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Tomato Firmness and Shelf-Life Increased by Application of Stimulated Calcium

Jolyon Dodgson *, Anna K. Weston and David J. Marks

Levity Crop Science, Crowhall Farm, Newsham Hall Lane, Woodplumpton, Preston PR4 0AS, UK
* Correspondence: jolyon@levitycropscience.com

Abstract: Tomato (*Solanum lycopersicum* L.) had almost 190 million tonnes produced in 2021. Tomato fruit suffer losses of up to 50% during harvest and transport, which causes financial hardship, reduces the amount of food available and causes environmental harm. Calcium plays an important role in cell wall strength. This work assessed the use of a calcium transport stimulant (MCAS) to increase the firmness of tomato fruit in the laboratory and the foliar application to tomato plants. Then, handling damage was simulated. In the laboratory, the calcium with MCAS significantly increased the fruit firmness compared to the untreated and calcium without MCAS, which were not significantly different. When calcium with MCAS was applied to tomato plants foliarly before harvest, the calcium with MCAS-treated fruit were significantly firmer than the untreated or calcium without MCAS-treated fruit for up to 10 weeks after harvest, and this was achieved by applying only 0.91 kg ha^{-1} calcium. Finally, when fruit were exposed to a simulated drop, the calcium with MCAS-treated fruit remained firmer than the undamaged fruit or dropped fruit with calcium without MCAS. Calcium with MCAS could significantly increase the fruit firmness and increase the shelf-life of tomatoes while applying less calcium.

Keywords: *Solanum lycopersicum* L.; MicroTom; cherry tomato; calcium transport stimulant; MCAS

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is a key vegetable for human consumption that has beneficial impacts due to its nutritional composition containing carbohydrates, protein and vitamins as well as its anti-oxidant, anti-inflammatory and anticancer effects [1]. In 2021, global tomato production covered 5,167,388 ha with a yield of almost 190 million tonnes [2]. Tomatoes are climacteric fruit, meaning they can be harvested before they are at the fully ripe stage and they will continue to ripen until the color is red, but they deteriorate rapidly after harvest [3]. Harvested tomatoes are susceptible to damage postharvest as they are soft, and much damage is caused by handling during transport and storage [4]. In developing countries, the storage, transport and packing of horticultural crops postharvest can cause 50% losses [5]. Fruit firmness is a key trait that enables the transport and storage of tomatoes as harder fruit will be able to better resist damage [6], while too much softening of fruit postharvest is the main factor impacting the shelf-life and reducing it [7]. When considering food supply chains for tomatoes and their environmental impacts, wastage accounted for up to 33% of their contribution to climate change [8], so being able to produce fruit that is firmer and better able to withstand postharvest handling will therefore reduce wastage and it could have a significant impact on tomatoes' environmental impact.

Calcium has been shown to be a key nutrient for the development and growth of plants as it is an essential nutrient that is also linked to cell wall strength and stability [9,10]. When calcium is present in cell walls, they have increased strength, which means the plant cells are able to better resist damage, especially in fruit that can become damaged once it has been harvested and is being handled and stored under suboptimal conditions. Calcium is taken up via the roots and moved to the leaves and shoots in the xylem [11]. This mass flow

of the calcium bypasses the young leaves and fruits as it mainly moves to the established mature leaves, so calcium deficiencies are often found in the new leaves and fruit [10]. Once the calcium has reached the mature tissue, there is only very limited remobilization of it to the young leaves and fruits via the phloem [10].

Previous work has assessed the effects of calcium chloride ($CaCl_2$), chitosan, hydrogen peroxide and ozonated water on the firmness of tomatoes linked to their quality and storage ability with chitosan and calcium chloride being the best treatments when applied postharvest [3]. Other work has also shown that foliar calcium application resulted in better growth, yield and quality of four tomato cultivars, thereby indicating that good nutrition with essential nutrients will increase tomato production [12]. Postharvest application of 2% (w/v) calcium lactate in distilled water could maintain the quality of tomato fruit when an ultrasound treatment was also used for between 15 and 40 min [13]. Postharvest dipping of tomato fruit in 0, 2 and 6% $CaCl_2$ for between 10 and 30 min was shown to increase fruit firmness under the 6% treatment for 20 and 30 min [14]. Additionally, it has been shown that the foliar application of calcium before harvest can also affect the postharvest physiology of tomatoes. When tomato plants were treated with foliar applications of $CaCl_2$ at 10 kg ha^{-1} 16, 8 and 3 d before harvest, it resulted in the fruit firmness being maintained at a higher level than in the untreated plants [15]. Other work assessed the source of calcium with calcium sulfate, calcium nitrate, calcium silicate, poultry manure and pressmud (sugarcane processing waste product) being used at application rates up to 80 kg ha^{-1} calcium. Poultry manure resulted in the greatest fruit firmness after harvest with the calcium sulfate, calcium nitrate and calcium silicate all resulting in similar firmness levels [16].

The previous work has shown that calcium application in a range of forms and at different times can improve the postharvest firmness and shelf-life, but the application rates are high, which is due to the way that calcium is transported through the plant and that it does not normally travel to fruits during their development, so large applications are needed for enough calcium to reach the fruit. Previously, a calcium transport stimulant (MCAS) has been developed that stimulate calcium transport into plant cells, as auxins do, but without the effect of being a plant growth hormone [17]. In strawberry, MCAS has been used to increase the fruit firmness and postharvest shelf-life while applying one-third of the amount of calcium as in the industry standard application [18], while in apple, it has reduced bitter pit, which is a disorder related to poor calcium transport [17].

This work aimed to assess the applicability of using calcium with an MCAS (Albina containing LoCal, Levity Crop Science, Preston, UK) to increase the postharvest firmness of tomato. To do this, calcium, with and without the MCAS, was applied to harvested fruit in the laboratory and to tomato plants in a glasshouse. The successful use of calcium and an MCAS to tomato would benefit growers by reducing the amount of calcium they would need to apply and increasing their saleable yields as the fruit would have better postharvest characteristics and shelf-life.

2. Materials and Methods

The work consisted of three separate experiments to show the impact of calcium applied with the MCAS vs. calcium applied without the MCAS, which are referred to as Ca+ai (active ingredient, the MCAS) and Ca–ai, respectively. Both the Ca+ai and Ca–ai treatments were identical apart from whether the MCAS had or had not been added.

2.1. Application to Harvested Tomatoes

Cherry tomatoes were purchased from a supermarket in the UK. Several punnets of cherry tomatoes were used in each repeat of the experiment, and for each repeat, they were selected to be the same variety, have the same best before date and to have come from the same supplier, and they were all purchased on the same day. All the cherry tomatoes were mixed, and fruit with similar weights were used. This part of the work aimed to determine the potential of using calcium with an MCAS to impact fruit firmness; therefore, as long

as the fruit had similar pre-purchase management, which we ensured was true by buying fruit of the same variety, from the same grower and with the same best before date, there would be limited impact of the precise agronomic management of the fruit production process not being known.

There were three treatments: untreated, misted with 5% Ca–ai and misted with 5% Ca+ai, which were applied once at the start of the experiment. For each repeat, 40 fruit were used for each treatment. To mist the 40 fruit in each of the Ca–ai and Ca+ai treatments, 5 mL of the 5% treatments was applied to each, and each treatment contained 0.5 g of calcium. A further 40 fruit had their weight and firmness tested on the day the experiment was set up to give initial values. The untreated, Ca–ai and Ca+ai-treated fruit had their individual weight (laboratory balance, model NVV2002, Scientific Laboratory Supplies Ltd., Nottingham, UK) and firmness recorded every three or four days for up to five times or until there were no differences between the treatments.

Firmness was recorded using a non-destructive penetrometer, Durofel (CTIFL Copa Technologie, Saint Etienne du Grès, France) with a bolt of 3 mm diameter (0.10 cm^2). Each cherry tomato had its firmness recorded twice, on opposite sides of the fruit, and an average was taken to give the firmness for each sampling date [19].

The experiment was repeated three times.

2.2. Application to Tomato Plants before Harvest

MicroTom tomato (*Solanum lycopersicum*) seeds were sown in plug trays filled with Sinclair's reduced peat compost and transplanted to 2 L pots containing the same compost at the four-leaf stage and grown in an experimental glasshouse located in Lancashire, UK. Plants were watered three times a week or as required to maintain pot moisture. An industry standard complete fertilizer was applied at the label rate every three weeks. At flower set, the plants were foliarly treated with Ca–ai or Ca+ai every 7 or 14 days at 1 L ha^{-1} in 200 L ha^{-1} water or untreated. The weekly treatments were applied nine times, and the fortnightly treatments were applied five times. When applying the Ca–ai or Ca+ai treatments at 1 L ha^{-1}, each application contained 181 g of calcium, so the weekly applications gave 1.6 kg ha^{-1} of calcium and the fortnightly treatments gave 0.91 kg ha^{-1} of calcium. In total, 50 plants were used: 10 for each of the five treatments.

As fruit became ripe, it was harvested six times over a one-month period. All harvested fruit was then kept at room temperature and scored for weight and fruit firmness weekly for 11 weeks following harvest.

Firmness was recorded using the same method as in Section 2.1.

2.3. Application to Harvested Tomatoes to Mitigate Handling Damage

This section used store-bought cherry tomatoes, and the method followed that in Section 2.1 closely for obtaining the fruit and measurement of the weight and firmness.

The fruit was treated to simulate it being dropped a moderate distance during harvest and handling. For this, we measured the weight of fruit and determined that a cherry tomato would weigh up to 18 g, and if it was dropped a distance of 0.3 m, it would experience an impact force of 0.053 J. Therefore, to ensure repeatability, the fruit was positioned on a bench, and an 11 g spherical hard object was dropped from a height of 0.5 m giving an impact force of 0.054 J. The fruit was kept stationary so that the impact point could be the same on all fruit rather than the fruit being dropped.

The treatments were undropped and untreated; dropped and untreated; dropped and treated with 5% Ca–ai mist; and dropped and treated with 5% Ca+ai mist applied once at the start of the experiment. As in Section 2.1, each treatment gave an application of 0.5 g calcium.

2.4. Statistical Analysis

Statistical analysis was undertaken using the Jamovi 1.6.23 for Windows software [20] using the $p < 0.05$ significance level. The normality of the data was determined using

a Shapiro–Wilk test followed by either ANOVA with an independent sample t-tests or ANOVA (non-parametric) with Dwass–Steel–Critchlow–Fligner pairwise comparisons.

3. Results

3.1. Application to Harvested Tomatoes

Cherry tomatoes were bought from a supermarket three times and had Ca–ai and Ca+ai or water applied as a mist. Figures 1 and 2 show the results from the three repeats of this experiment. Figure 1A–C shows the weights of the untreated fruit and those treated with Ca–ai and Ca+ai, and in all cases, there were no significant differences between the three treatments for each scoring date within each repeat. For each repeat, there was a significant decrease in the mean weight of all tomatoes from the first to last scoring dates (ANOVA (non-parametric) followed by Dwass–Steel–Critchlow–Fligner pairwise comparisons), with the weight in the first repeat decreasing by 15.6% (Figure 1A), the weight in the second repeat decreasing by 18.8% (Figure 1B), and the weight decreasing by 20.8% in the third repeat (Figure 1C).

Figure 2A–C shows the firmness values of the untreated fruit and those treated with Ca–ai and Ca+ai, and in all cases, the Ca+ai treatment gave significantly firmer fruit than the untreated or Ca–ai treatments, as indicated by an * in the figures for the scoring dates where this was the case. For the first repeat, the Ca+ai fruit were significantly firmer on 9, 13, 16 and 20 days after treatment (DAT) (Figure 2A), while for the second and third repeats, the fruit were significantly firmer on 11, 14 and 18 DAT and 10, 14 and 17 DAT, respectively (Figure 2B,C) (ANOVA followed by independent sample *t*-tests). For the first repeat, the Ca+ai-treated fruit statistically maintained their initial firmness until 20 DAT, while the untreated only maintained it until 9 DAT, and for the Ca–ai, it was until 13 DAT as indicated by the lowercase letters above the bars in the figure (ANOVA followed by independent sample *t*-tests) (Figure 2A). For the second repeat, the firmness of the Ca+ai-treated fruit decreased from treatment to 4 DAT but then remained statistically at the same level until 14 DAT, while both of the other treatments maintained their firmness until only 11 DAT (ANOVA followed by independent sample *t*-tests) (Figure 2B). For the third repeat, the Ca+ai treatment statistically maintained the same firmness from treatment to 10 DAT, while the other two treatments only maintained their firmness until 3 DAT (ANOVA followed by independent sample *t*-tests) (Figure 2C).

3.2. Application to Tomato Plants before Harvest

MicroTom tomatoes were grown in a glasshouse and were untreated or had Ca–ai or Ca+ai applied either weekly or fortnightly, which gave five treatments in total. As the fruit ripened, it was harvested, and its weight and firmness were recorded for 11 weeks after harvest (WAH). There were no significant differences between the treatments for the weight of the fruit for each WAH, but there was a significant decrease in the combined mean weight of the tomatoes from the first to last scoring dates of 39% (ANOVA (non-parametric) followed by Dwass–Steel–Critchlow–Fligner pairwise comparisons) (Table 1).

For the firmness, there was a significant decrease in the firmness from harvest (0 WAH) to 1 WAH, but there were no differences between the five treatments on each of these two scoring dates (ANOVA followed by independent sample *t*-tests) (Table 2). Throughout the experiment, there were no significant differences between the two application timings (7 or 14 d) within the Ca+ai or Ca–ai fruit for each scoring date (ANOVA followed by independent sample *t*-tests) (Table 2). On 3-9 WAH, there were significant differences between the two Ca+ai treatments and the untreated and two Ca–ai treatments, as indicated by an * in those rows (ANOVA followed by independent sample *t*-tests) (Table 2). The firmness of the fruit decreased slightly between 1 and 2 WAH, and then as indicated by the lowercase letters in the columns in the table, it decreased significantly following 2 to 3 WAH for the untreated or two Ca–ai treatments, while for the Ca+ai treatment, it statistically maintained the same firmness level until 5 WAH (ANOVA followed by independent sample *t*-tests) (Table 2).

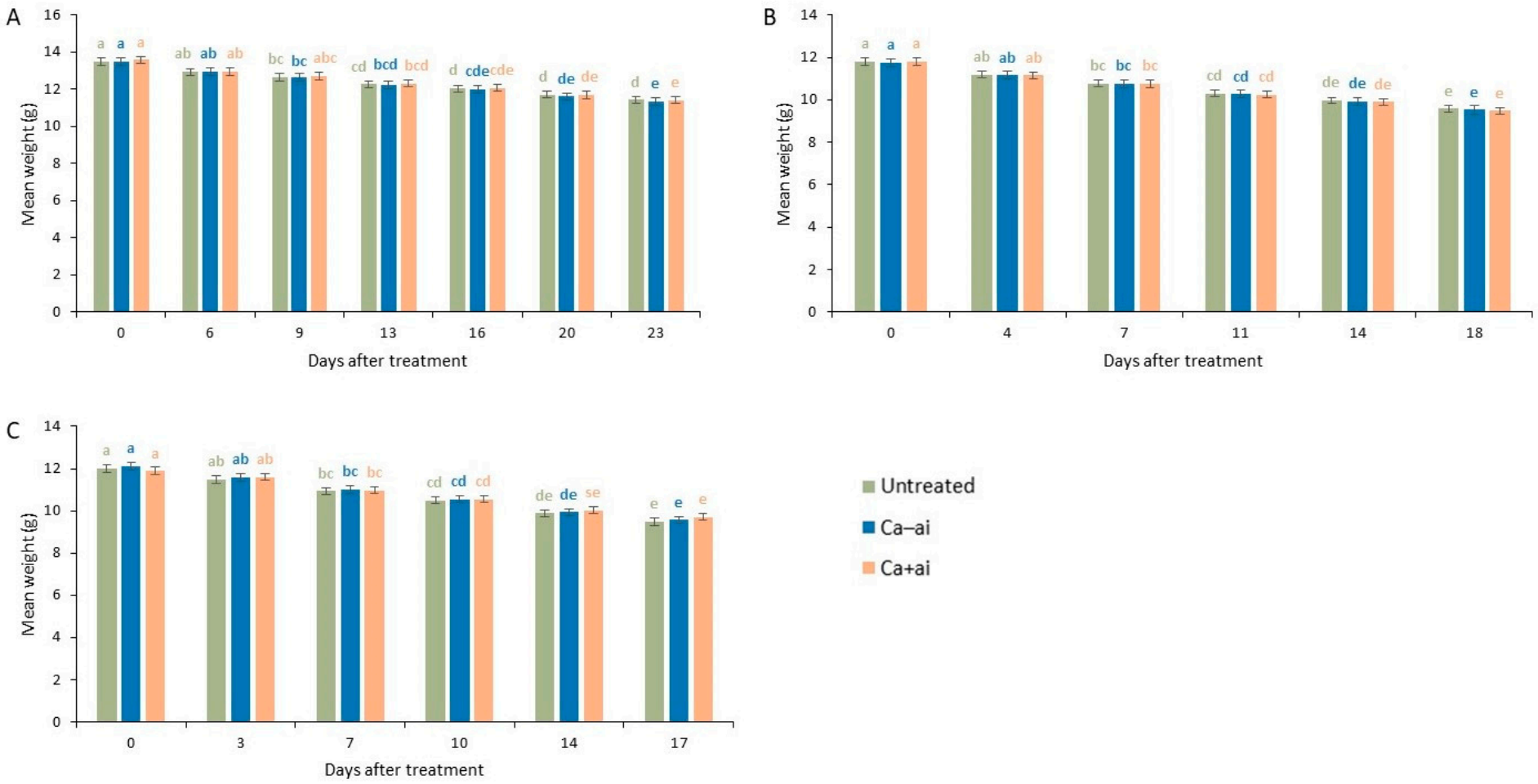

Figure 1. Mean weight (g) for three repeats (**A**–**C**) of experiment for cherry tomatoes brought from a UK supermarket for untreated, misted with 5% Ca–ai (active ingredient—MCAS) or misted with 5% Ca+ai treated once on first scoring date. There were no significant differences between treatments for each repeat, and lowercase letters indicate significant differences between combined weights for scoring dates (ANOVA (non-parametric) followed by Dwass–Steel–Critchlow–Fligner pairwise comparisons, $p < 0.05$). $n = 40$, bars indicate standard errors of means.

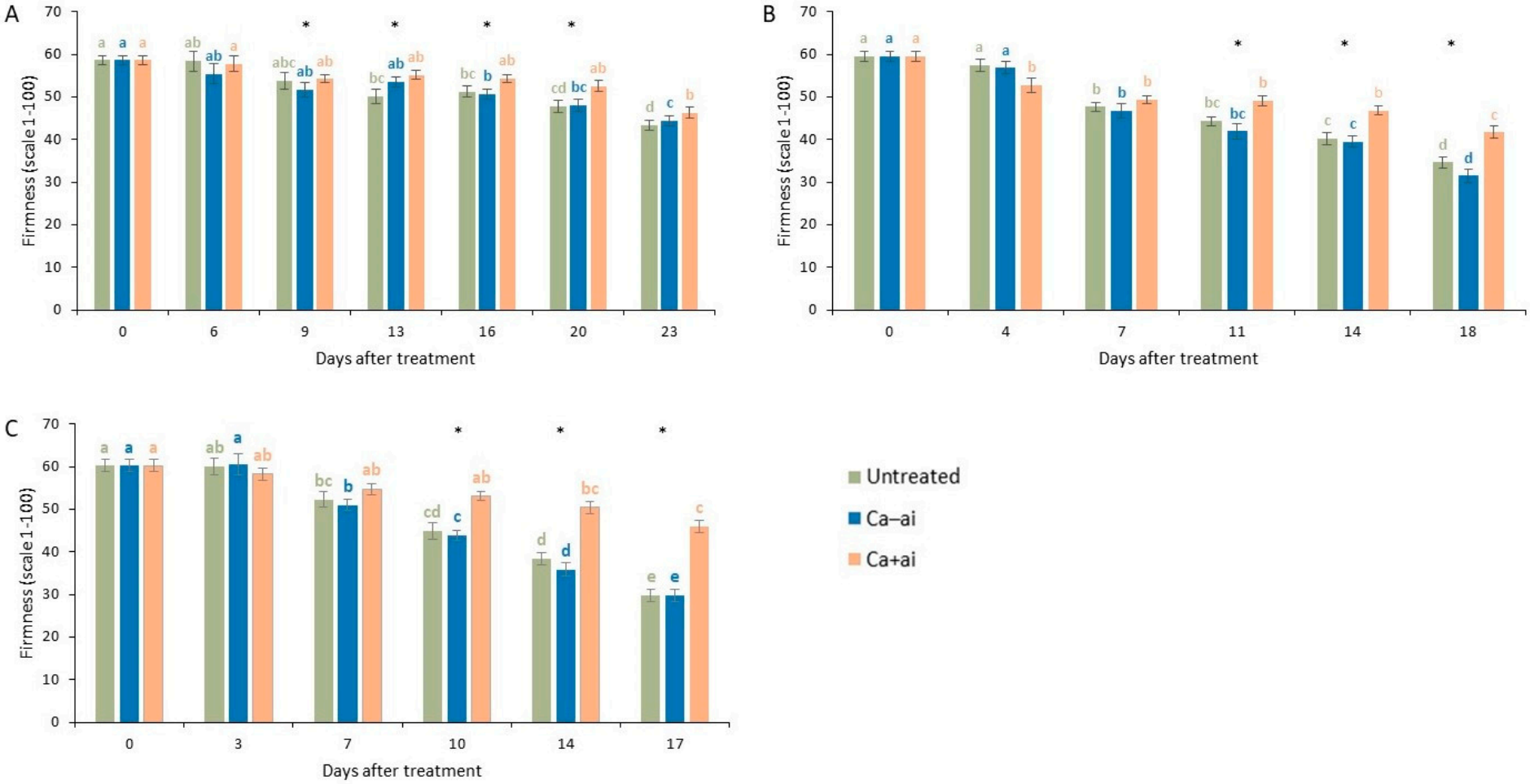

Figure 2. Mean firmness (unitless scale of 0 soft to 100 hard) for three repeats (**A**–**C**) of experiment for cherry tomatoes brought from a UK supermarket for untreated, misted with 5% Ca–ai (active ingredient—MCAS) or misted with 5% Ca+ai treated once on first scoring date. Lowercase letters above bars for each treatment indicate significant differences between scoring dates for that treatment (ANOVA followed by independent sample *t*-tests, $p < 0.05$). * indicates significant differences between the Ca+ai and the other two treatments for that sampling date (ANOVA followed by independent sample *t*-tests, $p < 0.05$). $n = 40$, bars indicate standard errors of means.

Table 1. Mean weight (g) ± standard error of means (n = 135) for 11 weeks postharvest for fruit harvested from MicroTom tomato (*Solanum lycopersicum*) grown in a glasshouse for untreated or foliarly sprayed with Ca–ai (active ingredient—MCAS) or Ca+ai either weekly or fortnightly at 1 L ha^{-1} in 200 L ha^{-1} water. There were no significant differences between treatments for each scoring date (ANOVA), and lowercase letters indicate significant differences between combined weights for scoring dates (within columns) (ANOVA (non-parametric) followed by Dwass–Steel–Critchlow–Fligner pairwise comparisons, $p < 0.05$).

Weeks after Harvest	Untreated	Ca–ai Weekly	Ca–ai Fortnightly	Ca+ai Weekly	Ca+ai Fortnightly
0	3.69 ± 0.13 a	3.69 ± 0.13 a	3.76 ± 0.12 a	3.81 ± 0.13 a	3.75 ± 0.12 a
1	3.52 ± 0.13 ab	3.51 ± 0.12 ab	3.59 ± 0.12 ab	3.65 ± 0.13 ab	3.59 ± 0.11 ab
2	3.39 ± 0.12 bc	3.37 ± 0.12 bc	3.45 ± 0.12 bc	3.52 ± 0.12 bc	3.46 ± 0.11 bc
3	3.26 ± 0.12 cd	3.25 ± 0.12 cd	3.31 ± 0.11 cd	3.39 ± 0.12 cd	3.33 ± 0.11 cd
4	3.13 ± 0.12 de	3.14 ± 0.11 de	3.19 ± 0.11 de	3.27 ± 0.11 de	3.21 ± 0.11 de
5	3.02 ± 0.11 e	3.08 ± 0.11 e	3.07 ± 0.11 e	3.25 ± 0.11 e	3.12 ± 0.1 e
6	2.93 ± 0.11 ef	2.98 ± 0.11 ef	2.99 ± 0.11 ef	3.1 ± 0.11 ef	3.01 ± 0.1 ef
7	2.72 ± 0.11 fg	2.78 ± 0.11 fg	2.77 ± 0.1 fg	2.88 ± 0.11 fg	2.84 ± 0.1 fg
8	2.63 ± 0.11 gh	2.68 ± 0.1 gh	2.64 ± 0.1 gh	2.77 ± 0.11 gh	2.72 ± 0.1 gh
9	2.48 ± 0.11 hi	2.49 ± 0.1 hi	2.47 ± 0.1 hi	2.62 ± 0.11 hi	2.56 ± 0.1 hi
10	2.35 ± 0.1 ij	2.37 ± 0.1 ij	2.32 ± 0.1 ij	2.45 ± 0.11 ij	2.39 ± 0.1 ij
11	2.26 ± 0.1 j	2.28 ± 0.1 j	2.22 ± 0.1 j	2.35 ± 0.1 j	2.28 ± 0.1 j

Table 2. Firmness (unitless scale of 0 soft to 100 hard) ± standard error of means (n = 135) for 11 weeks postharvest for fruit harvested from MicroTom tomato (*Solanum lycopersicum*) grown in a glasshouse for untreated or foliarly sprayed with Ca–ai (active ingredient—MCAS) or Ca+ai either weekly or fortnightly at 1 L ha^{-1} in 200 L ha^{-1} water. Lowercase letters indicate significant differences between scoring dates for that treatment (within columns) (ANOVA followed by independent sample t-tests, $p < 0.05$). * indicates significant differences between the Ca+ai weekly and fortnightly treatments and the other three treatments for that scoring date (within rows) (ANOVA followed by independent sample t-tests, $p < 0.05$).

Weeks after Harvest	Untreated	Ca–ai Weekly	Ca–ai Fortnightly	Ca+ai Weekly	Ca+ai Fortnightly
0	69.57 ± 0.4 a	68.76 ± 0.36 a	69.38 ± 0.37 a	69.04 ± 0.4 a	68.8 ± 0.4 a
1	57.57 ± 0.49 b	57.12 ± 0.45 b	57.62 ± 0.55 b	57.96 ± 0.58 b	56.97 ± 0.55 b
2	54.67 ± 0.57 c	55.58 ± 0.57 b	54.79 ± 0.71 bc	55.1 ± 0.68 c	54.76 ± 0.59 bc
3	51.02 ± 0.59 d	52.59 ± 0.67 c	51.06 ± 0.88 c	54.55 ± 0.79 c *	55.67 ± 0.77 bc *
4	46.81 ± 0.65 e	48.19 ± 0.64 d	46.09 ± 0.97 d	53.92 ± 0.72 c *	53.97 ± 0.79 bc *
5	41.2 ± 0.79 f	42.72 ± 0.75 e	40.47 ± 0.98 e	52.95 ± 0.76 cd *	52.15 ± 0.84 cd *
6	36.77 ± 0.7 g	38.98 ± 0.68 f	36.1 ± 1.03 ef	49.43 ± 0.9 de *	49.77 ± 0.89 de *
7	33.14 ± 0.6 h	34.85 ± 0.73 g	32.59 ± 1 fg	46.5 ± 1.03 ef *	46.8 ± 0.88 ef *
8	33.39 ± 0.68 h	34.66 ± 0.82 g	32.74 ± 0.95 g	42.42 ± 1.18 f *	42.45 ± 1.14 f *
9	31.77 ± 0.75 h	31.49 ± 0.87 gh	29.31 ± 1.11 gh	37.93 ± 1.38 fg *	37.32 ± 1.21 g *
10	26.02 ± 1.04 i	28.43 ± 0.98 hi	26.76 ± 1.21 hi	30.34 ± 1.61 gh	30.6 ± 1.37 h
11	23.88 ± 1.02 i	25.17 ± 1.03 i	22.42 ± 1.18 i	26.03 ± 1.53 h	26.35 ± 1.35 h

3.3. Application to Harvested Tomatoes to Mitigate Handling Damage

Another two batches of tomatoes were bought from the supermarket and had handling damage simulated (simulated drop) so the effect of Ca+ai could be determined. The treatments were undropped, dropped, dropped with Ca–ai and dropped with Ca+ai. For both repeats, there were no significant differences in the weights of the tomatoes between treatments for each scoring date (Figures 3A and 4A). There were significant decreases in the combined mean weight for all treatments from the first to last scoring dates with a 10.7% decrease for the first repeat (Figure 3A) and a 14.1% decrease for the second repeat (Figure 4A) (ANOVA (non-parametric) followed by Dwass–Steel–Critchlow–Fligner pairwise comparisons).

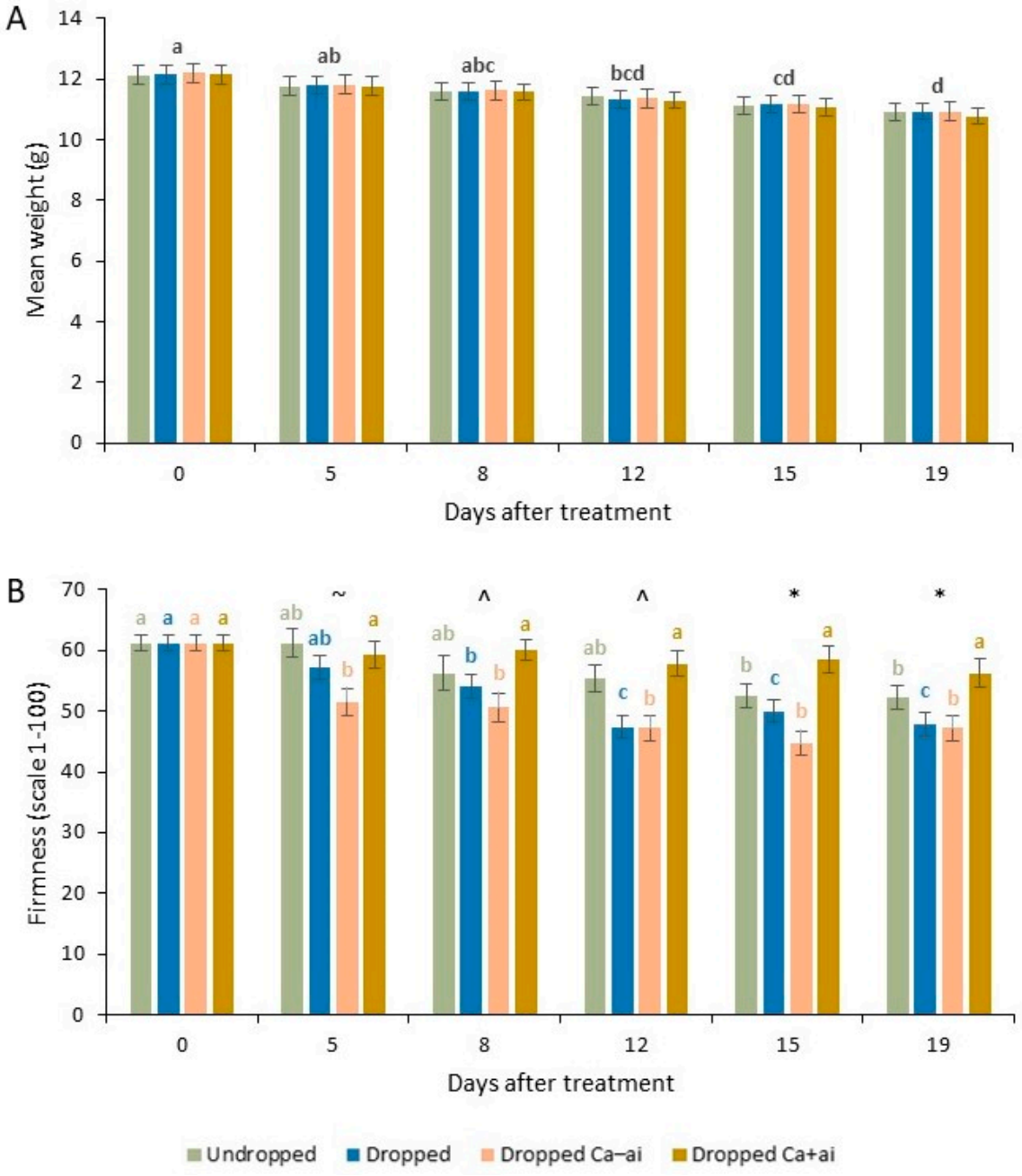

Figure 3. Mean weight (g) (**A**) and firmness (unitless scale of 0 soft to 100 hard) (**B**) for first repeat of experiment for cherry tomatoes brought from a UK supermarket for undropped and untreated (undropped), dropped and untreated (dropped), dropped and misted with 5% Ca–ai (active ingredient—MCAS) (dropped Ca–ai) or dropped and misted with 5% Ca+ai (dropped Ca+ai) treated once on first scoring date. (**A**) There were no significant differences between treatments for each sample date, and lowercase letters indicate significant differences between combined weights for scoring dates (ANOVA (non-parametric) followed by Dwass–Steel–Critchlow–Fligner pairwise comparisons, $p < 0.05$). (**B**) Lowercase letters above bars for each treatment indicate significant differences between scoring dates for that treatment (ANOVA followed by independent sample *t*-tests, $p < 0.05$). ~ indicates significant difference between dropped Ca+ai and dropped Ca-ai treatment, ^ indicates significant difference between dropped Ca+ai and dropped with dropped Ca-ai treatments and * indicates significant differences between the dropped Ca+ai and the other three treatments for that sampling date (ANOVA followed by independent sample *t*-tests, $p < 0.05$).

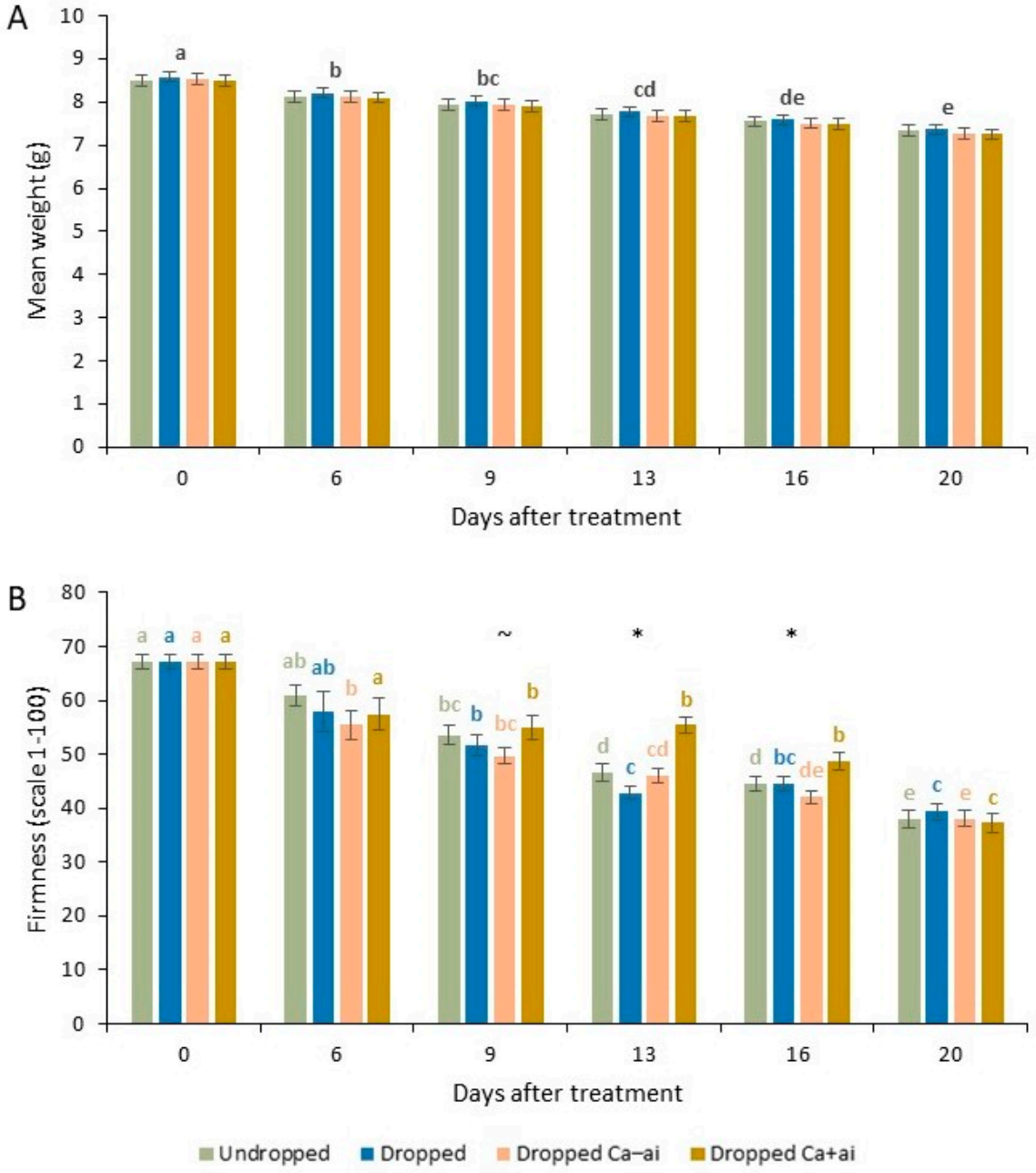

Figure 4. Mean weight (g) (**A**) and firmness (unitless scale of 0 soft to 100 hard) (**B**) for second repeat of experiment for cherry tomatoes brought from a UK supermarket for undropped and untreated (undropped), dropped and untreated (dropped), dropped and misted with 5% Ca–ai (active ingredient—MCAS) (dropped Ca–ai) or dropped and misted with 5% Ca+ai (dropped Ca+ai) treated once on first scoring date. (**A**) There were no significant differences between treatments for each sample date, and lowercase letters indicate significant differences between combined weights for scoring dates (ANOVA (non-parametric) followed by Dwass–Steel–Critchlow–Fligner pairwise comparisons, $p < 0.05$). (**B**) Lowercase letters above bars for each treatment indicate significant differences between scoring dates for that treatment (ANOVA followed by independent sample *t*-tests, $p < 0.05$). ~ indicates significant difference between dropped Ca+ai and dropped Ca–ai treatment and * indicates significant differences between the dropped Ca+ai and the other three treatments for that sampling date (ANOVA followed by independent sample *t*-tests, $p < 0.05$).

For the firmness of the first repeat, on 5 DAT, the dropped tomatoes treated with Ca+ai were significantly firmer than the dropped tomatoes treated with Ca–ai but the same as the

undropped and dropped tomatoes, on 8 and 12 DAT, the dropped tomatoes with Ca+ai were significantly firmer than the dropped tomatoes treated with Ca–ai and the dropped tomatoes but the same as the undropped tomatoes, while on 15 and 19 DAT, the dropped tomatoes treated with Ca+ai were significantly firmer than all the other treatments, as indicated by the symbols (~, ^ and *, respectively) above the scoring dates on the figure (ANOVA followed by independent sample *t*-tests) (Figure 3B). For the second repeat, on 9 DAT, the dropped tomatoes treated with Ca+ai were significantly firmer than the dropped tomatoes treated with Ca–ai but the same as the undropped and dropped tomatoes, and then on 13 and 16 DAT, the dropped tomatoes treated with Ca+ai were significantly firmer than all the other treatments, as indicated by the symbols (~ and *, respectively) above the scoring dates on the figure (ANOVA followed by independent sample *t*-tests) (Figure 4B).

For the first repeat of the experiment, the undropped fruit statistically maintained its starting firmness until 12 DAT, the dropped fruit maintained it until 5 DAT, the dropped treated with Ca–ai had its firmness decrease directly after the treatment, while the dropped fruit treated with Ca+ai statistically maintained its firmness until 19 DAT (ANOVA followed by independent sample *t*-tests) (Figure 3B). For the second repeat, the firmness of all the fruit decreased between the treatment and 6 DAT, and this level was statistically maintained by all the treatments apart from the Ca+ai until 9 DAT, while for the Ca+ai, it was maintained until 16 DAT (ANOVA followed by independent sample *t*-tests) (Figure 4B).

4. Discussion

In plants, calcium is generally transported from the roots to the new leaves, and it bypasses the developing and ripening fruit [10], despite it being a key element for cell wall strength in fruit [9] that is needed for increased postharvest robustness. Previous work has shown that the use of an MCAS and calcium can reduce calcium-related fruit disorders in apple [17] and increase the firmness of strawberries [18]. In this work, we assessed the application of calcium with and without the addition of an MCAS to tomatoes in the laboratory and glasshouse.

In the first part of this work, already harvested and ripe tomatoes were obtained from a supermarket in the United Kingdom and had calcium with and without the addition of the MCAS active ingredient misted onto their surface for 1 min and kept in a laboratory at room temperature. All three repeats of the experiment gave very similar results with the Ca+ai giving significantly firmer fruit (Figure 2A–C). The firmness of the harvested and ripe fruit, when kept at room temperature, was maintained for between 10 and 18 DAT when treated with Ca+ai compared to between 3 and 11 DAT for the untreated or Ca–ai treatments. This shows that the Ca+ai treatment was able to significantly improve the firmness of harvested and ripe fruit; the addition of the MCAS to calcium meant that it had impacts on the fruit that were not shown by just the calcium treatment without the MCAS added. This effect on the fruit was achieved by misting just 5 mL of the 5% product that contained only 0.5 g of calcium that was left on the fruit for only 1 min.

Previous work has shown mixed results from the postharvest application of calcium to increase tomato firmness and therefore increase the shelf-life. Calcium chloride (1%) was able to slow the rate at which the firmness reduced for fruit stored at 10 °C for up to 28 d with two days at room temperature, but the starting firmness of the untreated tomatoes was quite a bit less than that of the calcium-treated tomatoes [3], which can be compared to an extended storage time of up to seven days in this work, but the fruit was stored at room temperature, so the storage time increases are not directly comparable. In that work, the fruit was immersed in the 1% calcium chloride solution for 5 min, which could slow the harvesting and processing of the fruit much more than just misting the fruit, as was used in the current work. Another study used cherry tomatoes, as in this work, and a 2% calcium lactate treatment, but the treatment step involved using a sonicator at ultrasound energy densities of between 16 and 36 W L^{-1} at temperatures of between 15 and 35 °C for between 5 and 40 min for washing the cherry tomatoes in the calcium treatment [13]. The firmness was maintained when the energy density was 20 W L^{-1} at 15 °C for 15 min. The ultrasound

significantly increased the calcium content in the treated fruit, so the authors concluded that ultrasound and calcium lactate could be a suitable method for maintaining cherry tomato texture, but this seems quite an involved process compared to using an MCAS to increase the firmness of cherry tomatoes, as shown in the present study. Additionally, the authors only recorded the Ca content and firmness directly after treatment and did not keep any fruit to determine the impact of the treatment on the shelf-life, unlike in the current work, where the shelf-life was measured. Another study used a dipping method for the treatment of postharvest fruit where 0, 2 and 6% calcium chloride solutions were used for dipping fruit for 10, 20 and 30 min, with the 6% solution at 20 and 30 min giving significantly higher firmness levels for up to 12 d [14]. This study again used calcium to increase the firmness of tomatoes, but the application method would not be suitable for commercial application, as the fruit would need to be washed for up to 30 min and a 6% solution is needed, while in the current study, misting for only 1 min was used with 0.5 g of calcium applied. In a further study, tomatoes were treated in a hot water bath (40 or 50 °C) for two minutes before two minutes in a 2% calcium chloride solution, which was followed by storage at 10 °C for up to 14 d, which again showed that the loss of firmness could be controlled by calcium [21]. Again, this is a more complex treatment method than the one used in the current work, and the fruit were not stored at room temperature, as in the current work, so the increase in shelf-life of seven days at room temperature is not directly comparable to the 14-day increase the authors found, as tomatoes lose quality at room temperature much faster than when kept refrigerated. Another study dipped harvested tomatoes in 4, 8 and 12% calcium chloride solutions for 10 min with the 8% treatment giving the best result of the lowest loss of physicochemical characteristics [22]. This first application of calcium with MCAS to cherry tomatoes in the laboratory in the current study has shown that it can increase the firmness of the fruit while requiring a much simpler application method and smaller amount of calcium than in previously published work, as shown by the comparison of those previous studies with the key findings from this work in this section.

Once the initial laboratory work had shown that Ca+ai could increase the firmness in harvested fruit via misting, a glasshouse trial was conducted to assess the application of the Ca+ai foliarly and its impact on postharvest firmness and shelf-life. MicroTom tomatoes were grown in a glasshouse and were either untreated or had Ca–ai or Ca+ai applied either weekly or fortnightly. For both the Ca–ai and the Ca+ai treatments, there were no differences between the weekly or fortnightly applications (Table 2), which shows that the reduced application schedule would be suitable, thereby saving growers the time and money involved with more frequent applications. The Ca+ai treatment gave significantly firmer fruit than the untreated or Ca–ai from 3 to 9 WAH. This was in fruit that were stored at room temperature, so shelf-life and firmness could be maintained for even longer if cold storage was used. The application rate was 1 L ha^{-1} of product per application, which contained 181.2 g calcium per application. There were five applications for the fortnightly treatment schedule, so 0.91 kg ha^{-1} of calcium with the MCAS was applied to give the significantly firmer fruit in this work.

Previous work assessing the effect of foliar applications of calcium to fruit firmness has found that the firmness can be impacted. For example, 0.5, 1 and 1.5% calcium chloride concentrations were applied foliarly twice over the growing season with the fruit firmness increasing as the calcium concentration increased with the 1.5% concentration giving the greatest fruit firmness [12]. The application rate for the 1.5% calcium chloride treatment is not given in the work, but if it had been applied at 200 L ha^{-1}, as in the current study, that would have been 2.46 kg ha^{-1} calcium per application, so the two applications would apply 4.92 kg ha^{-1} calcium. Meanwhile, the current work only applied 0.91 kg ha^{-1} calcium, which could maintain the firmness when applied with an MCAS. Additionally, the study did not look at the storage characteristics of the fruit or the impact of the Ca application on the firmness after storage, so the current results offer new information over this previous study as they show that the application of Ca+ai increased fruit firmness for up to 9 WAH. Another study looked at different calcium sources (calcium sulfate, calcium nitrate, calcium

silicate, poultry manure and pressmud), which were all applied at rates up to 80 kg ha^{-1} calcium [16], which can be compared to the rate of 0.91 kg ha^{-1} used in this study. Their findings showed that the highest fruit firmness over 15 d monitoring postharvest was obtained from the 80 kg ha^{-1} calcium as poultry manure. Compared to the poultry manure, the calcium sulfate, calcium nitrate and calcium silicate all gave quite similar but lower firmness values for each application rate. In this work, the lowest rate tested, of 20 kg ha^{-1} calcium, was much higher than the 0.91 kg ha^{-1} calcium used in the current work, while the current study obtained firmness increases for up to 9 WAH rather than just 15 d. In an alternative piece of work, calcium chloride was applied foliarly at 10 kg ha^{-1} three times before harvest and compared to untreated plants [15], and the calcium treatment resulted in firmer fruit than the untreated for up to 21 d after harvest when kept at 12 °C. This improvement in the firmness from the calcium treatment was obtained by applying 30 kg ha^{-1}, which is more than the 0.91 kg ha^{-1} applied in the current study, and the increased fruit firmness was only obtained for 21 d rather than the 9 WAH that the current work has demonstrated. The current work has replicated the previous results and increased the postharvest firmness of tomatoes that were treated foliarly with calcium before harvest but when using a much lower application rate of calcium with MCAS, while the calcium treatment without the MCAS was not any better than the untreated treatment, which shows that the MCAS is the reason for the increased firmness.

Finally, we assessed the ability of calcium with MCAS to prevent or reduce the damage from postharvest bruising and improper handling. Tomatoes are a soft fruit that deteriorates quickly after harvest [3], especially due to handling during the transport of the fruit [4], and there can be losses of up to 50% in developing countries [5]. Calcium application postharvest to tomatoes has been shown to be critical for maintaining the quality [23]. Impact damage occurs to tomatoes during harvest, handling and processing [24]. To do this, we obtained harvested and ripe cherry tomatoes from a UK supermarket and simulated them being dropped 0.3 m. This was accomplished by keeping the tomato stationary and dropping a hard object onto it, so the impact force (0.054 J) was equivalent to a 0.3 m drop for an 18 g cherry tomato. Forces of 0.125 to 0.5 J have been used to simulate bruising and handling damage, for bruise modeling, to larger tomatoes previously [24], so the force used in this work was in line with pervious values used when considering the size of the tomato. In the first repeat, on 8 and 12 DAT, the dropped tomatoes treated with Ca+ai had the same firmness as tomatoes that had not been dropped or treated, while on 15 and 19 DAT, the Ca+ai-treated fruit were firmer than all the other fruit. Throughout this experiment, the firmness of the Ca+ai-treated tomatoes was not significantly different from the initial firmness (Figure 3). In the second repeat, on 9 DAT, the undropped and untreated tomatoes were the same as the dropped and Ca+ai-treated fruit with both being significantly firmer than the dropped and untreated or the dropped and Ca–ai-treated fruit, while on 13 and 16 DAT, the dropped and Ca+ai-treated fruit were firmer than the fruit from all other treatments (Figure 4). This shows that misting with 5 mL of product containing 0.5 g calcium could prevent the fruit softening that comes from damage during the harvest and transport of tomatoes.

There is limited previous research on using calcium applications to mitigate and prevent postharvest damage and bruising in tomatoes, so this work is one of the first to show this benefit of applying calcium that can be transported into tomato fruit. Calcium treatments have been shown to reduce and prevent bruises and damage, from handling, developing postharvest in apples [25] and lemons [26]. In tomato, when the fruit is diced for consumption either raw or canned, a calcium treatment has been shown to increase the firmness and ability to resist mechanical abuse [27]. In that work, a 600 mg L^{-1} calcium chloride solution was used to dip the tomato dices for 5 min at 24 °C either before or after the mechanical abuse. The current work used 500 mg L^{-1} calcium, which was less than this previous study, thereby providing a more efficient use of calcium. All samples exposed to calcium were firmer than the untreated samples, and there was no difference in the firmness between treatment before or after mechanical abuse. The current work supports

this, as we have found that postharvest treatment with calcium and MCAS can reduce and prevent postharvest damage in whole fruit, which will be very valuable knowledge for growers who face significant losses from postharvest handling and transport due to damaged fruit.

Calcium is an essential structural element for plant cells [9], and it is supplied to cells via ion channels linked to constitutive Ca^{2+} influx channels, which are linked to auxin [11] when new cells are forming, but once plant cells start to increase in size rather than number, the auxin level reduces, and so does the calcium supply. The MCAS in this work simulates the presence of auxin in mature cells, thereby enhancing the transport of calcium into the cells [17]. Calcium determines the strength and rigidity of cell walls as it forms cross-links in the middle lamella [10]; therefore, if there is limited calcium in plant cells, the cell walls will be weakened. This work has shown that when calcium is applied to tomatoes and tomato plants with the MCAS, the fruit are significantly firmer than when calcium is applied without the MCAS. As the MCAS enables the transport of calcium into mature plant cells, the cells will be firmer, as the presence of the extra calcium will enable the cell walls to be strengthened, which will result in firmer fruit.

In all the experiments presented here, there were no significant differences between the weights of the tomatoes for any of the treatments (Figures 1A–C, 3A and 4A and Table 1). The weights decreased over time but by a similar amount for all the tomatoes in each of the treatment groups. The treatment with calcium and MCAS did not impact weight loss in the current work.

5. Conclusions

This work has shown that a low level of calcium (5 mL containing 0.5 g), which does not impact the firmness of tomatoes when compared to untreated, can increase the firmness when applied with a calcium transport stimulant (MCAS) in the lab to harvested and ripe fruit. The fruit was purchased from the supermarket, so it had already been stored and transported to the shop from where it had been harvested. The method used in this work was much simpler than other methods presented in the literature, and so, it would be more applicable to growers who will be under time constraints to harvest, pack and ship the fruit to retailers. This work then proceeded to show that calcium with MCAS could be applied foliarly before harvest and that this would have a similar effect and significantly increase the firmness of the harvested fruit for several weeks over calcium without the MCAS that was similar to the untreated. The amount of calcium applied using the product in this work was much less than that used in previous studies to obtain similar effects. This reduction in the amount of calcium applied would benefit growers as they would need to purchase less product, increase the nutrient use efficiency and increase their saleable yield as the fruit would last longer. Finally, it has been shown that the application of calcium and MCAS to tomatoes can mitigate simulated impact damage that would bruise fruit and prevent it from being suitable for sale. The treated fruit maintained a firmness that was as good as if not better than undamaged fruit. This would prevent losses, thereby increasing the growers' profits, reduce food waste and reduce the environmental impact of tomato production as higher saleable yields could be obtained from the same land area while applying a lower rate of calcium.

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Conflicts of Interest: The authors are employees of a company that may be affected by the presented research. The mentioning of specific commercial products is only for providing information and does not provide a recommendation. Other similar products could be used to conduct similar research.

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