Uso del chitosano nella difesa antiperonosporica della vite e per il controllo dei marciumi di ortofrutticoli in pre e postraccolta

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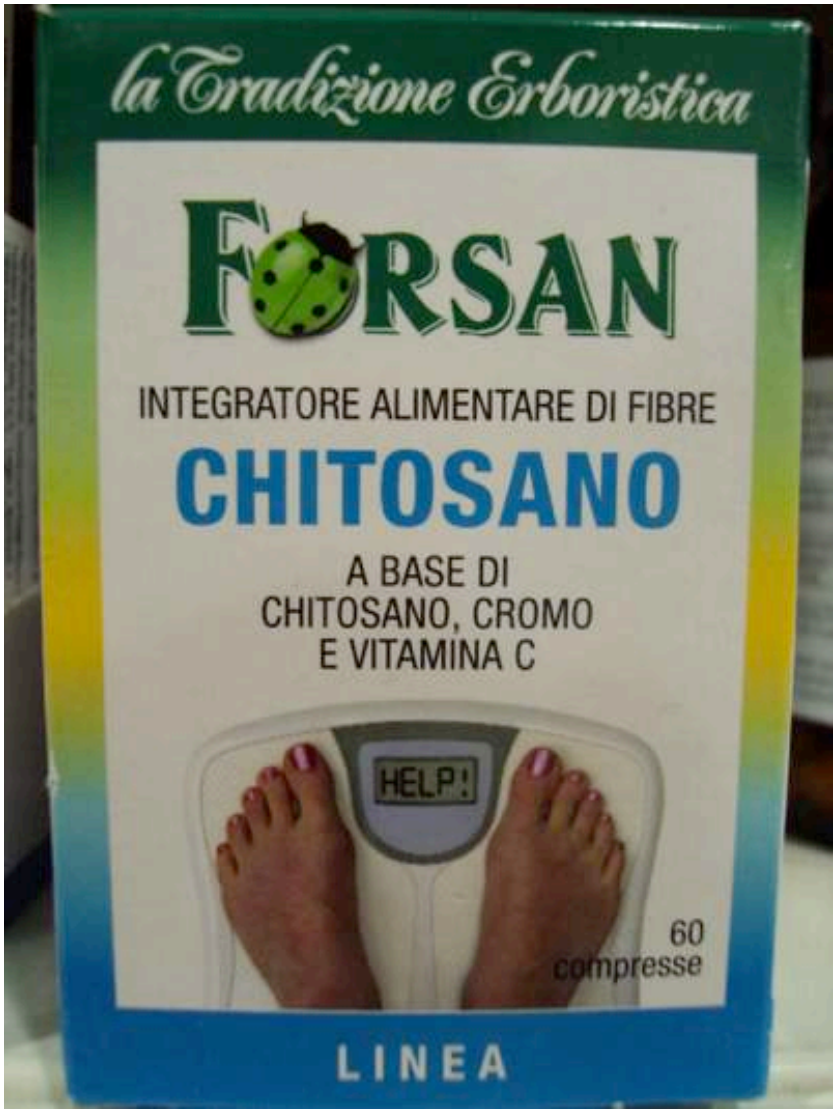
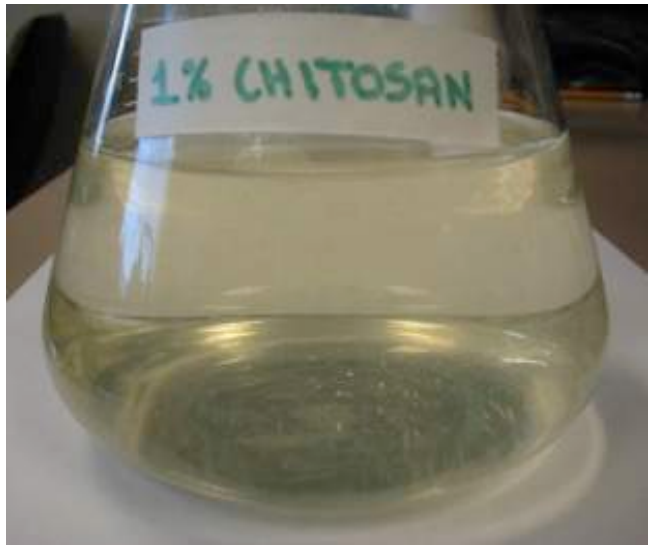
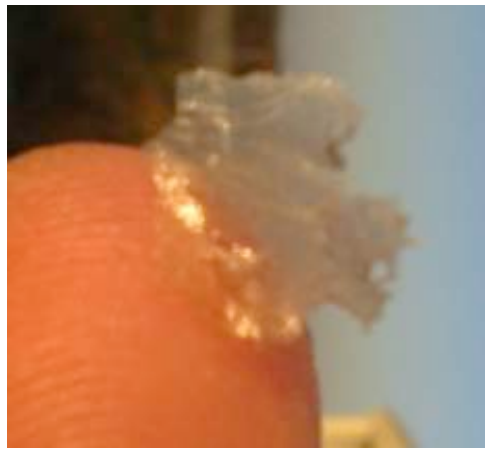
Cos’è il chitosano?





Dissolved in diluted acids



Attività del chitosano nei confronti della peronospora della vite

Evaluation of the characteristics of vineyard pruning residues for energy applications: effect of different copper-based treatments

Daniele Duca, Giuseppe Toscano, Andrea Pizzi, Giorgio Rossini, Sara Fabrizi, Giulia Lucesoli, Andrea Servili, Valeria Mancini, Gianfranco Romanazzi, Chiara Mengarelli

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Table 2. Comparison between copper distributed and copper registered (mean values) in pruning wood and soil.

Thesis	Copper absolute value (kg/ha)	Copper concentration in the vine pruning (mg/kg)	Copper concentration in the soil (mg/kg)
Bordeaux mixture	11.0	19.2	113.4
Copper hydroxide	5.8	10.6	102.8
Laminarin + copper hydroxide/copper oxide	1.8	8.5	81.0
Farm application	4.3	10.9	86.3
Control	-	7.0	88.3

Prova sperimentale

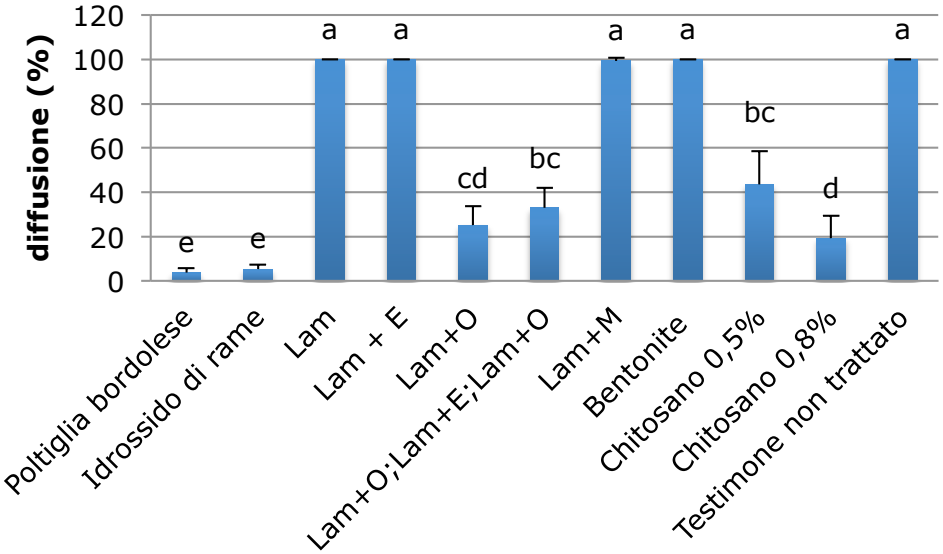
Vigneto di circa 0,5 ha della varietà Montepulciano, allevato a "Guyot", con un sesto di impianto di 0,8 × 2,2 m, non irrigato, ubicato nei pressi di Camerano (AN).

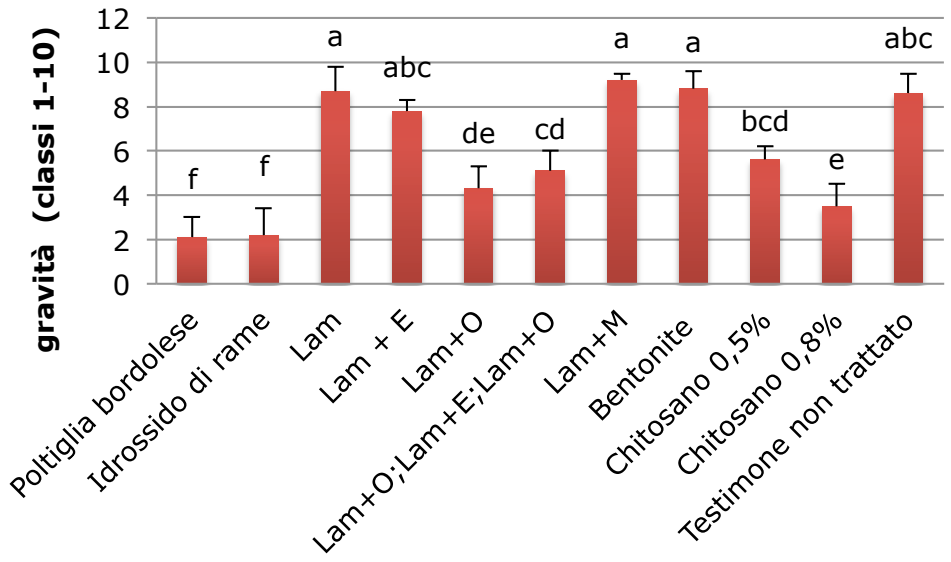
Tesi/Formulati (n. di applicazioni)	Principio attivo (%)	Dose d'impiego (ml o g/ha)	Anno prova
Poltiglia Disperss (11)	Poltiglia bordolese (20)	5000	2012, 2013
Funguran (11)	Idrossido di rame (19,2)	2800	2012, 2013
Frontiere (11)	Laminarina	1000	2012, 2013
Frontiere + Oomisine (11)	Laminarina + estratti microbici di Saccharomyces spp. (10), carbossilamine (10)	1000 + 2000	2012, 2013
Frontiere + Coptrel (11)	Laminarina + ossido/idrossido di rame (33)	1000 + 500	2012, 2013
Frontiere + Coptrel (1); Frontiere + Oomisine (7); Frontiere + Coptrel (3)	Laminarina + ossido/idrossido di rame (33); Laminarina + estratti microbici di Saccharomyces spp. (10), carbossilamine (10); Laminarina + ossido/idrossido di rame (33)	1000 + 500 1000 + 500 1000 + 500	2012, 2013
Frontiere + Micosat TAB fogliare (11)	Laminarina + Microorganismi (Glomus spp., Bacillus subtilis, Streptomyces spp., Trichoderma spp., Pichia pastoris)	1000 + 2000	2012, 2013
Bentotamnio (11)	Bentonite + K_2O (2,6), CaO (18,5), Mg (3,1)	14167	2012
Humixa polivalente (11)	Humus di lombrico (3,5)	6000	2013
Chito Plant (11)	Chitosano (99,9) + B (0,05) + Zn (0,05)	5000	2012, 2013
Chito Plant (11)	Chitosano (99,9) + B (0,05) + Zn (0,05)	8000	2012, 2013

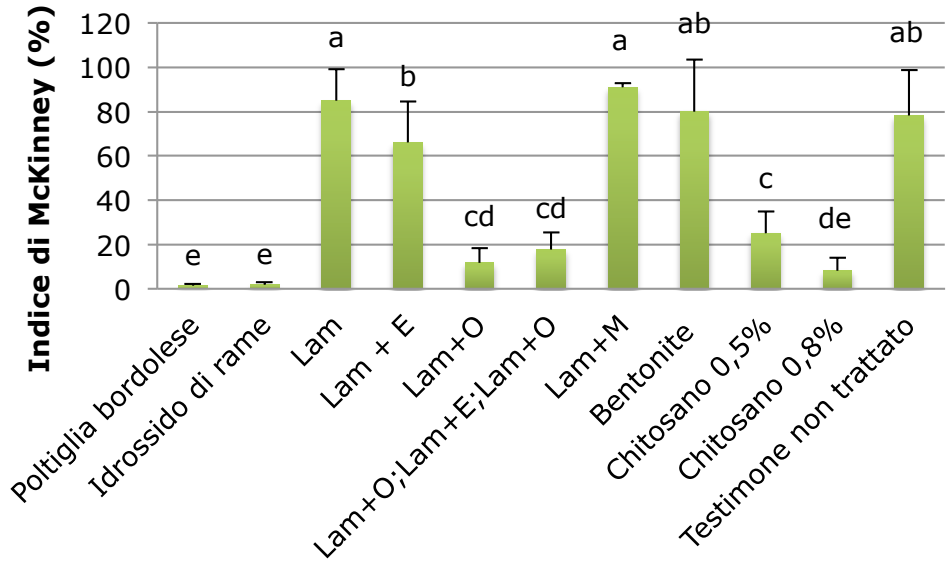
Schema sperimentale a blocchi randomizzati con quattro repliche

11 trattamenti effettuati con cadenza settimanale a partire da metà maggio fino a fine luglio

Risultati 24 settembre 2012







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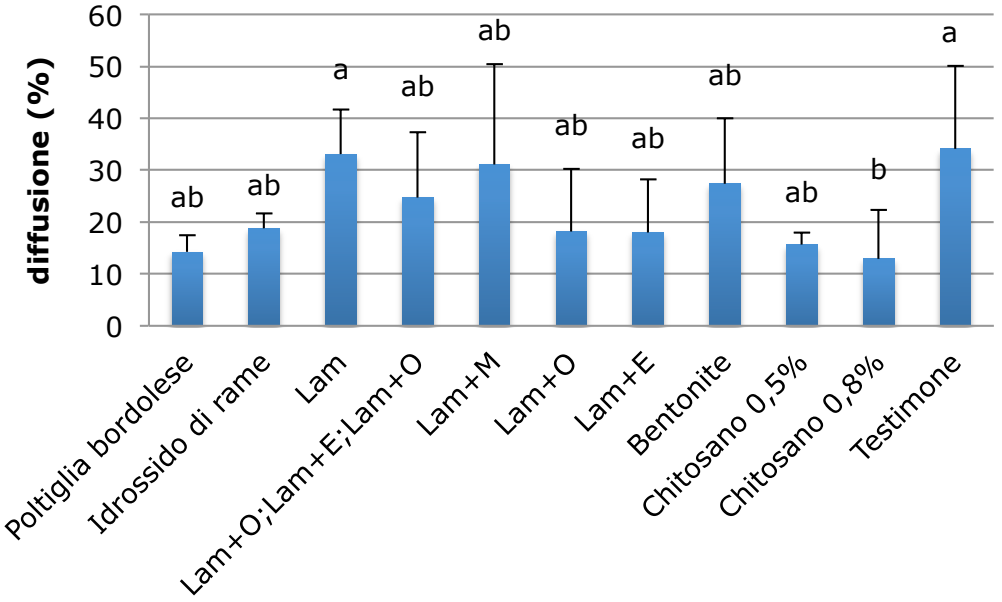

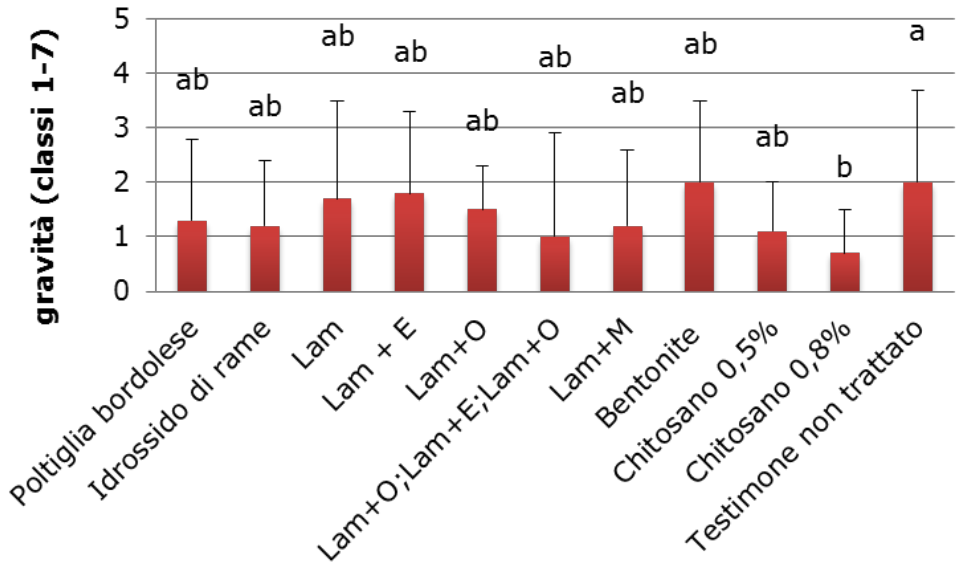

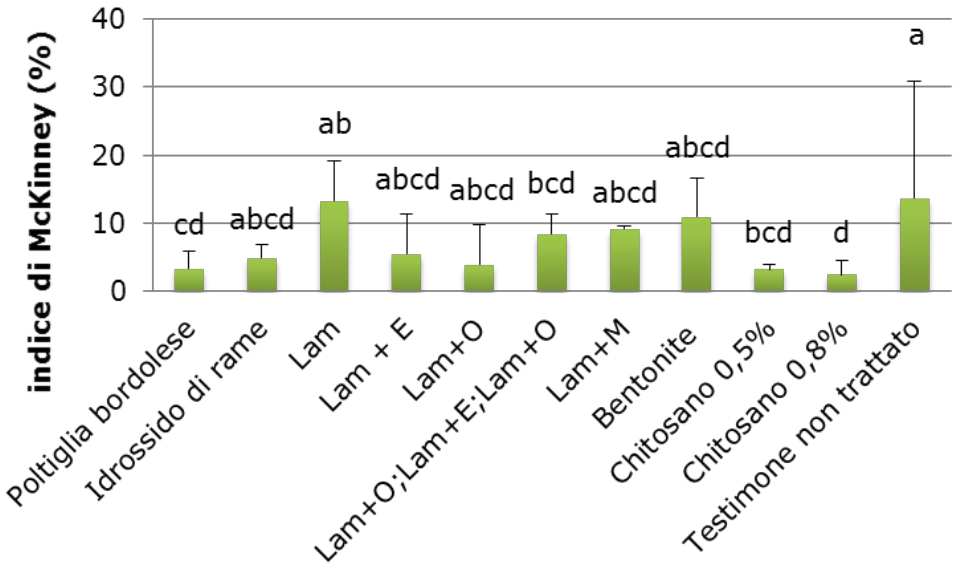

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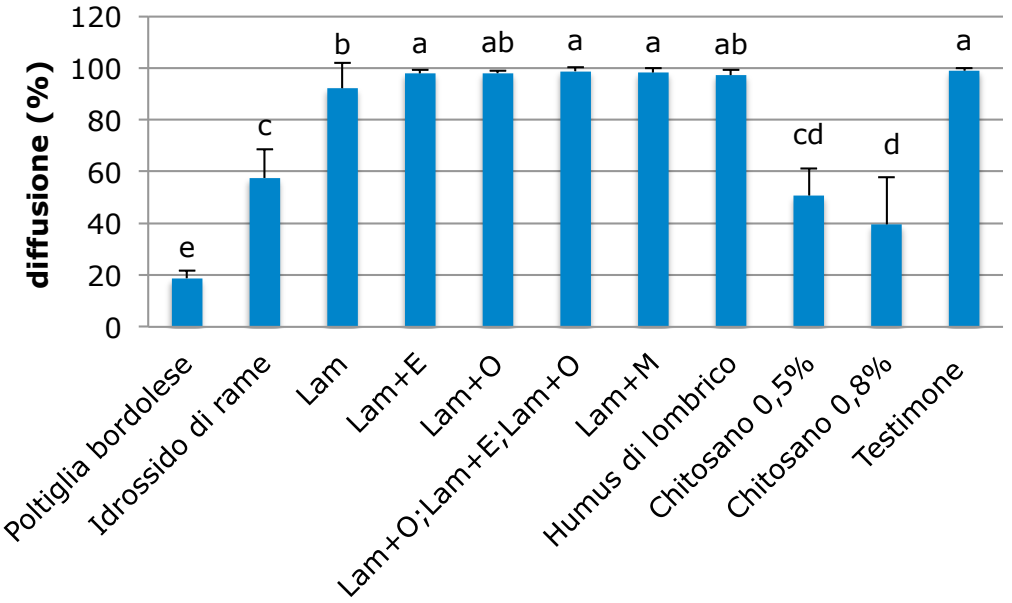
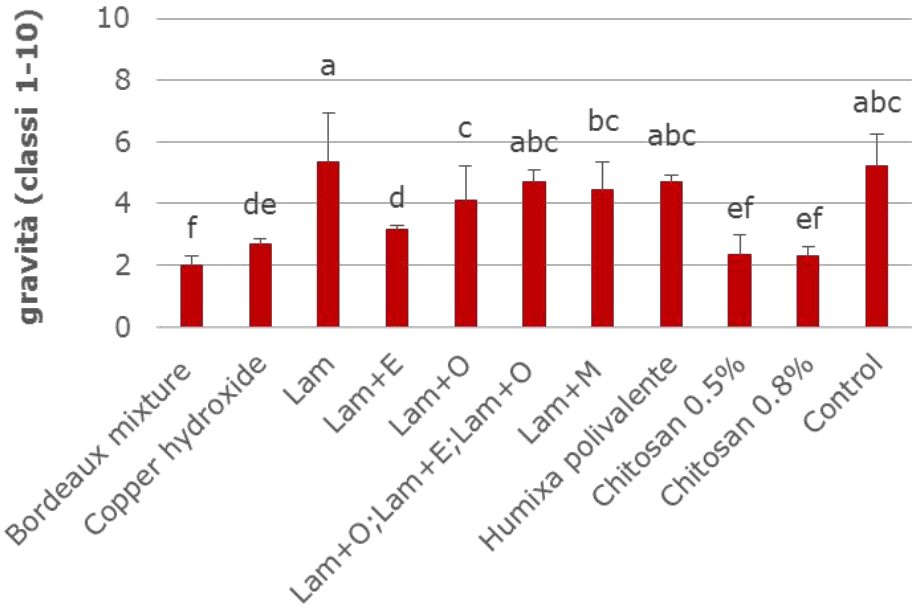
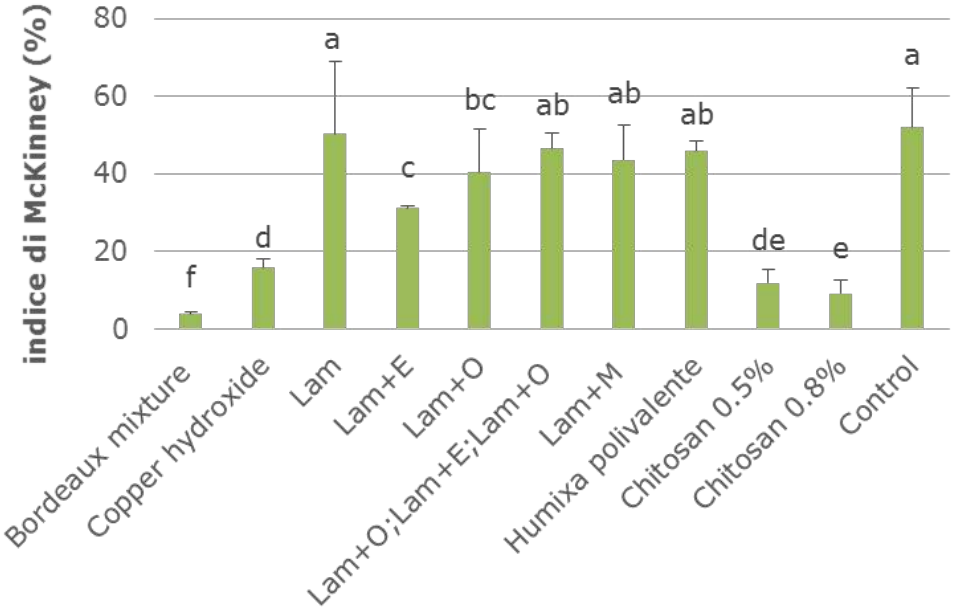
Risultati 31 luglio 2013







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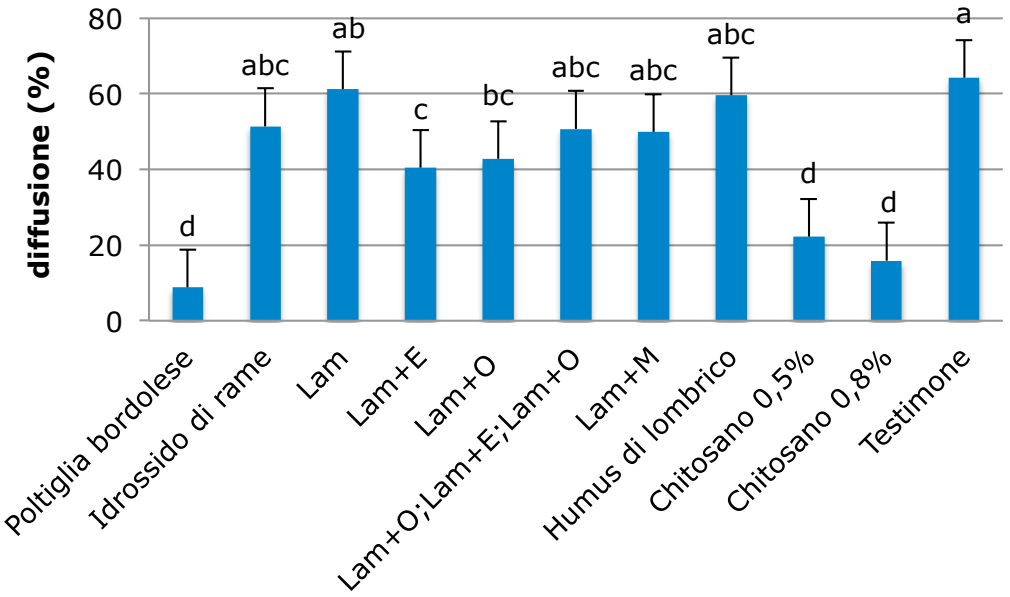

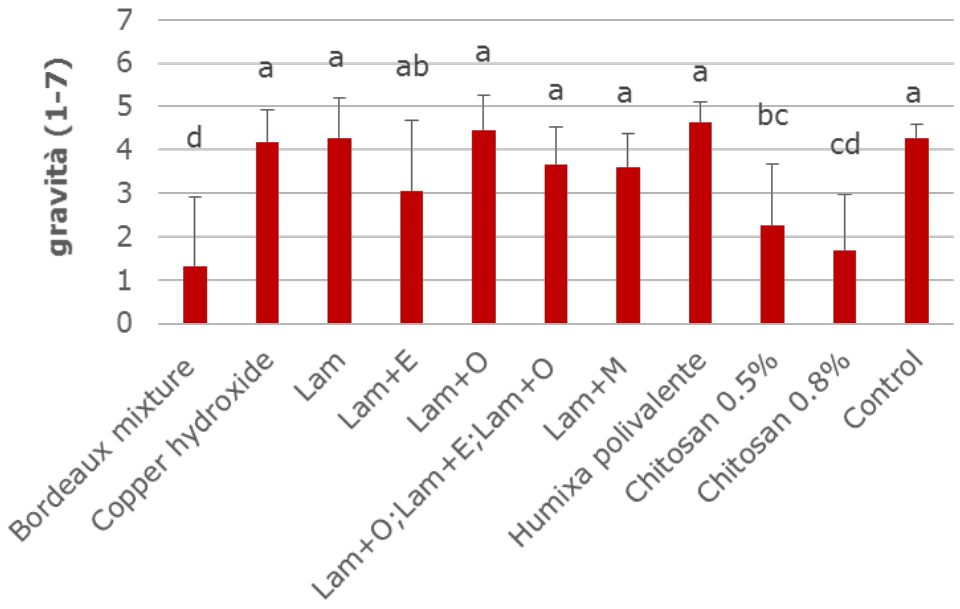

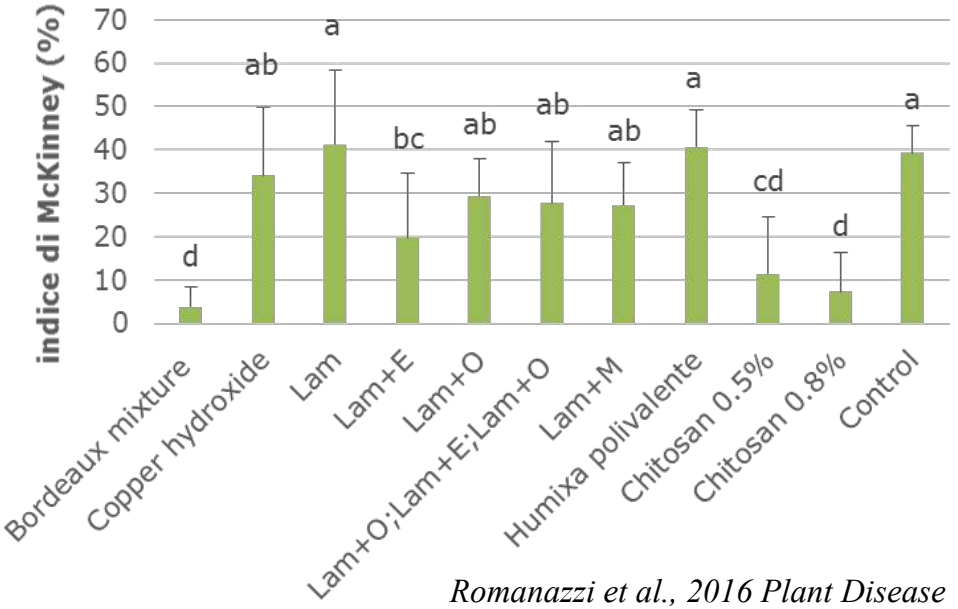

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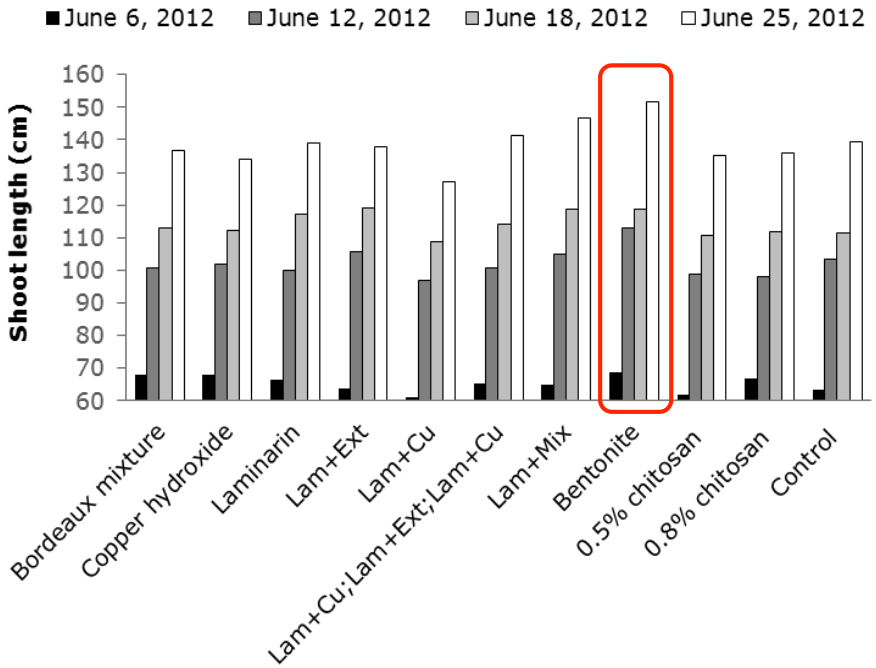
Late downy mildew symptoms in the vineyard of Camerano (AN) in the 1st year on September 24th

Growth parameters of vines

Shoot length

	Shoot length (cm)				
Treatments	**June 6**	**June 12**	**June 18**	**June 25**	**July 2**
Bordeaux mixture	68 ±22 a	101 ±20 b	113 ±21 a	137 ±23 ab	153 ±26 a
Copper hydroxide	67 ±22 a	102 ±17 b	112 ±17 a	134 ±26 ab	152 ±23 a
Laminarin	66 ±24 a	100 ±17 b	117 ±21 a	139 ±29 ab	154 ±34 a
Laminarin+Ext‡	65 ±24 a	105 ±13 ab	119 ±16 a	147 ±25 ab	156 ±25 a
Laminarin+Cu‡	61 ±26 a	97 ±21 b	109 ±21 a	127 ±24 b	145 ±32 a
Laminarin+Cu+Ext+Cu‡	65 ±23 a	101 ±15 b	114 ±18 a	141 ±27 ab	159 ±23 a
Laminarin+Mix‡	64 ±26 a	106 ±19 ab	119 ±20 a	138 ±22 ab	148 ±24 a
Bentonite	69 ±25 a	113 ±18 a	119 ±18 a	152 ±31 a	160 ±26 a
0.5% chitosan	62 ±23 a	99 ±14 b	111 ±19 a	135 ±24 ab	154 ±25 a
0.8% chitosan	67 ±24 a	98 ±21 b	112 ±26 a	136 ±40 ab	158 ±37 a
Control	63 ±26 a	103 ±15 b	111 ±16 a	139 ±26 ab	157 ±30 a



***Lam**, laminarin; **Ext**, microbial extract of* Saccharomyces *spp.; **Cu**, copper hydroxide/copper oxide; **Mix**, microorganisms (*Glomus *spp.*, Bacillus subtilis, Streptomyces *spp.*, Trichoderma *spp.*, Pichia pastoris*)*

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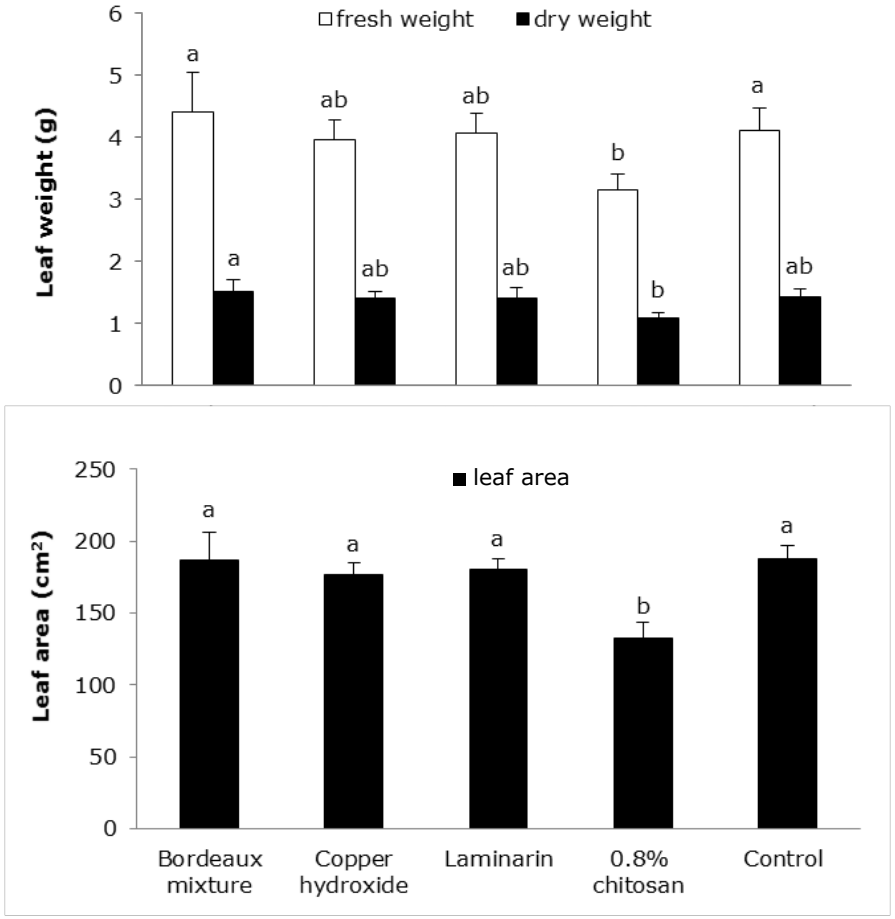
Growth parameters of vines

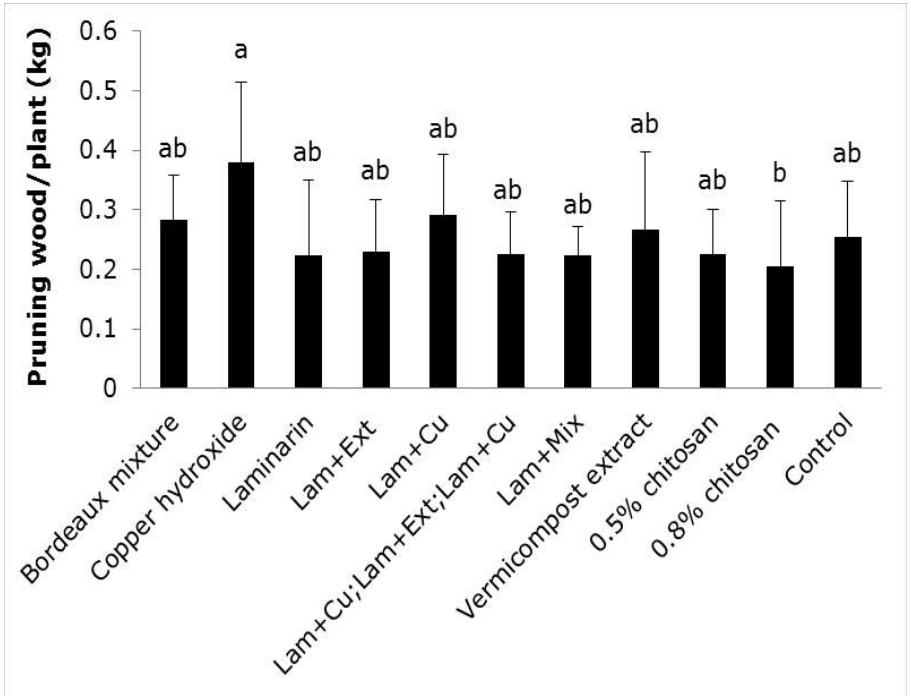
Leaf weight and area

- ✓ considering 40 leaves per plot on July in the 1st year
- ✓ measurements of the dry weight, putting leaves in an oven at 75 °C for 3 days
- ✓ measurements of the leaf areas, using a LI 3100 Area Meter



Pruning wood weight

- ✓ the weight of pruned branches was recorded for all of the plants for each treatment on March 2014 (2nd year)



***Lam**, laminarin; **Ext**, microbial extract of* Saccharomyces *spp.; **Cu**, copper hydroxide/ copper oxide; **Mix**, microorganisms (*Glomus *spp.,* Bacillus subtilis*,* Streptomyces *spp.,* Trichoderma *spp.,* Pichia pastoris*)*

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Growth parameters of vines

Quantity and quality of the grape production

Treatment	Production /plant (g)	Sugar content (%Brix)	Total acidity (g/L)	pH
Bordeaux mixture	2459 ±428	20.30 ±1.27	5.22 ±0.62	3.21 ±0.05 ab
Copper hydroxide	2194 ±253	20.80 ±0.00	5.18 ±0.27	3.19 ±0.00 ab
Laminarin	2055 ±458	20.65 ±0.49	5.73 ±0.35	3.27 ±0.05 a
Lam+Ext	1978 ±268	19.95 ±1.20	5.46 ±0.08	3.24 ±0.01 ab
Lam+Cu	2298 ±483	21.30 ±0.42	5.82 ±0.44	3.27 ±0.04 a
Lam+Cu;Lam+Ext;Lam+Cu	2357 ±141	20.70 ±0.42	5.50 ±1.35	3.19 ±0.08 ab
Lam+Mix	1815 ±277	20.20 ±1.13	4.89 ±1.40	3.25 ±0.01 ab
Bentonite	1950 ±555	19.85 ±1.06	4.65 ±0.97	3.21 ±0.05 ab
0.5% chitosan	2212 ±286	20.50 ±0.71	4.89 ±0.70	3.14 ±0.01 b
0.8% chitosan	2260 ±442	20.40 ±0.57	4.82 ±0.45	3.14 ±0.01 b
Control	1732 ±583	19.85 ±0.35	4.77 ± 0.81	3.21 ±0.01 ab

No treatment has negatively affect the quantitative and qualitative parameters of grapes

***Lam**, laminarin; **Ext**, microbial extract of* Saccharomyces *spp.; **Cu**, copper hydroxide/copper oxide; **Mix**, microorganisms (*Glomus *spp.,* Bacillus subtilis*,* Streptomyces *spp.,* Trichoderma *spp.,* Pichia pastoris*)*

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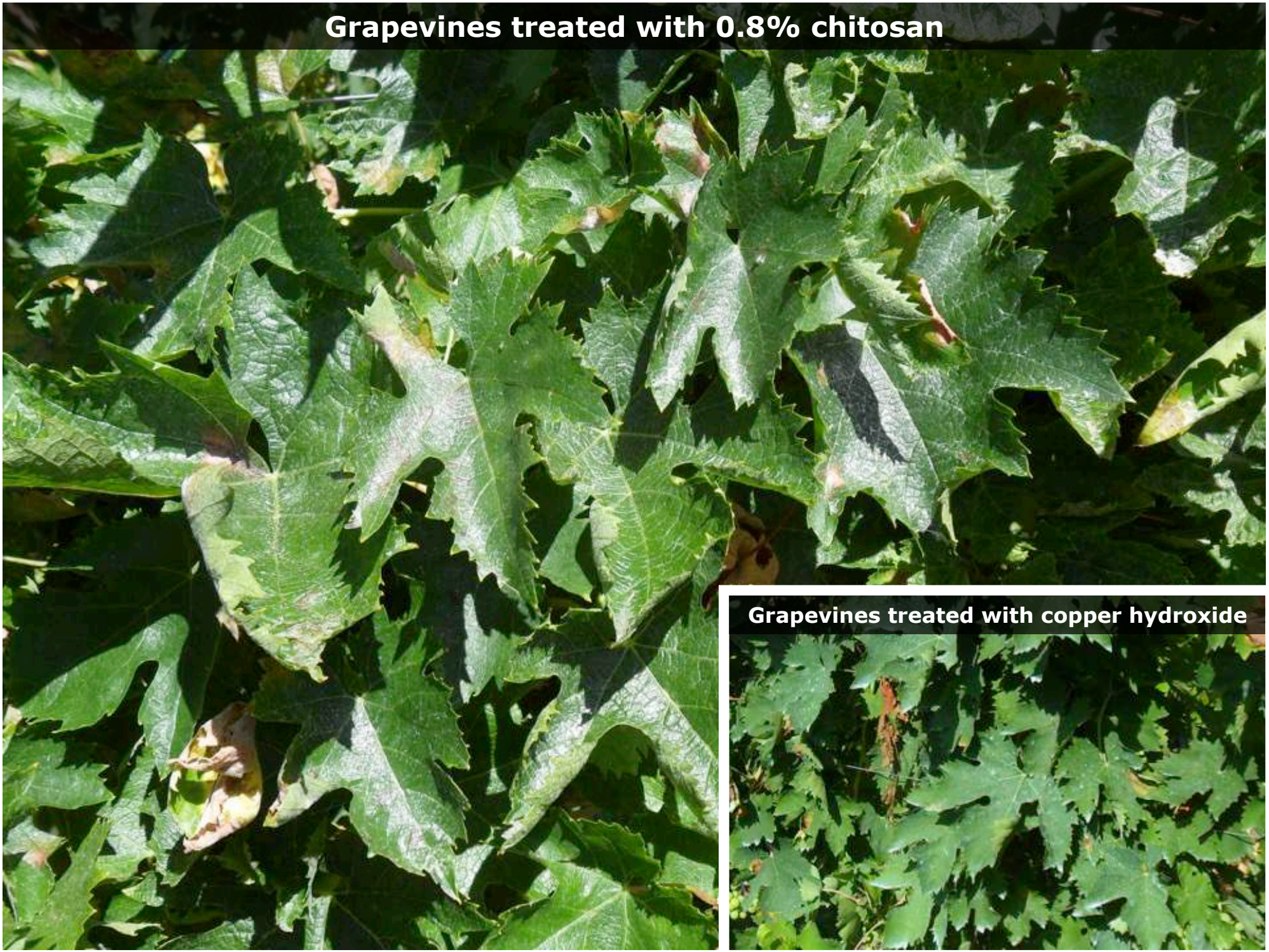
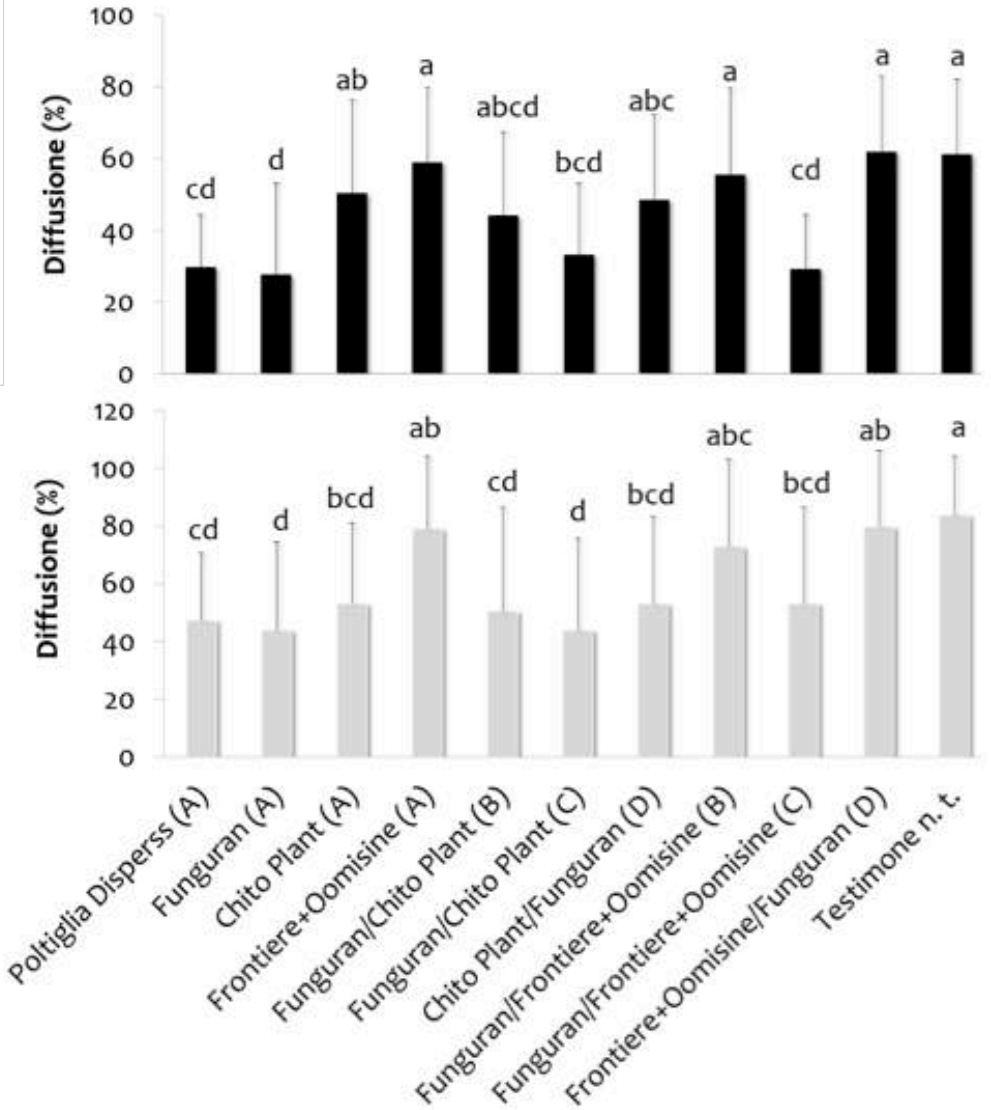

Grapevines treated with 0.8% chitosan
Grapevines treated with copper hydroxide

Risultati 2014



Strategia A = applicazione dello stesso formulato per tutta la stagione
Strategia B = applicazione alternata dei due formulati
Strategia C = applicazione di Funguran per la prima metà dei trattamenti e del prodotto alternativo per la seconda metà
Strategia D = applicazione del prodotto alternativo per la prima metà dei trattamenti e di Funguran per la seconda metà

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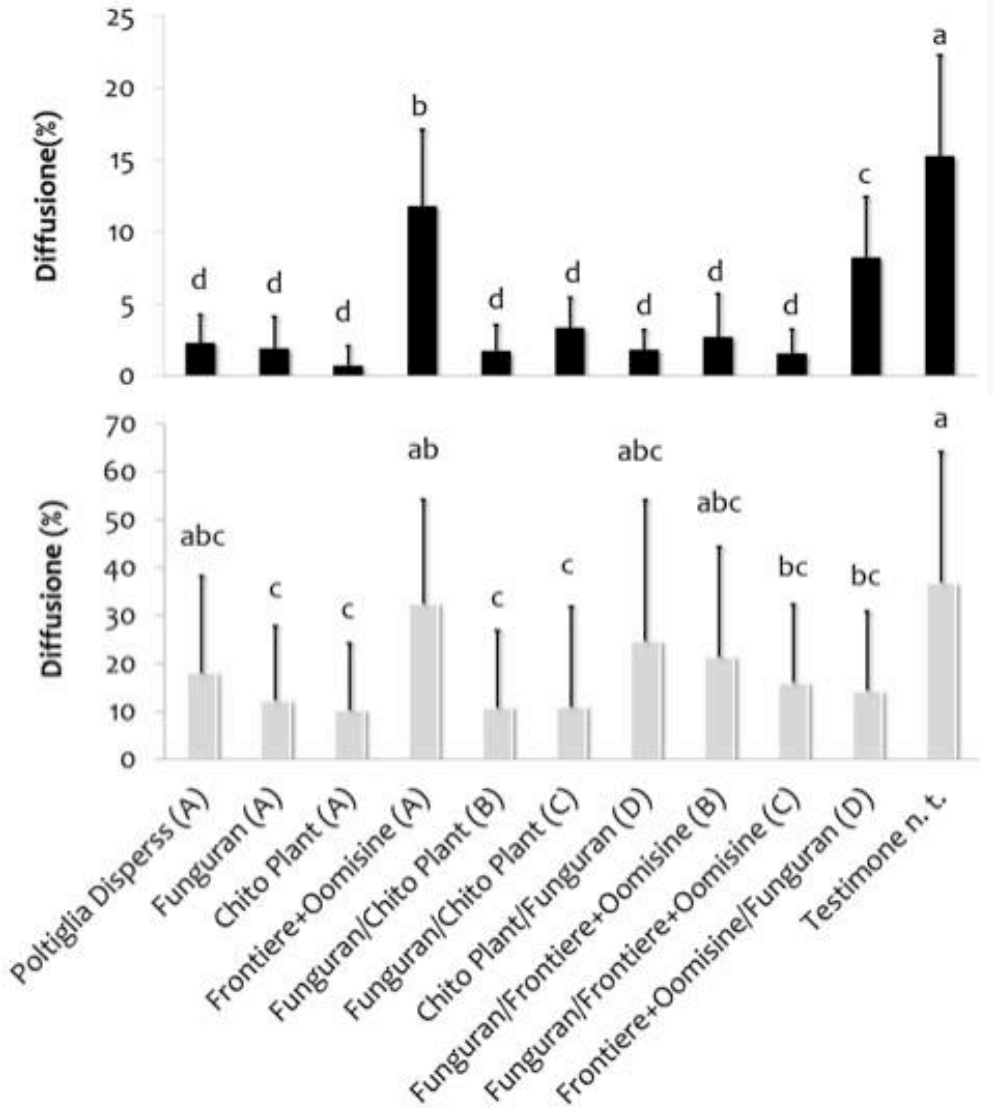
Atti Giornate Fitopatologiche

Risultati 2015



Strategia A = applicazione dello stesso formulato per tutta la stagione
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Strategia D = applicazione del prodotto alternativo per la prima metà dei trattamenti e di Funguran per la seconda metà

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Atti Giornate Fitopatologiche

Attività del chitosano nel controllo delle malattie postraccolta di ortofrutticoli

Postharvest Pathology and Mycotoxins

Antifungal Activity of Chitosan on Two Postharvest Pathogens of Strawberry Fruits

Ahmed El Ghaouth, Joseph Arul, Jean Grenier, and Alain Asselin

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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 β-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 β-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 2×10^5 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-

Effetto di trattamenti pre- e post-raccolta con chitosano sui marciumi della fragola in conservazione

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La conservabilità della fragola (*Fragaria × ananassa* Duch.) è molto limitata a causa della sua deperibilità ed elevata suscettibilità agli agenti di marciume. Le maggiori perdite sono dovute alla muffa grigia, causata da *Botrytis cinerea* Pers., ed al marciume acquoso, causato da *Rhizopus stolonifer* (Ehrenb.). La crescente richiesta da parte dei consumatori di un prodotto privo di residui di fitofarmaci, la necessità di raccogliere le fragole ad intervalli temporali ridotti (1-3 giorni), nonché la frequente comparsa di isolati di patogeni resistenti ai fungicidi orientano la ricerca verso mezzi di lotta alternativi ai trattamenti tradizionali con prodotti chimici. In tale contesto, una rilevante attenzione merita la lotta biologica, intesa in senso ampio, come recentemente definita da Wilson (1998), "la lotta contro una fitopatia mediante un processo biologico naturale o il prodotto di un processo biologico naturale".

Il chitosano, un componente dei gusci di crostacei e della parete cellulare di molti funghi, è un polimero della ß-1,4-D-glucosammina ottenuto per deacetilazione della chitina. È utilizzato in medicina umana in quanto policatione in grado di adsorbire i grassi ingeriti con gli alimenti, quindi consigliato nelle cure dimagranti (Nauss *et al.*, 1983); sembra possa anche ridurre il livello della frazione dannosa del colesterolo e favorire la guarigione delle ferite (Le Houx e Grondin, 1993; Muzzarelli e De Vincenzi, 1997). Inoltre, il chitosano è stato usato come conservante per succhi di mela (Roller e Covill, 1999). In patologia vegetale è nota la sua attività inibitoria nei confronti di numerosi batteri fitopatogeni (Pospieszny *et al.*, 1996) e funghi, fra cui alcuni dannosi per i prodotti ortofrutticoli (Allan e Hadwiger, 1979; El Ghaouth *et al.*, 1992a); inoltre, è risultato in grado di ritardare la maturazione e la senescenza di pomodori, pesche e pere, agendo come barriera alla diffusione dei gas (El Ghaouth *et al.*, 1992c; Du *et al.*, 1997). Il chitosano è riportato anche come induttore di resistenza. Infatti, in baccelli di pisello ha indotto la produzione di chitinasi (Mauch *et al.*, 1984) e della fitoalessina pisatina (Kendra *et al.*, 1989) ed è in grado di determinare resistenza nei confronti di virus e viroidi (Chirkov *et al.*, 1994; Pospieszny, 1997).

Fig. 1 - Veduta di uno dei tunnel nei quali sono state allevate le fragole sottoposte ai diversi trattamenti.

FRUTTICOLTURA - n. 5 - 2000 71

TAB. 1 - EFFETTO DEL CHITOSANO SULLO SVILUPPO DEI MARCIUMI POST-RACCOLTA IN FRAGOLE TRATTATE NELLO STADIO DI PIENA FIORITURA. LE FRAGOLE SONO STATE RACCOLTE 24 GIORNI DOPO I TRATTAMENTI E CONSERVATE A 0 ± 1 °C PER 10 GIORNI, SEGUITI DA 5 GIORNI DI "SHELF-LIFE" A 20 ± 1 °C.

Trattamenti	Frequenza dei frutti infetti (%)		Indice di infezione (%)	
	Botrytis cinerea	Marciumi totali	*Botrytis cinerea*	Marciumi totali
Chitosano 1,00%	59,9 c	77,3 a	43,7 B	58,6 a
Chitosano 0,50%	74,7 b	81,0 a	58,3 AB	63,8 a
Chitosano 0,10%	78,2 b	85,9 a	64,1 AB	72,6 a
Procymidone	74,3 b	88,7 a	51,2 AB	64,6 a
Testimone	93,1 a	93,7 a	71,3 A	73,9 a

Sulle colonne, i valori non seguiti da lettere uguali sono differenziati statisticamente ai livelli di probabilità $P \leq 0,01$ (lettere maiuscole) o $P \leq 0,05$ (lettere minuscole) secondo il test di Duncan.

TAB. 2 - EFFETTO DEL CHITOSANO SULLO SVILUPPO DEI MARCIUMI POST-RACCOLTA IN FRAGOLE TRATTATE NELLO STADIO DI FRUTTICINI VERDI. LE FRAGOLE SONO STATE RACCOLTE 9 GIORNI DOPO I TRATTAMENTI E CONSERVATE A 0 ± 1 °C PER 10 GIORNI, SEGUITI DA 6 GIORNI DI "SHELF-LIFE" A 20 ± 1 °C.

Trattamenti	Frequenza dei frutti infetti (%)		Indice di infezione (%)	
	Botrytis cinerea	Marciumi totali	*Botrytis cinerea*	Marciumi totali
Chitosano 1,00%	62,8 B	83,8 B	50,3 B	69,4 BC
Chitosano 0,50%	72,0 B	83,5 B	49,1 B	61,7 C
Chitosano 0,10%	75,2 B	98,0 A	65,4 AB	67,6 A
Procymidone	77,4 B	97,7 A	60,3 AB	82,0 AB
Testimone	98,1 A	98,8 A	85,7 A	86,7 A

Sulle colonne, i valori non seguiti da lettere uguali sono differenziati statisticamente al livello di probabilità $P \leq 0,01$ secondo il test di Duncan.

TAB. 3 - EFFETTO DEL CHITOSANO SULLO SVILUPPO DEI MARCIUMI POST-RACCOLTA IN FRAGOLE TRATTATE NELLO STADIO DI FRUTTI BIANCHI. LE FRAGOLE SONO STATE RACCOLTE 6 GIORNI DOPO I TRATTAMENTI E CONSERVATE A 0 ± 1 °C PER 10 GIORNI, SEGUITI DA 6 GIORNI DI "SHELF-LIFE" A 20 ± 1 °C.

Trattamenti	Frequenza dei frutti infetti (%)		Indice di infezione (%)	
	Botrytis cinerea	Marciumi totali	*Botrytis cinerea*	Marciumi totali
Chitosano 1,00%	37,9 C	47,9 B	22,4 C	31,1 c
Chitosano 0,50%	70,6 B	79,8 A	42,2 BC	51,4 b
Chitosano 0,10%	88,5 AB	91,7 A	53,4 B	56,7 b
Pyrimethanil	76,0 AB	86,9 A	44,8 B	56,6 b
Testimone	96,7 A	97,8 A	77,0 A	78,6 a

Sulle colonne, i valori non seguiti da lettere uguali sono differenziati statisticamente ai livelli di probabilità $P \leq 0,01$ (lettere maiuscole) o $P \leq 0,05$ (lettere minuscole) secondo il test di Duncan.

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Effect of pre-harvest chitosan sprays on post-harvest infection by *Botrytis cinerea* and quality of strawberry fruit

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Received 15 December 1999; accepted 23 April 2000

Abstract

The effect of pre-harvest sprays of chitosan on post-harvest decay and quality of strawberries stored at 3 and 13°C was investigated. Strawberry plants were sprayed with 2, 4 and 6 g l^{-1}, chitosan solutions as the fruit were turning red. A second spray was performed after 10 days. Fruit were picked 5 and 10 days after each spray. Harvested fruit from chitosan sprayed plants were challenged with *Botrytis cinerea*. Chitosan sprays significantly reduced post-harvest fungal rot and maintained the keeping quality of the fruit compared with control. The incidence of decay decreased with increased chitosan concentration and increased with storage period and temperature. The second spray of chitosan extended the protective effect against decay of fruit from subsequent picks. Fruit from chitosan sprayed plants were firmer and ripened at a slower rate as indicated by anthocyanin content and titratable acidity than berries from non-treated plants. Chitosan sprays were not phytotoxic at all the concentrations tested. Chitosan sprays at 6 g l^{-1} concentration performed twice, 10 days apart, protected the fruit from decay and kept the fruit quality at an acceptable level throughout the storage period of 4 weeks in fruit stored at 3°C. The protective effect of chitosan sprays was more pronounced for fruit from pick 1 than pick 2. Kinetic data on decay and ripening characteristics provided quantitative evidence that chitosan compensates for higher storage temperature and protects against deterioration of lower quality fruit from the second harvest.

Keywords: Strawberry; Chitosan; Pre-harvest spray; *Botrytis cinerea*; Post-harvest quality; Decay and ripening kinetics

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_2 are required in view of damage to bunches due to temperature increase, of hazards for human health, and of the difficulties in using SO_2 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; Schena 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 (10^4 spores ml^{-1}); in the spray-application experiments, concentrated stock suspension was added to achieve a final concentration of 10^5 spores ml^{-1}. 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

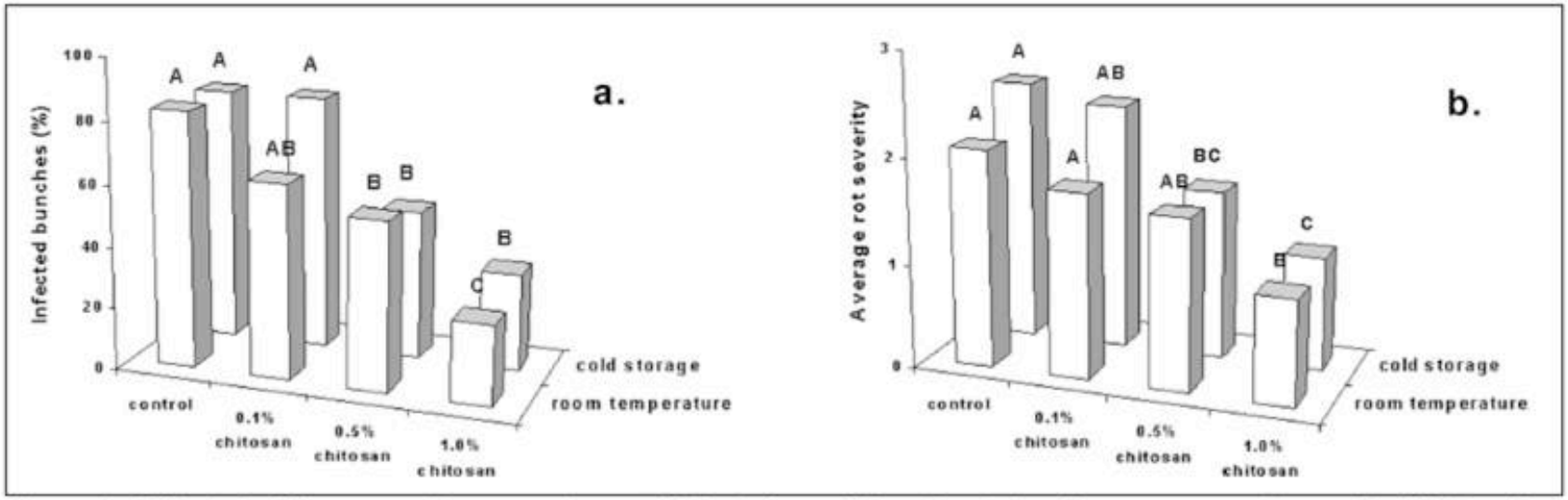

Figure 1—Effect of chitosan on the percentage of infected small bunches (a) and on average rot severity (b). Bunches were dipped in chitosan, sprayed with a *Botrytis cinerea* spore suspension (10^5 spores ml^{-1}) and stored for 20 d at room temperature or 15 d at 0 ± 1 °C, 95-98% RH, followed by a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

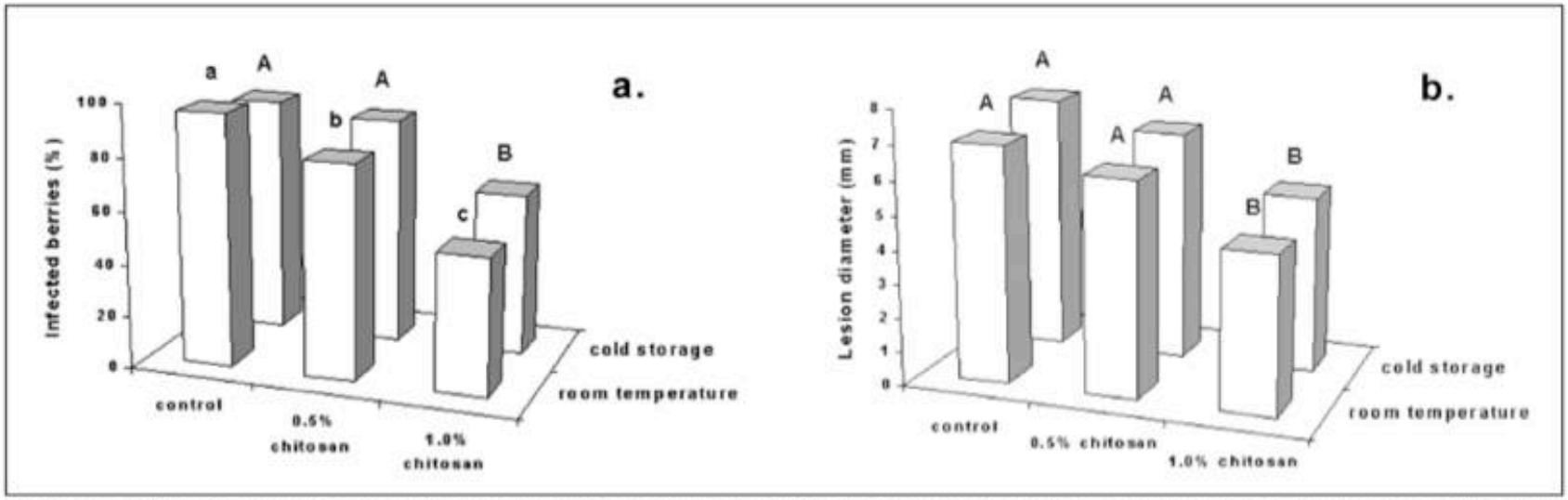

Figure 2—Effect of chitosan on the percentage of infected berries (a) and on lesion diameter (b) in berries artificially inoculated with *Botrytis cinerea*. Single berries were wounded, treated with chitosan (0.5 and 1.0%) or water (control) and inoculated with a spore suspension (10^4 spores ml^{-1})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

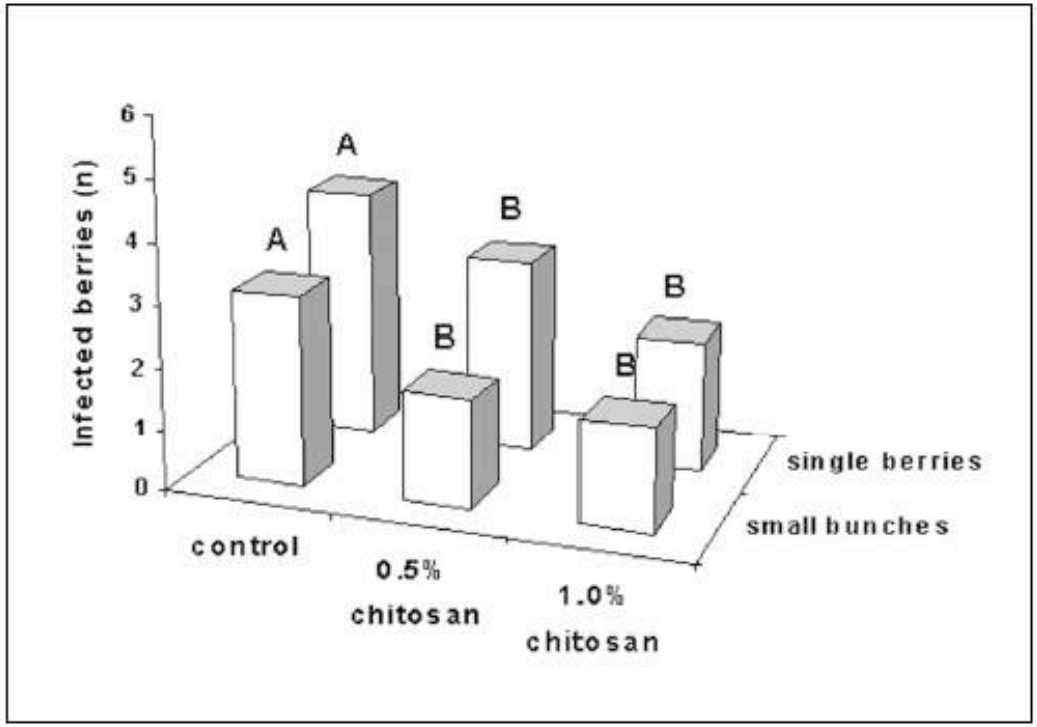

Figure 3—Effect of chitosan treatments on grey mold (nesting). Single berries and small bunches were dipped in chitosan solutions (0.5 and 1.0%) or in water (control); after air-drying, berries and bunches were arranged in plastic boxes and inoculated by placing a berry completely covered of grey mold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

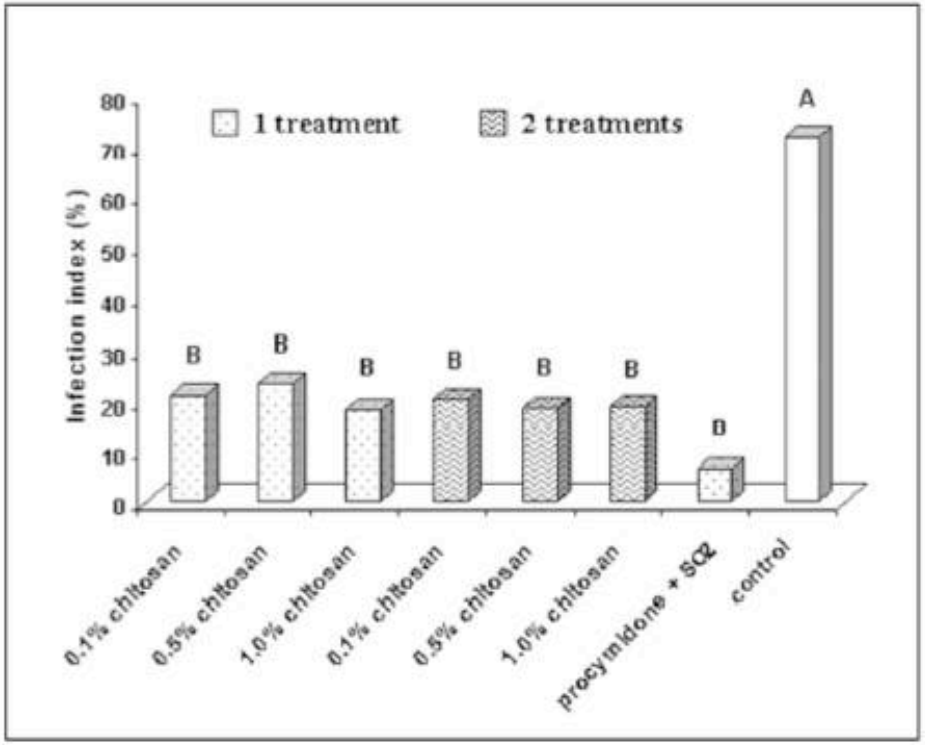

Figure 4—Effect of preharvest chitosan on the grey mold infection index of table grapes in storage. Bunches were sprayed once and twice (21 and 21 and 5 d before harvest). Table grapes treated with procymidone 21 d before the harvest and cold stored with sulphur dioxide (SO_2) is included for comparison. 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%.

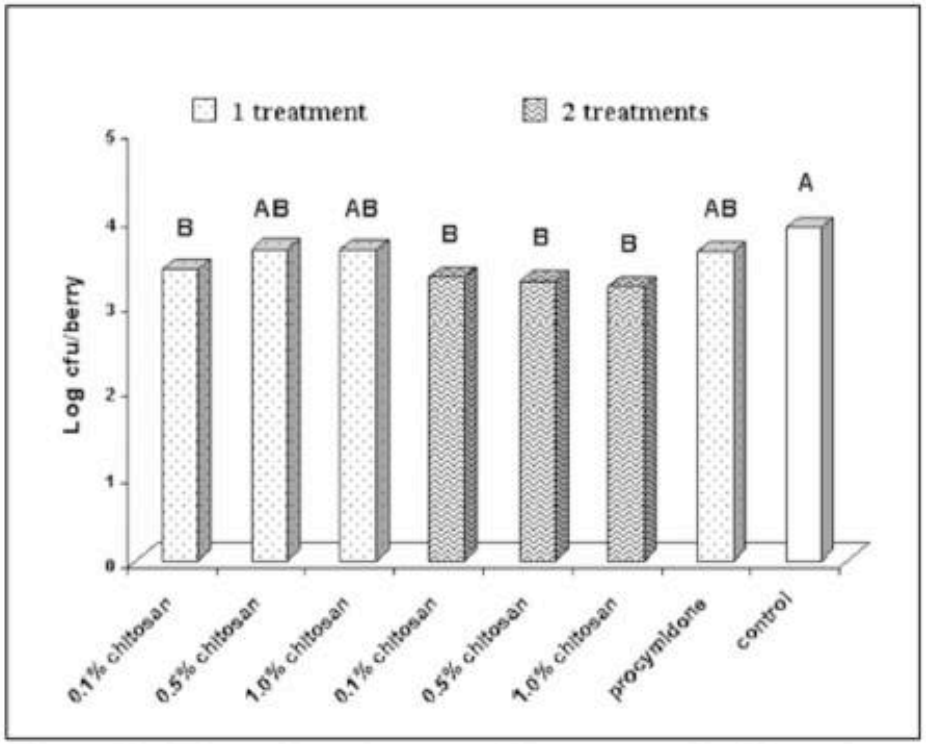

Figure 5—Effect of preharvest chitosan on the filamentous fungi population of table grape berries. Bunches were sprayed once and twice (21 and 21 and 5 d before harvest). The number of colonies was assessed at harvest time. Values marked with the same letter are not statistically different according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

MALATTIE POSTRACCOLTA DELLE CILIEGIE

Marciume da Alternaria

Marciume da Cladosporium

Muffa grigia

Marciume bruno

Marciume acquoso

Muffa blu

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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 ± 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.

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[a]	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_e)	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[a]	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.

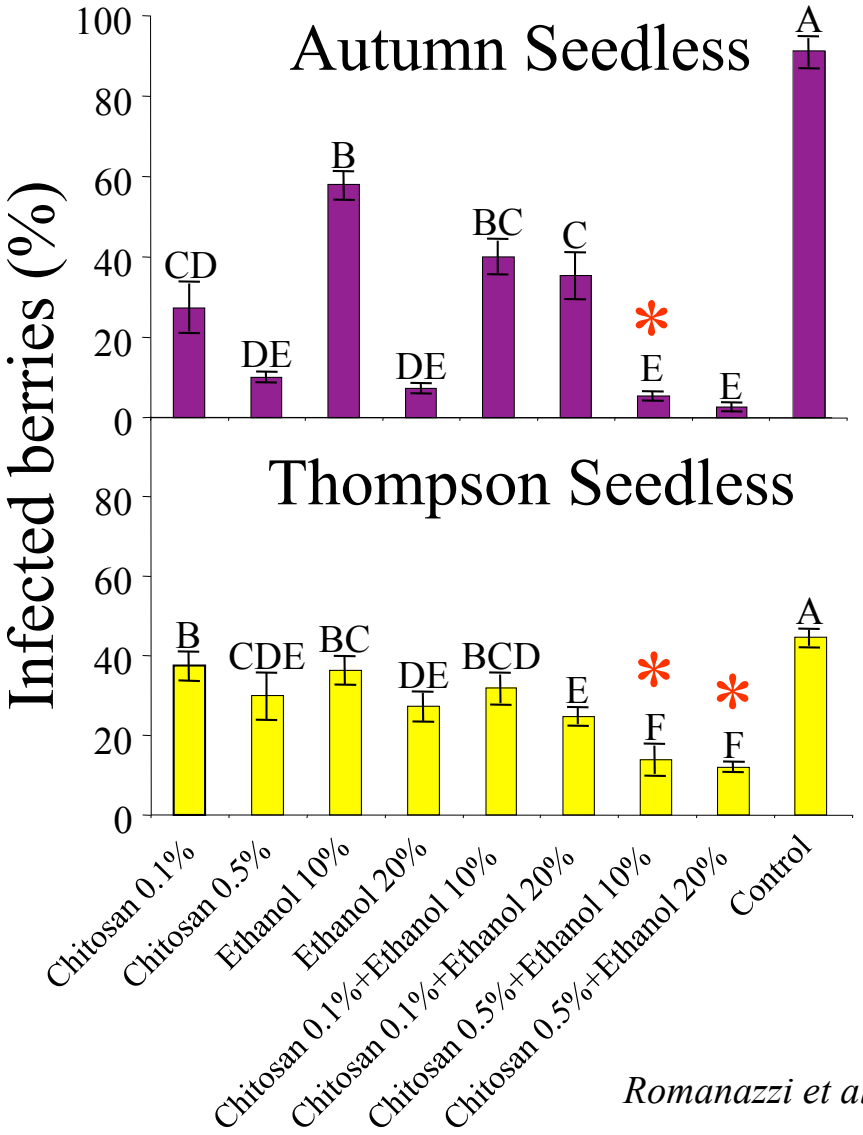
[a] Total rots include grey mould, brown rot, Rhizopus rot, Alternaria rot, blue mould and green rot. When the combination of the two agents produces any value of decay reduction (observed effect) greater than E_e (expected additive effect), according to Limpel's formula, then synergism exists (indicated with *). Limpel's formula is $E_e = X + Y - (XY/100)$, in which E_e 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


Leisch Schmidt
Monir Mansour
Dennis Margosan
Joe Smilanick
Franka Mlikota Gabler
Jennifer Gosoph

Single berries inoculated with *B. cinerea* and immersed in chitosan and ethanol solutions alone and in combination

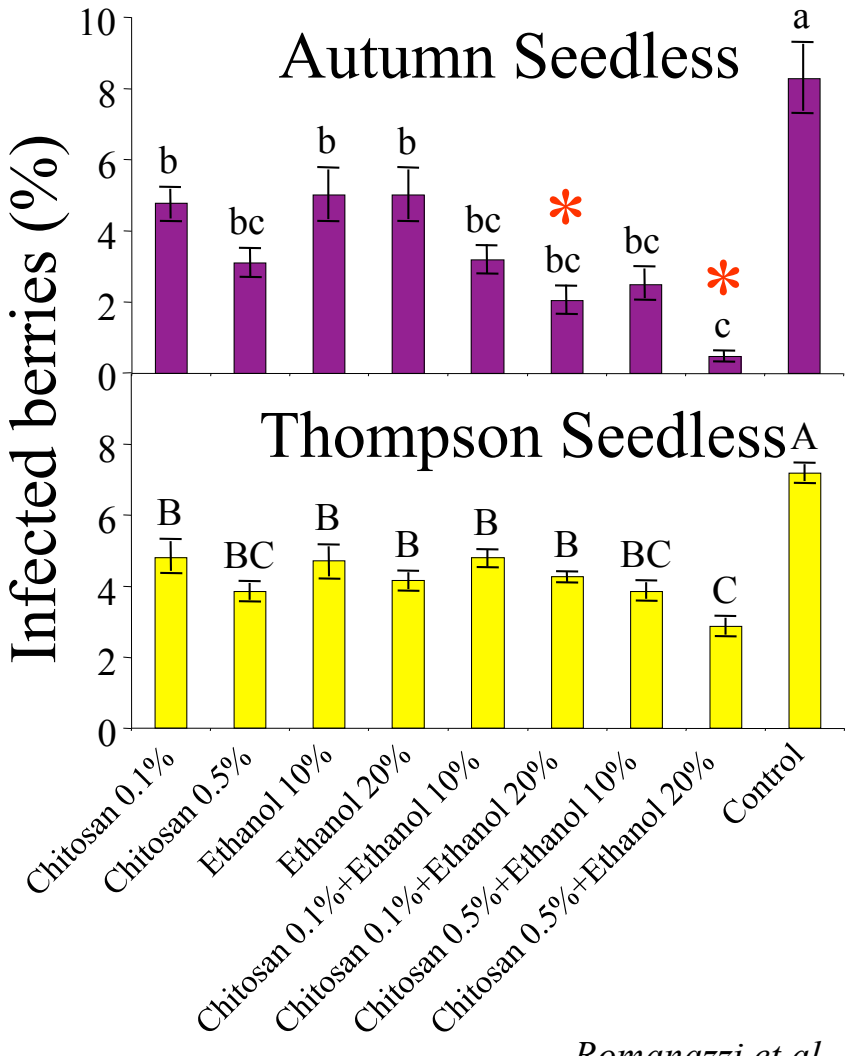
10 days
@ 15°C



*Synergistic effect, according to the Limpel's formula *(Richer, 1987)*

Romanazzi et al., 2007 Postharvest Biol. Technol.

Small clusters inoculated with *B. cinerea* and immersed in chitosan and ethanol solutions alone and in combination

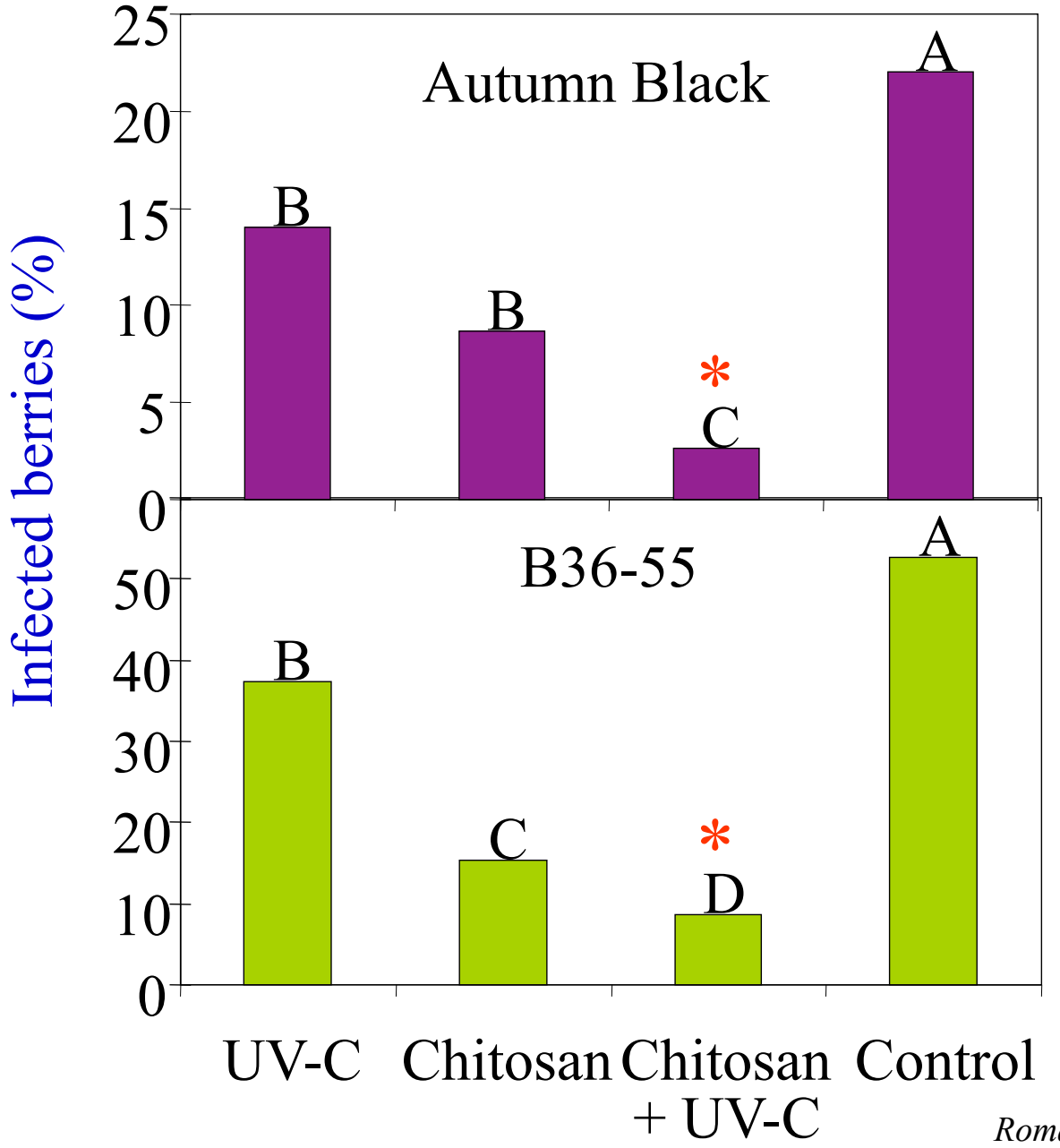


60 days
@ 0.5°C

*Synergistic effect, according to the Limpel's formula

Romanazzi et al., 2007 Postharvest Biol. Technol.

Combination of preharvest chitosan and UV-C treatments on gray mold



UV-C O.36 J/cm^2
Chitosan 1%

*Synergistic effect, according to the Limpel's formula

10 days @ 15°C

Romanazzi et al., 2006 Plant Dis.

Acids reported able to dissolve chitosan

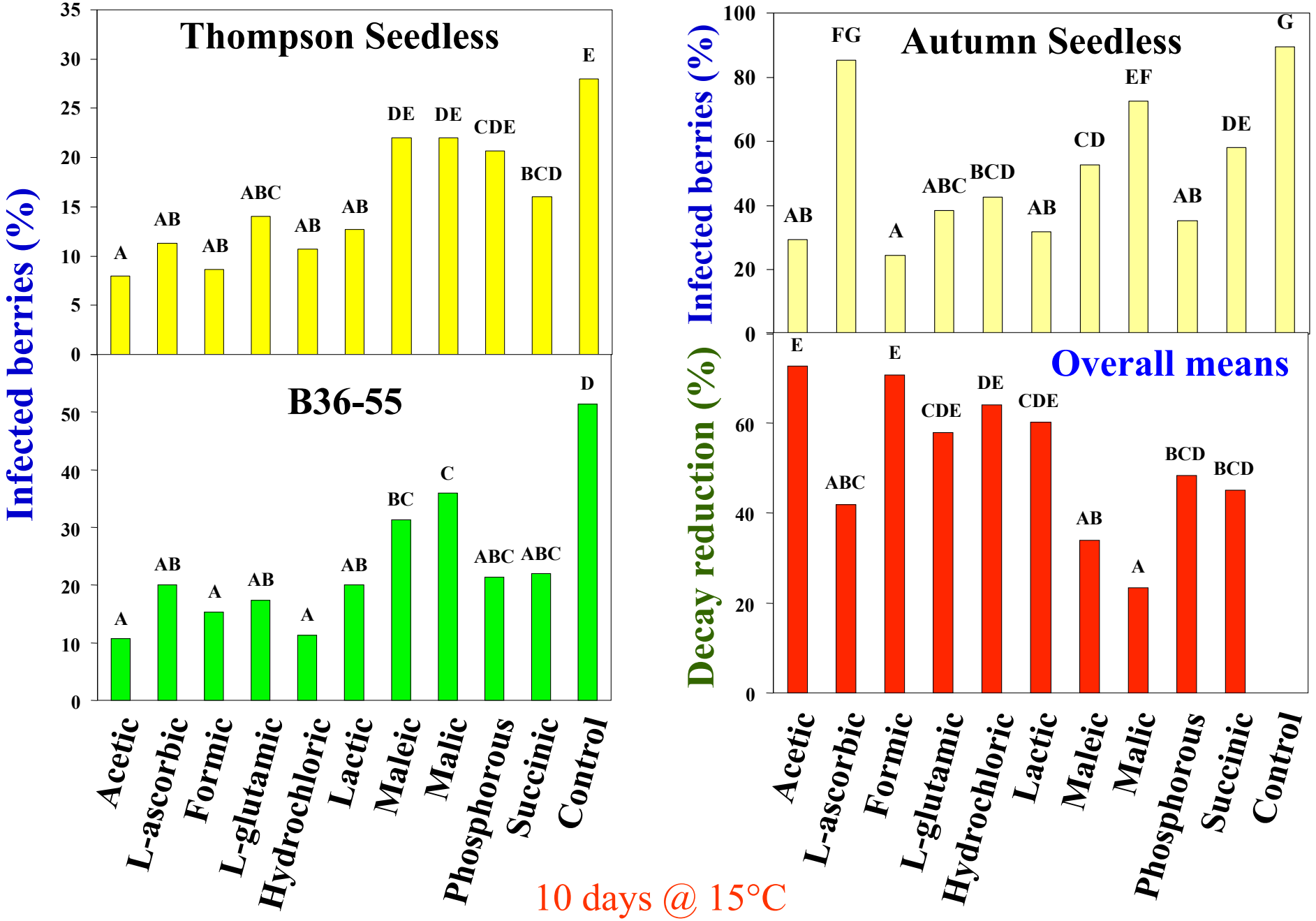
Acid	Concentration	Reference
Acetic	0.1 N	Allan and Hadwiger, 1979
	0.5%	Du et al., 1998
	1%	Kendra et al., 1989
	2%	Bégin and Van Calsteren, 1999
Citric	2%	Bégin and Van Calsteren, 1999
Formic	2%	Bégin and Van Calsteren, 1999
L-glutamic	1-2%	Zhang and Quantick, 1997
Lactic	0.5%	Devlieghere et al., 2004
	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

Romanazzi et al., 2009 Phytopathology

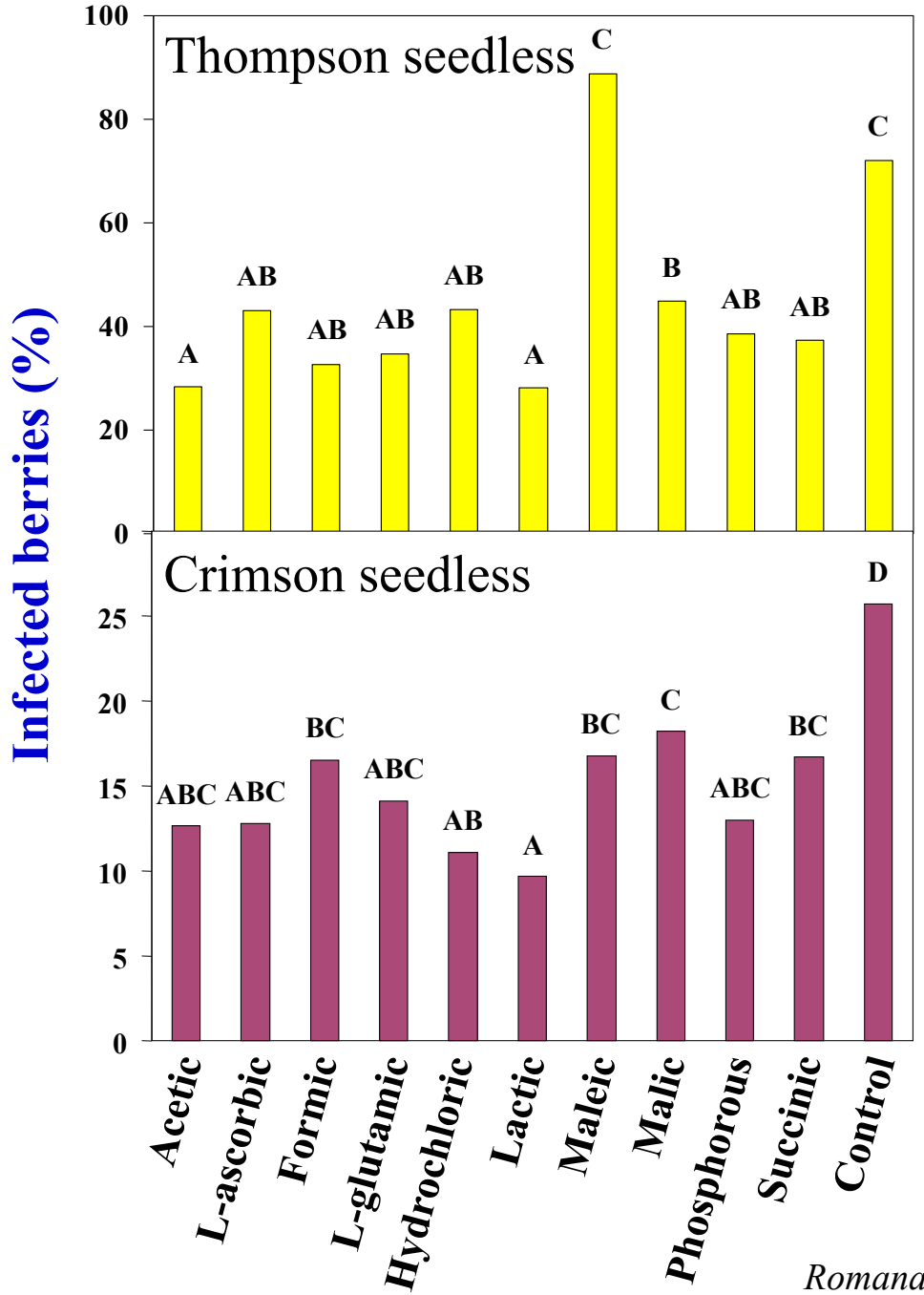
Ability of different acids to dissolve chitosan

Acid	pH 1%	Dissolve chitosan
Acetic	2.8	Yes
L-ascorbic	2.7	Yes
Boric	5.0	No
DL-α-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
Trans-Cinnamic	2.9	No

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

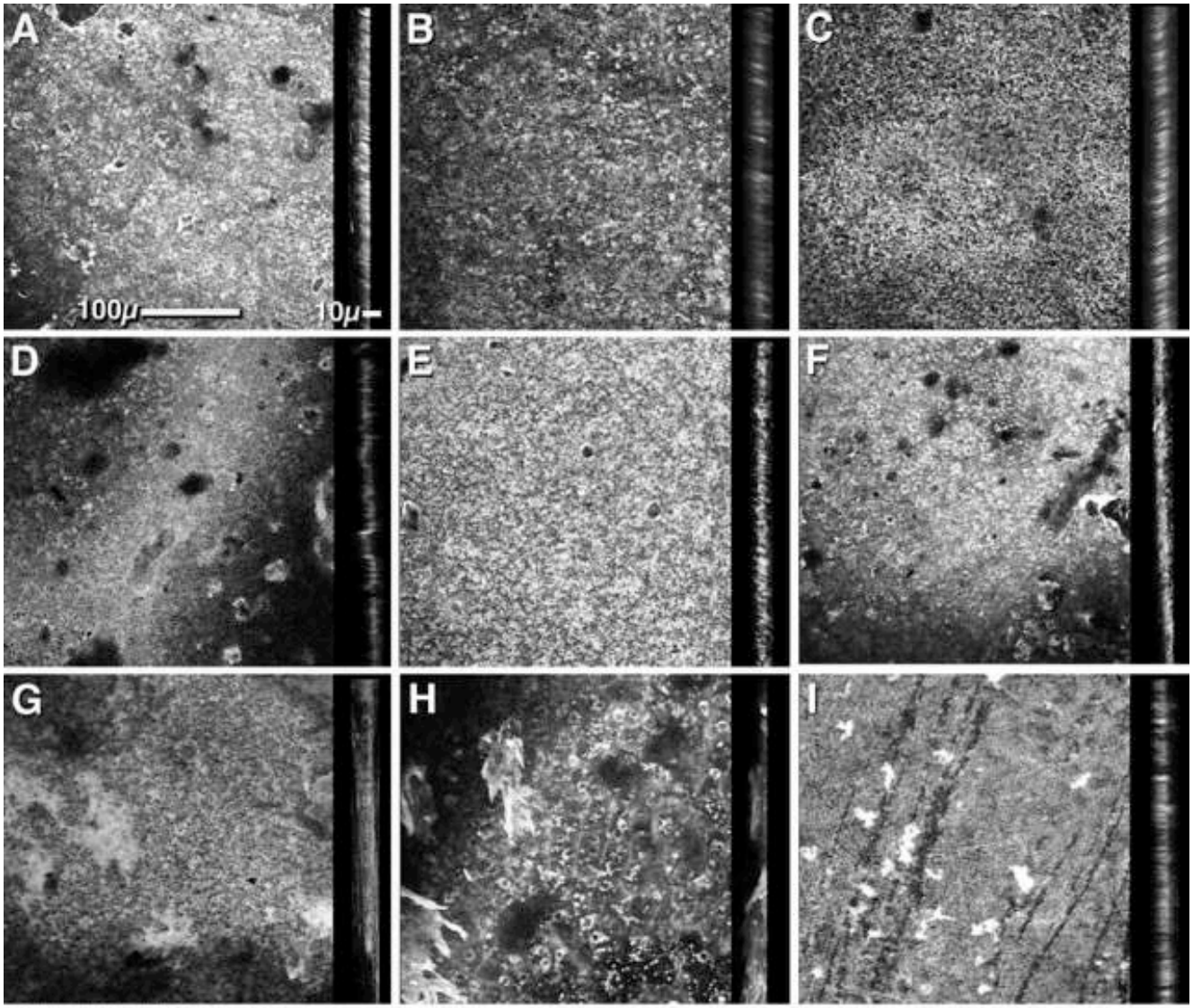


Small clusters inoculated with *B. cinerea* and immersed in chitosan solutions



60 days
@ 0.5°C

Romanazzi et al., 2009 Phytopathology



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.

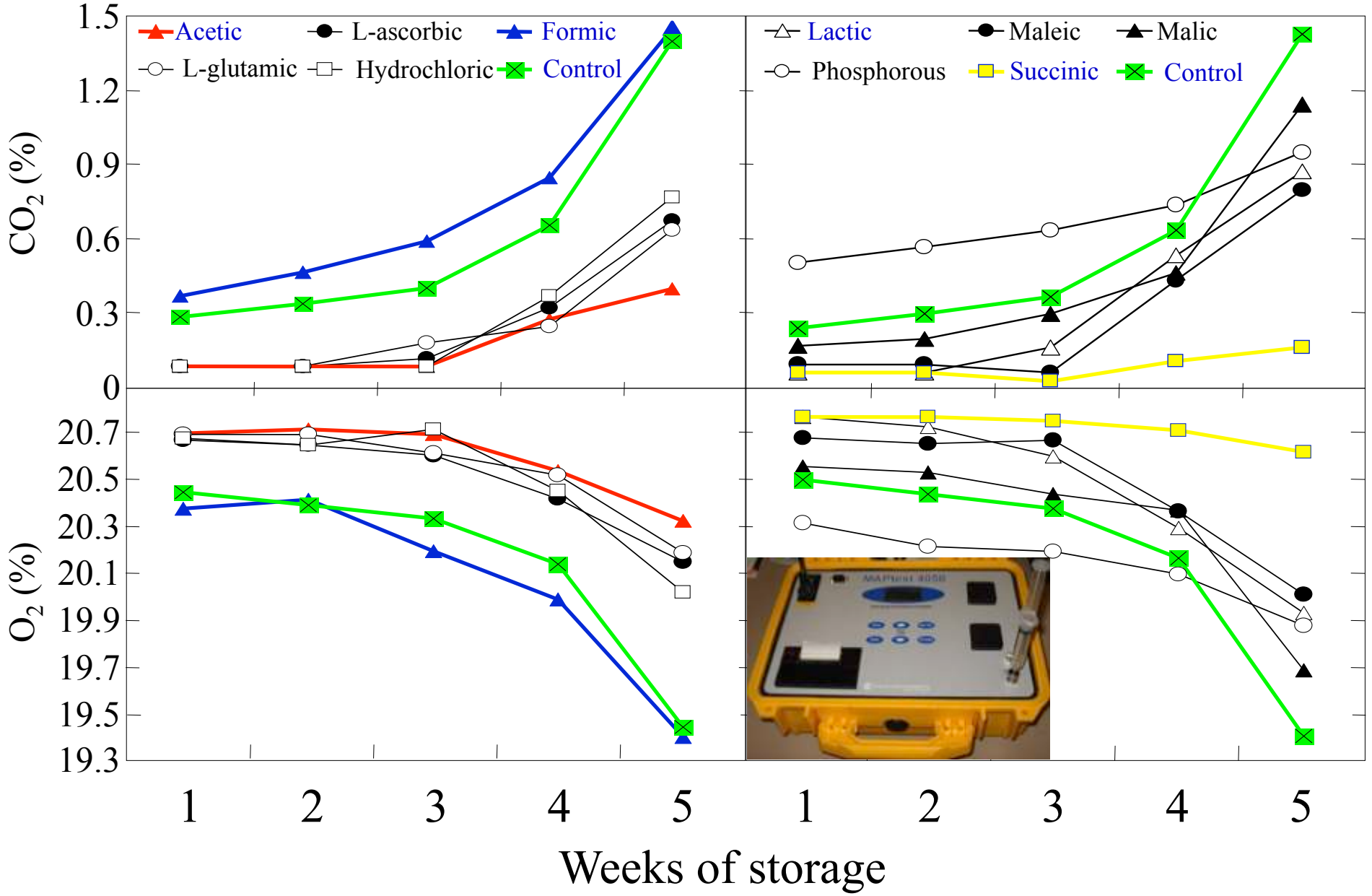
Romanazzi et al., 2009 Phytopathology

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

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

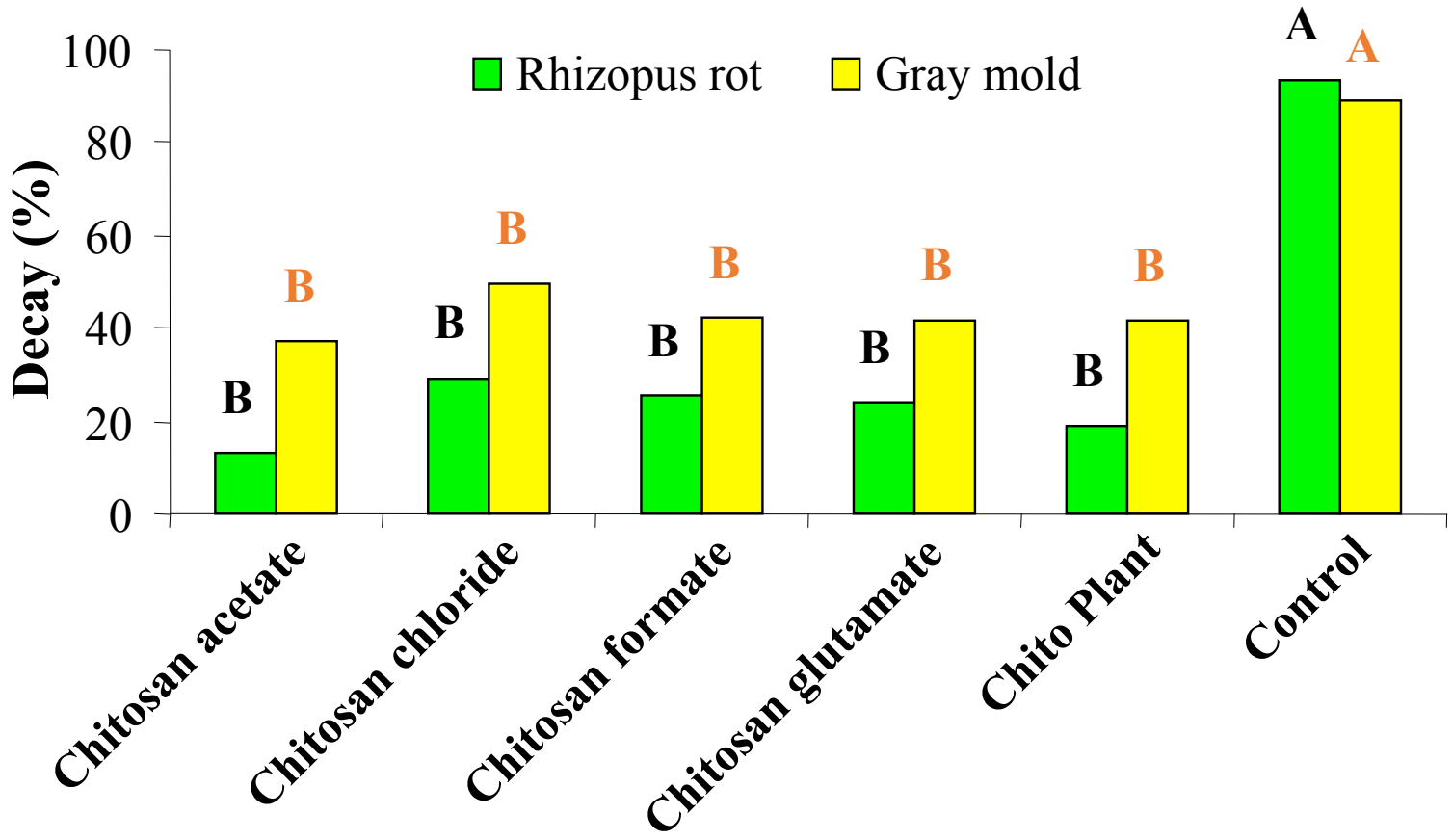
Romanazzi et al., 2009 Phytopathology

Respiration rate of grapes treated with chitosan solutions



Romanazzi et al., 2009 Phytopathology

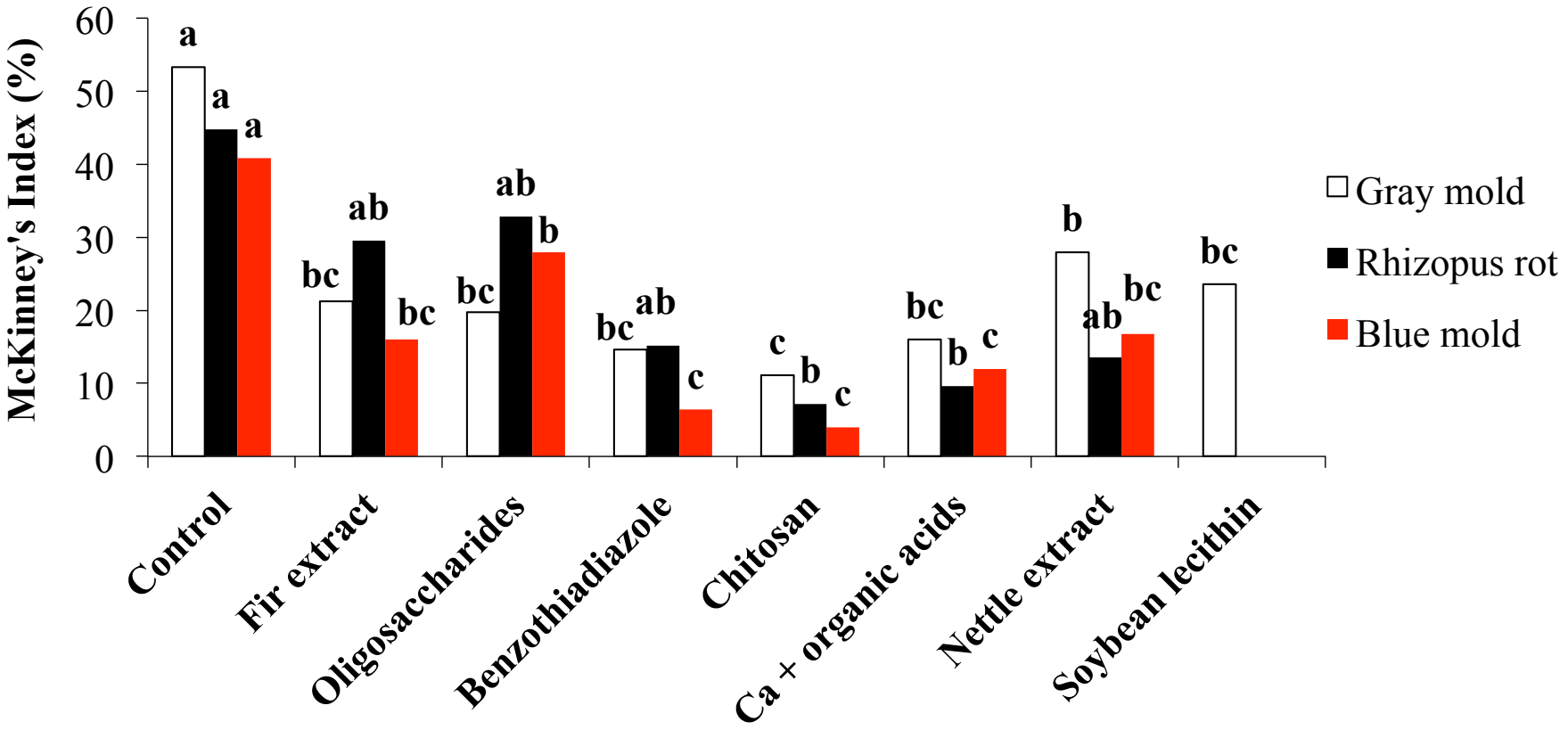
Effectiveness of postharvest chitosan treatment on gray mold and Rhizopus rot of strawberry



Romanazzi et al., 2013 PBT

POSTHARVEST TRIALS ON STRAWBERRY

McKinney's index of gray mold, Rhizopus rot and blue mold recorded on strawberries cv. Camarosa stored for 7 days at 0 ±1 °C and then exposed to 3 days of shelf life



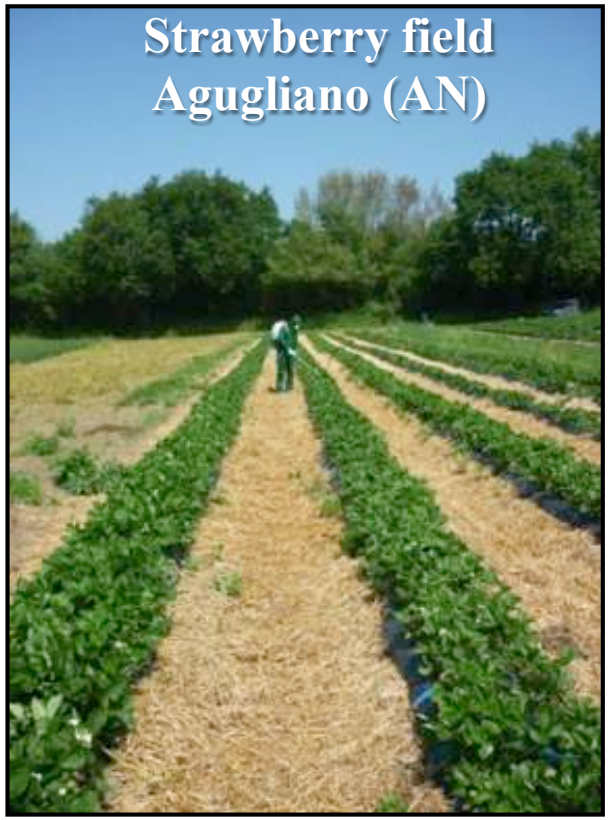
Values with the same letter are not different according to Duncan's MRT (P <0.05)

Romanazzi et al., 2013 PBT

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)



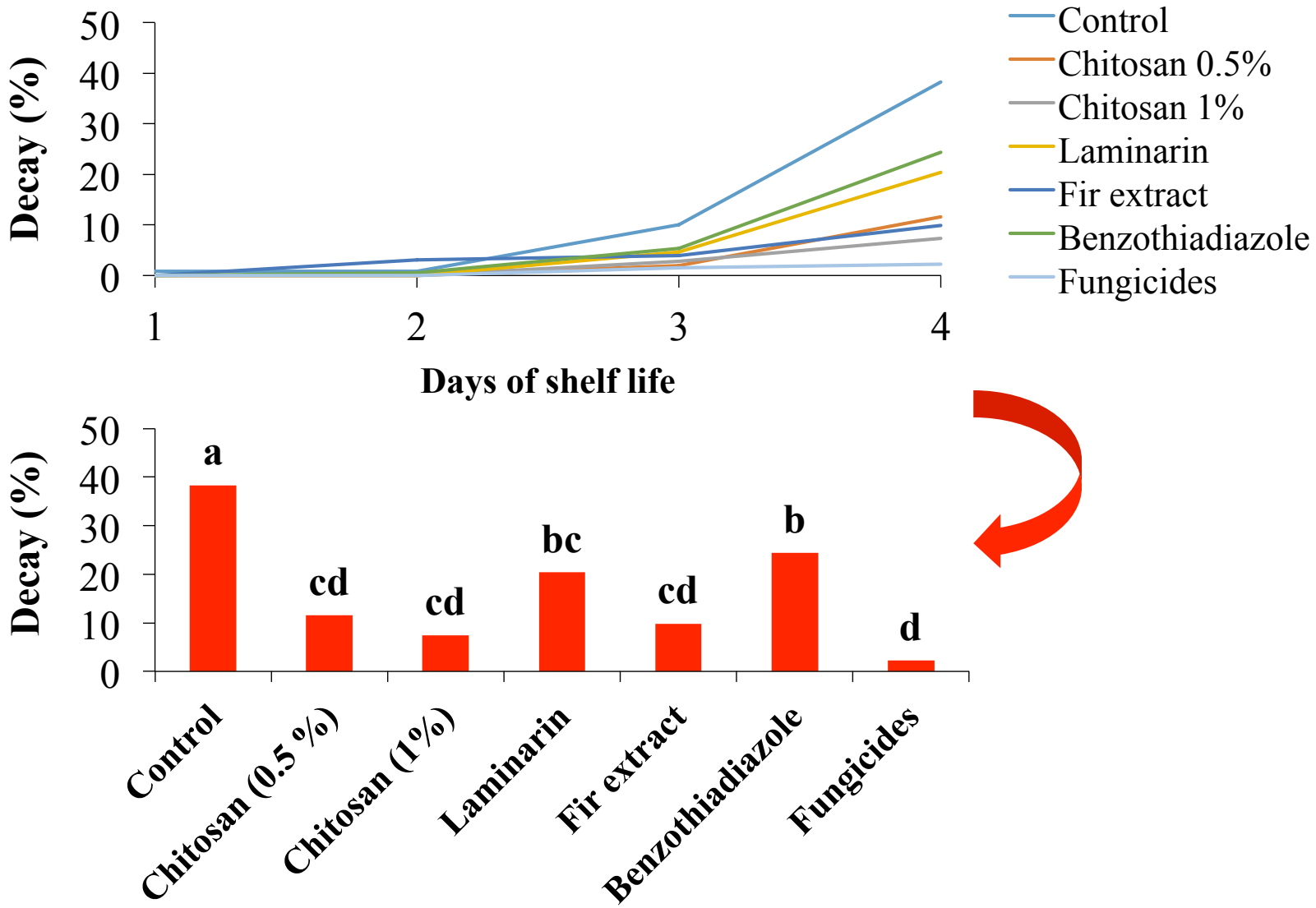
Chitosan (0.5%)	Laminarin
Laminarin	BTH
Fungicides	Fir extract
Control	Chitosan (0.5%)
Chitosan (1%)	Control
Fir extract	Fungicides
BTH	Chitosan (1%)
Laminarin	Fungicides
Fir extract	BTH
Fungicides	Chitosan (1%)
Chitosan (0.5%)	Fir extract
BTH	Control
Chitosan (1%)	Chitosan (0.5%)
Control	Laminarin

Treatment 5 times during season approximately every 5 days:

Flowering **End flowering** **Green fruit** **White fruit** **Red turning fruit**

PREHARVEST TRIALS ON STRAWBERRY

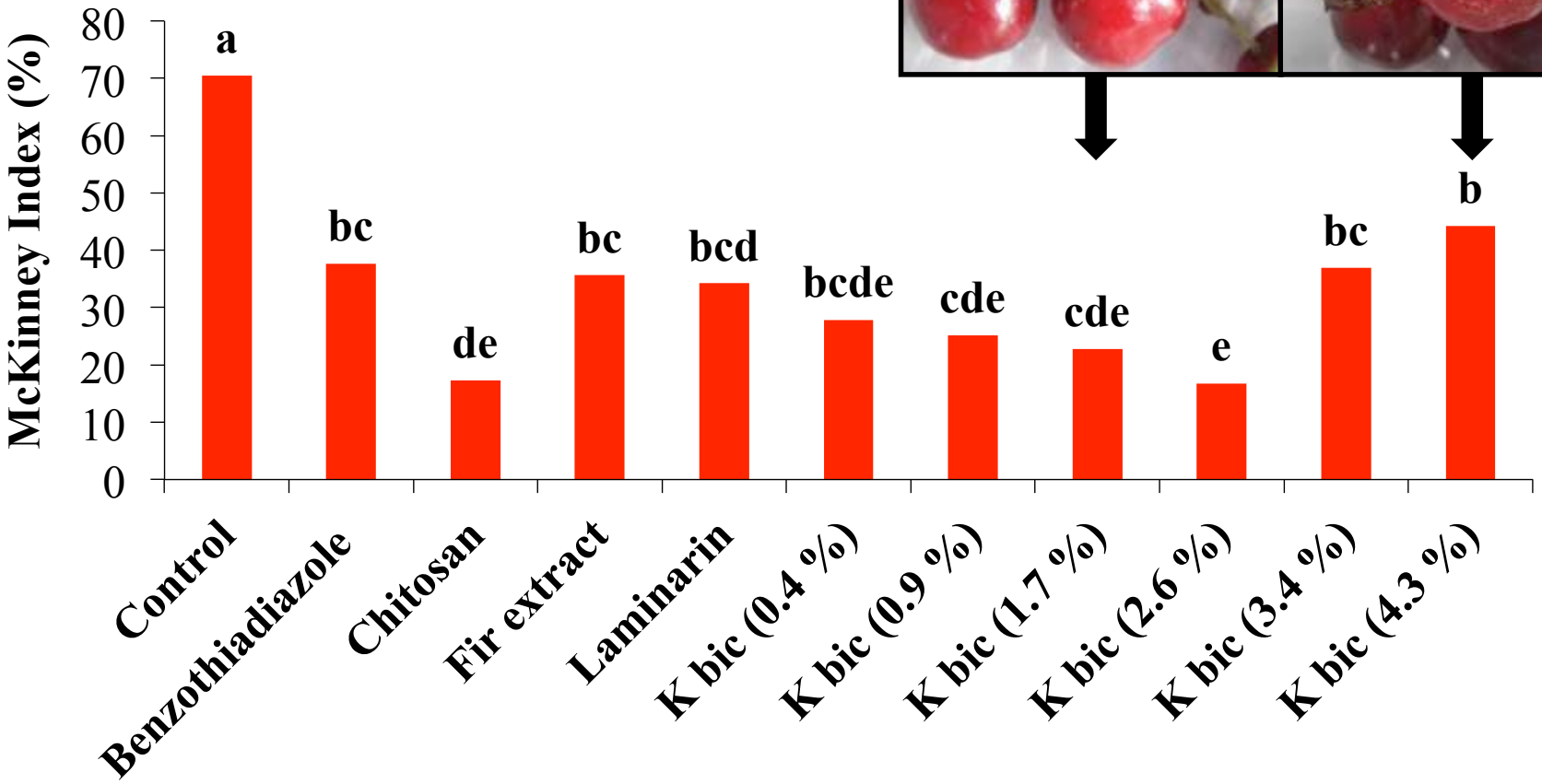
McKinney's Index of rots recorded on strawberries cv. ALBA treated for 5 times during the season, harvested and stored for 7 days at 0 ±1 °C and then exposed to shelf life



Feliziani et al., 2015 Carb Polym

POSTHARVEST TRIALS ON SWEET CHERRY

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

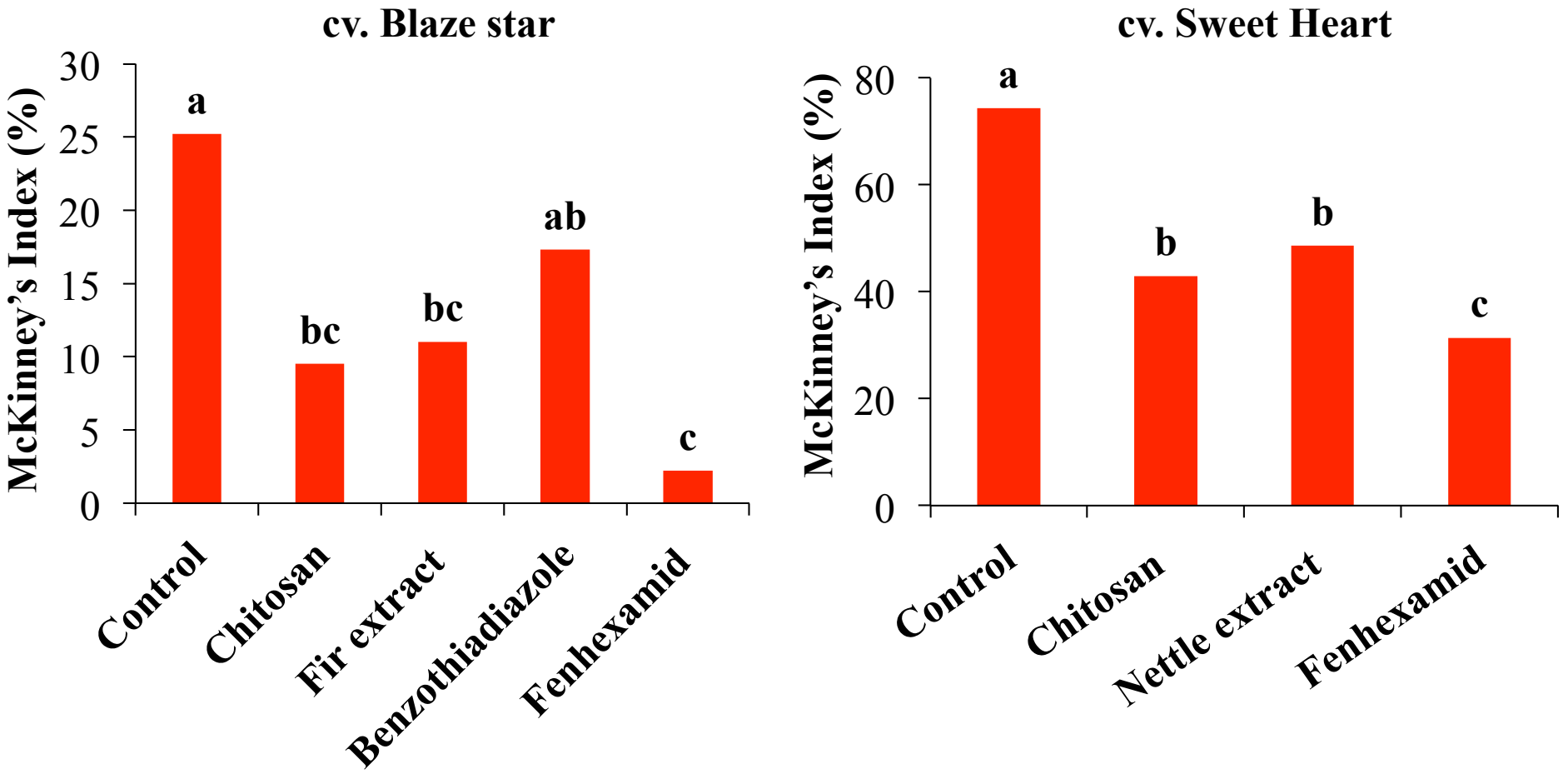


Values with the same letter are not different according Tukey HSD (P <0.05)

Feliziani et al., 2013 PBT

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)

Feliziani et al., 2013 PBT

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:

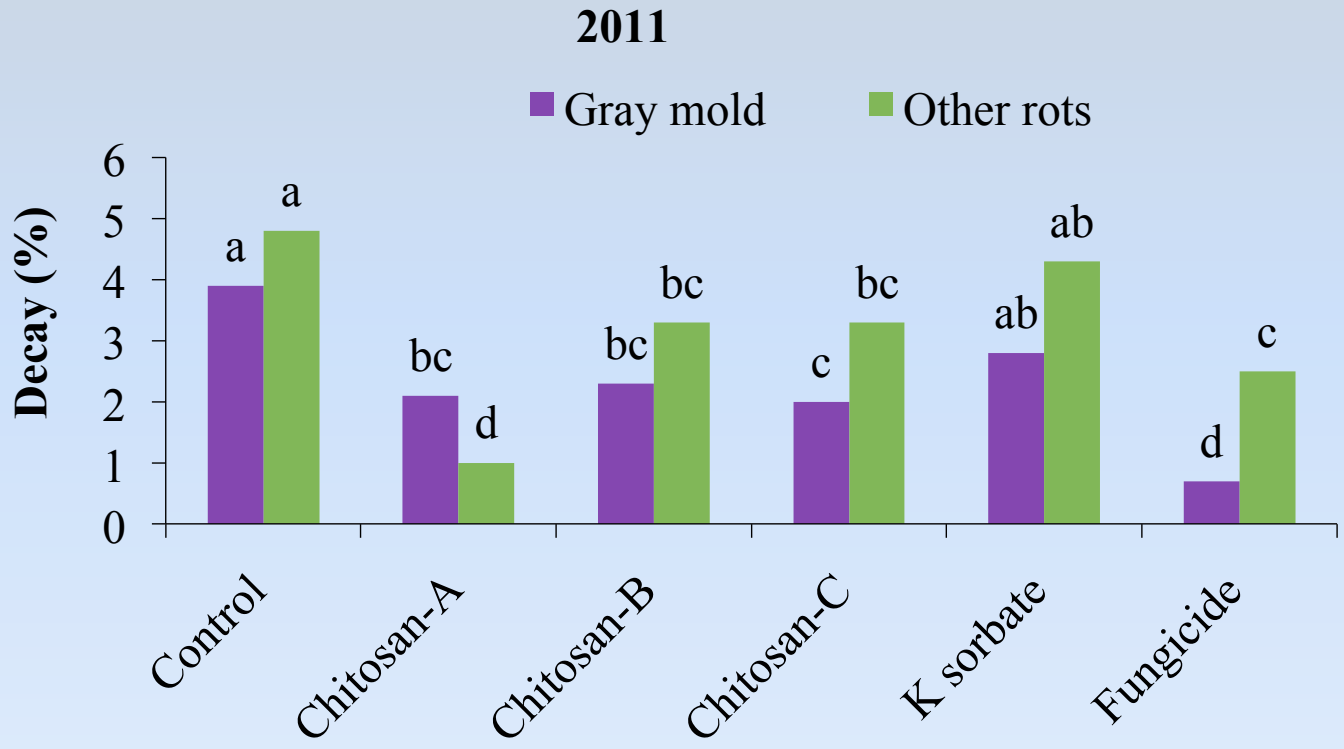
- **Water (control)**
- **Fungicides program**
  (1^ pyrimethanil,
  2^ cyprodinil + fludioxonil,
  3^ pyraclostrobin + boscalid,
  4^ fenhexamid)
- **K sorbate (1%)**
- **Chitosan-A:** OII-Ys
- **Chitosan-B:** Chito Plant
- **Chitosan-C:** Armour-Zen

3 commercial formulations at 1% chitosan

Feliziani et al., 2013 Plant Dis

POSTHARVEST ROTS FROM NATURAL INOCULUM

After 6 weeks of storage at 2°C



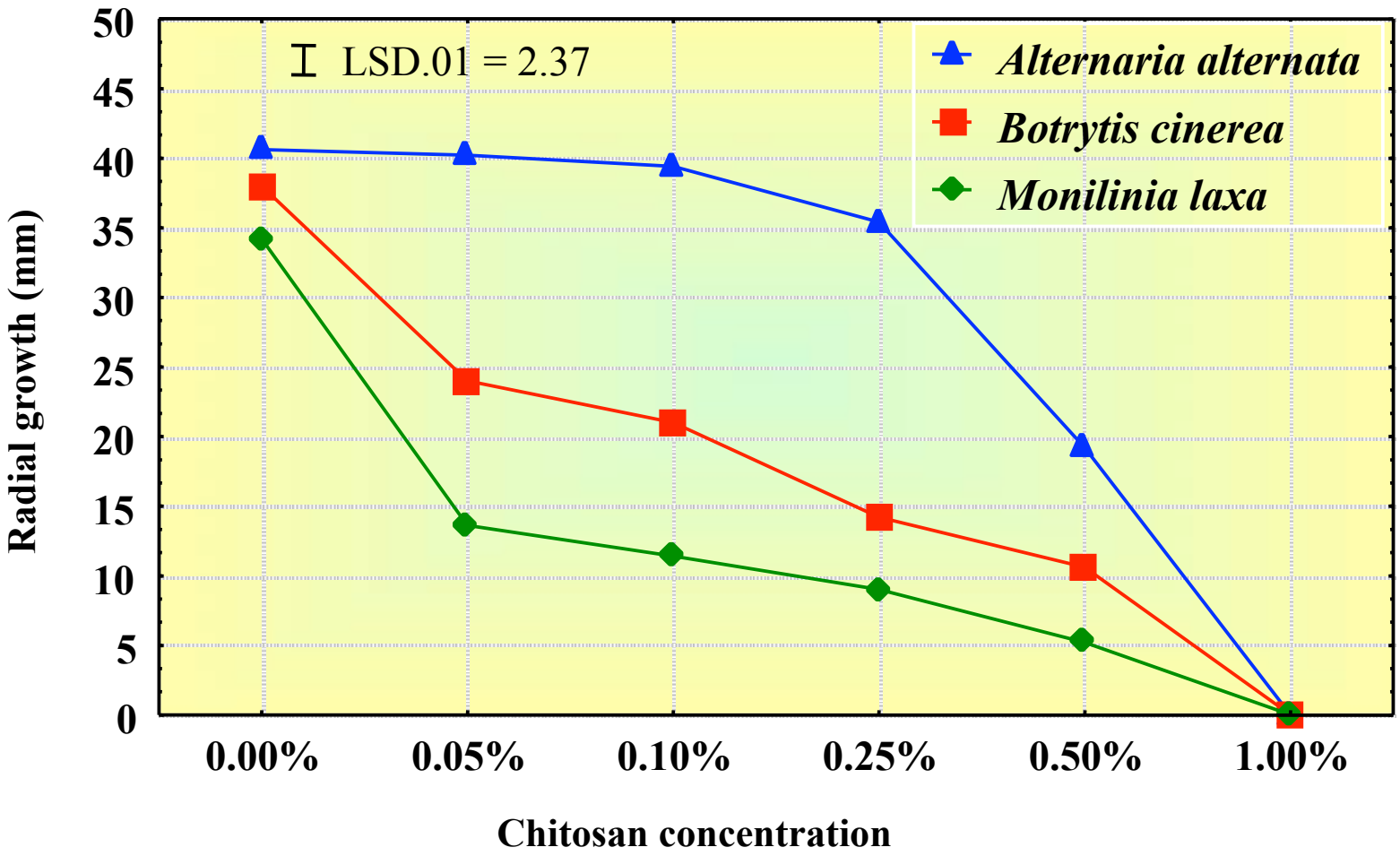
Other rots were caused mainly by *Alternaria* spp. and *Penicillium* spp.

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

Feliziani et al., 2013 Plant Dis

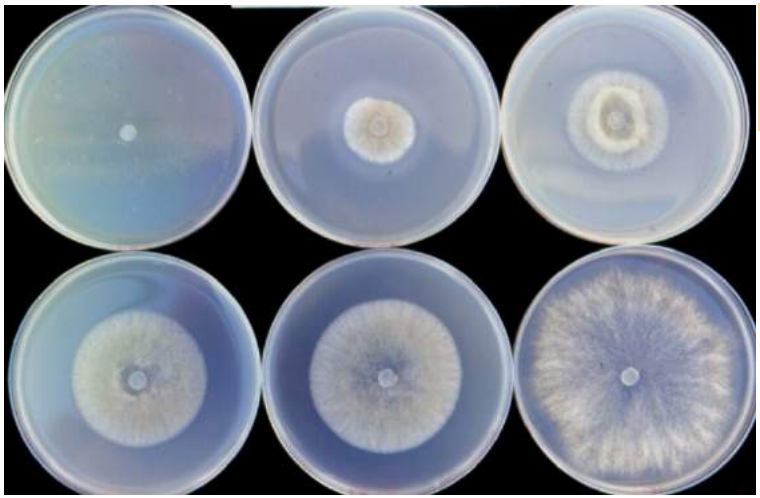
Which are the mechanisms of action of chitosan?

Growth of some decay-causing fungi

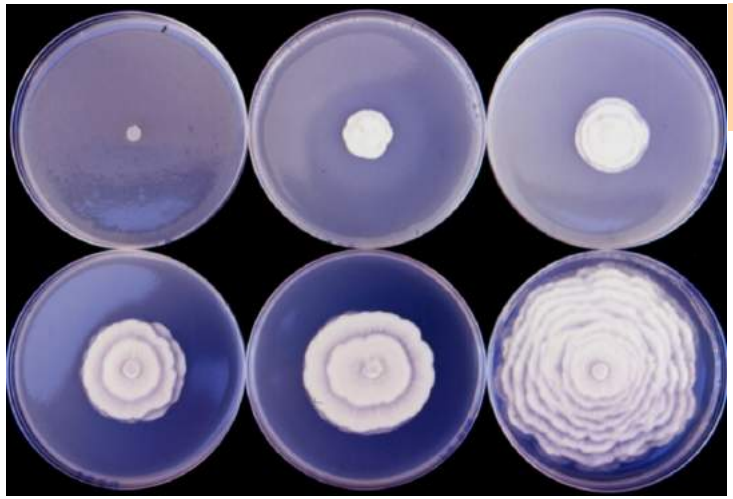


Romanazzi et al., 2001 Chitin Enzymology

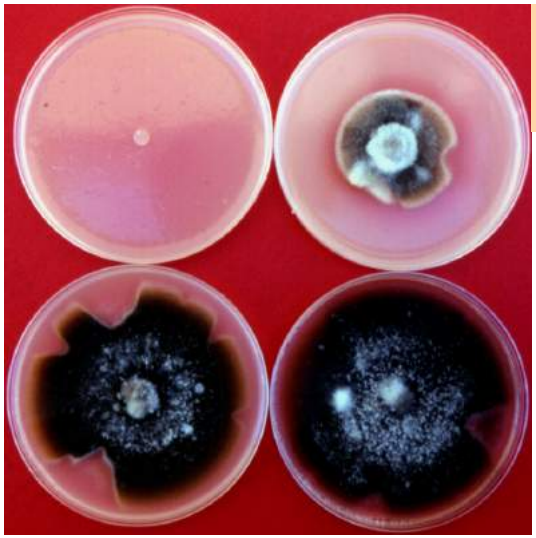
Effect of chitosan on radial growth of *Botrytis cinerea* (A), *Monilinia laxa* (B), and *Alternaria alternata* (C)



A

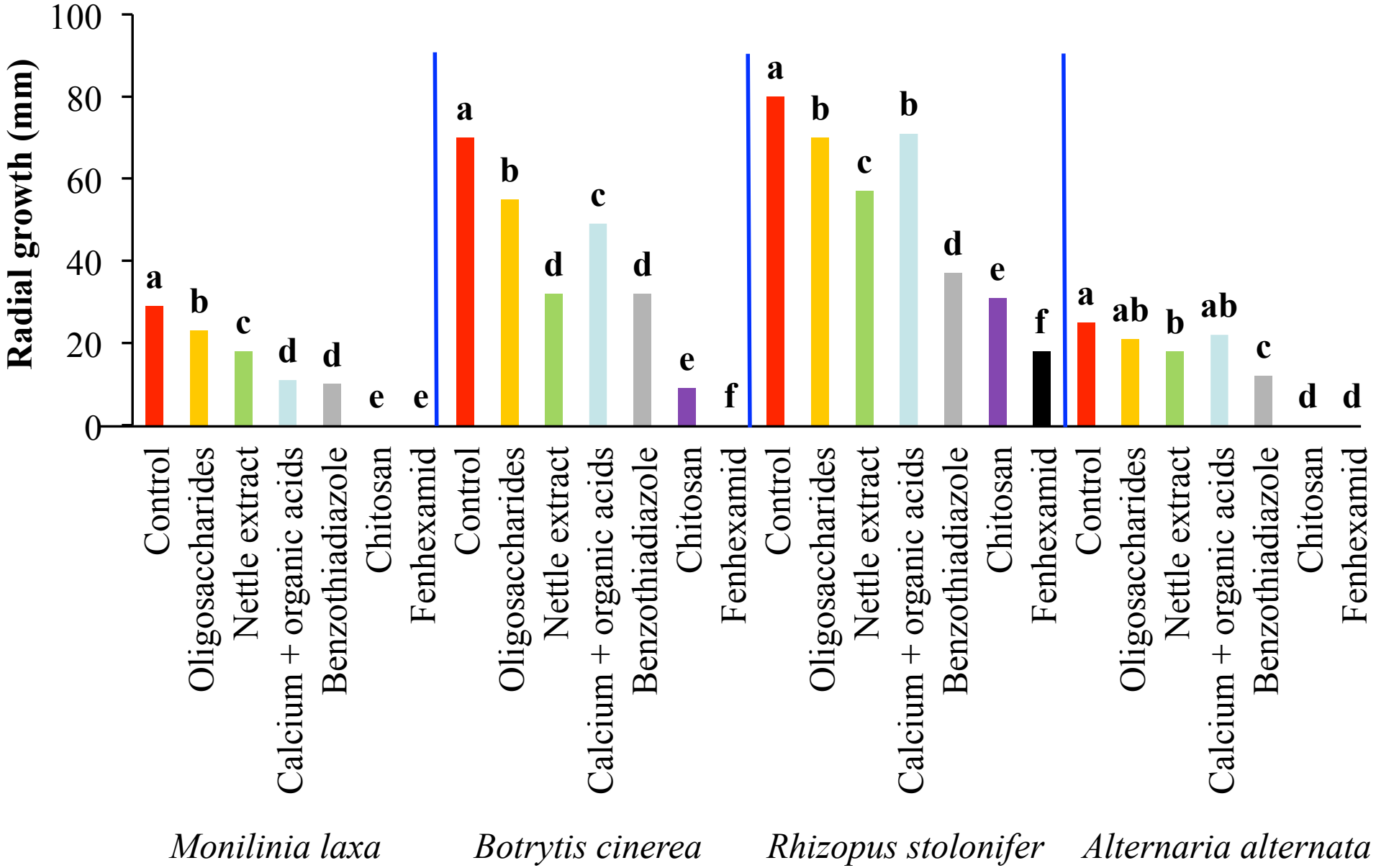


B



C

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



Feliziani et al., 2013 PBT

Antifungal activity of chitosan

Fungus	Infected species	Reference
Botrytis cinerea	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
Rhizopus stolonifer	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
***Penicillium* spp.**	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
***Aspergillus* spp.**	Pear	Cè et al., 2012; Plascencia-Jatomea et al., 2003
***Alternaria* spp.**	Tomato, pear	Sánchez-Domínguez et al., 2011; Meng, et al., 2010
***Cladosporium* spp.**	Litchi fruit, strawberry	Park et al., 2005; Sivakumar et al., 2005
***Colletotrichum* spp.**	Mango, papaya, banana, table grapes, tomato	Zahid et al., 2012; Abd-Alla and Haggar, 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
***Monilinia* spp.**	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)

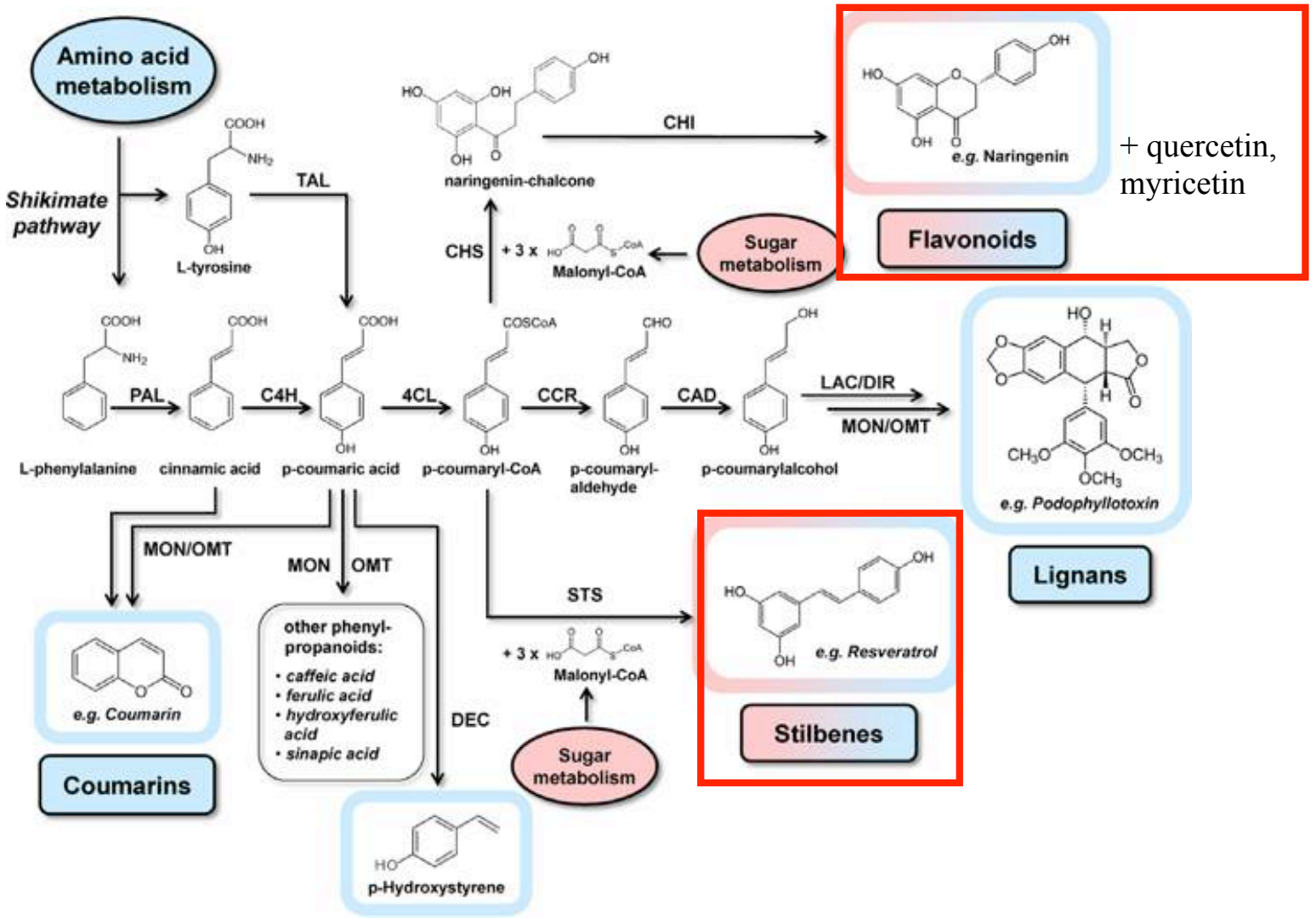
Trans-resveratrol and catechin content of berries treated with chitosan and exposed to UV-C

Treatment	Autumn Black		B36-55	
	Trans-resveratrol	Catechin	*Trans*-resveratrol	Catechin
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 µg/g fresh skin weight)

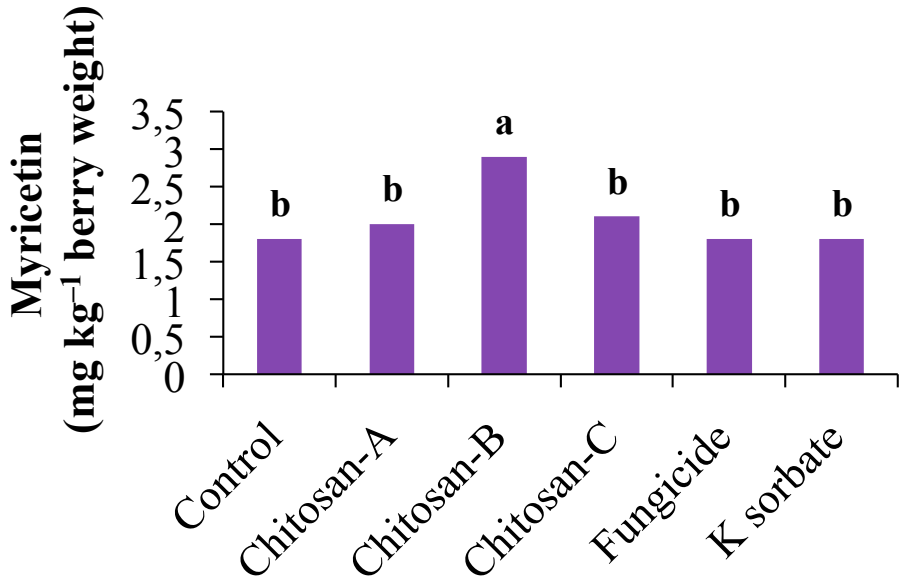
Romanazzi et al., 2006 Plant Dis.

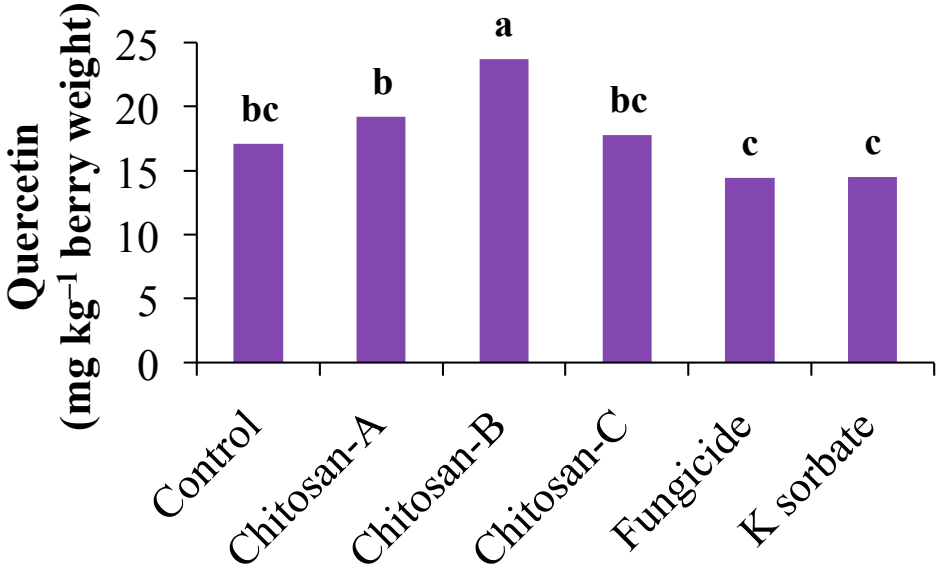
INDUCTION OF RESISTANCE

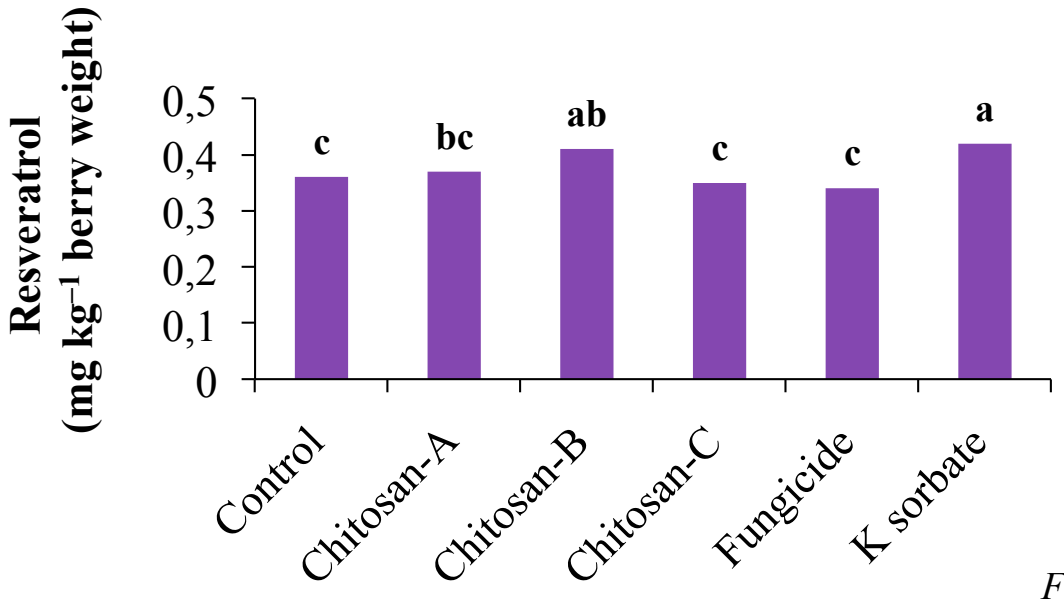


Marienhagen and Bott, 2013 Journal of Biotechnology

INDUCTION OF RESISTANCE



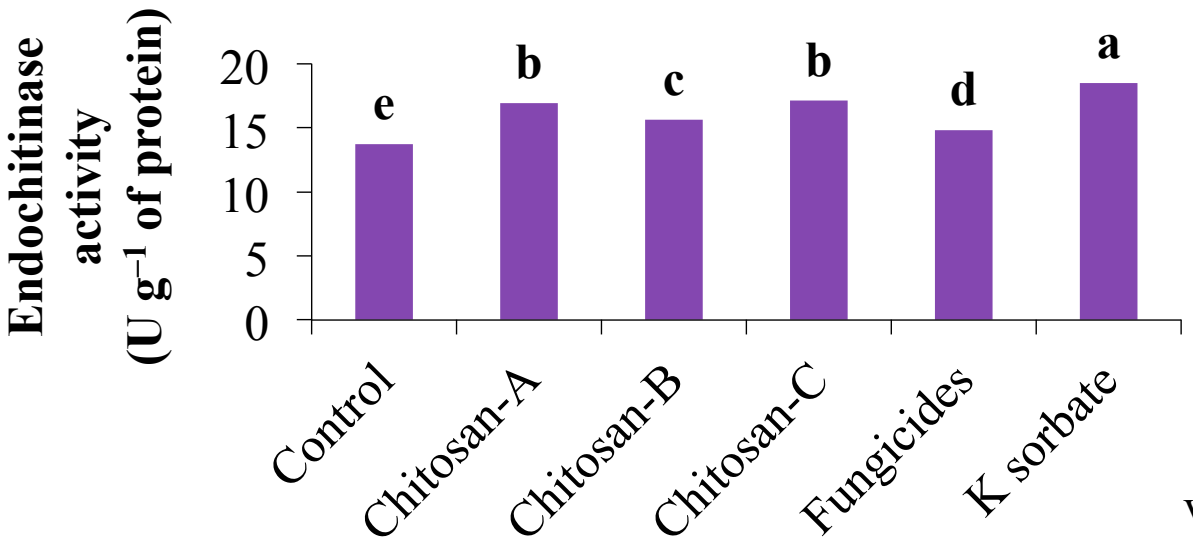


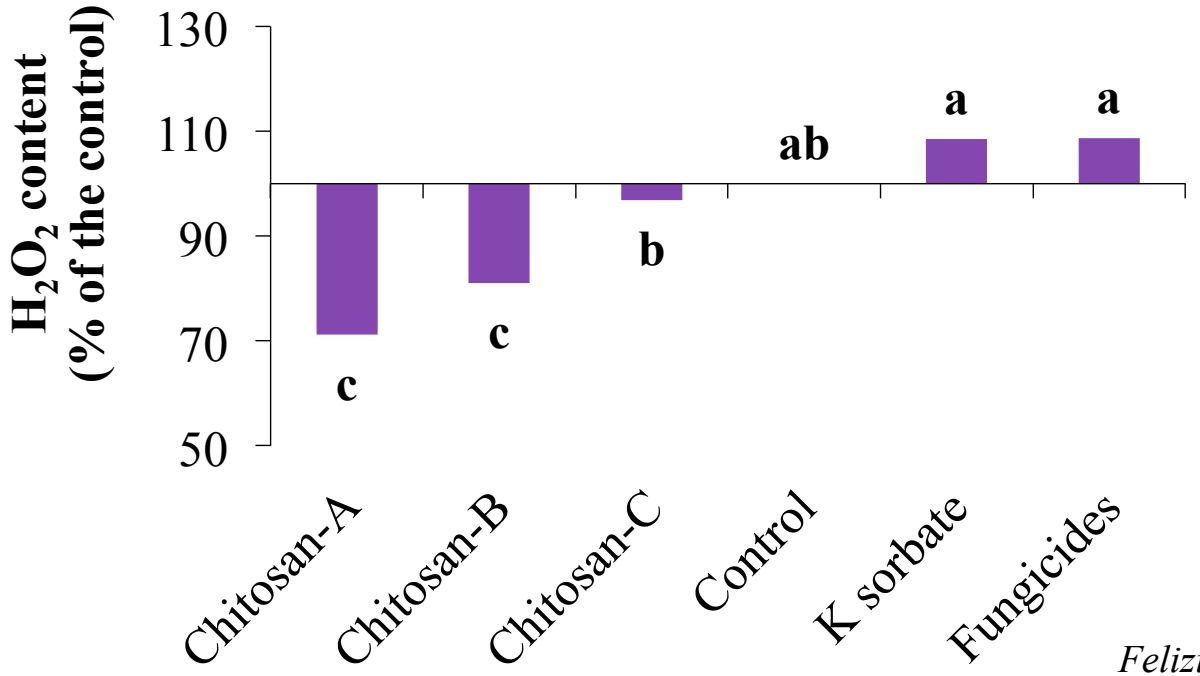


Values with the same letter are not different according Tukey HSD ($P < 0.05$)

Feliziani et al., 2013 Plant Dis

INDUCTION OF RESISTANCE

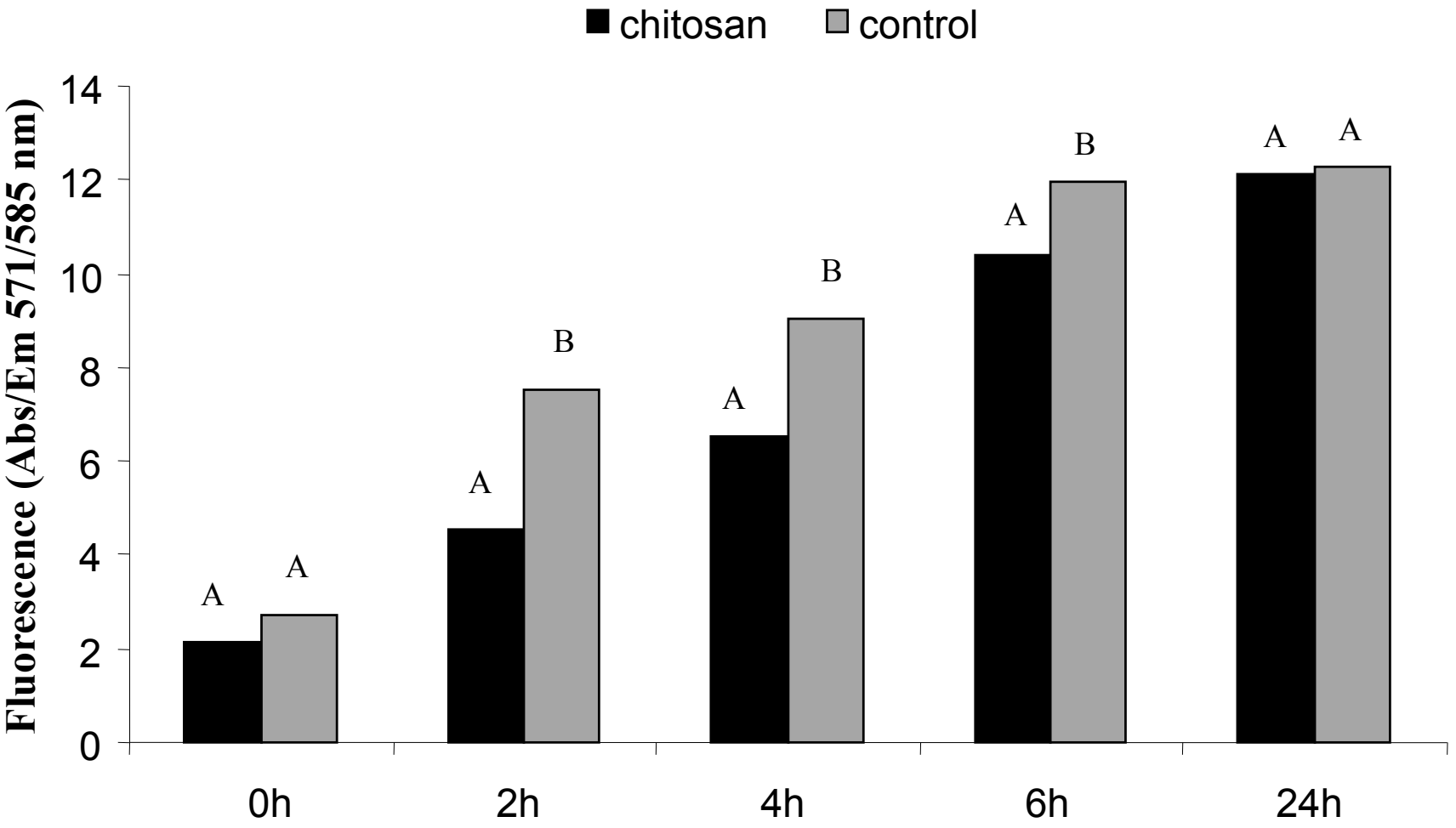




Values with the same letter are not different according Tukey HSD ($P < 0.05$)

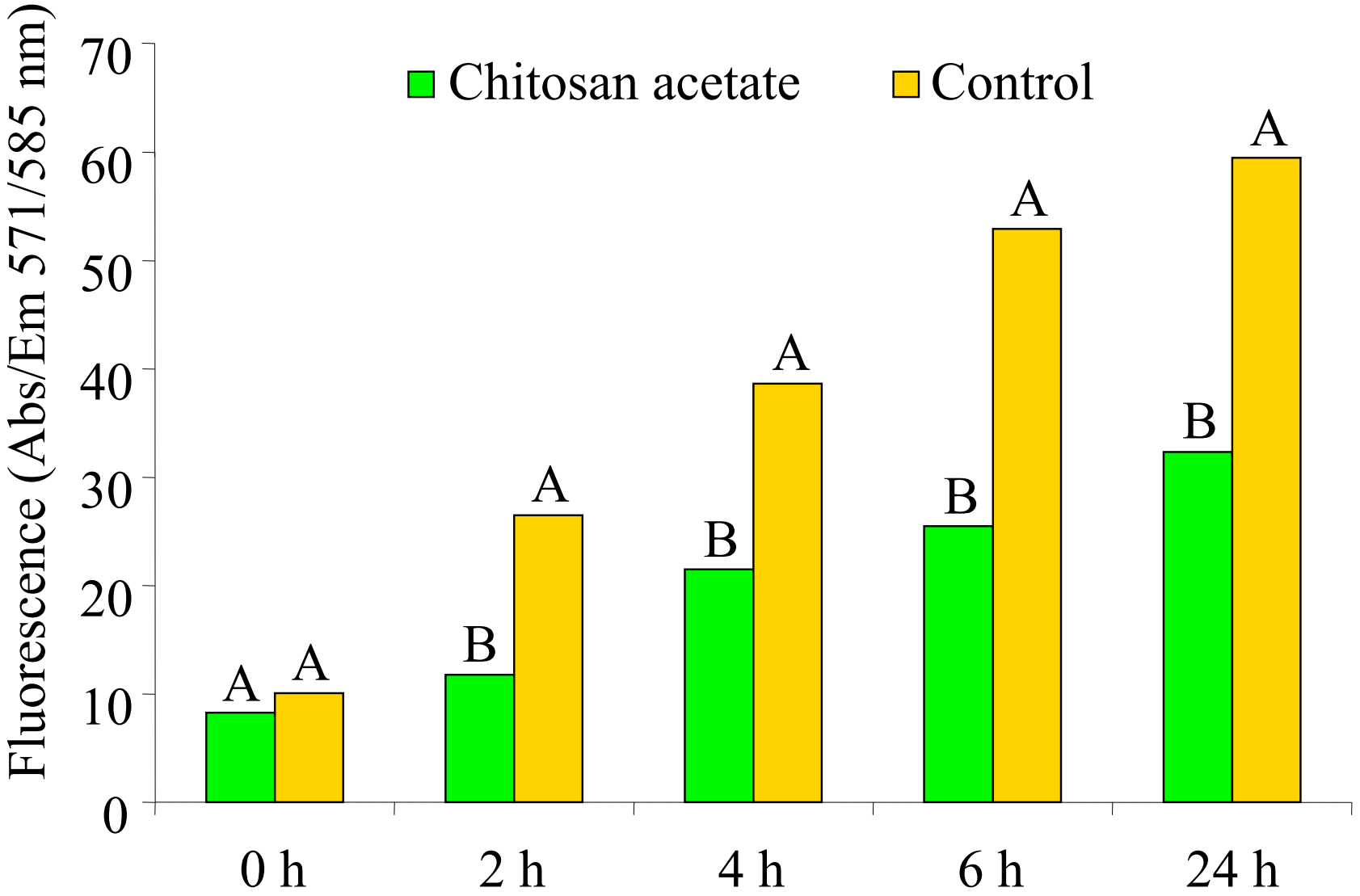
Feliziani et al., 2013 Plant Dis

Hydrogen peroxide production over time of strawberries treated with chitosan



Romanazzi et al., 2007 Proc. COST 924

Hydrogen peroxide production over time of table grape berries treated with chitosan



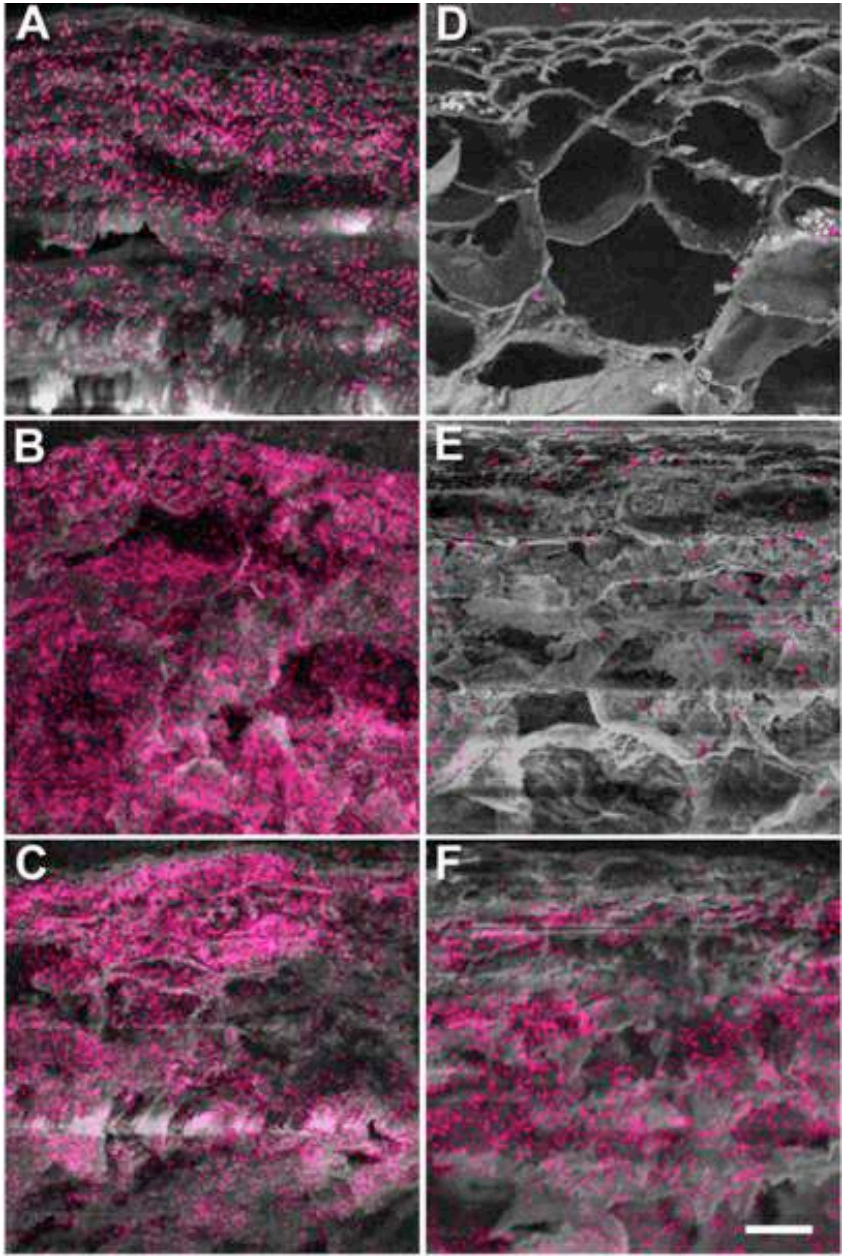
INDUCTION OF RESISTANCE

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

The berries were treated with:

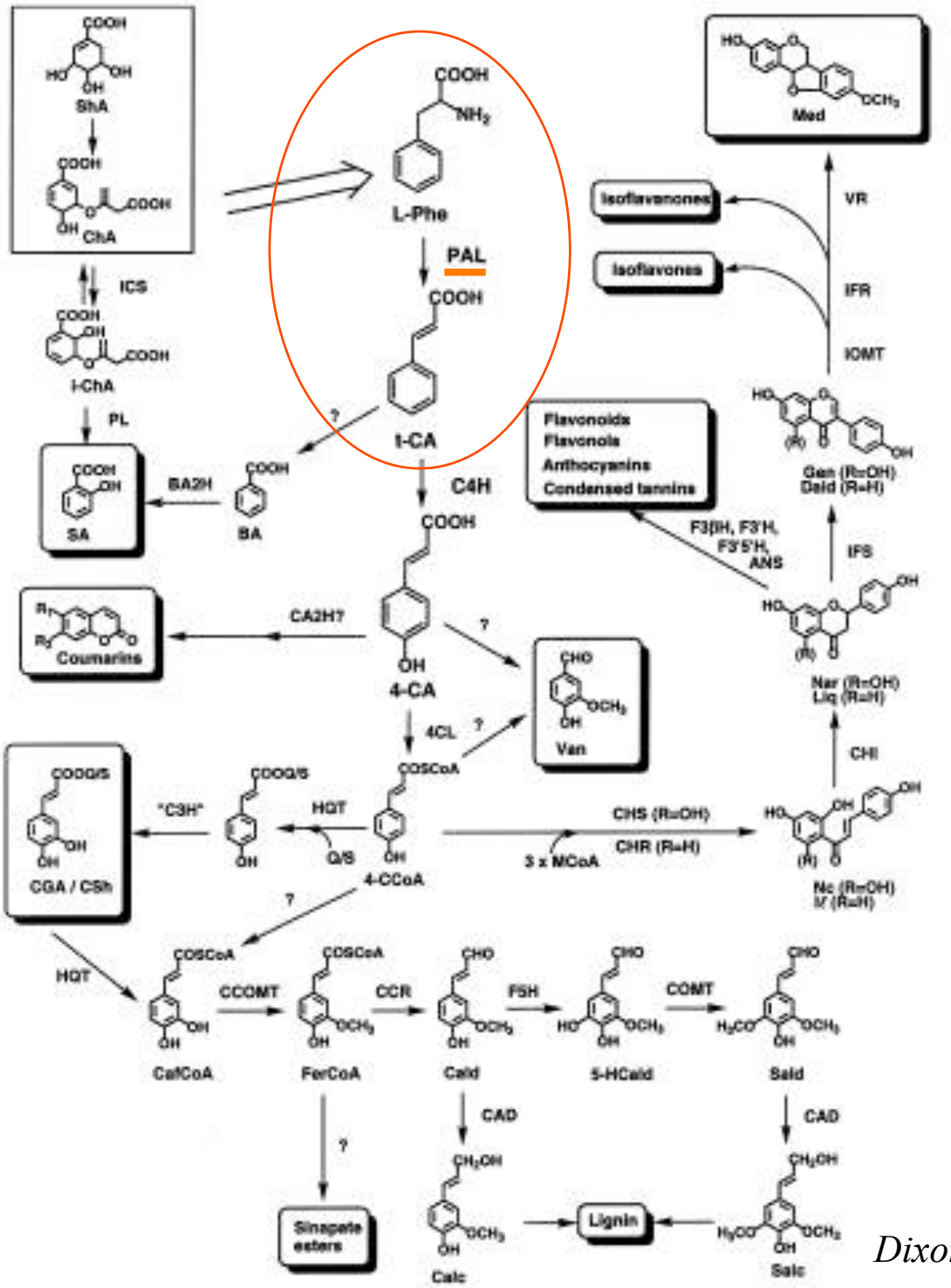
A – Water (control)
B – K sorbate
C – Fungicides
D – Chitosan-A (OII-YS)
E – Chitosan-B (Chito Plant)
F – Chitosan-C (Armour-Zen)

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

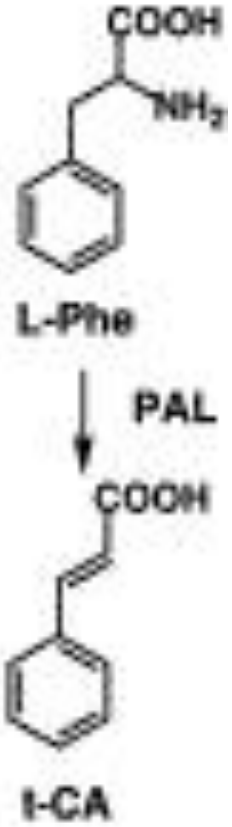


Feliziani et al., 2013 Plant Dis

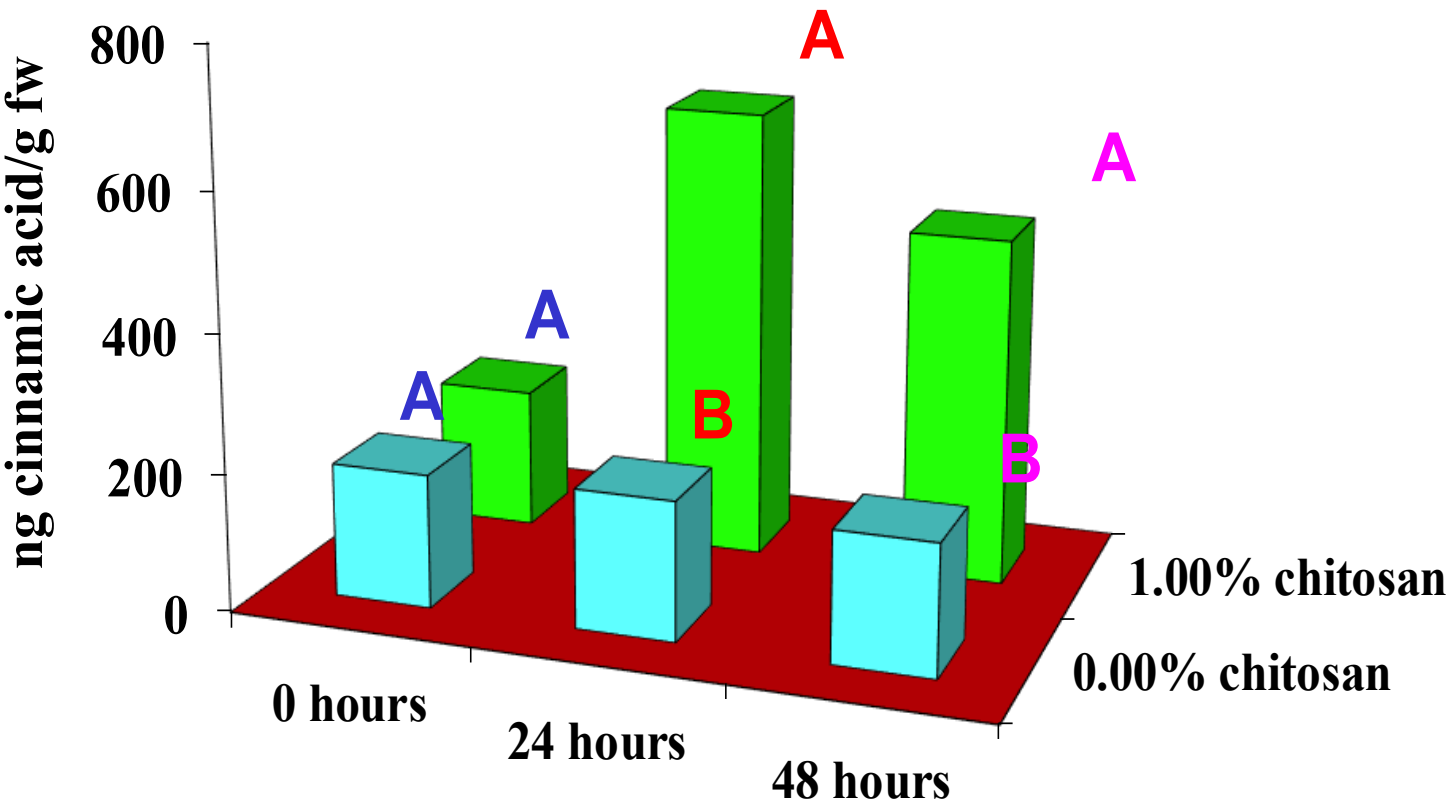
Phenylpropanoid pathway



Dixon et al., 2002. Molecular Plant Pathology

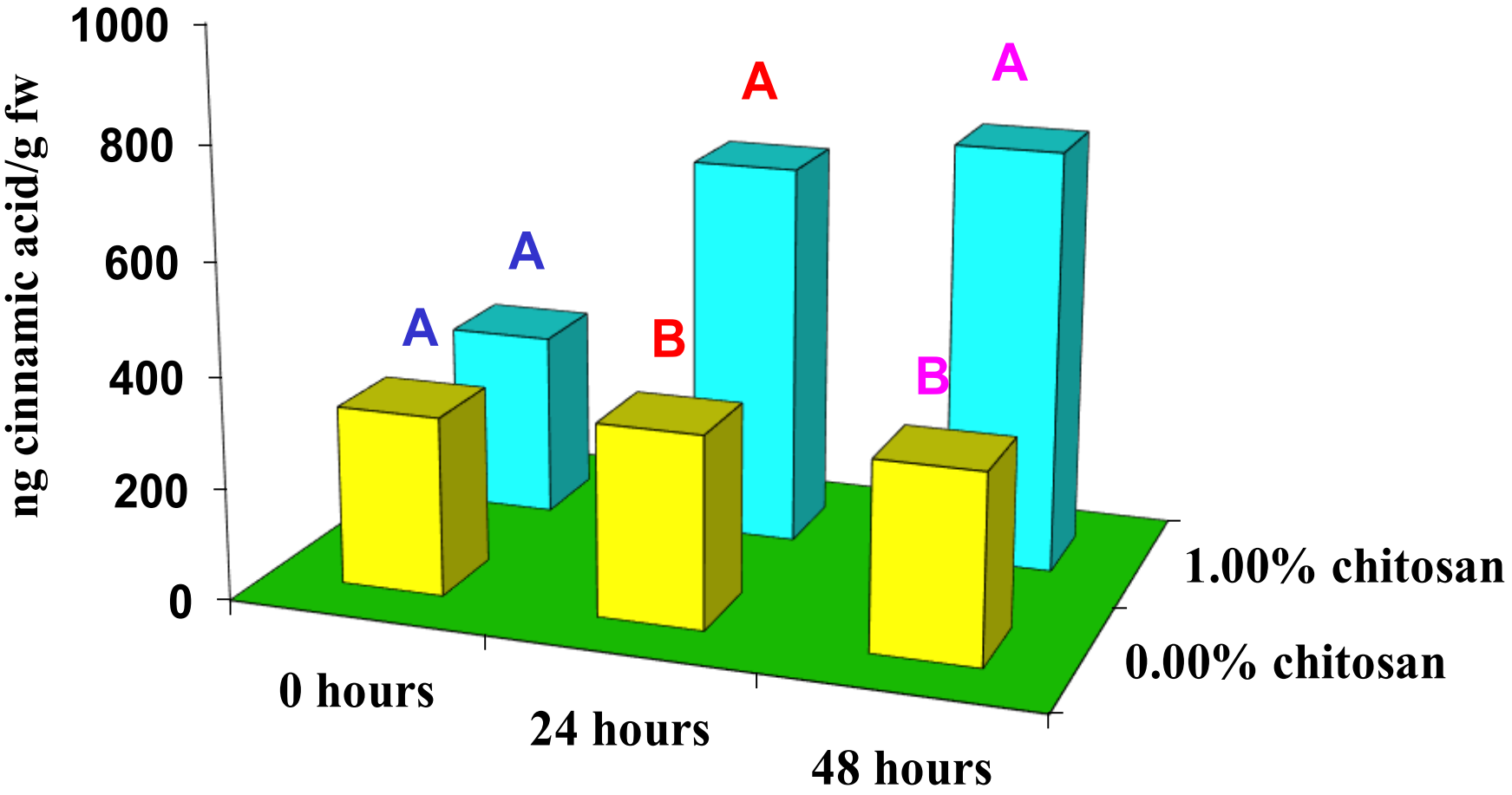


PAL activity on strawberries



Romanazzi et al., 2000 Frutticoltura

PAL activity on table grape berry skin



Romanazzi et al., 2002 J Food Sci

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

CHITOSAN
BTH
COA

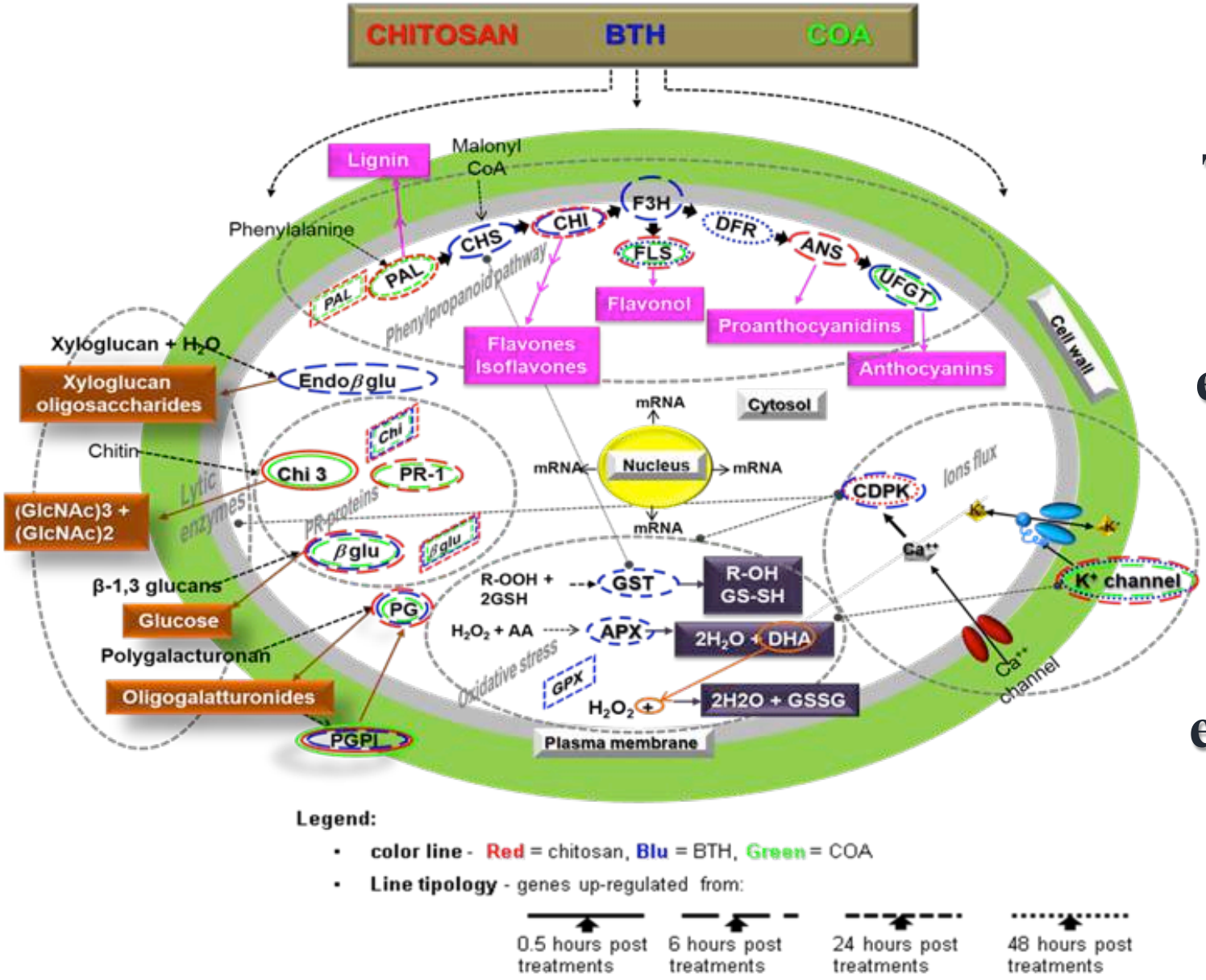
Postharvest treatments

Analysis in RT-qPCR of genes associated to:

- ✓ Ca^{2+} and K^{+} ion fluxes
- ✓ ROS cell responses
- ✓ phenylpropanoid pathway
- ✓ cell-wall degradation
- ✓ PR proteins

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

Landi et al., 2014 JAFC

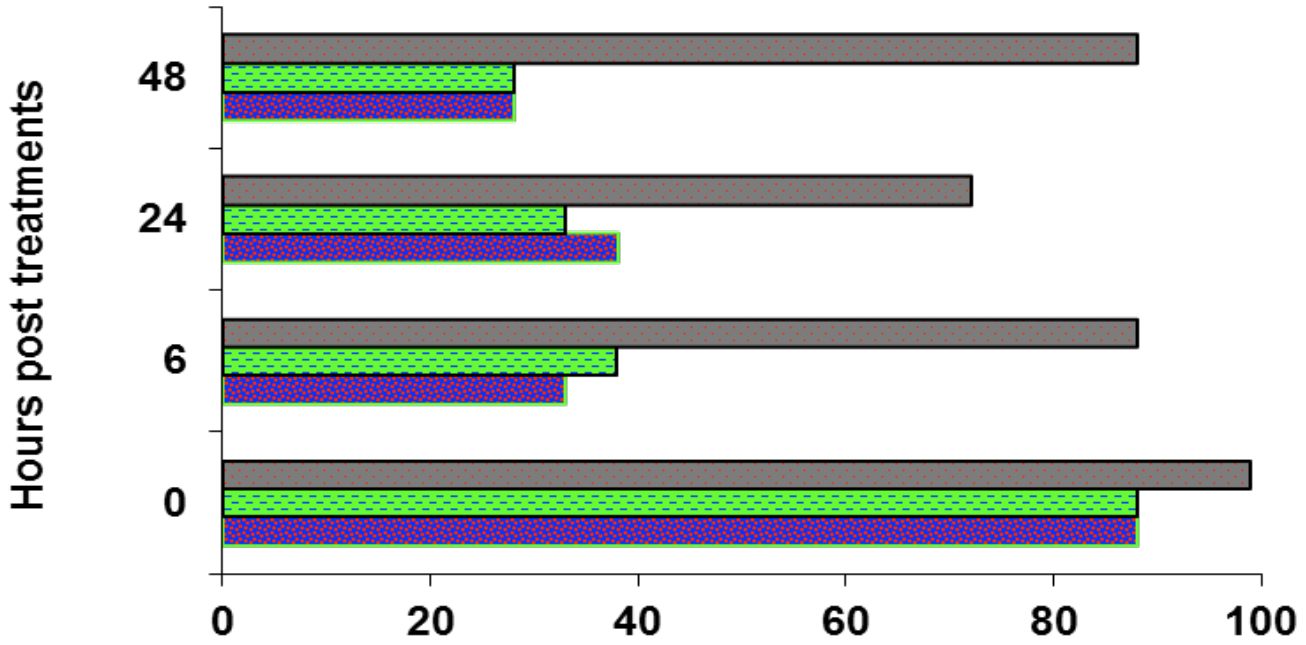


The resistance inducers triggered the expression of a large number of genes that lead to the physiological events involved in plant defense

This proof the induction of resistance in strawberry fruit

Landi et al., 2014 JAFC

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%

Landi et al., 2014 JAFC

Physiological changes induced in the plant tissues by chitosan

- **Higher quantity of phenolic**
  - **Myricetin**
  - **Quercetin**
  - **Resveratrol**
- **Higher activity of enzymes related to mechanism of plant defenses:**
  - **Phenylalanine ammonia-lyase**
  - **Peroxidase**
  - **Polyphenol oxidase**
  - **Superoxide dismutase**
  - **Chitinase**
  - **β-1,3-glucanase**

→ **Induction of plant defense**

- **Lower respiration rate**
- **Reduces weight loss**

→
- **Delay senescence**
- **Prolonged storage and shelf life**

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)

What happens to
chitosan treated fruit?

Chitosan on strawberries soon after dipping

ELSEVIER

Food Microbiology 21 (2004) 703–714

FOOD MICROBIOLOGY

www.elsevier.nl/locate/jnlabr/yfmic

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

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Received 3 September 2003; accepted 19 February 2004

Abstract

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

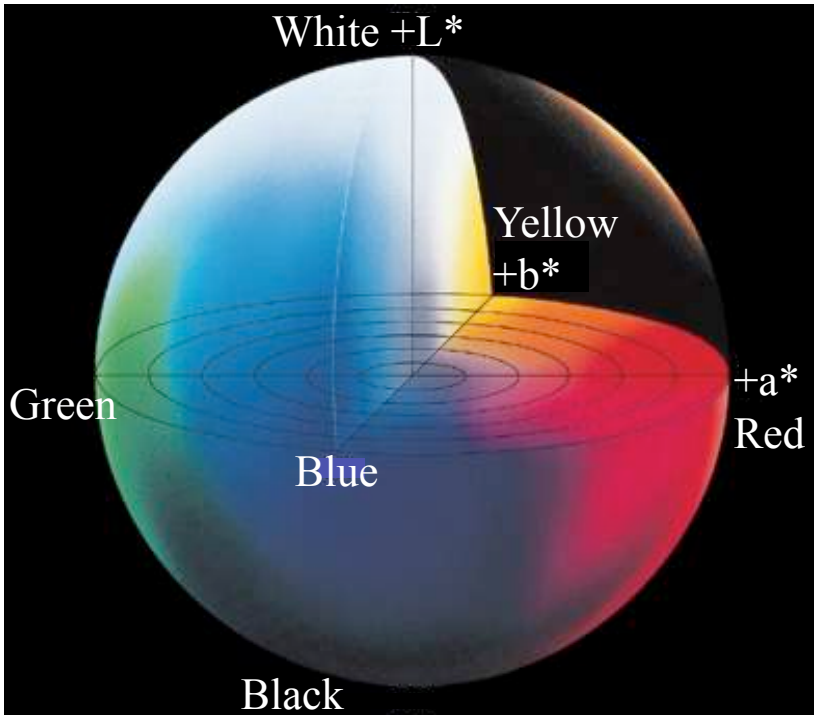
The aim of this study was threefold: (1) the quantification of th 94% and a molecular weight of 43 kDa on different psychrotrophi of the influence of different food components (starch, whey protein investigation of the effects of chitosan coatings on controlling dec lettuce). For the first aim several bacteria and yeast were exposed t Gram-negative bacteria seemed to be very sensitive for the applic positive bacteria was highly variable and that of yeast was interm one of these components added, were inoculated with *Candida la* reached the stationary phase. Starch, whey proteins and NaCl had no influence. For the third aim, the chitosan coating was form solution from which the pH was adjusted to the pH of the product packaged, stored at 7°C and during storage sensorially and micr applicable while on mixed lettuce the chitosan coating was n microbiological load on the chitosan-dipped samples was lower f disappeared after 4 days of storage, while it maintained on the st

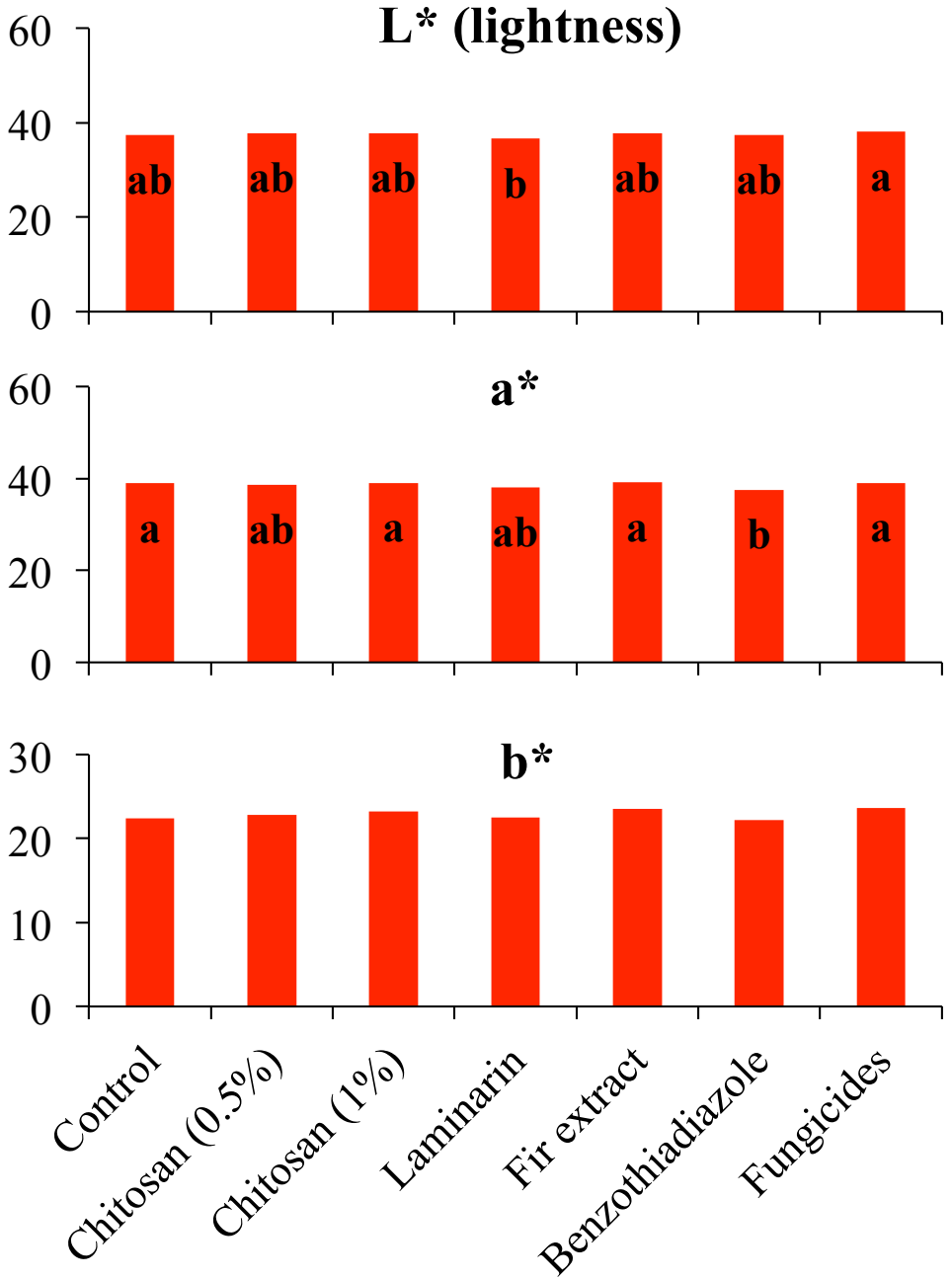
3.3.1.1. Analysis of the sensory quality. Sensorial analysis revealed that on the last day of the experiment (day 12) a small odor aberration appeared for all samples while the taste was still acceptable. The samples treated with chitosan were evaluated with a higher score for texture than the untreated 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 base 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





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

PREHARVEST TRIALS ON STRAWBERRY

FIRMNESS

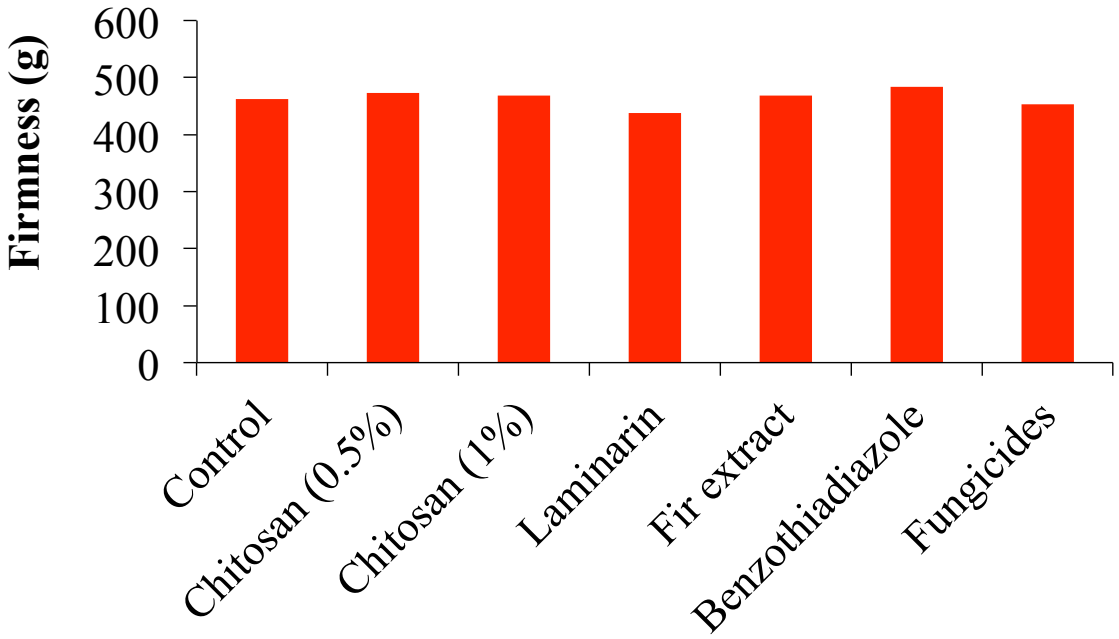


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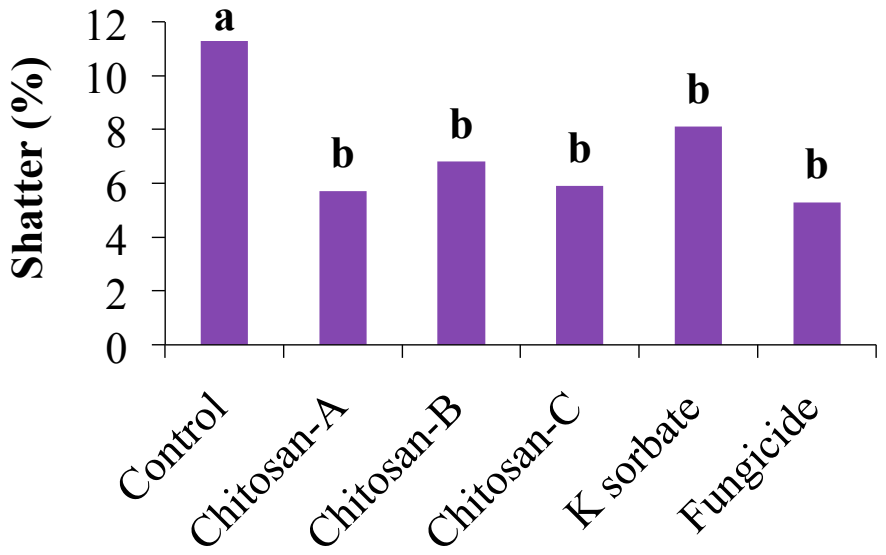
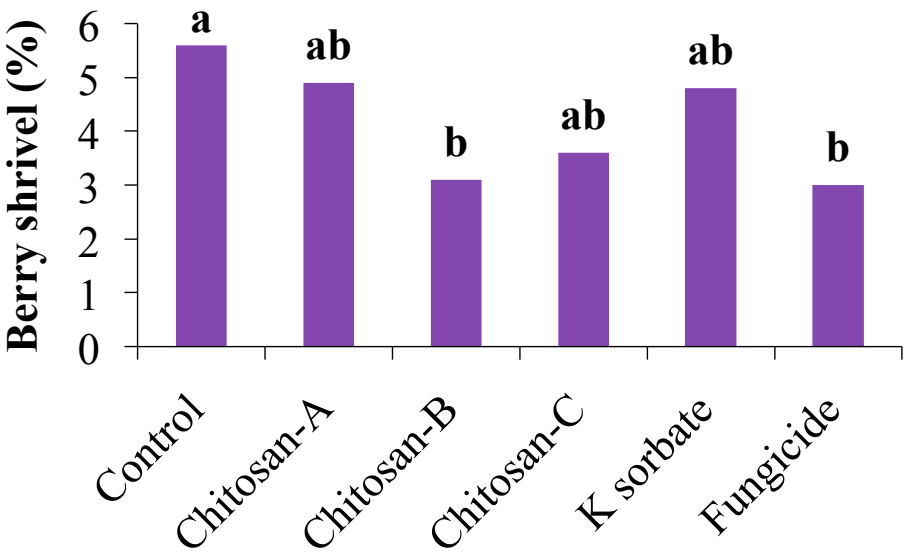
TABLE GRAPE QUALITY PARAMETERS

After 6 weeks of storage at 2°C





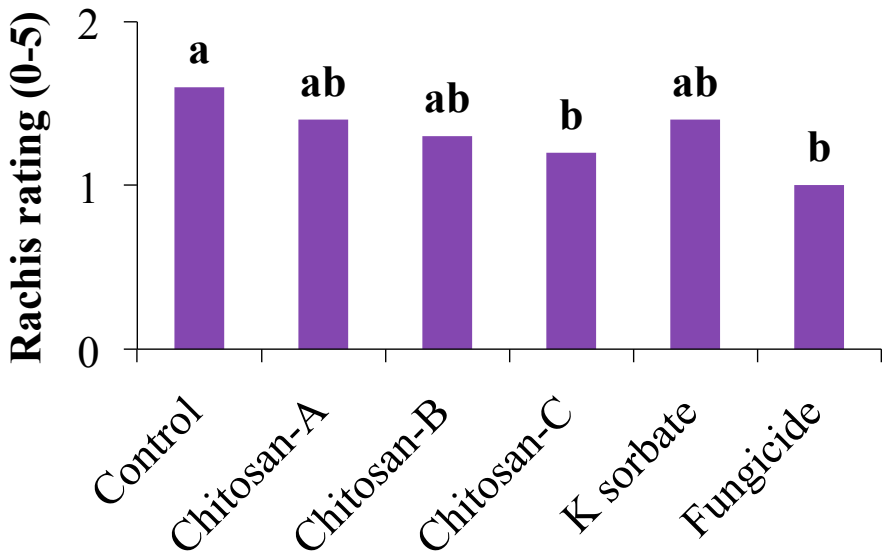


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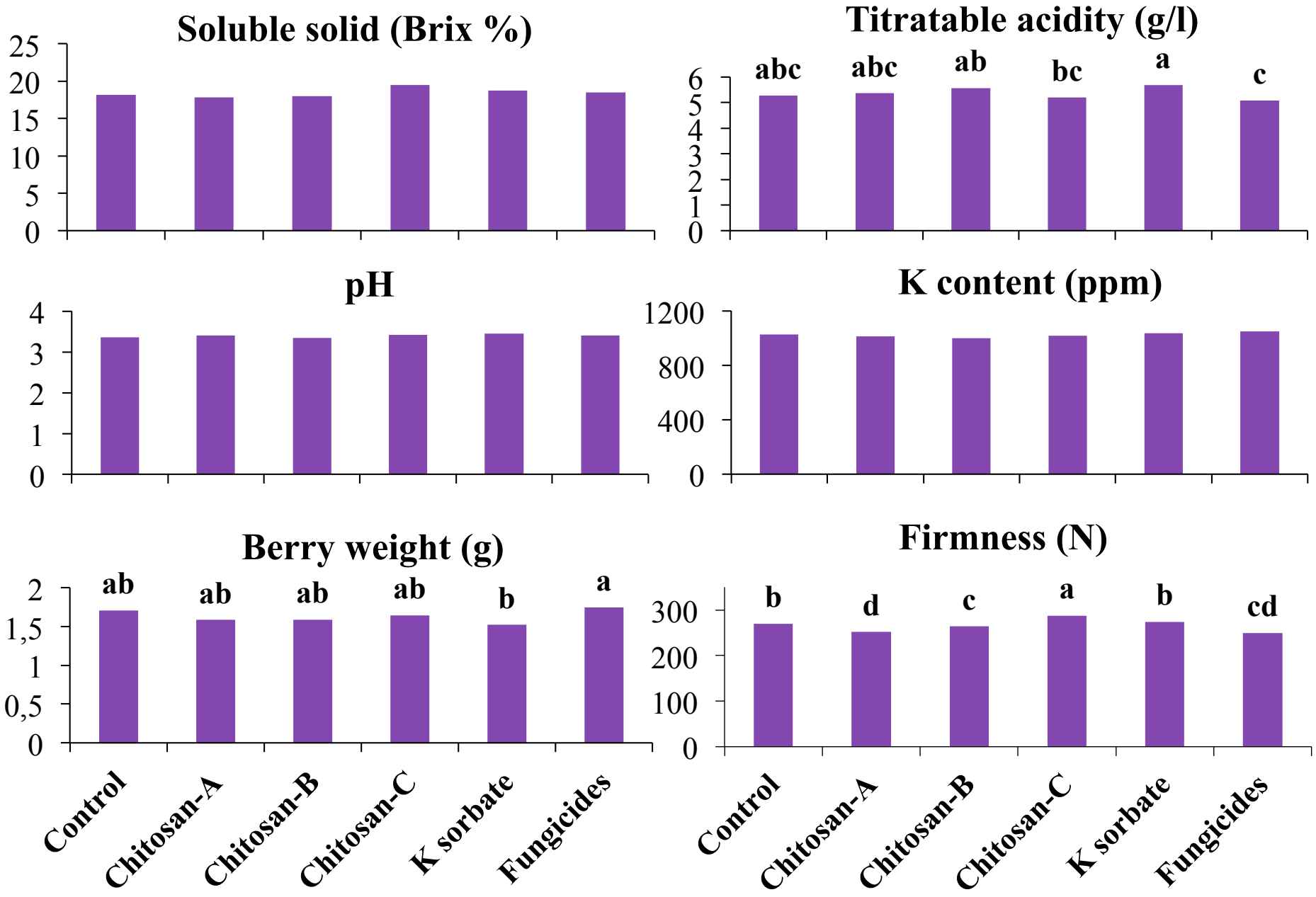
TABLE GRAPE QUALITY PARAMETERS



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CHITOSAN in the Preservation of Agricultural Commodities

EDITORS
Silvia Bautista-Baños
Gianfranco Romanazzi
Antonio Jiménez-Aparicio

Caratteristiche che deve avere un mezzo di lotta alternativo ai fungicidi di sintesi

1. efficacia equivalente o superiore a quella delle pratiche utilizzate al momento
2. assenza di danni o effetti fitotossici sul frutto
3. assenza di alterazioni delle caratteristiche organolettiche
4. assenza di rischi per l'uomo e l'ambiente
5. compatibilità con le pratiche attualmente utilizzate, economicità e facilità di implementazione
6. compatibilità con i principi dell'agricoltura biologica
7. beneficio per chi veicola la tecnologia affinché sia motore dell'innovazione

Romanazzi et al., 2012 PBT


Thanks for your attention
MacFrut 2016 - Rimini, 14 settembre 2016