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The application of chitosan and benzothiadiazole in vineyard (*Vitis vinifera* L. cv Groppello Gentile) changes the aromatic profile and sensory attributes of wine

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ABSTRACT

This work reports the effects of resistance inducers on wine aroma compounds and sensory attributes. Resistance inducers are a class of products able to elicit the plant defence mechanisms against pathogens, incurring lower toxicological risks than conventional agrochemicals. Among them, chitosan (CHT) and benzothiadiazole (BTH) are particularly effective in stimulating the biosynthesis of bioactive phytochemicals. They were used in a two-year survey conducted to assess experimental wines obtained from elicitor-treated grapes. Compared with conventional fungicides (penconazole and methyldinocap), in 2009, BTH increased total acetals and esters, while CHT raised the levels of total acetals and alcohols. Sensory analysis revealed that overall acceptance was higher in CHT than in BTH. In 2010, differences were not significant. Therefore, plant activators deserve attention beyond their efficacy in crop protection. In particular, in our experimental conditions, CHT improved the volatile profile, flavour and taste of Groppello wine.

1. Introduction

Aroma represents an essential quality trait of wine. It depends on more than a thousand volatile compounds (Polášková, Herszage, & Ebeler, 2008) belonging to heterogeneous chemical groups such as acetals, alcohols, aldehydes, esters, ketones and isoprenoids (or terpenes), the occurrence of which results from grape origin, winemaking techniques and wine ageing. These compounds are responsible for four different aromas: (1) varietal aroma, depending on genetic traits of grapes, environmental conditions and degree of ripeness; (2) pre-fermentative aroma, originating during grape processing, i.e. transport, crushing and maceration; (3) fermentative aroma, produced by yeasts and lactic acid bacteria during alcoholic and malolactic fermentation, respectively; (4) post-fermentative aroma, resulting from transformations occurring during conservation and ageing of wine (Vilanova & Oliveira, 2012). The available information about plant activator impact on volatile compounds that contribute to the aroma of wine is scanty. Therefore, in this study, we investigated the aromatic profile of experimental red wines (*Vitis vinifera* L. cv. Groppello Gentile) produced from chitosan (CHT) and benzothiadiazole (BTH) treated grapes and the effects of the same elicitors on sensorial attributes of wine. In agricultural practice, plant activators may represent an effective alternative to conventional agrochemicals (Buonaurio, Iriti, & Romanazzi, 2009). They do not exert a direct antimicrobial activity against phytopathogens, but are able to boost the plant innate immune systems, triggering a complex defence machinery known as systemic acquired resistance (SAR) (Iriti & Faoro, 2007; Spoel & Dong, 2012). Thus, in general, they seem to be environmentally-friendly products, even if their efficacy needs to be validated for each pathosystem (i.e. plant–pathogen interaction). Among SAR-inducers, BTH, a functional analogue of salicylic acid, and CHT, a deacetylated chitin derivative, deserve particular attention because of their wide use against grey mould (*Botrytis cinerea*) and powdery mildew (*Erysiphe necator*) infections in experimental vineyards. In addition, both plant activators increased the levels of bioactive phytochemicals, namely polyphenols, melatonin and phytosterols, in grapes and the corresponding experimental wines (Gómez-Plaza, Mestre-Ortuño, Ruiz-García, Fernández-Fernández,

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& López-Roca, 2012; Iriti, Rossoni, Borgo, & Faoro, 2004; Iriti, Rossoni, Borgo, Ferrara, & Faoro, 2005; Iriti et al., 2011; Ruggiero, Vitalini, Burlini, Bernasconi, & Iriti, 2013; Vitalini et al., 2011).

2. Materials and methods

2.1. Phytoiatric campaign

Open-field treatments with plant activators were performed in 2009 and 2010 on grapevines (*V. vinifera* L.) of Groppello Gentile, a Lombardy autochthonous cultivar grown in an experimental vineyard (Azienda Agricola San Giovanni) located at Raffa di Puegnago (Brescia, Italy). Groppello Gentile vines (420A rootstock) were trained with the Guyot system, spaced 2.3 × 0.9 m, on a grass-covered morainic soil, with 15% of gravel, sand-loamy. The vineyard was drip-irrigated. Vine rows ran N-S in an area characterised by loose and permeable land, rich in coarse soil of glacial origin and echoing a Mediterranean-style exposure, even at such extreme latitudes. The proximity of Lake Garda affects the local climate – temperate subcontinental – mitigating the winter rigours and reducing the annual and seasonal temperature ranges. The meteorological conditions during the two growing seasons were as follows: average temperature, humidity and wind velocity were 23.4 °C and 24.1 °C, 57.4% and 64.2%, 6.5 km/h and 6.6 km/h, in 2009 and 2010, respectively; days of rain, thunderstorm and fog were 37 and 49, 28 and 35, 1 and 2, in 2009 and 2010, respectively. In particular, in 2009, second half of August was hotter than normal, with maximum temperature that reached 34–37 °C for 5 days, while, during 2010, it was very hot for half of July, with 37 °C max for 3 days.

The treatments were: (i) 0.03% (w/v) chitosan (CHT, 76 kDa molecular weight and 85% deacetylation degree; Sigma–Aldrich, St. Louis, MO), (ii) 0.03% CHT in combination with 150 g/hL copper hydroxide, a dosage employed in organic agriculture (Kocide 3000; Du Pont, Wilmington, DE) (CHT/Cu) and (iii) 0.3 mM benzothiadiazole [benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester, BTH, trade name Bion®, Syngenta, Basilea, CH]. Grapevines treated with conventional fungicides (50 mg L^{-1} penconazole, a systemic triazole, and 250 mg L^{-1} methyldinocap, a phenyl crotonate) were used as positive control.

The trial was set up as a complete randomised block design in four replications, with 10 vines (a parcel) per treatment in each block. Plants were sprayed every 10 days approximately with a spray lance, according to the meteorological conditions, from the beginning of grape susceptibility to fungal diseases until the complete *véraison* (i.e. approximately from the half of April to the end of July).

2.2. Microvinification technique and standard analyses of experimental wine

Experimental Groppello wines were produced, by standard minivinification techniques, in the Centro Vitivinicolo Provinciale di Brescia (Italy), and stored at 4 °C until analysis. Minivinifications of 40 kg of grapes were carried out in the experimental cellar of Centro Vitivinicolo Provinciale di Brescia, following the EPPO (2010) guidelines for minivinifications of red wines, including the following steps: destemming, crushing, SO_2 addition (5 g/hL potassium metabisulfite), yeast addition, aeration of must, maceration (6 days), pigeage (1–2 days), clarification setting (no filtration), SO_2 addition after fermentation, bottling and storage. The obtained wines were analysed for their major components using standard oenological analytical techniques. In particular, they were examined for ethanol content (% v/v), reducing sugars (g L^{-1}), total titratable acidity (g tartaric acid L^{-1}), total volatile acidity (g acetic acid L^{-1}), pH and malic acid (g L^{-1}) by means of Fourier-transform infrared spectroscopy (FTIR) (Table 1).

2.3. Dynamic headspace sampling of volatile compounds

Volatiles were measured by using a dynamic headspace (DHS) technique system followed by gas-chromatography–mass spectrometry (GC–MS) analysis based on the procedure developed in our laboratories for different fruit products (Rapparini & Predieri, 2002; Rapparini & Rotondi, 2002). This procedure was optimised for determination of polar and nonpolar volatile compounds in the headspace of the wine samples and modified as follows. Optimal headspace parameters were: wine aliquots of 30 mL were transferred into a 50-mL glass container and placed in a thermostatic bath kept at 25 °C for 5 min to reach equilibrium conditions and to prevent alteration of the volatiles. DHS sampling was performed by fluxing 0 into the glass container.8 L of pure He at a rate of 100 mL min^{-1}, which is within the range recommended for the tube dimensions used and for Tenax (Ciccioli, Brancaleoni, Frattoni, & Maris, 2002). The gas inlet was located at 0.5 cm from the bottom of the extraction vessel. The outlet of the extraction system was connected to a sampling trap consisting of a glass tube filled with 0.120 g of Tenax TA® (Restek Corp., Bellefonte, PA),

Table 1
Standard analyses on microvinificates[a] obtained from red grapes (*Vitis vinifera* L. cv. Groppello) treated with different agrochemicals during the phytoiatric campaigns 2009 and 2010.

	Ethanol[b] (%, v/v)	Reducing sugars[b] (g L^{-1})	Total titratable acidity[b] (g tartaric acid L^{-1})	Total volatile acidity[b] (g acetic acid L^{-1})	pH[b]	Malic acid[b] (g L^{-1})	Total extract[b] (g L^{-1})
Vintage 2009							
CHT[c]	11.1	1.0	6.3	0.50	3.54	2.5	24.9
CHT + Cu[d]	10.7	1.6	5.3	0.62	3.62	2.7	23.4
BTH[e]	9.7	1.8	5.2	0.59	3.64	1.5	20.7
Conventional fungicides[f]	11.3	1.6	6.4	0.53	3.48	2.4	21.1
Vintage 2010							
CHT	10.7	<2	6.6	0.46	3.54	3.57	27.4
CHT + Cu	11.9	<2	6.3	0.42	3.51	3.10	26.5
BTH	11.4	<2	6.5	0.47	3.50	3.45	26.1
Conventional fungicides	12.4	<2	6.6	0.38	3.39	2.83	25.5

[a] Experimental wines were produced by standard microvinification techniques in the Centro Vitivinicolo Provinciale di Brescia (Italy).
[b] These parameters were determined by Fourier transform infrared spectroscopy (FTIR).
[c] 0.03% Chitosan.
[d] 0.03% Chitosan + 150 g hL^{-1} copper hydroxide.
[e] 0.3 mM Benzothiadiazole.
[f] Penconazole + methyldinocap (positive control).