Organic viticulture elements development

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Abstract. The results of a two-factor field experiment on the application of two elements of organic planting: sowing of inter-row spacing and introduction of biofertilizers – microbial preparations for increasing soil fertility, growth and productivity of grapes (Vitis vinifera L.) of Chardonnay cultivar on rootstock cultivar Berlandieri × Riparia Kober 5BB (Kober 5BB) are presented. Study was carried out in 2016-2018. The intercropping factor has two options: natural overgrowing (NO) and sowing a mixture of grasses (MG) – pasture ryegrass (Lolium perenne L.), alfalfa (Medicago sativa L.) and meadow bluegrass (Poa pratensis L.) in a ratio of 1:1:1. For the factor microbial preparations (MP): Diazofit – Agrobacterium radiobacter 204 strain, associative nitrogen-fixer, and a complex of microbial preparations (CMP). It has been established that use of organic methods positively influenced the growth, productivity and quality of Chardonnay grapes in the foothill zone of the Crimea. MP provided the most significant effect on these indicators against the intercropping with a mixture of grasses. The applied methods of biologization contributed to an increase in the sugar content of the grapes, to a greater extent the MP and reduced the acidity of the berries juice, where intercropping played a significant role. The increasing in soil fertility under the vineyard with the application of intercropping and MP on its background has been revealed. Model of Chardonnay variety productivity on rootstock cultivar Kober 5BB in the conditions of the foothill Crimea by organic viticulture methods was constructed.

1. Introduction

Vineyard cultivation by intensive technology is associated with high labor, material and energy costs. Prolonged monoculture and impoverishment of biodiversity leads to the development of specific pests and diseases. Control of them by chemical methods with the use of a significant amount of pesticides leads to contamination of the soil, groundwater and subsurface water with their residual quantities [1]. The same type of agriculture and high mechanical load on the soil causes its compaction, degradation of structure, water-physical properties and rapid mineralization of humus [2]. This can be worsened by the content of the soil in the rows of the fallow vineyard. High doses of mineral fertilizers, not fully consumed by grape plants, cause the leaching of mineral elements, especially nitrogen, beyond the soil profile and pollute the environment [3].

In alkaline soils, phosphates and some trace elements introduced with mineral fertilizers are non-exchangeable absorbed by the soil and become inaccessible to a plant. All this leads to a decrease in the growth and productivity of grapes, loss of soil fertility, reducing of production efficiency.
To overcome these negative consequences of intensification environmental experts propose to construct an agroecosystem on the principles of sustainable development. Adaptive biologized agroecosystems, based on making artificial agricultural lands be similar to natural ones, have been developing [4]. They provide reducing energy and chemical load on agrocenosis, increasing plant and microbial diversity by means of introduction of several layers of vegetation, and the use of efficient strains of microorganisms and return to the soil the maximum amount of plant residues [2, 5, 6].

However, such systems must be adapted to local environmental conditions and have a specific and varietal orientation. Under different climatic and soil conditions, as well as for certain species and even cultivars of plants, elements of biologized technology should be selected on the basis of related studies: agricultural plant – soil – climate – natural vegetation [7, 8]. Currently, the vineyards use biological plant protection, apply various soil content systems to improve its fertility [9]. MP to improve plant nutrition and increase their resistance to pathogens and adverse environmental conditions [10]. However, for the conditions of the foothill Crimea with its alkaline high-carbonate soils, difficult terrain and a set of cultivars and rootstocks, such studies are few in numbers [12, 13].

The objective of our research was to study the effect of grassing between the rows and the use of biofertilizers – microbial preparations on the growth, productivity and quality of Chardonnay grapes, as well as on the fertility of the soil under the vineyard.

2. Materials and methods

To achieve this objective, the experimental two-factor field experiment was laid out in the vineyard near Khmelnitsky village of Balaklava district of Sevastopol in the Valley of Chernaya River. The experiments were carried out in 2016-2018 in the planting of Chardonnay on rootstock of Berlandieri x Riparia Kober 5BB, founded in 2007 under the scheme is 2.5 x 0.9 m, pruning – Guyot training.

The first factor studied in the experiment was the treatment of plants with MP (A factor). We selected two MP: Diazofit, a strain of Agrobacterium radiobacter 204, an associative nitrogen fixer and the complex of microbial preparations (CMP). This preparation has an aggregated effect on the plant, since in addition to Diazofit it contains Phosphoenterin preparation on the basis of phosphate-mobilizing strain Enterobacter nimpressuralis 32-3, with phytohormonal and fungicidal effect [14], as well as Biopolitsid preparation created on the basis of the strain Paenibacillus polymyxa P, has fungicidal effect and nitrogen-fixing properties. Earlier on Muscat White cultivar in the same terroir, we found that these preparations were the best for the growth and productivity of grapes [13].

The preparations were introduced into the rhizosphere of the grape bush annually once during the growing season (June) with a drip irrigation dose of 200 ml of diluted suspension per bush. The concentration of the initial suspension of preparations was 0,1-10,8•10⁹ colony forming units in 1 milliliter. Dilution of the initial suspension of preparations was carried out in a ratio of 1:100. Control was without the use of MP. Initial suspension of MP was developed and provided by the Department of Agricultural Microbiology of Research Institute of Agriculture of Crimea.

A second factor in two-factor field experience (B factor) was the application of grassing with perennial herbs: natural overgrowing (NO), control 1 and sowing a mixture of graminea and legume grasses (MG), which included: perennial ryegrass (Lolium perenne L.), alfalfa (Medicago sativa L.) and meadow grass (Poa pratensis L.) in the ratio of 1:1:1. Grasses were sown in each row spacing of the vineyard in the fall of 2012. Until 2014 seeded grass covered the soil surface completely in the middle of the aisle stripe 1.5 m. None of the row spacing took place more slowly. At the beginning weeds were dominating: poppy, wild germander, bindweed, milkweed, rump, wheatgrass, etc. For 3-4 years after the termination of mechanical treatments a natural coenosis has developed, which was formed by segetal plants associations Convolvulo arvensis – Amaranthetum retroflexi Abrm. et Sakh. in Mirk. et al. 1986 and Cirsio – Lactucetum serriolae Mucina 1978, common for the agrophytocenosis of the vineyards in the foothill Crimea [15]. Mowing of herbs was carried out according to their growth up to 20-30 cm with the grinding and leaving crop residues in place as mulch. In the rows, the soil was kept black fallow by mechanical treatments.
The area of the simple plot of land is 45 m² (20 bushes of grapes). The experiment was repeated thrice. Arrangement of options is randomized. Care for the plants was carried out according to agro-instructions. To determine the condition of plants and their productivity, the numbers of main shoots, the numbers of clusters, the average mass of the clusters, the yield were identified according to the method of field experiments in the vineyard [16]. To assess the quality of the grapes and the berry juice, the mass concentration of sugars was performed by refractometer (GOST 27198-87); the mass concentration of acids by titration was performed according to GOST 25555-82.

Meteorological conditions in the years of research were favorable for the growing of grape plants. Soil moisture was maintained at 70-80 % – the least moisture capacity by drip irrigation. During the research period, mineral fertilizers were not applied to the soil. The soil at the site was meadow-alluvial carbonate, slightly humus and slightly clayish on recent carbonate alluvia. Soil samples for analysis were collected annually in a layer of 0-30 and 30-60 cm in June-July. The content of nitrate nitrogen was determined in the soil according to GOST 26951-86; mobile connections of phosphorus and potassium were determined by Machigin’s method (GOST 26205-91). Total carbonates were found by gas volumetric method [17], active lime – by Drouineau-Galle method [18], organic matter – by Tyurin method in the modification of Central Research Institute of Agrochemical Services (GOST 26213-91), the pH of the soil suspension was determined potentiometrically (GOST 26423-5).

Mathematical data processing was carried out by methods of variational statistics using ANOVA software, correlation, variance and regression analysis using Statistica 7 software package. A 5 % significance level was accepted as reliable.

3. Results and discussion
An important condition for increasing the productivity and quality of grapes is the formation of a strong bush with a sufficient number of fertile shoots. Because of research, it was established that the number of the main shoots of a grade of Chardonnay at the given forming in control on NO made 8.5 pieces (Figure 1). When using MP on this background of grassing, there was an increase in the number of shoots by 1.6-1.7 pcs or 19-20 % of the control (the difference is statistically insignificant).

The grassing with MG contributed to a significant increase in the number of shoots by 2.3 pcs (27 %). The use of MP in the background of MG even more greatly stimulated the shoots, significantly when applying Diazofit, which exceeded the control 1.6 times. This, apparently, was due to the release of bacteria growth-stimulating substances.

Naturally, with the increase in the number of shoots in the application of biological techniques, the number of clusters increased. On the background of NO there were 17.9 pcs in the control (Table 1). The MP application on the background of NO significantly and accurately increased the number of grapes by 20 %, approximately the same for both preparations compared to control 1.

The application of MG in the control also contributed to the increasing of the number of clusters, but it was insignificant. The joint effect of MG and Diazofit was higher (22 %) than the influence of Diazofit on the background of NO (20 %), CMP on the background of MG, was even slightly reduced their number compared with its effect on NO. Generally, the differences with the control were significant and most notable when using Diazofit, the share of this factor’s influence in the total variance was significant and amounted to 11.5 %. The factor in differences between the variants of grassing has not been revealed. Therefore, in the experiment the combination of the Diazofit and MG had the best effect on the number of clusters.
Current effect: $F(2, 36)=2.9592, p=0.06459$

Effective hypothesis decomposition

Vertical bars denote 0.95 confidence intervals

![Figure 1. The number of main shoots of Chardonnay grapes on rootstock Kober 5BB in the application of grassing and MP, 2016-2018](image)

An important indicator of the productivity of grapes is the mass of a cluster, which characterizes the genetic capabilities of the cultivar, as well as sensibly responsive to agricultural techniques and load with clusters. In the control of NO, this figure was 101 g, which is typical for this cultivar (Table 1). MP increased this figure by 7-24 %, especially Diazofit, by growth stimulation and enhancement of plants nutrition. The grassing with MG in the control only slightly increased the number of clusters, and the application of Diazofit on this background practically did not change their quantity in comparison with influence of this preparation on a background of NO. CMP on the background of MG was more effective than NO, but does not exceed Diazofit for its positive effects. Thus in general, on A factor both preparations had significant influence on the mass of the clusters, Diazofit’s impact was most significant and exceeded the control by 20 %, the share of factor influence in total variance was 37 %. The influence of B factor (grassing) was insignificant.

**Table 1.** Indicators of productivity of Chardonnay grapes on the rootstock Kober 5BB when grassing and MP’s application, 2016-2018, $n = 9$

| Variant       | Control | Diazofit | CMP     | Average of B factor |
|---------------|---------|----------|---------|---------------------|
| NO (control 1)| 17.9    | 21.5     | 21.4    | 20.3                |
| MG            | 19.0    | 21.9*    | 20.7    | 20.5                |
| Average of A factor | 18.5 | 21.7*    | 21.0*   |                      |
| NO (control 1)| 101     | 125*     | 108     | 111                 |
| MG            | 104     | 123*     | 115*    | 114                 |
| Average of A factor | 103 | 124*     | 112*    |                      |
| NO (control 1)| 8.4     | 10.2     | 9.8     | 8.2                 |
| MG            | 8.8     | 11.9*    | 9.7     | 10.1                |
| Average of A factor | 8.6 | 11.0*    | 9.8     |                      |

*Significance: $p<0.05$

The average yield of Chardonnay grapes for 3 years of experience in the control with NO was 8.4 t/ha. Use of MP contributed to the increase in plant productivity. Moreover, the use of Diazofit on the background of NO more increased yielding of grapes (21 %) compared to CMP (17 %). The
difference with control in both cases was insignificant. The grassing with MG of the vineyard in the control also contributed to some increase in yield by 0.4 t/ha or 5% of the crop per NO. On the background of MG, the maximum and a significant increase in grape yield observed after Diazofit application – 3.1 t/ha (35%) from the control with MG. Generally, on A factor Diazofit’s impact was the most significant and reliable, where the yield increased by 2.4 t/ha. Share of factor influence in total variance was substantial and amounted to 17.5%. The influence of B factor was inconsiderable, although quite significant (1.9 t/ha).

Thus, the applied methods of biologization influenced the growth and productivity of Chardonnay grapes. More significant role is played by the factor of MP and especially Diazofit on the background of MG. This combination contributed to the increase in the number of shoots and clusters, increases the weight of grapes and the grape yield by 20-35% from the control with NO.

The applied new agricultural techniques in the vineyard not only contributed to an increase in the number of grape berries, but also improved their quality, affecting the biochemical composition of the berries. So the mass concentration of sugars in the juice of grapes in the control 1 with NO is quite consistent with the maturity to receive sparkling wine-making materials (Table 2). The use of MP on this background of grassing led to a significant increase in the concentration of sugars, which allows collecting grapes at an early date.

Table 2. The quality of Chardonnay grapes with grassing and applying MP, 2016-2018, n = 9

| Variant          | Control | Diazofit | CMP | Average of B factor |
|------------------|---------|----------|-----|---------------------|
| Mass concentration of sugars, g/dm³, MSD₀₅ = 5 |
| NO (control 1)   | 175     | 180      | 180 | 178                 |
| MG               | 170     | 180      | 180 | 177                 |
| Average of A factor | 172     | 180      | 180 |                     |
| Acidity of juice, g/dm³, MSD₀₅ = 0,7 |
| NO (control 1)   | 9.1     | 8.9      | 9.2 | 9.1                 |
| MG               | 7.7     | 8.3      | 7.9 | 8.0                 |
| Average of A factor | 8.4     | 8.6      | 8.6 |                     |

*Significance: p<0.05

The grassing of the vineyard reduced the sugar content in the grape juice, which is probably due to a change in the microclimate in the vineyard – a decrease in soil temperature and an increase in air humidity. However, the use of MP on this background of grassing significantly increased the sugar content of berries by 6%, which was the same as when using MP on the background of NO. Generally, in A factor a significant increase in the mass concentration of sugars in grape juice is the same for both MP, the share of the influence of the factor in the total dispersion was 13%. B factor (grassing) showed no significant differences between the options.

Simultaneously with the increase in sugar content, the acidity of the juice decreased (Table 2). MP on the background of NO virtually had no influence on this indicator. The grassing with MG reduced this figure most significantly compared to NO (control 1) by 1.4 g/dm³ or 18%. The use of MP with MG insignificantly increased the acidity of the berries, but it was lower than in the same variants with NO, with CMP this change was significant. Generally, there were no essential differences in the variants of the experiment for A factor; the effect was significant for B factor, the acidity decreased by 14%. The proportion of the influence of the “grassing” factor in the total dispersion was significant and amounted to 14%.

Studies of agrochemical parameters of soil in the experiment showed that the soil in the vineyard was medium alkalinous – the figure of water pH in the control for both backgrounds of the grassing was high and approximately the same (Table 3).
Table 3. The pH value and the content of the main nutrients and humus in the soil (layer 0-60 cm) when grassing and application of MP, 2016-2018

| Grassing | MP     | pH   | Humus, % | N-NO₃, mg/kg | P-PO₄, mg/kg | K₂O, mg/kg |
|----------|--------|------|----------|--------------|--------------|------------|
| NO       | Control | 8.42 | 2.35     | 11.8         | 27.1         | 212        |
|          | Diazofit | 8.48 | 2.49     | 106          | 14.5         | 123        |
|          | CMP    | 8.30 | 2.75     | 117          | 12.6         | 107        |
| MG       | Control | 8.43 | 2.68     | 114          | 13.8         | 117        |
|          | Diazofit | 8.45 | 2.81     | 105          | 16.3         | 118        |
|          | CMP    | 8.29 | 2.94     | 110          | 16.8         | 122        |

- Difference with the control of the corresponding variant of intercropping is significant, p ≤ 0.05.
- Difference with the control 1 of NO is significant, p ≤ 0.05.

The use of Diazofit with any kind of grassing slightly increased the alkalinity, but the average difference was unreliable. CMP significantly reduced the value of water pH of soil suspension at 0.12-0.14, which is important for the absorption and mobility of many nutrients in alkaline soils.

The humus content in the control soil was rather low due to intensive and long-term use of the soil in horticulture and vegetable growing. MP on the background of NO increased its content by 6-17 relative percent, which is the most significant and reliable for CMP (Table 3). On the background of MG humus content in the soil increased significantly compared to NO (14 %). The use of MP on the background of MG led to a maximum increase in the humus content to 2.81-2.94 %, which is 0.46-0.59 % higher than that in the control 1 for NO (20-25 %).

The content of nitrate nitrogen in the soil of control 1 with NO was low, mobile forms of phosphorus and potassium was at an average level of security for grapes [19]. Diazofit on the background of the NO significantly increased the content of nitrate nitrogen in the soil by 23 %. The use of MG in the control also led to an increase in the content of this element by 17 % compared to the background of NO. The use of MP on background MG also increased the content of nitrate nitrogen by 18-22 % of the control with MG, the maximum figures were observed when using CMP.

The greatest increase in mobile phosphorus content in soil on the background of the NO has occurred under the action of Diazofit – 19 % of control 1. MG (control) also contributed to an increase in the concentration of this element in the soil compared to NO (control 1) by 15 %. The use of MP on MG created a tendency to increase the content of mobile phosphorus in comparison with the control, as much as possible under the action of CMP.

The concentration of exchangeable potassium in the soil under the action of MP on the background of grassing slightly decreased inaccurately in relation to the control 1. On the background of the MG there was only a slight increase in the K₂O content in the soil under the action of Diazofit by 4 % (difference from control is not significant).

Thus, the use of grassing and MP in ampelocenosis significantly increased the content of humus and nitrate nitrogen, mobile forms of phosphorus and potassium – slightly, and reduced its alkalinity.

The correlations dependence of growth and productivity of grapes on the main indicators of soil fertility were identified (Table 4). Correlations indicate the most significantly and positively impact to the number of primary shoots influenced by the humus content, the number of clusters, average cluster weight and sugar content of the juice content of nitrate nitrogen and mobile phosphorus in the soil. Acidity of juice decreased largely under the influence of increasing the content of mobile nitrogen and phosphorus in the soil.

The increase in yield was also determined by an increase in the content of nitrate nitrogen and phosphorus in particular, aided by the use of MP, largely when grassing with MG. From growth and productivity of the grape, harvest was significantly and most closely connected with the quantity of clusters (r = 0.76; n = 54) and an average weight of clusters (r = 0.60).
Table 4. Correlation between growth and productivity of Chardonnay grapes and soil fertility, 2016-2018, n = 54

| Indicators                        | N-NO<sub>3</sub> | P<sub>2</sub>O<sub>5</sub> | K<sub>2</sub>O | Humus |
|----------------------------------|------------------|-----------------|----------------|-------|
| Quantity of main shoots, pcs.    | −0.10            | −0.18           | −0.29          | 0.41  |
| Quantity of clusters, pcs.       | 0.35<sup>a</sup> | 0.46            | 0.37           | −0.28 |
| Average cluster weight, g        | 0.58             | 0.55            | 0.02           | −0.33 |
| Sugar content of berry juice, g/dm<sup>3</sup> | 0.58            | 0.49            | −0.13          | −0.32 |
| Acidity of berry juice, g/dm<sup>3</sup> | −0.74           | −0.65           | 0.12           | 0.36  |
| Yield, t/ha                      | 0.55             | 0.63            | 0.27           | −0.31 |

<sup>a</sup> Correlation is significant at $r \geq \pm 0.28$, $p < 0.05$.

A close multiple rectilinear dependence of yielding grape Chardonnay on a number of indicators of plants and soil, $R^2 = 0.775$, $p < 0.05$. The multiple regression equation is calculated as follows:

$$V_{yield, t/ha} = 0.32 \times x_1 + 0.03 \times x_2 + 0.03 \times x_3 + 0.23 \times x_4 - 3.54,$$

where $x_1$ – number of clusters, pcs; $x_2$ – average mass of a cluster, g; $x_3$ – content of P<sub>2</sub>O<sub>5</sub> in the soil, mg/kg; $x_4$ – number of main shoots, pcs.

Analysis of equation (1) shows that the greatest impact on the value of the grape yield has the number of clusters ($\beta = 0.552$). To a large extent the crop is also affected by the content of P<sub>2</sub>O<sub>5</sub> in the soil ($\beta = 0.317$); at least the value of the yielding was influenced by the average cluster mass and the number of main shoots ($\beta = 0.185-0.220$). The influence of all growth indicators and the state of ampelocenosis included in the equation was significant ($p < 0.05$). Thus, according to equation 1, the increase in the number of clusters on a bush per unit within 13-29 pcs will lead to an increase the grape yield by 320 kg/ha with other unchanged indicators and an increase in the content of mobile phosphorus by 1 kg/ha, within 3-38 kg/ha, will increase the yield by 30 kg. It is possible to model or predict the yield of grapes, knowing or contributing to changes in plant productivity or soil fertility, using equation 1.

4. Conclusion

It was found that the use of methods of organic farming positively influenced the growth, productivity and quality of Chardonnay grapes in the foothill Crimea. The most significant and reliable influence on these indicators was provided by MP on the background of grassing with a MG. Whereby the number of main grape shoots increased by 20-30 %, the number of clusters – by 20-22 %, the average cluster mass – by 20-24 %, the productivity of grapes – by 20-35 %. The most productive one was the use of Diazofit with a MG. The applied methods (MP to a remarkable degree) of biologization contributed to an increase in the sugar content of grapes and reduced the acidity of the juice of berries, where a significant role was played by grassing.

The increase in soil fertility under the vineyard with the use of grassing and MP was revealed. This significantly increased the content of humus by 20-25 % and nitrate nitrogen by 17-22 %, the change of concentration of mobile forms of phosphorus and potassium was low, there was a decrease of alkalinity of the soil, which was the most noticeable and reliable in the variant of CMP on the MG.

We found a significant correlation of growth and productivity of grapes from the fertility of the soil, a model of productivity of Chardonnay grapes on rootstock of Berlandieri x Riparia Kober 5BB under the conditions of the foothill Crimea during organic methods using. It allows one to simulate or to predict the yield of this cultivar, regulating the state of plants and soil fertility.

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