

**POSTHARVEST ANCONA 2024**  
**INNOVATIONS IN FOOD LOSS AND WASTE MANAGEMENT**

**Aula Magna - Department of Agricultural Food and Environmental  
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**INTEGRATED ALTERNATIVE  
CONTROL MEANS AGAINST  
POSTHARVEST DISEASES OF  
POMEGRANATES AND  
CITRUS FRUIT**

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# **WORK PACKAGE 1: Use of physical means to extend shelf-life of fruit, vegetables, and aromatic plants, and reduce waste**

**TASK 1.1** Application of electrolysed water to citrus and pomegranate fruit

**TASK 1.2** Application of ozone to citrus and pomegranate fruit

**LEAD BENEFICIARY:** DI.S.S.P.A. – UNIVERSITY OF BARI ALDO MORO - **UNIBA**



**Prof. Antonio Ippolito**  
**Dr. Annamaria Mincuzzi**



**CITRUS**



**POMEGRANATES**

# POMEGRANATE: POSTHARVEST DISEASES



Before harvest :

**18%**

**Abiotic damages**

- Bad handling
- Weather



From harvest till transportation :

**23%**

**LATENT PATHOGENS**

- In the field
- **65%**



From transportation to marketing :

**21%**

**POSTHARVEST FUNGAL DISEASES**

**WOUND PATHOGENS**

- Production chain
- 35%

# CITRUS: POSTHARVEST DISEASES



Francesca Garganese - Di.S.S.P.A. – Università degli Studi di Bari  
Aldo Moro

**BROWN SPOT**  
*Alternaria spp.*



**GREEN/BLUE MOLD**  
*Penicillium spp.*



**ANTHRACNOSE**  
*Colletotrichum spp.*

Production chain: incidence about yield losses  
are not up-dated

# POMEGRANATES: PREVIOUS TRIALS

## 1. Phytotoxicity tests:

- Potassium sorbate
  - Sodium bicarbonate
  - Sodium metabisulfite
  - Calcium propionate
- NEUTRAL ELECTROLYZED WATER  
(NEW)
- Gaseous ozone (various concentrations and exposure timings)
  - Ozonated water



Potassium sorbate, sodium bicarbonate, and sodium metabisulfite **phytotoxicity** at **1.25%**

## 2. In the field treatments:

- F1.** Red seaweed fertilizer (commercial product)
- F2.** *Aureobasidium pullulans* (DSM 14940 and DSM 14941 strains, commercial product)
- F3.** *Bacillus amyloliquefaciens* subs. *plantarum* (strain D747, commercial product).
- F4.** Hydrochloride chitosan (commercial product)



# POMEGRANATES: PREVIOUS TRIALS

## **3. Dipping:**

**D1. Ozonized H<sub>2</sub>O**

**D2. Neutral Electrolyzed H<sub>2</sub>O (NEW)**

**D3. NEW + 0.625% sodium metabisulfite**

**D4. NEW + 0.625% potassium sorbate**

**D5. NEW + 0.625% sodium bicarbonate**

**D6. NEW + 0.625% calcium propionate**

## **4. Gaseous O<sub>2</sub> shock treatments :**

- 750 ppb
- 4 h exposure time

**G1. 1 treatment per month every 30 days → 3 treatments.**

**G2. 2 treatments per month every 15 days → 6 treatments.**

**G3. 4 treatments per month every 7 days → 12 treatments.**



### **CRACKED FRUIT:**

**Access via for fungal wound pathogens (*Penicillium s.l.*)**

# POMEGRANATES: INTEGRATED APPROACH

best treatments of the previous trials

## PREHARVEST TRIAL: *Bacillus amyloliquefaciens subs. plantarum*

- 2 application during the **blooming stage (BA-2)**
- 4 application during the **blooming stage + 1 just before harvest (BA-5)**

- 4 months of cold storage + 2 weeks of shelf life

## POSTHARVEST TRIAL:

### Dipping

NEW + 0.625% calcium propionate (**CP**)

### Gaseous ozone treatment

750 ppb O<sub>3</sub> x 4 h exposure time. 4 treatments/month (**O3**)

## EVALUATION OF DECAY INCIDENCE AND SEVERITY

## EPIPHYTIC POPULATION

## QUALITATIVE ANALYSES (color, pH, TSS, TA)

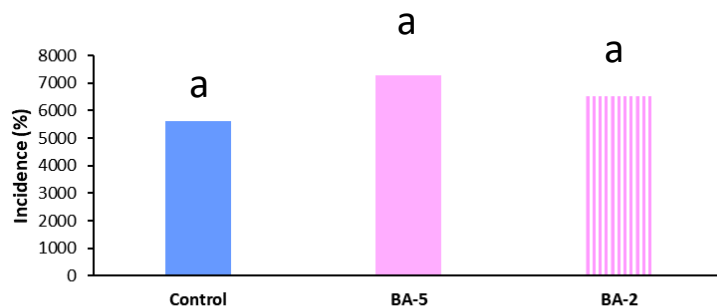
THESES	IN THE FIELD	NEW POSTHARVEST DIPPING	POSTHARVEST GASEOUS O <sub>3</sub>
1	Water control	Calcium propionate	O <sub>3</sub> exposure
2			No O <sub>3</sub>
3		Water control	O <sub>3</sub> exposure
4			No O <sub>3</sub>
5	Amylo-X5	Calcium propionate	O <sub>3</sub> exposure
6			No O <sub>3</sub>
7		Water control	O <sub>3</sub> exposure
8			No O <sub>3</sub>
9	Amylo-X2	Calcium propionate	O <sub>3</sub> exposure
10			No O <sub>3</sub>
11		Water control	O <sub>3</sub> exposure
12			No O <sub>3</sub>

Factorial ANOVA

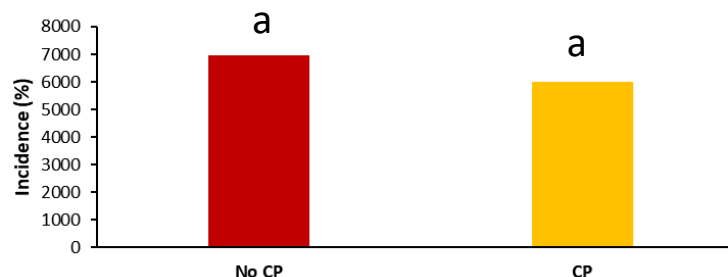
# 5. POMEGRANATES: INTEGRATED APPROACH

## EPIPHYTIC POPULATION

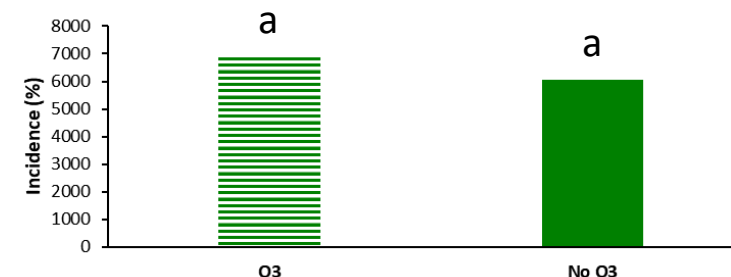
**In the field application**  
Filamentous fungi



**Calcium propionate**  
Filamentous fungi



**Ozone**  
Filamentous fungi



**BA-5** = *B. amyloliquefaciens*/ 5 treatments  
**BA-2** = *B. amyloliquefaciens*/ 2 treatments  
**CP** = NEW+Calcium propionate/postharvest dipping  
**O3** = gaseous ozone/exposure

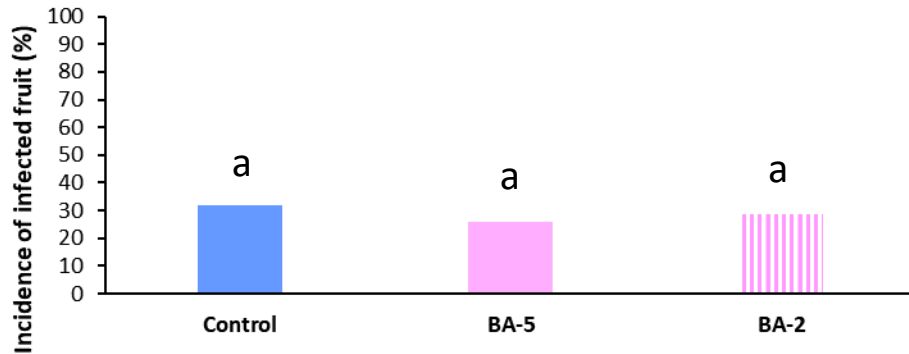
No significant differences



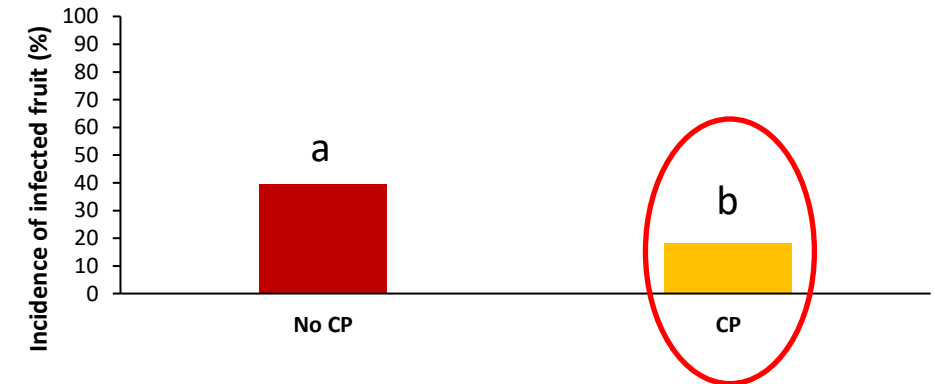
# 5. POMEGRANATES: INTEGRATED APPROACH

## DISEASE INCIDENCE AFTER 4 MONTHS OF COLD-STORAGE

**Pomegranate cold-storage**  
**In the field application**



**Pomegranate cold-storage**  
**Calcium propionate**



**Pomegranate cold-storage**  
**Ozone**



**BA-5** = *B. amyloliquefaciens*/ 5 treatments  
**BA-2** = *B. amyloliquefaciens*/ 2 treatments  
**CP** = NEW+Calcium propionate/postharvest dipping  
**O3** = gaseous ozone/exposure

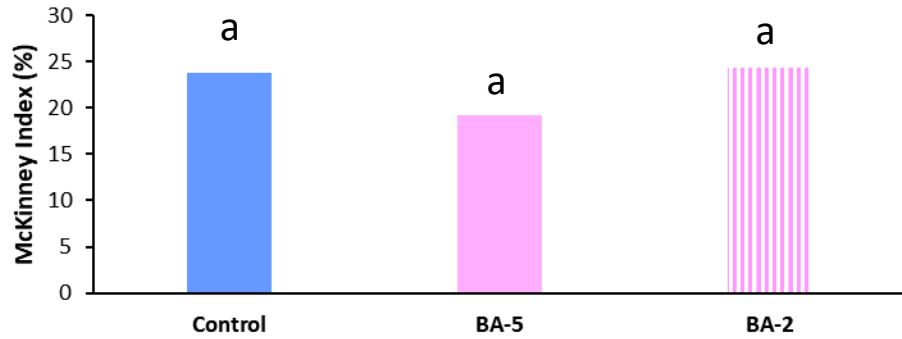


• Incidence

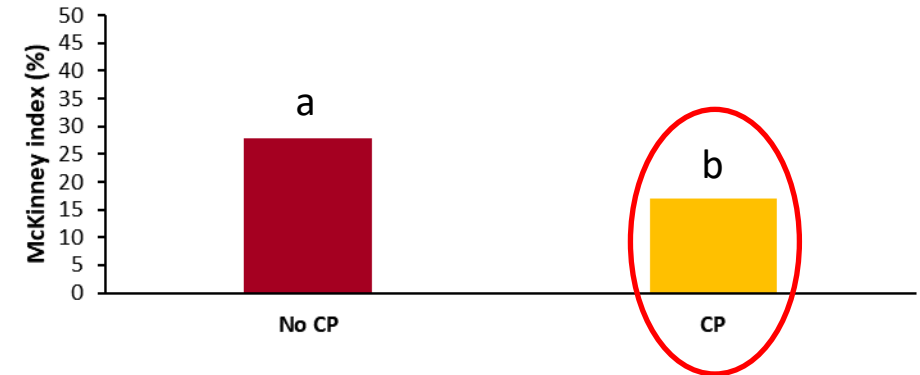
# 5. POMEGRANATES: INTEGRATED APPROACH

## DISEASE INCIDENCE AFTER 4 MONTHS OF COLD-STORAGE AND 2 WEEKS OF SHELF-LIFE

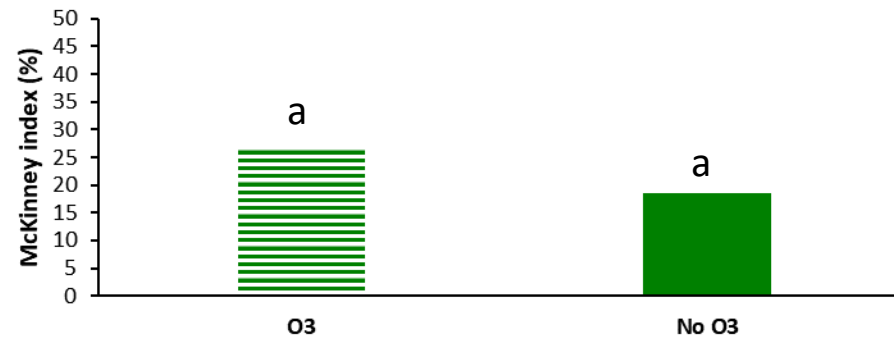
**Pomegranate shelf-life  
In the field application**



**Pomegranate shelf-life  
Calcium propionate**



**Pomegranate shelf-life  
Ozone**



**BA-5** = *B. amyloliquefaciens*/ 5 treatments  
**BA-2** = *B. amyloliquefaciens*/ 2 treatments  
**CP** = NEW+Calcium propionate/postharvest dipping  
**O3** = gaseous ozone/exposure

↓

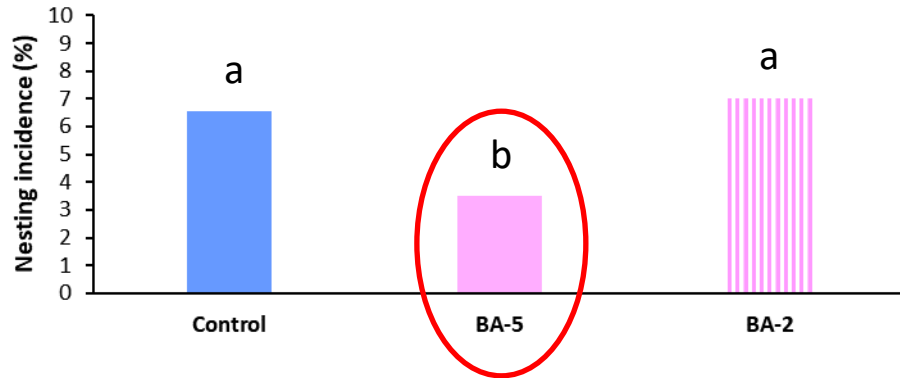
**-40%**

- Incidence
- Severity

# 5. POMEGRANATES: INTEGRATED APPROACH

## NESTING INCIDENCE AFTER 4 MONTHS OF COLD-STORAGE AND 2 WEEKS OF SHELF-LIFE

**Incidence of nesting  
In the field application**



**BA-5 = B.**  
*amyloliquefaciens*/ 5  
treatments

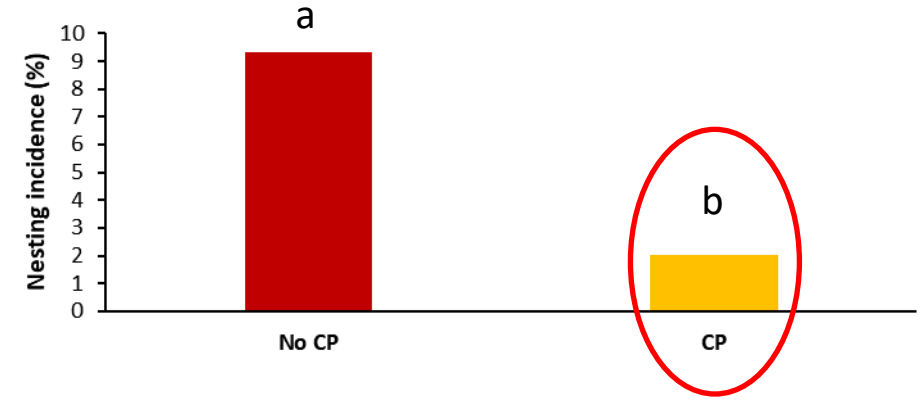
**BA-2 = B.**  
*amyloliquefaciens*/ 2  
treatments

**CP = NEW+Calcium**  
propionate/postharvest  
dipping

**O3 = gaseous**  
ozone/exposure

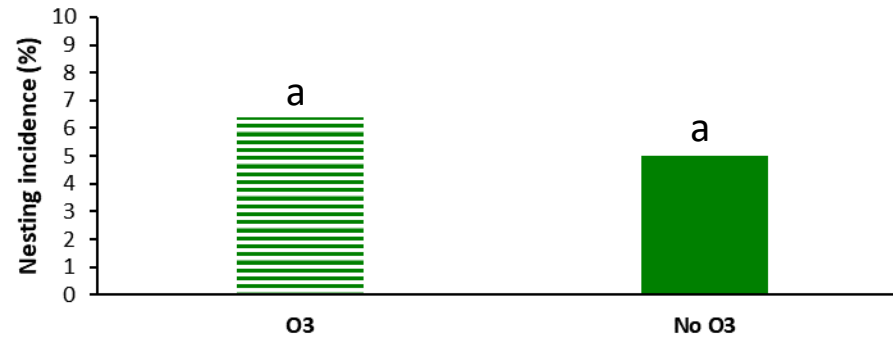
**-47%**

**Incidence of nesting  
Calcium propionate**

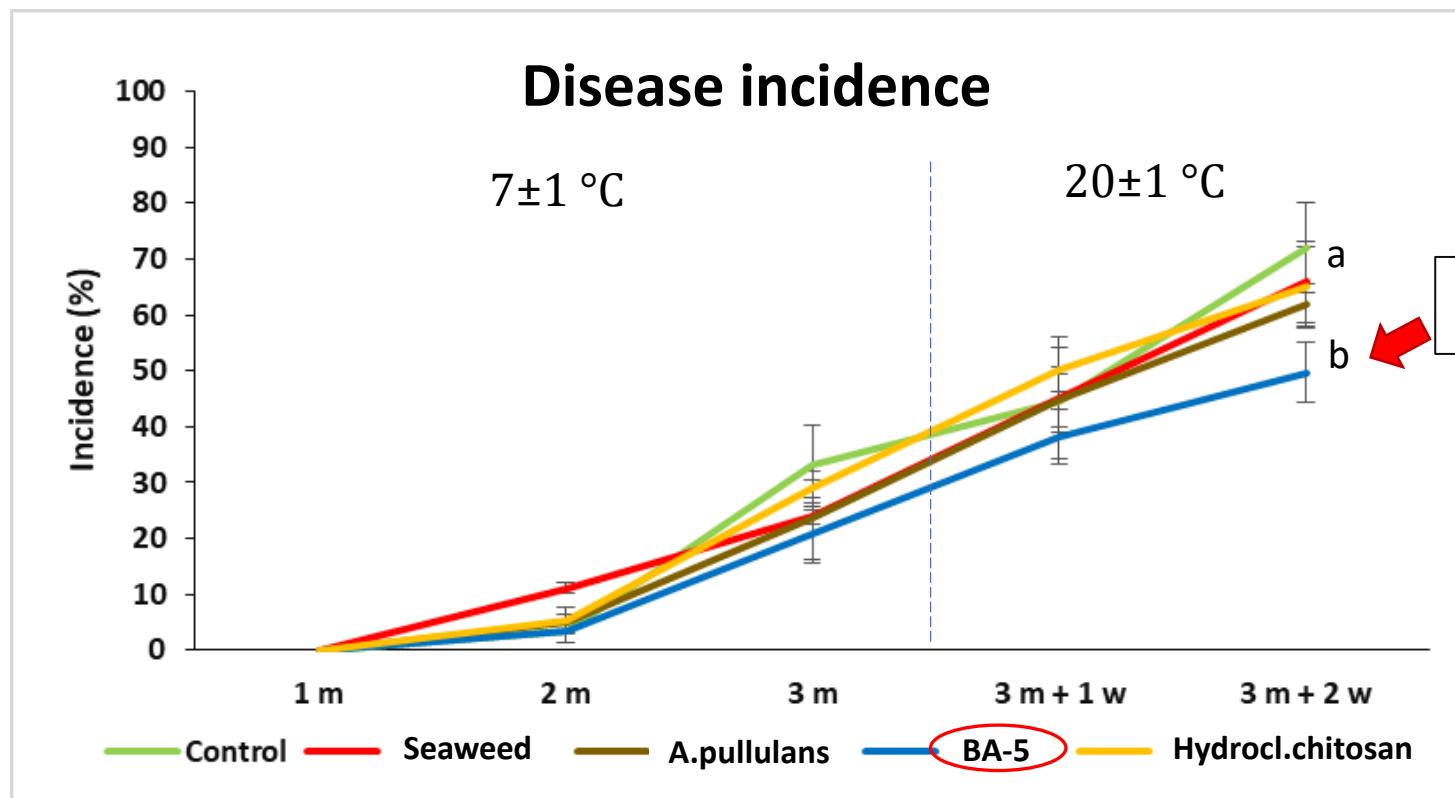


**-78%**

**Incidence of nesting  
Ozone**



# POMEGRANATES 2021: IN THE FIELD TRIAL



*B. amyloliquefaciens*

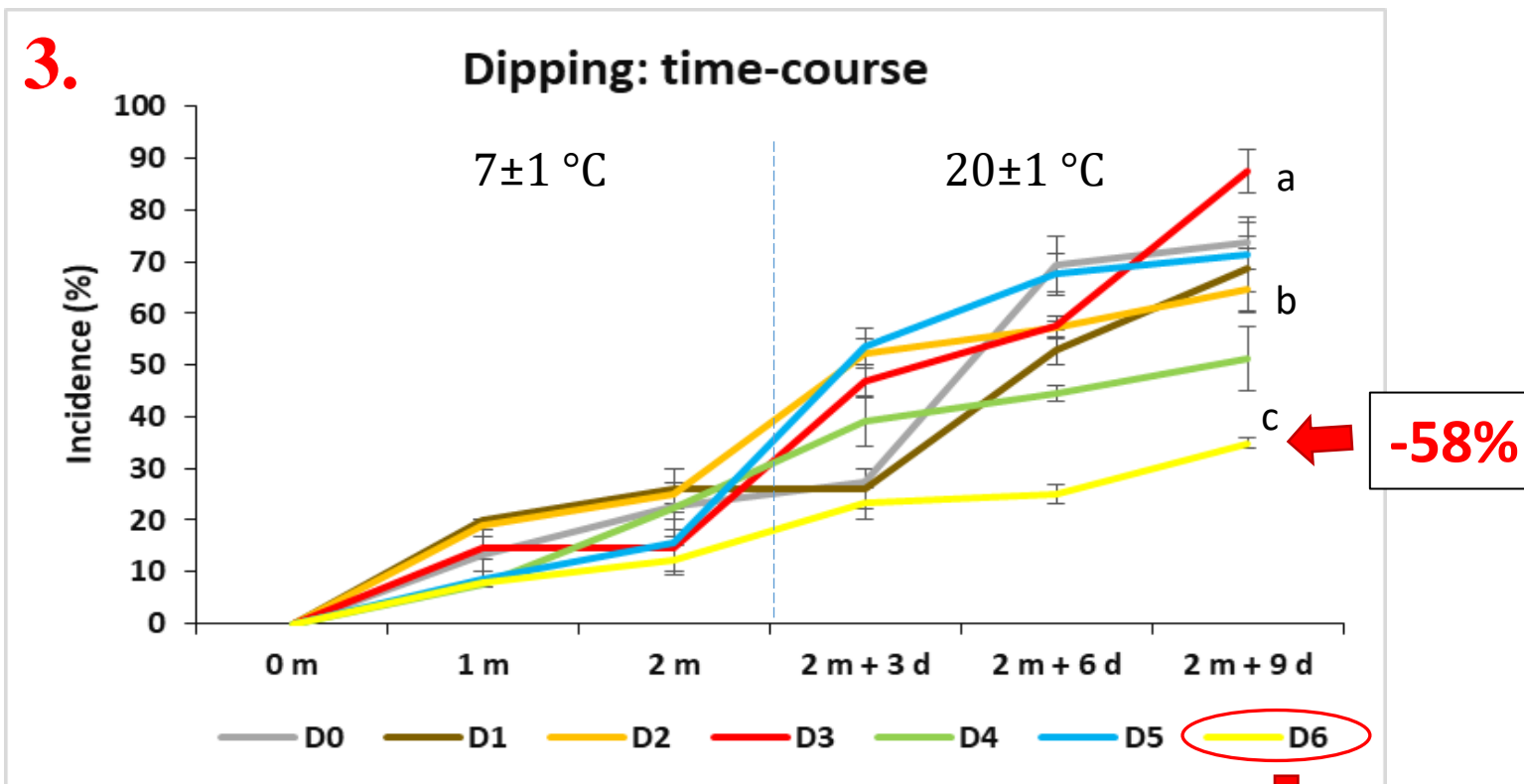


**-43%**

- Reduction by **43%** postharvest decays
- **Grey mold incidence** is more than **halved**.
- **Nesting** represents **20%** of decay incidence

# POMEGRANATES 2021: POSTHARVEST TRIAL

Effectiveness of **CP** previously confirmed by dipping trial with cracked fruit



To **prolong shelf-life** of second-class pomegranates

- D1 = Ozonized H<sub>2</sub>O
- D2 = Neutral Electrolyzed H<sub>2</sub>O (NEW)
- D3 = NEW + 0.625% sodium metabisulfite
- D4 = NEW + 0.625% potassium sorbate
- D5 = NEW + 0.625% sodium bicarbonate
- D6 = NEW + 0.625% calcium propionate

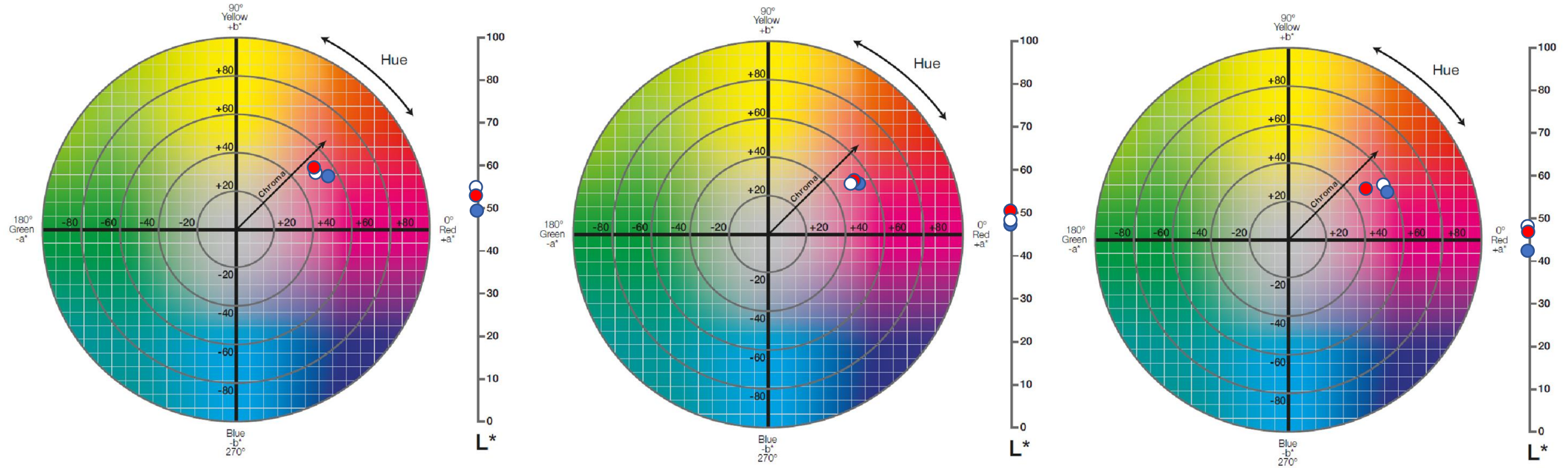
**Calcium propionate**



# 5. POMEGRANATES: INTEGRATED APPROACH

## QUALITY PARAMETERS: COLOR ASSESSMENT

*Before postharvest treatments*



Calyx area

Equatorial area

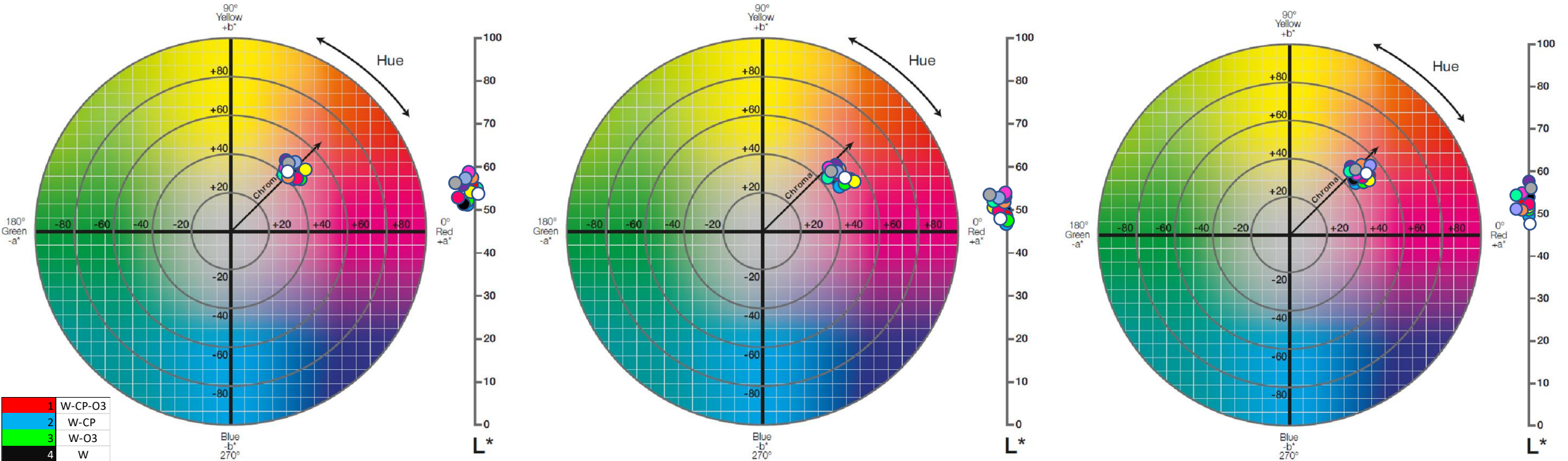
Peduncle area

- Water control
- *B. amyloliquefaciens* x 2
- *B. amyloliquefaciens* x 4 + 1

# 5. POMEGRANATES: INTEGRATED APPROACH

## QUALITY PARAMETERS: COLOR ASSESSMENT

*2 months of cold-storage*



Calyx area

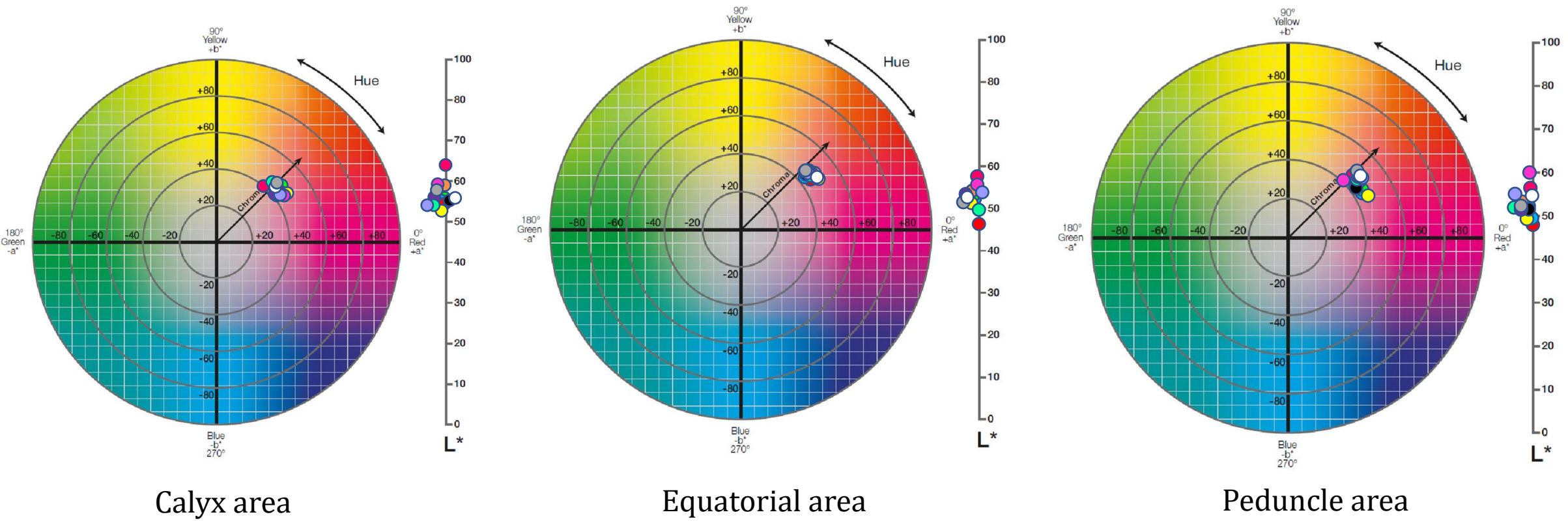
Equatorial area

Peduncle area

# 5. POMEGRANATES: INTEGRATED APPROACH

## QUALITY PARAMETERS: COLOR ASSESSMENT

*4 months of cold-storage*



**Steady values** over time and among treatments like pH, titratable acidity (TA), and total soluble solids (TSS)



# CITRUS: POSTHARVEST TRIALS

1) *In vitro*: **conidial survival** of *Penicillium digitatum* and *Penicillium italicum*:

- **continuous exposure**
- **1-min exposure**

2) *In vivo*: **injured citrus** (mandarinate [Fortune mandarin (2x) × Avana mandarin (4x)]):

- **continuous ozone**
- **3 gaseous ozone concentration (250, 500, 750 ppb)**
- **4 time of exposure (3, 6, 9, 12 h)**

3) *In vivo*: **injured citrus** (Navelina): **SEMI-COMMERCIAL SCALE**

- **3 ozone treatments/day**
- **7 g/h O<sub>3</sub>**

4) *In vivo*: **injured citrus** (clementines):

- **NEW amended with 0.625% calcium propionate**

5) *In vivo*: **injured citrus** (Femminello): **INTEGRATED APPROACH**



ARTIFICIAL WOUNDS  
&  
NATURAL INFECTIONS

# 5. CITRUS: INTEGRATED APPROACH

*best treatments*

*In vivo*: **injured citrus** ('Femminello' lemon):

Dipping

- 1) H<sub>2</sub>O with/without O<sub>3</sub>
- 2) Imazalil with/without O<sub>3</sub>
- 3) 0.625% calcium propionate with/without O<sub>3</sub>
- 4) NEW + 0.625% calcium propionate with/without O<sub>3</sub>
- 5) 0.625% sodium bicarbonate with/without O<sub>3</sub>
- 6) NEW + 0.625% sodium bicarbonate with/without O<sub>3</sub>

Gaseous ozone treatment

- 750 ppb O<sub>3</sub> x 3h exposure time
- Daily treatment (7 treat./week).

EVALUATION OF DECAY INCIDENCE AND SEVERITY

QUALITATIVE ANALYSES (color, pH, TSS, TA)

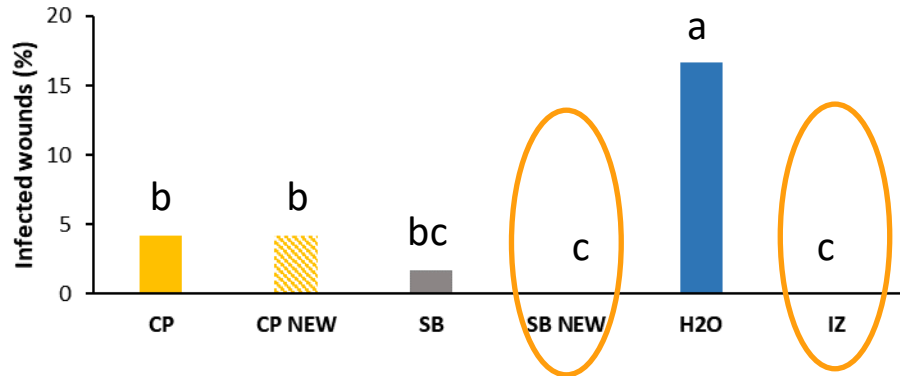
THESES	POSTHARVEST DIPPING	POSTHARVEST GASEOUS O <sub>3</sub>
T1.1	Calcium propionate	No O <sub>3</sub>
T2.1		O <sub>3</sub> exposure
T1.2	NEW + calcium propionate	No O <sub>3</sub>
T2.2		O <sub>3</sub> exposure
T1.3	Sodium bicarbonate	No O <sub>3</sub>
T2.3		O <sub>3</sub> exposure
T1.4	NEW + sodium bicarbonate	No O <sub>3</sub>
T2.4		O <sub>3</sub> exposure
T1.5	Water	No O <sub>3</sub>
T2.5		O <sub>3</sub> exposure
T1.6	Imazalil	No O <sub>3</sub>
T2.6		O <sub>3</sub> exposure

**Factorial ANOVA**

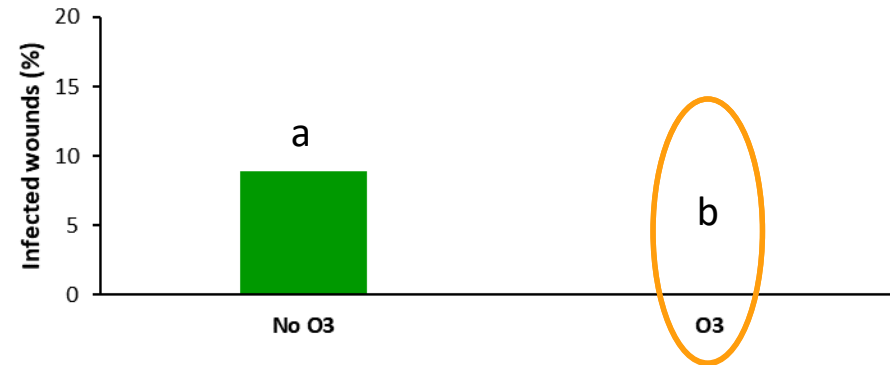
# 5. CITRUS: INTEGRATED APPROACH

## DISEASE INCIDENCE AFTER 2 WEEKS OF COLD-STORAGE

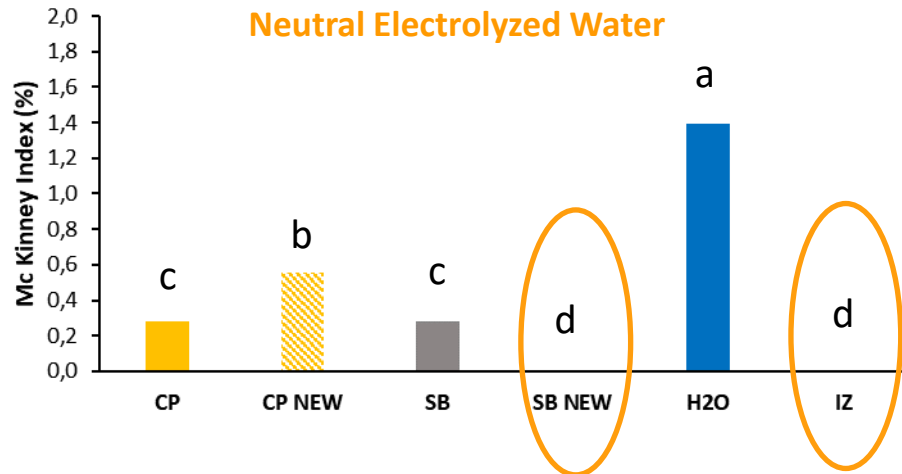
Citrus cold-storage  
Neutral Electrolyzed Water



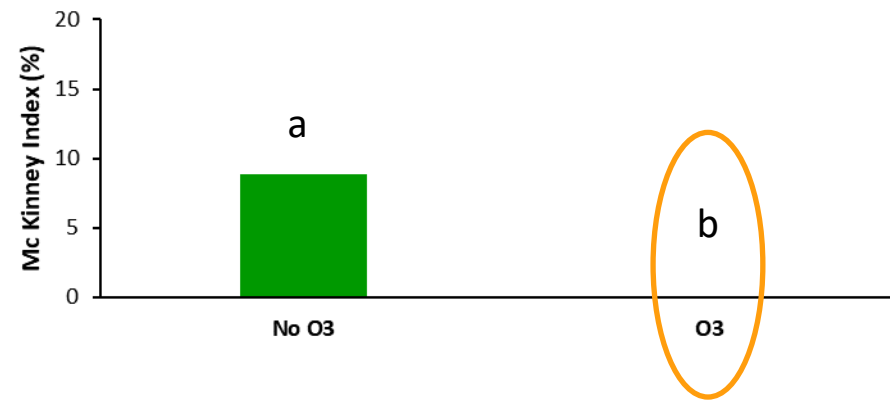
Citrus cold-storage  
Ozone



Citrus cold-storage  
Neutral Electrolyzed Water



Citrus cold-storage  
Ozone



- Rot incidence and severity were reduced by 70-90% in other treatments
- **NEW + SB** and **ozone**

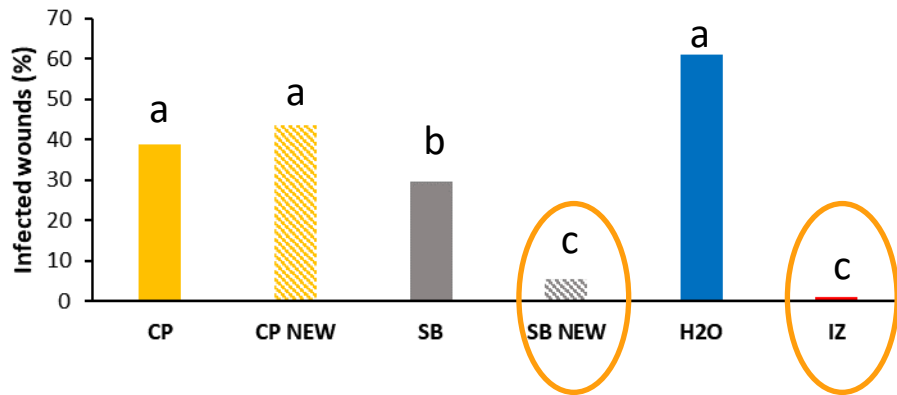
↓  
**-100%**

**CP** = Calcium propionate/postharvest dipping  
**SB** = Sodium bicarbonate/postharvest dipping  
**NEW** = Neutral Electrolyzed Water  
**IZ** = Imazalil//postharvest dipping  
**O3** = gaseous ozone/exposure

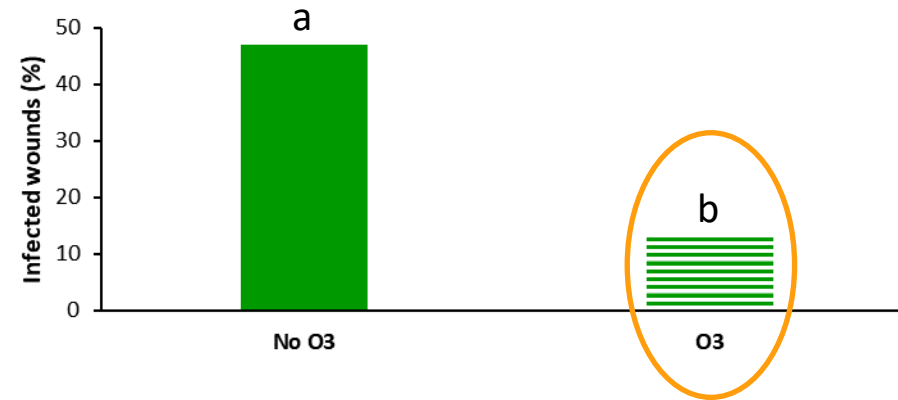
# 5. CITRUS: INTEGRATED APPROACH

## DISEASE INCIDENCE AFTER 2 WEEKS OF COLD-STORAGE AND 3 DAYS OF SHELF-LIFE

Citrus shelf-life  
Neutral Electrolyzed Water



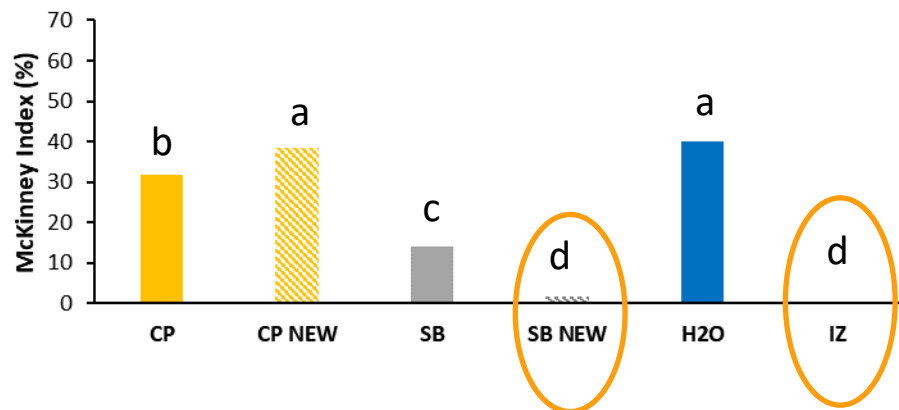
Citrus shelf-life  
Ozone



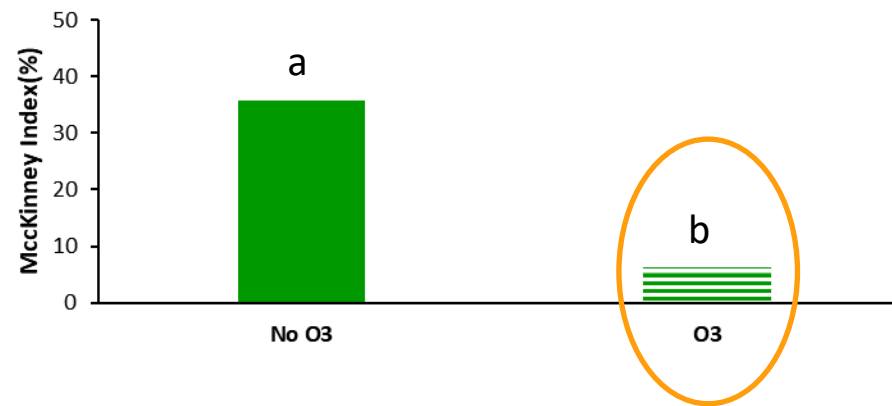
- **NEW + SB** reduced by **91-96%** disease incidence and severity

- **Ozone** reduced by **72-83%** disease incidence and severity

Citrus shelf-life  
Neutral Electrolyzed Water



Citrus shelf-life  
Ozone

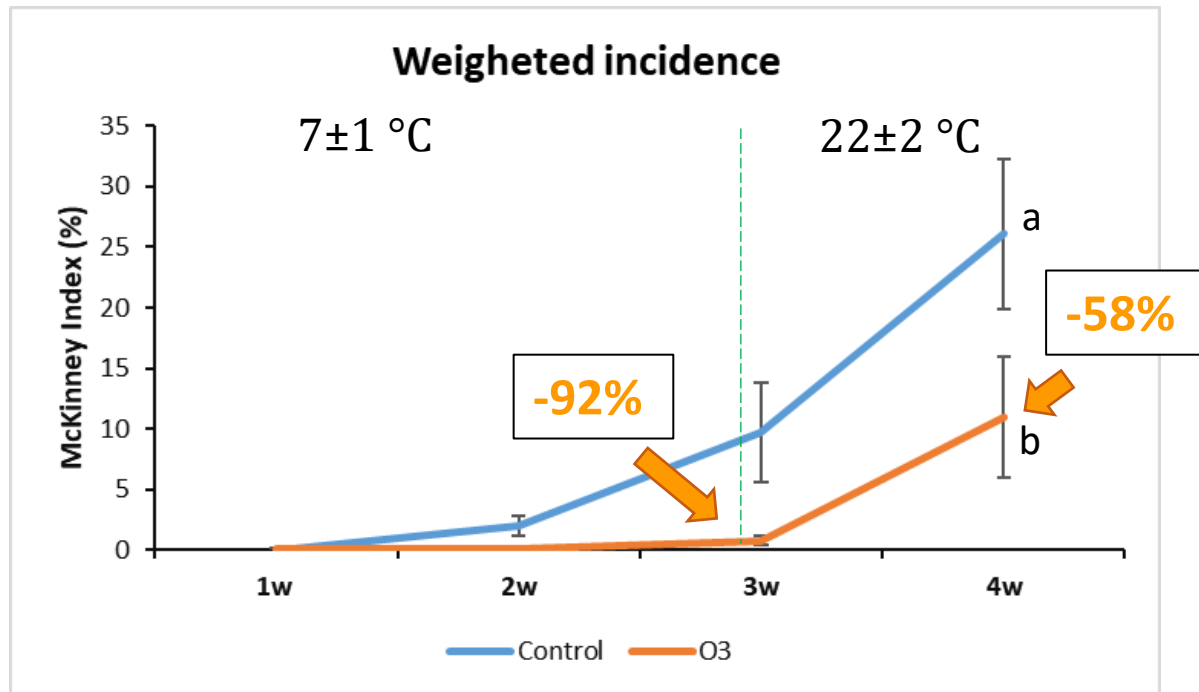


**CP** = Calcium propionate/postharvest dipping  
**SB** = Sodium bicarbonate/postharvest dipping  
**NEW** = Neutral Electrolyzed Water  
**IZ** = Imazalil//postharvest dipping  
**O3** = gaseous ozone/exposure

# CITRUS: POSTHARVEST TRIALS

## 3. *In vivo*: injured citrus (Navelina): SEMI-COMMERCIAL SCALE

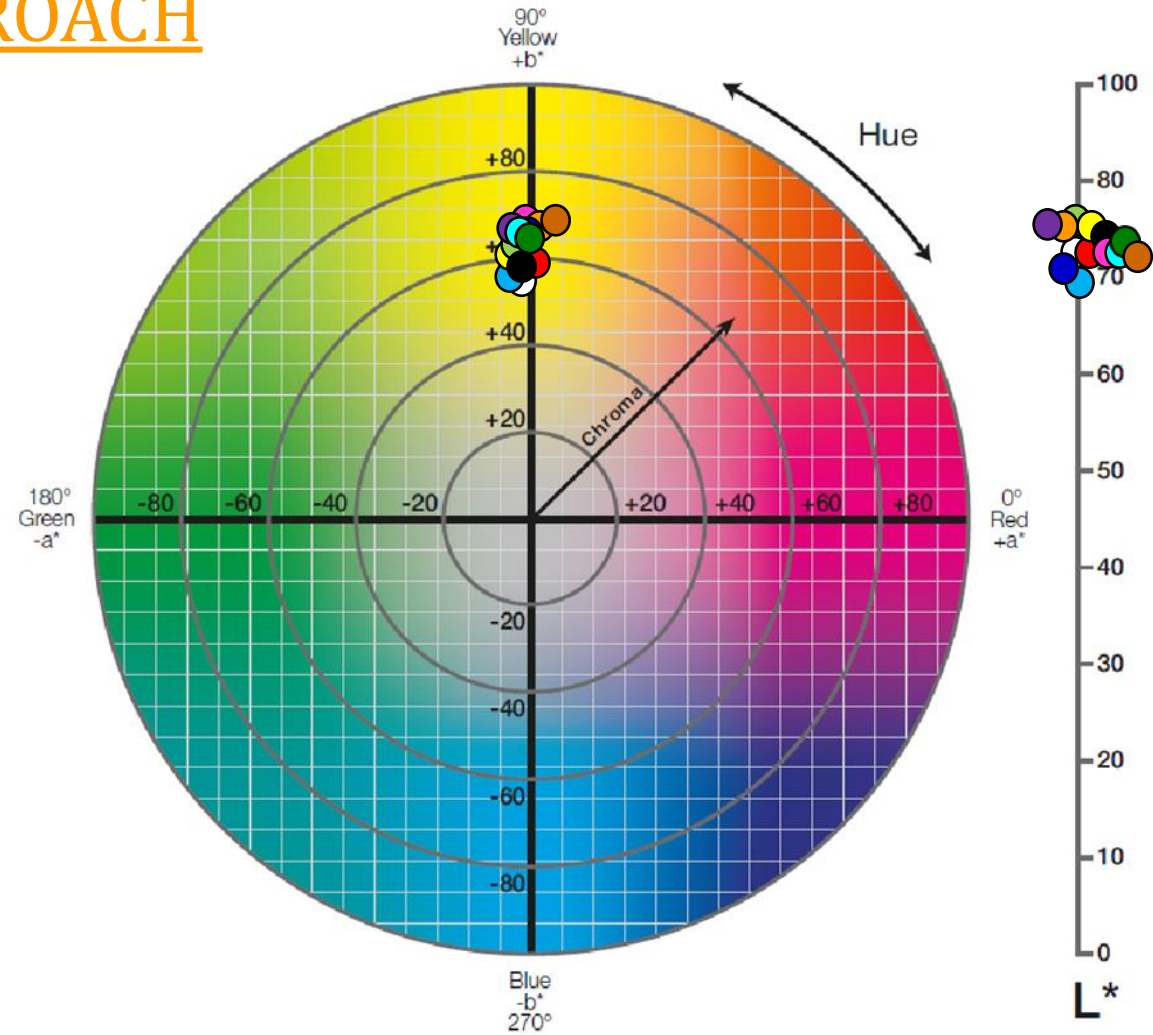
Effectiveness of **ozone** previously confirmed in the **semi-commercial scale trial**



O3 = gaseous ozone/exposure

## 5. CITRUS: INTEGRATED APPROACH

THESES	POSTHARVEST DIPPING	POSTHARVEST GASEOUS O <sub>3</sub>
<span style="color:red">●</span> T1.1	Calcium propionate	No O <sub>3</sub>
<span style="color:orange">●</span> T2.1		O <sub>3</sub> exposure
<span style="color:blue">●</span> T1.2	NEW + calcium propionate	No O <sub>3</sub>
<span style="color:blue">●</span> T2.2		O <sub>3</sub> exposure
<span style="color:yellow">●</span> T1.3	Sodium bicarbonate	No O <sub>3</sub>
<span style="color:purple">●</span> T2.3		O <sub>3</sub> exposure
<span style="color:green">●</span> T1.4	NEW + sodium bicarbonate	No O <sub>3</sub>
<span style="color:cyan">●</span> T2.4		O <sub>3</sub> exposure
<span style="color:black">●</span> T1.5	Water	No O <sub>3</sub>
<span style="color:green">●</span> T2.5		O <sub>3</sub> exposure
<span style="color:pink">●</span> T1.6	Imazalil	No O <sub>3</sub>
<span style="color:orange">●</span> T2.6		O <sub>3</sub> exposure

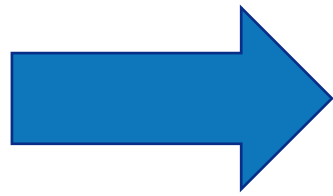


○ Standard values

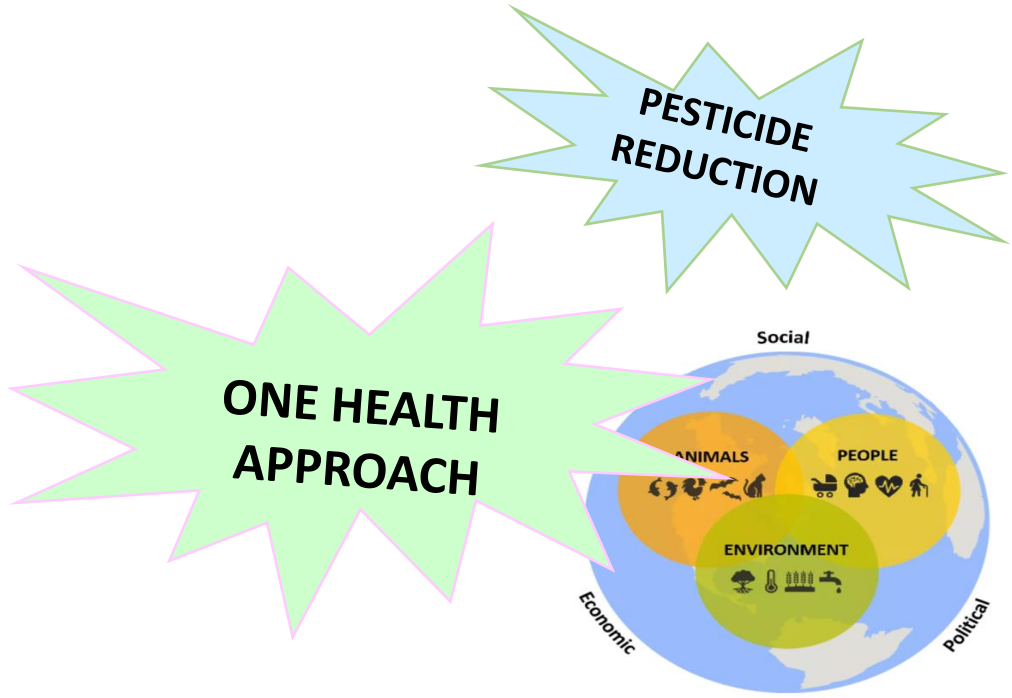
**Steady values** over time and among treatments like pH, titratable acidity (TA), and total soluble solids (TSS)

# INTEGRATED APPROACH

- I. In the field treatments**
- II. Postharvest treatments**



- I. Latent pathogens**
- II. Wound pathogens**



## DETAILING...

**1. *B. amyloliquefaciens* and NEW + Calcium propionate**



**1. Pomegranate postharvest diseases**

**2. NEW + Sodium bicarbonate and gaseous ozone**



**2. Citrus postharvest diseases**



**POMEGRANATES**

**CITRUS**





# Thank you!



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