The environmental impact of viticulture: analysis of the influence type of biofertilisers on wine quality and microbiology activity of soil

Impact environnemental de la viticulture : analyse de l'influence du type de bio-engrais sur la qualité du vin et l'activité microbiologique du sol

B. SIVCEV*, V. RAICEVIC,
N. PETROVIC, N. LEKIC and B. LALIC

Faculty of Agriculture, Belgrade-Zemun, Nemanjina 6,
Serbia and Montenegro
Corresponding author: bsivcev@agrifaculty.bg.ac.yu

Abstract: The trial was conducted in variety/rootstock Riesling/Kober 5 BB in the vineyard district of Vrsac. The vineyard was planted in 1996 on a south-facing slope, with rectangular type pruning of 3x1 m. The training system is of symmetric cordon type and mixed type pruning is practiced. At the beginning of vegetation of grapevine, in the vine row microbiological fertilizer (A-*Azotobacter chroococcum*, AH-*Azotobacter chroococcum*+humate, ABC-*Azotobacter chroococcum*+ *Bacillus megaterium*+*Bacillus circulans*, and ABC+H-*Azotobacter chroococcum*+*Bacillus megaterium*+*Bacillus circulans*+ humate incorporated in the top 20 cm of soil. Control treatment (K-control) was not fertilizer. In investigation years 2003, 2004 and 2005, it was only fertilizer in vineyard. Microbiological fertilizers were soluble in water. Analyses of the influence type of biofertilizers on total number of bacteria and input populations of bacteria were investigated in harvest period. Results point out that total number of bacteria increase, in comparator of control, in all combination of biofertilizers. Population of *Azotobakter choococcum* is more numerous than other bacteria. Analyses of chemical composition of wine and wine testing had shown differences between applied beofirtilizers.

Key words: microbiological fertilizer, yield, grape and wine quality

Introduction

Serbian soils for vinegrape are generaly have not low natural fertility with average organic mater levels and fragile to intensive agricultural practices. Our vineyards are on slope land and erosen eventualy lead to low soil productivity. These problemss are exacerbated when soils are depleted in organic matter (Wilkinson, 2004). In intensive viticultural production mineral fertilizers are largely used. Thereby high yields are provided with somewhat lower grape quality. The soil is maintained in loose condition - during vegetation it undergoes shallow treatment several times, whereby weed species are eliminated. In the phase of intensive growth of vine weeds were competitive with regard to water and mineral matters. Some of them can be potential « hosts » to viruses (Kamasi *et al.*, 1999). Inoculation of plants with certain groups of mocroorganisam offers a possibility of guiding the microbiological process in a desired direction. Beneficial effects of these plant growing-promoting rhizobacteria (PGRP) have been attributed to biological nitrogen fixtation and production of phytohornes that promote root development and proliferation, resulting in efficint uptake of water and nutritions (Hahtela *et al.*, 1990). Interest in the beneficial rhizobacteria associated with cereals has increased recently due to their potential use as biofertilizers (Okon and Labandera-Gonzales, 1994). Nevertheless, several National Institutions, as well as Internatinal Organizations, i.e. FAO, IAEA, WHO etc., have supported the use of biofertilizers (El Kholi *et al.*, 1988).
Biofertilizers - microbiological fertilizers which contain highly efficient kinds of bacteria, fungi and algae provide plants with biogenous elements: nitrogen, phosphorus and potassium (Govedarica *et al.*, 2002). Then there is no pollution of soil, water and atmosphere. *Azotobacter chroococcum* is present in the rhyzosphere of numerous plants, and in some plant species and genotypes it is coming close to associative nitrogen fixators (Raičević, 1996). *Bacillus megaterium* var. *phosphaticus* participates in the process of

amonification, i.e. with the effect of extracellular proteolytical and nucleolytical enzymes it dissolves nucleoproteides (Šutić and Radan, 2001). In that way phosphorus is transformed to inorganic form, available to the plant (Džamić and Stevanović, 2000). The needs of vine for potassium are great. The soil contains a silicate bacteria *Bacillus circullans* which dissolves alumosilicates from which potassium is released. The results of Raicevic *at al.* (2003) show that the total number of bacteria increase in comparation with the control, in the all combination of fertilizers. The greatest number of bacteria was recorded in hte case of inoculation with AH ($9.12x10^7$) and at the depth of 20-40 cm, where the lagest mass of active grapevine root-stock is situated.
The experiment are designed to comepare three microbiological fertilizers to ethance plant growth and nutrition with azote, phosphorus, pothasium and determined their influence on the yield and quality of grapes of the cv. Riesling.

Material and methods

The experiment was conducted in the region of south Banat, AD « Vrsacki vinogradi », at the bigest vinegrowing and winemaking company in Serbia.
Sandy chernozem in Serbia are found in chernozem regions in a transitional zone between chernozem on loess and aerolian sands, in the north-west part of the south Banat plateau, and on the boundary of the sandy region sount of Subotica and Horgos. This chernozem is also calcareous, beginnig from the surface and generally are medium thickness (70-80 cm). They are distinguished by their less expressed, crumbly structure, smaller humus content, smaller capacity and considerably lesser fertility.
In the work microbiological fertilizer - biological preparation prepared from the mixed populations *Azotobacter chroococcum, Bacillus megaterium* and *Bacillus circulans* was used with and without humat solution. The strain of microorganisms used for this research are from the collection from microoganisms of the Microbiology Department of the Faculty of Agriculture of Zemun. The bacterial starins were multiplied on adequate nutritions media and introdiced into sterile carrier-peat. 20 ml of bacterial inoculum (10^8 cells/ml) were introduced into each sterile bag (130 g). Biopreparation maked as A contents mixture of starins azotobacter *Azotobacter chroococcum* P-1, V-4, N-6, P-19 and ABC – starins azotobacter: *Azotobacter chroococcum, Bacillus megaterium* T-1, +*Bacillus circulans* strein V-1.
Preparation of humates – Humates were obtained from alkaline extractio of coal dust, followed by their separation with concentrated H_2SO_4. Filtration and rinsing were perfomed and followed by drying and dissolution in 1 N NaOH. The solution obtained wsa neutralized with diluted H_2SO_4. It contains 0.1% Na-humates (1gper liter). Treatment with mocrobiological fertilizers – Mocrobiological fertilizer was dissolved immediately prior to its use in ½ l water (treatment A and ABC) or in ½ Na-humate (treatments AH and ABCH), and introduced into soil at thedepth of 20 cm, at the begining of vegetation. Trestment C represent the control - without mocrobilogical fertilizer or humate upon the pruning of vinegrapes.
Cultivar Riesling on the root-stock Kober 5 BB was planted in the year 1991 on the lot where there used to be a vineyard. The training system is the bilateral cordon with mixed type of pruning and rectangular (3 x 1 m) arrangment of vines. During the experimantal period, the soil was plugged between rows and spaces inside the rows was treated by herbicides (combination by Basta 1.36-2.24 l/ha and Clinic 1.7/ha) and mineral fertilizers were not used. Grapes were picked in the phase of full maturity, the yield of grapes, number and mass of grapes, sugar contents and total acids were determined for the period 2003 to 2005. Microvinification according to variants was performed in 2003 and 2004. It includes the contents of ethanol, extracts, polyphenol, ashes, free and fixed sulphodioxide, evaporable and total acids in vine.

Results and discussion

Heat conditions, height and distribution of precipitation largely differ in the study period in comparison with perennial average. Mean annual temperature of air is approximately 11.7ºC in comparison with 12.2ºC in 2003. In the study period, August was the warmest month (25.8ºC) and by far the highest temperature was recorded in August (35.5ºC) the same year. Precipitations were approximately Rh=657.7 mm, of which Rh=427.5mm in the vegetation period. Precipitation and humidity in thered year of study were high and repainig of grape was unregular.

Tableau 1 - Average values of yield and quality of grapes of the cv. Riesling (Vrsac, 2003-2005)

Variant	Number of clusters per vine	Grape mass g	Grape yield per vine kg	Grape yield t per 1 ha	Veg. potential Vp	Sugar contents in must %	Total acids contents in must g/1	Maturity index
A	79.8	45	3.59	11.97	1.78	17.2	10.9	1.57
AH	69.5	56	3.89	12.96	1.79	17.4	10.9	1.60
ABC	77.9	53	4.13	13.77	2.00	16.9	11.1	1.52
ABCH	75.3	51	3.84	12.80	1.95	19.3	10.6	1.82
Control	72.7	52	3.78	12.60	2.14	17.8	10.8	1.65

Differences between the applied micrological fertilizers were greater in the number of clusters per vine in comparison with average grape mass. Greater effect was in the treatements *Acotobacter chroococcum* - A and *Azotobacter chroococcum + Bacillus megaterium +Bacillus circulans* - ABC. Grape yield per area unit was high both in the control variant and in the application of all three micrological fertilizers. With the use of pure nitrogen fixators the grape yield was the lowest, typical for cv. Riesling. Vegetative potential, comparing grape yield per vine and mass of discarted vine during mature cutting in this variant is between medium and low value. Results of *Corina et al.* (1999) point that the vegetative and production potential depends primarily on the method of soil maintenance, and then on the choice of vine stock. When the soil is treated constantly, vine is characterized by greater vegetative potential, sometimes even by expressed, high yield. However, vine is much more sensitive to being infected with *Botrutis cinerea* Pers.Ex Fr. according to the same author.

Sugar contents in must varies between 16.9%, which is the characteristic of the variant ABC - *Azotobacter chroococcum + Bacillus megaterium + Bacillus circulans*, and 19.3% in the variant ABCH - *Azotobacter chroococcum + Bacillus megaterium. + Bacillus circulans with humat.* Riesling grapes have a high acidity (10.6 to 11.1g/l as taratricacid) at harvest. Maturity index show that grapes were treatmented with fertilizer ABCH were in full repaining at harvest. High yield by treatment ABC was slowing wit the repaining of grape and after affect were low content of sugar and high acids in must

Table 2 - Some important indices of wine quality (Vrsac, 2003-2005)

Treatment	Ethanol vol %	pH	S mg/l free	S mg/l total	Evapor. acids g/l	Total acids g/l	Ashes g/l
A	12.32	2.90	11	77	0.65	8.3	1.57
AH	12.65	3.00	7	53	0.72	8.1	1.54
ABC	11.75	2.96	9	82	0.72	8.7	1.64
ABCH	12.92	3.02	5	80	0.75	7.8	1.53
K-control	12.17	2.96	7	70	0.70	8.2	1.55

The results shown in table 2 show that in treatment AH and ABCH treatment, the wine of better quality and sensory characteristics was obtained. In the control treatment, in which the application of multiplied bacteria was left out, and the green fertilizer was ploughed between rows, the wine was obtained with freshness and with typical fragrance of cv. Riesling (Jović *et al.,* 2000). Applied mocrobilogical fertilizers showed their unfluence on the content of sugar in the must and some important wine characteristics (figure 1). Content of sugar and in the must was the greatest in ABCH treatment , followed by the control, treatment AH, treatmen A and treatman ABC. The results of Sivcev *et al.* (2003 and 2005), where microbilogical fertilizers with out humats were used in the variety Riesling and microbilogical fertilizers with humat were used in the variey Merlot, show that rigsoil has simular reactions. The experiments with varieties Riesling and Merlot were in two different vone-growing region, of the same type of the soil formed on simular geological material.

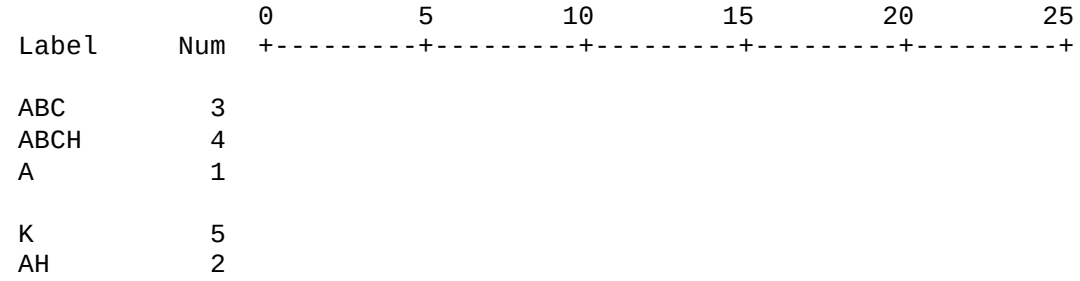

Figure 1 - Cluster analysis - dendogram of the grape (sugar and total acids) and wine characteristic

The results of Maigre and Aerny (2000) on the analysis and organoleptic characteristics of wine cv. Gamet Black point to the fact that by applying green fertilizers without mineral nitrogen, total level of nitrogen in must is decreased and the contents of higher alcohols phenyl-2 ethanol and 2- and 3-methyl-1-buthanol in wine are increased. Generally these wines are less typical and tannins are of poorer quality, but the fragrance is more prominent due to the increased nitrogen in must.

This experiment was on different types of soil: on chernozem regions in a transitional zone between chernozem on loess and aerolian sands, in the north-west part of the south Banat plateau. Rootstock Kober 5 BB and variety Riesling reacted the best on using micorobilocical fertilizers by humat.

References

Branislava Sivčev (1988): The results of investigations of white wine cultivars in the "Radmilovac" collection vineyard. *Review of Research Work at the Faculty of Agriculture. Vol. 43, No 2, pp 7-23*

Branislava Sivcev, S. Jovi', Vera Raicevic, A.Petrovic and B.Lalevic (2005): Apllication of microbiological fertilizers in viticulture: grape yield and quuality of wine cv. Riesling. *Journal of Agricultural ascience, Vol. 50, No 1 pp 19-26*. Belagrade

Drinkwater L.E., and Pettygrove G.S. (1999): Organic Soil Amendments and Fertilizers. Pennsylvania. Book 4-6.

El Kholi, A. Found, Galal Yehia, Gm. (1998(: Imapct of bilogical nitrigen fixation on agricultural and enviromental development. 16th World Congress of Siol Science, Montpelier, France, Scientific registration no. 2588, Symposium no. 12.

Govedarica M. and Mirjana Jarak (1995): Soil microbiology. Novi Sad. pp 152-155.

Govedarica M., Zora Jelačić, Mirjana Jarak, Nada Milošević, Nada Erić (2002): Bacterization as a measurefor increasing yield and microbiological activity in corn. Collection of Scientific works. Institute PKB Agroekonomik. Vol 8, no. 1, 105-109.

Jović S., Sanja Zagorac, Petrović A. (2000): The influence of maceration of crushed grapes in prefermentation phase on the chemical contents and sensory characteristics of wine of some clones cv. Chardonnay. Collection of works. V Conference of Alcoholic and Non-Alcoholic Beverages and Vinegar Industry. Vrnjačka Banja. 155-160.

Kamas J., Pool R., Lakso A., Dunst R., Fendinger A. (2001): Legumes and limited experience in eastern viticulture. New York State Agricultural Experimental Station, Cornell University, Geneva, 1-5.

Mairge D., Aerny J. (2000): Permanent grass cover and nitrogen fertilization trial on Gamay in western Switzerland. 2. Analytic and sensory results. Revue Suisse de Viticulture, Abiriculture Horticulture, no. 5, 279-285.

Šutić D. and Dragoslava Radan (2001): Microbiology - microorganisms in the life of plants, Belgrade, 250-258.

Vera Raicevic (1996): Capacity of Azotobacter genus to from associations with maize, Ph.D Thesis. Faculty of Agriculture, Belgrade-Zemun.

Vera Raičević, Vasić C., Snežana Djordjević, Tolmir M. (1998): The influence of inocculation of the seed of corn in strains Azotobacter on biological activity of soil Journal of scientific agriculture research. Belgrade. Vol. 59, No 209, 5-13.

Wilkinson K. (2004): Compost: a versatile soil managment tool for grapegrower. *The Australien and New Zeland Grapegrower and Winemaker*, Australie No 485, pp. 28-31.

Džamić Ružica i Stevanović D. (2001): Agrohemija Beograd.