EUBERRY

INTERNATIONAL BERRY SCHOOL (IBS)

Application of alternatives to synthetic fungicides for the control of postharvest decay of strawberries

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List of some of the most common fungicides to control gray mold

Active ingredient (%)	Trade name	Company	Chemical Group	FRAC code
Boscalid (70)	Cantus	Basf	Pyridine-carboxamides	7
Cyprodinil (37.5) + fluodioxonil (25)	Switch	Syngenta	Anilinopyrimidines + phenylpyrroles	9 + 12
Cyprodinil (75)	Vangard	Syngenta	Anilinopyrimidines	9
Fenhexamid (50)	Elevate/Teldor	Arysta LifeScience/Bayer CropScience	Hydroxyanilides	17
Fenpyrazamine (50)	Prolectus	Sumitomo Chemical Company	Amino-pyrazolinone	17
Fluazinam (40)	Omega	Syngenta	2,6-dinitro-anilines	29
Fluodioxonil (20.4)	Scholar	Syngenta	Phenylpyrroles	12
Fluodioxonil (50)	Geoxe	Syngenta	Phenylpyrroles	12
Fluopyram (50)	Luna Privilege	Bayer CropScience	Pyridinyl-ethyl-benzamides	7
Iprodione (50)	Rovral	Basf	Dicarboximides	2
Pyraclostrobin (12.8) + boscalid (25.2)	Pristine	Basf	QoI fungicides + pyridine-carboxamides	7+11
Pyraclostrobin (6.7) + boscalid (26.7)	Signum	Basf	QoI fungicides + Pyridine-carboxamides	7+11
Pyrimethanil (54.6)	Scala	Bayer CropScience	Anilinopyrimidines	9

Names highlighted in red are registered in Italy

From: Romanazzi and Feliziani, 2014. *Botrytis cinerea*. In: Postharvest Decay (Silvia Bautista-Baños Ed.)

Alternatives to synthetic fungicides for the control of postharvest decay of strawberries

- Natural fungicides
- Biocontrol agents
- Physical means

- Lower risk of fungal resistance
- Minor impact on human health and environment
- Possibility of application close to the harvest

Natural fungicides

ANTIMICROBIAL ACTIVITY

- **ESSENTIAL OILS AND PLANT EXTRACTS**

(Tripathi and Dubey, 2004; Antunes and Cavaco, 2010)

- **SALTS**

Bicarbonates, carbonates, chlorides

(Nigro et al., 2006; Khamis and Roberto, 2014)

- **DECONTAMINANS AGENTS**

Ethanol, acetic acid, hydrogen peroxide, ozone

(Romanazzi et al., 2007; Venditti et al., 2008; Cerioni et al., 2012; Feliziani et al., 2014)

RESISTANCE INDUCERS

- **CHITOSAN**

(Bautista Baños et al., 2006; Romanazzi et al., 2014)

- **BTH (Acibenzolar-S-methyl)**

(Terry and Joyce, 2004; Bi et al., 2007; Feliziani et al., 2013; Romanazzi et al., 2013)

- **BABA (β-Aminobutyric acid)**

(Jakab et al., 2001; Cohen et al., 2002)

Biological agents

ANTAGONIST MICROORGANISMS

(Janisiewicz and Korsten 2002; Sharma et al., 2009; Jamalizadeh et al., 2011; Nunes, 2012

- **COMPETITION FOR SPACE AND NUTRIENTS**
- **ANTIBIOSIS/PARASITISM**
- **INDUCTION OF RESISTANCE**

Aspire (*Candida oleophila*) and Bio-Save 110 (*Pseudomonas syringae*) are registered for postharvest application in the US

Fruit	Pathogen	Antagonist
Apple	*Botrytis cinerea*	*Candida oleophila*
Citrus	*Penicillium italicum*	*Pseudomonas syringae*
Apple	*Penicillium expansum*	*Cryptococcus laurentii*
Apple and citrus	*Penicillium digitatum*; *Botrytis cinerea*	*Candida satoiana*
Strawberry	*Botrytis cinerea*	*Trichoderma* spp.
Apple and peach	*Colletotrichum* spp.; *Penicillium digitatum*	*Muscodor albus*
Citrus	*Penicillium italicum*; *Penicillium digitatum*	*Bacillus subtilis*

Physical means

- **TEMPERATURE**

refrigeration, treatments with warm water

(Fan et al., 2008; Smilanick et al., 2008)

- **RADIATIONS**

UV-C, 254 nm

(Nigro et al., 1996; Romanazzi et al., 2006; Waje et al., 2008)

- **MODIFIED/CONTROLLED AMTOSPHERE**

low O_2, high CO_2, N_3, O_3

(Jayas and Jeyamkondan, 2002)

- **PRESSURE**

hypobaric or hyperbaric

(Romanazzi et al., 2001, 2003; Jiao et al., 2012; Hashmi et al., 2013)

CHITOSAN

Antifungal activities

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 et al., 2014. Critical Reviews in Food Science and Nutrition, 55: in press.

CHITOSAN

Applications on fruit and vegetables

Produce	Decay	Preharvest applications	Postharvest applications	References
Strawberry	Gray mold, Rhizopus rot, blue mold, Cladosporium rot	X	X	Romanazzi et al., 2013; 2000; Perdones et al., 2012; Vu et al., 2011; Mazaro et al., 2008; Hernández-Muñoz et al., 2006; 2008; Vargas et al., 2006; Park et al., 2005; Han et al., 2004; Reddy et al., 2000; Zhang and Quantick, 1998; El Ghaouth et al., 1991, 1992
Table grape	Gray mold, blue mold	X	X	Feliziani et al., 2013; Romanazzi et al., 2009; 2007; 2006; 2002; Meng and Tian, 2010; 2009; Xu et al., 2007
Sweet cherry	Brown rot	X	X	Feliziani et al., 2013; Romanazzi et al., 2003; 1999
Raspberry, blueberry	Gray mold, Rhizopus rot		X	Duan et al., 2011; Han et al., 2004; Zhang and Quantick, 1998
Apple	Blue mold, gray mold		X	Shao et al., 2012; Yu et al., 2007; De Capdeville et al., 2002; El Ghaouth et al., 2000
Pear	Blue mold		X	Yu et al., 2012
Peach	Brown rot		X	Casals et al., 2012; Li and Yu, 2000
Citrus fruit	Blue mold, black spot disease	X	X	Cháfer et al., 2012; Canale Rappussi et al., 2009; 2011; Chien and Chou, 2006; Fornes et al., 2005

Romanazzi et al., 2014. Critical Reviews in Food Science and Nutrition, 55: in press.

CHITOSAN

Physiological changes induced in the plant tissues:

- **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 et al., 2014. Critical Reviews in Food Science and Nutrition, 55: in press.

BTH

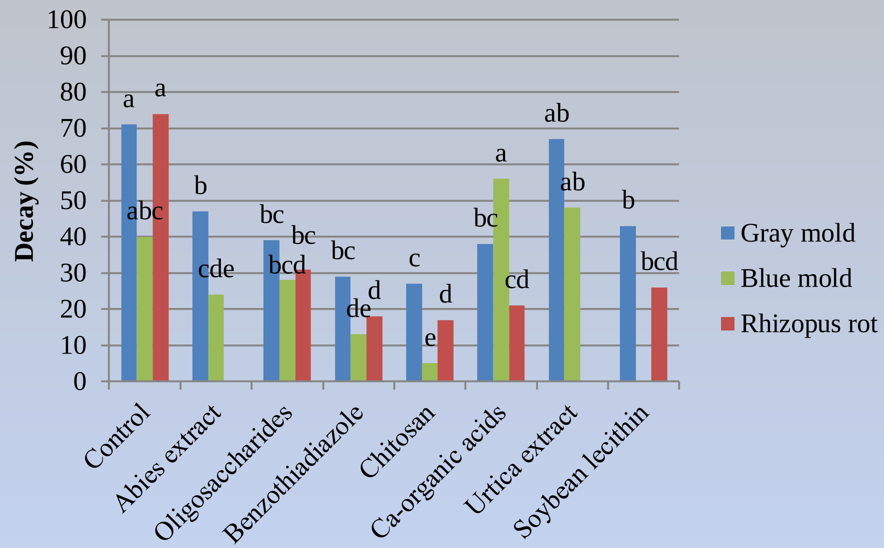
Applications on fruit and vegetables

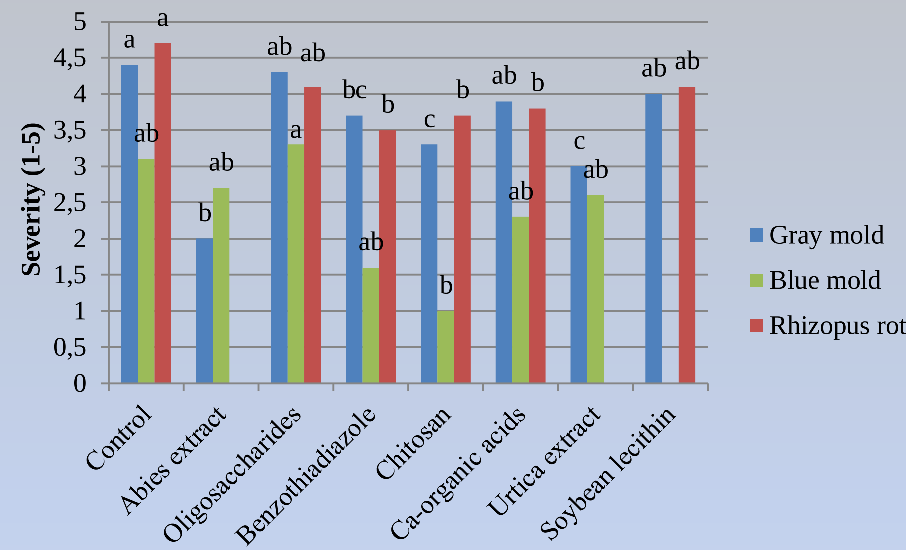
Produce	Decay	References
Strawberry	Gray mold	Romanazzi et al., 2013; Cao et al., 2011a; Terry and Joyce, 2000
Table grape	Gray mold	Muñoz and Moret, 2010
Sweet cherry	Brown rot	Feliziani et al., 2013
Peach	Blue mold	Cao et al., 2011b; Liu et al., 2005
Apple	Blue mold, gray mold	Spadaro et al., 2004
Pear	Blue mold, alternaria rot	Cao and Jiang, 2006; Cao et al., 2005
Tomato	Gray mold	Małolepsza, 2006
Melon	Rhizopus rot	Zhang et al., 2011; Bi et al., 2006; Huang et al., 2000

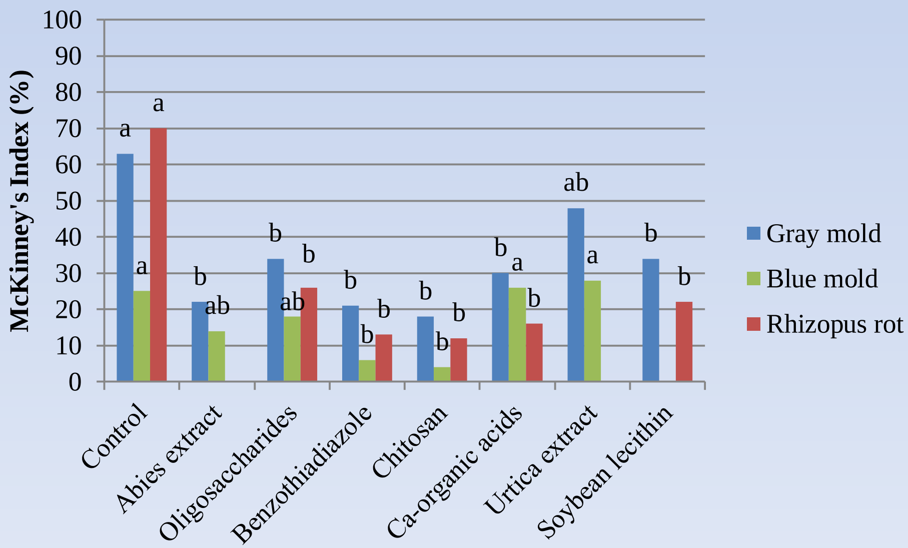
Experimental trials:

- STRAWBERRY
  - POSTHARVEST
  - PREHARVEST

POSTHARVEST TRIALS ON STRAWBERRY



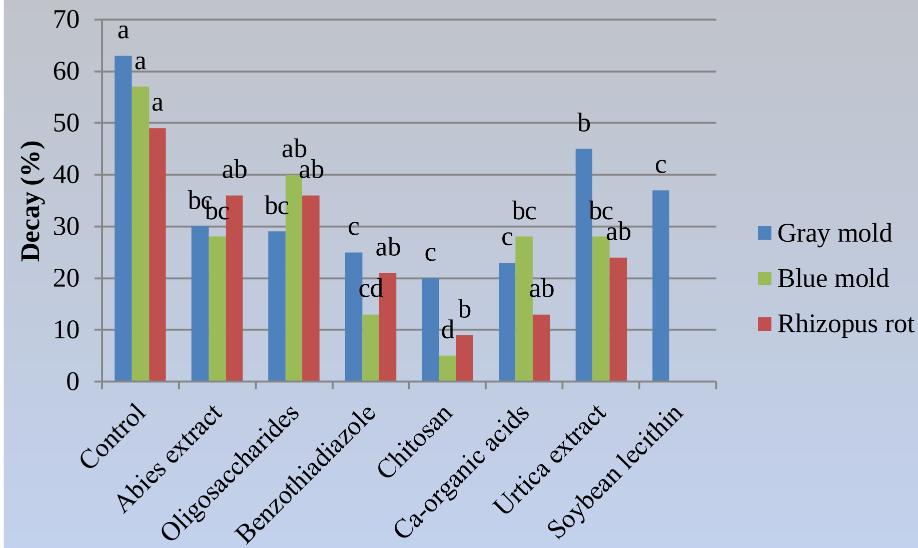


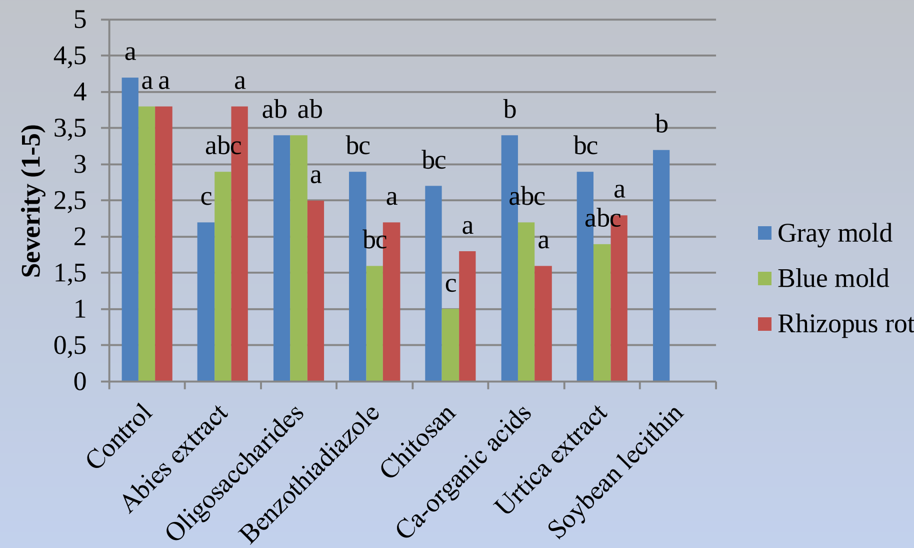


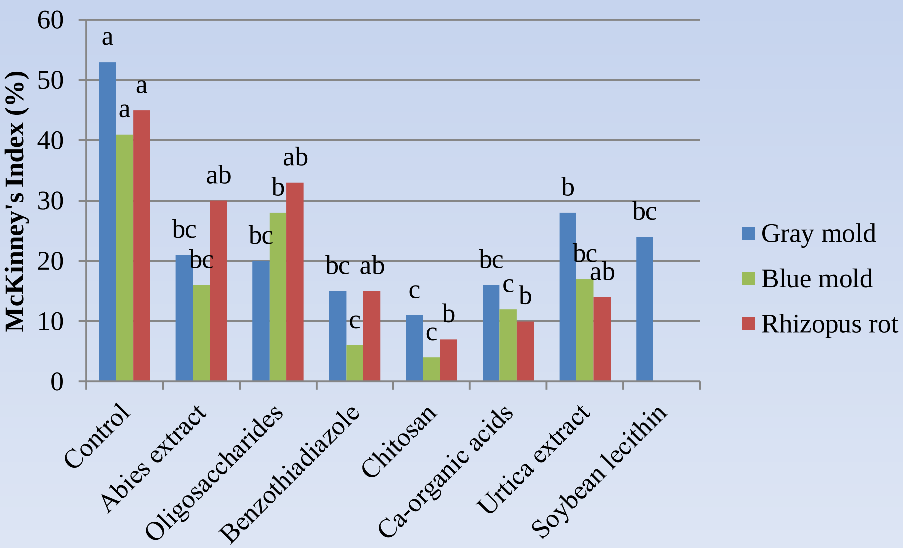
Decay, disease severity and McKinney index of gray mold, Rhizopus rot and blue mold recorded on “Camarosa” strawberries treated with commercial and experimental resistance inducers. The fruit were kept for **4 days at 20 ±1 °C, 95% to 98% RH**. Values with the same letter are not statistically different according to Duncan’s Multiple Range Test at $p < 0.05$.

Romanazzi et al., 2013 PBT

POSTHARVEST TRIALS ON STRAWBERRY





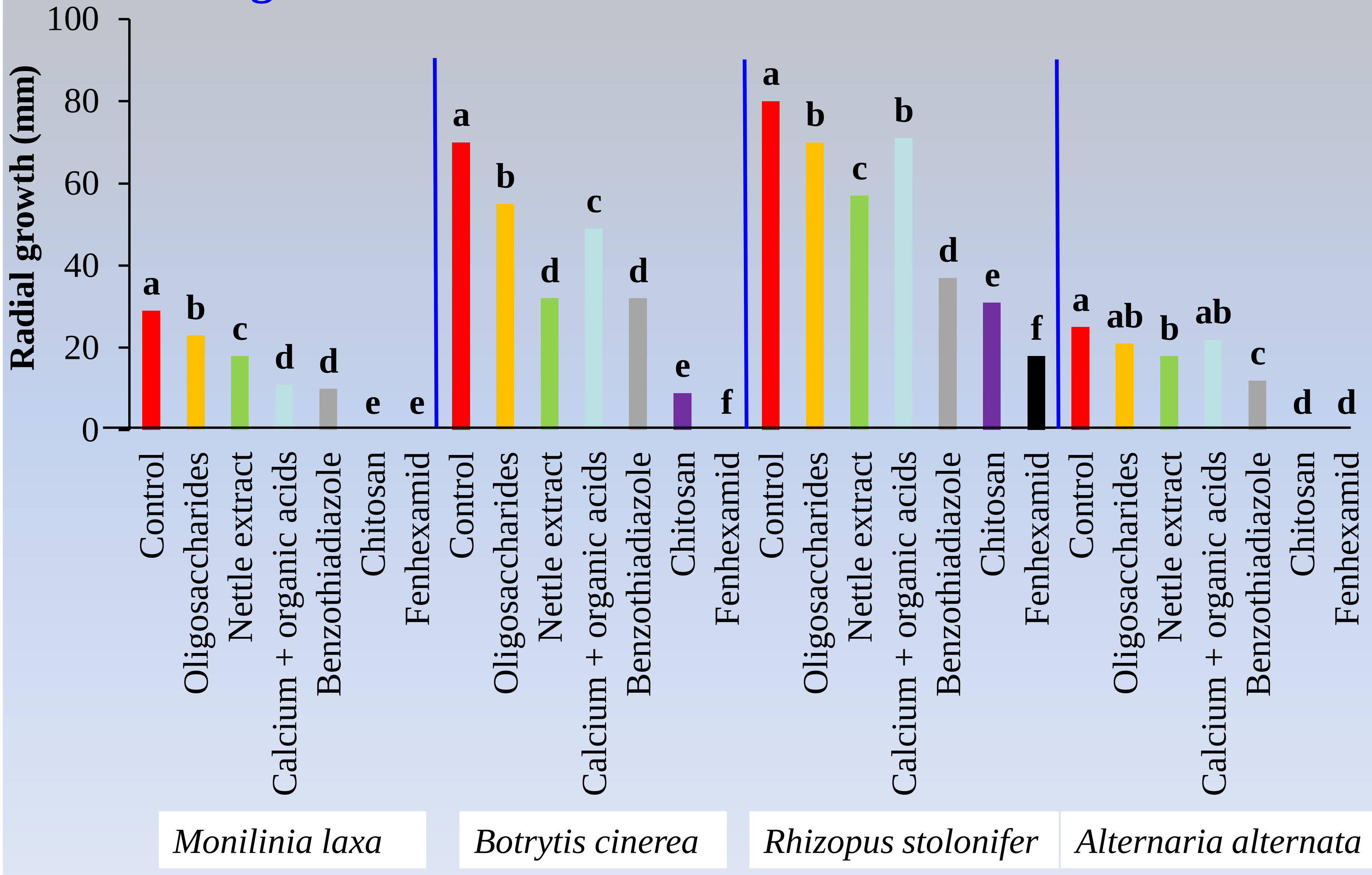


Decay, severity and McKinney index of gray mold, Rhizopus rot and blue mold recorded on strawberries treated with commercial and experimental resistance inducers. The fruit were stored for **7 days at 0 ±1 °C, 95% to 98% RH, followed by 3 days of shelf life at 20 ±1 °C, 95% to 98% RH**. Values with the same letter are not statistically different according to Duncan's Multiple Range Test at $p < 0.05$.

Romanazzi et al., 2013 PBT

Which are the mechanisms of action of these resistance inducers?

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

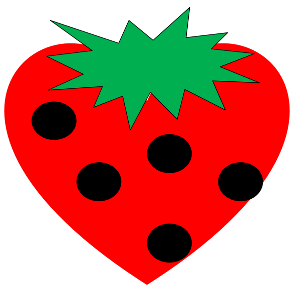


Feliziani et al., 2013 PBT

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

Resistance inducers

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

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Expression of Defense Genes in Strawberry Fruits Treated with Different Resistance Inducers

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ABSTRACT: The expression of 18 defense genes in strawberry fruit treated with elicitors: chitosan, BTH, and COA, at 0.5, 6, 24, and 48 h post-treatment was analyzed. The genes were up-regulated differentially, according to the elicitor. Chitosan and COA treatments promoted the expression of key phenylpropanoid pathway genes, for synthesis of lignin and flavonoids; only those associated with flavonoid metabolism were up-regulated by BTH. The calcium-dependent protein kinase, endo-β 1,4-glucanase, ascorbate peroxidase, and glutathione-*S*-transferase genes were up-regulated by BTH. The K^+ channel, polygalacturonase, polygalacturonase-inhibiting protein, and β-1,3-glucanase, increased in response to all tested elicitors. The enzyme activities of phenylalanine ammonia lyase, β-1,3-glucanase, Chitinase, and guaiacol peroxidase supported the gene expression results. Similarity of gene expression was >72% between chitosan and COA treatments, while BTH showed lower similarity (38%) with the other elicitors. This study suggests the relationship between the composition of the elicitors and a specific pattern of induced defense genes.

KEYWORDS: *benzothiadiazole, chitosan, elicitors, Fragaria × ananassa, gene expression*

INTRODUCTION

Strawberry (*Fragaria* × *ananassa*) is one of the most widely consumed berries, and it is a good source of natural antioxidants.[1] However, strawberry fruits are highly perishable and very susceptible to fungal decay in the field, and even more so during postharvest storage. This can result in severe crop losses. Application of natural compounds known as resistance inducers or elicitors is an innovative approach to prolong the shelf life of fresh fruit, through the reduction of disease incidence and with increased ecological security and safety for consumers. To reduce the postharvest decay of strawberries, the application of these natural compounds has been

The signaling pathways that control systemic resistance are multiple component networks with characteristic schemes that lead to plant resistance.[12] However, the transcription factors produced as a result of signal transduction can trigger the expression of a large number of genes, with the consequent physiological events usually involving changes in cell-wall composition, ion fluxes, de novo production of pathogenesis-related (PR) proteins, synthesis of phytoalexins, and reactive oxygen species (ROS) production.[13]

Several studies have shown the involvement of phenolic compounds[14,15] and cell-wall degradation enzyme activities[3,16] in the responses of strawberry fruit exposed to postharvest

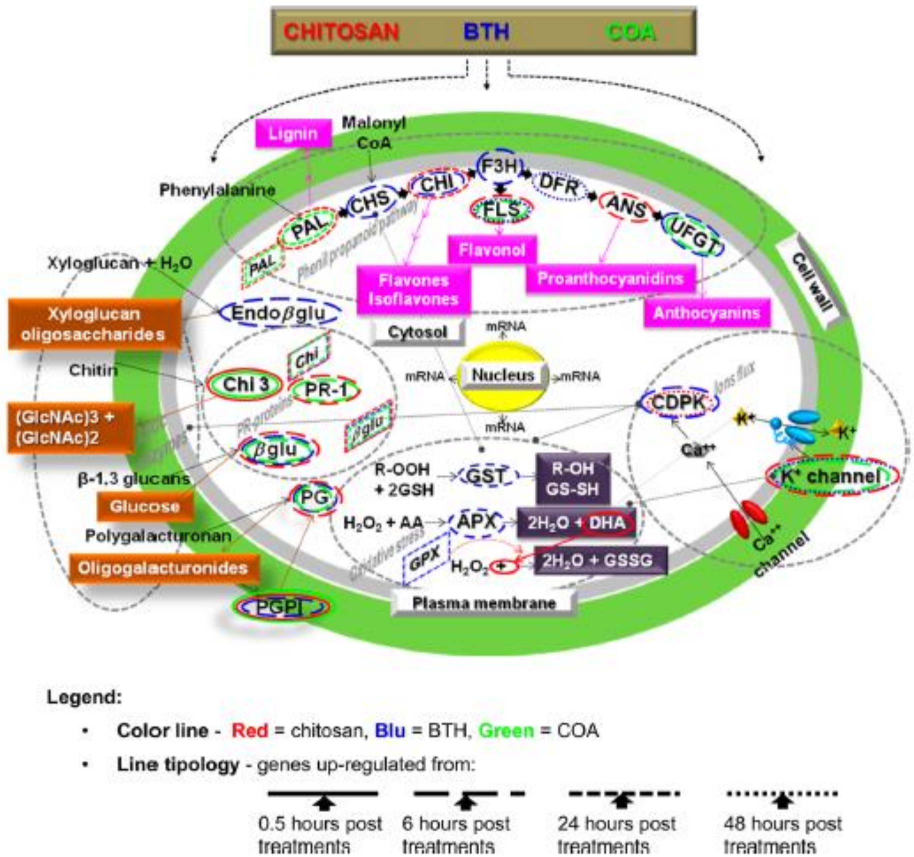


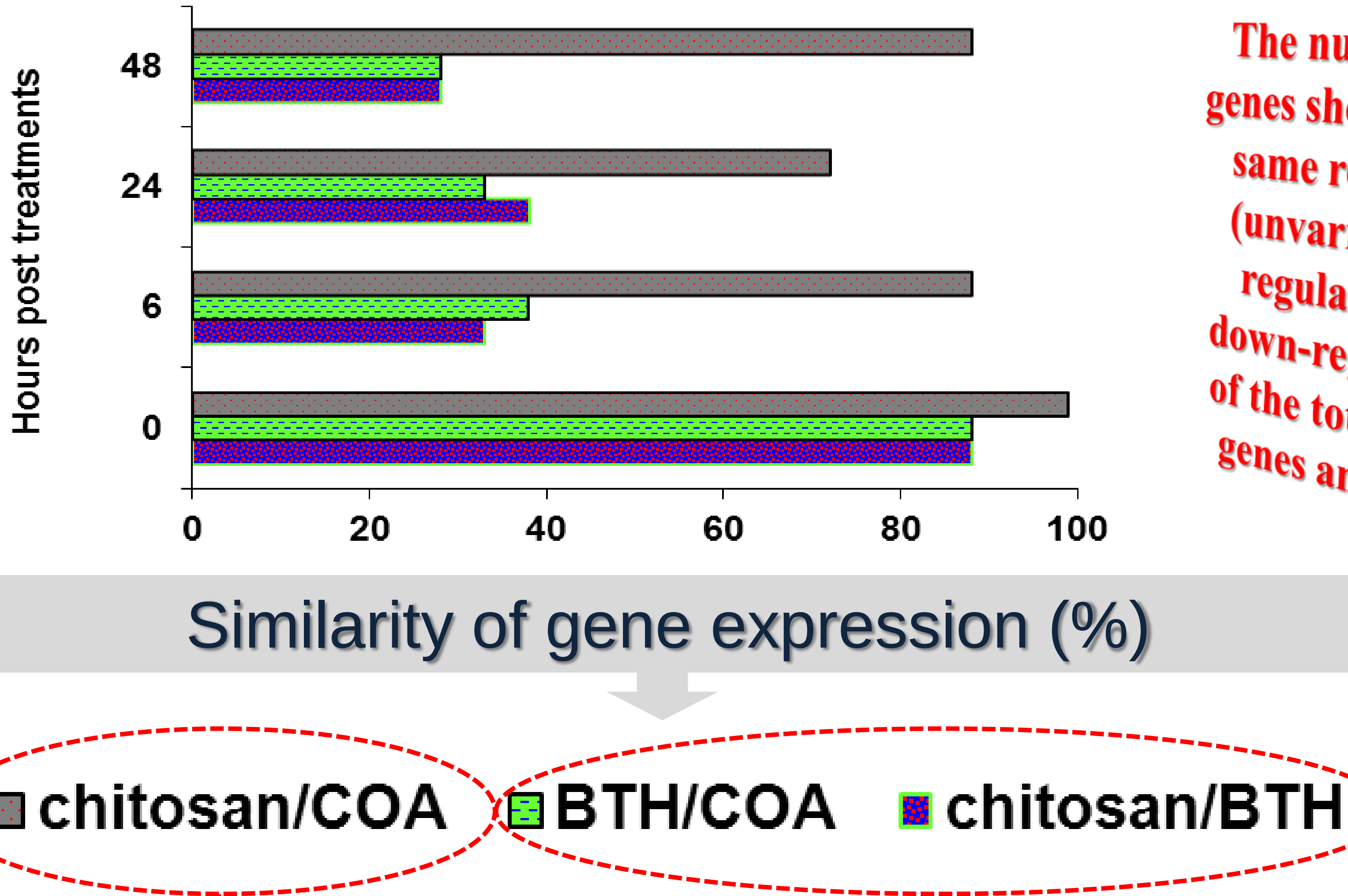
Figure 2. Gene expression and enzyme activity involved in resistance induced by chitosan, COA, and BTH (see legend). The metabolic roles of both the genes and enzymes analyzed are shown. Genes associated with ion fluxes, CDPK, the K^+ channel; genes associated with oxidative stress, GST, APX; genes associated with secondary metabolism, such as phenylpropanoid, PAL, CHS, CHI, F3H, FLS, DFR, ANS, and UFGT; genes associated with cell-wall degradation enzymes, endoβgluc, PG, and PGPI, some of which are known as PR proteins, as βglu, Chi3, and with the addition of the PR-1 protein. The up-regulation of genes by the resistance inducers and post-treatment times are shown with circular lines surrounding the abbreviated gene names, with different colors and typologies (see legend). The metabolic reactions of the analyzed enzyme activities are also shown: GPX, PAL, Chi, and βglu, surrounded by square lines (see legend).

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

This supports the effectiveness of these compounds for 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

>72% (chitosan/COA)

< 38% (BTH/COA, chitosan/BTH)

Landi et al., 2014 JAFC

CONCLUSIONS

- Treatment with some of the tested compounds contained postharvest decay of strawberry
- The effectiveness of chitosan and benzothiadiazole in decay control was the highest among the alternatives to synthetic fungicides
- The tested compounds had no negative effects on fruit quality, so they can be helpful in IPM
- Resistance inducers has antimicrobial activity on postharvest decay agents
- The application of resistance inducers elicited host defenses, with a different pattern involved between chitosan/COA and BTH