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Induction of fruit calcium assimilation and its influence on the quality of table grapes

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Abstract

Sprays containing soluble Ca, polypeptidic N and Ti ascorbate in several combinations were applied to cv. Crimson table grape vines *(Vitis vinifera* L.). Foliar spraying resulted in the accumulation of N, P, K, Ca, Mg, Fe, Zn, Cu and Ti in the leaves, but not of Na, Cl or Mn. In the berries, Ca, Fe, Zn and Cu concentrations increased in the skin and flesh. These berries were also larger than controls, firmer, had a deeper external red colour, and their weight loss during postharvest storage was reduced. The increase in the Ca and micronutrient content of the fruit is explained as a consequence of the beneficial effect of Ti on absorption, translocation and assimilation processes. In turn, improved Ca assimilation by the fruit was responsible for the beneficial effects seen on firmness and storage life.

Additional key words: colour, firmness, organic acids, plant nutrition, polypeptidic N, sugars, titanium

Resumen

Inducción de la asimilación de calcio en el fruto y su influencia sobre la calidad de la uva de mesa

Plantas de uva de mesa de la variedad Crimson *(Vitis vinifera* L.) se rociaron durante el ciclo de cultivo con disoluciones que contenían Ca soluble, N polipeptídico y ascorbato de Ti, en todas las combinaciones posibles. Las aplicaciones foliares indujeron un aumento de la concentración foliar de todos los elementos, salvo para Cl, Na y Mn. Sin embargo, la composición mineral de la piel y pulpa de los granos de uva sólo se vio afectada en lo que respecta a Ca, Fe, Zn y Cu, que aumentaron significativamente sus niveles. Asimismo, aumentó el tamaño del grano, su consistencia y color rojo, al tiempo que disminuyó la pérdida de peso durante el almacenamiento. La inducción de la concentración de Ca y de los tres oligoelementos en los tejidos del grano de uva se explica por el conocido efecto inductor de los bioactivadores, en especial del Ti, sobre los procesos de absorción, transporte y asimilación en el fruto. A su vez, el aumento de la cantidad de Ca metabolizado en piel y pulpa es responsable de los beneficios obtenidos en consistencia y vida útil del fruto.

Palabras clave adicionales: ácidos orgánicos, azúcares, color, consistencia, nitrógeno polipeptídico, nutrición vegetal, titanio.

Introduction

The postharvest quality of several fruits is closely related to a number of preharvest factors, including the environment in which they are grown and the cultivation practices to which they are subject. Seasonal growing temperatures, light conditions, the amount of rainfall and irrigation, mineral nutrition status and fertilization, pest management and maturity at the time of harvest can all affect postharvest quality, storage life and the susceptibility of crops to disorders and diseases (Wang, 1997).

N and Ca play important roles in all of aspects of plant physiology, including postharvest fruit quality. Fruit flavour ratings taken after several months of storage have been reported negatively correlated with leaf N, although lower N treatments result in smaller fruits and vegetables (Mattheis and Felman, 1999) and the use of N-containing growth regulators during cropping has been shown to negatively affect texture (Sams, 1999). Ca affects fruit softening since it is essential in the structure of the cell wall and also

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influences cell membrane integrity (Fallahi *et al.*, 1997). It has been reported that the postharvest infiltration of fruits with certain Ca^{2+} salts initially improves resistance against mechanical damage during storage, but later promotes decay, shortening storage life. Results have been better when the cation has been applied in-season, but the beneficial effects depend on the mode of application, salt type and period (Crisosto *et al.,* 2000). These difficulties in fruit Ca assimilation are probably due to the method by which Ca is absorbed, a process regulated in a manner different to that for other nutrients. This cation moves passively through the transpiration flux from the soil (Marschner, 1995) the fruit accumulating most of its Ca during the first 15-30 days after anthesis. After this time, fruit Ca assimilation is practically undetectable (Bernadac *et al.,* 1996). Fruits therefore commonly have low Ca concentrations and show high susceptibility to Ca deficiency problems. The use of preharvest Ca sprays on apple (Hickey *et al.,* 1995; Fallahi *et al.,* 1997), plum (Alcaraz-López *et al.,* 2003, 2004a), peach (Alcaraz-López *et al.,* 2004b) and nectarine (Alcaraz-López *et al.,* 2004c) do not increase total or cell wall Ca concentrations sufficiently to affect fruit firmness. However, it has been reported that when Ca is applied in combination with polypeptidic N, and especially with leaf assimilatable Ti^{+4} ascorbate, fruits show a significant increase in skin and flesh Ca, are firmer, and have a longer storage life (Alcaraz-López *et al.,* 2003, 2004a,b,c).

The aim of the present work was to study the effects of leaf applications of soluble Ca, polypeptidic N and Ti^{+4} ascorbate on cv. Crimson table grape vines in terms of Ca assimilation and fruit quality.

Material and Methods

Plant material and treatments

Four year-old table grape vines *(Vitis vinifera* L., cv. Crimson) growing under a drip fertirrigation regime in a 5 ha plot provided the experimental plant material. All vines were grown under the same environmental conditions, and received the same doses of irrigation water, fertilizer and crop protectants. Eight suitably distant sub-plots (four vines each x three replications) were selected and received the following leaf treatments per vine: 1.- Control: water spray only (5 L); 2.- Ca: 5 L of 0.1 mM soluble Ca (4 mg Ca L^{-1}, LIFE-Ca®); 3.- polypeptidic N (5 L of 0.1 mM soluble N; 1.4 mg N L^{-1}, LIFE-Mar®); 4.- Ti: 5 L of 0.042 mM Ti^{4+}ascorbate (2 mg Ti^{4+} L^{-1}); 5.- Ca+N (5 L of 0.1 mM Ca, 0.100 mM N); 6.- Ca+Ti (5 L of 0.1 mM Ca, 0.042 mM Ti^{4+}; 7.- N+Ti (5 L of 0.1 mM N, 0.042 mM Ti^{4+}); 8.- Ca+N+Ti (5 L of 0.1 mM Ca, 0.1 mM N, 0.042 mM Ti^{4+}).

Two leaf applications were made as follows in 2001: on the 5th April, five days after anthesis and with sufficient leaf development, and on the 20th April, when the leaves were fully developed.

Data collection and samplings

During the last week of November 2000, after pruning, two supposedly productive canes per vine, with similar developmental status and orientation, were selected and marked. All data for the next vegetative cycle were obtained from these branches. Leaves were sampled three times: on the 5th April, just before the treatments, on 30th March, and on 28th August (harvest) 2001. In addition, at harvest, all of the commercial clusters on each cane were sampled together to form a single sample (3×2×2 samples/treatment) for the determination of berry size (diameter, volume, weight), external colour, firmness, storage life and biochemical and mineral composition.

Determination of colour and firmness

Determination of colour variables. Colour was determined using the Hunter-Lab system with a Minolta CR200 colorimeter. Three determinations of colour variables were made along the equatorial axis of each fruit: L*: light (+) - dark (–); a*: red (+) - green (–); b*: yellow (+) - blue (–). The red grape colour index (RGCI = $[180 - (b^* \cdot a^{*-1})] \cdot [L^*+(a^{*2}+b^{*2})^{½}]^{-1}$; Carreño *et al.,* 1995) was obtained from these data.

All grape mechanical properties were determined using a Lloyd Universal Assay Machine (UAML), model LR5K (Lloyd Instruments, Segensworth, UK), interfaced with a computer.

Puncture assay. The force required to puncture the fruit was determined using a 2 mm diameter probe mounted on the UAML. After making contact with the skin, the needle continued to move at 20 mm min^{-1}

over a distance of 3 mm. Three measurements were made at the berry equator at an angle of 60° and the mean calculated. A bevelled holder prevented bruising on the opposite side. The results are expressed in Newtons (N).

Crushing assay. The force required to crush the fruit was determined using the UAML equipped with two parallel plates that approached one another at 20 mm s^{-1}. The maximum force and deformation were recorded at the moment of breaking. The results are presented as the means of 20 berries from each sample.

Analytical determinations

Mineral composition. The leaves were carefully washed three times with deionised water. The berries were similarly washed and the skin separated from the flesh. All leaf, berry skin and berry flesh samples were dried to a constant weight in a forced air oven (65 ± 3°C), pulverized in a stainless steel mill, and stored in thermosealed bags at 0 ± 3°C in darkness until analysis. Sample mineralisation was performed by the semimicro Kjeldahl procedure for total N, and by the nitric-perchloric acid method for the other elements. Phosphorus was determined by spectrophotometry of the phospho-molybdo-vanadate complex. Cations and metallic elements were determined by atomic absorbance spectrophotometry (AAS) and Ti by AAS using a graphite chamber device.

Grape juice collection. About 100 berries were squeezed and the collected juice centrifuged for 30 min at 25,000 rpm. The supernatant was diluted with double distilled water (1:2 v v^{-1}), filtered through a C18 sep-pak, and then through a 0.45 µm Millipore filter.

Titratable acidity. Potentiometric determinations of an aliquot of grape juice, suitably diluted with double distilled water, were undertaken using a METROHM potentiometer attached to a DOSIMAT 665 and a Titroprocessing 686 apparatus. The results are expressed as g of malic acid per 100 g of fresh flesh.

Soluble solid concentration. The soluble solid concentration was determined by refractometry (Warsawa refractometer, model RL2), using an aliquot of the grape juice. The results are expressed as °Brix.

Sugars and organic acids. Sugar and organic acid concentrations were determined by HPLC (Hewlett-Packard®, DAD1, Sig=210.16 Ref.=360.100) using an aliquot of grape juice. The mobile phase was 0.1% phosphoric acid (0.5 ml min^{-1}). Results are expressed as g or mg per 100 ml of grape juice.

Statistical analysis. All analyses were performed using the SAS statistical software package. Means were compared using Tukey's HSD test.

Results and Discussion

Table 1 shows the mineral composition of the leaves at each of the sampling points. No significant differences were seen before the application of the sprays. However, the treatments induced significant increases in the concentrations of most of the elements studied. Only leaf concentrations of Cl, Na and Mn remained unaffected. These effects on leaf mineral balance are in agreement with results obtained in other plants after the application of Ti (Pais, 1983; Carvajal and Alcaraz, 1998a; Wojcik and Wojcik, 2000; Alcaraz-López *et al.,* 2003, 2004a,b,c).

The addition of Ti improved mineral absorption. This agrees with that observed in other studies in which this trace element improved the production of biomass and nutrient absorption, especially that of N, P, K, Ca and Mg (Ram *et al.,* 1983; Frutos *et al.,* 1996; López-Moreno *et al.,* 1996).

All treatments affected fruit growth, except for the Ca treatment. In addition, all treatments increased the number of clusters obtained over that of control vines (data not shown). All of the Ti-treated vines showed a statistically significant increase in berry size (Fig. 1). The best results were obtained with treatments 3 (polypeptidic-N), 4 (Ti^{4+}-ascorbate), 7 (N + Ti), and especially 8 (Ca+N+Ti).

Some increases were seen in the N, P, K and Mg concentrations of the skin and flesh of the treated vines, although these were not significant compared to controls (Table 2). No differences in Cl, Na, Ti or Mn concentration were seen in either fruit tissue between the control and treated plants, while the concentration of Ca, Fe, Cu and Zn was significantly higher in both tissues in Ti-treated plants (compared to controls). This increase in fruit Fe concentration induced by Ti agrees with previous results reported for vegetables and fruit trees (Carvajal and Alcaraz, 1995, 1998a,b; Carvajal *et al.,* 1995a; Wojcik and Wojcik, 2001; Alcaraz-López *et al.,* 2003, 2004a,b,c). The beneficial effect of Ti

Table 1. Effects of foliar applications of sprays containing soluble Ca, polypeptidic N and Ti ascorbate on leaf mineral composition of table grapes (cv. Crimson). Each value is the mean of 12 samples (dry matter)

	Sampling	Treatment							
		Control	Ca	N	Ti	Ca+N	Ca+Ti	N+Ti	Ca+N+Ti
N, g kg^{-1}	Before treatments	32.4	32.3	34.3	32.1	32.2	32.2	34.5	32.4
	After treatments	20.6a	29.1bc	30.1bc	30.4bc	29.3bc	29.0b	30.8c	29.0bc
	At harvest	15.6a	16.4a	21.8c	25.7d	18.4b	26.7de	27.5e	27.8e
P, g kg^{-1}	Before treatments	1.02	0.97	1.02	1.06	0.99	0.98	1.00	1.07
	After treatments	0.48a	0.52a	0.62bc	0.66cd	0.59b	0.66cd	0.69cd	0.70d
	At harvest	0.49a	0.53a	0.65b	0.78cd	0.73c	0.82de	0.85de	0.86e
K, g kg^{-1}	Before treatments	8.3	8.2	8.0	8.9	8.1	8.6	8.5	8.2
	After treatments	8.5a	9.1ab	9.6bc	10.8cd	9.6b	10.9d	11.9de	12.8e
	At harvest	13.1a	15.2b	16.4bc	17.0cd	15.7bc	16.8bc	17.3c	16.4bc
Ca, g kg^{-1}	Before treatments	2.14	2.15	2.15	2.05	2.12	2.16	2.07	2.15
	After treatments	3.54a	4.40c	3.95b	4.32c	4.56c	4.99d	4.40c	5.21d
	At harvest	4.11a	4.96bc	4.82b	5.03bc	5.08bcd	5.29bcd	5.36cd	5.53d
Mg, g kg^{-1}	Before treatments	0.32	0.33	0.35	0.32	0.32	0.33	0.32	0.34
	After treatments	0.56a	0.66b	0.69bcd	0.67bc	0.67bc	0.69bcd	0.71cd	0.72d
	At harvest	0.59a	0.61a	0.68b	0.68bc	0.68b	0.72bc	0.75bc	0.77c
Cl, mg kg^{-1}	Before treatments	0.80	0.82	0.79	0.82	0.82	0.79	0.82	0.82
	After treatments	0.78	0.73	0.73	0.71	0.72	0.72	0.79	0.78
	At harvest	0.71	0.73	0.78	0.80	0.70	0.80	0.78	0.80
Na, mg kg^{-1}	Before treatments	53	56	51	53	53	51	53	54
	After treatments	50	47	47	46	48	47	48	49
	At harvest	49	48	50	49	46	49	51	49
Fe, mg kg^{-1}	Before treatments	150	142	149	147	155	140	149	151
	After treatments	184a	194ab	218c	223cd	207bc	220cd	237d	258e
	At harvest	191a	198a	212b	239cd	224b	246c	272de	297e
Mn, mg kg^{-1}	Before treatments	207	201	201	207	198	206	207	209
	After treatments	180	182	179	167	177	187	169	170
	At harvest	156	162	160	159	160	165	155	167
Zn, mg kg^{-1}	Before treatments	9	9	11	10	9	11	11	10
	After treatments	11a	13b	16c	22e	15c	18d	22e	32f
	At harvest	13a	14a	18b	29cd	20b	29c	32c	37d
Cu, mg kg^{-1}	Before treatments	49	49	48	51	49	51	49	50
	After treatments	64a	69b	85cd	86cd	80c	90de	94ef	99f
	At harvest	58a	61a	74b	79bc	76b	86c	96de	99e
Ti, mg kg^{-1}	Before treatments	2.1	1.8	2.0	2.4	1.7	2.1	2.3	2.2
	After treatments	2.0a	1.6a	2.1a	16.2b	1.9a	15.1b	15.7b	15.6b
	At harvest	2.5a	2.8a	2.3a	19.3b	2.4a	18.8b	20.4b	19.7b

For each element and sampling, values followed by the same letter, or without a letter, are not significantly different at $p < 0.05$.

Table 2. Effects of foliar application of sprays containing soluble Ca, polypeptidic N and Ti ascorbate on berry mineral composition of table grapes (cv. Crimson) at harvest. Each value is the mean of 12 samples (dry matter)

		Treatment							
		Control	**Ca**	**N**	**Ti**	**Ca+N**	**Ca+Ti**	**N+Ti**	**Ca+N+Ti**
N, g kg^{-1}	Berry	14.9	14.5	15.5	15.6	15.1	15.0	15.5	15.5
	Skin	12.5	12.3	13.5	13.1	13.0	12.6	13.3	13.2
	Flesh	15.5	15.1	16.0	16.2	15.7	15.6	16.1	16.1
P, g kg^{-1}	Berry	1.28	1.14	1.05	1.21	1.25	1.08	1.17	1.17
	Skin	1.59	1.51	1.58	1.60	1.55	1.43	1.70	1.66
	Flesh	1.20	1.05	0.91	1.11	1.17	0.99	1.03	1.04
K, g kg^{-1}	Berry	16.2	15.8	16.4	16.6	17.2	15.8	16.6	16.3
	Skin	12.9	13.1	13.1	13.3	13.1	13.0	13.3	13.4
	Flesh	17.0	16.6	17.2	17.5	18.3	16.6	17.5	17.1
Ca, g kg^{-1}	Berry	1.64a	1.70a	1.78ab	2.11bc	2.04b	2.23cd	2.30d	2.53e
	Skin	2.78a	2.89ab	3.03bc	3.59cd	3.48cd	3.79de	3.91ef	4.30f
	Flesh	1.34a	1.39ab	1.46b	1.72cd	1.67bc	1.82d	1.88d	2.06e
Mg, g kg^{-1}	Berry	0.89	0.80	0.90	0.87	0.88	0.81	0.83	0.88
	Skin	0.84	0.83	0.85	0.85	0.85	0.84	0.87	0.88
	Flesh	0.90	0.79	0.91	0.88	0.88	0.80	0.82	0.88
Cl, mg kg^{-1}	Berry	141	129	133	137	150	134	151	132
	Skin	168	154	159	164	179	160	180	158
	Flesh	134	122	126	130	142	127	143	125
Na, mg kg^{-1}	Berry	90	80	83	91	98	84	96	83
	Skin	108	96	99	109	117	100	115	99
	Flesh	85	76	79	86	93	80	91	79
Fe, mg kg^{-1}	Berry	35a	44ab	48bc	58c	51bc	55cd	61d	59d
	Skin	42a	53a	80b	96bc	81b	97bc	107bc	111c
	Flesh	33a	34a	40ab	48b	43b	45b	50b	46b
Mn, mg kg^{-1}	Berry	11	10	10	11	11	10	11	10
	Skin	17	15	17	17	16	15	17	15
	Flesh	10	9	8	9	9	9	10	9
Zn, mg kg^{-1}	Berry	14a	13a	21b	24b	24b	25b	27b	27b
	Skin	17a	17a	22ab	30bc	24b	30bc	34c	36c
	Flesh	13a	12a	21b	23b	24b	23b	25b	25b
Cu, mg kg^{-1}	Berry	16a	16a	17a	23b	21ab	23b	25b	25b
	Skin	19a	20a	22a	30b	25ab	29b	31b	31b
	Flesh	15a	15a	16a	22b	20ab	22b	23b	23b
Ti, mg kg^{-1}	Berry	0.25	0.30	0.27	0.25	0.24	0.27	0.24	0.23
	Skin	0.28	0.34	0.30	0.28	0.27	0.30	0.27	0.26
	Flesh	0.24	0.29	0.26	0.24	0.23	0.26	0.23	0.22

For each element and tissue, values followed by the same letter, or without a letter, are not significantly different at $p < 0.05$.

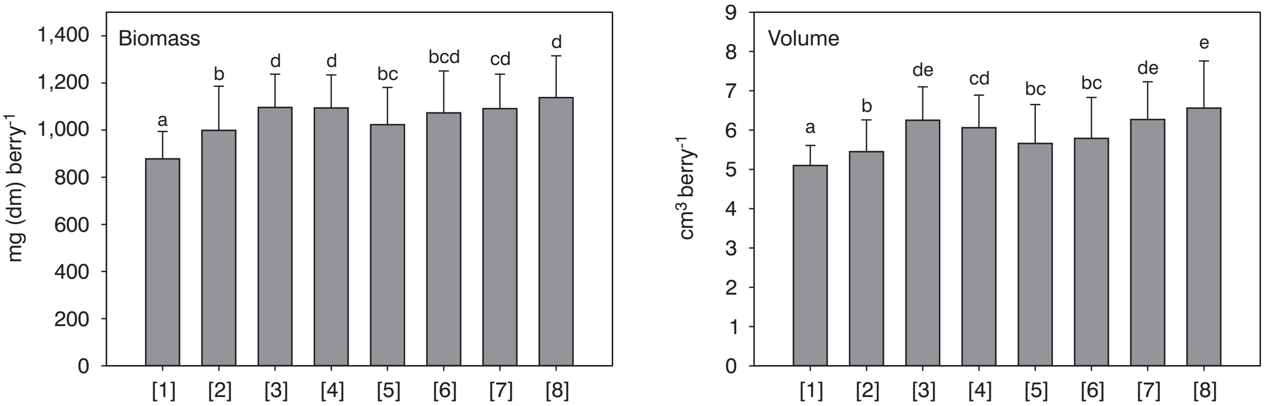


Figure 1. Effect of foliar applications of sprays containing Ca, polypeptidic N and Ti ascorbate on berry size. For each graph, bars with the same top letter are not significantly different at $p < 0.05$. Treatments: [1] Control; [2] Ca; [3] Polypeptidic N; [4] Ti ascorbate; [5] (2+3); [6] (2+4); [7] (3+4); [8] (2+3+4). dm: dry matter.

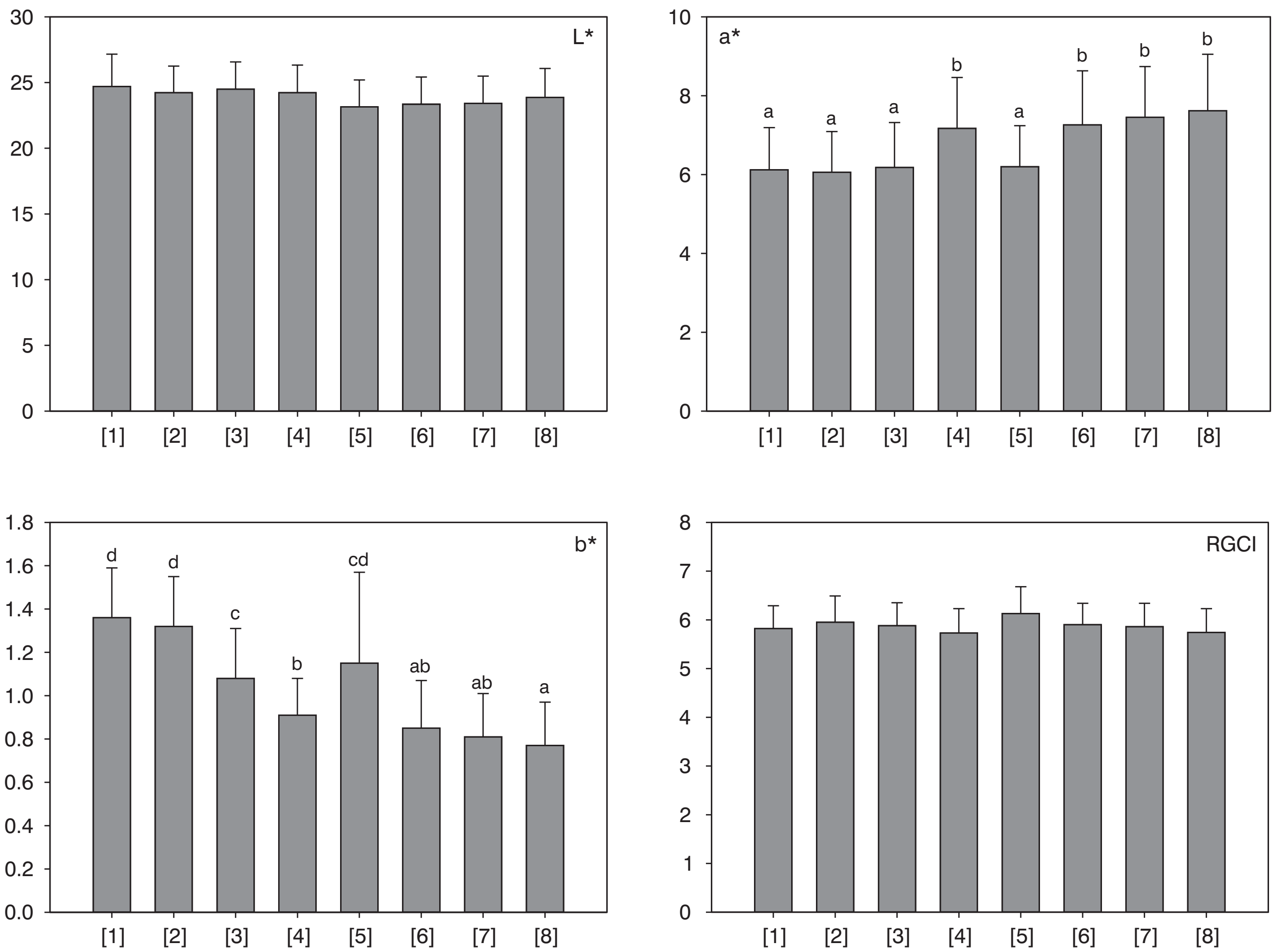


Figure 2. Effect of foliar applications of sprays containing Ca, polypeptidic N and Ti ascorbate on berry skin colour. For each graph, bars with the same top letter (or without letter) are not significantly different at $p < 0.05$. Treatments: [1] Control; [2] Ca; [3] Polypeptidic N; [4] Ti ascorbate; [5] (2+3); [6] (2+4); [7] (3+4); [8] (2+3+4).

therefore occurs through the activation of iron in leaf chloroplasts and fruit chromoplasts.

No significant differences were seen in the external colour of the berries at harvest (Fig. 2) although in general the treatments including Ti increased the values of variable a* (red colour) and reduced those of b* (yellow colour). From these results, it might be expected that the berries from Ti-sprayed vines would ripen earliest. However, no differences were seen in the RGCI between control and Ti-treated samples. Given the effect of Ti leaf sprays on the fruits of other plants (Martínez-Sánchez *et al.,* 1993; Carvajal *et al.,* 1994a,b, 1995b, 1998; Carvajal and Alcaraz, 1995, 1998a,b), Ti would seem to promote a certain increase in the synthesis of berry pigments (and consequently in the intensity of the red colour) without accelerating the ripening process. This is supported by the ripeness index data shown in Table 3.

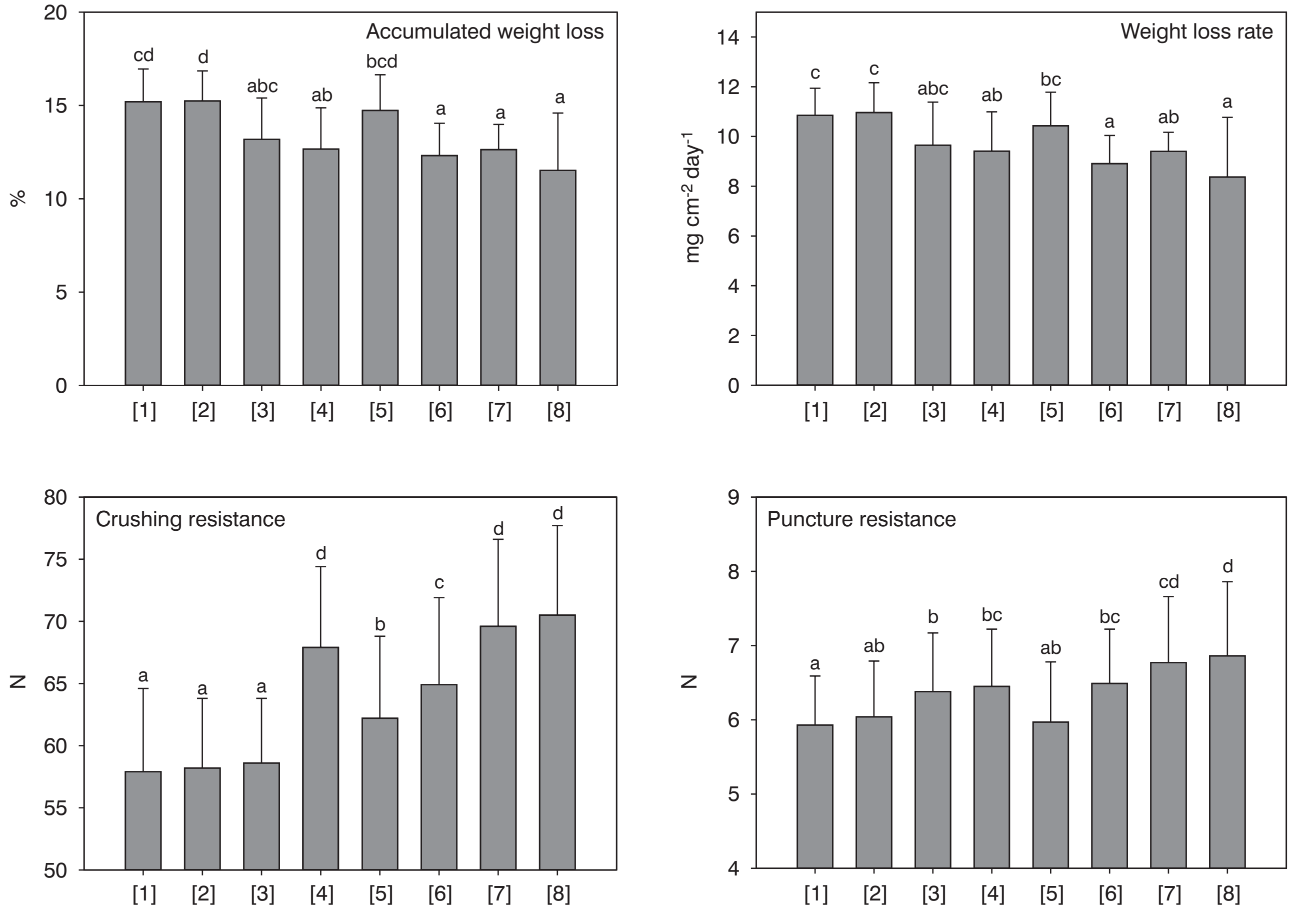
All of the Ti-treatments (Treatments 4, 6, 7 and 8) induced a significant increase in fruit firmness (Fig. 3), as confirmed by the crushing and puncture resistance data. The behaviour of clusters during storage at room temperature (RT) was also affected by treatments containing Ti. Weight loss during a three week storage period at RT was minimal when the vines were thus treated, the smallest loss occurring when Ti, polypeptidic N and soluble Ca were applied together. Most of the weight lost was due to the evaporation of water, a phenomenon directly related to the external surface area, which in turn is a function of volume. Thus, the maintenance of fruit weight was influenced by the larger size of the berries produced in the Ti treatments.

No significant differences were seen in the biochemical composition of the grape juice (titratable acidity, soluble solids concentration, sugar and organic acid concentrations); only a few metabolites in minor concentration had values higher than those of the controls (Table 3).

Taking these results together, the physical quality improvements induced by Ti can only be attributed to differences in the Ca concentration of the berries. Compared to control plants, the Ti-treated vines had berries with about 45% more Ca in the skin and flesh, which showed better mechanical resistance. These improvements in Ca absorption can only be interpreted

Table 3. Effects of foliar application of sprays containing soluble Ca, polypeptidic N and Ti ascorbate on the biochemical composition of juice of table grapes (cv. Crimson) at harvest. Each value is the mean of 20 samples

	Treatment							
	Control	**Ca**	**N**	**Ti**	**Ca + N**	**Ca + Ti**	**N + Ti**	**Ca + N + Ti**
Titrat. acidity, mg $100ml^{-1}$	0.61b	0.58ab	0.53ab	0.53ab	0.56ab	0.52ab	0.50ab	0.49a
Soluble solids conc., ºBrix	18.4	17.2	19.0	19.2	18.2	19.4	19.6	19.9
Ripeness index, SSc Ta^{-1}	30.16a	29.66a	35.85b	36.23b	32.50ab	37.31b	39.20b	40.61b
Sugars, g $100ml^{-1}$								
Sucrose	0.02a	0.02a	0.05b	0.09d	0.07c	0.09d	0.13e	0.13e
Glucose	8.85	7.60	7.57	8.16	8.20	8.14	9.08	9.08
Fructose	8.88b	7.45a	7.77a	8.41ab	8.21ab	8.35ab	9.26b	9.36b
Sorbitol	0.15cd	0.12a	0.12a	0.14bc	0.13ab	0.14bc	0.16d	0.16d
Total sugars	17.90	15.18	15.52	16.79	16.61	16.71	18.63	18.73
Organic acids, g $100ml^{-1}$								
Citric	0.032b	0.027ab	0.027ab	0.028ab	0.024a	0.027abc	0.030ab	0.025ab
Fumaric	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Malic	0.688b	0.540a	0.539a	0.603ab	0.540a	0.541a	0.654b	0.678b
Oxalic	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Tartaric	0.234a	0.218a	0.224a	0.265ab	0.244ab	0.281b	0.264ab	0.262ab
Total organic acids	0.957b	0.787a	0.792a	0.898ab	0.809a	0.875ab	0.950b	0.967b



For each variable or metabolite, values followed by the same letter, or without a letter, are not significantly different at $p < 0.05$.

as a consequence of the beneficial effect of Ti on Ca absorption, translocation and assimilation processes since the treatments were performed during the period in which Ca can move passively to the berry. This would allow the biological activation of berry cell iron, improving Ca integration into the cell wall, and consequently increasing berry firmness.

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