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Research Article

# Enhancement of bud dormancy release and development, leaf nutrition, flower and fruit quality of kiwifruit cv. 'Hayward' induced by BUD 14 biostimulant

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## Abstract

The aim of this research was to investigate the influence of a foliar fertilization program, consisted of the BUD 14 nitrogen-calcium commercial formulation (N: 14% w/w, CaO: 5.5% w/w) as a biostimulant, on bud development percentage, flowering rate, classification of flowers into open, closed and triple, flower and pollen quality traits, fruit quality attributes, and leaf nutritional status of the 'Hayward' kiwifruit cultivar. The study was realized during a two-year experimental period in Naoussa, Central Macedonia, Greece. The results showed that BUD 14 induced synchronization in bud development relative to different vegetative stages including initiation of bud expansion, appearance of leaf apices covered by hair and deployment of 2-8 leaves and increased the flowering rate of open flowers. Pedicel length, ovary fresh weight, and dry weight, dry matter and length in female flowers as well as maximum pollen grain diameter and area in polar view in male flowers were significantly enhanced in the BUD 14 treatment. Fruit quality characteristics like average weight and dry mass were significantly augmented, and a 1.5-fold and 2-fold increase was recorded in canes length and number of kiwifruits per cane. In addition, leaf nutrient Ca and Mg concentrations were significantly enhanced, compared to the control. The efficacy of BUD 14 as a more target-oriented and environmentally friendly alternative method of supplying plants with smaller and controlled amounts of nutrients for breaking bud dormancy and improving their development was demonstrated, enhancing flower and fruit quality, leaf nutrition, kiwifruit developmental characteristics, and finally the total production per fruit per tree.

***Keywords:*** biostimulant; bud development; flower-fruit quality; foliar sprays; kiwifruit; leaf nutrition

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## Introduction

According to the Food and Agriculture Organization of the United Nations, the kiwi fruit has seen a significant rise in global production over the years, and even though native to China, it is now cultivated in several countries (China: 2,380,304 tons, New Zealand: 603,523 tons, Italy: 523,120 tons, Greece: 320,270 tons, Iran: 294,571 tons, Chile: 114,534 tons, Turkey: 100,772 tons, Portugal: 52,920 tons, France: 47,120 tons, United States: 33,110 tons) across the globe, contributing to an annual global production of approximately 4.5 million tons. Countries like Greece (30.5 kg per person) and Italy (9.02 kg per person) also feature prominently in per capita production as compared to New Zealand (116.06 kg per person), reflecting their roles as significant producers despite smaller overall outputs compared to China (FAOSTAT, 2024; Worldostats, 2024). The distinct flavor, sweet and slightly acidic flesh, the increased level of ascorbic acid – vitamin C (Ferguson, 2011) as the most prominent and advantageous bioactive compound, other secondary metabolites such as carotenoids, phenolics, flavonoids, and chlorophyll (Jesion *et al*., 2013) as well as minerals (Na, K, Ca, Mg, Mn, Fe, Cu, Zn) (Latocha *et al*., 2021) have been identified globally as the most attractive and valuable characteristics of kiwifruits for consumption (Jesion *et al*., 2013). Globally, 'Hayward' is still the most popular and common kiwifruit variety marketed commercially by the industry, developed by the growers and demanded by the consumers, because of the high-quality characteristics of its fruits such as taste, size, and storage (Ward and Courtney, 2013). The 'Hayward' accounts for about 60% of the kiwifruit grown commercially and 90% of the kiwifruit traded internationally (Ferguson 2011) and in Greece it has been the dominant kiwifruit cultivar since 1973 with a total annual fruit production of 40,000 t and an acreage of 4400 ha (FAO, 2004). Despite the existence of various kiwifruit cultivars in Greece such as 'Tsechelidis' (Sotiropoulos *et al*., 2009), 'Abbot', 'Bruno' and 'Monty' (Koukouryannis, 1990), the industry depends on a single cultivar, the 'Hayward' (Koukouryannis and Vasilakakis, 1997). Greek kiwis are considered a high-value export product, with 95% of production headed to international markets, in fact, in 2017-2018 exports amounted to 151,287,722 euros, an increase of about 77.18%. Most of Greek production is of the Hayward cultivar and comes mainly from Pieria (approximately 45% of total yield), while Thessalia, Kavala and Arta are among the most suitable areas in Greece for kiwi fruit growing (ELSTAT, 2018).

The size of kiwifruit at harvest is an important factor that determines the economics of the farm, and growers' cultivation practices during the growing season aiming at maximizing fruit weight. Final fruit weight results from the combined effect of both pre-floral and post-floral factors on cell division and tension of the various tissues. Important post-floral factors include pollination, the ratio of leaves to fruit, the existence of sufficient lighting in the canopy through pruning, carbon economy, irrigation water availability, tree nutrition, crop load, and variety, among others (Patterson *et al*., 1999; Sharma *et al*., 2018). Quality, firmness and storage life of kiwifruit during cultivation and especially at the time of harvest or at the end of storage have been shown to be highly dependent on several coefficients from which the essential and balanced nutrition especially of the mineral elements is of the utmost significance (Huang *et al*., 2020; Williams *et al*., 2003).

Besides kiwifruit quality parameters and nutrient status, bud development and subsequent flowering in the shoot cane is not uniform and depends on climatic factors (Guedon *et al*., 2001). The percentage of development ranges from 0% near the base to 100% near the tip of the shoot cane (Mcpherson *et al*., 1994). Bud development that produces annual flowering shoots is over 50% when cold winter weather conditions prevail, while it is below 20% when winters are mild (Costa *et al*., 1995). In order for the buds to develop in the spring, they must receive cold hours of 0-7.2 °C (cold hours' model) in the winter. Kiwi requires 900 hours of temperatures of 0-7.2 °C to break bud dormancy (Wall *et al*., 2008). Different strategies and chemical compounds have been tested worldwide to deal with incomplete bud development when winter chilling

requirements are not met in subtropical conditions but also in marginal zones of kiwi cultivation due to climate change (Di Tommaso *et al*., 2011; Marques *et al*., 2021; Pichakum *et al*., 2018).

Although the high adaptability of the kiwi to climate change, it is highly prone to environmental alterations including temperature, rainfall and humidity mainly during growth, strong winds, and frost, among others, leading to a decline in kiwi productivity, yield and quality (Gurbuz *et al*., 2024). It has been reported that the qualitative and quantitative attributes of the kiwifruits are adversely affected by variations in temperature, precipitation and sunshine exposure period (Malhi *et al*., 2021). Moreover, kiwifruits when continuously exposed to heat/ high temperatures are subjected to sunburns, loss of texture and decrease of nutritional value (Parajuli *et al*., 2019). In the context of climate change induced-stresses (*i.e.* drought, salinity, temperature) and future food security, potential adaptation and mitigation strategies to counterbalance the negative impact on agriculture and contribute to ecology restoration is the use of biostimulants as an integrated and sustainable way of crop production (Bhupenchandra *et al*., 2022; Yakhiu *et al*., 2017).

Plant growth and development can be enhanced by the application of commercial fertilizers as an integrated nutrient management (INM) treatment due to the enriched composition of these formulations in polysaccharides, biostimulants, organic calcium and urea as nitrogen sources, stimulating the effectiveness of the fertilizer owing to their water-soluble ability and non-toxic nature (Chiaregato *et al*., 2012; Tripathi *et al*., 2014). Except bud development, flowering and subsequent plant growth, crop quality traits and nutrient concentration of kiwifruits can be reinforced by applying several plant biostimulants, aiming at increasing nutrient uptake, abiotic stress tolerance, market value and further presuming upon the pharmaceutical properties of the organically produced fruits (Jardin, 2015; Khachi *et al*., 2015; Park *et al*., 2012; Park *et al*., 2013). The requirement of trees in nutrients can be fulfilled by fertilizer application through foliar sprays as a more target-oriented and environmentally friendly way of supplying plants with smaller and controlled amounts of nutrients (Fernadez and Eichert, 2009). In addition to the usefulness of nitrogen especially during fruit growth, the foliar application of calcium fertilizers via sprays has been shown to enhance fruit quality at the post-harvest stage due to its higher uptake by the plant, and the analogous relationship between Ca concentration in the fruit and its flesh firmness (Antunes *et al*., 2007; Vajari *et al*., 2018).

In the framework of the aforementioned, this study was undertaken to investigate the feasibility of using the BUD 14 biostimulant, as a nitrogen-calcium (N: 14% w/w, CaO: 5.5% w/w) commercial formulation in the cultivation of kiwi. The specific objectives of the study were to compare the effectiveness of BUD 14 with the non-fertilized control treatment regarding bud development status (percentage) in different vegetative stages, flowering rate, flower and pollen quality characteristics, fruit quality attributes (*i.e.* average fruit weight, soluble solids, acidity, dry matter, flesh resistance to pressure) and leaf nutrient concentrations. For this reason, the results of a two-year experimental period (2020-2021), carried out in a commercial orchard located in Naoussa, Central Macedonia, Greece (geographical coordinates: longitude 2201200 0 ' E; latitude 400 290040 ' N; elevation 350 m), were presented in this research.

## Materials and Methods

The experiment was carried out for two years on 6-year-old kiwi orchard of the 'Hayward' variety, formed in a T bar trellis and planted at distances of 3×4 m. Soil samples from the experimental orchard were collected from a depth of 0-60 cm and analyzed (Page *et al*., 1982). The mechanical texture of the soil samples was determined based on the soil organic matter devoid of the inorganic matrix of sand, silt, and clay after grinding or ball-milling to destroy soil aggregates and increase the surface area available to the extracting reagent for wetting and solubilization by conversion of acidic components to ions and subsequent formation of a physical solution of the ions in water. Soil pH measured as the activity of ionized H ($H^+$) - the intensity factor (index) in the soil solution. The quantitative method used for $CO_2$ determination released from carbonates

was the Schollenberger vacuum-distillation. Organic matter was determined spectrometrically by multiplying the value of organic-C obtained after soil wet-combustion and decomposition by chromic acid and $H_2SO_4$ with the conventional "Van Bemmelen factor" of 1.724. The soil of the orchard was of medium mechanical texture, with a pH of 7.5, organic matter of 4.1% and total calcium carbonate 4.2%.

*First experimental period – cultivation year of 2020*

In the first experimental period (2020), plants were sprayed with BUD 14 about 40 days before the estimated bud development. The nutritional composition of the BUD 14 formulation is as follows: 14% total nitrogen, 2% nitrogen (ureic), 5% nitrogen (ammoniacal), 7% nitrogen (nitric), and 5.5% calcium (CaO) enriched with hydrolyzed protein of plant origin (Nature S.A., Nea Efessos, Pieria, Greece). More specifically, 100 plants were sprayed with the BUD 14 formulation on 4/2/20 with a concentration of 150 L per ton of water [150 L $t^{-1}$ suggested fertilizer dose, 15 L/100 L of the final solution (15 L BUD 14 + 85 L water)], while 100 plants were used as controls (non-sprayed).

During the periods 8-4-20 and 16-4-20 bud development measurements were made and their development stages were recorded. The percentage (%) of bud development in each phenological stage was calculated. During the flowering period, the flowering percentage (%) was measured and the flowers were classified into open, closed and triple. During the harvest stage, the average fruit weight and fruit quality characteristics were measured. Average length of cane and average number of kiwifruits per cane during the fruit set period in the two treatments (control, BUD 14) were also recorded.

*Second experimental period – cultivation year of 2021*

In the second experimental period (2021), the spraying with the BUD 14 formulation was carried out on 4/2/2021 (about 40 days before the estimated bud development) with a concentration of 150 L per ton of water (150 L BUD 14 + 850 L water per 1 ton). During the spraying period, approximately 700 hours of temperatures of 0-7.2 °C had been completed. During the periods 10-4-21 and 20-4-21 measurements and recording of the bud phenological stages were carried out based on the BBCH scale. Four stages were recorded: 01 (initiation of bud swelling), 07 (initiation of bud expansion), 09 (appearance of leaf apices covered by hairs) and 18 (two to eight leaves unfolded but not yet fully grown) (Salinero *et al*., 2009).

During the period of full flowering, four canes were selected from each plant (two from each side of the crown). A developing shoot was selected from the middle of each cane and two flowers in full bloom were marked. Flowers were selected from positions 4-6. One flower was collected in order to perform flower quality measurements and the other flower was later used to determine fruit development. During the flowering period, the flowering rate was measured and the flowers were classified into open, closed and triple. The flowering rates (%) of open, closed and triple flowers were calculated using the following equation: number of buds with development of open, closed and triple flowers, respectively/ total – initial number of buds x 100%), so as the total flowering rate per fertilization treatment, which represents the sum of open, closed and triplet flowering rates, to be 100%. In female flowers, ovary fresh weight, dry weight, dry matter, diameter and length, as well as pedicel fresh weight, length and thickness were measured. The diameter, perimeter and area of the pollen grains were measured in the male flowers. Flower quality was determined with a digital caliper (Finder) and pollen grain dimensions were measured using an Olympus BX40 microscope from an oil immersion lens (×1000), through the Olympus DP-Soft 3.0 program software.

During the vegetative period, fruit weight measurements were made at regular intervals from fruit set to harvest (*i.e.* 0, 40, 55, 70, 85, 100, 115, 130 and 145 days from full blossom). During the harvest stage, the average fruit weight and fruit quality characteristics were measured. One hundred fruits were collected from the five trees of each replication, and therefore 20 fruits per tree were collected. Fruits were sampled at commercial maturity. Fruits from all treatments were harvested at the same time, based on total soluble solids concentration. Fruits were transported immediately to the laboratory for analyses. Quality characteristics of the fruits were measured such as: average fruit weight, total soluble solids with an Atago PR-1 electronic

refractometer (Atago Inc., Bellevue, WA, USA), acidity by titration with 0.1 N NaOH (Ough and Amerine, 1988), the pressure resistance of the flesh with an Effegi penetrometer with an 8-mm tip (Effegi Model FT 327, Alfonsine, Italy) and the dry matter content according to a previous published method (Schotsmans *et al*., 2007). During the summer period, leaf samples collected manually were taken for chemical-nutritional status analysis (*i.e.* total N, P, K, Ca, Mg, B, Mn, Zn, Fe, Cu). 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 twice with distilled water, and then they 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). Total nitrogen was determined by the method of Kjeldahl (Chapman and Pratt, 1961) and B by the azomethine-H method (Wolf, 2008).

*Statistical analysis*

The adopted experimental design was a randomized block with five replications of two fertilization treatments (five canes per replication were used). The statistical analysis of the experimental data between the two fertilization treatments (control, BUD 14), related to flowering rates, flowering and pollen quality parameters, fruit quality characteristics, average length of cane, average number of kiwi fruits per cane, and leaf nutrients concentration, was performed with the statistical program MSTAT-C version 1.41, while the comparison of the averages with the Fischer's method ($P \leq 0.05$).

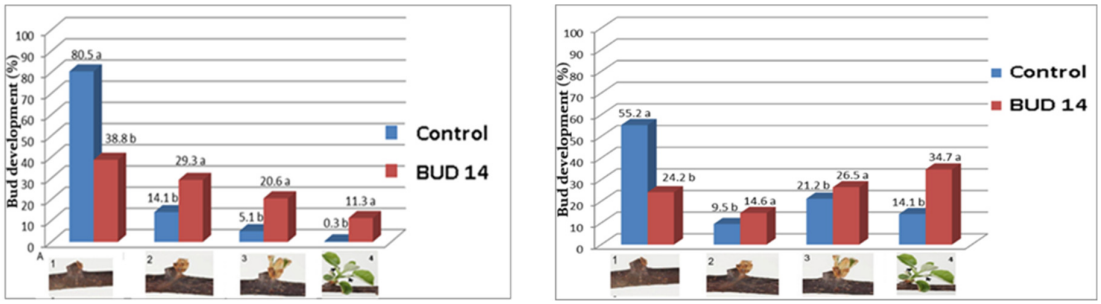
Regarding the percentage (%) of bud development status, a 2 × 2 × 2 full factorial experimental design was applied for combined statistical analysis including two treatments (Control, BUD 14), two cultivation years (2020 and 2021) and two sampling dates per year (8/4/2020 and 16/4/2020, 10/4/2021 and 20/4/2021), thus comprised of eight combinational treatments. In addition, statistical analyses were conducted either between the two treatments per sampling date and per year, or among the two fertilization treatments and the two sampling dates per year (2 x 2 combined experimental design, four combinational treatments). In the case of the 4 × 2 × 2 × 2 multifactorial experimental design (*i.e.* four bud development stages × two cultivation years × two sampling dates per year × two fertilization types) consisted of 32 combinational treatments in total, the effect of the main factors [bud development stage (A), cultivation year (B), sampling date per cultivation year (C), fertilization type (D)] and theirs amongst interactions (A*B, A*C, A*D, B*C, B*D, C*D, A*B*C, A*B*D, B*C*D, A*B*C*D) was evaluated. The different statistical analyses were performed to identify the main effect of factors (bud development stage, cultivation year, sampling date, fertilizer treatment) and theirs among interactions using the statistical program MSTAT-C version 1.41, Analysis of Variance (ANOVA), General Linear Model and the Fischer's method ($P \leq 0.05$). The mean value of percentage (%) bud development status in every combinational treatment was comprised of 100 different plants.

Related to quality traits of kiwifruits [*i.e.* average fruit weight (g), soluble solids (%), acidity (% citric acid), flesh resistance to pressure (kg $cm^{-2}$), and dry matter (%)], statistical analyses were performed either between the two fertilization treatments (control, BUD 14) per cultivation year (2020 and 2021) or among the two fertilization treatments and the two cultivation years (four combinational treatments, 2 x 2 experimental design) to evaluate the effect of the main factors (fertilization treatment, cultivation year) and their interaction using the statistical program MSTAT-C version 1.41, ANOVA, General Linear Model and the Fischer's method ($P \leq 0.05$). Each combinational treatment consisted of 100 different fruits (5 trees × 20 fruits/tree). Data presented in Tables and Figures show mean ± standard deviation (SD).

## Results

*Effect of the BUD 14 biostimulant on bud development*

During the first cultivation year of 2020, on 8/4/2020 in the BUD 14 treatment fewer buds were found at stage 1 (*i.e.* initiation of bud swelling) compared to the control and more at the remaining 2-4 stages (*i.e.* initiation of bud expansion, appearance of leaf apices covered by hairs, 2-8 leaves unfolded but not yet fully grown), *i.e.* more buds had developed in the application with BUD 14 in this period (Figure 1A; Figure 2; Table 1). At the next measurement on 16/4/2020 in the BUD 14 treatment also fewer buds were found at stage 1 compared to the control and more at the remaining stages (Figure 1B; Figure 2; Table 1).




(A) (B)

**Figure 1.** Percentage (%) of bud development on two different sampling dates of the first cultivation year of 2020 in the two fertilization treatments (control, BUD 14) relative to the vegetative stage including initiation of bud swelling (1), initiation of bud expansion (2), appearance of leaf apices covered by hairs (3), and 2-8 leaves unfolded but not yet fully grown (4); (A) 8/4/2020; (B) 16/4/2020. In each diagram, means followed by different letters per fertilization treatment and per phenological stage of bud development denote significant differences (Fischer's test, $p \leq 0.05$)


**Figure 2.** Bud development status of kiwi plants during the first cultivation year of 2020 in two sampling periods (8/4/2020 and 16/4/2020) under the two fertilization treatments (Control, BUD 14)

In both cultivation years (2020, 2021) and sampling dates (8 and 10/4/2020, 16 and 20/4/2021) per cultivation year, the initiation of bud swelling during the first developmental stage was significantly higher (55.2-80.5%, 58.6-78.2%) in the control treatment than in BUD 14 (24.2-38.8%, 26.6-40.1%), being maximized (78.2-80.5%) under the first sampling date of both cultivation years (8/4/2020, 10/4/2021) without differing significantly. In contrast to the initial bud swelling (1st) stage, the subsequent bud development stages (2nd, 3rd, and 4th) including the initiation of bud expansion, appearance of leaf apices covered by hairs, and deployment of two to eight leaves but not yet fully grown were better promoted under treatment with BUD 14 as compared to the untreated control, regardless cultivation year and sampling date per year. In particular, a 1.5-2-fold increase was recorded in the initiation percentage of bud expansion when plants treated with BUD 14 biostimulant in relation to the control, being optimized (26.2-29.3%) in the first sampling date of both cultivation years (8/4/2020, 10/4/2021). In the 3rd bud developmental stage, the percentage of leaf apices appearance covered by hairs was significantly higher (26.5-26.9%) by 1.5-5 times in BUD 14 treated plants in the second sampling date of both cultivation years (16/4/2020, 20/4/2021) than in the control untreated plants (5.1-21.2%). Under BUD 14, the percentage of treated buds with deployment of two to eight leaves (4th stage) was significantly the highest (34.7%) in the second sampling date of the first cultivation year (16/4/2020), being 2-10 times raised to the control (0.3-14.1%). The comparison among the four bud development stages, the two cultivation years, the two sampling dates per year and the two fertilization types showed that the percentage of bud development status was significantly higher 78.2-80.5% in the control non-sprayed plants in both sampling dates of the first cultivation year 2020 at stage 1, 29.3% at stage 2 in BUD 14 sprayed plants in the first sampling date – 8/4/20 of the 2020 first cultivation year, 26.9% at stage 3 in BUD 14 sprayed plants in the second sampling date – 20/4/21 of the 2021 second cultivation year, and 34.7% at stage 4 in BUD 14 sprayed plants in the second sampling date – 16/4/20 of the 2020 first cultivation year. Analysis of variance (ANOVA) and General Linear Model revealed that the main effect of all four factors involved (bud development stage, cultivation year, sampling date, fertilization type) and theirs amongst interactions on % of bud development status was varied (Table 1).

Similar bud development status percentage results to the first experimental period (2020) (Supplementary Figure 1A-1D) were also obtained during the second experimental period (2021) (Supplementary Figure 1E-1H), for both sampling dates (Table 1).

**Table 1.** Percentage (%) of bud development status during two different years (2020 and 2021), two different sampling dates per year (2020: 8/4/20 and 16/4/20, 2021: 10/4/21 and 20/4/21) and four different phenological stages relative to the vegetative stage based on the BBCH scale under BUD 14 and non-sprayed control kiwifruit plants

| | **Experimental year 2020** | | | | **Experimental year 2021** | | | |
|---|---|---|---|---|---|---|---|---|
| Sampling date | 8-4-20 | | 16-4-20 | | 10-4-21 | | 20-4-21 | |
| Bud phenological stages | Control | BUD 14 | Control | BUD 14 | Control | BUD 14 | Control | BUD 14 |
| 2-8 leaves unfolded but not yet fully grown (4 or 18) | 0.3 ± 0.0 b D (F) *r* | 11.3 ± 0.6 a C (D) *lmn* | 14.1 ± 0.7 b B (C) *jkl* | 34.7 ± 1.7 a A (A) *e* | 2.0 ± 0.1 b D (E) *q* | 14.8 ± 0.7 a B (C) *jk* | 11.7 ± 0.6 b C (D) *lmn* | 30.2 ± 1.5 a A (B) *f* |
| Appearance of leaf apices covered by hairs (3 or 09) | 5.1 ± 0.3 b D (G) *p* | 20.6 ± 1.0 a C (BC) *i* | 21.2 ± 1.1 b B (B) *i* | 26.5 ± 1.3 a A (A) *h* | 7.2 ± 0.4 b C (D) *op* | 18.9 ± 0.9 a B (C) *i* | 19.3 ± 1.0 b B (C) *i* | 26.9 ± 1.3 a A (A) *gh* |
| Initiation of bud expansion (2 or 07) | 14.1 ± 0.7 b B (BC) *jkl* | 29.3 ± 1.5 a A (A) *fg* | 9.5 ± 0.5 b C (D) *no* | 14.6 ± 0.7 a B (B) *jk* | 12.6 ± 0.6 b C (C) *klm* | 26.2 ± 1.3 a A (A) *h* | 10.4 ± 0.5 b D (CD) *mn* | 16.3 ± 0.8 a B (B) *j* |
| Initiation of bud swelling (1 or 01) | 80.5 ± 4.0 a A (A) *a* | 38.8 ± 1.9 b C (C) *d* | 55.2 ± 2.8 a B (B) *c* | 24.2 ± 1.2 b D (D) *h* | 78.2 ± 3.9 a A (A) *a* | 40.1 ± 2.0 b C (C) *d* | 58.6 ± 2.9 a B (B) *b* | 26.6 ± 1.3 b D (D) *h* |
| p-values (32 combinational treatments, 4 × 2 × 2 × 2 multifactorial design, General Linear Model) | | | | | | | | |
| Bud development stage (A): 0.000*** | | | | | | | | |
| Cultivation year (B): 0.773 ns | | | | | | | | |
| Sampling date (C): 0.733 ns | | | | | | | | |
| Fertilization type (D): 0.733 ns | | | | | | | | |
| (A)*(B): 0.075 ns | | | | | | | | |
| (A)*(C): 0.000*** | | | | | | | | |
| (A)*(D): 0.000*** | | | | | | | | |
| (B)*(C): 0.733 ns | | | | | | | | |
| (B)*(D): 0.733 ns | | | | | | | | |
| (C)*(D): 0.733 ns | | | | | | | | |
| (A)*(B)*(C): 0.000*** | | | | | | | | |
| (A)*(B)*(D): 0.898 ns | | | | | | | | |
| (A)*(C)*(D): 0.000*** | | | | | | | | |
| (B)*(C)*(D): 0.773 ns | | | | | | | | |
| (A)*(B)*(C)*(D): 0.004** | | | | | | | | |

Means ± standard deviation (SD) within rows related to different fertilization treatments (Control, BUD 14), sampling dates (8/4/20, 16/4/20, 10/4/21, 20/4/21) and cultivation years (2020, 2021) per bud phenological stage followed by different letters denote significant differences (Fischer's test, $p \leq 0.05$). Small letters denote differences between the two fertilizer treatments per sampling date and per year. Capital letters within a row denote differences among the two fertilization treatments and the two sampling dates per year (2 × 2 experimental design, 4 combinational treatments). Capital letters in parenthesis within a row denote differences among the two fertilization treatments, the two sampling dates and the two cultivation years (2 × 2 × 2 experimental design, 8 combinational treatments). Small letters in italics within columns and rows denote differences among the four bud phenological stages, the two fertilization treatments, the two sampling dates and the two cultivation years (4 × 2 x 2 x 2 experimental design, 32 combinational treatments).

*Effect of the BUD 14 biostimulant on flowering rates and flower quality characteristics*

In the second experimental period (2021), the flowering rates of open and closed flowers were significantly higher (88.6%) and lower (7.2%), respectively in the BUD 14 treatment than in the control; however, the flowering rates (4.2-4.4%) in triple classified flowers were similar in both fertilization treatments (control, BUD 14) with non-significant difference. In the case of either open or closed flowers, the effect of the fertilization type on flowering rate was significant. General Linear Model demonstrated the interactive effect

of flower type (open, closed, triple) with fertilization type (control, BUD 14) and that of flower type as main factor (Figure 3).

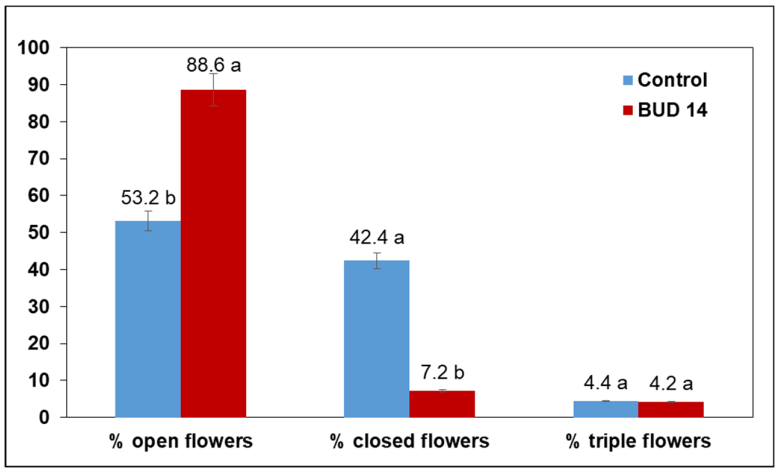



**Figure 3.** Flowering rates (%) and classification into open, closed and triple flowers between the two fertilization treatments (control, BUD 14) in the second cultivation year of 2021. Error bars are standard deviations. Means followed by different letters between the two fertilization treatments (control, BUD 14) separately for flowering rates of open-, closed- and triple-flowers denote significant differences (Fischer's test, $p \leq 0.05$). P-values [open or closed flowers: 0.000, triple flowers: 0.288, flower type (A): 0.000, fertilization type (B): 1.000, A*B: 0.000]

In 2021, inflorescence measurements on female flowers revealed the following: fresh weight, dry weight, dry matter and length of the ovary, as well as the length of the pedicel were increased in the BUD 14 treatment compared to the control, while pedicel thickness and fresh weight as well as maximum ovary diameter were not significantly affected. In male flowers, the maximum diameter of the pollen grain and area in polar view were larger in the BUD 14 treatment compared to the control, while its perimeter did not change significantly (Table 2).

**Table 2.** Flower and pollen quality parameters under control and BUD 14 fertilization treatments during the second experimental cultivation year of 2021

| Flower type | Flower and pollen quality parameters | Control | BUD 14 | p-value |
|---|---|---|---|---|
| Female flowers | Ovary fresh weight (mg) | 271.0 ± 9.0 b | 300.0 ± 10.0 a | 0.045* |
| | Ovary dry weight (mg) | 43.0 ± 2.0 b | 51.0 ± 2.6 a | 0.013* |
| | Ovary dry matter (%) | 16.0 ± 0.2 b | 17.0 ± 0.3 a | 0.039* |
| | Maximum ovary diameter (mm) | 9.0 ± 0.5 a | 9.0 ± 0.5 a | 0.936 ns |
| | Ovary length (mm) | 8.0 ± 0.2 b | 9.0 ± 0.3 a | 0.028* |
| | Pedicel fresh weight (mg) | 156.0 ± 7.8 a | 174.0 ± 8.7 a | 0.056 ns |
| | Pedicel length (mm) | 55.0 ± 2.8 b | 69.0 ± 3.5 a | 0.006** |
| | Pedicel thickness (mm) | 2.0 ± 0.1 a | 2.0 ± 0.1 a | 1.000 ns |
| Male flowers | Maximum pollen grain diameter (μm) | 21.0 ± 0.2 b | 22.0 ± 0.3 a | 0.024* |
| | Pollen grain perimeter in polar view (μm) | 67.0 ± 3.4 a | 69.0 ± 3.5 a | 0.521 ns |
| | Pollen area in polar view ($\mu m^2$) | 300.0 ± 3.0 b | 317.0 ± 6.9 a | 0.043* |

Means ± SD followed by different letters on the same row denote significant differences (Fischer's test, $p \leq 0.05$). ns: non-significant difference at a 5% value ($p > 0.05$), * significant difference at a 5% level ($p \leq 0.05$), ** significant difference at a 1% level ($p \leq 0.01$)

*Effect of the BUD 14 biostimulant on fruit quality characteristics*

During the harvest period of each cultivation year (2020, 2021), the average fruit weight in the BUD 14 treatment was found to be significantly increased as compared with the control (Table 3; Figure 4), however fruits soluble solids content and acidity (%) as well as flesh resistance to pressure were not influenced significantly by fertilization treatment in each cultivation year (2020, 2021) (Table 3). Dry matter of kiwi fruits was significantly enhanced by BUD 14 but only in the second (2021) cultivation year in relation to the control (Table 3). Thus, application of BUD 14 in the second cultivation year of 2021 yielded simultaneously the best fruits quality characteristics (*i.e.* 119.2 g average weight, 6.7% soluble solids, 1.14% acidity, 6.8 kg $cm^{-2}$ firmness, 19.4% dry matter) (Table 3; Figure 4). Combined statistics between the two treatments (control, BUD 14) and the two cultivation years (2020, 2021) revealed that quality characteristics of kiwi fruits were superior in both experimental periods (2020 and 2021) of plants treated with BUD 14, except of dry matter (%) being optimum at 2021 (Table 3).

**Table 3.** Quality characteristics of the kiwi fruits in the two treatments (Control, BUD 14) during the harvest period of two successive cultivation years (2020, 2021)

| Cultivation year | Treatment | Average fruit weight (g) | Soluble solids (%) | Acidity (% citric acid) | Flesh resistance to pressure (kg $cm^{-2}$) | Dry matter (%) |
|---|---|---|---|---|---|---|
| 2020 | Control | 108.1 ± 5.4 b B | 6.5 ± 0.3 a A | 1.23 ± 0.06 a A | 6.6 ± 0.3 a A | 17.3 ± 0.9 a B |
| | BUD 14 | 122.3 ± 6.1 a A | 6.8 ± 0.3 a A | 1.19 ± 0.06 a A | 7.0 ± 0.4 a A | 18.3 ± 0.9 a AB |
| 2021 | Control | 105.0 ± 5.3 b B | 6.7 ± 0.3 a A | 1.18 ± 0.06 a A | 6.6 ± 0.3 a A | 18.2 ± 0.9 b AB |
| | BUD 14 | 119.2 ± 6.0 a A | 6.7 ± 0.3 a A | 1.14 ± 0.06 a A | 6.8 ± 0.3 a A | 19.4 ± 1.0 a A |
| p-value (2020 year) | | 0.040* | 0.380 ns | 0.460 ns | 0.205 ns | 0.239 ns |
| p-value (2021 year) | | 0.037* | 0.837 ns | 0.460 ns | 0.485 ns | 0.196 ns |
| p-value (combined statistics, General Linear Model) | | | | | | |
| Cultivation year (A) | | 0.371 ns | 0.900 ns | 0.187 ns | 0.571 ns | 0.099 ns |
| Fertilization type (B) | | 0.003** | 0.602 ns | 0.282 ns | 0.145 ns | 0.049* |
| (A)*(B) | | 0.995 ns | 0.418 ns | 1.000 ns | 0.605 ns | 0.869 ns |

Means ± SD followed by different small letters in the same column between the two fertilization treatments (Control, BUD 14) for each cultivation year (2020, 2021) separately denote significant differences (Fischer's test, $p \leq 0.05$). Means ± SD followed by different capital letters in the same column among the two treatments and the two years denote significant differences (Fischer's test, $p \leq 0.05$). ns: non-significant difference at a 5% value ($p > 0.05$), * significant difference at a 5% level ($p \leq 0.05$), ** significant difference at an 1% level ($p \leq 0.01$)

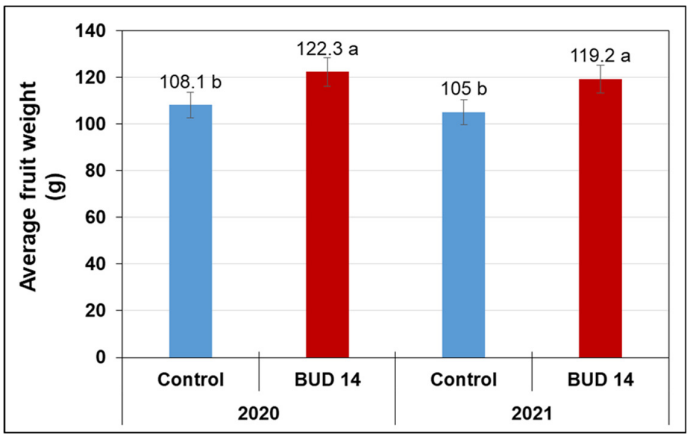



**Figure 4.** Average fruit weight (g) of kiwi plants in two different cultivation years under two different fertilization treatments. Error bars are standard deviations. Means followed by different letters between the two fertilization treatments (control, BUD 14) for each cultivation year (2020, 2021) denote significant differences (Fischer's test, $p \leq 0.05$)

In the second cultivation year (2021), fruit weight showed an increase in the BUD 14 treatment compared to the control starting from the fruit set stage, maintained throughout the vegetative period until harvest (0-145 days from full blossom) (Figure 5). Macroscopically, a slight increase was observed in the vertical and horizontal meridians of the fruits treated with BUD14 as compared with the control treatment (Supplementary Figure 2).

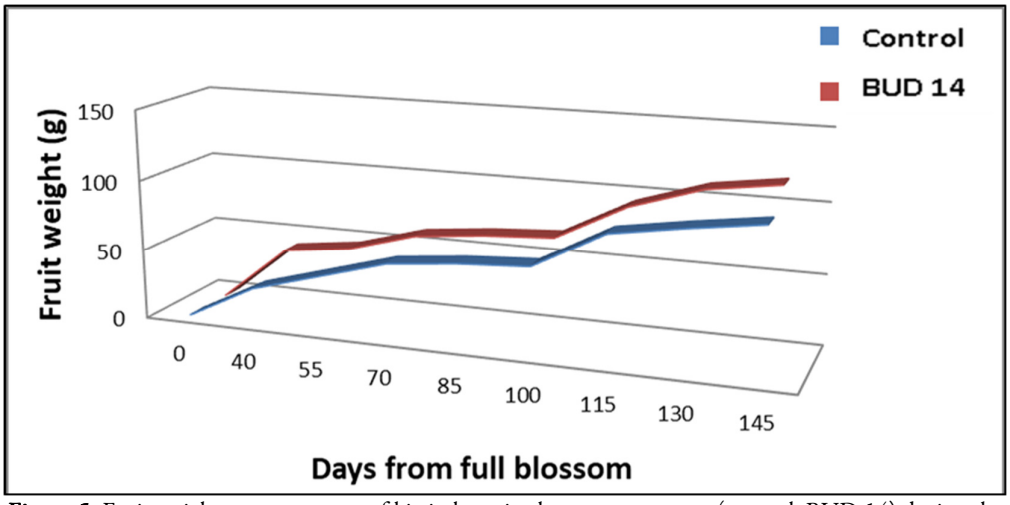



**Figure 5.** Fruit weight measurements of kiwi plants in the two treatments (control, BUD 14) during the vegetative period of the second cultivation year of 2021 made at regular intervals from fruit set to harvest (*i.e.* 0, 40, 55, 70, 85, 100, 115, 130 and 145 days from full blossom)

*Effect of the BUD 14 biostimulant on length of cane and number of fruits per cane*

In the first cultivation year of 2020, specifically on 13/5/2020 the average length of the cane in the control treatment was 65 cm (Figure 6, Supplementary Figure 3A) while on BUD 14 it was 90 cm (Figure 6; Supplementary Figure 3B). On 20/6/2020 and during the fruit set period, the average number of fruits per cane in the control treatment was 35 (Figure 6; Supplementary Figure 3C) while in the BUD 14 treatment it was 62 (Figure 6, Supplementary Figure 3D). Both the length of cane and number of fruits/cane were significantly higher in the plants subjected to BUD 14 treatment compared to the control (Figure 6; Supplementary Figure 3A-3F). The effect of fertilization treatment both on length of cane and number of fruits was significant.

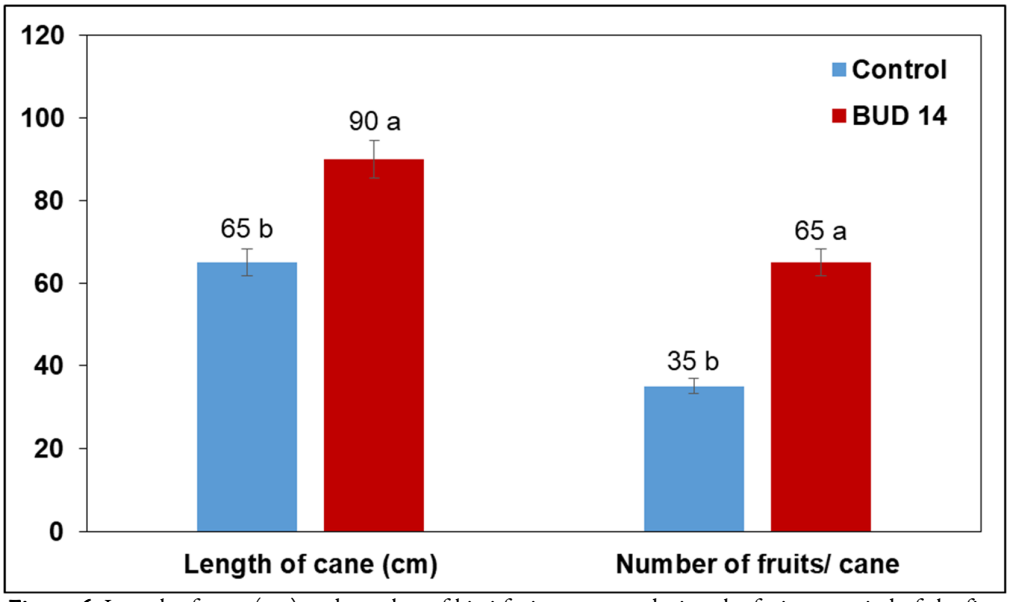



**Figure 6.** Length of cane (cm) and number of kiwi fruits per cane during the fruit set period of the first cultivation year of 2020, in the two fertilization treatments (control, BUD 14). Error bars are standard deviations

Means followed by different letters between the two treatments for each parameter denote significant differences (Fischer's test, $p \leq 0.05$).

*Effect of the BUD 14 biostimulant on leaf nutrient concentrations*

In the second experimental period (2021), leaf Ca (2.89% d.w.) and Mg (1.20% d.w.) concentrations were significantly increased in BUD 14 treated plants, compared to the control (2.47% d.w. Ca and 0.86% d.w. Mg) ($p= 0.017$ for Ca and 0.001 for Mg $\leq 0.05$); however non-significant differences were observed between the treatments (control, BUD 14) regarding the other nutrient concentrations (N, P, K, B, Mn, Zn, Fe, Cu) ($p= 0.053$-$1.000 > 0.05$) (Table 4).

**Table 4.** Determination of inorganic nutrients, consisted of macronutrients including total nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), and magnesium (Mg) expressed as percentage (%) of dry weight (D.W.), and micronutrients including boron (B), manganese (Mn), zinc (Zn), iron (Fe), and copper (Cu) expressed as mg $Kg^{-1}$ of dry weight (D.W.), in kiwi plant leaves during the second cultivation year of 2021 under BUD 14 and non-sprayed control treatments

| Nutrients content | Fertilization treatment | | p-value |
|---|---|---|---|
| | Control | BUD 14 | |
| Total N (%) | 2.65 ± 0.13 a | 2.60 ± 0.13 a | 0.662 ns |
| P (%) | 0.20 ± 0.01 a | 0.23 ± 0.02 a | 0.053 ns |
| K (%) | 2.08 ± 0.10 a | 2.14 ± 0.11 a | 0.523 ns |
| Ca (%) | 2.47 ± 0.12 b | 2.89 ± 0.14 a | 0.017* |
| Mg (%) | 0.86 ± 0.04 b | 1.20 ± 0.06 a | 0.001** |
| B (mg $kg^{-1}$) | 66.00 ± 3.30 a | 68.00 ± 3.40 a | 0.505 ns |
| Mn (mg $kg^{-1}$) | 62.00 ± 3.10 a | 59.00 ± 2.95 a | 0.291 ns |
| Zn (mg $kg^{-1}$) | 35.00± 1.75 a | 35.00± 1.75 a | 1.000 ns |
| Fe (mg $kg^{-1}$) | 60.00 ± 3.00 a | 58.00 ± 2.90 a | 0.453 ns |
| Cu (mg $kg^{-1}$) | 7.00 ± 0.55 a | 6.00 ± 0.45 a | 0.055 ns |

Means ± S.D. followed by different letters in the same row denote significant differences (Fischer's test, $p \leq 0.05$). ns: non-significant difference at a 5% value ($p > 0.05$), * significant difference at a 5% level ($p \leq 0.05$), ** significant difference at a 1% level ($p \leq 0.01$)

## Discussion

Unlike inorganic fertilizers that provide nutrients, biostimulants have been shown to improve nutrient uptake and translocation, induce resistance to specific biotic and abiotic stress through activation of enzymes activity, and corroborate the photosynthetic apparatus, thus enhancing total plant yield, productivity and quality traits (*i.e.* sugar and protein contents, fruit color, seed formation, shelf life, nutrient use efficiencies) (Du Jardin, 2012).

Research has shown that calcium acts as a carrier that signals the onset of bud dormancy. Moreover, there are very interesting studies according to which certain organic nitrogenous compounds such as amino acids (e.g. arginine, tryptamine), proteins and peptides, while being at relatively low levels during bud dormancy, their levels gradually increase from the onset of dormancy break until its complete release. Exogenous nitrogen availability has been shown to help increase the concentration of these organic nitrogenous compounds in the buds and break dormancy. In addition, the rapid accumulation of reactive oxygen species and reactive nitrogen species play a crucial role in the process of breaking bud dormancy (Pang *et al*., 2007). The special composition of the BUD 14 product, used in kiwi plants through foliar sprays that allows very high rates of uptake by the trees during the period of its application and the high content of different forms of nitrogen and calcium, effectively supports the above-mentioned mechanisms of breaking bud dormancy.

A possible explanation for the higher percentage of open flowers in association with the improved flower, pollen and kiwifruit attributes in the BUD 14 treatment compared to the control could be that the first flowers on each plant usually have larger ovaries with more cells than the last, and at harvest the fruits from the first flowers are larger (Lai *et al*., 1990). In addition, flowering time affects flower size and their potential to produce larger fruits (Cruz-Castillo *et al*., 2002). The increased fruit weight has been linked to better flower quality, particularly of larger ovaries (Mcpherson *et al*., 1994). The assertive relationship between fruit size (*i.e.* kiwifruit average weight) at harvest and pedicel length under BUD 14 treatment herein could be attributed to the increased translocation of carbohydrates to the kiwifruit as the pedicel size in kiwifruit canes may constitute a countermeasure of the fruit to natural conditions or cultural practices that enhance fruit sink strength (Cruz-Castillo and Woolley, 2006), for instance augmented pedicel length and flower size were achieved by chilling period extension in sweet cherry (Mahmood *et al*., 2000). In contrast with the results presented in this study, none of the tested commercial Ca products plus B affected mean fruit weight of kiwi fruit cv. Tsechelidis compared with the control for the two years cultivation (Koutinas *et al*., 2010).

An effective way for increasing the productivity of kiwifruit orchards is through application of long-term fertilization (Liu *et al*., 2020), particularly of inorganic fertilizers that lead to elevated fruit yields, stem diameter, leaf number and area (Zhang *et al*., 2020). Optimum plant growth and development is highly hinge on nutrients balance (Peuke, 2010), mainly of N, P, K and S that perform a strong linear interactive effect (Ogidi *et al*., 2018). The Ca formulation used and application synchronization schedule conduce to the quality attributes of kiwifruits (Sotiropoulos *et al*., 2021). The increased amount of Ca in the kiwi leaves under BUD 14 treatment herein could be ascribed to the stimulating impact of Ca on cell wall stability and structure, presumably leading to less fruit mass loss and greater preservation of kiwifruit physicochemical attributes (Sotiropoulos *et al*., 2023) since the key factor for Ca uptake is the fruit growth (*i.e.* kiwifruits of higher dry mass after fertilization including organic Ca-formulated biostimulants) (Sotiropoulos *et al*., 2021). In agreement also with the findings obtained herein, foliar sprays with fertilizer containing organic Ca increased the leaf Ca levels in the kiwifruit cultivar 'Tsechelidis' (Sotiropoulos *et al*., 2021). The higher leaf Ca and Mg levels in the BUD 14 treated plants herein could be due to the enhancing effect of this biostimulant on plants' moisture status, increasing Ca and Mg nutrients uptake and accumulation; the fact that Mg was also increased after BUD 14 application (containing CaO 5.5% w/w) could be ascribed to synergistic interaction between Ca and Mg, as was also revealed by other studies (Koutinas *et al*., 2010; Zhu *et al*., 2011). Then, the enhanced Ca and Mg levels might have led to increased chlorophyll contents and net photosynthetic rate (Pn) (Chatzistathis

and Papaioannou, 2019), thus improving fruit quality characteristics such as average weight and dry matter content, both considered as important quality criteria, determining the market prices of kiwifruit. Magnesium (Mg) is actively involved in the photosynthetic apparatus function (Pn), chlorophyll production and carbohydrate metabolism, while Ca is implicated in the biological membranes of organelles and Pn via regulation activity of phosphatase enzymes, which participate in the carbon-reduction-cycle (Shahid and Liu, 2022). In agreement with our findings with kiwi fruit cv. Hayward treated with the BUD14 formulation, N, P, Fe, Mn, and Zn concentrations of leaves in kiwi fruit cv. 'Tsechelidis' were also not influenced by the foliar sprays of all Ca-containing compounds for both cultivation years (Koutinas *et al.,* 2010). In kiwi fruit cv. 'Tsechelidis', all Ca products increased Ca concentration but hardly affected Mg concentration in the leaves (Koutinas *et al.,* 2010), which is partly in line with our results in cv. Hayward since both Ca and Mg concentrations were increased under the BUD 14 treatment. Supplementing some commercial products with N and amino acids has been reported to influence foliar absorption due to the fact that urea facilitates the simultaneous influx of other nutrients (*i.e.* Ca, Mg) at both the cuticular and cellular levels (Weinbaum, 1988).

The advantageous impact of BUD 14 herein on growth parameters, including the increase in cane length, number of fruits per cane, and average kiwifruit weight could be ascribed either to: (i) the activity of the contained plant growth regulators in this nitrogen-calcium formulation such as cytokinins, auxins, gibberellins, and mineral nutrients by boosting cell division and cell elongation and lead in turn to a higher photo-assimilate supply to the fruits (Babita and Rana, 2015; Colla *et al*., 2014; Rana *et al*., 2023), or to: (ii) the increased photosynthetic rate and chlorophyll production, linked to the increased concentration of photo-assimilates in the fruits, raising as a consequence average fruit weight (Khan *et al*., 2012). Finally, the positive impact of BUD 14 on plant growth could also be ascribed to the production of reactive oxygen/nitrogen species and hormonal signaling biostimulants-induced (Kerch, 2015).

Flesh resistance of kiwi fruits to pressure herein was not influenced significantly by the BUD 14 fertilization in contrast to the increase recorded in kiwifruits of cv. Hayward subjected either to different Ca fertilization treatments and collected at the harvest period (Sotiropoulos *et al*., 2021) or sprayed up to three times during fruit development with $CaCl_2$ (Gerasopoulos *et al*., 1996). Cicco *et al.* (2007) reported that kiwifruit firmness throughout the postharvest period could be linked with Ca content. Even though total soluble solids and total acidity of kiwifruits cv. 'Hayward' herein were not affected by the BUD 14 fertilization treatment, soluble solids and acidity content of kiwifruits cv. 'Hayward' were decreased and increased, respectively after foliar sprays with $CaCl_2$ (Gerasopoulos *et al*., 1996). A possible explanation for the non-significant differences between the two treatments (control, BUD 14) regarding the content of the kiwifruits in soluble solids, acidity, and flesh resistance to pressure could be: an immoderate Ca concentration in the soil (Scudellari, 1998), absence of correlation between leaf Ca levels and fruit quality traits (Vilhena *et al*., 2002), the reduction in the degradation of total sugars present in fruits during storage (Soppelsa *et al*., 2018), an inadequate absorption of macronutrients in plant tissues including kiwifruits due to the depressed catalytic action of micronutrients functioning as boosters of the macronutrients uptake (Rana and Rana, 2003) (*i.e.* BUD 14: high N+Ca macronutrient/low micronutrients ratio), and/or the balanced sugar buildup, sugar delivery into fruit tissues, and conversion of organic acids to sugars in both treatments (Soppelsa *et al*., 2018). Even though not all impacts of Ca on fruit quality appear to be assertive, it is evident that Ca formulations, their rate, and timing of application influence the effectiveness of Ca on various fruit quality parameters (Sotiropoulos et al., 2010).

The significance of the nitrogen-calcium BUD 14 biostimulant applied through foliar sprays in bud dormancy release and development, leaf nutrition, flower and fruit quality of kiwifruit cv. 'Hayward' as a useful biostimulant to encounter climate change challenges related to food security and environmental degradation, is foreseen. Nitrogen use efficiency is a sustainable practice for promotion of productivity and quality consisted of crop diversification, alternate land-use management, and agricultural intensification due to nitrogen involvement in short- and long-term global warming and cooling effects (Zhang *et al*., 2015; Zhang *et al*., 2016).

Calcium might expedite bud breaking effect by actively intervening into the GA and ABA signaling routes, specifically triggering GA biosynthesis and signalling overpowering those of ABA (Gai *et al*., 2024). In addition, Ca included in BUD 14 is an essential nutrient linked to fruit quality in kiwifruit (*e.g.* premature kiwifruit softening under low Ca concentrations) since minor amounts of Ca are concentrated in the fruit related to those of other nutrients presumably owing to Ca immobility in kiwifruit plants (Otero *et al*., 2007). It seems that the composition and the form of Ca (organic, inorganic, etc.) and N (urea, ammonium ions, nitrate ions) of different commercial products (BUD14 in this study) play a key role in the absorption and transportation of other nutrients in the different tissues of the plant (Ca and Mg in the leaves, herein). The variations observed between the 2 years of the study on the effectiveness of the BUD14 may have been probably attributed to the impact of the first-year treatment on the second, on the crop load (elevated in the second year), the climatic conditions (temperature, relative humidity, rainfall, sunshine hours), etc. (Koutinas *et al*., 2010). In comparison to previous studies in different cultivars of the kiwi fruit plant, the novelty of this study lays in the simultaneous use of a commercial production, the BUD14, besides fruit quality and plant tissue nutrition, for bud dormancy release and development, flowering precocity and flower quality improvement.

## Conclusions

The outcomes of our study displayed that the BUD 14 biostimulant, applied through foliar sprays, increased the percentage of developed buds, induced synchronization in bud development and raised flowering rate. In addition, BUD 14 increased the percentage of open flowers, improving simultaneously specific flower and pollen quality traits, increased the length of cane and number of fruits per cane, fruit weight and dry matter content, and finally the total production per plant of the kiwifruit cv. 'Hayward'. Its foliar-based nutrition, revealed its potential to break bud dormancy and boost flowering, flower quality and tree productivity. Especially, bud dormancy breaking is of high importance due to climate change and global warming as an adaptation process to the environment. The advantageous foliar application of the nitrogen-calcium BUD 14 in kiwifruit 'Hayward' on bud breaking dormancy and development, flower and fruit quality, and total production can be described as a climate and dormancy modelling, physiology and agronomy tool that could alleviate or even abolish the adverse repercussions of climatic change on fruit production. Besides elevated plant growth and productivity, BUD 14, both as a fertilizer and as a bio-control can upgrade plant's physiological rejoinder concerning nutrients accumulation and amplify nutrient absorption competence. Over and above all the aforementioned, BUD 14 as a biostimulant displays the prospective of reinforcing kiwifruit plant resilience in the face of adversity associated with climate change, to develop more refined products.

## Authors' Contributions

Conceptualization: TS, AV and DT; Data curation: TC, VS and AB; Formal analysis: VS; Funding acquisition: AV; Investigation: TS, IM and MD; Methodology: TS, AV, DT, and IM; Project administration: TS, AV and DT; Resources: TS; Software: TC, MD, AB and IM; Supervision: TS, DT and AV; Validation: TS, DT and AV; Visualization: TS, AV and DT; Writing - original draft: TS, AV, DT, TC and VS; Writing - review and editing: TS, TC and VS. All authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Not applicable.

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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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