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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.