the flesh firmness in kiwifruits, due to the excessive Ca concentration in soil (Scudellari 1998), and there was no correlation between Ca concentration in leaves and fruit quality parameters in persimmon fruits (Vilhena *et al.*, 2002). The formulation of Ca and the timing of application contribute to the quality characteristics of kiwifruits (Sotiropoulos *et al.*, 2021). The key factor for Ca uptake is the fruit growth. Regarding the content of dry mass in kiwifruits, which is a common quality index, it was observed that all treatments had higher values compared to control, for both years (Figures 1 and 2). The relative quantity of carbon in different parts of the vine is evaluated by dry weight measurements without indicating the partitioning of photosynthates within the plant (Cassano *et al.*, 2006).

From the data of Table 1 it is concluded that sufficient to high levels of soil nutrient concentrations were determined. In addition, soil pH was alkaline (7.7), while sufficient levels of organic matter were determined (2.7%). Similar results for the soil properties of Central Shaanxi province (the largest kiwifruit producing region in the world), in China, were also found in the study of Wang *et al.* (2019). More specifically, in that study, it was verified the calcareous origin of soils (pH 7.54), while approximately over a quarter of the soils in that region were deficient in organic matter, which is partially in contrast to the data existing in our study region, where the organic matter levels can be characterized as sufficient (2.7%).

The results of 2019 showed that the concentrations of N, P, K and B in leaves were higher compared to the control, without significant differences among the treatments (Table 6). On the other hand, the different treatments did not affect Mg, Mn, Zn, Fe and Cu concentrations. Ca concentration in leaves was higher among all treatments compared to control in two years (Tables 6 and 7). The highest value was recorded in A4 (A4>A3>A2>A1>C). In 2020, notable are the results of total N, the lowest values were found in treatments applied only with root irrigation (B4 and B5) while the highest were observed in treatments applied with both foliar and root irrigation (B1, B2 and B3) (Table 7). The available N in soil had an important effect on the N fruit content. Xylem flux is negligible to *Actinidia* fruit after the cell division phase (Clark and Smith, 1992). Nitrogen partitioning in the late stages of development depends almost solely to the phloem influx from the leaves (Tagliavini *et al*., 2000), which upon maturity contain important amounts of labelled nitrogen (Ferguson, 1980). The absorption of minerals with foliar application happened via the cuticle (Schreiber, 2005). The differences among plants regarding the cuticle thickness and composition is the reason of the different rates of absorption (Schreiber, 2005; Eichert and Coldbach, 2008). The type of anion salt, the number of foliar applications and the stage of plant growth controls the effectiveness of foliar application (Val *et al*., 2008; Wojcik and Borowik, 2013; Fernandez *et al*., 2020). Foliar and soil application of Ca chloride increased the Ca content of 'Golden delicious' and 'Braeburn' apple varieties (Torres *et al*., 2017). The nutrient range values for kiwi leaves are in accordance with our findings (Taraksioglu et al., 2007).

Table 6. Effect of foliar sprays on nitrogen(N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), boron (B), manganese (Mn), zinc (Zn), iron (Fe) and copper (Cu) concentration of kiwifruit cv. 'Hayward' leaves during 2019

Treatment	Total N % d.w	P % d.w	K % d.w	Ca % d.w	Mg % d.w	B mg kg^{-1} d.w	Mn mg kg^{-1} d.w	Zn mg kg^{-1} d.w	Fe mg kg^{-1} d.w	Cu mg kg^{-1} d.w
C	2.01±0.24b*	0.39±0.05 b	1.75±0.19 c	2.31±0.29 e	0.60±0.07 a	51±5.90 b	55±6.22 a	24±2.92 a	85±9.2 a	8±0.98 a
A1	2.59±0.29 a	0.52±0.06 a	2.41±0.32 a	2.85±0.33 d	0.58±0.06 a	72±8.13 a	52±6.03 a	22±2.41 a	87±9.6 a	8±1.01 a
A2	2.63±0.30 a	0.55±0.06 a	2.26±0.30 ab	3.19±0.35 c	0.56±0.06 a	75±8.62 a	57±6.61 a	25±3.06 a	89±10.2a	7±0.94 a
A3	2.68±0.32 a	0.57±0.07 a	2.29±0.34 ab	3.70±0.41b	0.63±0.07 a	78±9.17 a	56±6.25 a	26±3.25 a	85±9.5 a	8±1.02 a
A4	2.70±0.31 a	0.55±0.06 a	2.15±0.29 ab	3.97±0.46 a	0.61±0.08 a	75±8.24 a	53±6.90 a	24±2.82 a	89±10.4 a	7±0.89 a

*No significant difference in mean values (± SD) with the same letter in the same column (Duncan's multiple range test, P≤ 0.05)

Table 7. Effect of foliar sprays on nitrogen(N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), boron (B), manganese (Mn), zinc (Zn), iron (Fe) and copper (Cu) concentration of kiwifruit cv. 'Hayward' leaves during 2020

Treatment	Total N% d.w	P % d.w	K % d.w	Ca % d.w	Mg % d.w	B mg kg^{-1} d.w	Mn mg kg^{-1} d.w	Zn mg kg^{-1} d.w	Fe mg kg^{-1} d.w	Cu mg kg^{-1} d.w
C	2.08±0.24 c	0.50±0.07 b	1.77±0.19 d	3.19±0.38 d	0.75±0.09 a	48±5.6 b	48±5.3 b	25±2.9 b	75±8.4 b	7±0.9 b
B1	3.38±0.41 a	0.55±0.07 ab	2.66±0.29 a	4.61±0.52 c	0.76±0.09 a	72±8.4 a	50±5.7 a	28±3.1 a	85±9.2 a	8±1.2 ab
B2	3.31±0.38 a	0.59± 0.08a	2.43±0.26 b	4.82±0.55 b	0.70±0.08 ab	75±8.4 a	53±5.9 a	25±2.8 b	87±9.7 a	8±1.4 ab
B3	3.26±0.36 a	0.56±0.06 a	2.20±0.26 c	4.9±30.55 a	0.56±0.07 b	74±8.6 a	52±6.1 a	30±3.7 a	89±10.7 a	9±1.5 a
B4	2.73±0.32b	0.57±0.07 a	2.60±0.30 a	4.69±0.53 c	0.72±0.09 a	50±5.9 b	49±5.7 ab	27±3.4 ab	86±10.4 a	7± 1.0 b
B5	2.66±0.30 b	0.54±0.06 ab	2.41±0.27 b	4.86±0.58 b	0.65±0.08 ab	47±5.4 b	48±5.3 b	26±3.2 b	83±10.8 ab	8±1.1 ab

*No significant difference in mean values (± SD) with the same letter in the same column (Duncan's multiple range test, P≤ 0.05)

From all the results presented in the Tables 6 and 7, it is concluded that, with the exception of N (its optimum range of adequacy should be from 2.30 to 3.0%), K in the control, which was 1.75% (the optimum range of foliar K should vary from 2.0 to 3.0%) and the Mn levels in the control and B5 for the year 2020 (their levels were below 50 mg kg^{-1}), all the other nutrients concentrations were determined within the optimum levels of adequacy for good agronomic yields. Our data are partially in disagreement with those of Wang *et al.*

(2019), who found that more than 65% of the kiwi orchards analysed were deficient in leaf N, P and K concentrations, implying low N, P and K efficiency in the Central Shaanxi region, China. From this comparison between our study area (Naoussa, Central Macedonia, Northern Greece) and that of Central Shaanxi, in China, it can be concluded that low efficiency exists only for N and K, in the control plots (Tables 8 and 9). In all the other (A and B) plots normal levels of nutrient adequacy were determined, thus high nutrient efficiency exist.

Table 8. Effect of foliar sprays on nitrogen(N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), boron (B), manganese (Mn), zinc (Zn), iron (Fe) and copper (Cu) concentration of kiwifruits cv. 'Hayward' during cultivation in 2020

Treatment	Total N% d.w	P % d.w	K % d.w	Ca % d.w	Mg % d.w	B mg kg^{-1} d.w	Mn mg kg^{-1} d.w	Zn mg kg^{-1} d.w	Fe mg kg^{-1} d.w	Cu mg kg^{-1} d.w
C	1.14±0.14 b*	0.62±0.08 a	1.48±0.18 c	0.20±0.03 d	0.13±0.02 a	14±0.18 b	6±0.9 a	7±0.9 a	14±0.18 a	5±0.8 a
A1	1.32±0.16 a	0.64±0.09 a	2.03±0.25a	0.29±0.04 c	0.12±0.02a	18±0.20 a	5±0.7 a	7±1.0 a	13±0.16 a	4±0.6 a
A2	1.33±0.17 a	0.65±0.09 a	1.75±0.21ab	0.37±0.05 b	0.11±0.01 a	19±0.20 a	5±0.8 a	7±0.9 a	13±0.15 a	5±0.8 a
A3	1.35±0.19 a	0.63±0.10 a	1.78±0.23 ab	0.39±0.05 b	0.12±0.02 a	20±0.25 a	5±0.9 a	6±0.8 a	13±0.16 a	5±0.9 a
A4	1.37±0.20 a	0.59±0.09 a	1.64±0.19 b	0.48±0.07 a	0.11±0.01 a	18±0.21 a	5±0.8 a	6±0.9 a	12±0.15 a	4±0.7 a

*No significant difference in mean values (± SD) with the same letter in the same column (Duncan's multiple range test, P≤0.05).

Table 9. Effect of foliar sprays on nitrogen(N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), boron (B), manganese (Mn), zinc (Zn), iron (Fe) and copper (Cu) concentration of kiwifruits cv. 'Hayward' during cultivation in 2020

Treatment	Total N% d.w	P % d.w	K % d.w	Ca % d.w	Mg % d.w	B mg kg^{-1} d.w	Mn mg kg^{-1} d.w	Zn mg kg^{-1} d.w	Fe mg kg^{-1} d.w	Cu mg kg^{-1} d.w
C	0.97±0.11 c	0.49±0.58 a	1.49± 0.19 b	0.19±0.02 c	0.20±0.03 a	11±1.4 b	6±0.8 a	8±1.0 a	15±1.9 a	7±0.9 a
B1	1.36±0.18 a	0.54±0.61 a	1.75±0.25 a	0.35±0.04 ab	0.16±0.02 ab	17±2.2 a	6±0.9 a	11±1.4a	19±2.4 a	8±1.1 a
B2	1.34±0.18 a	0.50±0.68 a	1.71±0.24 ab	0.39±0.05 ab	0.14±0.02 b	19±2.5 a	5±0.8 a	9±1.1 a	17±2.3 a	7±0.8 a
B3	1.30±0.16 a	0.49±0.57 a	1.60±0.21 ab	0.46±0.07 a	0.12±0.02 b	18±2.6 a	6±0.8 a	10±1.5 a	19±2.6 a	8±1.2 a
B4	1.18±0.22 b	0.54± 0.63 a	1.80±0.24 a	0.32±0.05 b	0.16±0.02 ab	19±2.8 a	6±0.9 a	9±1.3 a	18±2.4 a	7±1.1 a
B5	1.20±0.26 b	0.52±0.62 a	1.66±0.20 ab	0.44±0.05 a	0.13±0.01 b	18±2.4 a	7±1.0 a	10±1.2 a	19±2.7 a	7±1.0 a

*No significant difference in mean values (± SD) with the same letter in the same column (Duncan's multiple range test, P≤ 0.05)

Regarding the analysis in kiwifruits, the concentrations of N and B increased compared to control and no significant differences were recorded among the different treatments (Table 8). The concentrations of P, Mg, Mn, Zn, Fe and Cu did not show any differences among treatments. Potassium and Ca concentrations in kiwifruits were significantly higher in all treatments compared to the control. The highest value of K concentration was found in A1 treatment while the highest Ca concentration was observed in A4 treatment (Table 8). In treatments which had a higher proportion of Ca, a decreasing trend of K concentration was observed due to the antagonism of K and Ca. Therefore, treatments that contained Ca contributed to the increase of its concentration and consistency of kiwifruits. A similar pattern was observed in the results of 2020 measurements except of Mg concentration that was higher in control (Table 9) due to the antagonistic effect of Ca to Mg and vice versa (Paiva *et al.*, 1998).

During fruit growth nutrient accumulation varies, reducing the Ca content of the fruit after 2 months. Nitrogen, phosphorous and magnesium did not follow this pattern. In this study, foliar sprays with fertilizer containing organic Ca increased the concentrations of Ca in leaves and agreed with the results of 'Tsechelidis' cultivar (Koutinas *et al.*, 2010). The boron concentration of leaves increased with the highest application of Ca fertilizer. Boron plays a cooperative role with Ca in the construction of the plant cell wall (Yamaguchi *et al.*, 1986). Calcium and B applications did not affect the total content of soluble solids, but they reduced the accumulation of boron and increased the fruit calcium content (Xu *et al*., 2015). The higher levels of Ca in fruit tissue of two varieties of pears were observed to delay fruit softening (Allegra *et al*., 2022).

Conclusions

A balanced fertilization program enriched with fertilizers containing Ca produced high added value fruits. The organic Ca-fertilizer 'THEOCAL' resulted in increased flesh firmness, total soluble solids, and dry mass percentage. Additionally, the high Ca concentration in kiwifruits offers post-harvest perseverance. The optimum concentration of 'THEOCAL' was 2-4 Kg/t^{-1} per ha and this product could be applied as foliar sprays during the growing season starting from fruit set up to prior to harvest. Biostimulants have a positive effect to kiwi cv. 'Hayward' cultivation. Foliar application with THEOFAST resulted to the elongation of the stigmas area in kiwi fruits.

Authors' Contributions

Conceptualization: T.S. and G. T.; Methodology: T.S. and G.T.; Software, O.D.; Validation, I.M., T.S. N.K. and T.C.; Formal Analysis, I.M. and T.C.; Investigation, T.S., I.M. and G.T.; Resources, T.S. and N.K.; Data Curation, T.C and I.M.; Writing—original draft preparation: T.S. and O.D.; Writing—review and editing: T.S., G.T. and T.C.; Visualization: T.S.; Supervision: T.S.; Project administration: T.S. and G.T. All authors read and approved the final submitted manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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