UDC 663.952:663.14:663.2
ISSN 1330-9862

original scientific paper

(FTB-1286)

Influence of Quinoxyfen Residues on *Saccharomyces cerevisiae* Fermentation of Grape Musts

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Received: December 2, 2003
Accepted: April 15, 2004

Summary

The effect of Quinoxyfen, a new pesticide against powdery mildew, on the fermentation of *Saccharomyces cerevisiae* has been evaluated. When vines (Montepulciano d'Abruzzo, Trebbiano and Sangiovese) were treated with doses recommended by the producer (30 mL/hL of a suspension concentrate 250 g/L), Quinoxyfen was detected up to the concentration of 0.014 mg/L in the must. The *S. cerevisiae* growth parameters, μ_{max} and lag phase, were not affected by this residual level during fermentation. However, in must fortified with Quinoxyfen to obtain the concentrations of 0.5, 1.0, 2.0 mg/L, a decrease in the lag phase was observed. The fermentation kinetics did not show any significant differences between the different treatments and control musts. Moreover, the production of volatiles during fermentation, determined with solid phase microextraction – capillary gas chromatography (SPME-GC), was not affected by the residual level of Quinoxyfen. Principal component analysis (PCA) showed that the samples could be clustered according to the different yeast strains, regardless of the pesticide treatment.

Key words: Quinoxyfen, pesticide residues, fermentation, aroma compounds, SPME-GC

Introduction

The use of fungicides in viticulture is a procedure of major importance for vineyard protection. At present, preventive spray programs are applied for disease control against powdery mildew (*Uncinula necator*), downy mildew (*Plasmopara viticola*) and grey mould (*Botrytis cinerea*).

Grape maceration, pressing, racking, and must clarification and filtration can influence the content of fungicide residues that can be removed or degraded (*1–5*). Factors such as the initial concentration of pesticide residues in harvested grapes and the physico-chemical characteristics of the product exert an important role in their disappearance. Pesticide residues can negatively affect the growth, viability and fermentative activity of yeasts (*6–9*) or stimulate the yeast to produce more alcohol (*10*) due to a higher sugar concentration in healthy grapes with respect to the contaminated ones. More-

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over, some fungicides can reduce the concentration of secondary compounds of fermentation (*11*), affecting the biosynthesis of ergosterol or amino acids and mitochondrial electron transport chain (*12–17*).

A new fungicide product, 5,7-dichloro-4-(4-fluorophenoxy)-quinoline (IUPAC), having specific activity towards powdery mildews and commonly known as Quinoxyfen™ (Dow-Agrosciences, USA), has recently been developed. It has been shown that Quinoxyfen displays its activity during the pre-infection developmental stages by suppressing germination, early germ tube development and/or appressoria formation (*18*). Control of powdery mildew of grapes using Quinoxyfen has been reported (*19–22*), but not its influence on must fermentation and wine quality.

In this paper we studied the influence of Quinoxyfen treatment on the kinetics of *S. cerevisiae* during three different vintages (1999–2001) and its effects on the production of some secondary products of fermentation.

Materials and Methods

The trials were carried out in experimental vineyards, located in three different areas of Italy. A random-block scheme was used, with four replications for each test. The treatments were performed in the spring of 1999, 2000 and 2001, according to the following experimental program:

*Experiment 1: **Montepulciano d'Abruzzo***

In order to evaluate the effect of the dose of Quinoxyfen applied in the field on the must fermentation, in 1999 a preliminary study was carried out on fifteen-year-old grapevine plants (cv. Montepulciano d'Abruzzo) located in Montelabbate (Pesaro). Plots consisted of 20 plants grown on a cordon trellis with a planting distance of 4 m between rows and 1.5 m in a row. Between each treatment plot, two rows were left as a buffer zone. A total of nine applications of Quinoxyfen (250 g/L) at doses of 20, 30 and 40 mL/hL was applied. The treatment began in spring, 15–20 days after the bud break, when the shoots were 25–30 cm long, and continued throughout the season at 14–15 day intervals; untreated plots were used as control.

The musts obtained from these vines were inoculated with three different strains of *S. cerevisiae*: SC635, SC632 and SC404.

*Experiment 2: **Sangiovese and Trebbiano***

In 2000, the field trials on the Sangiovese cultivar were set up in Pontassieve, a hilly area in Tuscany. The field trials for the Trebbiano variety were established in the area of Argenta, located in the Po Valley (Ferrara, Italy). Plants were grown on a cordon trellis with a planting distance of 4 m between rows and 1.5 m in a row. Experimental design was a randomised complete block with 4 replications; plots consisted of 30 plants. Between each treatment plot, two rows were left as a buffer zone. In order to protect the grapes against pest attack, both Sangiovese and Trebbiano grapevines were sprayed with a commercial formulation of fungicides. A water suspension of commercial Quinoxyfen (250 colloidal suspension) at a concentration of 30 mL/hL for each application was used against powdery mildew. Five applications of the commercial formulation of the fungicides were used with a 14-day interval beginning in spring and continuing throughout the season. Untreated plots were used as control. The treatment was suspended 28 days before harvesting. In each treatment, the application of the product was carried out by using a senior knapsack sprayer of 10 L with a conic opening of 2 mm and with a pressure level of 3 atm.

*Experiment 3: **Effect of pesticide residues on the production of secondary aroma compounds***

Secondary aroma compounds of the fermentation produced by five different strains of *S. cerevisiae* were studied on must derived from the vines treated in 2001. With this aim, two different experiments (3A and 3B) were conducted in a ten-year-old vineyard (cv. Sangiovese) located at Lucca (Tuscany). Blocks and planting distance were the same as those used in Experiment 2. The experiment is shown in Table 1. The control samples were untreated vines. In both studies, the commercial Quinoxyfen 250 colloidal suspension diluted at the concentration of 30 mL/hL was used for each application. The treatments began in spring.

Table 1. Quinoxyfen treatment during year 2001

Experiment 3A	Experiment 3B
Last Quinoxyfen treatment before the harvest/day	Number of treatments during the season
28	1 (May)
21	2 (May, June)
14	3 (May, June, July)
7	4 (May, June, July, August)

In the third experiment (3C), *S. cerevisiae* SC635 fermentation was evaluated in pasteurised Sangiovese musts, derived from the untreated vines, fortified with aliquots of Quinoxyfen (pure compound) in order to obtain final concentrations of 0.5, 1.0 and 2.0 mg/L. Untreated must was used as control.

Microvinifications

For vinifications of both white and red grapes from Experiments 1 and 2, four replicates were obtained from four subplots randomly selected in the field. After crushing, small-scale fermentations were carried out using 450 mL of must with added 80 mg/L of potassium metabisulfite. The must was inoculated with a 48-h preculture of *S. cerevisiae* (5 %) and incubated at (21±1) °C. The fermentation kinetics was obtained by plotting daily mass loss due to CO_2 evolution (g/100 mL) versus time. The data were analysed according to the Gompertz equation as modified by Zwietering *et al.* (*23*):

$$y = A \cdot \exp\left\{-\exp\left[\left(\frac{\mu_{max} \cdot e}{A}\right) \cdot (\lambda - t) + 1\right]\right\}$$

where y is CO_2 percentage at time t (hours), A (as g/100 mL of must) represents the maximum integrated value for CO_2 production (when $t \rightarrow \alpha$) expressed as percentage, μ_{max} is the maximum CO_2 production rate (as %/(h·100 mL)), λ is the lag time (h) for CO_2 production and t is the fermentation time in hours.

The strains of *Saccharomyces cerevisiae*, SC635, SC632, SC692, CV41 and SC404, used in the microvinifications belonged to the collection of DIPROVAL (Dipartimento di Protezione e Valorizzazione Agroalimentare, University of Bologna, Italy).

After 21 days of fermentation, the wines were filtered and stored at –30 °C until the analytical determinations.

Chemical analyses of musts

The must samples were analysed for pH, reducing sugars, tartaric acid, citric acid, malic acid and total acidity, according to the methods described in the EU Official Gazette (*24*).

Quinoxyfen extraction

The fungicide standard Quinoxyfen was supplied by Dow-AgroSciences. Quinoxyfen was purified from the samples according to Khoshab *et al.* (*25*). A volume of 10 mL of the sample was placed into a 50-mL vial, to which 10 mL of sodium bicarbonate solution (5 g/100 mL) and 10 mL of hexane were added. The solution was shaken for 15 min and centrifuged for 5 min at 2000 rpm. An aliquot (2 g) of sodium sulphate was introduced into the vial to prevent the formation of gel. The hexane fraction was transferred into a 30-mL vial and the extraction was repeated. The extracts were evaporated in a heating block at 40 °C under a gentle nitrogen stream. The residue was reconstituted in 2 mL of 0.1 % corn oil in trimethylpentane and sonicated for 1 min. This solution (1 μL) was injected in GC-MS.

Gas chromatography – mass spectrometry (GC-MS) determination of Quinoxyfen

A 3400 GC (Varian, Italy) equipped with an ITS40 mass spectrometry detector (Finnigan, United Kingdom) was used. The fused silica capillary column (30 m x 0.25 mm i.d.) was a ZB-5 Phenomenex (Torrance, USA), coated with 5 % phenyl-polysiloxane (0.25 μm film thickness). The samples were splitlessly injected; the splitting valve was opened for 30 s after the injection. The carrier gas flow (helium) was 1.40 mL/min. The temperature of the injector and the transfer line were set at 300 °C. The oven temperature was maintained at 60 °C for 1 min and then raised to 220 °C at a rate of 8 °C/min. After an isothermal step at 220 °C for 3 min, the temperature was increased to 300 °C and the column was purged for 5 min. The mass detector was used in the electronic impact (70 eV) mode. The emission current was 10 μA and the scan rate was 1 scan/s; a solvent delay of 180 s was used before the acquisition started.

A calibration curve was obtained by injecting standard solutions with Quinoxyfen at different concentrations: 0 (blank solution), 10, 100, 333, 667 and 1000 μg/L. Each solution was injected in four replications except the blank solution (injected in three replications). The coefficient of determination (r^2) for the regression line was 0.9470 for 23 injections. The detection limit for Quinoxyfen was 2 μg/L and quantification limit was 6 μg/L.

Solid phase microextraction – gas chromatography (SPME-GC) analysis of volatile compounds

Volatile compounds were determined with solid phase microextraction (SPME) coupled with gas chromatography according to De la Calle-Garcia *et al.* (*26*) and Vas *et al.* (*27*). The fiber used for SPME was coated with a polyacrylate layer of 8.5 μm thickness (Supelco, USA). For quantitative determination, a CP 380 capillary gas chromatograph equipped with an 8200 autosampler SPME III (Varian, Italy) was used. The fused silica capillary column was a CP-Wax 52 CB (50 m x 0.32 mm) by Chrompack (The Netherlands), coated with polyethyleneglycol (film thickness 1.2 μm), as stationary phase. The injector and FID temperature was 250 °C. The temperature program was the following: initial temperature (50 °C) held for 2 min; first ramp, 1 °C/min to 65 °C (0 min hold); second ramp, 10 °C/min to 150 °C (10 min hold); third ramp 10 °C/min to 200 °C (1 min hold). The carrier gas (N_2) flow rate was 2.5 mL/min.

The aroma compounds were identified by comparing the retention time of standards and their identification was confirmed by using GC-MS. The quantitative analysis of wine aroma compounds was carried out on the basis of the relative peak area (Qi) calculed from head space SPME (HS/SPME) gas chromatograms after the addition of known amounts of analyte standards, as well as the internal standard according to De la Calle-Garcia *et al.* (*26*) and Vas *et al.* (*27*).

Statistical analysis

One-way analysis of variance and least significant difference (LSD) were used to analyse mean differences in mean values, if any, at 95 and 99 % accuracy level. Principal component analysis (PCA) was used in order to group homogeneous samples and determine the most significant variables affected by Quinoxyfen residual level.

Results and Discussion

Influence of Quinoxyfen on the must

A preliminary research was performed on Montepulciano d'Abruzzo in Montelabbate (Pesaro, Italy) in 1999. Various grape blocks were subjected to treatments with different Quinoxyfen concentrations (20, 30 and 40 mL/hL) and the musts were inoculated with *S. cerevisiae* strains SC635, SC632 and SC404. The sugar content in control musts or musts obtained from the grapes treated with a low Quinoxyfen dose (20 mg/hL) was lower than in those treated with high doses (30 and 40 mg/hL). However, the fermentation performances were not affected by the treatment doses. In fact, the fermentation dynamics showed only negligible differences, which could be attributed to different sugar concentrations (Table 2).

In the vintage 2000, higher sugar concentrations in musts from Trebbiano and Sangiovese cultivated in the Po Valley and in Tuscany, respectively (Table 3), were

Table 2. Gompertz parameters obtained from the fermentation of *S. cerevisiae* in treated and untreated Montepulciano d'Abruzzo must

	20 mL/hL	30 mL/ hL	40 mL/ hL	Untreated
γ(sugar)/(g/L)	14.27 ± 2.45	15.57 ± 3.43	15.49 ± 2.79	14.37 ± 2.86
S. cerevisiae 404				
λ/h	21.86 ± 1.25	25.30 ± 1.98	25.30 ± 1.30	24.42 ± 1.54
A/(g/100 mL)	10.02 ± 0.54	10.52 ± 0.23	10.75 ± 0.61	9.81 ± 0.24
μ_{max}/(%/(h·100 mL))	0.59 ± 0.09	0.70 ± 0.10	0.60 ± 0.10	0.56 ± 0.07
S. cerevisiae 632				
λ/h	26.65 ± 2.07	38.59 ± 1.47	33.57 ± 2.55	34.15 ± 2.89
A/(g/100 mL)	9.49 ± 0.34	10.46 ± 0.26	11.22 ± 0.28	9.60 ± 0.18
μ_{max}/(%/(h·100 mL))	0.39 ± 0.01	0.55 ± 0.07	0.64 ± 0.05	0.52 ± 0.05
S. cerevisiae 635				
λ/h	26.67 ± 1.87	32.70 ± 2.03	70.03 ± 2.31	30.91 ± 1.67
A/(g/100 mL)	9.60 ± 0.65	10.50 ± 0.78	11.22 ± 0.43	10.44 ± 0.61
μ_{max}/(%/(h·100 mL))	0.57 ± 0.06	0.72 ± 0.05	0.67 ± 0.01	0.53 ± 0.05

Mean and standard deviation from 4 determinations

Table 3. Composition of must derived from treated and untreated Sangiovese and Trebbiano Romagnolo vines

	Trebbiano Romagnolo must		Sangiovese must	
	Treated	Untreated	Treated	Untreated
pH	3.17 ± 0.07* a	3.21 ± 0.03a	3.12 ± 0.03b	3.09 ± 0.05b
γ(reducing sugars)/(g/L)	184 ± 6.1a	172 ± 7.3b	209 ± 9.60c	199 ± 11.0d
γ(tartaric acid)/(g/L)	5.16 ± 0.1a	6.16 ± 6.2b	5.33 ± 0.04c	5.46 ± 0.06c
γ(citric acid)/(g/L)	0.20 ± 0.05a	0.27 ± 0.03b	0.15 ± 0.02a	0.14 ± 0.04a
γ(malic acid)/(g/L)	2.50 ± 0.3a	3.05 ± 0.15b	1.63 ± 0.1c	1.72 ± 0.15d
γ(total acidity)/(g/L)	7.50 ± 0.2a	7.20 ± 0.1b	6.23 ± 0.04a	6.36 ± 0.06b
γ(Quinoxyfen residual level)/(mg/L)	< 0.002**	< 0.002	0.012	<0.002

* Mean value of 4 samples; ** detection limit of the method
Numbers in one row with different letters are significantly different at p>0.05 according to the Fisher's LSD mean comparison

correlated with healthy grapes from which the powdery mildew was absent. Significant differences, observed in malic and tartaric acid contents of treated and untreated must, were not correlated with the residual Quinoxyfen content (p<0.05). In fact, it is well known that the natural chemical composition of must and the relative ratios between ripening markers (sugars and acids) can notably vary in relation to pedoclimatic, agronomical, varietal and technological factors (*28,29*). In Trebbiano musts, Quinoxyfen residues were not detectable (<0.002 mg/L), whereas in Sangiovese musts, they were present at a concentration of 0.012 mg/L, thus lower than the limit (MRL 0.5 mg/L) prescribed by Italian legislation. After vinification of these musts, the Quinoxyfen residues in the wines were under the detection limit. Cabras *et al.* (*30*) reported a $t_{½}$ of 7.2 days for Quinoxyfen in grapes. On the other hand, the residual fungicide concentration in musts has been reported to be related to the physico-chemical properties of the active compound, to its solubility in a hydroalcoholic solution and also to the wine-making procedure (*10,30–32*). In particular, Quinoxyfen tends to be distributed in the solid fraction (skins and lees). It has also been suggested that Quinoxyfen content should decrease in wine because it can be partially degraded by *S. cerevisiae* and significantly absorbed by yeasts deposited in lees (*30*).

Effect of Quinoxyfen on fermentation

The strain of *S. cerevisiae* SC635, which showed a good fermentation performance in Experiment 1, was used as inoculum for fermentation in the must obtained from grapes treated with Quinoxyfen and from the control grapes from the vintage 2000 (Experiment 2). The results are reported in Table 4.

The regression coefficients of the growth curve ranged between 0.987 and 0.998. The treatment of vines with Quinoxyfen did not significantly affect the fermentation dynamics of *S. cerevisiae*: the lag phase and growth rate were similar in all the musts. The maximum CO_2 production (A_{max}) in the treated Sangiovese musts was significantly higher (p<0.05) than that in the untreated

Table 4. Gompertz parameters obtained from the fermentation of *S. cerevisiae* SC635 in treated and untreated Sangiovese and Trebbiano Romagnolo must

	Sangiovese must		Trebbiano Romagnolo must	
	Treated	Untreated	Treated	Untreated
A/(g/100 mL)	13.33 ± 1.00a	12.52 ± 1.1b	12.60 ± 1.10c	11.42 ± 1.20d
μ_{max}/(%/(h·100 mL))	0.31 ± 0.05a	0.36 ± 0.03a	0.14 ± 0.04b	0.13 ± 0.07b
λ/h	44.53 ± 5.80a	45.77 ± 6.40a	30.45 ± 9.60b	32.28 ± 5.40b

* Mean and standard deviation from 4 determinations
A: (g/100 mL); μ_{max}: maximum growth rate (%/(h·100 mL)); λ: lag phase length (h)
Numbers in one row with different letters are significantly different at p>0.05 according to the Fisher's LSD mean comparison

must, probably due to higher sugar content (Table 4). A similar trend was observed in Trebbiano must.

In the vintage 2001, the sugar content in control must was lower than in musts treated with Quinoxyfen (Tables 5 and 6), probably due to the decreased powdery mildew attack on the treated vines. The μ_{max} and λ values of *S. cerevisiae* showed significant differences depending on the strains, but not related to the residue level. Cabras *et al.* (*10*) reported that the fermentation was regular for yeasts and lactic acid bacteria starter cultures in the presence of Quinoxyfen.

Influence of Quinoxyfen on the secondary compounds of fermentation

Regarding the production of secondary aroma compounds such as higher alcohols (isobutanol, *n*-propylalcohol, isoamyl alcohols, dodecanol, undecanol, and 2-phenyl ethanol) and esters (ethyl octanoate and decanoate) during the fermentation of *S. cerevisiae* SC635 inoculated in Sangiovese and Trebbiano musts (Experiment 2), the statistical analysis did not show any significant differences in the resulting wines (data not shown). In any case, the higher alcohol content did not exceed 350 mg/L.

Table 5. Influence of Quinoxyfen treatment number on the Gompertz parameters obtained from the fermentation of different *S. cerevisiae* strains in treated and untreated Sangiovese must

Number of applications of Quinoxyfen	γ(reducing sugar) g/L	γ(Quinoxyfen residual) mg/L	SC632		SC635		SC692		SC404		CV41	
			μ_{max} %/(h·100 mL)	λ h	μ_{max} %/(h·100 mL)	λ h	μ_{max} %/(h·100 mL)	λ h	μ_{max} %/(h·100 mL)	λ h	μ_{max} %/(h·100 mL)	λ h
control	159.3a	<0.002*	0.010a	11.76a	0.021a	15.62a	0.019a	21.34a	0.013a	14.19a	0.013a	14.57a
1	166.2ac	222.27a	0.008b	12.24a	0.015bc	16.74a	0.013b	20.58a	0.007b	15.07a	0.009b	20.34b
2	150.4a	178.42b	0.009b	11.97a	0.017ac	17.45a	0.026c	21.81a	0.007b	10.53b	0.009b	18.52bc
3	183.0b	252.34c	0.013ab	12.81a	0.016c	15.85a	0.013b	22.63a	0.011a	18.39d	0.013a	16.20ac
4	175.9bc	307.52d	0.010ab	15.59b	0.018ac	15.24a	0.013b	21.58a	0.015a	15.81a	0.010a	18.9bdc

* Mean value of 3 determinations
μ_{max}: maximum growth rate (%/(h·100 mL)); λ: lag phase length (h)
Numbers in one row with different letters are significantly different at p>0.05 according to the Fisher's LSD mean comparison

Table 6. Influence of the last Quinoxyfen treatment before the harvest on the Gompertz parameters obtained from the fermentation of different *S. cerevisiae* strains in treated and untreated Sangiovese must

t(last treatment before harvest) days	γ(reducing sugar) g/L	γ(Quinoxyfen residual) mg/L	SC632		SC635		SC692		SC404		CV41	
			μ_{max} %/(h·100 mL)	λ h	μ_{max} %/(h·100 mL)	λ h	μ_{max} %/(h·100 mL)	λ h	μ_{max} %/(h·100 mL)	λ h	μ_{max} %/(h·100 mL)	λ h
untreated	155a	n.d	0.031a	14.52a	0.014a	10.35a	0.017a	23.35a	0.020a	10.61a	0.027a	22.72a
28	170b	0.191a	0.023b	15.07ac	0.019b	8.58b	0.021b	23.29a	0.025a	16.44b	0.027a	23.90a
21	205c	0.254b	0.020bc	12.24ab	0.018b	10.93a	0.023bc	14.57b	0.032b	14.67c	0.028a	20.83b
14	159a	0.292c	0.018c	14.45a	0.014a	7.89b	0.026c	13.49b	0.019a	15.21bc	0.016b	20.45b
7	147d	0.196a	0.034a	15.83ac	0.031c	11.90a	0.018ab	16.60c	0.021a	14.58c	0.016b	28.23c

Mean value of 3 determinations
μ_{max}: maximum growth rate (%/(h·100 mL)); λ: lag phase length (h)
Numbers in one row with different letters are significantly different at p>0.05 according to the Fisher's LSD mean comparison

Although higher alcohols constitute a relatively lower percentage of the total components in wine, they may undoubtedly affect wine sensory quality. When their concentration exceeds 400 mg/L, higher alcohols are considered as a negative factor (*33*).

In order to group the wines with respect to both treatment and yeast strains used for the fermentation, five strains of *S. cerevisiae* were inoculated in the musts obtained in Experiments 3A and 3B of the vintage 2001. For this purpose, the gas chromatographic data concerning several secondary compounds of fermentation were analysed using PCA.

The results from the Experiment 3A showed that two principal components accounted for 99 % of the variance (Fig. 1). The wines obtained using the strain SC635 appeared as a distinctive group from those obtained by using other strains, regardless of the fungicide treatment. This strain produced a great amount of volatile compounds, namely isoamyl alcohol, ethyl octanoate and 2-phenyl-ethanol. In the wines fermented with SC635 strain, the level of isoamyl alcohol was lower in controls (50E) and in the samples derived from grapes subjected to one treatment only (51E), compared to the remaining wines.

When the strain SC635 was excluded from the PCA, a very homogeneous group characterised by a low production of volatiles was related to the strain CV41, as can be seen in Fig. 2. However, a relation between Quinoxyfen concentration and volatile production was not observed.

Similar results were obtained with musts from the Experiment 3B, considering the effect of the last treatment of vines before the harvest on the secondary compounds of fermentation. The PCA scatter plot of the first two principal components is reported in Fig. 3. The model considering two principal components accounted for 90 % of the variance. In this experiment, two strains were well characterised. The strain CV41 (samples from 1D to 5D) was clearly differentiated from the other strains due to its low production of volatiles. The strain SC632 (samples from 1B to 5B) produced high amounts of volatiles.

Previous studies performed with other pesticides showed that secondary compounds of fermentation were affected by pesticide treatments; in particular, the levels of ethanol and higher alcohols decreased (*11*). On the contrary, we can assume that Quinoxyfen residues, at the levels detected in this work, did not influence the metabolic pathways of higher alcohols in *S. cerevisiae* by direct action. However, literature data show that great differences are observed in the production of volatile compounds, among yeast species and, within each species, among different strains (*34–37*).

Fermentations in fortified must

In order to evaluate the effect of unusually high residue levels of Quinoxyfen (above the minimum residue level, MRL) on fermentation, different amounts of the pesticide (to obtain final concentration of 0.5, 1.0 and 2.0 mg/L) were added to the pasteurised must derived from the untreated vines and the obtained musts were inoculated with *S. cerevisiae* SC635.

In Table 7, the growth parameters obtained from the different fermentations are reported. The statistical analysis showed that the pesticide treatments did not remarkably affect A_{max} and μ_{max}. However, significant differences among the batches were observed in the duration of lag phase, which decreased when Quinoxyfen concentrations increased. These results are interesting, showing that cells adjust to their new environment by induced or repressed enzyme syntheses and activity during the lag

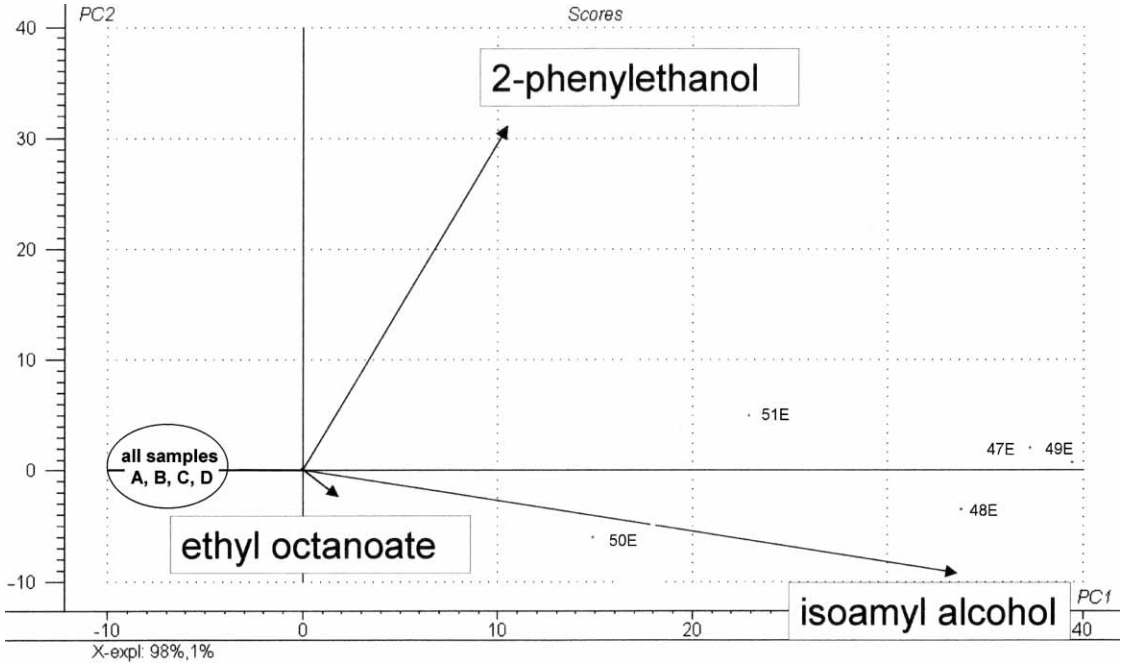

Fig. 1. Principal components analysis of the volatile components of Sangiovese wine obtained from the fermentation of different strains of *S. cerevisiae* in the Experiment 3A. **A**: Strain SC404; **B**: Strain SC632; **C**: Strain SC692; **D**: Strain CV41; **E** Strain SC635. **47:** 4 treatments**;** **48**:3 treatments; **49**: 2 treatments; **50**: 1 treatment; **51**: untreated

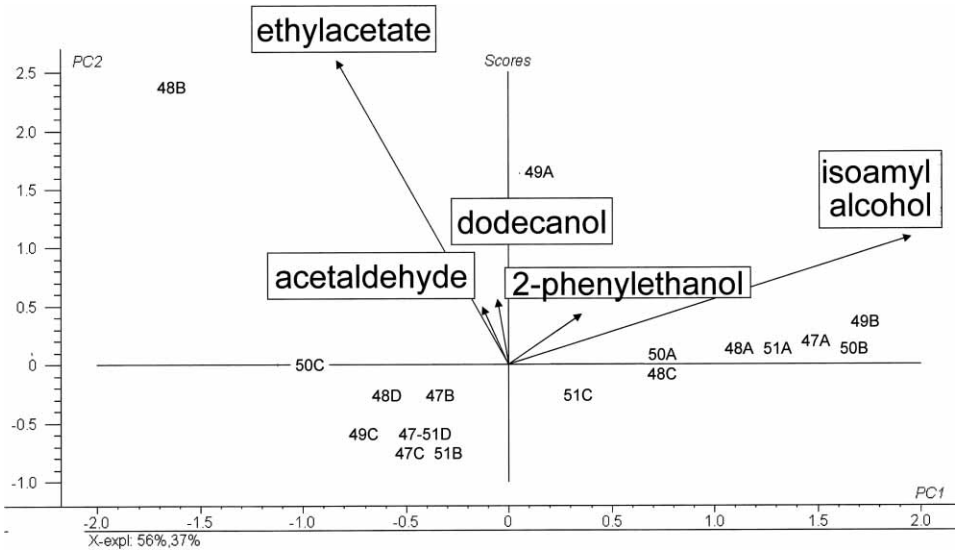

Fig. 2. Principal components analysis of the volatile components of Sangiovese wine obtained from the fermentation of different strains of *S. cerevisiae* in the Experiment 3A: the Strain SC635 was excluded from the elaboration. Same abbreviations as in Fig. 1.

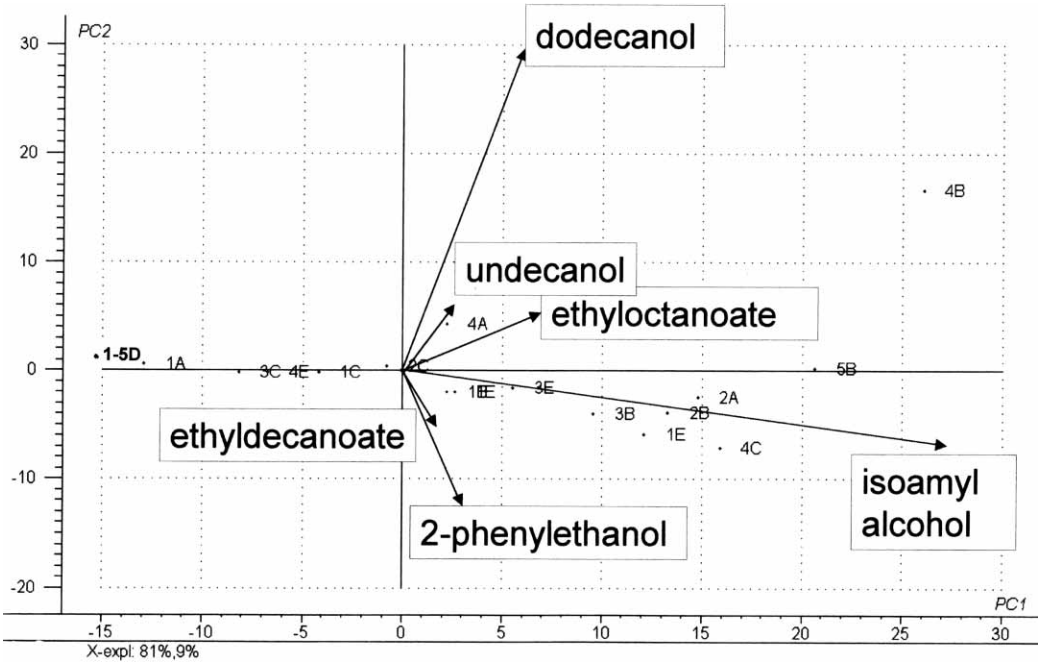

Fig. 3. Principal components analysis of the volatile components of Sangiovese wine obtained from the fermentation of different strains of *S. cerevisiae* in the trial 3B. **A**: Strain SC404; **B**: Strain SC632; **C**: Strain SC692; **D**: Strain CV41; **E** Strain SC635. Last treatment before harvest (day)**: 1**: 28; **2**: 21; **3**: 14; **4**: 7; **5**: control

Table 7. Gompertz parameters obtained from the fermentation of *S. cerevisiae* SC635 in Sangiovese must fortified with different Quinoxyfen levels

γ(Quinoxyfen)/(mg/L)	A_{max}	μ_{max}/(%/(h·100 mL))	λ/h	φ(ethanol)/(mL/100 mL)
control	54.78 ± 1.1a	0.38 ± 0.01a	33.54 ± 1.2a	12.07 ± 0.08a
0.5	56.39 ± 1.0b	0.33 ± 0.01b	28.64 ± 2.1b	11.70 ± 0.1a
1	54.74 ± 0.05a	0.34 ± 0.01b	20.34 ± 1.4c	12.12 ± 0.1a
2	54.78 ± 5.8a	0.36 ± 0.01a	19.99 ± 3.6c	11.92 ± 0.04a

Mean value and standard deviation of 4 determinations
A_{max}: (g/100); μ_{max}: maximum growth rate (%/(h·100 mL)); λ: lag phase length (h)
Numbers in one column with different letters are significantly different at p>0.05 according to Fisher's LSD mean comparison

phase. Thus, at these concentrations, it seems that Quinoxyfen shortened the time taken by yeasts to start multiplication. On the other hand, Cabras *et al.* (*10*) found that the presence of pesticides seems to stimulate a higher production of alcohol from yeasts. This evidence was observed by the same author particularly in the case of *Kloeckera apiculata*, for which two- or three-fold increments of alcohol were detected in the presence of pesticides.

Conclusions

The levels of Quinoxyfen in must from different vintages (1999, 2000 and 2001) did not show any detrimental effects on the growth kinetics of *S. cerevisiae*, not even at concentrations normally found in grapes in field experiments. However, a reduction of the lag phase was observed in the treated must, with concentrations four times higher than those permitted by the Italian legislation (0.5 mg/L), suggesting that Quinoxyfen might induce some modifications of yeast metabolism. With regard to the amounts of secondary compounds of fermentation, the data obtained were similar in the musts produced from treated and untreated vines. In fact, the PCA analysis of the aroma compounds revealed that the production of volatiles during yeast fermentation was more dependent on the yeast strains used as starters than on the Quinoxyfen treatment calendar and residue values in must.

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Utjecaj ostataka Quinoxyfena na fermentaciju *Saccharomyces cerevisiae* u moštu

Sažetak

Ispitan je utjecaj Quinoxyfena, novog pesticida protiv pepelnice, na fermentaciju *Saccharomyces cerevisiae*. Nakon što je, prema uputama proizvođača, loza (sorte Montepulciano d´Abruzzo, Trebbiano i Sangiovese) prskana sa 30 mL/hL suspenzije Quinoxyfena (250 g/L), u moštu je ustanovljena njegova koncentracija od 0,014 mg/L. Ta količina nije utjecala na parametre rasta *Saccharomyces cerevisiae* (μ_{max} i lag faza). Međutim, u moštu obogaćenom Quinoxyfenom do koncentracije od 0,5, 1,0 i 2,0 mg/L opaženo je skraćivanje lag faze tijekom fermentacije. U usporedbi s kontrolnim moštom kinetika fermentacije nije se bitno razlikovala pri različitim postupcima fermentacije. Nadalje, na proizvodnju hlapljivih spojeva tijekom fermentacije, određenih kapilarnom mikroekstrakcijom čvrste faze u plinskom kromatografu, nije utjecala količina Quinoxyfena zaostala u moštu. Analiza glavnih sastojaka pokazala je da se bez obzira na prisutnost pesticida uzorci mogu podijeliti prema različitim sojevima kvasca.