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Influence of the use of fungicides on the volatile composition of Monastrell red wines obtained from inoculated fermentation

J. Oliva [a], A.M. Martínez-Gil [b], C. Lorenzo [b], M.A. Cámara [a], M.R. Salinas [b], A. Barba [a], T. Garde-Cerdán [c,*]

[a] Dpto. Química Agrícola, Geología y Edafología, Universidad de Murcia, Campus de Espinardo s/n, 30100 Murcia, Spain
[b] Cátedra de Química Agrícola, E.T.S.I. Agrónomos, Universidad de Castilla-La Mancha, Campus Universitario, 02071 Albacete, Spain
[c] Instituto de Ciencias de la Vid y del Vino (Gobierno de La Rioja-CSIC-Universidad de La Rioja), Ctra. Mendavia-Logroño NA 134, Km. 90, 26071 Logroño, La Rioja, Spain

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ABSTRACT

The influence of six fungicides (famoxadone, fenhexamid, fluquinconazole, kresoxim-methyl, quinoxyfen and trifloxystrobin) on the volatile composition of red wines obtained from inoculated fermentation was studied. Although treatments were carried out under critical agricultural practices (CAP), the residues in the wines were below their maximum residue limit (MRL). Ethyl decanoate was the compound most influenced by these fungicides, while diethyl succinate, decanoic acid, β-ionone, and citronellol concentration were not changed with any of the treatments. The treatment of grapes with trifloxystrobin induced changes in only one volatile compound, and the variation in volatile composition of wines from grapes treated with fenhexamid, fluquinconazole and quinoxyfen compared to control wines was almost negligible invaluable. The treatment with famoxadone influenced more volatile compounds than the other ones, except for wine from grapes treated with kresoxim-methyl, which was the only wine that showed a big change in its aromatic composition.

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1. Introduction

The use of fungicides for the control of pest in the vineyard shows the risk of residues of these compounds in grapes, and their transfer to wines elaborated with these grapes, implying a health hazard. For this reason, their maximum residue limits (MRLs) are controlled by the current legislation. Moreover, these residues can affect the yeast involved in the fermentative process. Hence, their effect on yeast population and fermentation have been studied by several authors (Calhelha, Andrade, Ferreira, & Estevinho, 2006; Comitini & Ciani, 2008; Cus & Raspor, 2008; González-Rodríguez et al., 2011a; Noguerol-Pato, Torrado-Agrasar, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2014; Oliva, Navarro, Barba, Navarro, & Salinas, 1999; Zara et al., 2011). Some suggest that depending on the type of fungicides the effect will be different, as they show specificity for certain species (Cadez, Zupan, & Raspor, 2010). Thus, some types of fungicides can slow down fermentation (Cabras et al., 1999; Navarro, García, Navarro, Oliva, & Barba, 1997), and in extreme cases they may even stop it (González-Álvarez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2012), while other types of fungicides do not affect it (Cabras & Angioni, 2000; Oliva et al., 2007; Ubeda, Briones, & Izquierdo, 1996).

The aroma of a wine is one of the most important characteristics in defining its quality. Since several wine volatile compounds are produced during fermentation, the potential impact of fungicide residues on their biosynthesis, and so on the total wine aroma, is a matter of a great concern. In fact, new phytosanitary products used to control fungal diseases should be completely inactive against fermentative microflora (González-Álvarez et al., 2012). To our knowledge, there are few data about the influence of new-generation fungicides on aroma biosynthesis (Noguerol-Pato et al., 2014), on volatile composition of white wines (García et al., 2004; González-Rodríguez, Noguerol-Pato, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2011b; González-Álvarez et al., 2012), and red wines (Noguerol-Pato, González-Rodríguez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2011; Oliva, Zalacain, Payá, Salinas, & Barba, 2008).

For these reasons, the aim of this work was to study the influence of the use of several fungicides widely used in the vineyard on the volatile composition of red wines obtained from inoculated fermentation. Individual treatments at the recommended doses were performed with the selected fungicides under critical agricultural practices (CAP).

* Corresponding author. Tel.: +34 941291383; fax: +34 941291392.
E-mail address: Teresa.GardeCerdan@gmail.com (T. Garde-Cerdán).

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2. Materials and methods

2.1. Chemicals

Analytical standards of the fungicides (purity ⩾ 95%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany); stock standard solutions of 1 mg/l were prepared by accurately weighing individual analytical standards in volumetric flaks and dissolving and diluting them to volume with acetonitrile.

The active ingredient used (Table 1) at the doses recommended by the manufacturer in the experiments were: Famoxadone [3-anilino-5-methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione] with effective preventive effects and broad fungicidal spectrum. Fluquinconazole [3-(2,4-dichlorophenyl)-6-fluoro-2-(1H-1,2,4-triazol-1-yl)quinazolin-4(3H)-one] with protective, eradicative and systemic properties; it is used to control *Uncinula necator*. Kresoxim-methyl [methyl (E)-methoxyimino[2-(o-tolyloxymethyl)phenyl] acetate] is an oximinoacetate (strobilurin type) with protective, curative, eradicative and long residual disease control: it is used to control *U. necator* in grapes. Quinoxyfen (5,7-dichloro-4-quinolyl-4-fluorophenyl ether); it is a mobile, protective fungicide; it is used in grapes for the control of powdery mildew (*U. necator*). Fenhexamid (2,3-dichloro-4-hidroxy-1-methylcyclohexanecarboxanilide) with protective action and is not translocated; it is used to control *Botrytis cinerea* in grapes. Trifloxystrobin [methyl(E)-methoxyimino-{(E)-α-[1-(α,α,α-trifluoro-m-tolyl)ethyl ideneaaminooxy]-0-tolyl}acetate] is the main active ingredient for treating downy and powdery mildews that can be present in grapes and wines.

2.2. Plant materials

Red grapes *Vitis vinifera* var. Monastrell were harvested in an experimental plot in Jumilla, Murcia (SE Spain). The nutritional state and physiological conditions of the grape were suitable to give quality wines.

2.3. Fungicide treatments and sampling

Seven experimental plots of 225 m^2 were selected (one control and six for the individual treatments with the fungicides under study). All treatments were performed under critical agricultural practices (CAP), i.e., six hours before grape collection. Despite this, the final results in wines for the commercially products used were famoxadone 0.12 mg/l, trifloxystrobin < 0.05 mg/l; fluquinconazole < 0.05 mg/l, quinoxyfen < 0.01 mg/l; fenhexamid 0.89 mg/l, and kresoxim-methyl 0.08 mg/l, all of these under their MRL (Table 1). Table 1 also shows the application doses. The experimental plots had not received previous treatment with the fungicides studied and at the time of application, grapes were exempt from any pesticide residues.

Grape samples (15 kg) were destemmed and crushed, and then 80 mg/l of SO_2 was added. Musts were inoculated with active dry yeast UCLM S377 (Anfiquimica SL) following the protocol of the manufacturer. Samples were introduced in the fermenters and maintained in dynamic maceration for 8 days at controlled temperature (24–28 °C). After alcoholic fermentation, wines were decanted, clarified and filtered. All the vinifications were carried out in duplicate.

Wine samples (1 l) of each of the selected plots were collected and wine samples were frozen at −30 °C until analysis.

2.4. Analysis of volatile compounds by gas chromatography

The wine volatile compounds were extracted by stir bar sorptive extraction (SBSE) according to Lorenzo, Garde-Cerdán, Pedroza, Alonso, and Salinas (2009) and analysed by GC–MS. The volatile compounds were extracted from wines by introducing the polydimethylsiloxane (PDMS) coated stir bar (0.5 mm film thickness, 10 mm length, Twister, Gerstel, Mülheim and der Ruhr, Germany) into 10 ml of sample, to which 100 μl of internal standards γ-hexalactone and 3-methyl-1-pentanol solution at 1 μl/ml, both in absolute ethanol (Merck, Darmstadt, Germany) was added. Samples were stirred at 500 rpm at room temperature for 60 min. The stir bar was then removed from the sample, rinsed with distilled water and dried with a cellulose tissue, and later transferred into a thermal desorption tube for GC–MS analysis. In the thermal desorption tube, the volatile compounds were desorbed from the stir bar under the following conditions: oven temperature, 330 °C; desorption time, 4 min; cold trap temperature, −30 °C; helium inlet flow 45 ml/min. The compounds were transferred into the Hewlett–Packard LC 3D GC–MS (Palo Alto, USA) with a fused silica capillary column (BP21 stationary phase 30 m length, 0.25 mm i.d., and 0.25 μm film thickness; SGE, Ringwood, Australia). The chromatographic program was set at 40 °C (held for 5 min), raised to 230 °C at 10 °C/min (held for 15 min). The total time analysis was 36 min. For mass spectrometry analysis, electron impact mode (EI) at 70 eV was used. The mass range varied from 35 to 500 a, and the detector temperature was 150 °C. The analysis of volatile compounds in the wines was done in duplicate, and since the fermentations were done in duplicate, the results shown for these compounds were the mean of 4 analyses. Identification was carried out using the NIST library and by comparison with the mass spectrum and retention index of chromatographic standards designed by us and data found in the bibliography. Quantification was based on five-point calibration curves of respective standards (Aldrich, Gillingham, England) (R^2 > 0.94) in a 12% ethanol (v/v) solution at pH 3.6.

2.5. Analysis of fungicide residues

Analytical determination of fluquinconazole was performed with GC-ECD, while that of famoxadone was with LC-DAD, after extraction with acetone and ethyl acetate–hexane (1:1 v/v) (Oliva et al., 2007). The other fungicides were determined using GC–MS/MS and LC–MS/MS, after extraction for the modified version of the QuEChERS method (Payá et al., 2007).

2.6. Statistical analysis

The statistical elaboration of the data was performed using SPSS Version 17.0 statistical package for Windows (SPSS, Chicago, IL).

Table 1
Fungicide treatments, dose, pre-harvest interval (PHI) and maximum residue limit (MRL).

Fungicide	Commercial name	Manufacturers	Dose (kg/ha)	PHI (days)	MRL (EU) (mg/kg)
Famoxadone	Equation Pro GR (22.5%)	Dupont Ibérica	0.4	28	2
Fenhexamid	Teldor WG (50%)	Bayer Hispania	1	14	5
Fluquinconazole	Castellan GD (25%)	Argos Schering AgrEvo	0.4	21	0.5
Kresoxim-methyl	Stroby WG (50%)	BASF	0.2	35	1
Quinoxyfen	Arius SC (25%)	Dow Agro Science	0.3	28	1
Trifloxystrobin	Flint WG (50%)	Bayer Cropscience	0.15	28	5

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