

The Effect of pesticide usage on grape yeast

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Abstract. For the conventional vineyard, reducing the pesticide usage is the most important issue for converting to organic vineyard. In order to study the effect of pesticide usage on grape yeast, an investigation were designed in vintage 2020, ten vineyards (include two organic vineyards) with different treatment frequency index (TFI) were chosen, and the yeast population counted by two method: cultivate method and the direct epifluorescence technique (DEFT). The results show, the grape yeast from Moldova exist the viable but non-culturable (VBNC) state. In organic vineyards, less proportion yeast reach the VBNC, however the opposite conclusion draw from conventional vineyards. High TFI with high level VBNC yeast population, inversely, less culturable yeast. No matter in which yeast state, the treatment of powdery mildew is the most influential factor on yeast population.

1 Introduction

Microbes, especially yeast, is an important role in process of wine fermentation. Temperature, rainfall et.al climatic factors impact on yeast population, the farming method also changes the microbial diversity [1].

There is a trend that more and more vineyards chose to convert to organic farming. For converting the farming method, the most important issue is reducing pesticide usage. Reducing pesticides results higher pests and disease but lower yield. For the microbes, the effect of pesticide is controversial. An investigation from Italy [2] compare one conventional vineyard and one organic vineyard, the results show the fungicide with positive effect on yeast population. But a large-scale three-year study in Portugal [3] came to a completely opposite conclusion. The treatment frequency index (TFI) were extensively used in agriculture section, recently research also use TFI as a index to investigate reducing pesticide in vineyard [4].

Roszak and Colwell [5] described a state of bacteria as VBNC, in the natural condition because of the variety of physical factor and chemical factor, bacteria can reach a VBNC state, which can act as decrease of population if counted by cultivate method, but the direct epifluorescence technique (DEFT) can observed them. The VBNC state microorganisms aren't "dead", the metabolism still work, the VBNC state microorganism can recovery under some conditions. Most previous research focus on the influence of sulfur dioxide

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addition on the VBNC state of wine microbes, but in the process of fermentation, the wild yeast also should be considered, especially in some bio or natural wine.

The main idea of this study is to insight the effect of pesticide usage on grape yeast in two different state, and find out what causes the difference.

2 Material and method

2.1 Samples

The samples are collected in harvest time of vintage 2020. There are ten samples from ten vineyards located in three IGP regions of Moldova. The sampling process under the sterile conditions. The samples and location information in Table1. For each sample, 1 kg grapes were took.

Table 1. Characteristics of samples.

Code	Region	Sampling place
C1	IGP Codru	(E47.41,N27.98)
C2		(E47.06,N28.51)
C3		(E47.22,N28.52)
V1	IGP Vadul lui Traian	(E46.39,N28.73)
V2		(E46.19,N28.63)
V3		(E45.65,N28.47)
S1	IGP Stefan Voda	(E46.53,N29.87)
S2		(E46.48,N29.94)
S3		(E46.53,N29.87)
S4		(E46.53,N29.86)

300g grape berries were washed by 500 mL solution, which containing 2ml/L Tween 80 and 10g/L bacto Soytone. The washing process lasted three hours at 30°C with slow shaking. Before transferring to test tube, the washing solution was filtered.

2.2 Yeast counting

2.2.1 Yeast counting by cultivate method:

For culturable yeast, the method followed V. Renouf^[6], in brief: each sample was plated out on yeast counting plates, in which containing 25g/L agar, 20g/L glucose, 10/L Bactotryptone and 10g/L yeast extrac, using prthophosphoric acid adjusted pH to 5.0. The medium was supplemented with biphenyl (0.015%) and chloramphenicol (0.01%) to inhibit mould development and bacterial growth, respectively. The addition of 0.1% cycloheximide eliminated the saccharomyces spp. And allowed the numeration of the non-saccharomyse yeast population. At 25°C, incubation lasted 5 days for counting the culturable yeast.

2.2.2 Yeast counting by DEFT:

The total viable yeast are counted using a protocol and materials developed by the company Chemunex. Depending on the suspected microbial load, between 0.1 mL and 10 mL of sample are filtered through a membrane (Chemfilter CB04) which is then incubated (30 minutes in the dark and at 30°C) in the presence of the reagents (550µL of buffer, Chemsol B16, supplemented with 15µL of substrate, fluorescein acetate, (ChemChrome V6). The

initially non-fluorescent substrate is cleaved by a cellular enzymatic system and releases a fluorochrome. The latter excited by light radiation of appropriate wavelength (ultraviolet at 480nm) emits green fluorescence. The level of fluorescence intensity is directly related to the integrity of cell membranes and the metabolic activity of cells. When the cells are dead no fluorescence is emitted. Placed between slide and coverslips, the membrane is observed using an epifluorescence microscope (Olympus BX51), using the magnification of 1000 and an appropriate filter (Olympus 467803).

2.2.3 IFT calculation:

Treatment Frequency Index (TFI) is commonly used to measure the pesticide use. TFI, developed by Denmark to monitor intensity of use and not simply volumes of pesticides. It refers to the number of pesticide application per hectare, based on reference doses of commercial products [7]:

$$TFI = \sum \frac{\text{applied_dose}}{\text{reference_dose}} * \frac{\text{treated_area}}{\text{total_area}}$$

TFI were divided in to 3 groups, the detail shows in Table 2.

Table 2. Characteristic of TFI.

Variable	Description
TFI (M)	Fungicide for downy mildew
TFI (F)	Fungicide for powdery mildew
TFI (I)	Pesticide for insects

2.3 Statistical analysis

One-way analysis of variance (ANOVA) and PCA analysis were used, p<0.05 is regarded significant, all statistical analysis were performed by R.4.0.4 for IOS.

3 Results and discussion

The yeast population counted by cultivate method is written as PCM, and PDM is the yeast population counted by DEFT. The general results show in Table 3. There is not so big a total TFI value in Moldova, considering that an average of 20.1 treatments per year in France [8]. The treatment for powdery mildew TFI(F) is higher than others, few pesticide for insect were used.

It should be noted, C2 and SV4 are organic vineyards.

Table 3. Results of TFI and yeast population.

Code	TFI (M)	TFI (F)	TFI (I)	Total TFI	PCM	PDM
C1	5.23	11.12	2.72	19.07	1E+06	2.00E+07
C2	5.60	6.86	0.00	12.46	5E+06	1.00E+06
C3	2.00	4.00	0.00	6.00	1E+06	4.80E+06
VLT1	5.23	11.12	2.72	19.07	8E+05	2.00E+07
VLT2	2.07	8.33	1.11	11.51	1E+06	1.40E+07
VLT3	4.58	5.33	1.00	10.91	4E+06	8.20E+06
SV1	4.71	7.08	2.08	13.87	6E+05	5.10E+06

SV2	0.80	5.00	0.00	5.80	2E+05	9.30E+05
SV3	6.00	8.13	1.00	15.13	2E+06	2.30E+07
SV4	0.00	0.00	0.00	0.00	8E+06	9.10E+05

For the cultivate method, only the culturable yeast can be counted, but in DEFT all viable yeast were counted. Although, different method gives different results, the VBCN exist on grape surface can be confirm by the great difference of yeast population^[9].

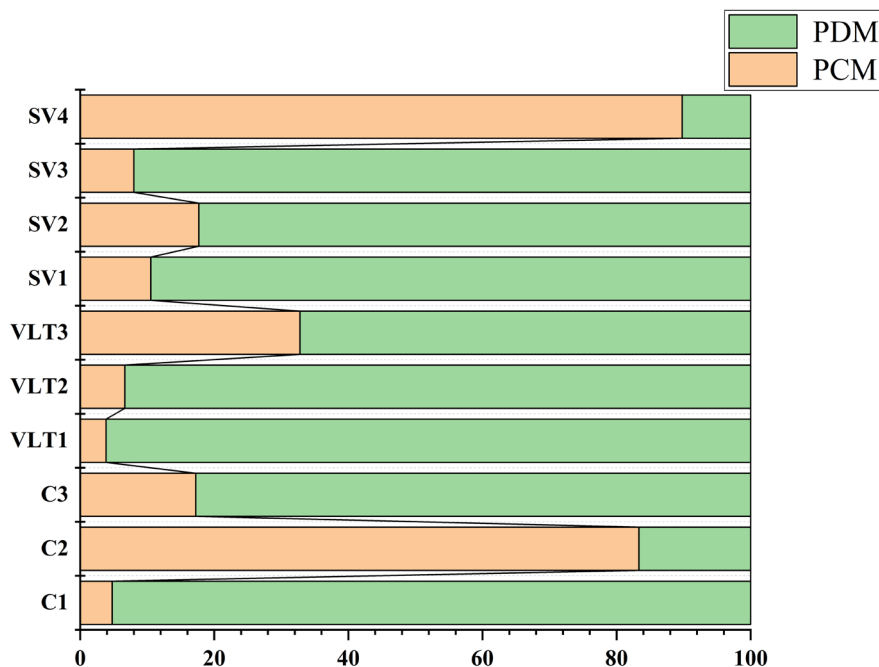


Fig. 1. The yeast ration between DEFT and media.

From Fig.1. The organic vineyards SV4 and C2 show counting from media is obviously bigger than DEFT, it means more yeast can cultivate in this condition. In conventional vineyards sulfur and copper basic fungicide are comment used, although a part of microbes are tolerated, the toxicity can lead yeast to VBNC state [10].

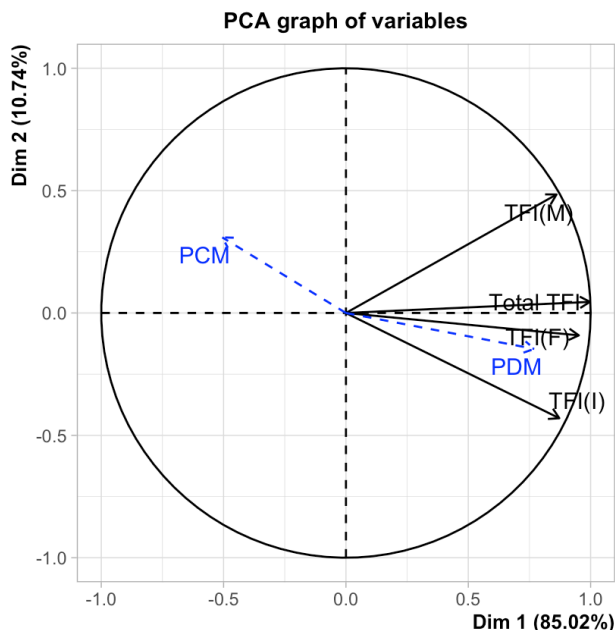


Fig. 2. PCA analysis of TFI impact on yeast population.

From the PCA analysis, all pesticides have positive influence on population by DEFT, but negative influence on cultivate method (except itfi, $P=0.6$). The pesticide usage frequently the less cultivate yeast population is. Sulfur based fungicide is a powerful tool to deal with powdery mildew, it's not surprising that itff has a strong influence on cultivate yeast. For the analysis of DEFT results, all the TFI are relevant with population of yeast, especially TFI(F).

4 Conclusion

In organic vineyards, the culturable yeast have a big proportion in total viable yeast than conventional vineyards. The frequently pesticide usage, the less culturable yeast is, but the more VBNC yeast is. However, fungicide for powdery mildew has the obviously effect on population of yeast, no matter the state of yeast.

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