2024 Citrus Australia
AUSTRALIAN CITRUS CONGRESS
SUNSHINE COAST, QUEENSLAND
5 March | AGM & Welcome Event
6 & 7 March | Congress

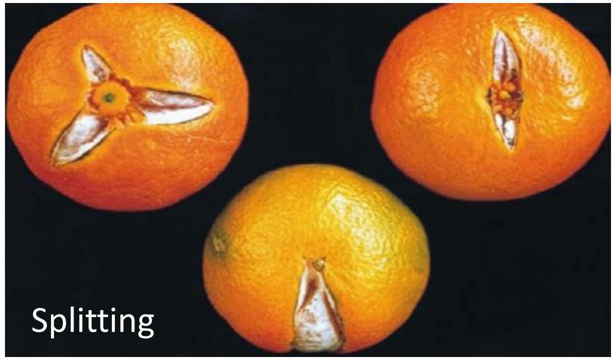
Preharvest treatments to extend shelf life in citrus fruit

Rubén Pascual

LOSS OF FRUIT QUALITY

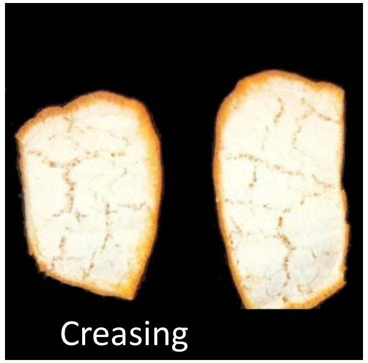
SENESCENCE OF THE FRUIT AND ASSOCIATED DAMAGES

UNFAVOURABLE ENVIRONMENTAL CONDITIONS THAT CAUSE DETERIORATION OF THE RIND



- Changes in relative humidity.
- Abundant rains after a period of drought.
- Irregular irrigation

- Low temperatures in pre and/or post harvest



- Changes in relative humidity.
- Abundant rains after a period of drought.
- Irregular irrigation

- Harvest in high humidity conditions
- Dry and hot winds

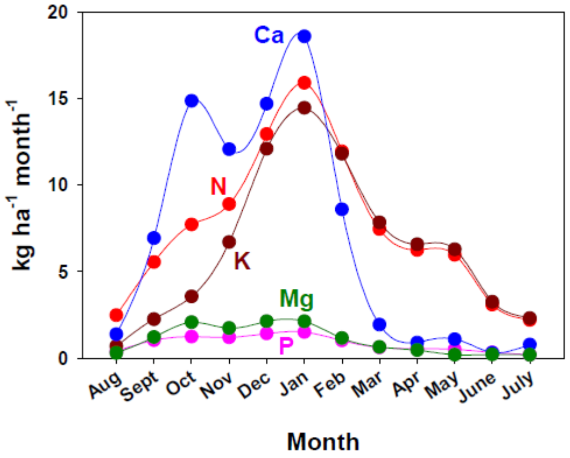
DETERIORATION OF THE RIND

LOSS OF THE INTEGRITY OF CELL WALL AND MEMBRANE ACCELERATES DETERIORATION

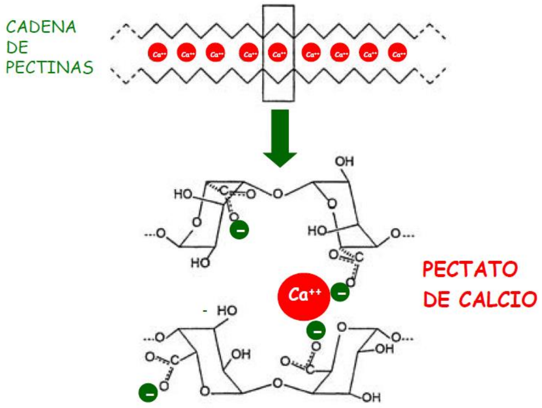
Adaptado de: Primo-Millo, E., Zaragoza, S., Agustí, M. (2021). *La práctica del cultivo de los cítricos en el área mediterránea.* PHYTOMA

STRUCTURAL CALCIUM

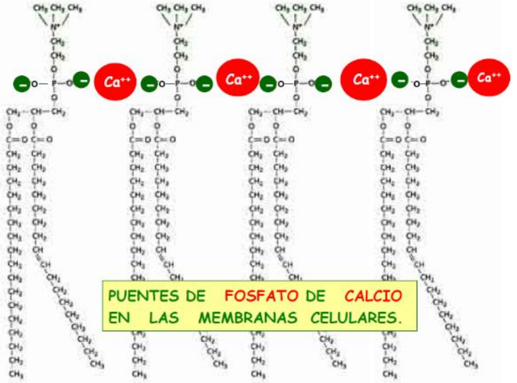
- Regulation of physiological processes. **Ca secondary messenger.**
- **Stomatal regulation** at the membrane.
- **Cell division and growth**
- **Structural calcium:** component of the cell wall, middle lamina, and plasma membrane.



CALCIUM PECTATE
(middle lamina and cell wall)

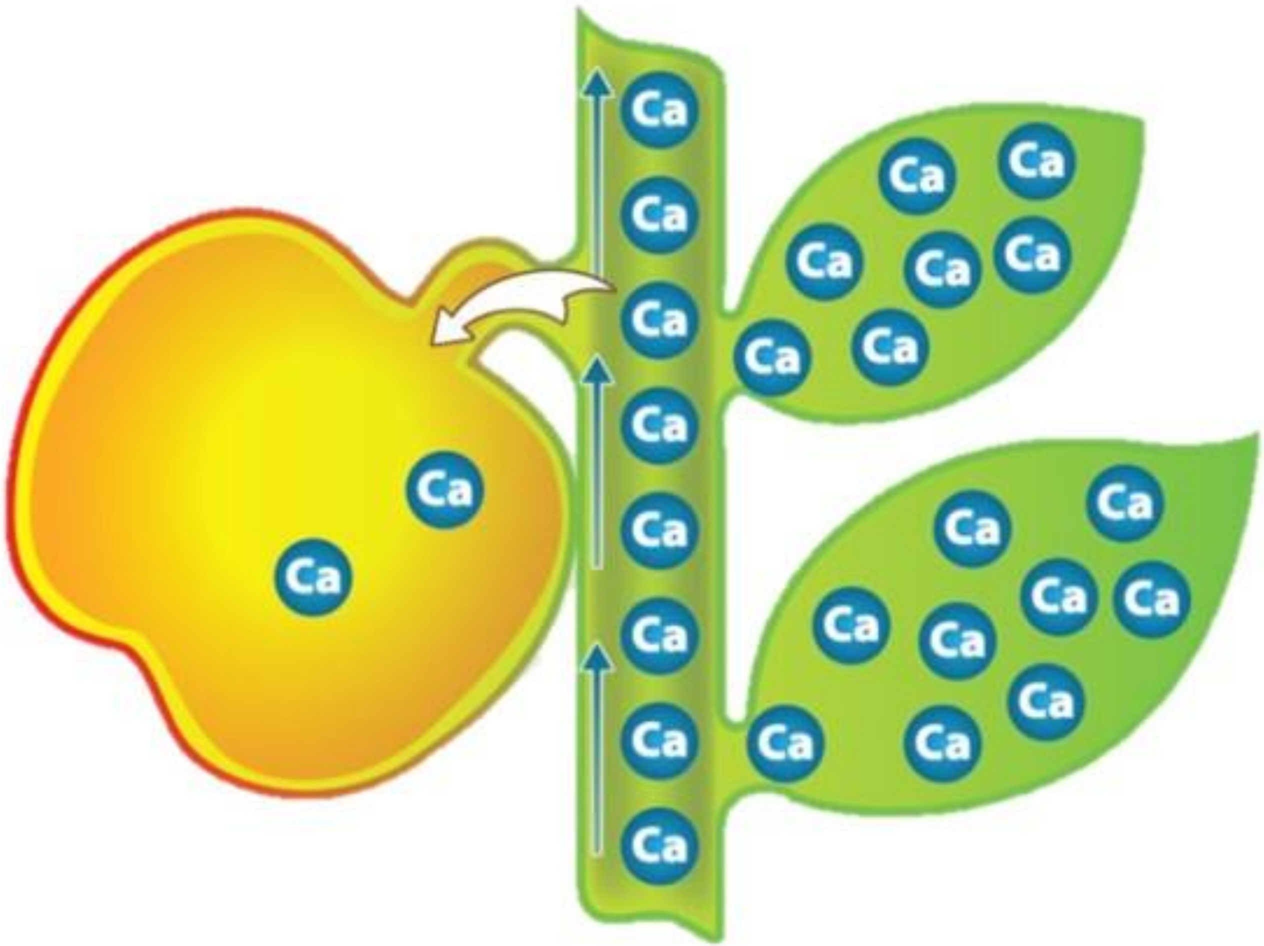


CALCIUM PHOSPHATE
(Plasma membrane phospholipids)



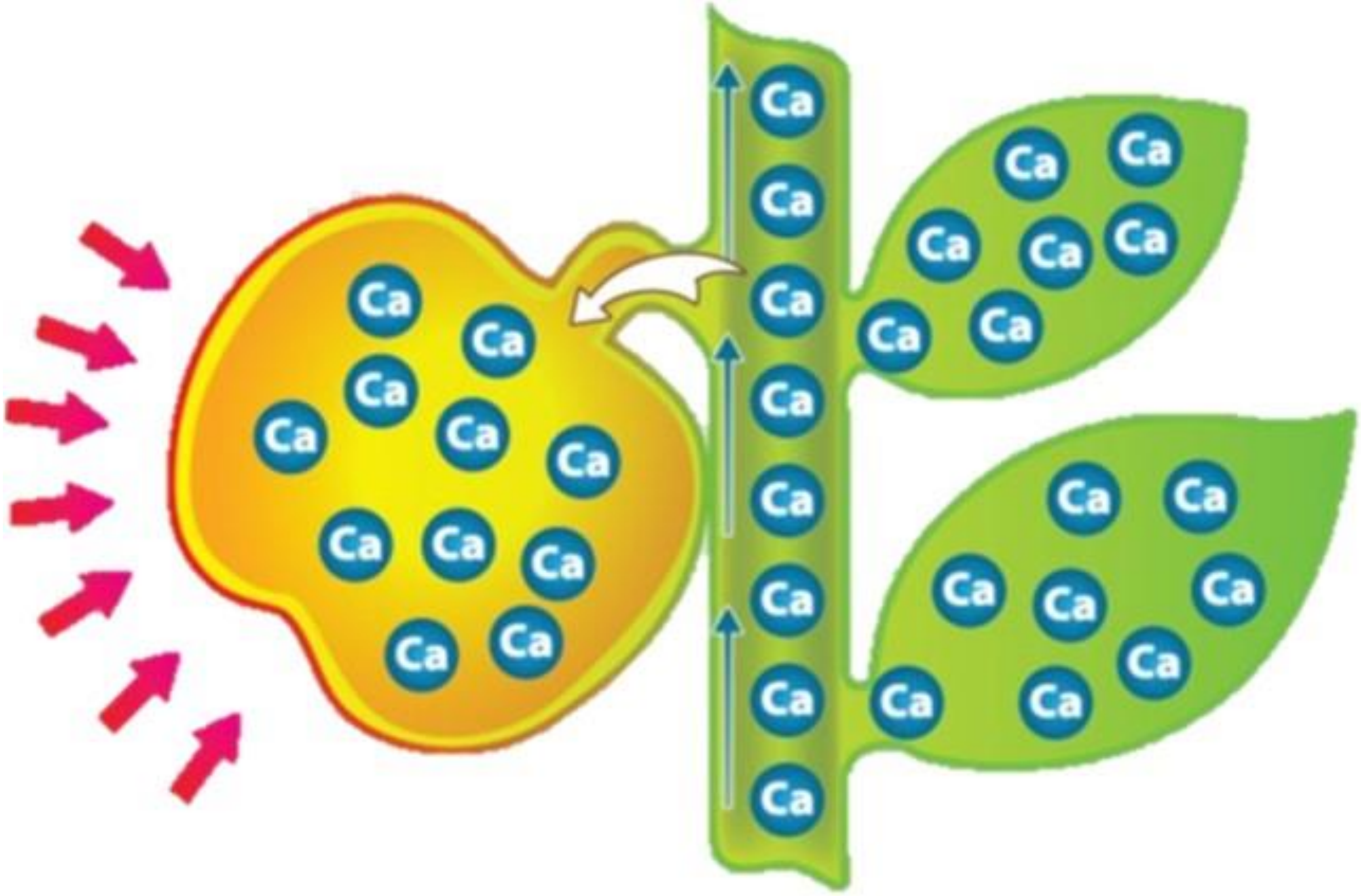
CALCIUM ABSORPTION

THE CALCIUM CONTENT IN FRUIT IS REGULATED BY TRANSPIRATION



CALCIUM ABSORPTION

FOLIAR CALCIUM APPLICATIONS DIRECTLY TO THE FRUIT



CALCIUM ABSORPTION

ABSORPTION OF CALCIUM (^{45}Ca) APPLIED BY THE ROOTS

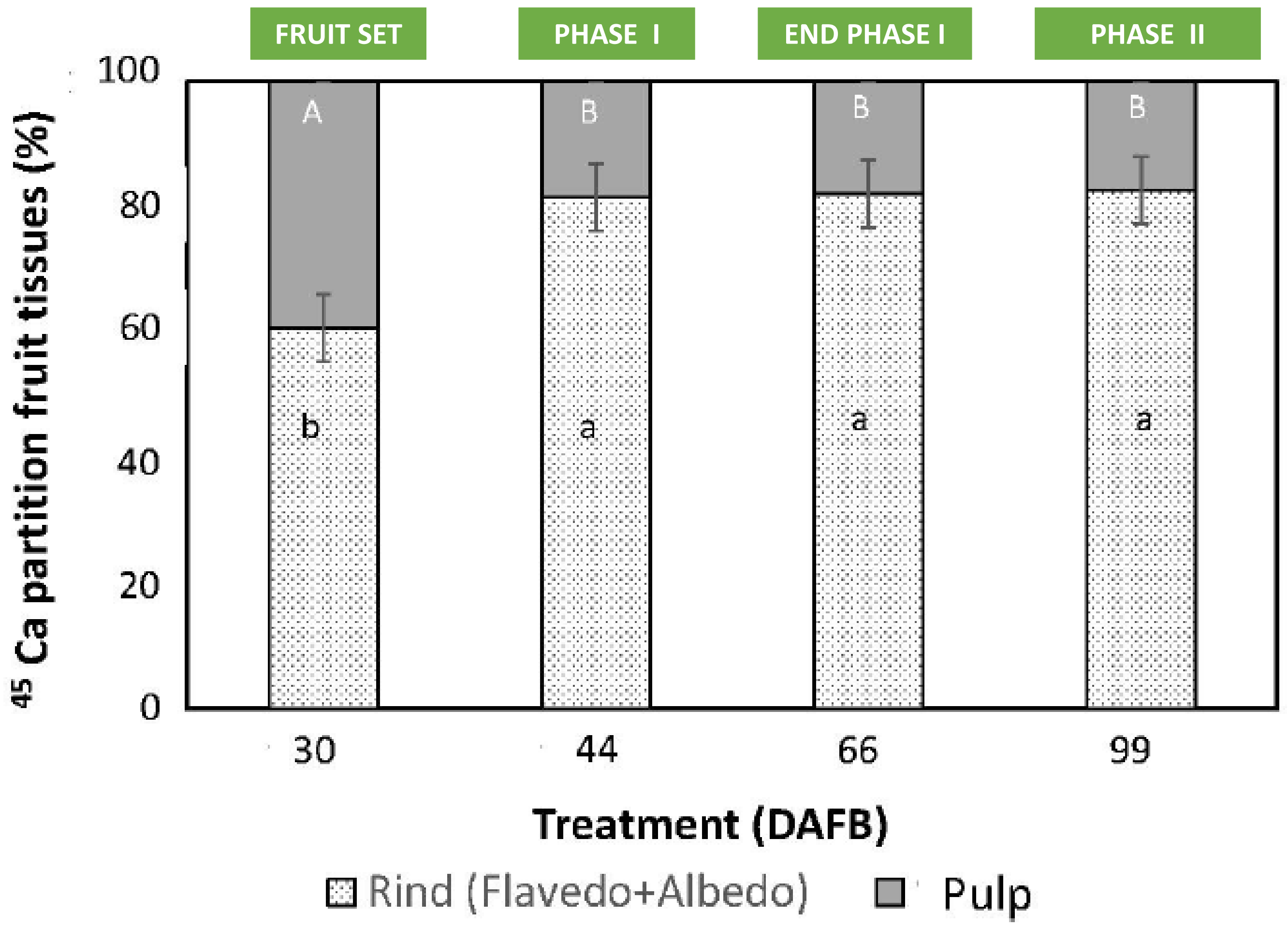
	^{45}Ca activity	
Tissue sample	DPM g^{-1}	%
Soil	6902.1 ± 828.9 a	56.0 ± 6.7 a
Roots	2406.0 ± 777.35 ab	19.5 ± 6.3 b
Trunk	1796.8 ± 231.3 c	14.6 ± 1.8 b,c
Shoots	1184.4 ± 259.1 d	9.6 ± 2.1 d
Fruits	25.9 ± 3.17 e	0.3 ± 0.1 3

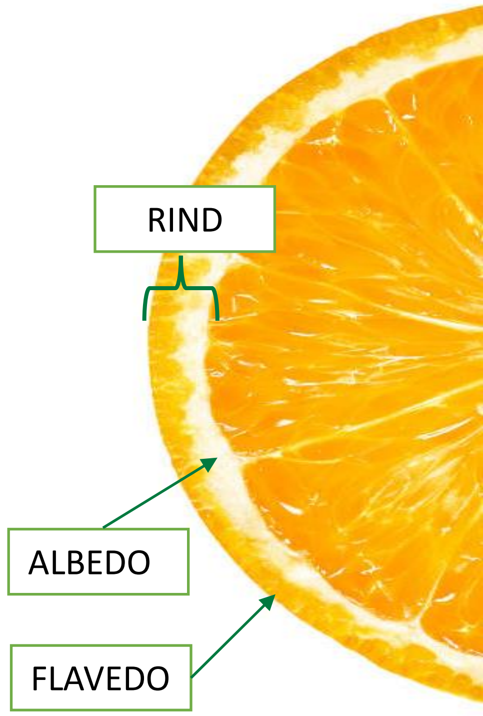
Root application of calcium ($^{45}CaCl_2$) in Clemenules 45 DAFB

Bonomelli *et al*. (2020). *J Sci Food Agric* 100: 4643-4650

CALCIUM ABSORPTION

ABSORPTION AND DISTRIBUTION OF CALCIUM (^{45}Ca) APPLIED BY FOLIAR IN DIFFERENT STAGES OF FRUIT DEVELOPMENT

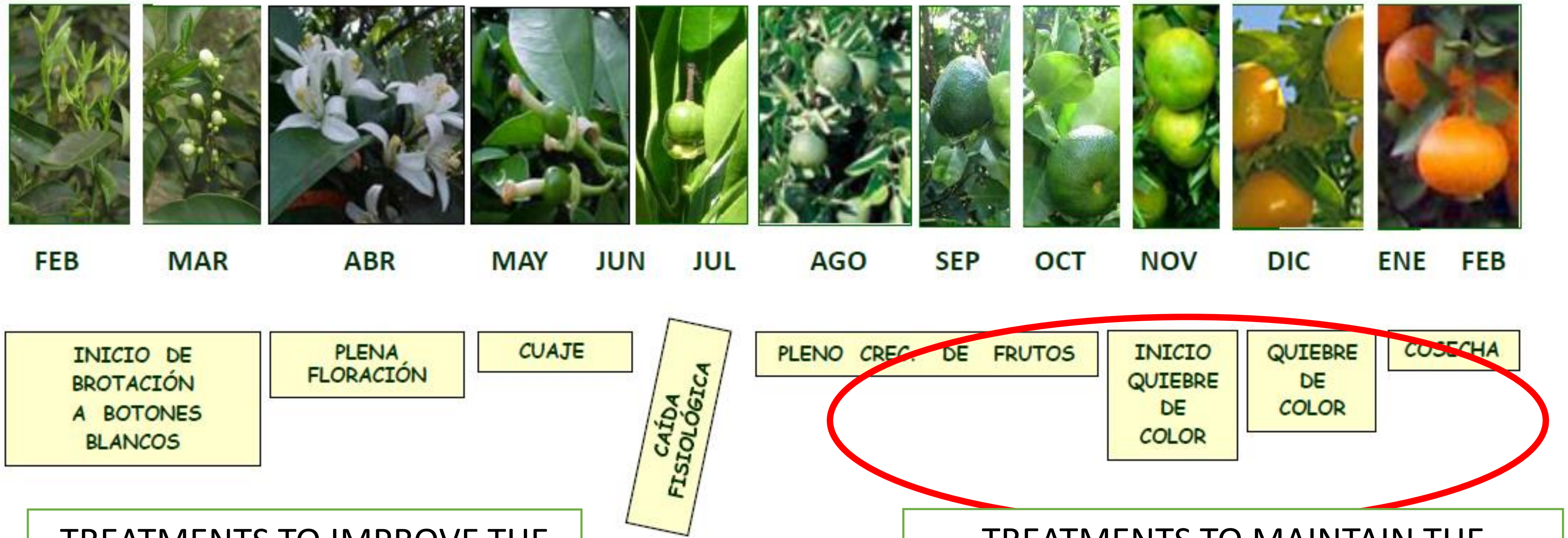




Bonomelli *et al*. (2022). *Agronomy*, 12, 150.

CALCIUM ABSORPTION

NUTRITION MANAGEMENT ACCORDING TO PHENOLOGICAL STATUS



TREATMENTS TO <u>IMPROVE</u> THE FORMATION OF CELL WALLS AND MEMBRANES

TREATMENTS TO <u>MAINTAIN</u> THE INTEGRITY OF CELL WALLS AND MEMBRANES

¿CALCIUM APPLICATIONS?

Mendoza, H. (2008)

CALCIUM ABSORPTION

The application of **calcium nitrate** just **before the color break** reduced **(33-81% less)** the percentage of Fortune mandarins affected by **peel-pitting**.

Table 2. Effectiveness of calcium nitrate [$Ca(NO_3)_2.4H_2O$], GA_3 and their mixture on the percentage of 'Fortune' mandarin fruits affected by peel-pitting.

Expt. Nº.	Control	$Ca(NO_3)_2.4H_2O$	GA_3	$Ca(NO_3)_2.4H_2O + GA_3$	Signif.
I	43 b	20 a	29 ab	-	5%
II	67 b	45 a	-	-	5%
III	63 b	-	46 a	-	5%
IV	60 b	23 a	48 b	38 ab	5%
V	13 c	5 a	10 b	11 b	5%
VI	31 b	6 a	-	-	1%
VII	69 b	38 a	-	54 ab	5%

Source: Zaragoza *et al.*, 1996.

Calcium loss from cell walls during ripening causes solubilization of pectins and **accelerates senescence.**

CALCIUM ABSORPTION

DIFFERENT ABSORPTION DEPENDING ON THE SOURCE OF CALCIUM USED

In citrus Ca foliar absorption from **$Ca(NO_3)_2$ or Ca formate was more active than that from $CaCl_2$ or Ca acetate**. In conclusion, $Ca(NO_3)_2$ and **Ca formate are recommended for the foliar application of Ca in citrus in order to increase absorption of Ca.**

CALC

Table 4. Ca absorptions depending on its co tomato and citrus leaves.

Ca compounds†	Absorption rates♦
	Citrus†
$CaCl_2$	7.0[b]
$Ca(NO_3)_2$	45.8[a]
$(HCOO)_2Ca$	31.2[ab]
$(CH_3COO)_2Ca$	6.6[b]

† ^{45}Ca-labeled calcium compound solutions were applied to leaves. Leaves were detached 7 days after application and ^{45}Ca activities were measured.

♦ Absorption rates were determined by dividing ^{45}Ca radioactivities in

H

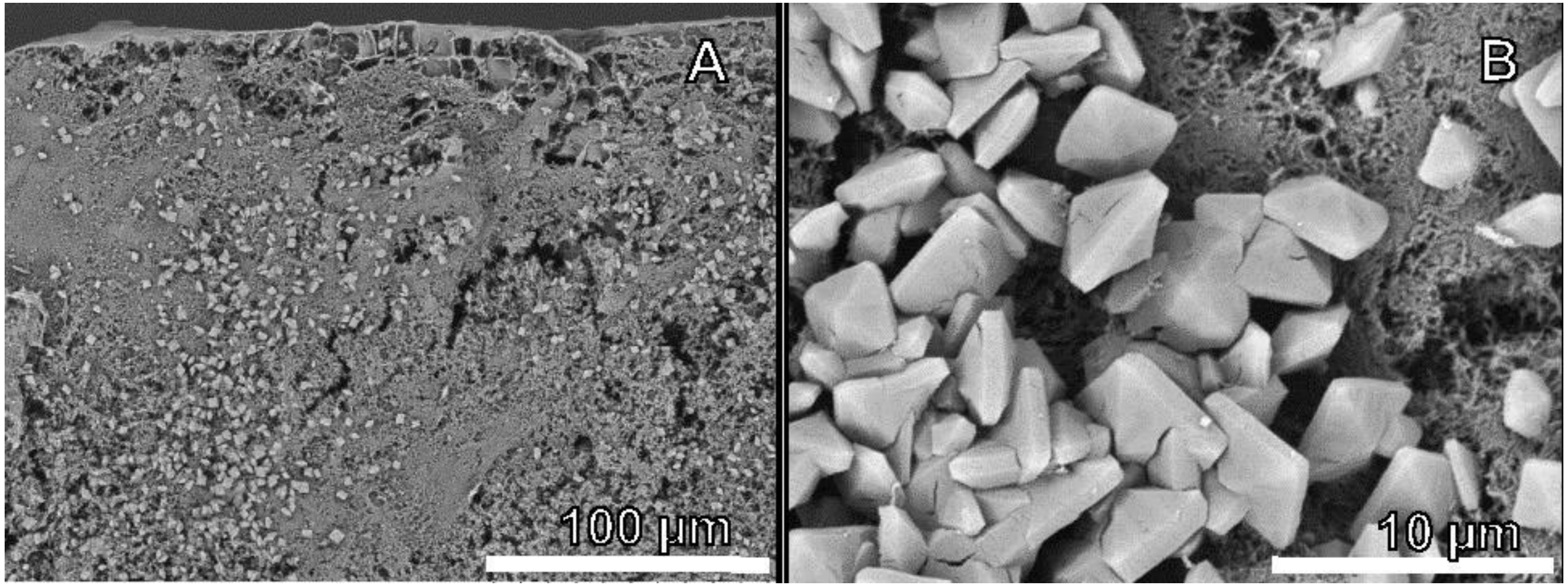
E NITROGEN OR

Sung-Jun *et al*. (2006)*.Korean J. Soil. Sci. Fert* Vol.39, No. 2, 80-85

CALCIUM OXALATE

FRUITS WITH HIGH CALCIUM CONCENTRATIONS SHOW DEFICIENCY SYMPTOMS (FIRMNESS, SHELF LIFE, CRACKING...)

THE CALCIUM CONTENT IS IN THE FORM OF **OXALATE** WHICH IS **PHYSIOLOGICALLY INACTIVE**



Formation of oxalate crystals in the rind of the fruit

Bonomelli *et al*. (2022). *Agronomy,* 12, 150.

CALCIUM OXALATE

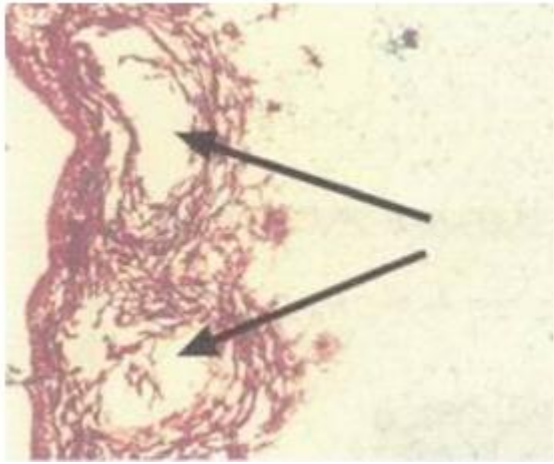
OXALIC ACID

↓

CALCIUM OXALATE

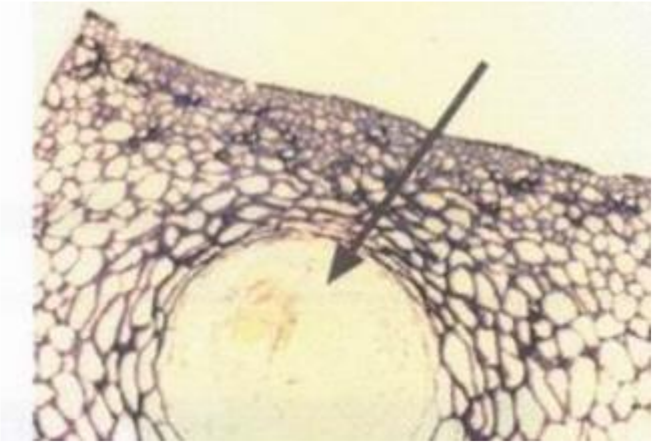
OXALIC ACID
REMOVES CALCIUM
FROM THE CELL WALL!

CALCIUM OXALATE



DAMAGED TISSUE (WITH SYMPTOMS OF PETECA)

CALCIUM OXALATE: 33,6 g/100 g dry mat.

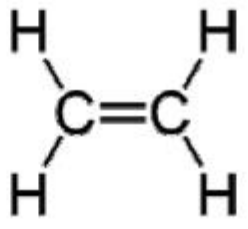


HEALTHY TISSUE (WITHOUT SYMPTOMS OF PETECA)

CALCIUM OXALATE: 16,2 g/100 g dry mat.

Undurraga et al (2.002) Simiente 72 (1-2): 35-40

ABIOTIC STRESS



ETHYLENE

⬆

ABIOTIC STRESS

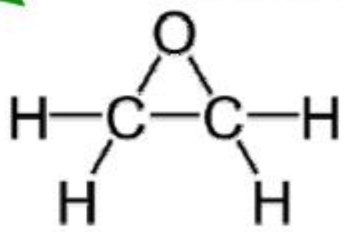
TEMPERATURE
UV RADIATION
DROUGHT
ROOT ASPHYXIA
FROST
WIND
SALINITY

⬇

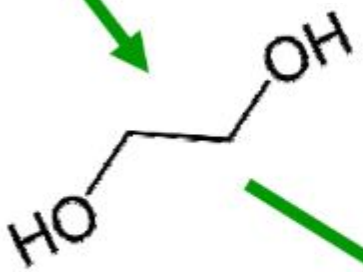
REACTIVE OXYGEN SPECIES (ROS)

LIPID PEROXIDATION OF THE MEMBRANE (MDA)

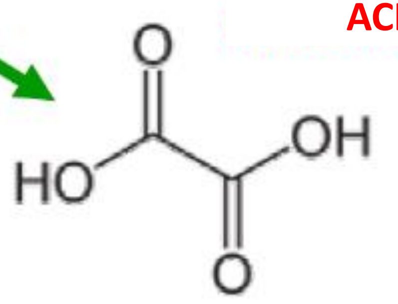
ETHYLENE OXIDE



ETHYLENE GLYCOL



OXALIC ACID

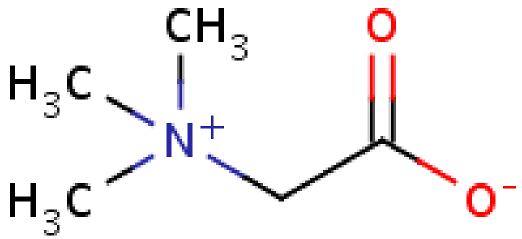


Mendoza, H. (2008). *Factores nutricionales que determinan calidad y condición en cítricos* (73)

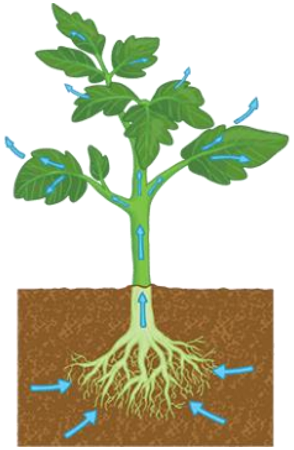
GLYCINE BETAINE (GB)

Glycine betaine (GB) is a major **organic osmolyte** that accumulate in a variety of plant species **in response to <u>environmental stresses</u>.**

Is thought to have positive effects on **<u>enzyme and membrane integrity</u>** along with adaptive roles in mediating osmotic adjustment in plants grown under stress conditions.

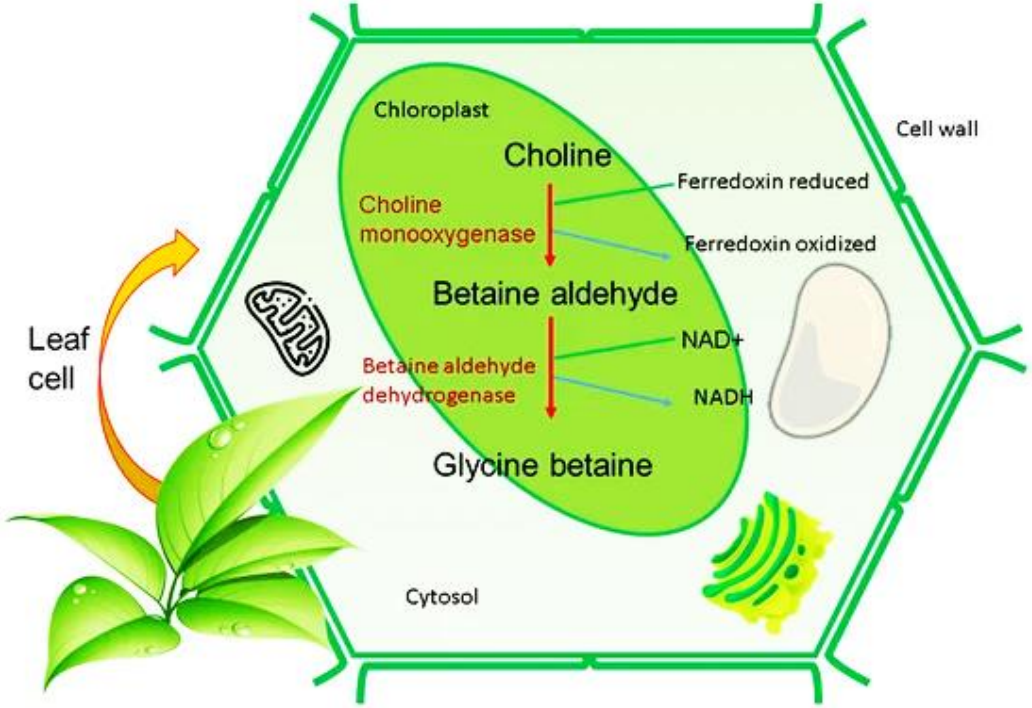


Maintains active transpiration flow increasing **photosynthesis**



GLYCINE BETAINE (GB)

BIOSYNTHESIS



The natural accumulation of GB is insufficient to improve the adverse effects of dehydration caused by various environmental stresses.

↓

INCREASE GB IN THE CROP BEFORE THE STRESS

CALFORMATE

CALCIUM FORMATE WITH BORON

- Calcium Formate (Ca) 24.0% w/w
- Boron (B): 0.9% w/w
- Glycine betaine 12%

Strengthens cell wall and membrane structures

Improves the firmness of the fruit.

Reduces skin wounds

Activate metabolic pathways that maintain the integrity of the cell wall

OSMOSHIELD

GLYCINE BETAINE OF NATURAL ORIGIN

- Glycine betaine 97%

providing an osmoregulation effect in the fruit cell.

Increases the activity of antioxidant enzymes (↓ROS).

Maintains the integrity of the all membrane.

Decreases fruit dehydration and loss of firmness

"Evaluation of CALFORMATE® and OSMOSHIELD® in preharvest treatments on the quality and shelf life of NADORCOTT "

Department of Food Technology, EPSO,
University Miguel Hernández, Orihuela, Alicante, Spain

Dr. Daniel Valero

Dra. María Emma García Pastor

EXPERIMENTAL DESING

LOCATED: Alicante (Spain)

CULTIVAR: NADORCOTT

ROOTSTOCK: *C. Macrophylla*

CALFORMATE

OSMOSHIELD

EXPERIMENTAL DESIGN. TREATMENTS

TREATMENT	ACTIVE MATTER	PRODUCT	RATE
1	Control		
2	15 mM GB	OSMOSHIELD	1,45 g/l
3	15 mM Calcium formate 2,5 mM GB 0,2 mM B	CALFORMATE	2,5 g/l
4	15 mM Calcium formate 15 mM GB 0,2 mM B	CALFORMATE + OSMOSHIELD	2,5 g/l 1,45 g/l

EXPERIMENTAL DESIGN. EVALUATIONS

Randomized block design

4 Preharvest treatments

- Untreated
- GB 15 mM
- CaO-B
- CaO-B-GB

6 blocks of 2 trees
12 trees per treatment

At harvest

21,945 fruits
1,972 kg

10 fruits per tree
120 fruits per treatment

Postharvest storage

30 days at 1 ºC
+10 days at 20 ºC

60 days at 1 ºC
+10 days at 20 ºC

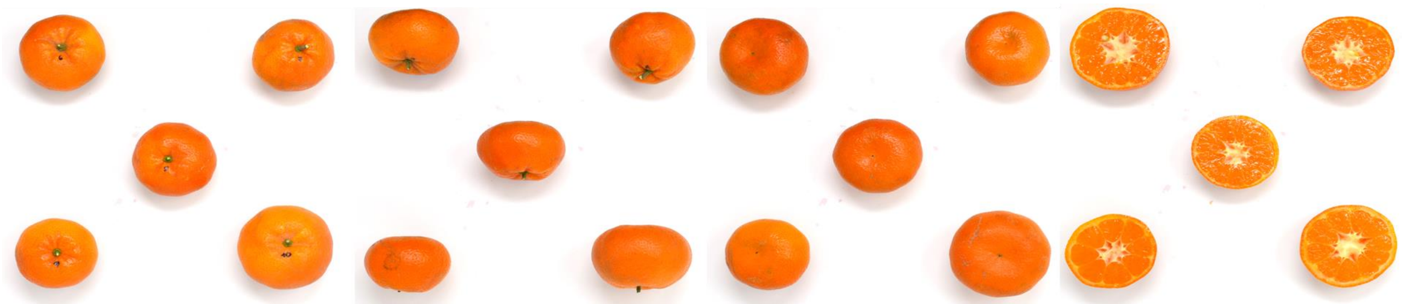
4 fruits per replicate (n=6)
24 fruits per treatment

Evaluation of CALFORMATE® and OSMOSHIELD® in preharvest treatments on the quality and shelf life of NADORCOTT

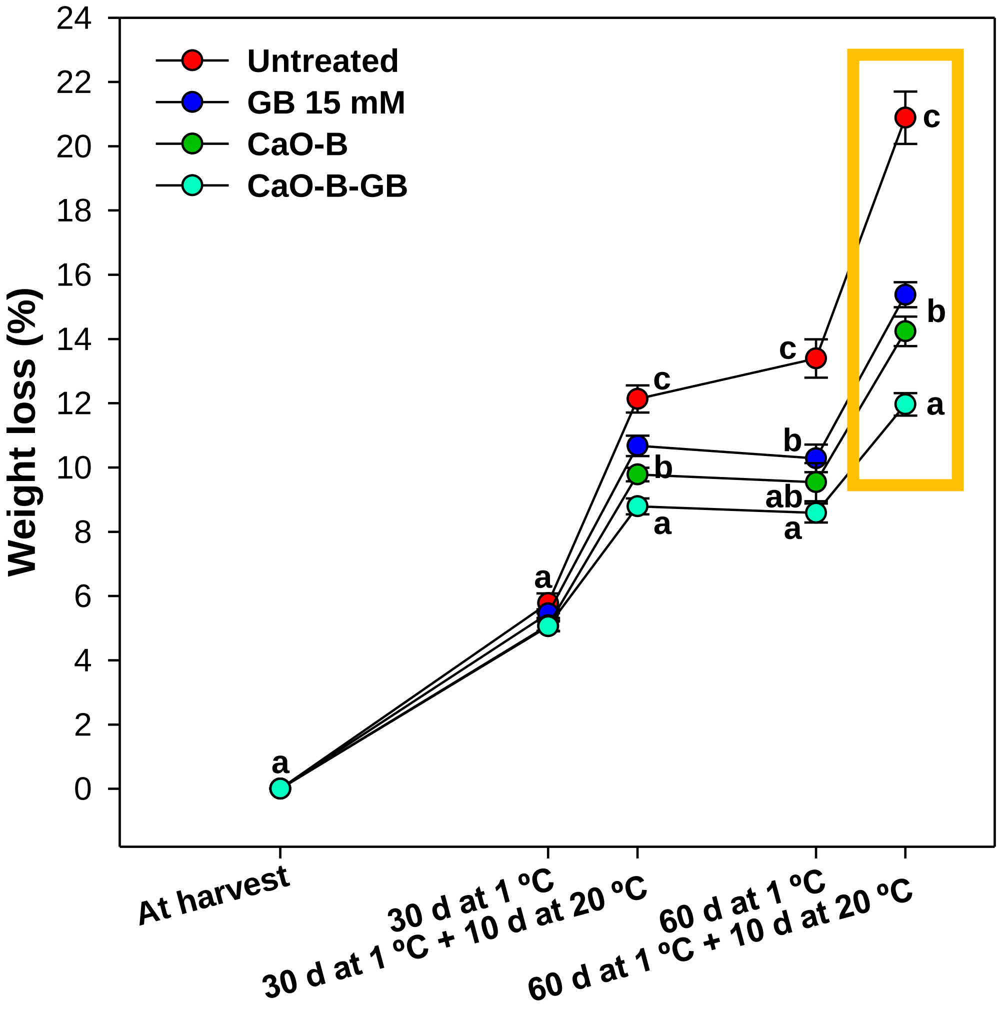
TREATMENT 1
Beginning colour break
2-November

TREATMENT 2
Colour break
29-November

TREATMENT 3
7 days before harvest
9-Genuary



RESULTS. WEIGHT LOSS %

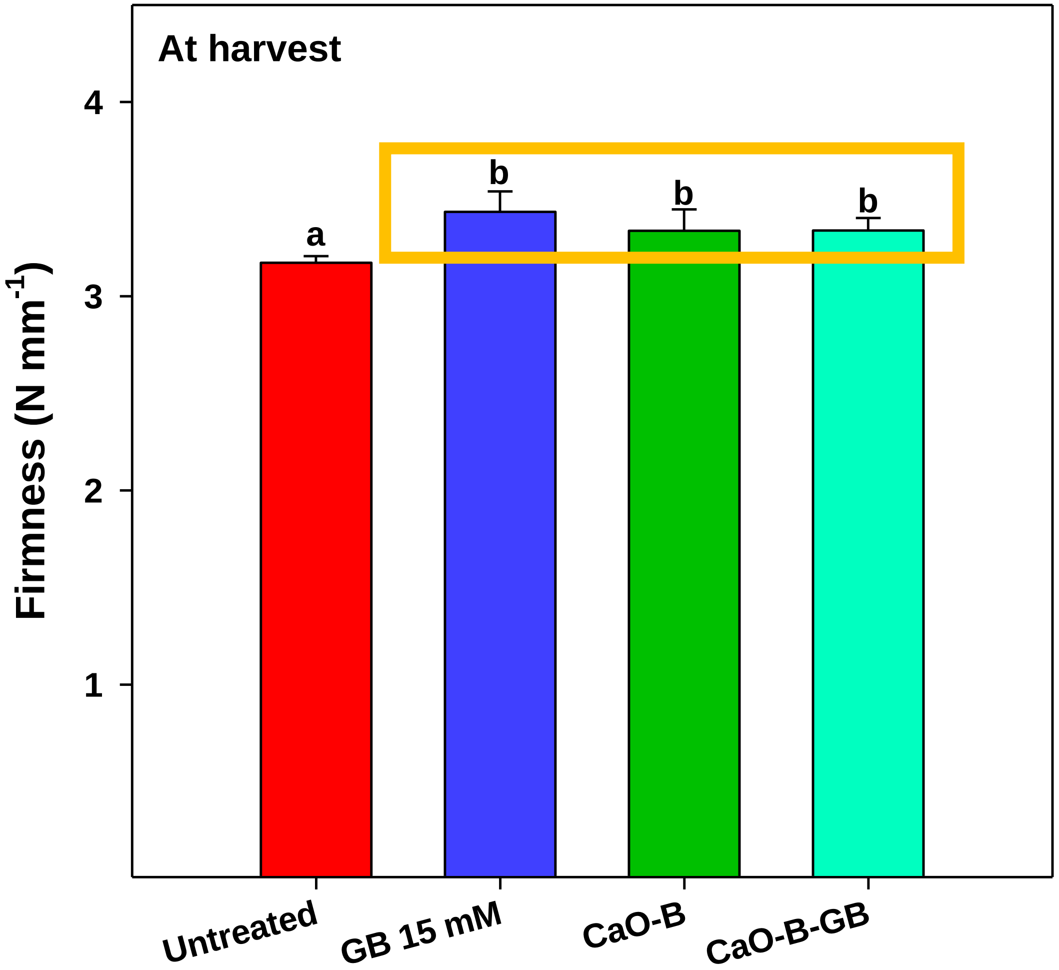


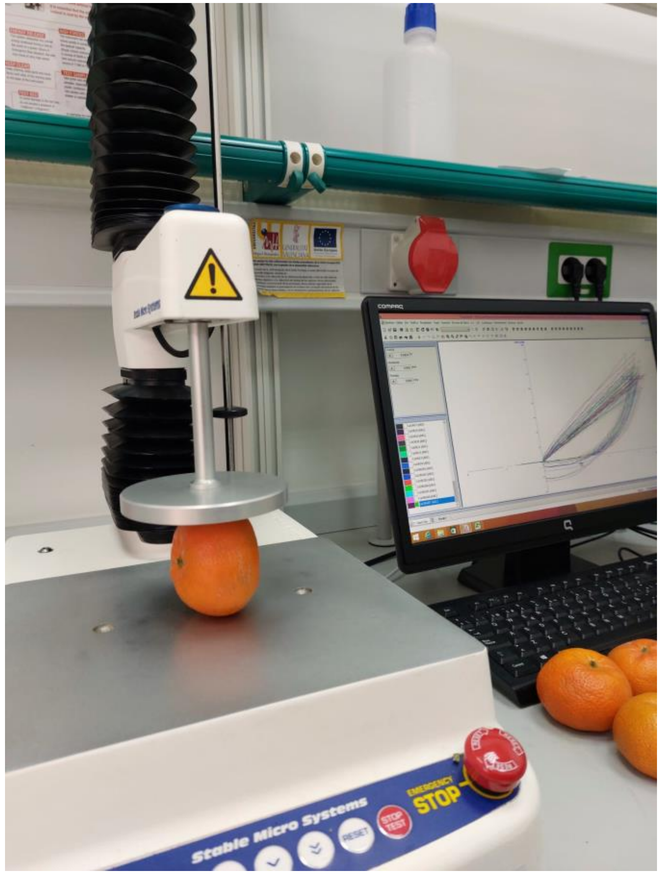
The treated fruits had less weight loss during their storage and shelf life. The combination of OSMOSHIELD + CALFORMATE has obtained the best results.

-9% (abs.)

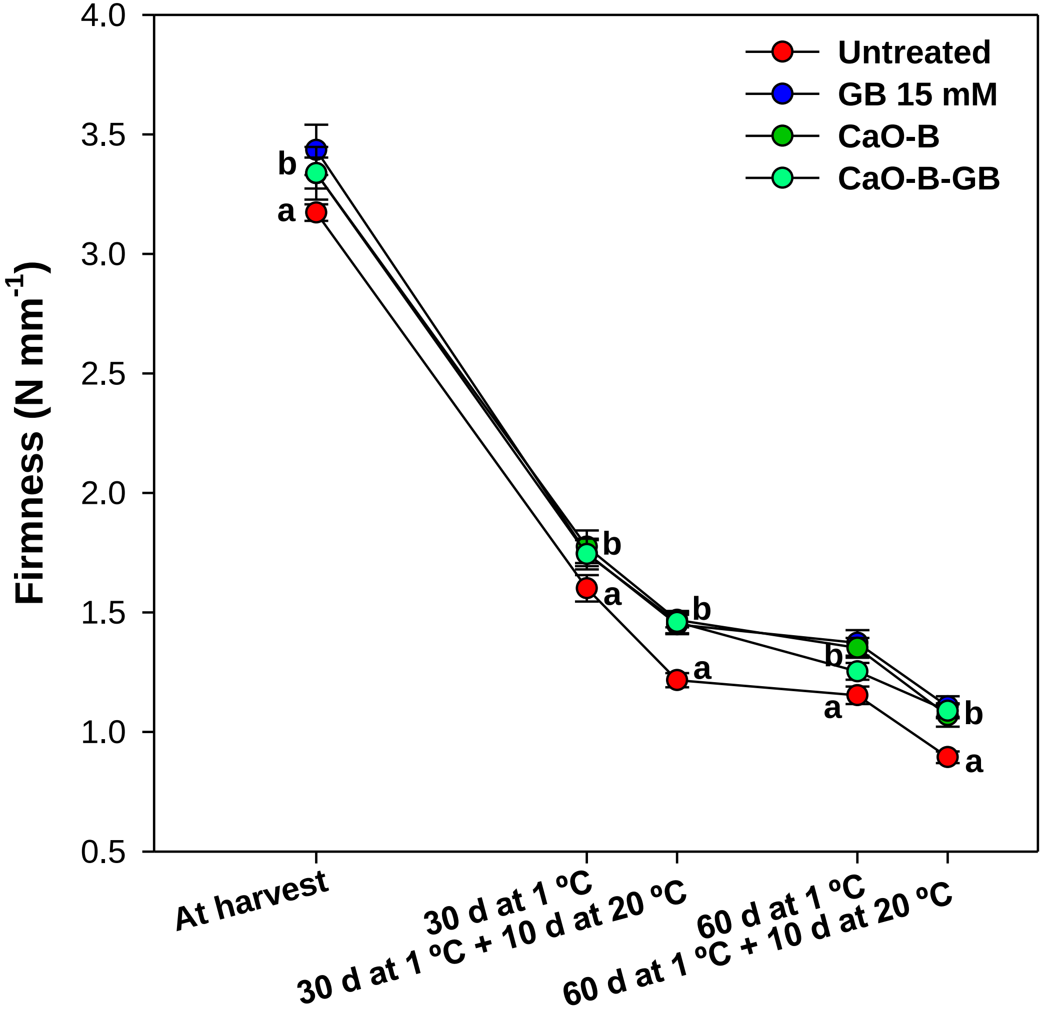
-45% (rel.)

RESULTS. FIRMNESS





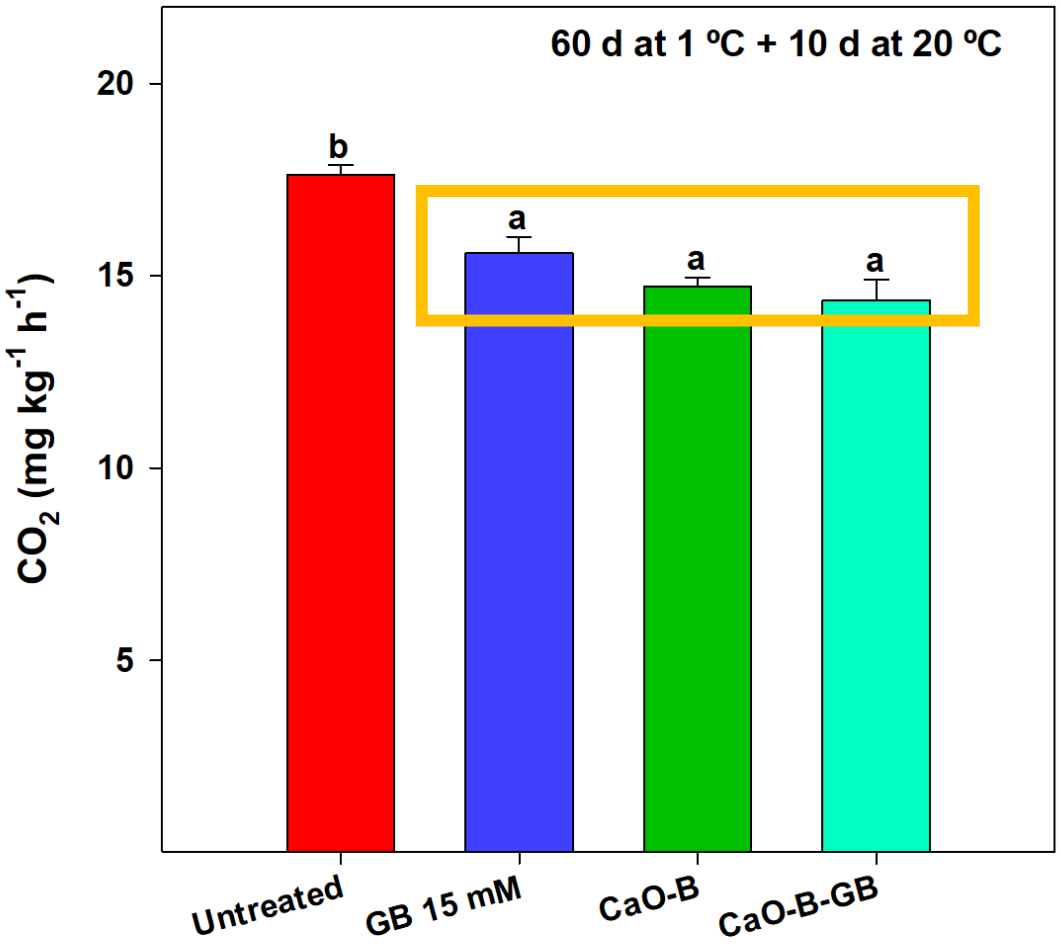
RESULTS. FIRMNESS



The firmness of the fruit at harvest was higher in the treated fruits. This difference is maintained during storage and shelf life

CALFORMATE + OSMOSHIELD
+22%

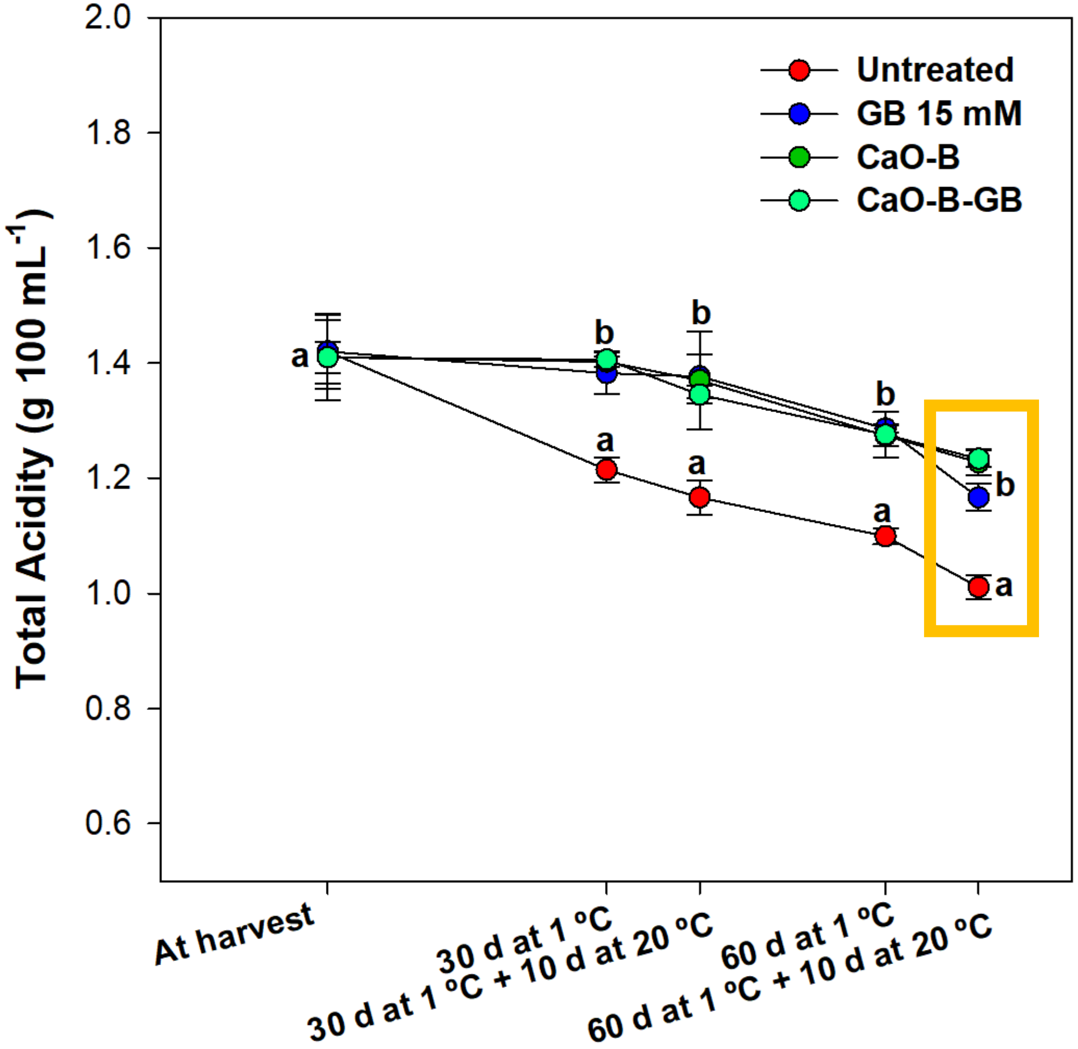
RESULTS. FRUIT RESPIRATION RATE

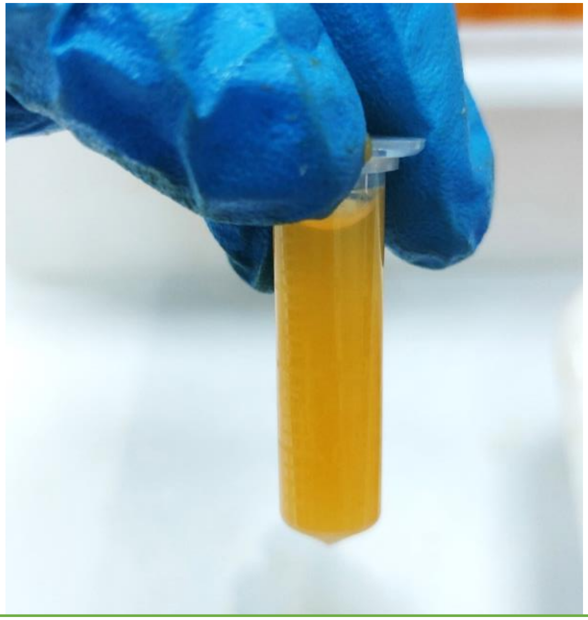


At the end of storage and shelf life, CALFORMATE® and/or OSMOSHIELD® reduced CO2 by 20% compared to the untreated, then slowing down the senescence process.

-19%

RESULTS. TOTAL ACIDITY

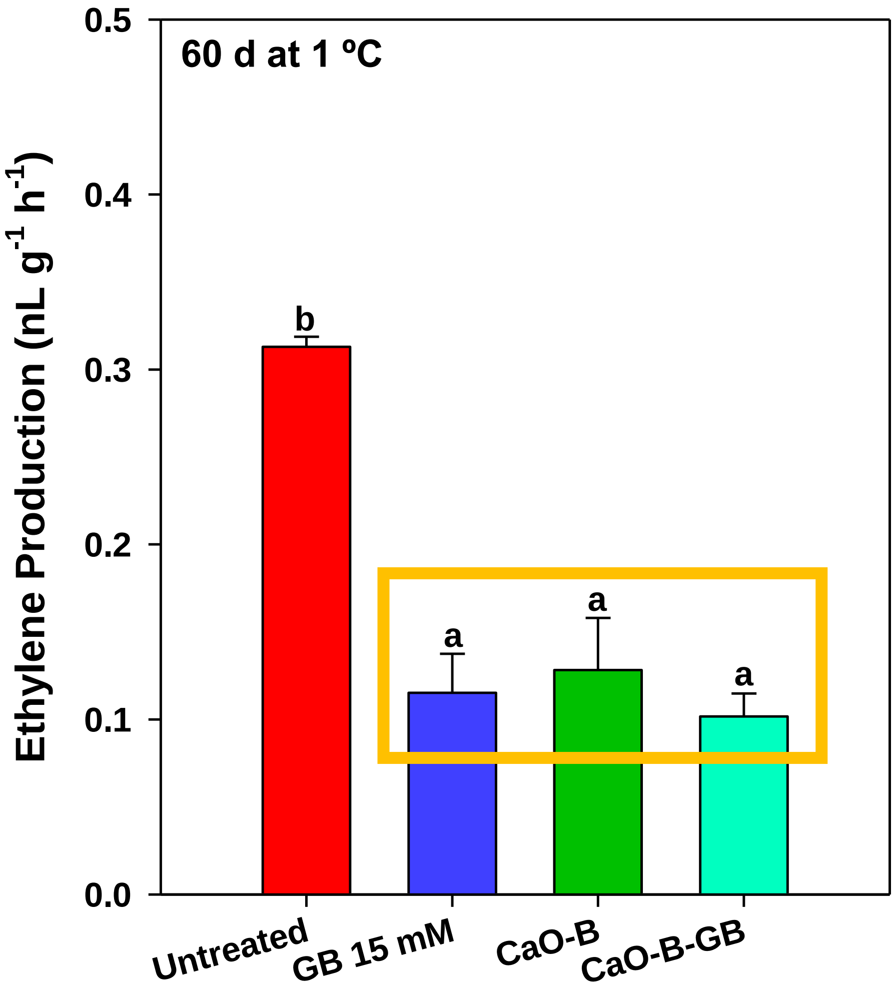


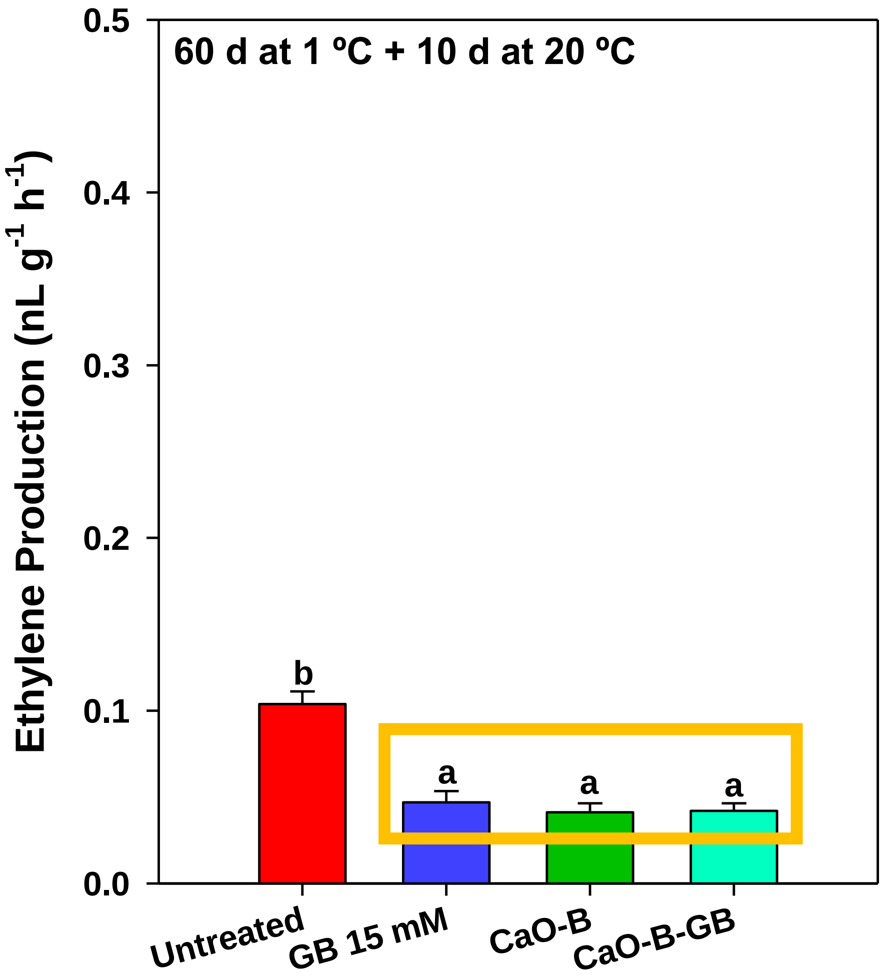


The treated fruits maintain better their acidity and maturity ratio, thus improving their shelf life and minimising any over mature taste and off flavours.

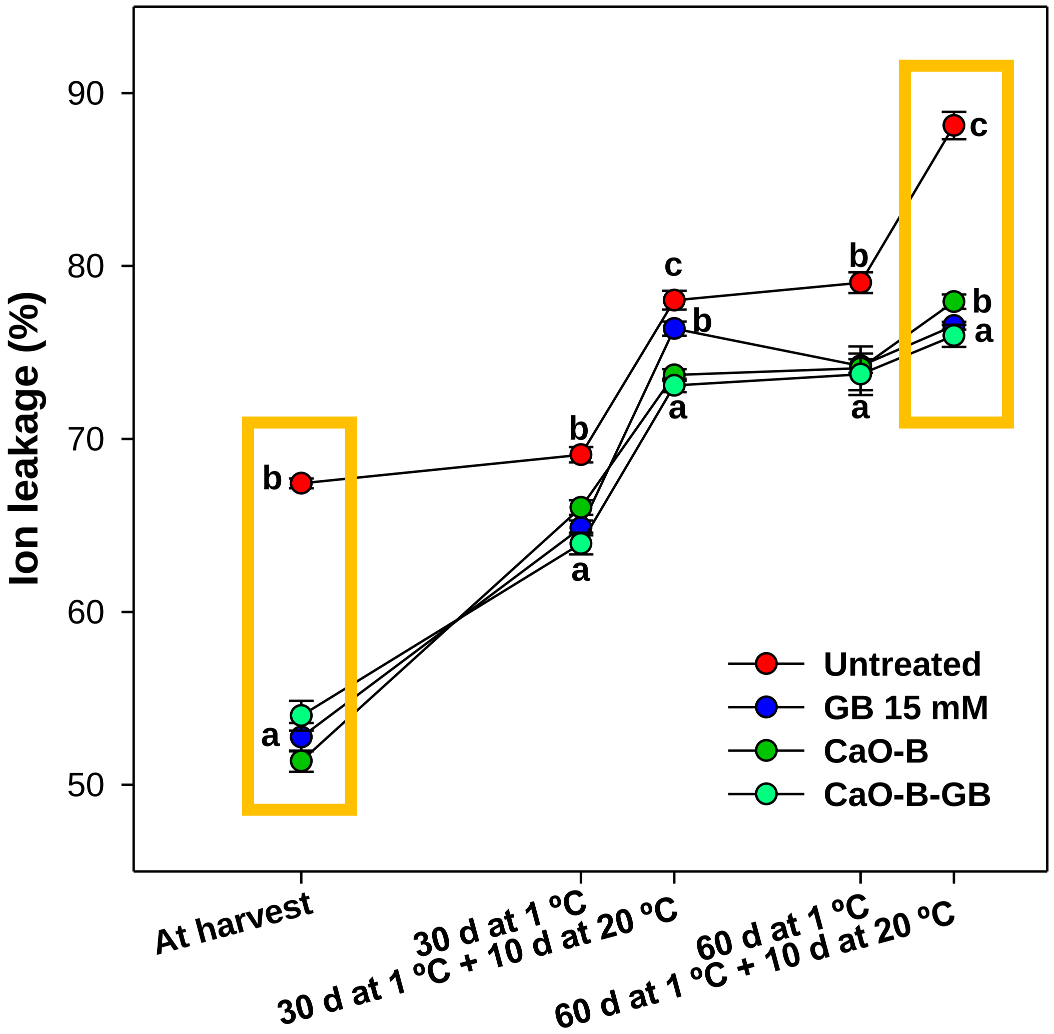
+22%

RESULTS. ETHYLENE PRODUCTION





RESULTS. ION LEAKAGE %



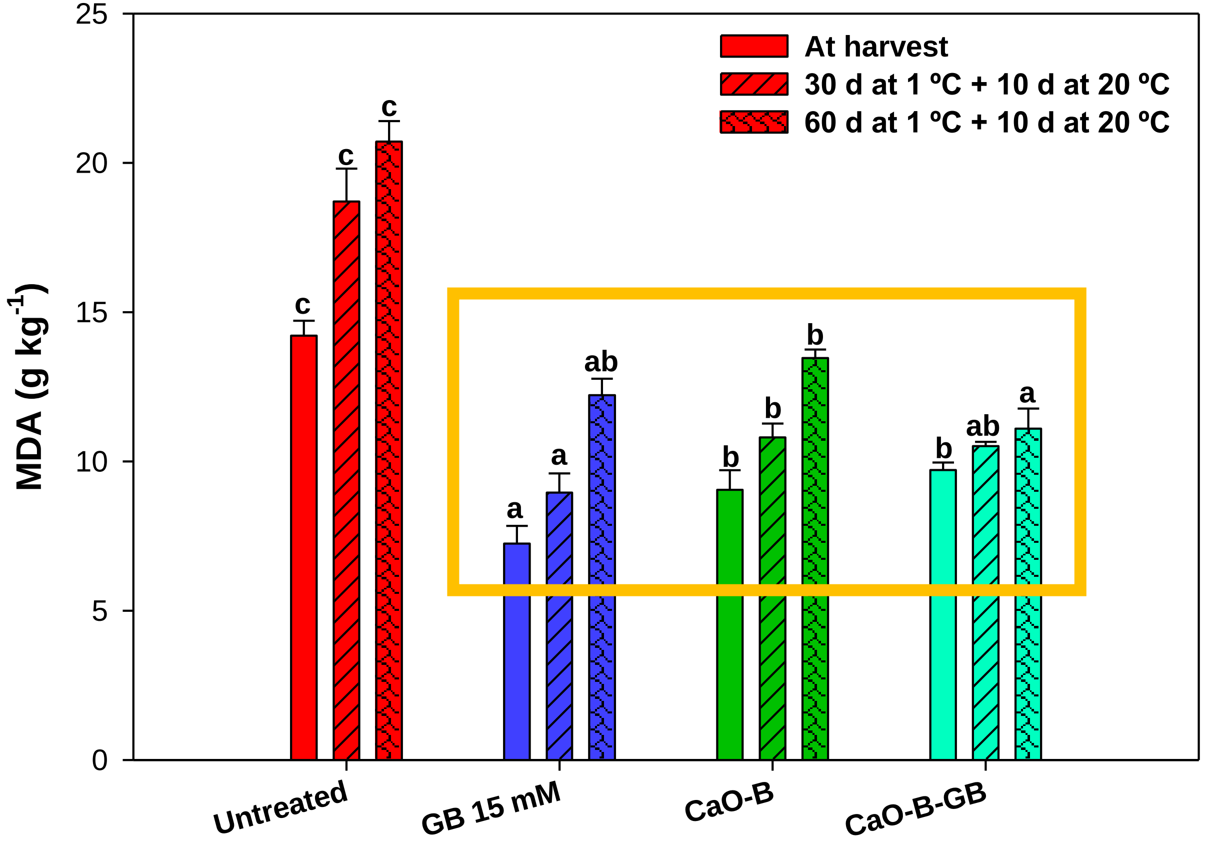
The ion - electrolyte leakage (EL) index measures the integrity of the fruit all membrane.
Higher % means higher ions concentration outside of the membrane and,
therefore, a more damaged cell membrane

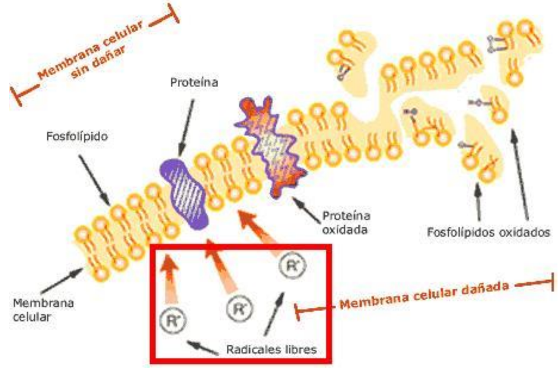
Harvest: -20%

60 d: -14%

Evaluation of CALFORMATE® and OSMOSHIELD® in preharvest treatments on the quality and shelf life of NADORCOTT

RESULTS. LIPID PEROXIDATION (MDA)

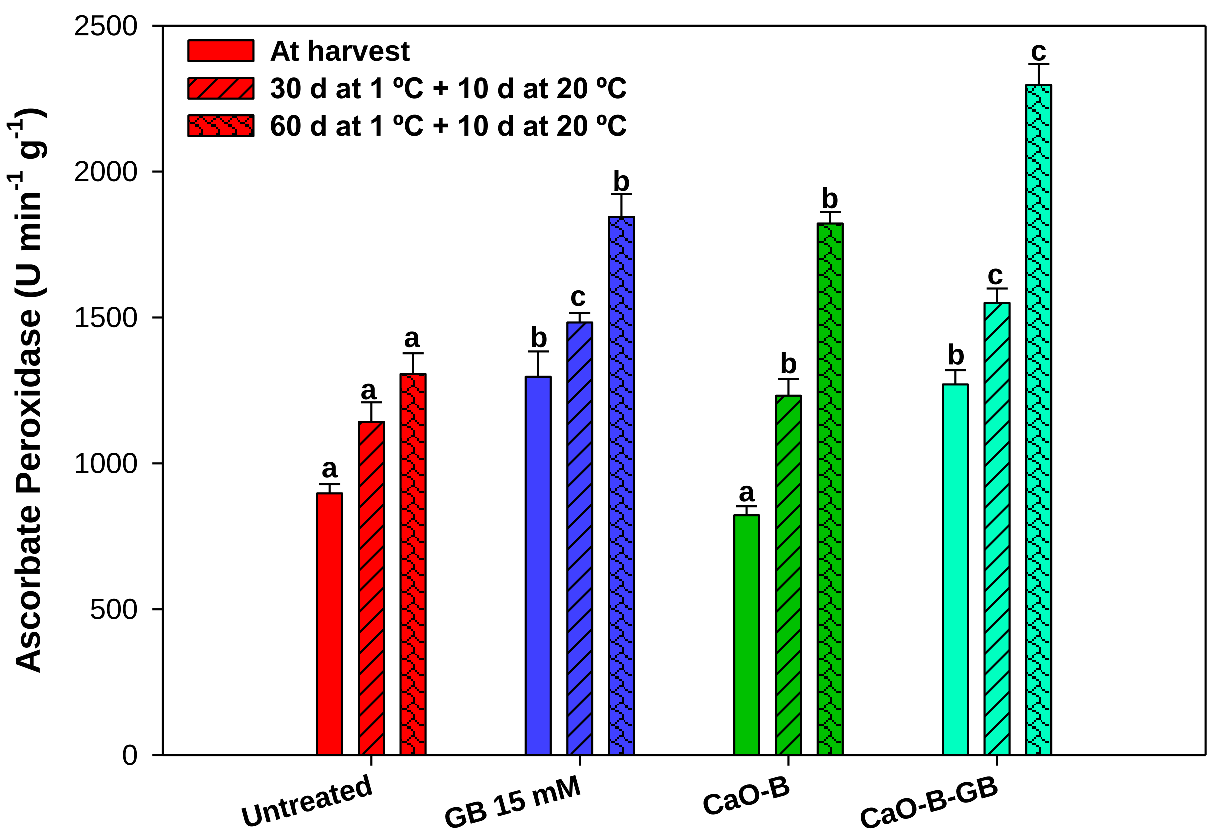




Malondialdehyde content (MDA) is a biomarker of lipid peroxidation produced by reactive oxygen species (ROS). A lower MDA content shows a greater integrity of the cell membrane.

-47%

RESULTS. Ascorbate Peroxidase (APX)

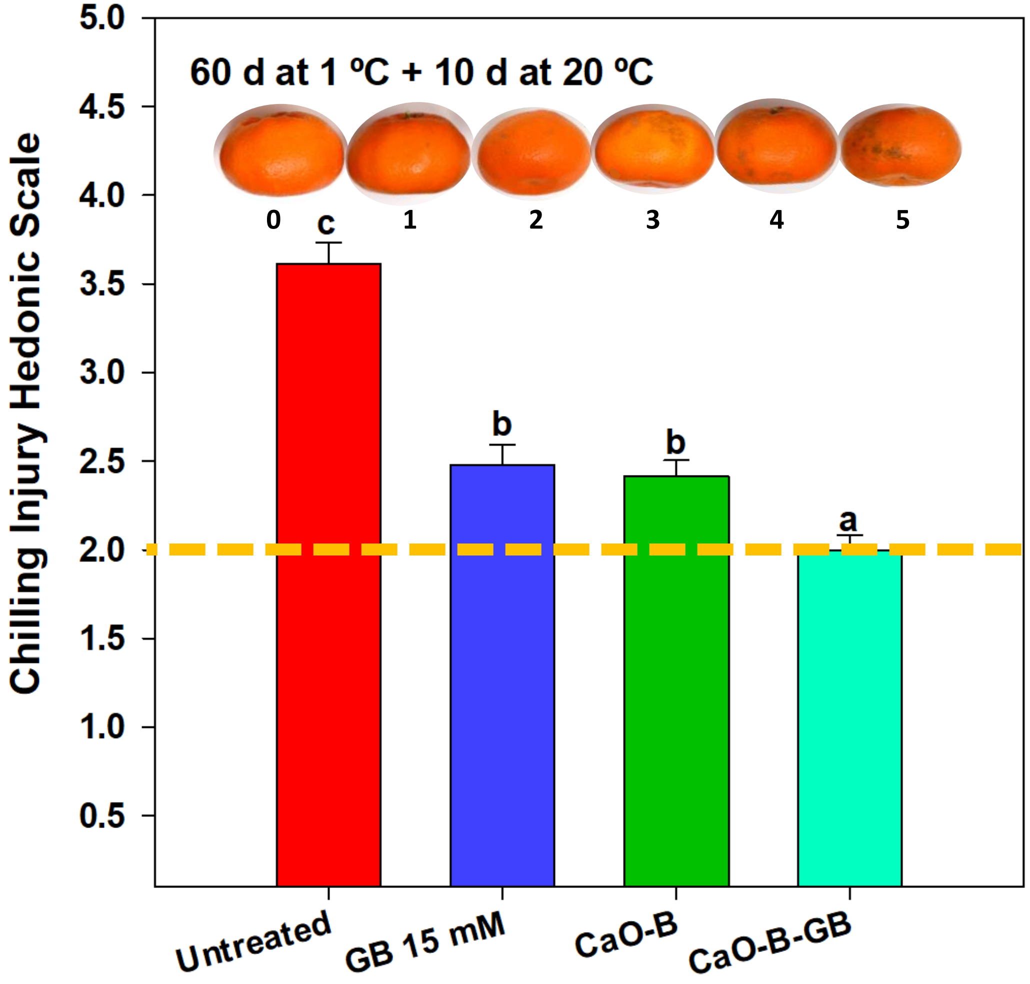


Ascorbate peroxidase (APX) is a key antioxidant enzyme that scavenges/reduces reactive oxygen species (ROS) in plant cells under stress conditions.(Asada, 1999; Mittler et al., 2004).

The combination of CALFORMATE + OSMOSHIELD increases APX levels

+76%

RESULTS. Chilling injury



Chilling injury reduction of:

-45%

Evaluation of CALFORMATE® and OSMOSHIELD® in preharvest treatments on the quality and shelf life of NADORCOTT

CONTROL | 60 d at 1 °C | CALFORMATE + OSMOSHIELD

Evaluation of CALFORMATE® and OSMOSHIELD® in preharvest treatments on the quality and shelf life of NADORCOTT

CONTROL	60 d at 1 °C	CALFORMATE + OSMOSHIELD

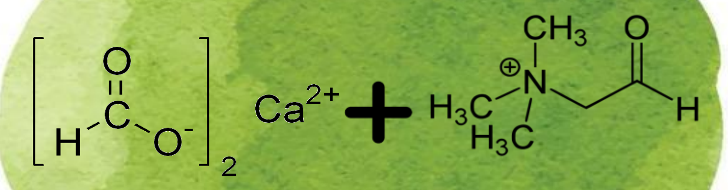
MODE OF ACTION

CALFORMATE

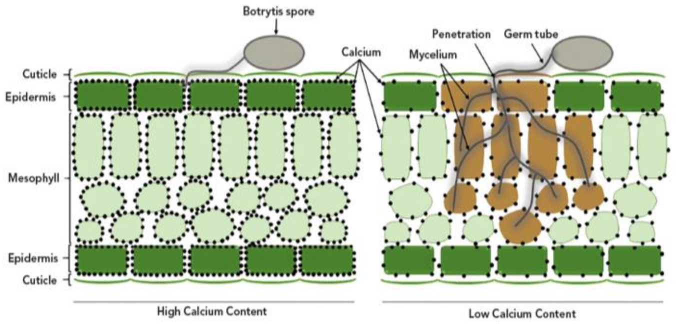
- ✓ Maintains the integrity of the cell wall.
- ✓ Improves the firmness of the fruit.
- ✓ Fruit less susceptible to disease.

OSMOSHIELD

- ✓ It stimulates the enzymatic activity of APX.
- ✓ It maintains the integrity of the cell membrane.
- ✓ Reduces firmness losses.
- ✓ Increases tolerance to chilling injury.



Leaf Cross Section



Strong leaf surface structure vs **Weak** leaf surface when Botrytis spore attack