



# Chitosan treatment for the control of postharvest decay of fruit

**Gianfranco Romanazzi**

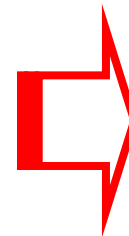
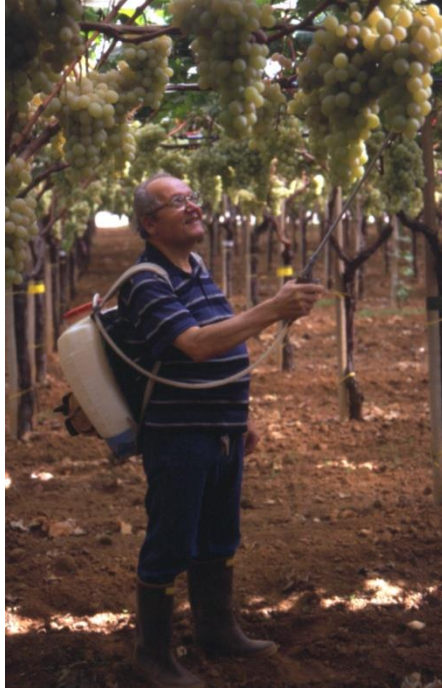
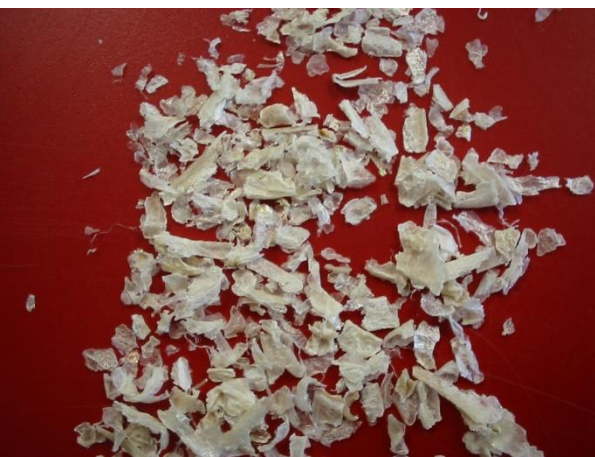
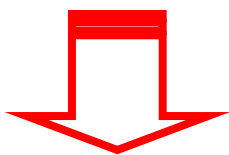
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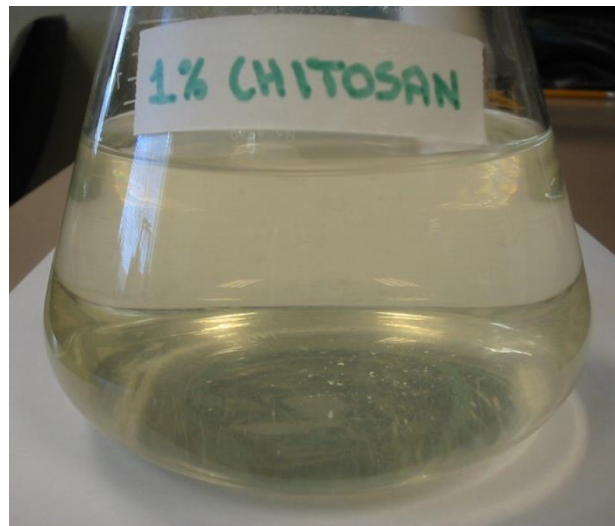
 *GianfRomanazzi*

# What's chitosan?





2 weeks after the spray



Dissolved in diluted acids



**... for its properties, chitosan  
can be an ideal coating for  
fruit and vegetables**



***(Riccardo Muzzarelli,  
University of Ancona, 1986)***



Postharvest Pathology and Mycotoxins

## Antifungal Activity of Chitosan on Two Postharvest Pathogens of Strawberry Fruits

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### ABSTRACT

El Ghaouth, A., Arul, J., Grenier, J., and Asselin, A. 1992. Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits. *Phytopathology* 82:398-402.

Effect of chitosan coating on decay of strawberry fruits held at 13 C was investigated. Strawberry fruits were inoculated with spore suspensions of *Botrytis cinerea* or *Rhizopus stolonifer* and subsequently coated with chitosan solutions (10 or 15 mg/ml). After 14 days of storage, decay caused by *B. cinerea* or *R. stolonifer* was markedly reduced by chitosan coating. Decay was not reduced further when the concentration of chitosan coating was increased from 10 to 15 mg/ml. Coating intact strawberries with chitosan did not stimulate chitinase, chitosanase, or  $\beta$ -1,3-glucanase activities in the tissue as revealed by polyacrylamide gel

assays. Chitosan, when applied on freshly cut strawberries, however, stimulated acidic chitinase activity. Chitosan was very effective in inhibiting spore germination, germ tube elongation, and radial growth of *B. cinerea* and *R. stolonifer* in culture. Furthermore, chitosan at a concentration greater than 1.5 mg/ml induced morphological changes in *R. stolonifer*. Mechanisms by which chitosan coating reduced the decay of strawberries appear to be related to its fungistatic property rather than to its ability to induce defense enzymes such as chitinase, chitosanase, and  $\beta$ -1,3-glucanase.

*Additional keywords:* *Fragaria* sp., glucanohydrolase, gray mold.

## MATERIALS AND METHODS

**Materials.** Crab-shell chitosan was purchased from ICN Biochemical Inc. (Cleveland, OH) and ground to a fine powder. The purified chitosan was prepared by dissolving chitosan in 0.25 N HCl, and the undissolved particles were removed by centrifugation (15 min, 10,000 g at 24 C). The viscous solution was then neutralized with 2.5 N NaOH (pH 9.8). Precipitated chitosan was collected by centrifugation, washed extensively with deionized water to remove the salts, and subsequently lyophilized.

**Decay.** Chitosan solutions (10 and 15 mg/ml) were prepared by dissolving chitosan in 0.25 N HCl and adjusting the pH to 5.6 with 2 N NaOH. Strawberry fruits were inoculated by dipping in a solution of 0.1% (v/v) Tween 80 containing  $2 \times 10^5$  conidia per milliliter of *B. cinerea* or *R. stolonifer* and were allowed to air dry at 20 C for 2 h. Inoculated berries were then individually dipped either in the chitosan solution (10 or 15 mg/ml) with 0.1% (v/v) Tween 80 or in sterile deionized water (pH 5.6) containing 0.1% (v/v) Tween 80. Treatments consisted of four repli-

# Effects of Pre- and Postharvest Chitosan Treatments to Control Storage Grey Mold of Table Grapes

G. ROMANAZZI, F. NIGRO, A. IPPOLITO, D. DI VENERE, AND M. SALERNO

**ABSTRACT:** The effectiveness of pre- and postharvest treatments with chitosan (0.1, 0.5, and 1.0%) to control *Botrytis cinerea* on table grapes was investigated. In postharvest treatments, small bunches dipped in chitosan solutions and inoculated with the pathogen showed a reduction of incidence, severity, and nesting of grey mold, in comparison with the control. Single berries artificially wounded, treated with the polymer, and inoculated with *B. cinerea* showed a reduced percentage of infected berries and lesion dia. Higher chitosan concentrations demonstrated greater decay reduction. All preharvest treatments significantly reduced the incidence of grey mold, as compared to the control. Table grapes treated with 1.0% chitosan showed a significant increase of phenylalanine ammonia-lyase (PAL) activity. Consequently, besides a direct activity against *B. cinerea*, chitosan produces other effects contributing to reduce decay.

**Keywords:** *Botrytis cinerea*, postharvest decay, PAL activity, sulphur dioxide, microflora

## Introduction

GREY MOLD, INDUCED BY *BOTRYTIS CINEREA* PERS., CAUSES HEAVY losses of table grapes in the field and is a major obstacle to their long-distance transport and storage. The pathogen is able to develop at low temperature, shortening the length of storage and marketing (Ippolito and others 1998). In Italy, no synthetic fungicides are licensed to control decay of table grapes after harvest; sulphur dioxide is permitted as an adjuvant and is effective in reducing grey mold development during storage. However, alternatives to SO<sub>2</sub> are required in view of damage to bunches due to temperature increase, of hazards for human health, and of the difficulties in using SO<sub>2</sub> with colored grapes (Nelson and Richardson 1967). Considerable progress has recently been made in developing alternatives to synthetic fungicides for the control of postharvest diseases of fruit and vegetables (Wilson and Wisniewski 1994; Sclena and others 1999; Ippolito and Nigro 2000; Romanazzi and others 2001a). The use of a natural substance such as chitosan, a high molecular weight cationic polysaccharide present in fungal cell walls and arthropod exoskeletons, has been considered as a valid alternative. In fact, chitosan is an ideal preservative coating for fresh fruit and vegetables because of its film-forming and biochemical properties (Muzzarelli 1986); it prolongs storage life and controls decay of strawberries (El Ghaouth and others 1991; Romanazzi and others 2000a), litchi (Zhang and Quantick 1997), and apples (Du and others 1998). Chitosan reduces the growth of many phytopathogenic bacteria and fungi (Allan and Hadwiger 1979). Moreover, it elicits phytoalexin formation (Reddy and others 1999) and induces the production of antifungal hydrolases (Fajardo and others 1998; Zhang and Quantick 1998; Hirano 1999). Chitosan has generally been applied in postharvest treatments (Baldwin and others 1995; Cheah and others 1997), and there are very few examples of preharvest application (Reddy and others 2000; Romanazzi and others 2000a, 2000b).

The objective of this study was to investigate the effectiveness of pre- and postharvest chitosan treatments in controlling

grey mold storage rot of table grapes. In addition, the influence of chitosan on the naturally-occurring microflora and on phenylalanine ammonia-lyase (PAL) activity of the treated berries was evaluated.

## Materials and Methods

### Fruits

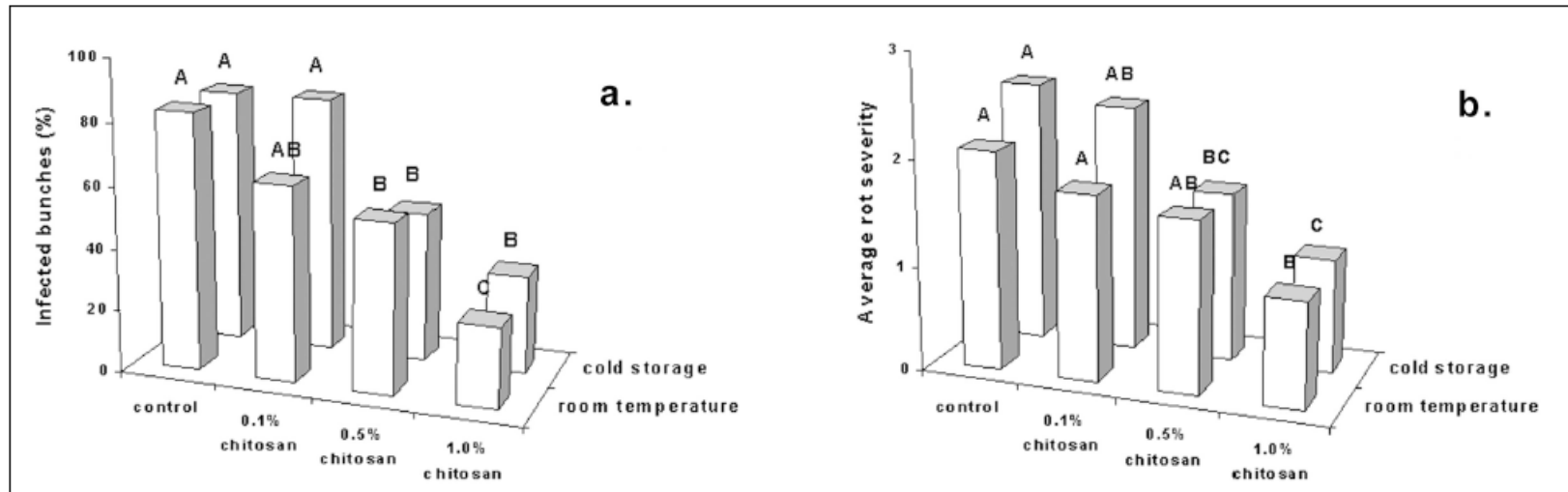
Trials were carried out on table grapes (*Vitis vinifera* L., cv Italia) grown in commercial groves located at Rutigliano (Province of Bari), Southern Italy. Vines, cultivated according to standard cultural practices, were covered with plastic sheets in the 2nd half of August to protect bunches from rainfall and to delay the harvest.

### Pathogens

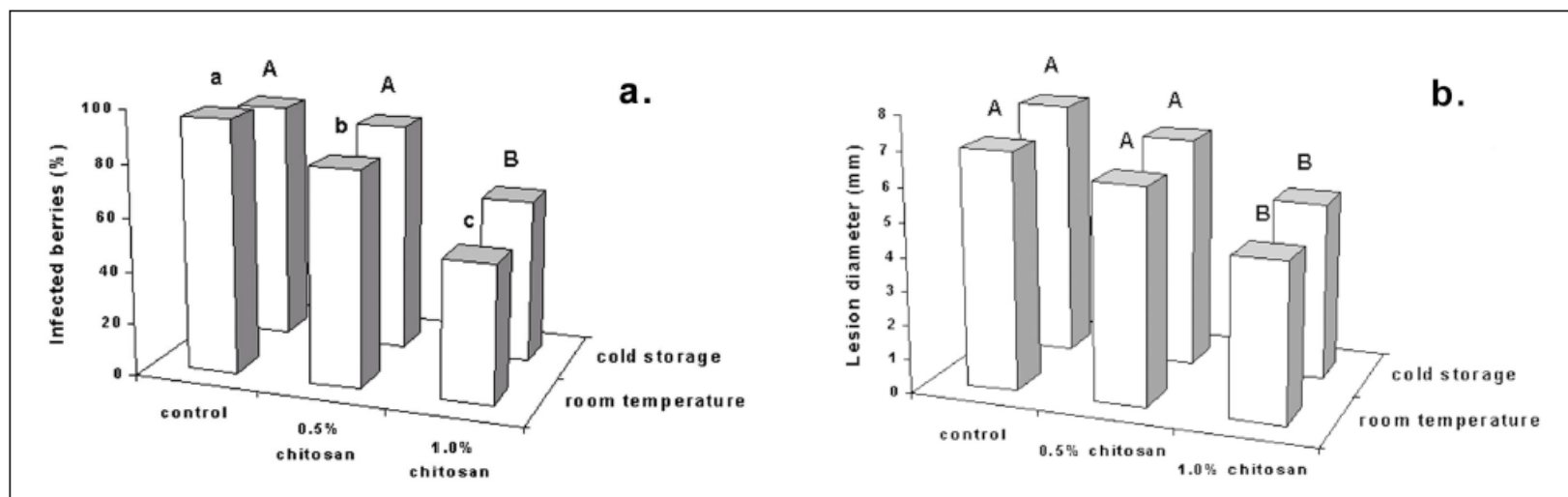
*B. cinerea*, strain 69, had been isolated from a cold-stored table grape berry and maintained on potato dextrose agar (PDA) slants at 5 ± 1 °C, with annual inoculation and re-isolation from berries to maintain virulence. In the drop-inoculation experiments, the inoculum consisted of aqueous spore suspension (10<sup>4</sup> spores ml<sup>-1</sup>); in the spray-application experiments, concentrated stock suspension was added to achieve a final concentration of 10<sup>5</sup> spores ml<sup>-1</sup>. The spore suspension was prepared by flooding a 12-d old culture of *B. cinerea*, grown at 20 ± 2 °C, with 10 ml of sterile distilled water containing 0.1% (v/v) Tween 80 (Eastman Chemical, Kingsport, Tenn., U.S.A.) gently agitated to remove the spores.

### Chitosan

Crab-shell chitosan, purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.), was ground to a fine powder (particle size smaller than 1 mm) by extensive grinding in a mortar, washed 3 times in distilled water (20 ml of water per g of chitosan), pelleted by low-speed centrifugation and air-dried at room temperature. The purified chitosan was prepared as described by Benhamou and others (1994). For experimental use the stock

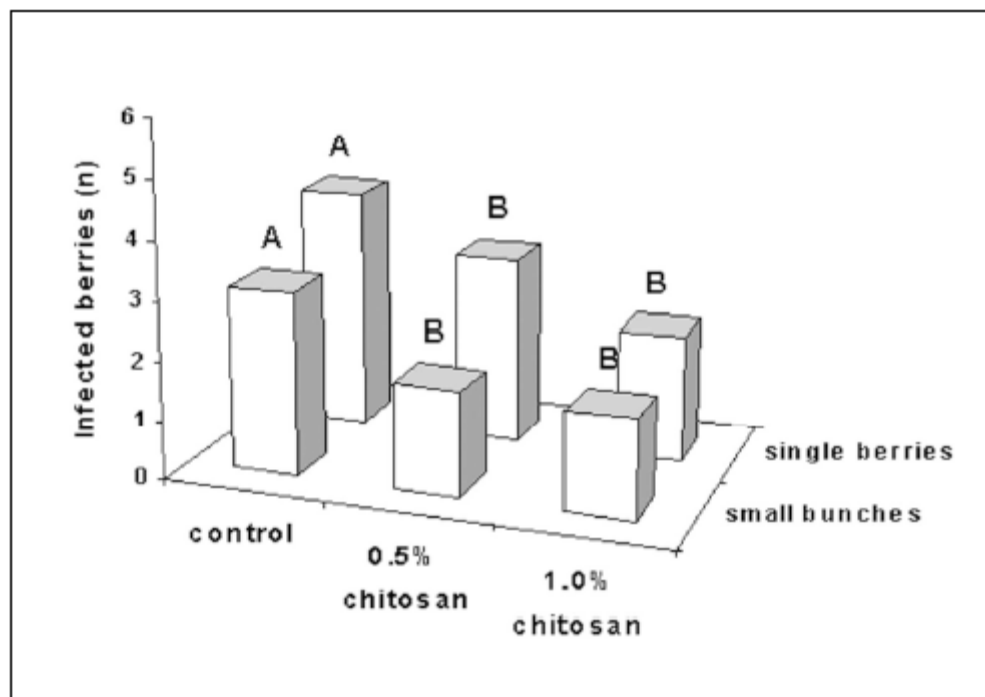


**Figure 1—Effect of chitosan on the percentage of infected small bunches (a) and on average rot severity (b). Bunches were dipped in chitosan, sprayed with a *Botrytis cinerea* spore suspension ( $10^5$  spores  $\text{ml}^{-1}$ ) and stored for 20 d at room temperature or 15 d at  $0 \pm 1^\circ\text{C}$ , 95-98% RH, followed by a 10-d shelf life at  $20 \pm 2^\circ\text{C}$ . Values marked with the same letter are not statistically different according to DMRT at 1%.**

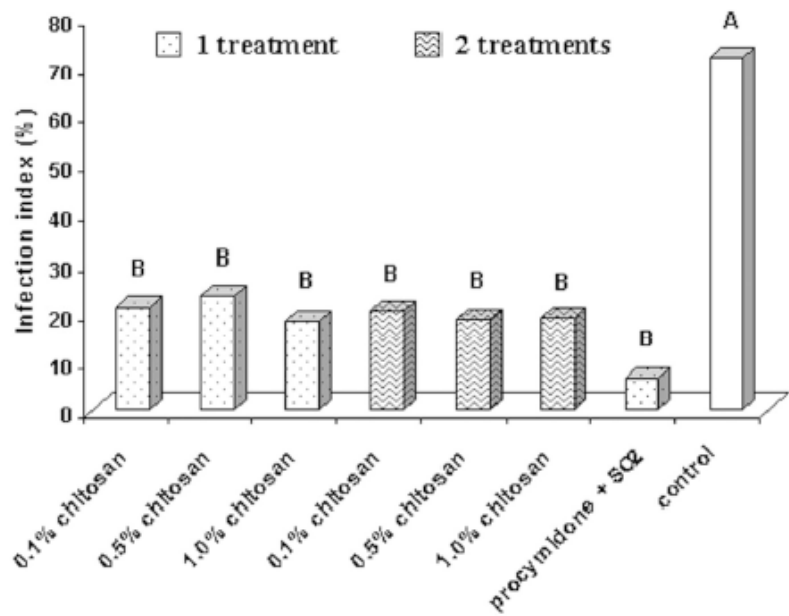


**Figure 2—Effect of chitosan on the percentage of infected berries (a) and on lesion diameter (b) in berries artificially inoculated with *Botrytis cinerea*. Single berries were wounded, treated with chitosan (0.5 and 1.0%) or water (control) and inoculated with a spore suspension ( $10^4$  spores  $\text{ml}^{-1}$ ) of the pathogen; after drying, berries were stored for 5 d at room temperature or 15 d at  $0 \pm 1^\circ\text{C}$ , 95-98% RH, followed by a 2-d shelf life at  $20 \pm 2^\circ\text{C}$ . Values marked with the same letter are not statistically different, according to DMRT at 5% (small letters) or 1% (capital letters).**

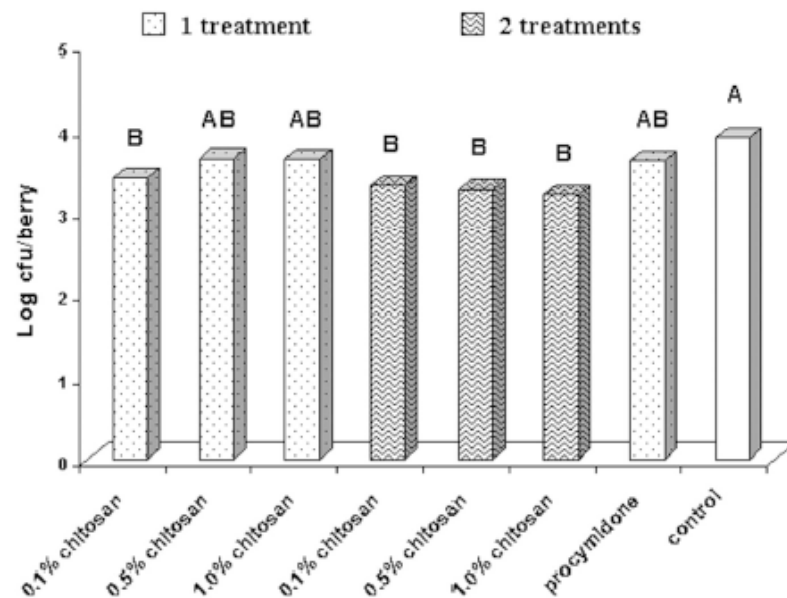




**Figure 3—Effect of chitosan treatments on grey mold (nesting). Single berries and small bunches were dipped in chitosan solutions (0.5 and 1.0%) or in water (control); after air-drying, berries and bunches were arranged in plastic boxes and inoculated by placing a berry completely covered of grey mold in the middle. Storage was 15 d at  $0 \pm 1$  °C, 95-98% RH, followed by a 7-d shelf life at  $20 \pm 2$  °C. On the column, values marked with the same letter are not statistically different according to DMRT at 1%.**



**Figure 4—Effect of preharvest chitosan on the grey mold infection index of table grapes in storage.** Bunches were sprayed once and twice (21 and 21 and 5 d before harvest). Table grapes treated with procymidone 21 d before the harvest and cold stored with sulphur dioxide (SO<sub>2</sub>) is included for comparison. Bunches were stored for 30 d at 0 ± 1 °C, 95-98% RH, followed by a 4-d shelf life at 20 ± 2 °C. Values marked with the same letter are not statistically different according to DMRT at 1%.



**Figure 5—Effect of preharvest chitosan on the filamentous fungi population of table grape berries.** Bunches were sprayed once and twice (21 and 21 and 5 d before harvest). The number of colonies was assessed at harvest time. Values marked with the same letter are not statistically different according to DMRT at 1%.

# POSTHARVEST DISEASES OF SWEET CHERRY

**Brown rot**



**Gray mold**

**Cladosporium rot**



**Alternaria rot**

**Blue mold**



**Rhizopus rot**

## Short hypobaric treatments potentiate the effect of chitosan in reducing storage decay of sweet cherries

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### Abstract

The effectiveness of chitosan and short hypobaric treatments, alone or in combination, to control storage decay of sweet cherries, was investigated over 2 years. In single treatments, chitosan was applied by postharvest dipping or preharvest spraying at 0.1, 0.5, and 1.0% concentrations; hypobaric treatments at 0.50 and 0.25 atm were applied for 4 h. In combined treatments, sweet cherries were dipped in 1.0% chitosan and then exposed to 0.50 and 0.25 atm, or sprayed with chitosan (0.1, 0.5, and 1.0%) 7 days before harvest and exposed to 0.50 atm soon after harvest. Untreated sweet cherries kept at normal pressure (near 1.00 atm) were used as controls. Rot incidence was evaluated after 14 days storage at  $0 \pm 1$  °C, followed by a 7 day shelf life. In both years, chitosan and hypobaric treatments applied alone significantly reduced brown rot, grey mould, and total rots, the latter also including blue mould, *Alternaria*, *Rhizopus* and green rots. A combined treatment with 1.0% chitosan and 0.50 atm was the best in controlling decay, showing in the first year, a synergistic effect in the reduction of brown rot and total rots. The results indicate that the combination of hypobaric and chitosan treatments is a valid strategy for increasing the effectiveness of the treatments in controlling postharvest decay of sweet cherries.

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*Keywords:* Chitosan; Hypobaric treatments; Integrated treatments; Sweet cherries; Synergism; Postharvest decay

Table 1

Combined effect of chitosan and hypobaric treatments on the percentage of sweet cherries (cv Ferrovia) affected by brown rot, grey mould and total rots in the first year of trials

| Disease                 | Pressure level (atm) | Chitosan concentration (%) |         | Average |
|-------------------------|----------------------|----------------------------|---------|---------|
|                         |                      | 1.0                        | 0.0     |         |
| Brown rot               | 0.25                 | *11.3 de                   | 44.0 b  | 27.6 B  |
|                         | 0.50                 | *6.0 e                     | 35.3 c  | 20.6 C  |
|                         | 1.00                 | 15.3 d                     | 55.3 a  | 35.3 A  |
| Average                 |                      | 10.9 B                     | 44.9 A  |         |
| Grey mould              | 0.25                 | 6.0 b                      | 7.8 b   | 6.9 B   |
|                         | 0.50                 | 4.0 b                      | 7.5 b   | 5.7 B   |
|                         | 1.00                 | 8.7 b                      | 28.0 a  | 18.3 A  |
| Average                 |                      | 6.2 B                      | 14.4 A  |         |
| Total rots <sup>a</sup> | 0.25                 | 26.7 d                     | 49.3 b  | 38.0 B  |
|                         | 0.50                 | *13.3 e                    | 42.0 bc | 27.6 B  |
|                         | 1.00                 | 30.7 cd                    | 78.7 a  | 54.7 A  |
| Average                 |                      | 23.6 B                     | 56.7 A  |         |

\* Synergistic effect, according to Limpel's formula.

Table 2

Effect of chitosan and hypobaric treatment on the percentage reduction of sweet cherries (cv Ferrovia) infected by brown rot, grey mould, and total rots in the first year of trials

| Disease                 | Treatment              | Decay reduction (%)                |                 |
|-------------------------|------------------------|------------------------------------|-----------------|
|                         |                        | Expected additive effect ( $E_c$ ) | Observed effect |
| Brown rot               | 1% chitosan + 0.25 atm | 77.97                              | 79.52*          |
|                         | 1% chitosan + 0.50 atm | 82.30                              | 89.16*          |
| Grey mould              | 1% chitosan + 0.25 atm | 91.90                              | 78.58           |
|                         | 1% chitosan + 0.50 atm | 91.15                              | 85.71           |
| Total rots <sup>a</sup> | 1% chitosan + 0.25 atm | 75.55                              | 66.10           |
|                         | 1% chitosan + 0.50 atm | 79.17                              | 83.06*          |

Total rots include grey mould, brown rot, Rhizopus rot, Alternaria rot, blue mould, and green rot.

<sup>a</sup> Total rots include grey mould, brown rot, Rhizopus rot, Alternaria rot, blue mould and green rot. When the combination of the two agents produces any value of decay reduction (observed effect) greater than  $E_c$  (expected additive effect), according to Limpel's formula, then synergism exists (indicated with \*). Limpel's formula is  $E_c = X + Y - (XY/100)$ , in which  $E_c$  is the expected effect from additive response of two treatments and  $X$  and  $Y$  are the percentages of decay reduction relative to each agent used alone.



*Parlier, June 2004 - Jan 2005*

# Acids reported able to dissolve chitosan

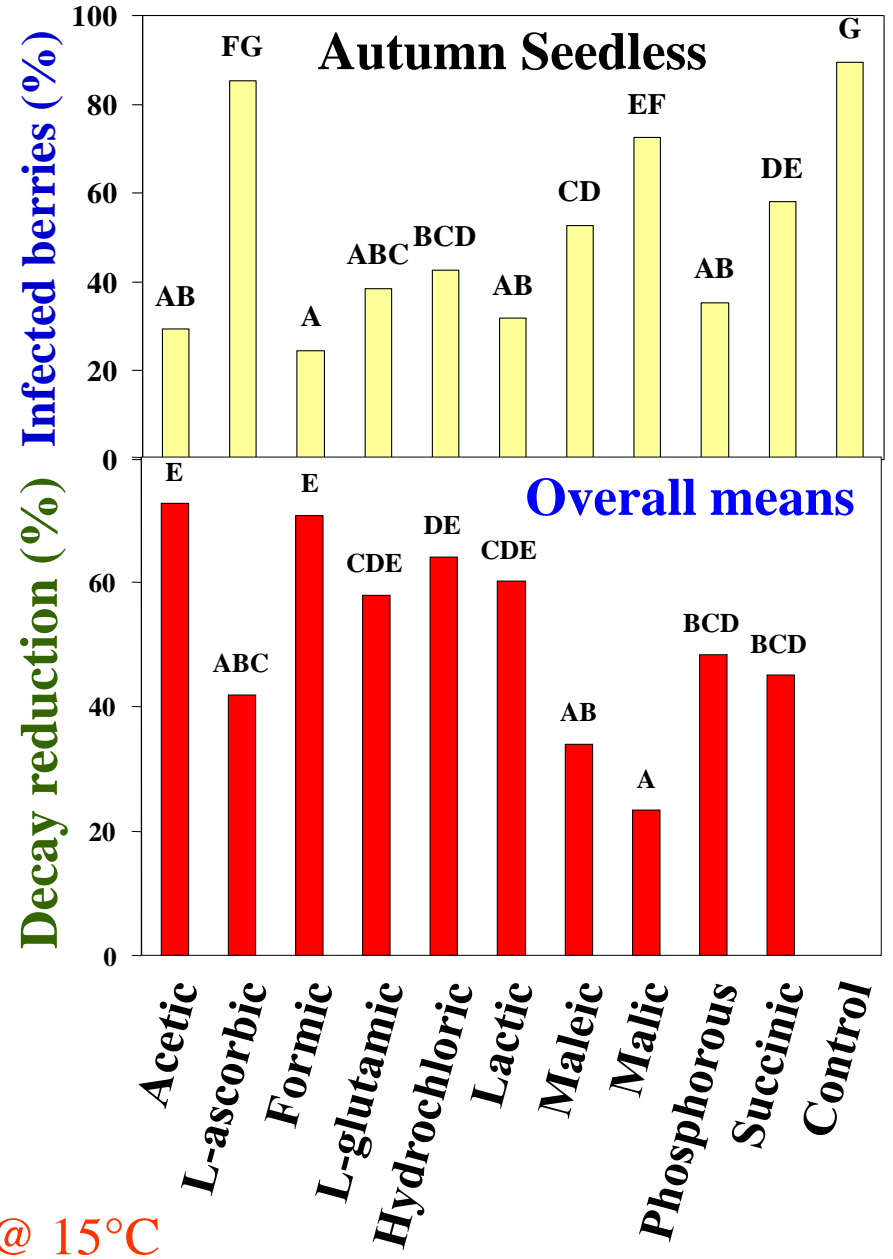
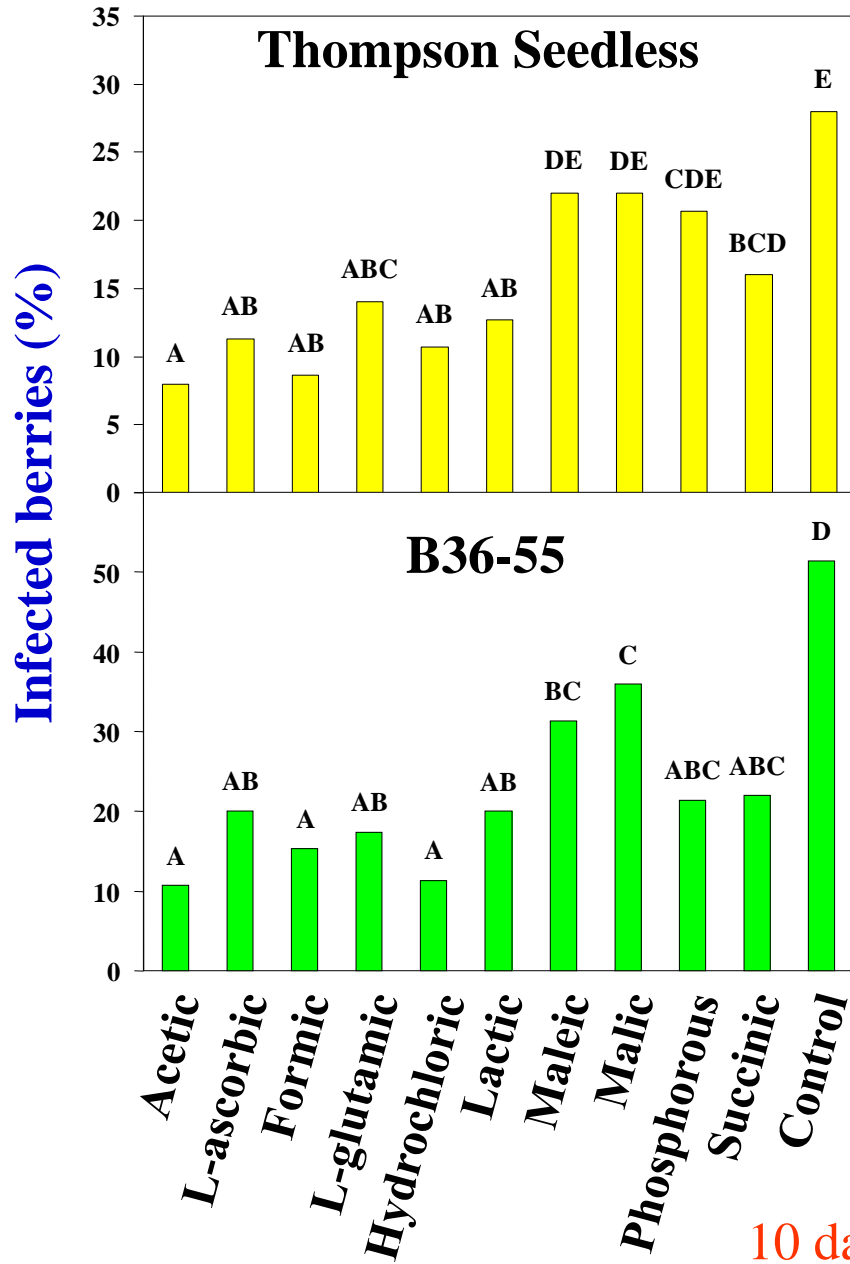
| <b>Acid</b>  | <b>Concentration</b> | <b>Reference</b>              |
|--------------|----------------------|-------------------------------|
| Acetic       | 0.1 N                | Allan and Hadwiger, 1979      |
|              | 0.5%                 | Du et al., 1998               |
|              | 1%                   | Kendra et al., 1989           |
|              | 2%                   | Bégin and Van Calsteren, 1999 |
| Citric       | 2%                   | Bégin and Van Calsteren, 1999 |
| Formic       | 2%                   | Bégin and Van Calsteren, 1999 |
| L-glutamic   | 1-2%                 | Zhang and Quantick, 1997      |
| Lactic       | 0.5%                 | Devlieghere et al., 2004      |
|              | 2%                   | Bégin and Van Calsteren, 1999 |
| Hydrochloric | 10 N                 | El Ghaouth et al., 1991       |
|              | 0.25 N               | El Ghaouth et al., 1992       |
|              | 0.1%                 | Bégin and Van Calsteren, 1999 |
| Malic        | 0.5-2%               | Du et al., 1997               |

# Ability of different acids to dissolve chitosan

| Acid                       | pH 1% | Dissolve chitosan |
|----------------------------|-------|-------------------|
| Acetic                     | 2.8   | Yes               |
| L-ascorbic                 | 2.7   | Yes               |
| Boric                      | 5.0   | No                |
| DL- $\alpha$ -aminobutyric | 5.4   | No                |
| Formic                     | 2.2   | Yes               |
| Gallic                     | 2.9   | No                |
| L-glutamic                 | 2.6   | Yes               |
| Hydrochloric               | 0.6   | Yes               |
| Lactic                     | 2.4   | Yes               |
| Maleic                     | 1.5   | Yes               |
| Malic                      | 2.3   | Yes               |
| Phosphorous                | 1.4   | Yes               |
| Polygalatturonic           | 3.0   | No                |
| Succinic                   | 2.6   | Yes               |
| <i>Trans</i> -Cinnamic     | 2.9   | No                |

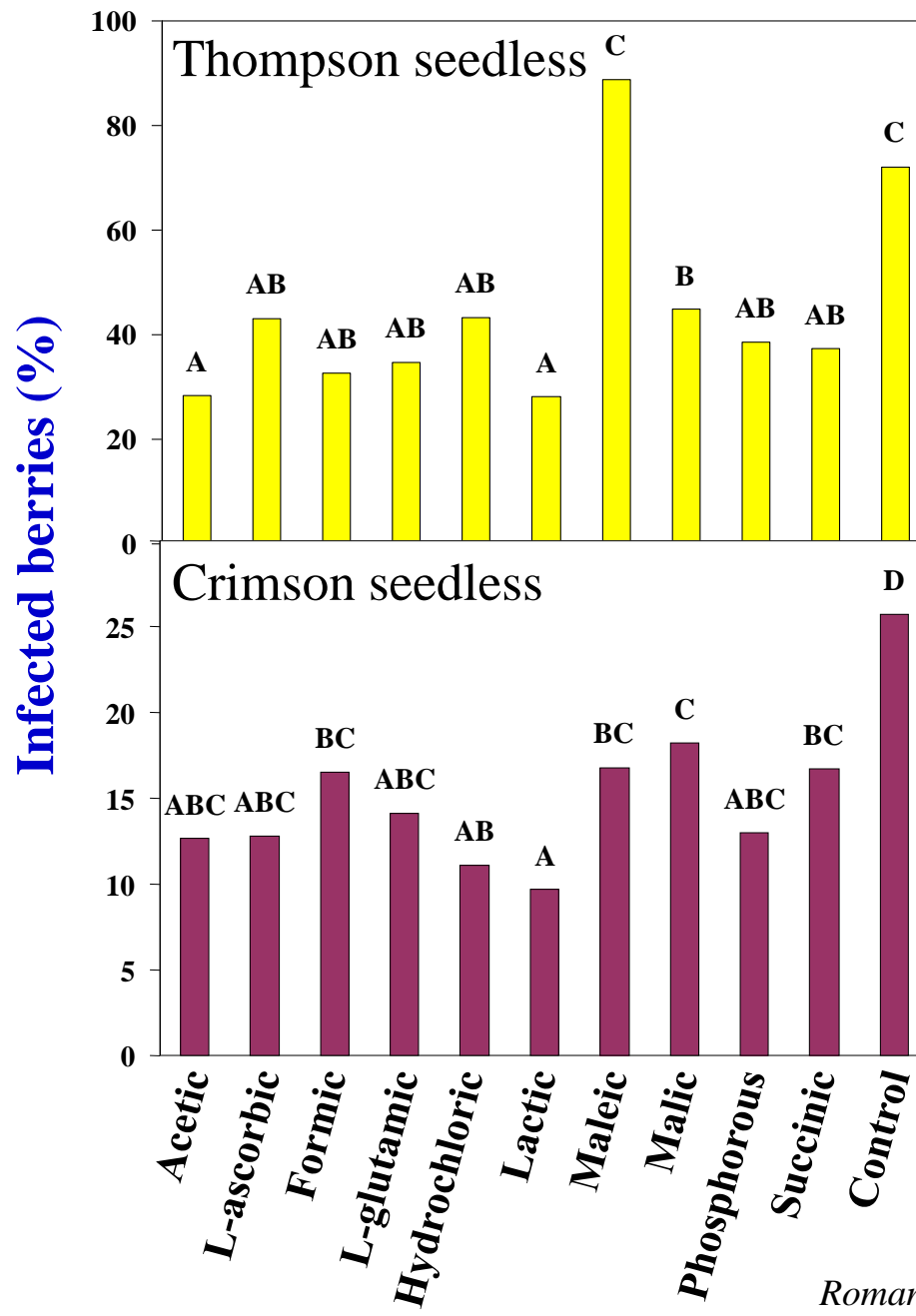


# Single berries inoculated with *B. cinerea* and immersed in chitosan solutions

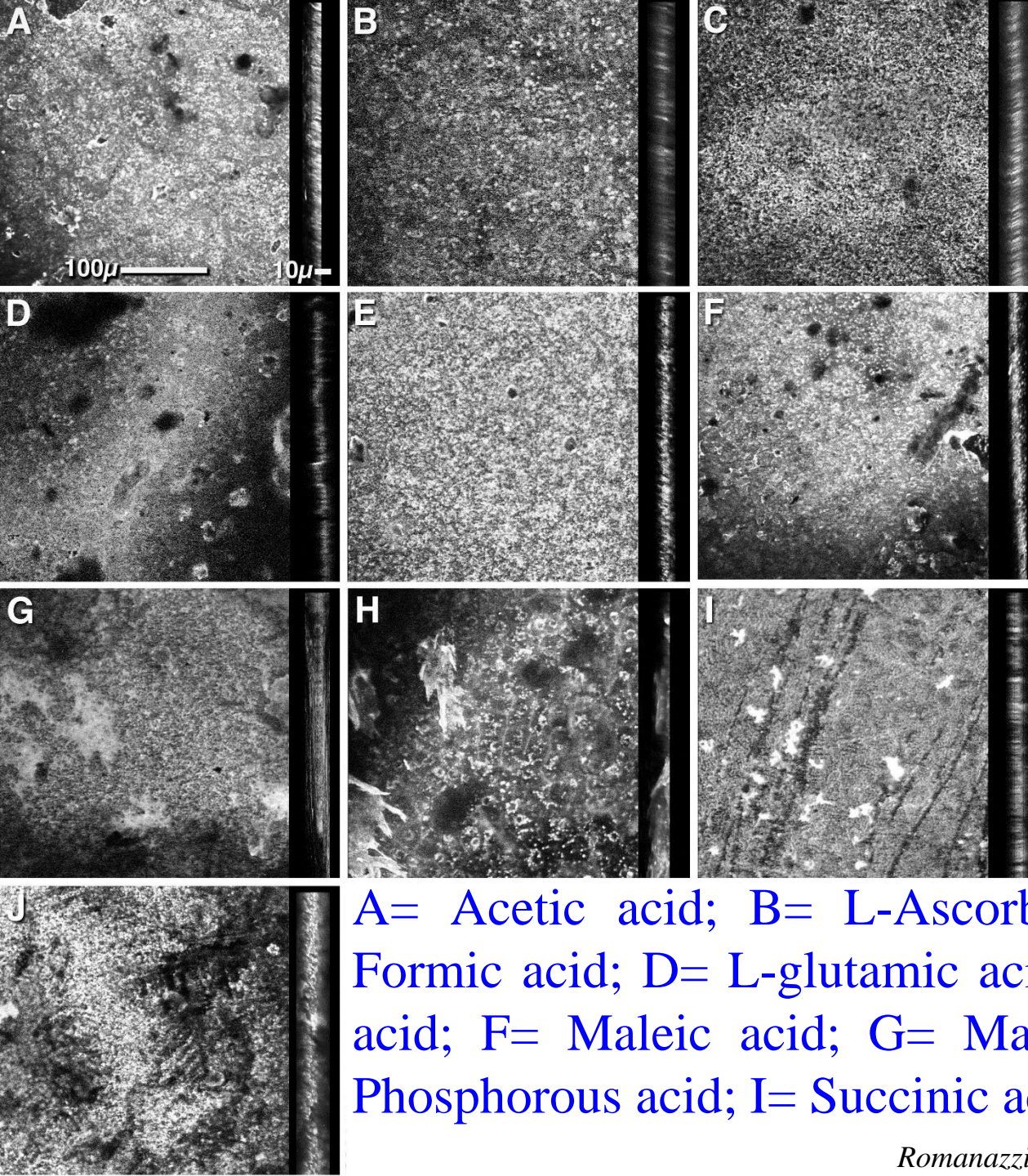


10 days @ 15°C

# Small clusters inoculated with *B. cinerea* and immersed in chitosan solutions



60 days  
@ 0.5°C



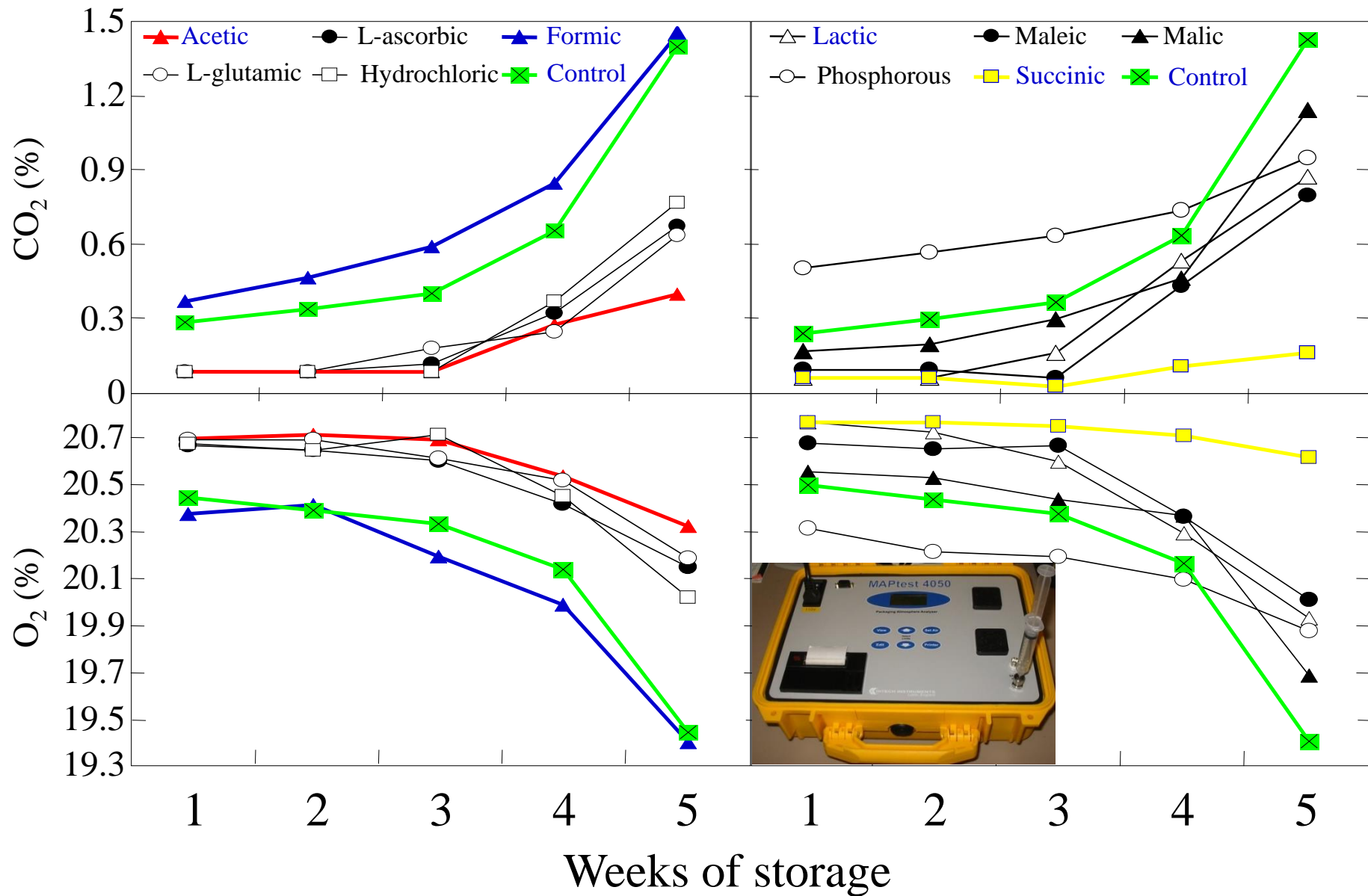
## Chitosan coating on table grape berries

A= Acetic acid; B= L-Ascorbic acid; C= Formic acid; D= L-glutamic acid; E= Lactic acid; F= Maleic acid; G= Malic acid; H= Phosphorous acid; I= Succinic acid.

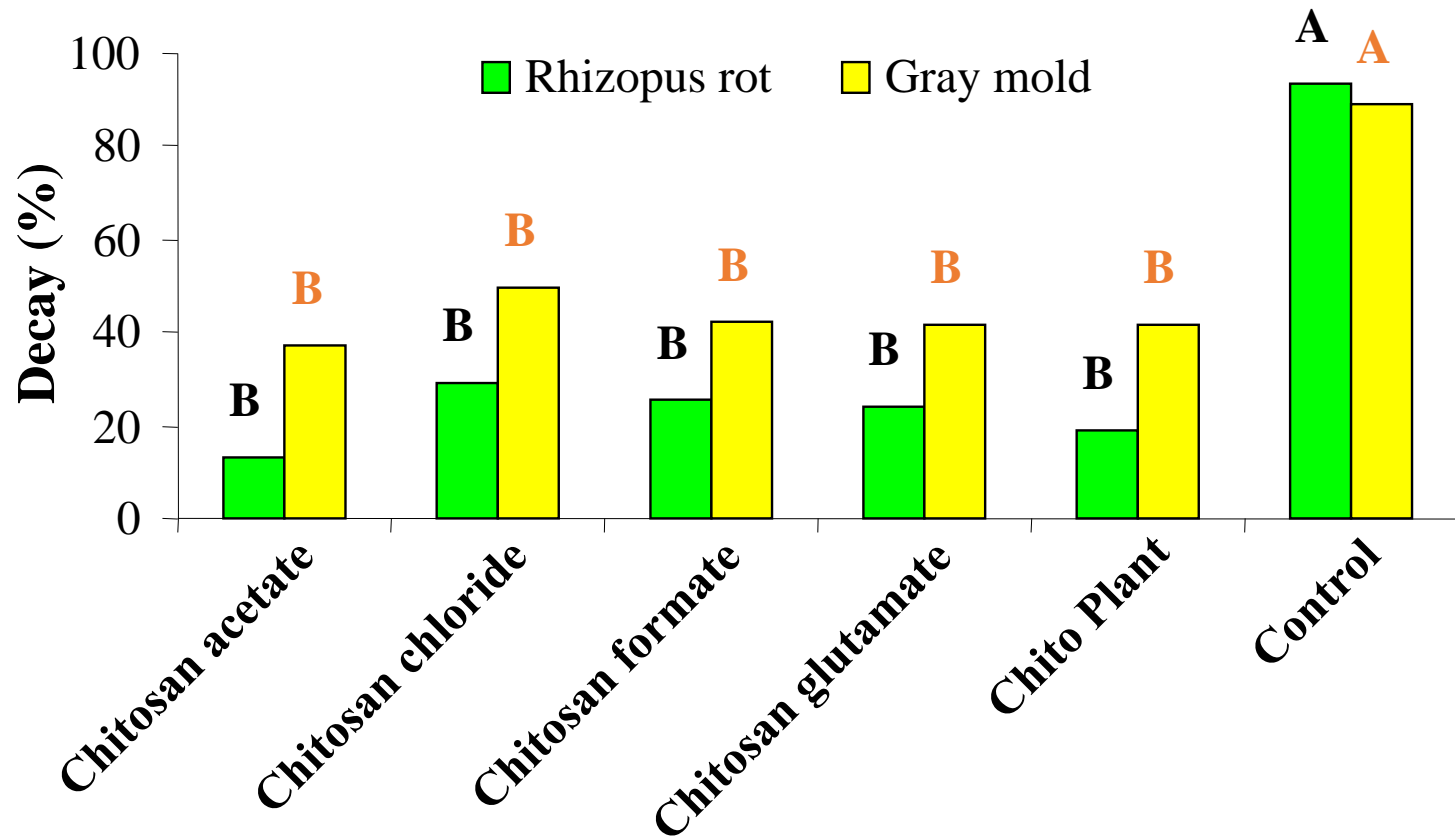
# Thickness of chitosan film on the berries and viscosity of chitosan solutions

| Dissolving acid | Coating thickness ( $\mu\text{m}$ ) | Viscosity (cp)         |
|-----------------|-------------------------------------|------------------------|
| Acetic          | 6.3 ( $\pm 1.91$ )                  | 43.47 ( $\pm 4.47$ )   |
| L-Ascorbic      | 13.1 ( $\pm 2.80$ )                 | 1.91 ( $\pm 0.25$ )    |
| Formic          | 9.8 ( $\pm 1.82$ )                  | 234.89 ( $\pm 21.23$ ) |
| L-Glutamic      | 9.9 ( $\pm 1.87$ )                  | 23.78 ( $\pm 2.71$ )   |
| Hydrochloric    | 11.2 ( $\pm 2.26$ )                 | 3.94 ( $\pm 0.56$ )    |
| Lactic          | 9.7 ( $\pm 1.95$ )                  | 102.95 ( $\pm 11.10$ ) |
| Maleic          | 9.1 ( $\pm 3.22$ )                  | 306.41 ( $\pm 8.56$ )  |
| Malic           | 10.7 ( $\pm 1.25$ )                 | 148.38 ( $\pm 10.10$ ) |
| Phosphorous     | 9.6 ( $\pm 1.10$ )                  | 178.13 ( $\pm 13.14$ ) |
| Succinic        | 7.4 ( $\pm 2.61$ )                  | 12.91 ( $\pm 2.05$ )   |

# Respiration rate of grapes treated with chitosan solutions



# Effectiveness of postharvest chitosan treatment on gray mold and Rhizopus rot of strawberry



# PREHARVEST TRIALS ON STRAWBERRY

- Treatment with:

**Water (control)**

**Chitosan (0.5%)**

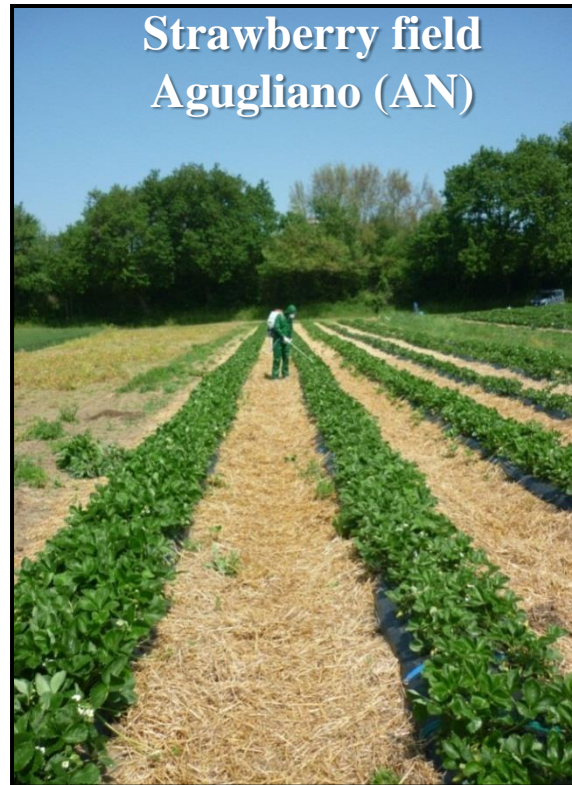
**Chitosan (1%)**

**Laminarin (1%)**

**Fir extract (1%)**

**Benzothiadiazole (0.2%)**

**Fungicides (cyprodinil + fludioxonil, pyrimethanil)**



|                 |                 |
|-----------------|-----------------|
| Chitosan (0.5%) | Laminarin       |
| Laminarin       | BTH             |
| Fungicides      | Fir extract     |
| Control         | Chitosan (0.5%) |
| Chitosan (1%)   | Control         |
| Fir extract     | Fungicides      |
| BTH             | Chitosan (1%)   |
| Laminarin       | Fungicides      |
| Fir extract     | BTH             |
| Fungicides      | Chitosan (1%)   |
| Chitosan (0.5%) | Fir extract     |
| BTH             | Control         |
| Chitosan (1%)   | Chitosan (0.5%) |
| Control         | Laminarin       |

Treatment 5 times during season approximately every 5 days:

**Flowering**

**End flowering**

**Green fruit**

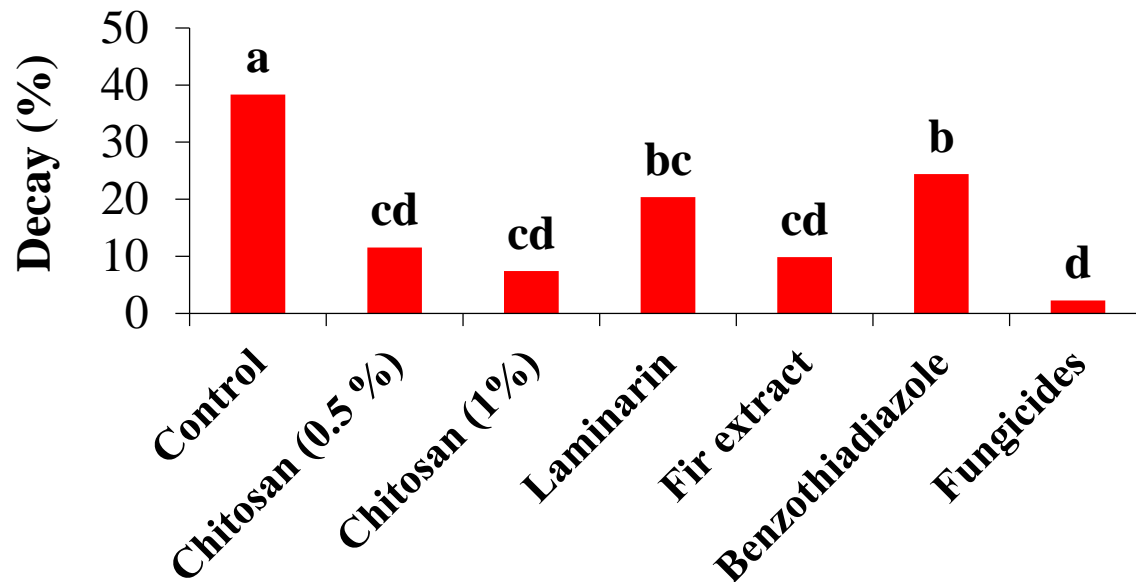
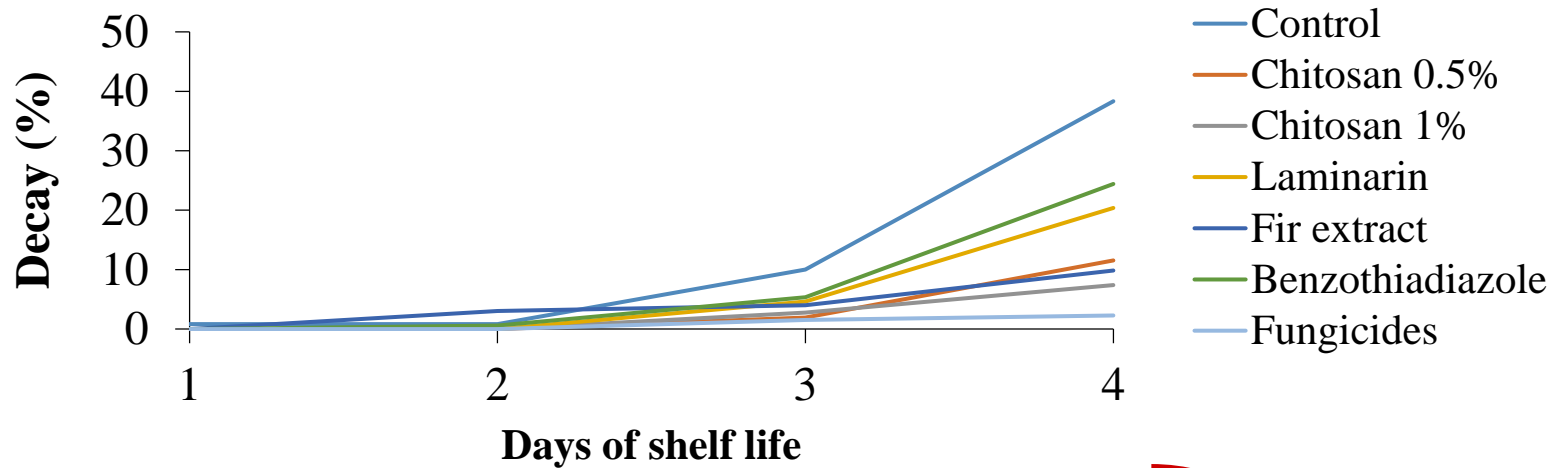
**White fruit**

**Red turning fruit**



# PREHARVEST TRIALS ON STRAWBERRY

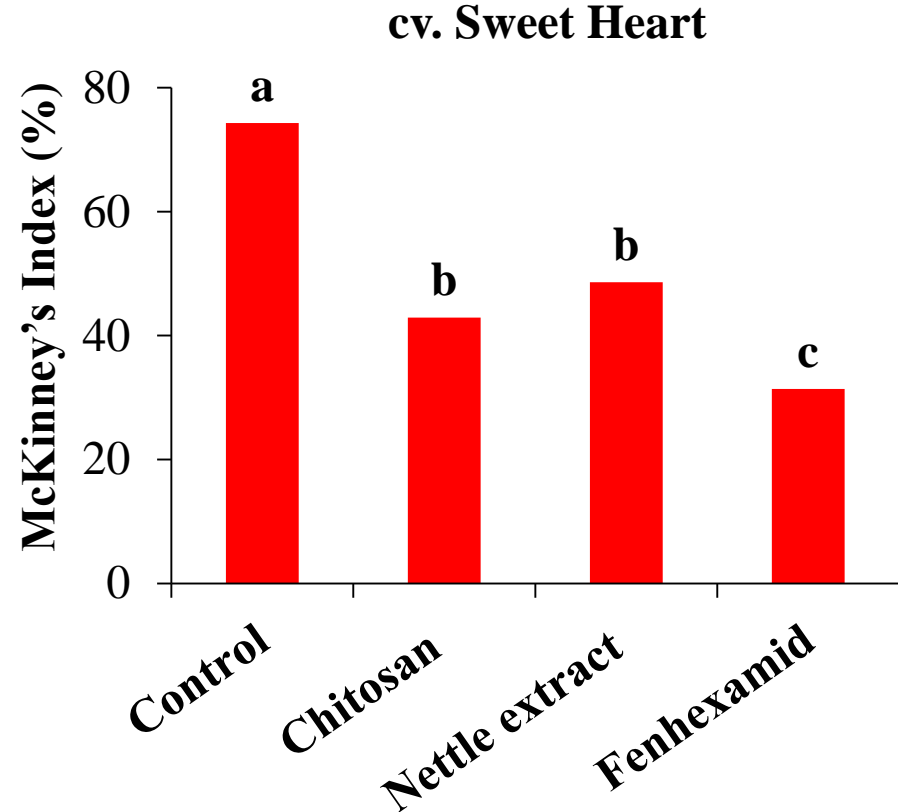
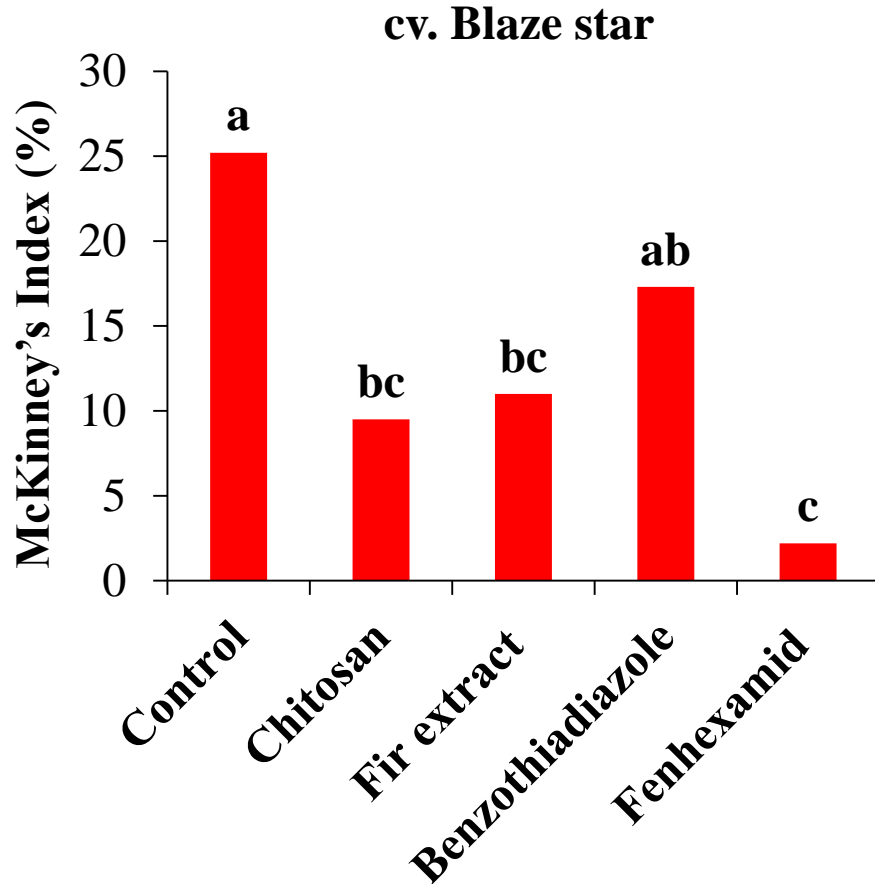
McKinney's Index of rots recorded on strawberries cv. ALBA treated for 5 times during the season, harvested and stored for 7 days at  $0 \pm 1$  °C and then exposed to shelf life





# PREHARVEST TRIALS ON SWEET CHERRY

McKinney's Index of total rots that include brown rot and gray mold of sweet cherries stored for 14 days at 0.5 °C and then exposed to shelf life



Values with the same letter are not different according Tukey HSD ( $P < 0.05$ )

# PREHARVEST TRIALS ON TABLE GRAPES

## THOMPSON SEEDLESS TABLE GRAPES In Parlier, CALIFORNIA

Treatments **4 times** during the season:

- Berry set
- Pre-bunch closure
- Veraison
- 2/3 weeks before harvest



# PREHARVEST TRIALS ON TABLE GRAPES

In 2011

Treatments with:

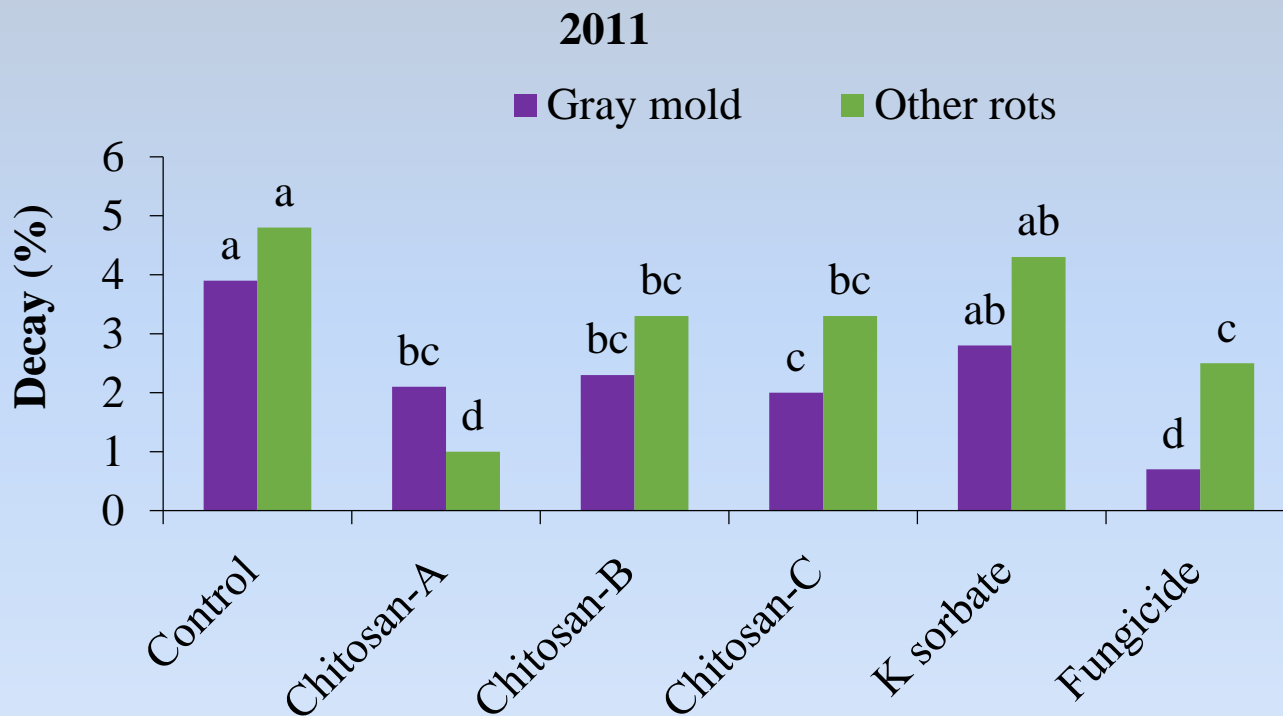
- Water (control)
- Fungicides program
  - (1<sup>^</sup> pyrimethanil,
  - 2<sup>^</sup> cyprodinil + fludioxonil,
  - 3<sup>^</sup> pyraclostrobin + boscalid,
  - 4<sup>^</sup> fenhexamid)
- K sorbate (1%)
- Chitosan-A: OII-Ys
- Chitosan-B: Chito Plant
- Chitosan-C: Armour-Zen



**3 commercial  
formulations at 1%  
chitosan**

# POSTHARVEST ROTTS FROM NATURAL INOCULUM

After 6 weeks of storage at 2°C

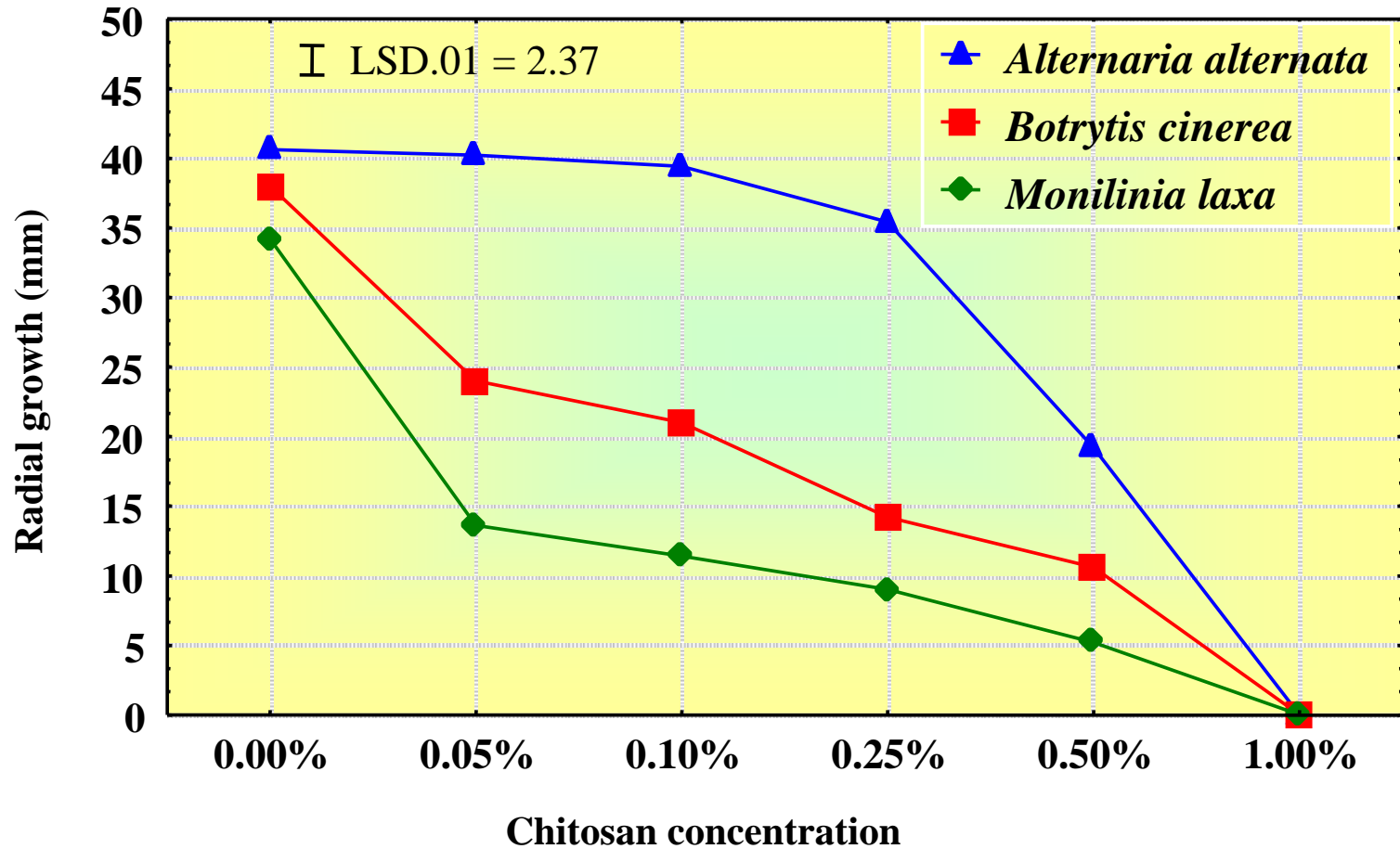


Other rots were caused mainly by *Alternaria* spp. and *Penicillium* spp.

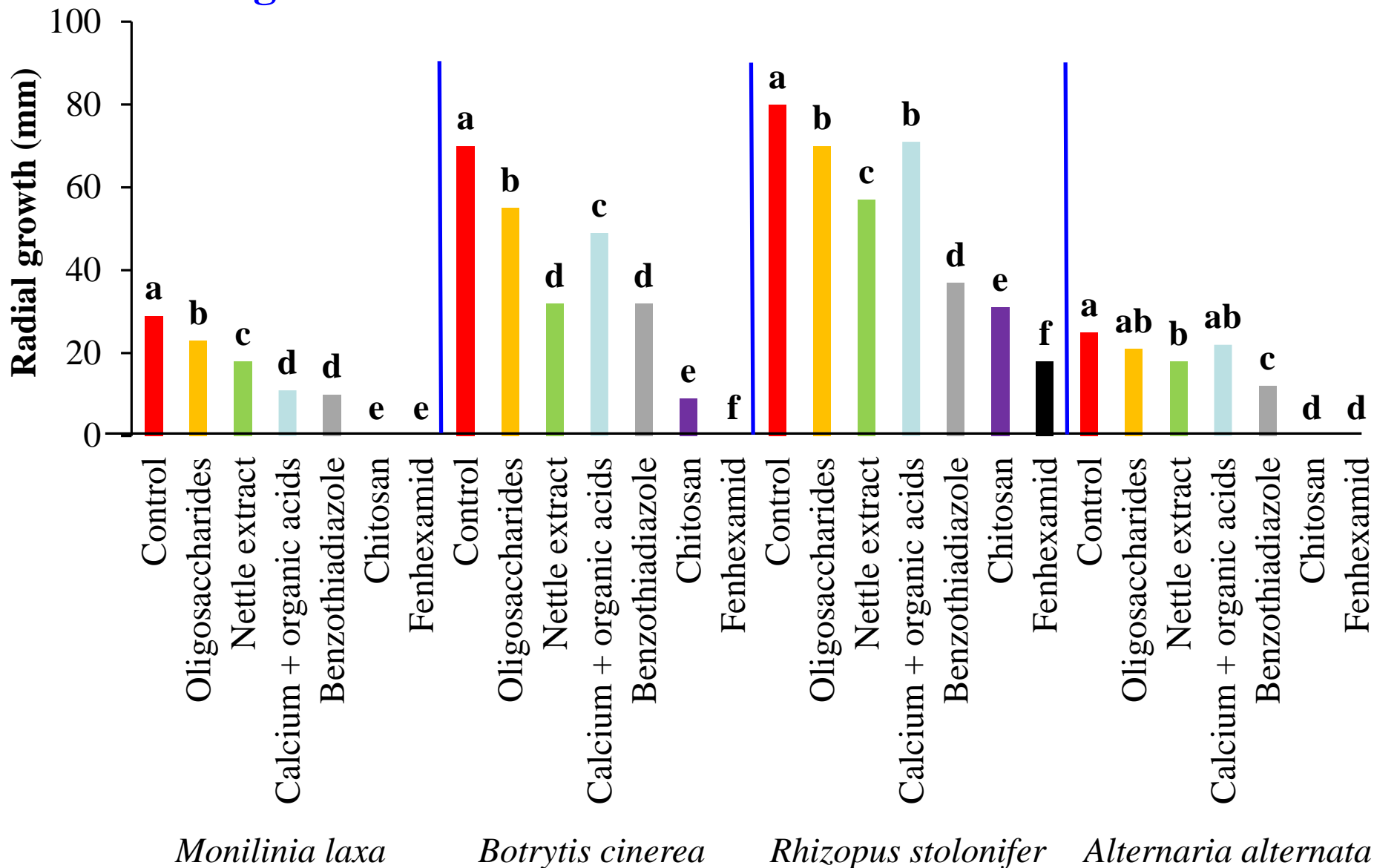
Values with the same letter are not different according Tukey HSD (P <0.05).

**Which are the  
mechanisms of action of  
chitosan?**

# Growth of some decay-causing fungi



# Radial mycelial growth of fungal colonies of decay causing fungi on PDA amended with resistance inducers



# Antifungal activity of chitosan

| Fungus                     | Infected species  | Reference  |
|----------------------------|---|--|
| <i>Botrytis cinerea</i>    | Tomato, potato, bell pepper, cucumber, peach, strawberries, table grapes, pear, apple, citrus fruit | Rabea and Badawy, 2012; Badawy and Rabea, 2009; Liu et al., 2007; Xu et al., 2007; Chien and Chou, 2006; Lira-Saldivar et al., 2006; Elmer and Reglinski, 2006; Ait Barka et al., 2004; Badawy et al., 2004; Ben-Shalom et al., 2003; Romanazzi et al., 2002; El Ghaouth et al., 2000; 1997; 1992; Du et al., 1997 |
| <i>Rhizopus stolonifer</i> | Peach, strawberries, papaya, tomato   | Ramos García et al., 2012; García Rincón et al., 2010; Hernández-Lauzardo et al., 2010; Guerra-Sánchez et al., 2009; Park et al., 2005; Bautista Baños et al., 2004; El Ghaouth et al., 1992   |
| <i>Penicillium spp.</i>    | Strawberry, apple, pear, tomato, citrus fruit, jujube, litchi fruit                                 | Cè et al., 2012; El-Mougy et al., 2012; Xing et al., 2011; Liu et al., 2007; Yu et al., 2007; Chien and Chou, 2006; Sivakumar et al., 2005; Bautista Baños et al., 2004; El Ghaouth et al., 2000   |
| <i>Aspergillus spp.</i>    | Pear  | Cè et al., 2012; Plascencia-Jatomea et al., 2003   |
| <i>Alternaria spp.</i>     | Tomato, pear  | Sánchez-Domínguez et al., 2011; Meng, et al., 2010   |
| <i>Cladosporium spp.</i>   | Litchi fruit, strawberry  | Park et al., 2005; Sivakumar et al., 2005  |
| <i>Colletotrichum spp.</i> | Mango, papaya, banana, table grapes, tomato   | Zahid et al., 2012; Abd-Alla and Hagggar, 2010; Ali et al., 2010; Maqbool et al., 2010a, 2010b; Hewajulige et al., 2009; Muñoz et al., 2009; Ali and Mahmud, 2008; Jitareerat et al., 2007; Win et al., 2007; Sivakumar et al., 2005; Bautista Baños et al., 2003  |
| <i>Monilinia spp.</i>      | Apple, peach, sweet cherry  | Feliziani et al., 2013; Yang et al., 2012; 2010  |

Romanazzi G., Feliziani E., Bautista Baños S., Sivakumar D., 2017. Shelf life extension of fresh fruit and vegetables by chitosan treatment. *Critical Reviews in Food Science and Nutrition* (in press)

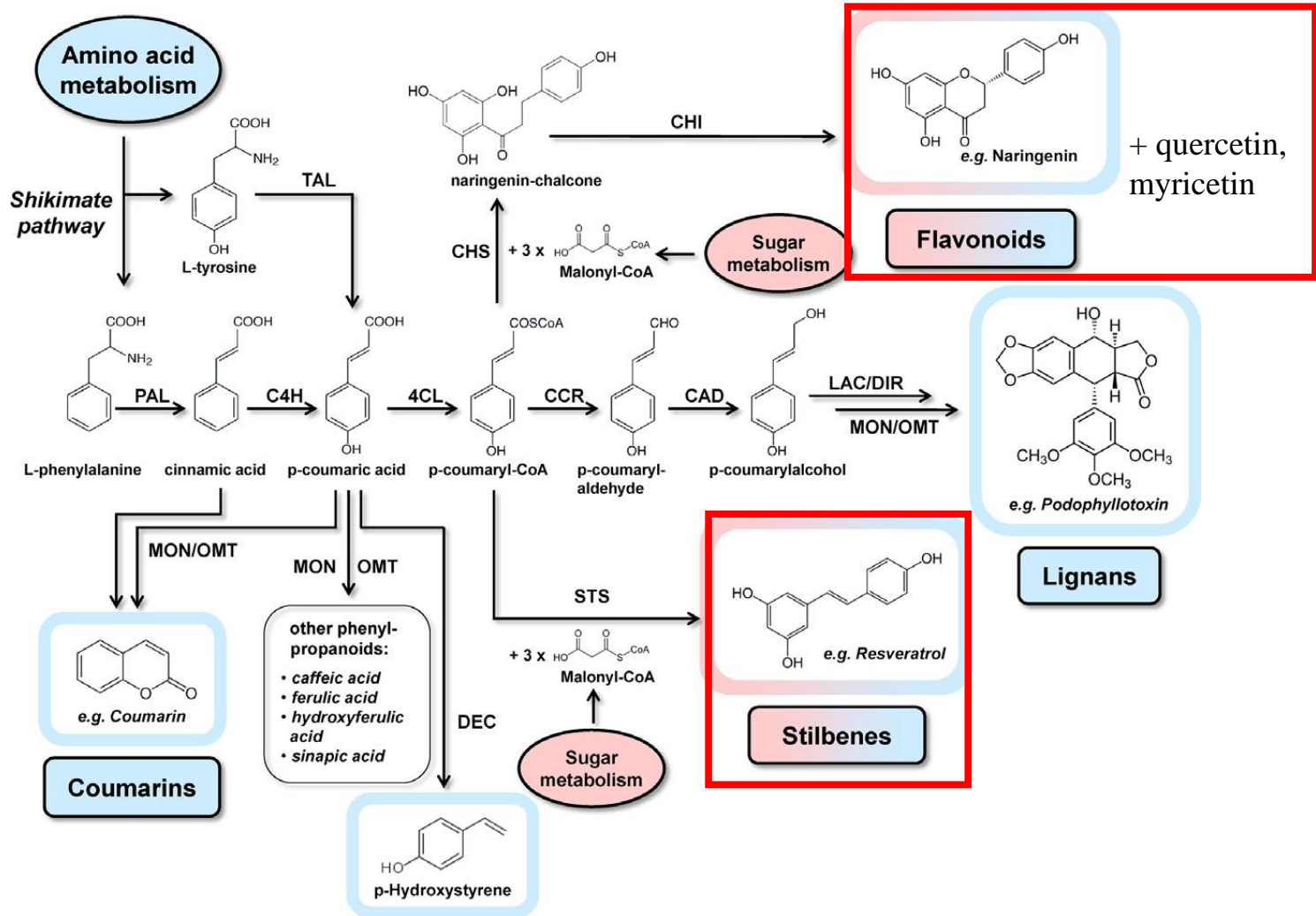


# Trans-resveratrol and catechin content of berries treated with chitosan and exposed to UV-C

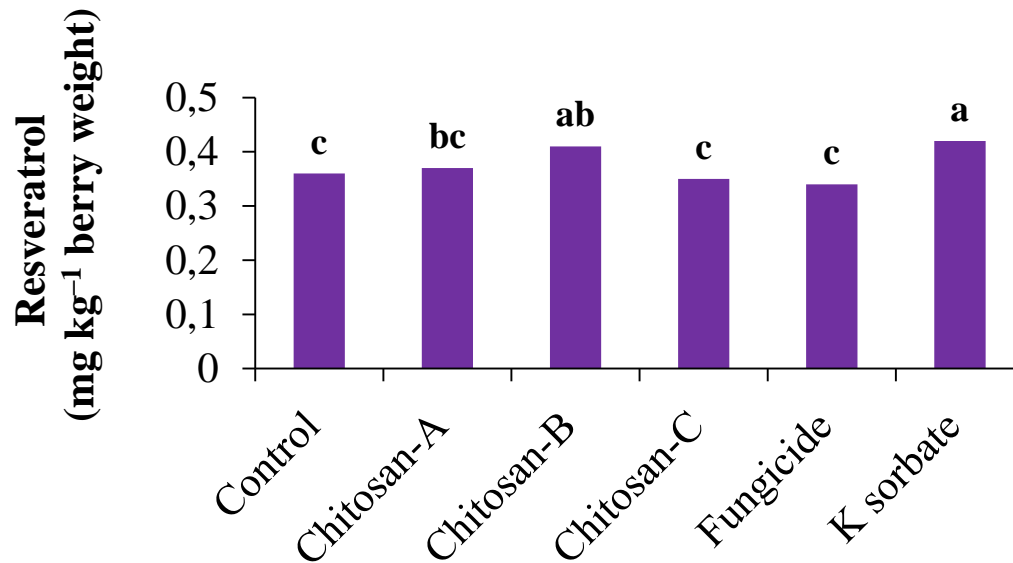
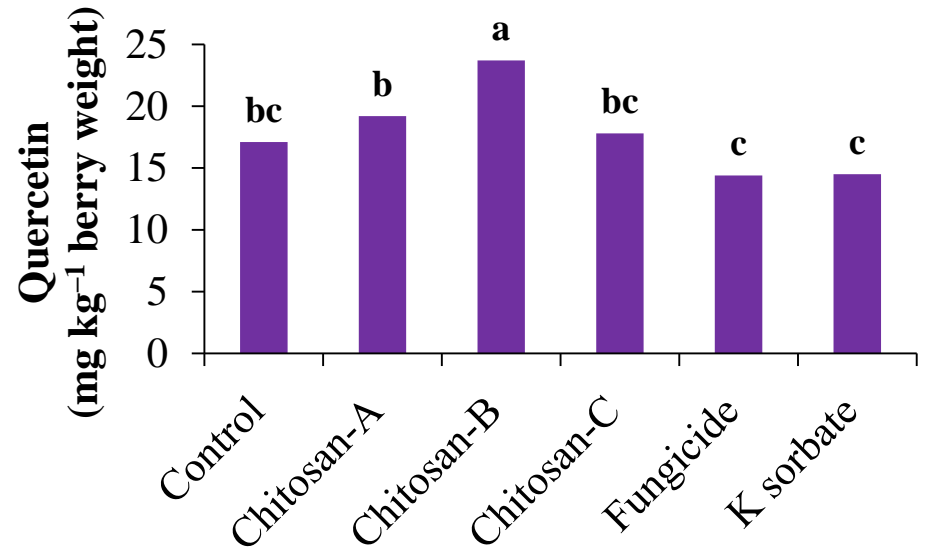
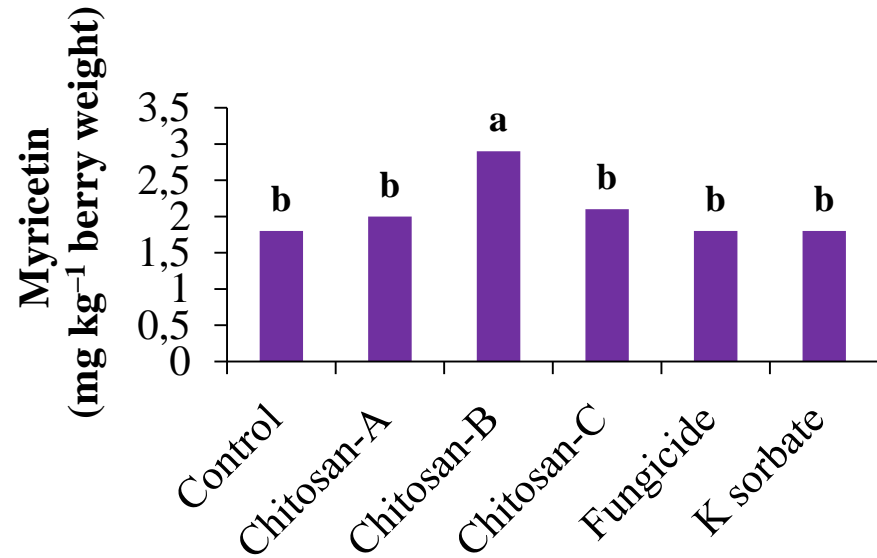
| <b>Treatment</b> | <b>Autumn Black</b>      |                 | <b>B36-55</b>            |                 |
|------------------|--------------------------|-----------------|--------------------------|-----------------|
|                  | <i>Trans-resveratrol</i> | <i>Catechin</i> | <i>Trans-resveratrol</i> | <i>Catechin</i> |
| Chitosan         | ND*                      | ND              | 1.90 C                   | ND              |
| UV-C             | 17.47 b                  | 1.41 b          | 18.12 B                  | ND              |
| Chitosan + UV-C  | 23.15 a                  | 2.56 a          | 22.00 A                  | ND              |
| Control          | ND                       | ND              | 1.84 C                   | ND              |

\*ND = Below the detection limit (0.2  $\mu\text{g/g}$  fresh skin weight)

# INDUCTION OF RESISTANCE

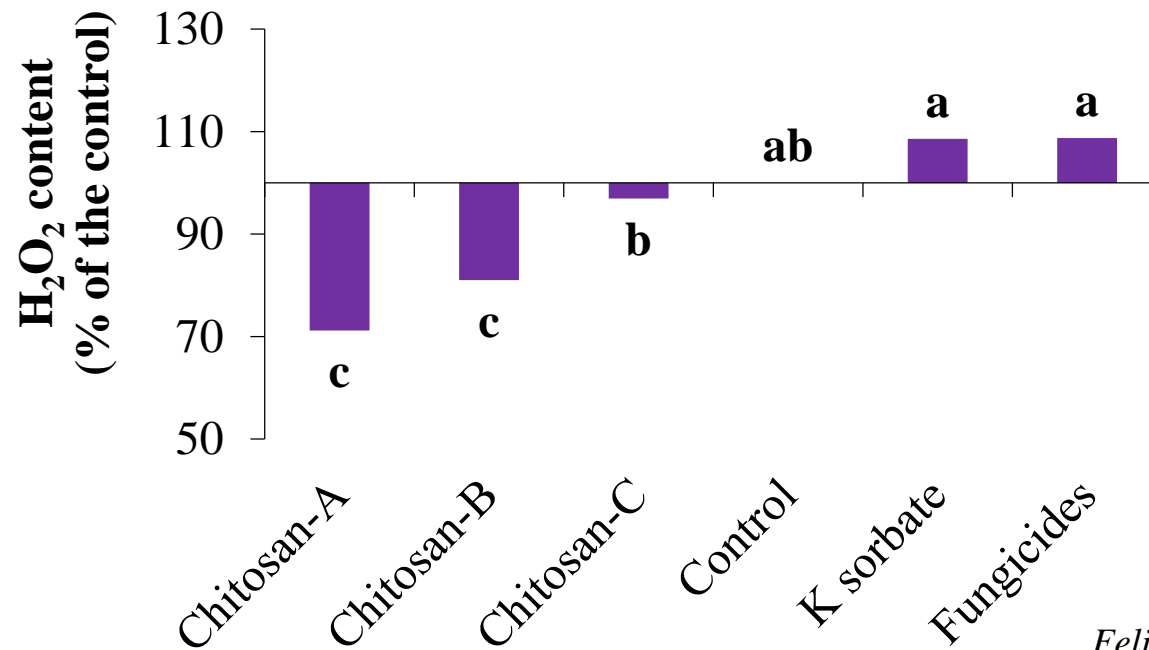
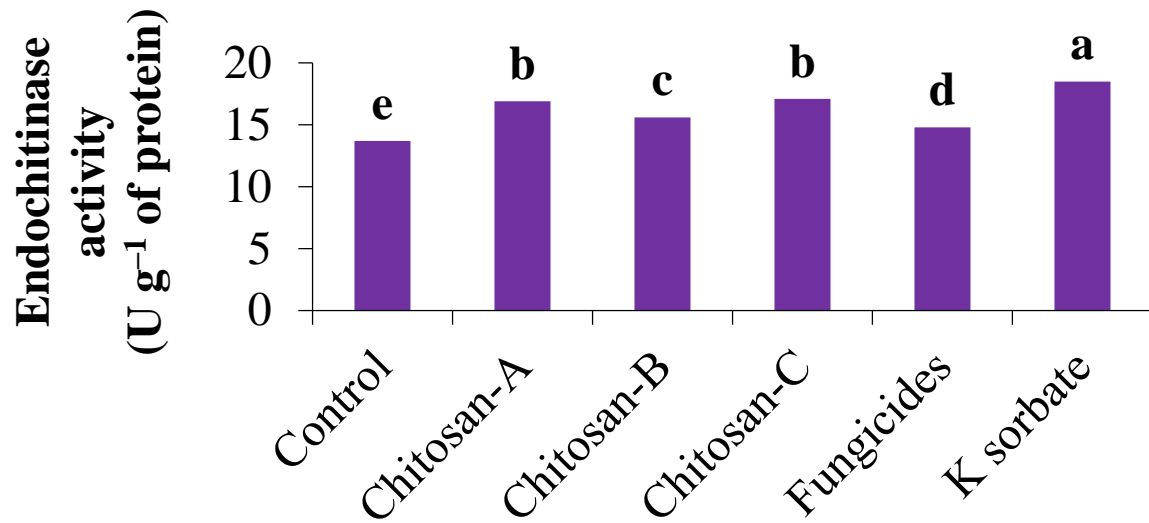


# INDUCTION OF RESISTANCE



Values with the same letter are not different according Tukey HSD (P < 0.05)

# INDUCTION OF RESISTANCE



Values with the same letter are not different according Tukey HSD (P < 0.05)

# INDUCTION OF RESISTANCE

Location and content of hydrogen peroxide in mature 'Thompson Seedless' grape berry tissue as shown by scanning electron microscope

The berries were treated with:

A – Water (control)

B – K sorbate

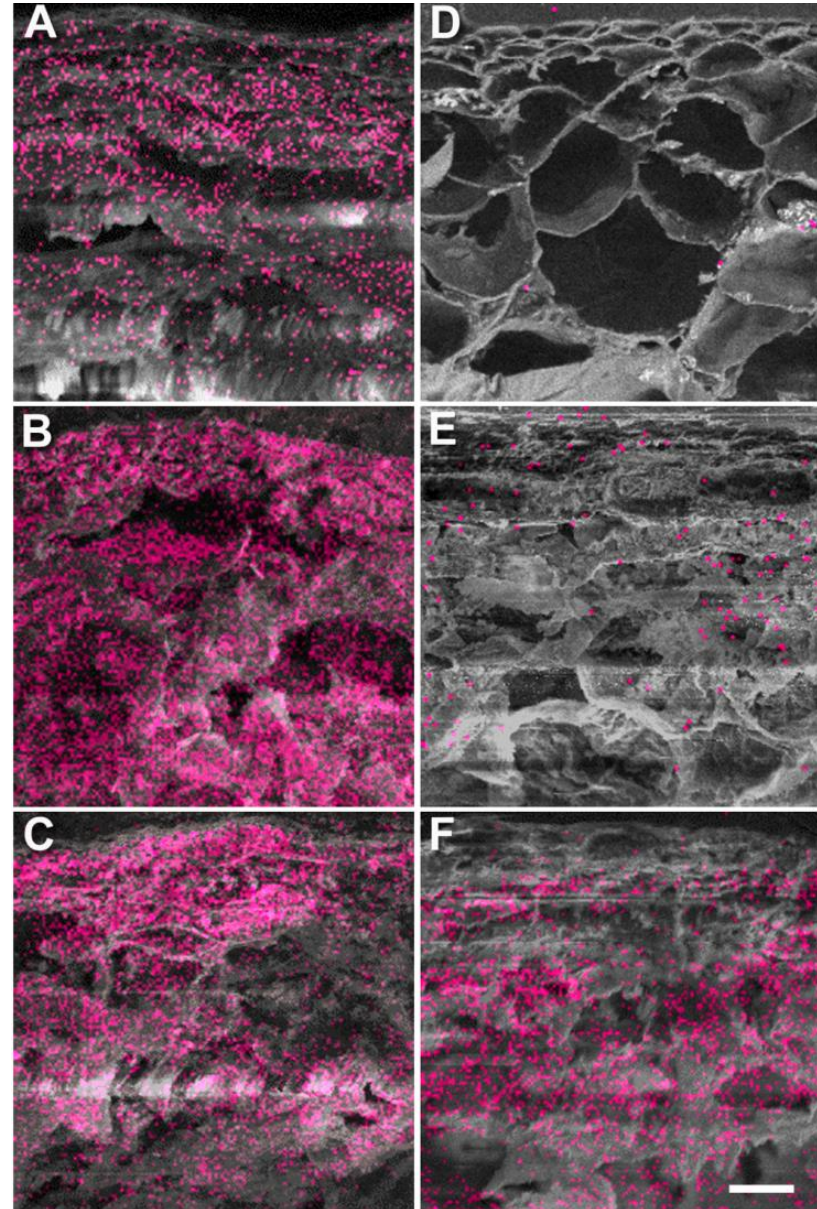
C – Fungicides

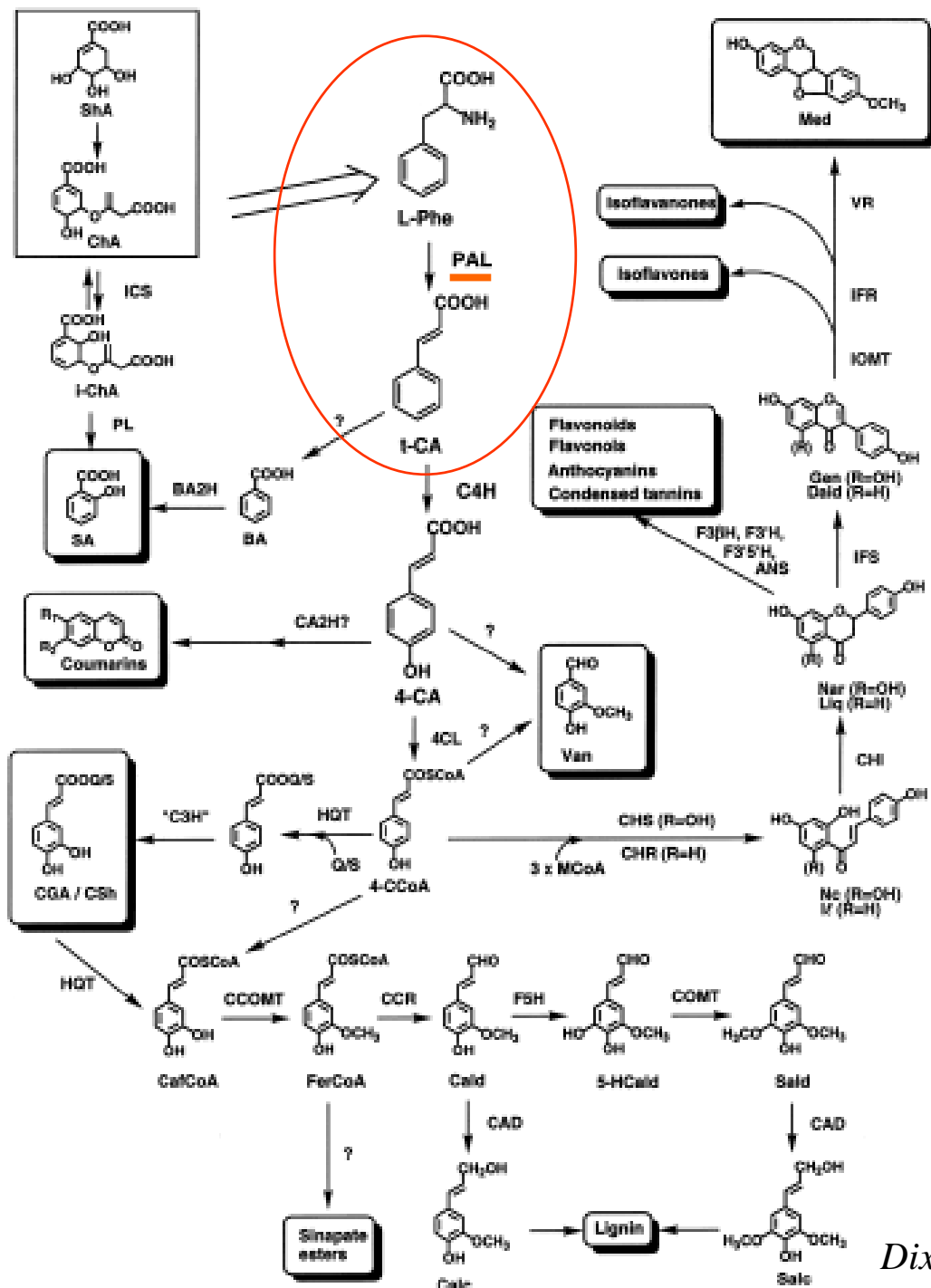
D – Chitosan-A (OII-YS)

E – Chitosan-B (Chito Plant)

F – Chitosan-C (Armour-Zen)

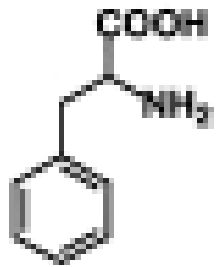
The reaction product of hydrogen peroxide and cerium chloride is cerium hydroxide, that is highlighted by the pink pixels





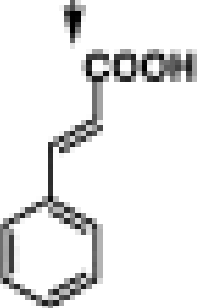
# Phenylpropanoid pathway

# PAL activity on strawberries

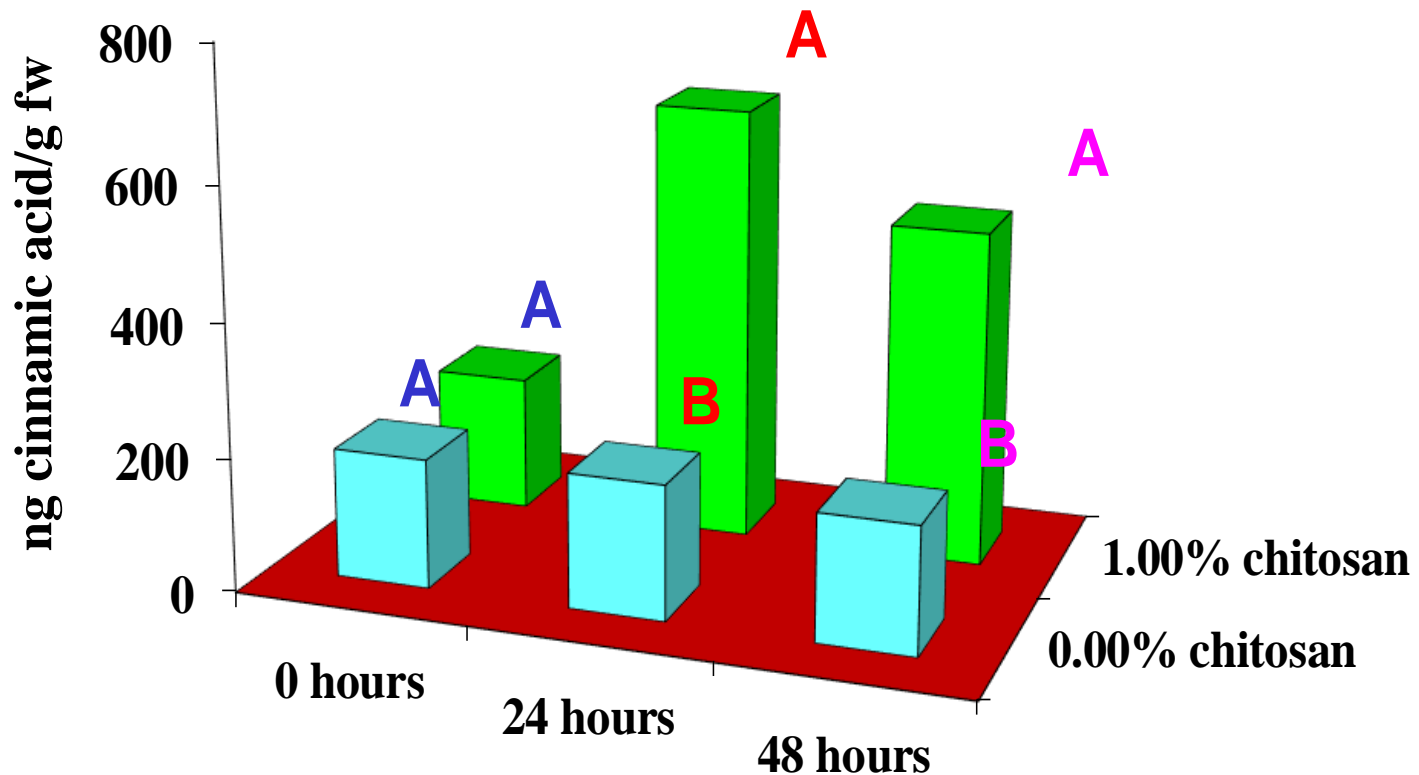


L-Phe

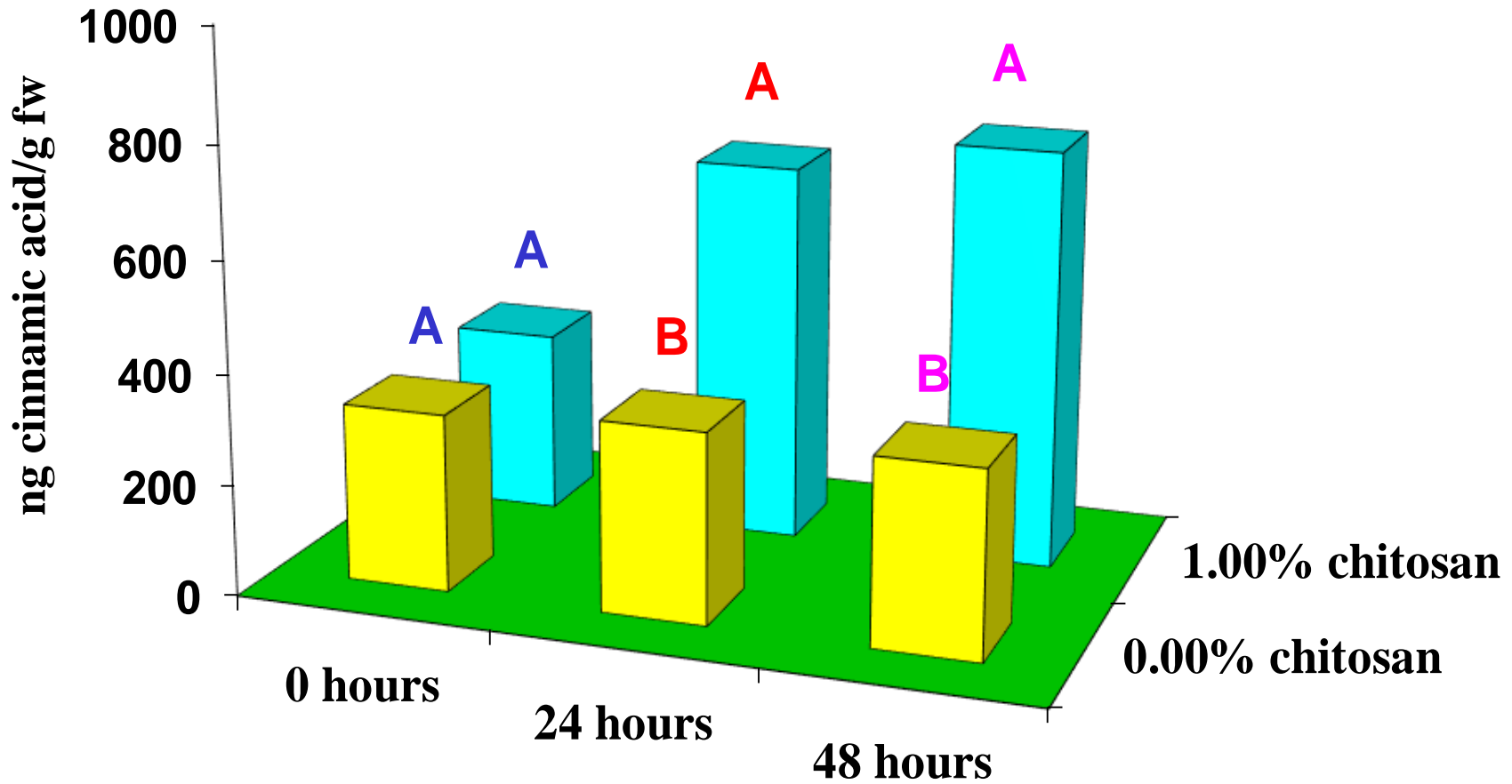
PAL



I-CA



# PAL activity on table grape berry skin





Which gene associated to defense mechanisms is involved in induced resistance?

**CHITOSAN  
BTH  
COA**

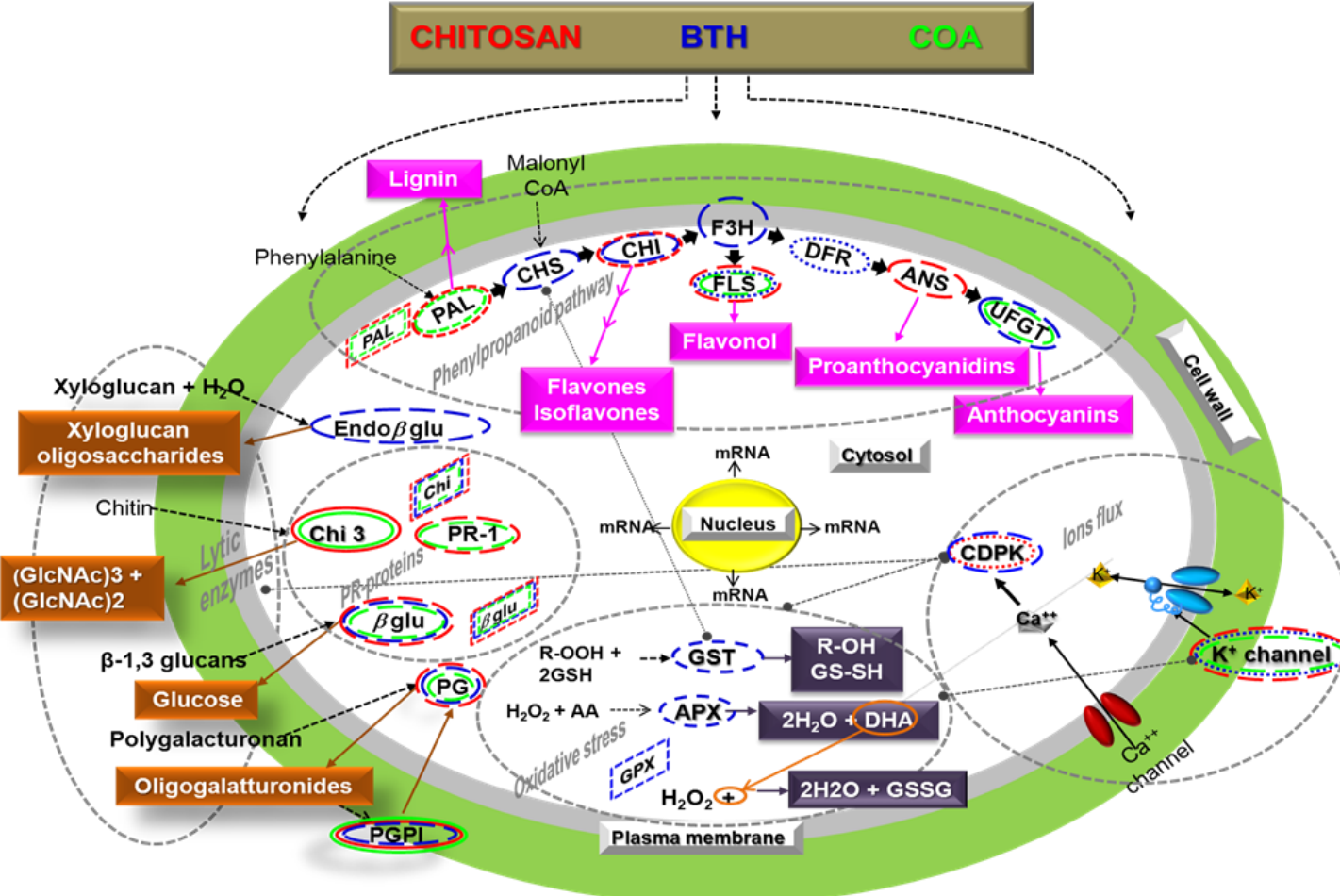


**Postharvest treatments**

**Analysis in RT-qPCR of genes associated to:**

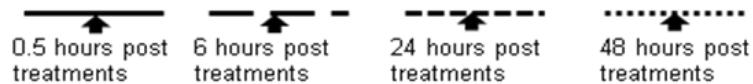
- ✓ **Ca<sup>2+</sup> and K<sup>+</sup> ion fluxes**
- ✓ **ROS cell responses**
- ✓ **phenylpropanoid pathway**
- ✓ **cell-wall degradation**
- ✓ **PR proteins**

*At 0.5, 6, 24, 48 hours post treatments*



**Legend:**

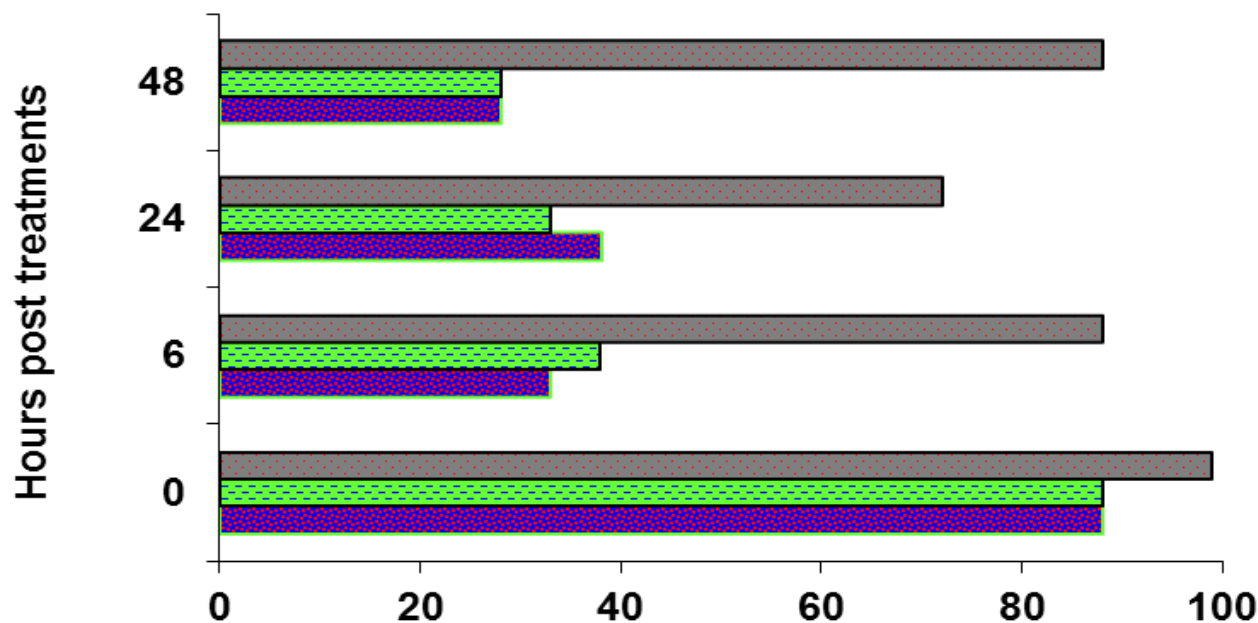
- color line - Red = chitosan, Blu = BTH, Green = COA
- Line tipology - genes up-regulated from:



The resistance inducers triggered the expression of a large number of genes that lead to the physiological events involved in plant defense

*This proof the induction of resistance in strawberry fruit*

# THE ELICITOR COMPOSITION AFFECTS SPECIFIC PATTERN OF INDUCED DEFENSE GENES



The number of genes showing the same response (unvaried, up-regulated, or down-regulated) of the total of 18 genes analyzed

Similarity of gene expression (%)

chitosan/COA

BTH/COA

chitosan/BTH

>72%

<38%

# Physiological changes induced in the plant tissues by chitosan

- Higher quantity of phenolic

Myricetin

Quercetin

Resveratrol



- Induction of plant defense

- Higher activity of enzymes related to mechanism of plant defenses:

Phenylalanine ammonia-lyase

Peroxidase

Polyphenol oxidase

Superoxide dismutase

Chitinase

$\beta$ -1,3-glucanase

- Lower respiration rate

- Reduces weight loss



- Delay senescence
- Prolonged storage and shelf life

What happens to  
chitosan treated fruit?

## Chitosan on strawberries soon after dipping



# Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables

F. Devlieghere\*, A. Vermeulen, J. Debevere

Laboratory of Food Microbiology and Preservation, Department of Food Technology and Nutrition, Ghent University, Coupure Links 653, Ghent 9000, Belgium

Received 3 September 2003; accepted 19 February 2004

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## Abstract

Chitosan has recently gained more interest due to its applicative activity of chitosan has been pointed out as one of its most interesting

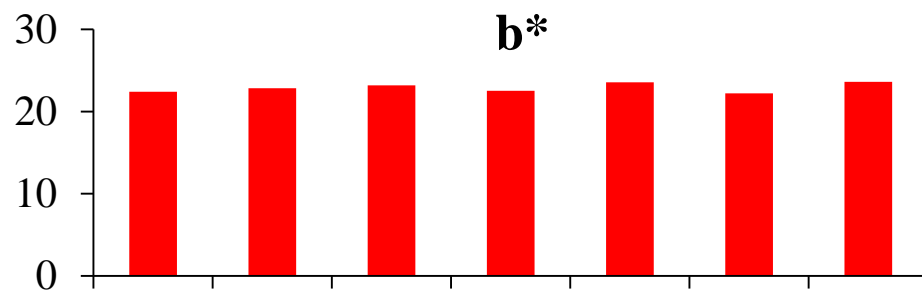
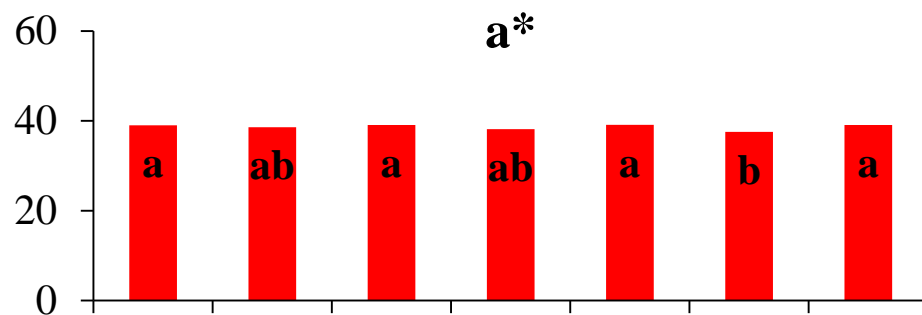
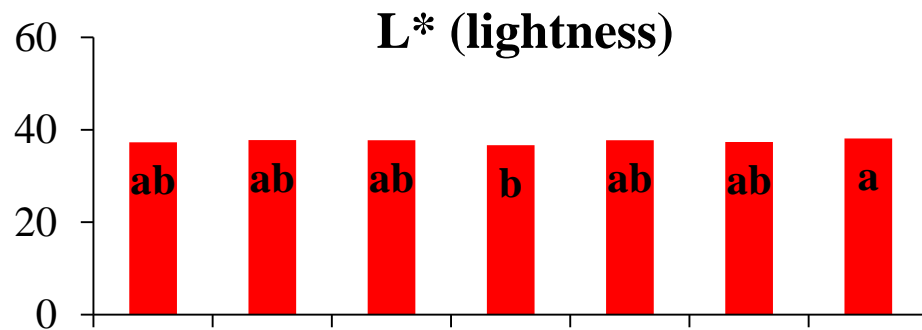
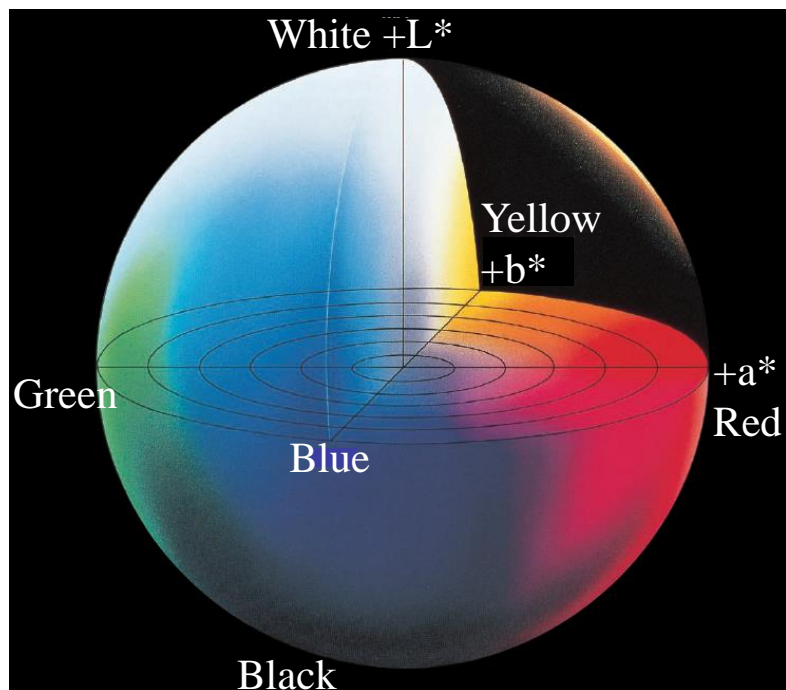
The aim of this study was threefold: (1) the quantification of the influence of different food components (starch, whey protein investigation of the effects of chitosan coatings on controlling dec lettuce). For the first aim several bacteria and yeast were exposed to Gram-negative bacteria seemed to be very sensitive for the application positive bacteria was highly variable and that of yeast was intermediate one of these components added, were inoculated with *Candida lus* reached the stationary phase. Starch, whey proteins and NaCl had no influence. For the third aim, the chitosan coating was formulated solution from which the pH was adjusted to the pH of the product packaged, stored at 7°C and during storage sensorially and microscopically applicable while on mixed lettuce the chitosan coating was not microbiological load on the chitosan-dipped samples was lower for disappeared after 4 days of storage, while it maintained on the surface © 2004 Elsevier Ltd. All rights reserved.

*3.3.1.1. Analysis of the sensory quality.* Sensorial analysis revealed that on the last day of the experiment (day 12) a small odor aberration appeared for all samples while the taste was still acceptable. The samples treated with chitosan were evaluated with a higher score for texture than the untreated samples and those dipped in the lactic acid/Na-lactate solution. Also the juiciness and the color remained optimal during the whole storage period for the three different treatments. On day 0 the strawberries with the chitosan film tasted bitter, but this abnormality disappeared after 3 days of storage at 7°C. Even during further storage, there was no difference between the three treatments on the basis of sweetness, sourness and bitterness. The chemical and aberrant tastes were also evaluated, the former was weak to very weak during the whole storage period and the latter was absent for both the untreated and chitosan treated samples.

# PREHARVEST TRIALS ON STRAWBERRY

## COLOR

Representation of color solid for  $L^*a^*b^*$  color space



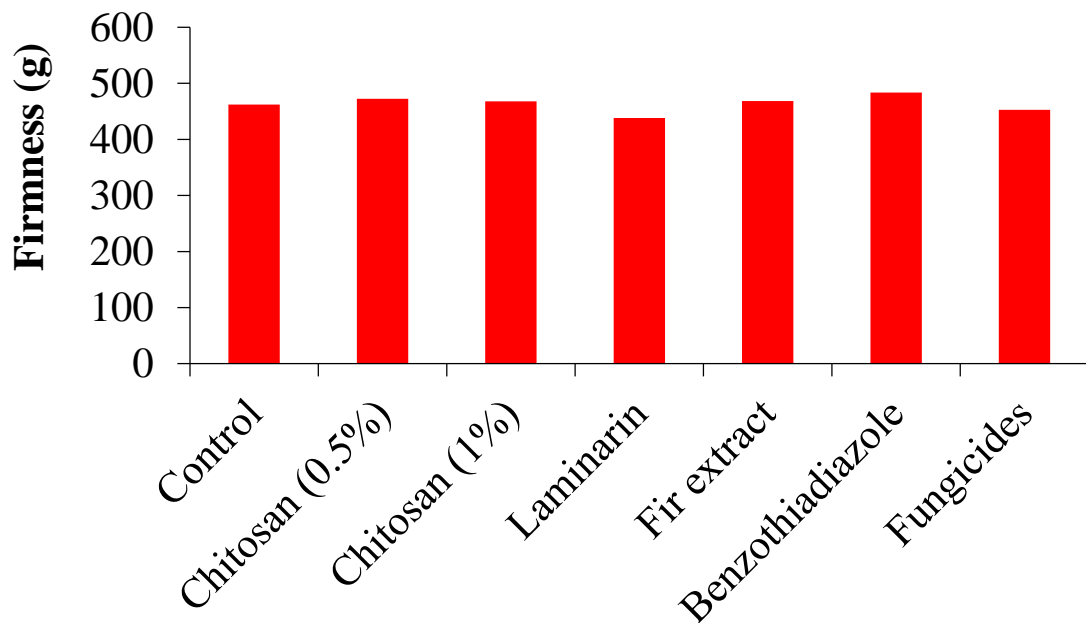
Control  
Chitosan (0.5%)  
Chitosan (1%)  
Laminarin  
Fir extract  
Benzothiadiazole  
Fungicides

Values with the same letter are not different according TUKEY HSD ( $P < 0.05$ ).



# PREHARVEST TRIALS ON STRAWBERRY

## FIRMNESS

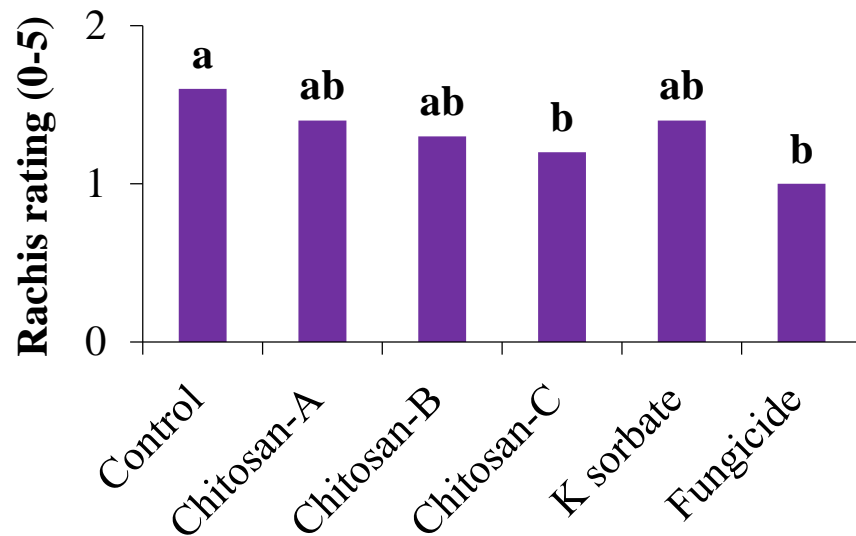
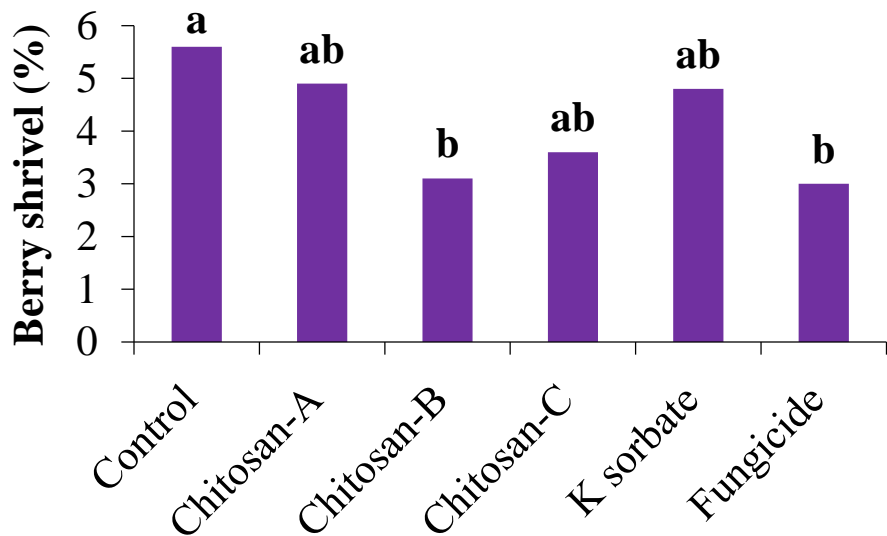
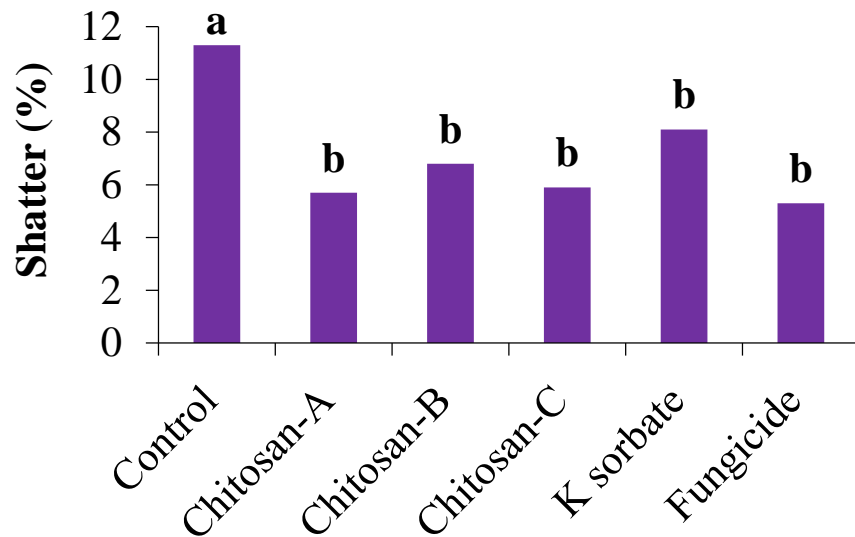


Values with the same letter are not different according TUKEY HSD ( $P < 0.05$ ).



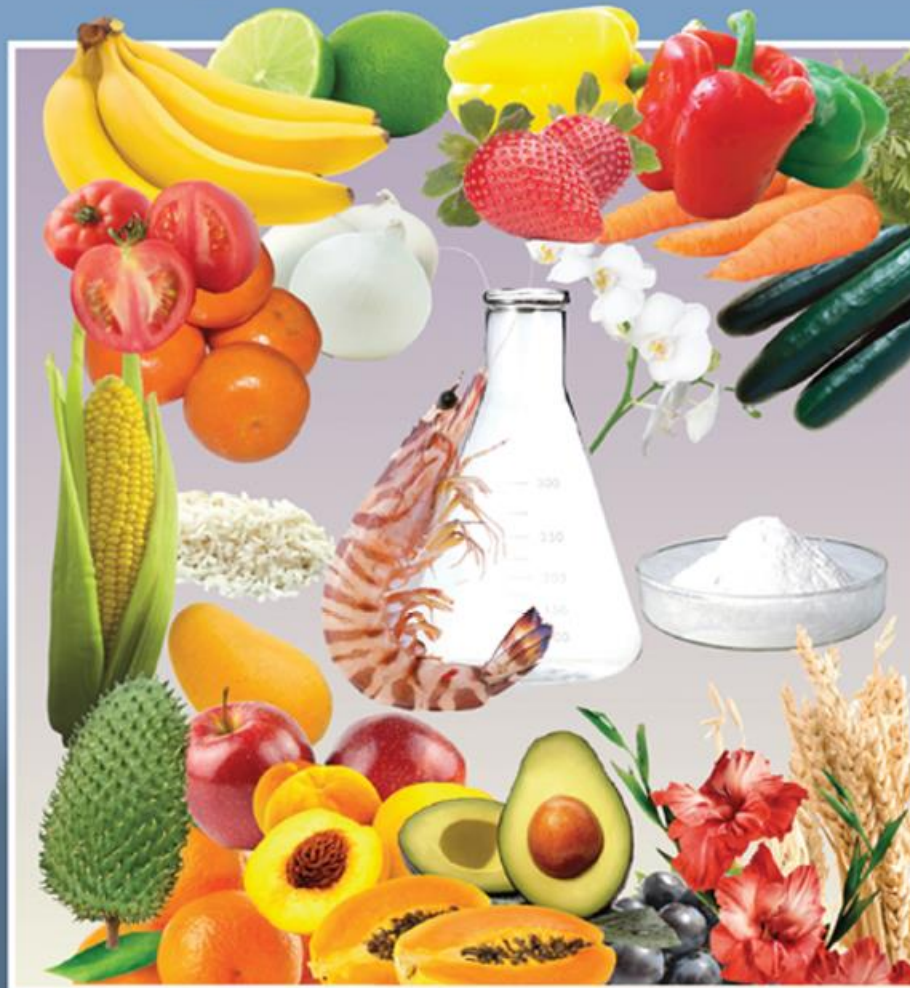
# TABLE GRAPE QUALITY PARAMETERS

After 6 weeks of storage at 2°C



Values with the same letter are not different according Tukey HSD ( $P < 0.05$ ).

# CHITOSAN in the Preservation of Agricultural Commodities



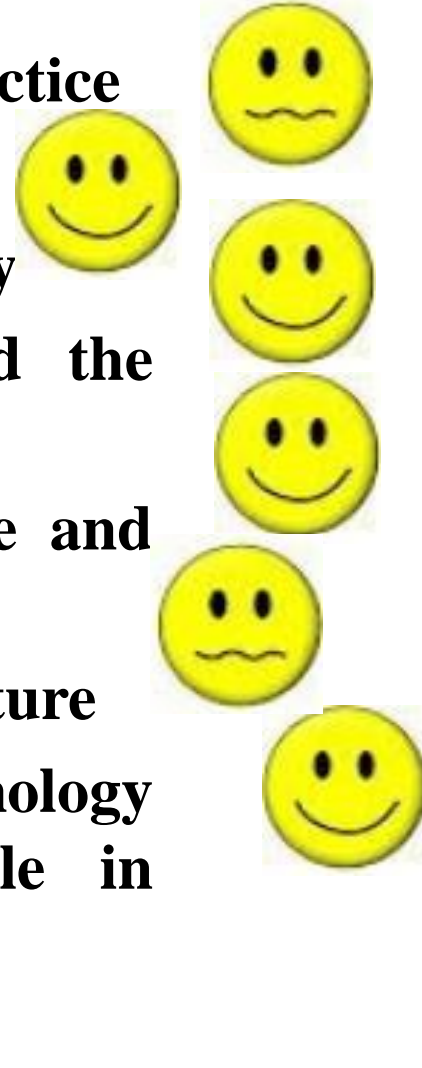
EDITORS

Silvia Bautista-Baños  
Gianfranco Romanazzi  
Antonio Jiménez-Aparicio



## Properties that alternative means to control postharvest diseases of fruit need to meet

1. efficacy equivalent or better than the current practice
2. will not injure or cause phytotoxic effects
3. will not compromise the fruit organoleptic quality
4. will not be a threat to human health and the environment
5. compatible with standard practices, affordable and easy to implement
6. compatible with the principles of organic agriculture
7. offer substantial benefits to the technology manufacturer which often play a pivotal role in commercialization of novel treatments





*Thanks for your attention*

**Nanoinnovations, Roma, 20-23 September 2016**