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Preharvest treatments with chitosan and other alternatives to conventional fungicides to control postharvest decay of strawberry

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ABSTRACT

The effectiveness of the control of postharvest decay of strawberry (*Fragaria* × *ananassa*, 'Alba' and 'Romina' cvs.) fruit following field applications of chitosan, laminarin, extracts of *Abies* spp., *Polygonum* spp., and *Saccharomyces* spp., an organic acids and calcium combination, and benzothiadiazole, were compared with a fungicide strategy. These compounds were sprayed every 5 days on the strawberry canopy, from flowering to ripening, in 2012 and 2013. The treatments with alternative compounds provided ~30% reduction in postharvest decay of strawberry compared to the water-treated controls, mainly against gray mold and Rhizopus rot, and without negatively affecting fruit color and firmness. Chitosan and benzothiadiazole were the most effective alternative treatments. Preharvest spraying with these alternative treatments can complement the use of conventional fungicides in the control of postharvest decay of strawberry fruit, especially when disease pressure is low.

1. Introduction

Strawberry (*Fragaria* × *ananassa*) is a perishable fruit that easily undergoes fungal spoilage after harvesting. The main pathogen that affects strawberry during storage is *Botrytis cinerea*, a saprophytic fungus that is the causal agent of gray mold (Snowdon, 1990). Pathogen infection occurs during strawberry cultivation, while symptoms develop mainly after harvesting, and the infection can easily move to nearby fruit, a phenomenon known as nesting (Maas, 1998). Usually, to prevent this postharvest rot, fungicides are sprayed several times on the canopy of the strawberry plants through the season, from flowering to harvest. However, the normative restrictions and the growing concern of consumers regarding fungicide residues on the fruit have led to the search for alternatives to the use of conventional fungicides. Furthermore, fungicide resistance has been detected in *B. cinerea* isolates exposed to fungicides that were constantly applied in the field to control gray mold (Fillinger et al., 2008; Weber, 2011).

Many of the alternative compounds to fungicides are nontoxic for human health and the environment, have no negative effects on the quality of the fruit, and might complement or improve current productive practices (Romanazzi, Licther, Mlikota Gabler, & Smilanick, 2012). Alternative compounds to conventional fungicides are characterized by antimicrobial activities against the main postharvest pathogens that cause fruit rot, or they are resistance inducers that activate the plant defenses, to simulate the presence of a pathogen. Resistance inducers can be pathogen or plant constituents, or their analogs. Chitosan is a natural biopolymer in the cell wall of many pathogenic fungi, derived from chitin (Benhabiles et al., 2012; Romanazzi, Feliziani, Bautista-Baños, & Sivakumar, 2015; Synowiecki & Al-Khateeb, 2003), and laminarin is an oligosaccharide that is one of the main components of algal tissue (Rioux, Turgeon, & Beaulieu, 2007; Wu, 2014). These compounds have been reported to both stimulate plant defenses and prevent disease development (Aziz et al., 2003; Landi, Feliziani, & Romanazzi, 2014). Benzothiadiazole is an analog of salicylic acid that has been applied to plant tissues as an activator of systemic acquired resistance (Lawton et al., 1996). Plant or microbial extracts can also be considered as useful alternatives to conventional fungicides in the management of postharvest rot of fruit and vegetables (Feliziani & Romanazzi, 2013).

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The effectiveness of such alternative compounds to control strawberry gray mold have been tested in preliminary studies carried out at the postharvest stage, by dipping strawberry fruit in solutions of these compounds (Benhabiles, Drouiche, Lounici, Pauss, & Mameri, 2013; Cao, Hu, Zheng, Yang, & Lu, 2011; Romanazzi, Feliziani, Santini, & Landi, 2013), or at the preharvest stage under controlled conditions in plastic tunnels (Bhaskara Reddy, Belkacemi, Corcuff, & Castaigne, 2000; Mazaro et al., 2008; Terry & Joyce, 2000). Romanazzi, Nigro, and Ippolito (2000) applied practical grade chitosan either at postharvest or under field conditions, spraying 0.1%, 0.5% or 1.0% chitosan on strawberry plants at the growth stages of full bloom, green fruit and whitening fruit. Chitosan reduced postharvest rot caused by *B. cinerea* and *R. stolonifer*, with the greatest reductions seen for 1.0% chitosan applied to the strawberry fruit at the whitening stage.

For the present study, we selected a list of promising compounds and tested them under field conditions with two strawberry varieties over two seasons, with repeated treatments from strawberry flowering to fruit maturity. The aim of this study was to determine the effectiveness in the control of postharvest decay of strawberry fruit using field applications of: chitosan (0.5%, 1.0%); laminarin alone, or in a mixture with extracts of *Saccharomyces* spp. (yeast) or *Polygonum* spp. (knotweed); *Abies* spp. (fir) extract alone, or in combination with organic acids and calcium; and benzothiadiazole. The effectiveness of these compounds was compared to the control that was treated with water, and to the spraying of a fungicide strategy that is currently used in conventional agriculture, as a combination of cyprodinil, fludioxonil, pyrimethanil, and fenehexamid. Furthermore, the strawberry fruit quality parameters, including color and firmness, were recorded.

2. Materials and methods

2.1. Climate data

The environmental parameters of the area where the experimental trials were carried out were kindly provided by a local weather station (Agenzia Servizi Settore Agroalimentare delle Marche, Ancona, Italy), with the recording of the minimum, maximum, and mean monthly temperatures (°C) and rainfall (mm). In addition, the local weather station provided the climate data for the historical series of 1961–2000 for the area of interest.

2.2. Treatments

The trials were carried out in the years 2012 and 2013 in an experimental strawberry field in a flat area of central-eastern Italy (Agugliano; 43°31′60″N, 13°22′60″E). The strawberry plantlets were the 'Alba' and 'Romina' varieties, and they were planted under field conditions using the plastic hill culture production system, as twin rows 30 cm apart and plantlets at intervals of 30 cm, with each twin row separated by 1 m from the next. Through the season, the plants were irrigated using a drip system.

For the trial, different treatments of the strawberry plants were compared. Table 1 summarizes the products that were sprayed in 2012 and 2013.

In 2012, the strawberry plants were treated with: water (control); chitosan at two different concentrations; laminarin; an extract from the fir *Abies sibirica*; benzothiadiazole; or a fungicide strategy of cyprodinil and fludioxonil for two initial applications, followed by pyrimethanil for three applications. In 2013, the strawberry plants were treated with: water (control); chitosan at two different concentrations; laminarin mixed with a microbial extract of *Saccharomyces* spp. or with a vegetal extract of *Polygonum* spp.; the extract form the fir *A. sibirica* for the three initial applications, and then organic acids and calcium for the final three applications; benzothiadiazole; or a fungicide strategy of cyprodinil and fludioxonil for two initial applications, followed by pyrimethanil for three applications, and finally fenhexamid. The commercial chitosan formulation here used has a deacteylation degree of 80–90%, a viscosity of 0.08–0.12 Pa s (1% w/v solution), the molecular weight of monomer of D-glucosamine hydrochloride is 215.62 g/mol and the molecular weight of monomer of *N*-acetyl-D-glucosamine hydrochloride is 257.66 g/mol. The extract form the fir *A. sibirica* is mainly composed by triterpenic acids, a emulsifier agent, and distiller water.

A randomized block design with four replicates was used, and the treatments were assigned to plots using a random-number generator (Excel; Microsoft Corp., Redmond, WA, USA). Along the twin rows, each plot was 6.5 m in length, which corresponded to ~45 plants per plot. The plots were divided from each other by 0.5 m of untreated plants.

The treatments were distributed by spraying a volume equivalent to 1000 l/ha using a motorized backpack sprayer (GX 25, 25 cm^3, 0.81 kW; Honda, Tokyo, Japan). To indicate the flowers that were completely opened and with five petals, just before the first treatment, a tag was put on their stems. The first treatments were carried out approximately in mid-April in both years, at flowering, and further treatments followed every 5 days, for a total of 5 treatments in 2012, and 6 treatments in 2013.

The harvests were carried out from mid-May to the end of May, ≥5 days after the plants had received the last treatment. At harvest times, only the ripe strawberry fruit in each plot that had the tags on the stems and were red over ≥2/3 of their surface were picked, to be sure that they had received all of the treatments from flowering to maturity.

After harvesting, the strawberry fruit were selected for absence of defects and uniformity of color and shape. The strawberry fruit harvested from each plot were randomly divided into groups of six fruit that were placed into small boxes, which were then placed into large covered boxes. To create the humid conditions of storage, a layer of wet paper was placed in the bottom of the large boxes. The strawberry fruit were stored for 7 days at 0.5 ± 1 °C, and then exposed to a shelf life at 20 ± 1 °C and 95% to 98% relative humidity, for 4 days.

For each treatment on each cultivar, there were four blocks in the field, and for each block at least six replicates, each of six strawberry fruits that were harvested and tested.

2.3. Decay evaluation

After the shelf-life period, the percentages of decayed strawberry fruit (i.e., the decay incidence) were recorded. Decay severity was also recorded according to an empirical scale with six degrees: 0, healthy fruit; 1, 1% to 20% fruit surface infected; 2, 21% to 40% fruit surface infected; 3, 41% to 60% fruit surface infected; 4, 61% to 80% fruit surface infected; 5, ≥81% fruit surface infected and showing sporulation (Romanazzi et al., 2000). The infection index (or McKinney index), which incorporates both the incidence and severity of the decay, was expressed as the weighted means of the decay as a percentage of the maximum possible level (McKinney, 1923). This was calculated using the formula:

$$I = \left[\sum \frac{(d \times f)}{(N \times D)}\right] \times 100 \quad (1)$$

where d is the category of rot intensity scored on the fruit, and f is its frequency, N is the total number of examined fruit (i.e., healthy and infected), and D is the highest category of decay intensity that occurred on the empirical scale.

Table 1
Commercial names and sources of the formulations containing the active ingredients used in the experimental trials. The formulations were sprayed over the strawberry canopy according to the doses and the number of applications indicated, during the 2012 and/or 2013 seasons.

Formulation commercial name	Source (country)	Active ingredient (%)	Application dose (g or ml/l)	Number of applications per season	Season of trial
Chito Plant	ChiPro GmbH (D)	Chitosan (99.9)	5	5; 6	2012; 2013
Chito Plant	ChiPro GmbH (D)	Chitosan (99.9)	10	5; 6	2012; 2013
Bion	Syngenta (CH)	Benzothiadiazole (50)	2	5; 6	2012; 2013
Frontiere	K&A (IT)	Laminarin	10	5	2012
Frontiere + Botrisine	K&A (IT); K&A (IT)	Laminarin + extract of *Polygonum* spp.	1 + 3	6	2013
Frontiere + Oomisine	K&A (IT); K&A (IT)	Laminarin + extract of *Saccharomyces* spp.	1 + 3	6	2013
Abies	Agritalia (IT)	Fir extract	10	5	2012
Abies Fitocalcio	Agritalia (IT) Agrisystem (IT)	Fir extract; Organic acids and calcium	10 10	3 3	2013
Switch Scala	Syngenta (CH) Bayer (D)	Cyprodinil (37.5) + fludioxonil (25); Pyrimethanil (37.4)	0.8 1.5	3 2	2012
Switch Scala Teldor	Syngenta (CH) Bayer (D) Bayer (D)	Cyprodinil (37.5) + fludioxonil (25); Pyrimethanil (37.4); Fenexamid (42.8)	0.8 1.5 1	3 2 1	2013

D, Germany; IT, Italy; CH, Switzerland.

2.4. Determination of fruit quality parameters

At harvest, per plot, 10 strawberry fruit that did not show any deformity and had uniformity of size and degree of maturation were randomly selected. In 2012, the fruit were transported to the laboratory and their color and firmness were determined, while in 2013 these measurements were carried out after the strawberries were cold stored for one week. The fruit color was measured on two sides of each fruit, using a colorimeter (Chroma Meter CR 400; Konica Minolta, Tokyo, Japan). The instrument provided the parameters of L^*, a^* and b^*, which relate to the luminescence, red tone, and yellow tone, respectively, of the fruit color. The fruit firmness was measured with a penetrometer (Fruit Pressure Tester 327; Effegì, Ravenna, Italy), on the same strawberry fruit used for the color analysis, and the data were expressed in g. These measurements were carried out for 'Alba' in both years and for 'Romina' in 2013.

2.5. Statistical analysis

The data were analyzed statistically by two-way ANOVA, followed by Fisher's protected least significant difference (LSD) or Tukey's honestly significant difference (HSD) tests, at $P = 0.05$ (Statsoft, Tulsa, OK, USA). In the statistical analysis of the randomized complete block design, the block was considered as a second factor. Data from two or more harvests within the same season and same cultivar were pooled, and the statistical analysis to determine the homogeneity of the variance was tested using Levene's tests. When the range of percentages was greater than 40%, the percentage data were arcsine transformed before analysis, to improve the homogeneity of the variance. The actual values are shown.

3. Results

3.1. Climate date

The environmental parameters included rain (mm) and mean temperature (°C). These were provided weekly by the local weather station that was located in the area where the trials were carried out. These data are illustrated in Fig. 1.

The reported environmental data are from April 17 to June 4 for both years, as 2012 and 2013, which corresponded approximately to the period when the trials were carried out. For 2012 and 2013, the average rainfall and the average mean temperatures were 10 mm and 23 mm, and 17 °C and 17 °C, respectively. May 2013 was characterized by abundant rain, with 130 mm in total, which, according to the local weather station records, was twice that in May over the historical series of 1961–2000 for the area of interest. In addition, the number of rainy days during May 2013 was approximately double that for the same historical series of 1961–2000. This greater than usual rainfall in 2013 was concomitant to the harvest, although the strawberry fruit were harvested only when they were dry.

3.2. Decay evaluation

The treatments with these alternative compounds to conventional fungicides generally reduced the development of strawberry fruit decay after the 4 days of shelf life, which was mainly gray mold, followed by Rhizopus rot. In particular, compared to the control, for the 'Alba' cv. in 2012, the treatments with 0.5% chitosan, 1.0% chitosan, benzothiadiazole, fir extract, laminarin, and the fungicide strategy significantly decreased decay incidence by 36%, 29%, 36%, 14%, 15% and 84%, respectively (Table 2). Similarly, the McKinney's index of decay was significantly decreased compared to the control, by 50%, 49%, 53%, 23%, 23% and 89%, respectively. The severity of this strawberry fruit postharvest decay was also significantly reduced by both chitosan treatments (28%, 33%, by 0.5% and 1% chitosan),

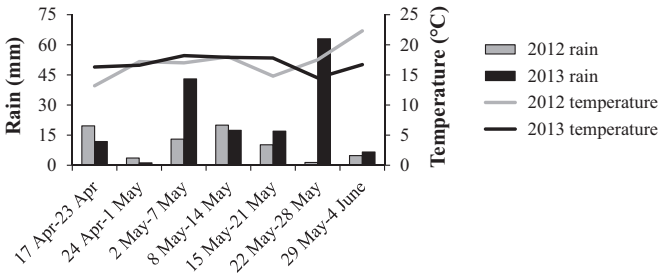

Fig. 1. Environmental parameters, including mean temperatures (°C) and rainfall (mm), of the area where the experimental trials were carried out in 2012 and 2013, as provided by a local weather station.

Table 2
Incidence, severity, and McKinney's index of postharvest decay of the 'Alba' strawberry fruit. Strawberry plants were treated during the 2012 season. After harvest, the strawberries were stored for 7 days at 0.5 ± 1 °C, and then exposed to 4 days of shelf-life at 20 ± 1 °C and 95% to 98% relative humidity.

Treatment	Incidence (%)	Severity (1–5)	McKinney's index (%)
Control	92.0a	3.9a	71.8a
Chitosan 0.5%	59.2cd	2.8cd	36.1c
Chitosan 1.0%	65.6c	2.6d	36.8c
Benzothiadiazole	58.5d	2.7d	33.7c
Fir extract	78.9b	3.5ab	55.0b
Laminarin 1.0%	77.8b	3.3bc	55.2b
Fungicide strategy	14.6e	1.0e	7.7d

Values followed by different letters are significantly different ($P = 0.05$; Fisher's protected LSD).

Table 3
Incidence, severity, and McKinney's index of postharvest decay of the 'Romina' strawberry fruit. Strawberry plants were treated during the 2012 season. After the harvest, the strawberries were stored for 7 days at 0.5 ± 1 °C, and then exposed to 4 days of shelf-life at 20 ± 1 °C and 95% to 98% relative humidity.

Treatment	Incidence (%)	Severity (1–5)	McKinney's index (%)
Control	93.3a	2.7a	51.6a
Chitosan 0.5%	79.5b	2.5ab	39.5bc
Chitosan 1.0%	93.5a	2.5ab	47.4ab
Benzothiadiazole	69.2bc	2.6ab	37.2bcd
Fir extract	75.0b	2.0b	31.1cd
Laminarin 1.0%	59.4c	2.3ab	26.5d
Fungicide strategy	8.9d	1.0c	1.8e

Values followed by different letters are significantly different ($P = 0.05$; Fisher's protected LSD).

benzothiadiazole (31%), laminarin (15%) and the fungicides (74%), but not by the fir extract.

As with the 'Alba' cv., for the 'Romina' fruit in 2012, the treatments with the alternative compounds generally reduced the development of postharvest strawberry fruit decay after 4 days of shelf life. Compared to the control, the treatments with 0.5% chitosan, laminarin, fir extract, benzothiadiazole and the fungicide strategy significantly decreased decay incidence, by 15%, 26%, 20%, 36%, and 90% (Table 3).

Similarly, the McKinney's index of decay was significantly decreased compared to the control, by 23%, 28%, 40%, 49%, and 97%, respectively. However, the severity of this strawberry fruit postharvest decay was significantly reduced only by 0.5% chitosan (26%) and the fungicides (63%), and not by the other treatments. Thus, again, the treatments with the fungicides provided the greatest protection of the strawberry fruit from postharvest decay during the shelf life.

In 2013, the treatments with these alternatives to synthetic fungicides were generally effective in reducing postharvest decay of strawberry fruit, although their effects were lower compared to 2012. In particular, the decay incidence with the 'Alba' cv. was reduced by benzothiadiazole (15%) and by the fungicides strategy (96%), and the decay severity was reduced by 1.0% chitosan (18%) and by the fungicides (71%) (Table 4), but not by the other treatments. Compared to the control, the treatments with chitosan at both concentration (0.5% and 1.0%), benzothiadiazole, laminarin mixed with the vegetal extract of *Polygonum* spp., and the fungicide strategy significantly decreased the McKinney's index, by 21%, 20%, 28%, 20%, and 97%, while the mixture of the laminarin with microbial extracts of *Saccharomyces* spp., and the fir extract combined with organic acids and calcium did not reduce postharvest decay.

In 2013, with the 'Romina' cv., these treatments reduced the development of strawberry fruit decay in most cases. In particular, compared to the control, the treatments with 0.5% chitosan, 1.0% chitosan, benzothiadiazole, fir extract in combination with organic acids and calcium, laminarin in the mixture with the vegetal extract of *Polygonum*, and the fungicide strategy significantly decreased disease incidence by 22%, 24%, 15%, 22%, 23% and 84%, respectively (Table 5); while the mixture of laminarin with the microbial extracts of *Saccharomyces* did not differ from the control. Similarly, the McKinney's index of the strawberry fruit decay was significantly decreased compared to the control by chitosan at both concentrations, laminarin mixed with the vegetal extract of *Polygonum* spp. or mixed with microbial extracts of *Saccharomyces* spp., and the fungicides strategy, by 27%, 34%, 33%, 29%, and 93%, respectively. The severity of the postharvest disease was significantly reduced by laminarin mixed with the vegetal extract of *Polygonum* spp. (22%) and the fungicides (57%), but not by the other treatments.

Table 4
Incidence, severity, and McKinney's index of postharvest decay of the 'Alba' strawberry fruit. Strawberry plants were treated during the 2013 season. After harvest, the strawberries were stored for 7 days at 0.5 ± 1 °C, and then exposed to 4 days of shelf-life at 20 ± 1 °C and 95% to 98% relative humidity.

Treatment	Incidence (%)	Severity (1–5)	McKinney's index (%)
Control	79.5a	3.4a	55.8a
Chitosan 0.5%	69.8ab	3.0ab	44.3b
Chitosan 1.0%	77.6ab	2.8b	44.8b
Benzothiadiazole	67.2b	2.9ab	39.9b
Fir extract/organic acids and calcium	73.3ab	3.3ab	49.6ab
Laminarin 0.1% + *Saccharomyces* spp. extract	73.0ab	3.1ab	46.7ab
Laminarin 0.1% + *Polygonum* spp. extract	71.4ab	3.0ab	44.8b
Fungicide strategy	3.4c	1.0c	1.8c

Values followed by different letters are significantly different ($P = 0.05$; Fisher's protected LSD).

Table 5
Incidence, severity, and McKinney's index of postharvest decay of the 'Romina' strawberry fruit. Strawberry plants were treated during the 2013 season. After harvest, strawberries were stored for 7 days at 0.5 ± 1 °C, and then exposed to 4 days of shelf life at 20 ± 1 °C and 95% to 98% relative humidity.

Treatment	Incidence (%)	Severity (1–5)	McKinney's index (%)
Control	82.1a	2.3a	38.2a
Chitosan 0.5%	64.2b	2.1ab	27.7b
Chitosan 1.0%	62.6b	2.0ab	25.2b
Benzothiadiazole	69.6b	2.3a	31.8ab
Fir extract/organic acids and calcium	64.1b	2.4a	29.5ab
Laminarin 0.1% + *Saccharomyces* spp. extract	63.0b	2.0ab	25.7b
Laminarin 0.1% + *Polygonum* spp. extract	72.8ab	1.8b	27.0b
Fungicide strategy	13.2c	1.0c	2.6c

Values followed by different letters are significantly different ($P = 0.05$; Fisher's protected LSD).

Table 6
Fruit surface color described by the parameters of L^*, a^* and b^*, which relate to their luminescence, red tone, and yellow tone, respectively. The data were recorded for the 'Alba' strawberries in the 2012 season.

Treatment	L^*	a^*	b^*
Control	37.3ab	39.0a	22.4a
Chitosan 0.5%	37.8ab	38.6ab	22.8a
Chitosan 1.0%	37.7ab	39.1a	23.2a
Benzothiadiazole	37.4ab	37.5b	22.2a
Fir extract	37.7ab	39.1a	23.6a
Laminarin 1.0%	36.7b	38.2ab	22.5a
Fungicide strategy	38.1a	39.1a	23.6a

Values followed by different letters are significantly different ($P = 0.05$; Tukey's HSD).

3.3. Analysis of strawberry quality parameters

Almost all of the strawberry fruit quality parameters showed no significant changes from the controls for all of these field treatments, with the measurements of the firmness, and the color luminescence (L^*), red tone (a^*), and yellow tone (b^*). In 2012, the only treatment that resulted in any color change of the fruit skin was with benzothiadiazole, which significantly decreased the a^* values of the 'Alba' strawberry fruit (Table 6).

In 2013, the color of the 'Alba' strawberry fruit was affected by the benzothiadiazole and chitosan treatments, while none of the other compounds that were applied altered the external appearance of the strawberry fruit, compared to the control (Table 7). In particular, the L^* parameter was reduced by chitosan and the a^* value was reduced by both benzothiadiazole and chitosan. The firmness of the strawberry fruit in both years and for both cultivars was not altered by any of these preharvest treatments, as compared to the control (data not shown).

4. Discussion

The present study shows that preharvest treatment with a range of alternative compounds to conventional fungicides can reduce the development of postharvest rots of strawberry fruit. The effectiveness for decay reduction obtained by these treatments was less than that of the conventional fungicides.

Weather conditions can have great influence on the performance levels of these alternative compounds, as they are mainly natural formulations that are easily biodegradable. Indeed, considering the weather conditions of the seasons when the experimental trials were carried out, spring in 2013 was characterized by abundant rain compared to the previous year, which was approximately double the historical mean rain for the area for the month of May.

In 2012, the disease reduction of the alternative compounds was higher compared to 2013, and this higher effectiveness was concomitant with the low amounts of rain.

In the present trials, the experimental conditions were a close simulation of the scenario of strawberry fruit production for commercial purposes. These were very different from the conditions of postharvest applications carried out in other experimental trials, where the performance of alternative compounds for the reduction of postharvest fruit disease were more consistent because of the uniform environmental conditions that occurred during fruit storage (Romanazzi et al., 2015).

The chitosan treatments at both concentrations were overall effective in the control of gray mold. As the higher concentration of 1.0% chitosan did not always show better performance than that of 0.5% chitosan, this effectively hid any dose-dependent effects of chitosan here, which have instead been observed in other trials (Bhaskara Reddy et al., 2000), where the incidence of strawberry fruit disease decreased with increased chitosan concentrations. In previous studies, treatments with chitosan also reduced the postharvest disease of strawberry fruit when they were applied at the postharvest stage, either alone (Bhaskara Reddy et al., 2000; El Ghaouth, Arul, Ponnampalam, & Boulet, 1991; Romanazzi et al., 2013) or when mixed with other compounds (Hernández-Muñoz, Almenar, del Valle, Velez, & Gavara, 2008; Perdones, Sánchez-González, Chiralt, & Vargas, 2012; Vargas, Albors, Chiralt, & Gonzalez-Martinez, 2006). Similarly, benefits were seen when chitosan was applied at the preharvest stage under controlled conditions in plastic tunnels (Bhaskara Reddy et al., 2000; Mazaro et al., 2008), and under field conditions (Romanazzi et al., 2000; Lopes, Zambolim, Costa, Liparini Pereira, & Finger, 2014). Preharvest chitosan sprays significantly reduced post-harvest fungal rot and maintained the quality of the strawberry fruit compared with the untreated control (Bhaskara Reddy et al., 2000). The preharvest chitosan treatments delayed strawberry maturation, increased the flesh firmness, and decreased the fruit weight loss (Mazaro et al., 2008). Chitosan sprays were not phytotoxic to the plants or the fruit at the concentration of 1% or lower (Bhaskara Reddy et al., 2000; Lopes et al., 2014; Mazaro et al., 2008; Romanazzi et al., 2000). With respect to previous preharvest trials, which used practical grade chitosan, in the present study the commercial formulation for chitosan was used, as it can be easily dissolved in water. The effectiveness of the practical grade chitosan has been reported to be the same as for commercial formulations in the control of postharvest gray mold and Rhizopus rot of strawberry fruit (Romanazzi et al., 2013).

The effectiveness of chitosan has been ascribed to its antifungal activity against *B. cinerea* (Badawy, Rabea, & Taktak, 2014; Muñoz & Moret, 2010; Silva Jùnior et al., 2014) and to its triggering of plant defenses, such as compounds, enzymes, or genes related to pathogenesis, cell-wall degradation, or the phenylpropanoid pathway, both in strawberry tissues (Landi et al., 2014; Wang & Gao, 2013) and in other fruit (Burketova, Trda, Ott, & Valentova, 2015; Feliziani et al., 2013b; Lu et al., 2014; Muzzarelli et al., 2012). Furthermore, the formation of a semi-permeable film by chitosan over the fruit

Table 7
Fruit surface color described by the parameters of L^*, a^* and b^*, which relate to the luminescence, red tone, and yellow tone, respectively. The data were recorded for the 'Alba' and 'Romina' strawberries in the 2013 season.

Treatment	'Alba'			'Romina'		
	L^*	a^*	b^*	L^*	a^*	b^*
Control	33.9a	39.6a	23.6abc	34.3ab	39.0a	24.4a
Chitosan 0.5%	32.8ab	37.6b	22.5bc	33.8ab	39.5a	24.5a
Chitosan 1.0%	32.3b	40.0a	23.7abc	33.6ab	39.9a	24.5a
Benzothiadiazole	32.8ab	37.8b	22.2c	34.0ab	39.3a	24.5a
Fir extract/organic acids and calcium	33.0ab	39.7a	24.5ab	32.9b	39.0a	23.1a
Laminarin 0.1% + *Saccharomyces* spp. extract	34.0a	40.8a	25.3a	34.7a	40.0a	24.5a
Laminarin 0.1% + *Polygonum* spp. extract	32.5ab	39.1ab	23.3abc	33.9ab	39.9a	23.8a
Fungicide strategy	33.1ab	40.3a	24.3abc	33.5ab	40.0a	24.0a

Values followed by different letters are significantly different ($P = 0.05$; Tukey's HSD).

surface has been reported to decrease postharvest strawberry fruit weight loss and gaseous exchange, which helps to slow the fruit metabolism and senescence (Hernández-Muñoz et al., 2008). In addition, chitosan can reduce the microbial load that fruit harbor, which includes the microorganisms that are responsible for food-borne diseases (Tsai, Zhang, & Shieh, 2004).

Treatments with the oligosaccharide laminarin partially reduced the postharvest strawberry fruit gray mold, both when it was used alone at 1.0% and when used at 0.1% in a mixture with a microbial extract of *Saccharomyces* or a vegetal extract of *Polygonum*. The strawberry fruit of the 'Romina' cv. appeared to benefit more from the treatments with laminarin than those of the 'Alba' cv. Laminarin is a resistance inducer that can activate plant defense systems (Trouvelot et al., 2014). It was shown to reduce postharvest diseases of sweet cherry (Feliziani, Santini, Landi, & Romanazzi, 2013a), and to elicit defense responses in grapevine, where it can induce protection against *B. cinerea* and *Plasmopara viticola* (Aziz et al., 2003). The reduction of decay by the microbial extract of *Saccharomyces* used here associated to laminarin 0.1% can be ascribed to possible resistance induced in the host tissues. On the other hand, extracts of the plant *Polygonum cuspidatum* have been shown to have antifungal effects against some plant pathogen fungi, including those responsible for postharvest rots of fruit (Hussain et al., 2010).

Benzothiadiazole is a molecule that has been defined as a functional analog of salicylic acid, which is a signal mediator in systemic acquired resistance (Vallad & Goodman, 2004). Application of benzothiadiazole to strawberry fruit has been reported to decrease the development of disease compared to control fruit, by enhancing the strawberry antioxidant system, free-radical-scavenging capability, and expression of genes associated with secondary metabolites (Cao et al., 2011; Landi et al., 2014). As well as its action as a resistance inducer, benzothiadiazole also has antimicrobial properties against some of the fungi that can cause postharvest decay, including *B. cinerea* (Feliziani, Santini, Landi, & Romanazzi, 2013a; Terry & Joyce, 2000). In the present study, benzothiadiazole indeed reduced the postharvest disease of the strawberry fruit. Similar results have been obtained in other studies with benzothiadiazole treatments carried out postharvest (Romanazzi et al., 2013) and preharvest (Mazaro et al., 2008; Terry & Joyce, 2000).

Likewise, the treatments with the fir extract reduced postharvest rot of the strawberry fruit, compared to the control. The antimicrobial activity of these fir extracts appears to be ascribable to certain of its constituents, which include the terpenoids and phenols (Yang, Li, Shen, & Zhang, 2008), and which have been reported to be active in plant defenses (Lattanzio, Lattanzio, & Cardinali, 2006). In a similar way, in other studies, the immersion of strawberry fruit in a fir extract solution reduced postharvest gray mold and blue mold (Romanazzi et al., 2013). In the 2012 season, the fir extract was used alone, while in the following year it was used in combination with a formulation based on organic acids and calcium. This commercial formulation is registered as a fertilizer that can reinforce the vegetal structure, as calcium ions can strengthen the pectins of the cell wall, by binding to them. On the other hand, the organic acids can act as resistance inducers (El-Hendawy, Shaban, & Sakagami, 2010; Landi et al., 2014).

In the results relating to the two strawberry varieties, there were differences in the effectiveness of the preharvest treatments in controlling postharvest decay. Indeed, with the cv. 'Alba' strawberries the highest decay reductions were obtained after benzothiadiazole treatments, while with 'Romina', laminarin and chitosan provided the best performances. Differences in treatment responses among varieties were observed by Lopes et al. (2014) as well. In their study, chitosan application to strawberry plants in the field in combination with postharvest dipping of fruits was an effective strategy for controlling gray mold in strawberry in 'Oso Grande' but was not effective in the variety 'Camarosa' (Lopes et al., 2014). In the same way, in our study the effectiveness of preharvest treatments with resistance inducers in controlling postharvest decay largely varied among the two strawberry varieties. A molecular study on strawberry tissue response to elicitor applications revealed that there is a relationship between the composition of the elicitor and the specific pattern of induced defense genes that it triggers: the levels of involvement of genes associated with plant defense depend on the resistance inducer applied (Landi et al., 2014). Although chitosan and benzothiadiazole activated different pathways, they were both effective in reducing postharvest decay of 'Camarosa' strawberry (Romanazzi et al., 2013). When these treatments are applied to the varieties 'Alba' and 'Romina', a different level of effectiveness can be expected for possible activation of a cultivar-specific pathway, as observed for the elicitor-specific pathway observed in the cv. 'Camarosa' (Landi et al., 2014).

Color and firmness of strawberry fruit are the quality parameters that are fundamental for consumer acceptability (Hernández-Muñoz et al., 2008; Hernanz, Recamales, Meléndez-Martínez, González-Miret, & Heredia, 2008). In the present study, none of the treatments negatively affected the external appearance of the strawberry fruit. Indeed, the slight changes recorded compared to the control for the L^* and a^* parameters after the preharvest treatments did not have any detrimental effects on the appearance of the strawberry fruit, as the color parameters were in line with previous analyses (Capocasa, Scalzo, Mezzetti, & Battino, 2008). These parameters were also within the range of 6 units to 7 units, which is the usual color tolerance that takes into account the natural variability of this fruit (Perdones et al., 2012). In particular, the color of the strawberry fruit was affected by the benzothiadiazole and chitosan treatments, while none of the other compounds that were applied altered the external appearance of the strawberry fruit compared to the control. These data are in agreement with previous studies that have reported that preharvest chitosan treatments of strawberry plants reduced the fruit content of anthocyanin (Bhaskara Reddy et al., 2000), which is the pigment that contributes to the red color of the strawberry fruit (Nunes, Brecht, Morais, & Sargent, 1995).

In conclusion, preharvest treatments with alternatives to conventional fungicides can reduce postharvest disease of strawberry fruit without negatively affecting the quality parameters of the fruit, such as the color and firmness. The strategy based on conventional fungicides remained the most effective here, although it cannot be used in organic agriculture. Thus, in a context of integrated decay management, as planned by European Directive 2009/128/EC for the sustainable use of pesticides, the preharvest treatments with these alternative compounds might complement the use of conventional fungicides in the control of postharvest decay of strawberry fruit, especially when the disease pressure is low.

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