

Physiological Response of Three Grapevine Cultivars Grown in North-Western Poland to Mycorrhizal Fungi

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West Pomerania (Poland) is located near the northern boundary of the range of viticulture (the coldest zone A). Unfavourable weather conditions can pose a serious threat to the cultivated vines. One of the treatments used to increase the tolerance of plants to abiotic and biotic stresses is inoculation with symbiotic soil microorganisms. This paper focuses on the influence of mycorrhization on the changes in soil microbiology, the degree of colonization of roots by mycorrhizal fungi, and on selected physiological parameters of three grapevine cultivars ('Pinot Noir' on SO4 rootstock, 'Regent' on 5BB rootstock, and 'Rondo' on 125AA rootstock). The applied inoculation had a stimulating effect on the colonization of roots by arbuscular mycorrhizal (AM) fungi, as evidenced by higher mycorrhizal frequency and intensity in the mycorrhized plants. The mycorrhizal treatment increased the intensity of CO₂ assimilation and transpiration. Mycorrhization reduced the efficiency of photosynthetic water use and increased stomatal conductance for water in the grapevines tested. The mycorrhizal treatment did not affect the concentration of assimilation pigments in vine leaves. The mycorrhization of grapevines had no effect on the values of initial fluorescence, maximum fluorescence, the maximum potential efficiency of photochemical reaction in PS II, the size of the pool of reduced electron acceptors in PS II, nor on the value of the PS II vitality index.

INTRODUCTION

West Pomerania (Poland) is located near the northern boundary of the range of viticulture (the coldest zone A). On a global scale, Poland is currently an insignificant producer of wine. However, grape growing is becoming increasingly popular due to the good prices achieved by Polish wines (Stój *et al.*, 2017). The most commonly grown cultivars of red grapes are 'Regent', 'Rondo' and 'Pinot Noir' (Wilk, 2011).

Mycorrhization of fruit plants is performed in order to improve vegetative growth, yield quality parameters, increase the tolerance of plants to abiotic and biotic stresses, and to reduce the use of chemicals in the environment. Because of these considerations, inoculation of plants with symbiotic soil microorganisms is a very important treatment used not only in organic farming, but also in integrated and conventional crop cultivation. The action of mycorrhizal fungi results in physical and chemical stabilization of the soil and practical possibility of limiting fertilization. Mycorrhizal mycelium increases the absorptive surface of roots and phosphorus availability to plants, which makes it a

very important component of the rhizosphere of fruit plants (Sas Paszt *et al.*, 2010). Mycorrhiza can indirectly affect the intensity of photosynthesis, and thus the productivity of plants, by increasing the stomatal conductance to CO₂ and improving the efficiency of photochemical processes. This phenomenon is observed especially in the case of plant growth under stressful conditions (Borkowska, 2005; Wu and Zou, 2010).

Soil moisture is important for the mycorrhizal frequency in grapevine roots. The water content of the soil, weather conditions and its frequency or lack of irrigation significantly affects the mycorrhizal frequency in grapevine roots. Holland *et al.* (2014) had observed that irrigating every few days significantly increased the number and size of arbuscules found in the roots, while daily irrigation increase the number of vesicles. Donkó *et al.* (2014) studied the effect of soil moisture on the degree of colonization of grapevine roots by AM fungi. The most extensive colonization of roots by mycorrhizal fungi was shown by plants growing on the most elevated site, where there was never any stagnant water.

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The fungal colonization there ranged from 64% to 81%, depending on the year and season. The lowest mycorrhizal frequency (from 46% to 76%) was found in the vines growing in a periodically flooded location. Water deficiency affects the mycorrhizal frequency in plant roots to a lesser extent than its excess, which is a factor that considerably restricts the colonization of roots (Deepika & Kothamasi, 2015). In addition, mycorrhizal frequency is also affected by soil pH, being lower at a low pH (Wang *et al.*, 1993).

The aim of the study was to assess the influence of mycorrhization on the changes in soil microbiology, the degree of colonization of roots by mycorrhizal fungi, and on selected physiological parameters of three cultivars of red grapevines grafted onto different rootstocks, grown in the conditions of West Pomerania (Poland).

MATERIALS AND METHODS

The study was conducted in the Turnau vineyard near Baniewice (53°03'38" N, 14°35'59" E) located in Northwestern Poland. A two-factor experiment was established in a random block design in three replications. One replication consisted of 5 plants planted at 2.5 × 1 m. Grapevine plantlets were planted in a clayey-sandy soil in 2012. The soil pH in KCL was 6.10. Soil nutrient availabilities (mg·100g⁻¹), determined in the soil samples collected from 0 to 60 cm of the soil depth, were: N-NH₄ 0.13, N-NO₃ 0.20, P 7.8, K 13.8 and Mg 5.5. In the first year, a multi-component fertilizer Suprofos 25 NPK (Ca, Mg, S) 5:10:25 (2.5:2:13) was applied at the dose of 400 kg/ha. In the second year, no mineral fertilization was used to fertilize the plants. The first experimental factor was the inoculation of plant roots with mycorrhizal fungi. Plants were treated with mycorrhizal substrate (produced by Mykoflor, Końskowola, Poland), containing 1000 propagules of arbuscular mycorrhizal fungi per 1 g of substrate: *Rhizophagus irregularis*, *Glomus mosseae*, *Claroideoglomus etunicatum* on an organic carrier medium, once with a special applicator for subsurface placement, in 2012, one month after planting. The inoculum vaccine was used in the form of an aqueous solution with a hydrogel, at a dose of 30 ml/plant (3000 propagules per plant). The following variants of the first factor were used: control, without mycorrhiza (variant M0), and with mycorrhiza (variant M1). The second experimental factor was the cultivars of red grapevines grafted onto different rootstocks. The following variants of the second factor were used: 'Pinot Noir' on SO4 rootstock (PNR variant), 'Regent' ('Diana' ('Silvaner' x 'Muller-Thurgau') x 'Chambourcin') on 5BB rootstock (REG variant), and 'Rondo' ('ZaryaSever' ('Seyanets Malengra' x *V. amurensis*) x 'St. Laureate') on 125AA rootstock (RON variant).

Microbiological analysis of soil

Soil samples were collected at the beginning of November. They were mixed thoroughly and 5 g of each sample was transferred to 100 mL Erlenmeyer flasks containing 45 g of sterile distilled water. The suspended samples were homogenized at 190 rpm for 45 minutes. A series of tenfold dilutions (10⁻², 10⁻³ ... 10⁻⁵) were prepared from each suspension. Soil dry weight was determined by oven drying the soil samples at 90°C for four days.

Sterile Petri dishes were inoculated with 100 µL aliquots of each dilution prepared from the soil suspensions. The inoculated dishes were flooded with a liquid agar medium at a temperature of approx. 50°C. All analyses were made in triplicate. To estimate the number of microorganisms, the following microbial media were used: for estimation of the total number of culturable bacteria – 10% Tryptic Soy Agar (TSA) (Biocorp, cat. number: PS22); for estimation of the total number of spore-forming bacteria (to obtain the bacterial spores, the soil samples were incubated at 80°C for 30 minutes before being transferred into Petri dishes) – 10% TSA (Biocorp, cat. number: PS22); for estimation of the number of filamentous fungi – Rose Bengal Chloramphenicol Agar (Biocorp, cat. number: PS66); and for estimation of the number of fluorescent pseudomonads – S1 (Gould *et al.*, 1984).

For estimation of total number of spore forming bacteria and microscopic fungi, inoculated plates were incubated at 26°C for 5-7 days. For evaluation of bacteria population, the inoculated plates were incubated at 26°C for 10-14 days and for evaluation of fluorescent pseudomonads, the inoculated Petri plates were incubated at 26°C for 3 days.

To estimate the number of bacterial or fungal colonies, Petri dishes containing between 30 and 300 colonies were selected. The results were calculated as colony forming units per 1 g of dry weight of soil (Schinnerk *et al.*, 1995).

Assessment of root colonization by arbuscular mycorrhizal fungi The roots of grapevines (10 g from each replication), collected in November, were stained according to the method developed in the Department of Microbiology of the Research Institute of Horticulture (Derkowska *et al.*, 2015). Microscopic specimens were prepared and examined with a Nikon 50i microscope (objectives with magnifications of 20×, 40×, 60×, 100×), and photographic records of the observed mycorrhizal structures were produced. The assessment of the degree of colonization of the roots by arbuscular mycorrhizal fungi was performed by the Trouvelot method (Trouvelot *et al.*, 1986). Based on the results, mycorrhizal frequency (F%), relative mycorrhizal intensity (M%) and absolute mycorrhizal intensity (m%) were calculated using the computer program MYCOCALC, available from the website: <http://www2.dijon.inra.fr/mychintec/Mycocalcprg/MYCOCALC.EXE> (Table 2).

Gas exchange parameters of plants The parameters of gas exchange of plants (CO₂ assimilation intensity – A, transpiration – E, stomatal conductance for water – g_s, and CO₂ concentration in the intercellular spaces – c_i) were measured twice in the growing season (at the veraison stage – 1st test date, and during fruit ripening – 2nd test date), with a TPS-2 (PP Systems) portable gas analyzer (with standard settings) equipped with a PLC4 measuring chamber operating in an open system. The results were read off the screen of the gas analyzer after the values had stabilized. The measurements were performed on healthy, fully grown vine leaves situated on the opposite side of the second or third cluster of grapes (counting from the tip of the shoot) in 12 replications (2 leaves per replication were analyzed, with each leaf coming from a different plant). On the basis of the results of CO₂ assimilation intensity and transpiration, the photosynthetic water-use efficiency (ω_w) was calculated,

which was estimated by the ratio of assimilation intensity to transpiration (Candolfi-Vasconcelos & Koblet, 1991).

Concentrations of assimilation pigments in leaves

The amounts of chlorophyll 'a', 'b' and total chlorophyll in leaves were determined by the method of Arnon *et al.* (1956) modified by Lichtenthaler and Wellburn (1983), while the concentrations of carotenoids in leaves were determined by the method of Hager and Mayer-Berthenrath (1966). The concentration of assimilation pigments was determined in 6 replications on the same test dates and the same leaves on which gas exchange measurements were made. Extracts of the pigments were obtained by grinding samples of fresh leaf mass, about 0.05 g, in a mortar with 10 cm³ of 80% acetone. The homogenates were then centrifuged at 1500 rpm for 10 min. The optical density of samples was determined using a Marcel Mini spectrophotometer at wavelengths of $\lambda = 440$, 465 and 663 nm.

Chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters were recorded in 2014, in the third year after mycorrhization, using a Handy PEA (Hansatech) spectrofluorometer, based on the standard apparatus procedure (3 x 650 nm LEDs, maximum actinic light intensity 3000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The measurements were made twice during the grapevine growing season, in 18 replications (3 leaves per replication were analyzed, each of them coming from a different plant), on the same test dates and the same leaves on which the other physiological characteristics were determined. Leaves were shaded 20 minutes before measurement with factory clips (illuminated area with a diameter of 4 mm). The following parameters of chlorophyll fluorescence induction were measured and calculated using the spectrofluorometer: F_0 – initial fluorescence (zero), excitation energy loss index in power antennas; F_M – maximum fluorescence, after reduction of acceptors in PS II and after dark adaptation; $F_V = F_M - F_0$ – variable fluorescence, determined after dark adaptation, a

parameter dependent on the maximum quantum yield of PS II; F_V / F_M – the maximum potential photochemical reaction efficiency in PS II determined after dark adaptation and after reduction of acceptors in PS II (Bolhár-Nordenkampf & Öquist, 1993); T_{FM} – increase in time of chlorophyll fluorescence from the beginning of measurement to the maximum (F_M); P I - PS II vitality index for the overall viability of this system; A_M (Area) – surface area above the chlorophyll fluorescence curve and between F_0 and F_M points proportional to the size of the reduced plastoquinone acceptors in PS II (Kalaji & Łoboda, 2007).

Statistical analysis of results The results were statistically analyzed using multivariate analysis of variance in the system of random blocks. Multiple comparisons of the means for the combinations were performed with Tukey's test, at a significance level of $\alpha = 0.05$, using STATISTICA v.10 software package. For the numerical data on the gas exchange parameters, concentration of assimilation pigments and chlorophyll fluorescence parameters, the analysis of variance was performed separately for each measurement date.

Meteorological conditions during the experiment During the growing season in 2013, the most rainfall was recorded in the period from around mid-May to the first 10 days of June and from around mid-June to the first 10 days of July. The greatest rainfall shortages occurred in the first 20 days of July, from around mid-August to the first 10 days of September, and from the last ten days of September to the first ten days of October. The weather conditions in 2013 were favourable for grapes to undergo veraison (colour change) and ripening. The year 2014 was characterized by considerable rainfall, practically throughout the entire growing season, with the exception of one prolonged period of rainfall deficit that occurred in June. Adverse weather conditions occurred both during the veraison and fruit ripening stages (Fig. 1). Meteorological data from the Szczecin-Dąbie Meteorological Station (WMO 12205) were used.

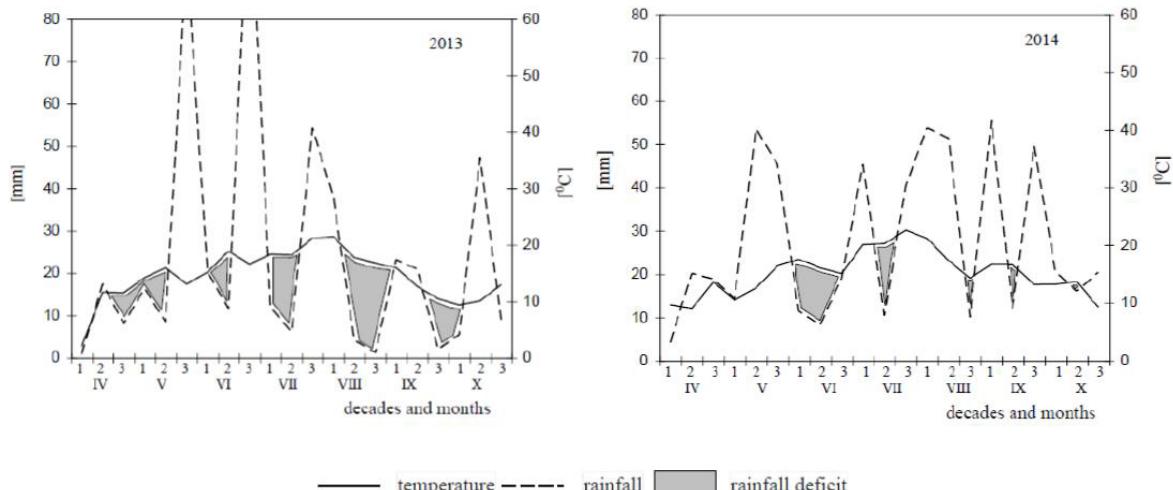


FIGURE 1

Climatogram of the growing season (April-October) in 2013 and 2014 according to Walter and Lieth, modified by Gregorczyk (1995), in a 10-day step system.

RESULTS AND DISCUSSION

The applied treatments modified the bacterial population inhabiting the soil in the growing seasons of 2013 and 2014 (Table 1). In the cultivar 'Regent', mycorrhization was found to have an effect on increasing the total bacterial count in the soil in both years of the experiment. In the cultivar 'Rondo' in 2014, a reduction in the total number of bacteria in the soil was observed. In 2014, mycorrhization contributed to a significant reduction in the total number of spore-forming bacteria in the soil in the cultivar 'Regent'. In the case of the mycorrhized plants of the cultivar 'Pinot Noir', an increase in the number of spore-forming bacteria in the soil was observed in the second year of the experiment. Increased population of *Pseudomonas* bacteria in the mycorrhizal soil may indicate the existence of mutual interrelations between these two groups of microorganisms. The bacteria *Pseudomonas fluorescens* are characterized by a high ability to colonize live mycorrhizal mycelium (Toljander *et al.*, 2005). However, the explanation of the possible relationships between *Pseudomonas* bacteria and AM fungi requires further investigations (Table 1).

In the mycorrhized cultivar 'Regent', the total number of filamentous fungi in the soil was reduced in both years of the experiment. In 2014, an increase in the population of filamentous fungi was observed in the soil of the mycorrhized cultivars 'Pinot Noir' and 'Rondo'. In addition, there was a reduction in the total number of filamentous fungi in the 2014 growing season in comparison with the growing season of 2013 (Table 1). In 2014, a positive effect of mycorrhization on increasing the population of *Pseudomonas* bacteria was observed in all grapevines varieties. The obtained results indicate that the changes in soil microbiology were influenced by several factors, including mycorrhization, grapevine cultivar and weather conditions prevailing during the growing season. In 2014, a smaller overall number of isolated bacteria and filamentous fungi was observed (Table 1). This phenomenon could have been related to the meteorological conditions because in 2014 there was much more rainfall, compared with 2013, when rainfall shortages were observed.

In the soil environment, there are many interactions between arbuscular mycorrhizal fungi and bacteria, which can be of

TABLE 1

Effect of grapevine mycorrhization on the population of isolated microorganisms in 1 g of dry weight of substrate (DW_s).

Factor I/ Factor II	M0	M1	mean	M0	M1	mean
	2013					
Total number of isolated bacteria x 10 ⁷ cfu/g DW _s						
PNR	163.8 a*	163.5 a	163.3**	10.70 cd	11.90 de	11.30
REG	164.4 a	233.7 b	199.1	4.80 a	7.10 b	5.95
RON	n.d.***	n.d.	n.d.	13.00 e	8.80 bc	10.90
Mean	164.1	198.6		9.50	9.30	
Total number of isolated spore-forming bacteria x 10 ⁵ cfu/g DW _s						
PNR	168.9 a	194.5 ab	181.7	59.7 b	96.8 c	78.3
REG	207.8 b	241.6 c	224.7	166.4 e	38.5 a	102.5
RON	n.d.	n.d.	n.d.	141.9 d	124.9 d	133.4
Mean	188.4	218.1		122.7	86.7	
Total number of isolated fluorescent <i>Pseudomonas</i> x 10 ⁵ cfu/g DW _s						
PNR	n.d.	n.d.	n.d.	1.30 a	2.80 c	2.10
REG	n.d.	n.d.	n.d.	1.90 b	2.50 c	2.20
RON	n.d.	n.d.	n.d.	1.40 ab	2.80 c	2.10
Mean	n.d.	n.d.	n.d.	1.50	2.70	
Total number of isolated filamentous fungi x 10 ⁴ cfu/g DW _s						
PNR	80.4 b	76.2 b	78.3	36.8 a	56.8 b	46.8
REG	84.7 b	57.4 a	71.1	61.8 b	40.9 a	51.35
RON	n.d.	n.d.	n.d.	52.8 b	74.3 c	63.55
Mean	82.6	66.8		50.5	57.3	

* means for the interaction of experimental factors marked with the same letters do not differ significantly at the significance level of $\alpha = 0.05$,

** main effects were only compared where the interactions were not statistically significantly different,

*** n.d. – not determined

M0 – without mycorrhiza, M1 – with mycorrhiza

PNR – 'Pinot Noir' on SO4, REG – 'Regent' on 5BB, RON – 'Rondo' on 125AA

TABLE 2

Effect of mycorrhization on parameters of mycorrhizal colonization in the roots of grapevine plants. Analysis of the results, November 2013 and 2014.

Factor I/ Factor II	M0	M1	mean	M0	M1	mean
	2013			2014		
F [%] – mycorrhizal frequency						
PNR	20.00 b*	26.67 d	23.34**	23.33 a	27.78 b	25.56
REG	14.44 a	22.22 bc	18.33	23.33 a	31.11 c	27.22
RON	23.34 c	27.78 d	25.56	21.11 a	32.22 c	26.67
Mean	19.26	25.56		22.59	30.37	
M [%] – relative mycorrhizal intensity						
PNR	0.20 ab	0.27 b	0.24	0.23 a	0.28 b	0.26
REG	0.14 a	0.22 ab	0.18	0.23 a	0.31 c	0.27
RON	0.23 ab	0.28 b	0.26	0.21 a	0.32 c	0.27
Mean	0.19	0.26		0.22	0.30	

* means for the interaction of experimental factors marked with the same letters do not differ significantly at the significance level of $\alpha = 0.05$,

** main effects were only compared where the interactions were not statistically significantly different

M0 – without mycorrhiza, M1 – with mycorrhiza

PNR – 'Pinot Noir' on SO4, REG – 'Regent' on 5BB, RON – 'Rondo' on 125AA

importance in agriculture, such as adherence of bacterial cells to fungal spores, production of volatile substances by bacteria and degradation of fungal cell walls (Miransari, 2011). Arbuscular mycorrhizal fungi and soil bacteria can interact synergistically and stimulate plant growth by facilitating the uptake of nutrients by plants and by reducing fungal soil pathogens. Such interactions are of great importance in sustainable agricultural systems based on the use of biological processes to maintain soil fertility and good plant health. Although there have been many studies on the interaction between arbuscular mycorrhizal fungi and bacteria, including fluorescent *Pseudomonads*, spore-forming bacteria and filamentous fungi, the mechanisms of interaction are not well understood and require further experimentation to optimize the composition of microbial consortia for use in agriculture (Artursson *et al.*, 2006).

After inoculation with the biopreparation Mykoflor, more extensive colonization of roots by arbuscular mycorrhizal fungi was observed in all the cultivars tested, both in the first and second year of the study. The authors mainly observed numerous mycorrhizal vesicles in the grapevine plant roots, which are documented in the photos (Fig. 2-7). The stimulating effect of the biopreparation Mykoflor on the colonization of roots by AM fungi is evidenced by the higher mycorrhizal frequency and intensity observed in the mycorrhized plants during the two years of the experiment (Table 2). Absolute mycorrhizal intensity shows no significant differences between cultivars during the duration of the experiment. The degree of root colonization by mycorrhizal fungi after inoculation, in 2013 and 2014, was the highest for the cultivar 'Rondo' (combination M1RON) and was F% 27.78 and F% 32.22, respectively (Table 2). The lowest mycorrhizal frequency in the inoculated plants in the first year of the experiment was observed in the roots of 'Regent' grapevines

(F% 22.22), and in the second year of the experiment in the roots of 'Pinot Noir' grapevines (F% 27.78). The degree of colonization of grapevine roots by arbuscular mycorrhizal fungi was higher in the second year of the study. Despite the stimulating effect of the biopreparation Mykoflor on the colonization of grapevine roots by arbuscular mycorrhizal fungi, the observed mycorrhizal frequency was low (22.22% to 32.22%) compared with higher values of mycorrhizal frequency for grapevine plants observed by Petit & Gubler (2006). These authors observed colonization of roots at 48.3% in *Vitis rupestris* grapevine plants inoculated with the fungus *Rhizophagus intraradices*, and at 54.5% in plants inoculated with *R. intraradices* and the pathogenic fungus *Cylindrocarpon macroditymum*. A similar, low mycorrhizal frequency had been recorded for apple trees (6.6% to 36.7%) growing in the Pomological Orchard of the Research Institute of Horticulture in Skierniewice (Derkowska *et al.*, 2013). A low mycorrhizal frequency had also been observed in the roots of apple trees (2.5% to 25.8%) and blackcurrant plants (0 to 11.1%) treated with organic mulches, growing in the same research facility (Sumorok *et al.*, 2011). The degree of colonization of plant roots by AM fungi may be due to soil properties, such as phosphorus content and competition from other soil microorganisms (Treseder, 2013; van Overbeek & Saikkonen, 2016).

An important factor affecting mycorrhizal frequency in the roots of grapevine plants is the type of rootstock used. Karagiannidis *et al.* (1997) observed that mycorrhizal frequency, the number of mycorrhizal structures in the roots and the number of spores in the substrate depended on the rootstock used and on the cultivar grafted onto it. In their experiments, they examined 4 rootstocks onto which 9 grapevine cultivars were grafted. They found the highest mycorrhizal frequency, from 68% to 87%, in the

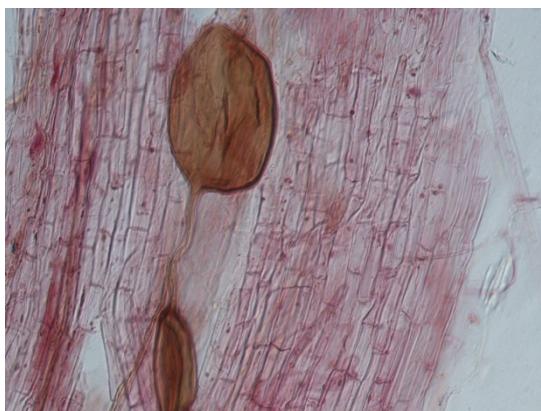


FIGURE 2
Vesicles in the control roots of 'Rondo' grapevine grafted onto 125AA rootstock (Derkowska E., IO Rhizosphere Laboratory, 2014).



FIGURE 3
Vesicles in the mycorrhized roots of 'Rondo' grapevine grafted onto 125AA rootstock (Derkowska E., IO Rhizosphere Laboratory, 2014).



FIGURE 4
Vesicles in the control roots of 'Pinot Noir' grapevine grafted onto SO4 rootstock (Derkowska E., IO Rhizosphere Laboratory, 2014).

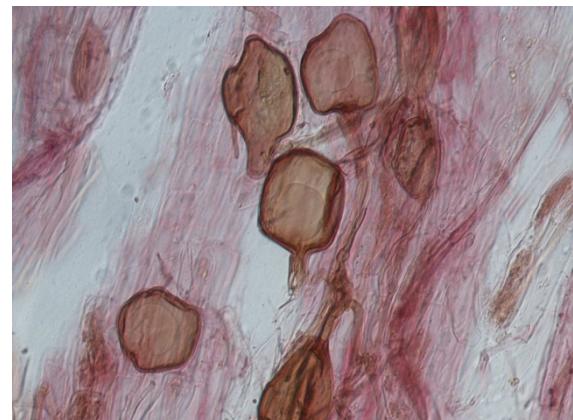


FIGURE 5
Vesicles in the mycorrhized roots of 'Pinot Noir' grapevine grafted onto SO4 rootstock (Derkowska E., IO Rhizosphere Laboratory, 2014).



FIGURE 6
Vesicles in the control roots of 'Regent' grapevine grafted onto 5BB rootstock (Derkowska E., IO Rhizosphere Laboratory, 2014).



FIGURE 7
Vesicles in the mycorrhized roots of 'Regent' grapevine grafted onto 5BB rootstock (Derkowska E., IO Rhizosphere Laboratory, 2014).

roots of rootstock 1103 P, while the values determined for other rootstocks were in the ranges: 36% to 55% for 110 R, 41% to 62% for 140 Ru, and 57% to 74% for the 41B rootstock. Similar relations were noted in the number of observed spores, which occurred in the largest numbers in the rhizosphere of the 1103 P rootstock, with the lowest numbers found in the rhizosphere of the 110 R rootstock. In the tests conducted in 2013, the highest average mycorrhizal frequency was found for the cultivar 'Rondo' on the 125AA rootstock (25.56%) and for the cultivar 'Pinot Noir' on the SO₄ rootstock (23.34%), while the lowest mycorrhizal frequency was observed for the cultivar 'Regent' on the 5BB rootstock (18.33%) (Table 2). In 2014, no statistically significant differences in the average mycorrhizal frequency were found among the cultivars.

According to Borkowska (2010), the phenomenon of mycorrhiza does not directly affect the functioning of processes, such as electron transport during the light phase of photosynthesis and regeneration of the Rubisco enzyme involved in binding CO₂, responsible for the activity of the photosynthetic apparatus of plants. Nevertheless, it can affect them indirectly by providing plants with water and a better supply of phosphorus necessary, *inter alia*, in photosynthetic phosphorylation. In addition, the plant receives nitrogen and magnesium through the fungus, and also microelements such as iron, copper and manganese – these elements perform important functions in photosynthesis (Kaschuk *et al.*, 2009). The current study showed that the mycorrhized grapevine plants were characterized by a greater CO₂ assimilation intensity – this relationship was found on one of the measurement dates in each year of the study. Similarly, according to Zhu *et al.* (2012), mycorrhization with the species *Claroideoglomus etunicatum* increased the rate of net photosynthesis in maize plants growing in the conditions of both drought and optimal soil moisture. An increase in the intensity of CO₂ assimilation in grapevines of the cultivar 'Crimson' and *Citrus tangerina* under the influence of mycorrhization with fungi of the genus *Glomus* was also demonstrated by Nicolás *et al.* (2015) and Wu & Xia (2006), respectively.

In the first year of the study, on the second measurement date, the highest intensity of CO₂ assimilation was shown by the grapevines 'Rondo' and 'Regent'. In the second year of the study, the highest intensity of CO₂ assimilation was also observed in the cultivar 'Rondo' on the second measurement date, compared to 'Pinot Noir'. After analyzing the interaction of the experimental factors, it can be stated that in the first year of the study, on the second measurement date, the highest intensity of CO₂ assimilation was shown by the grapevines of the cultivars 'Rondo' and 'Regent', both the mycorrhized and control ones. In the second year of the study, the highest intensity of this process was exhibited by the grapevines from the combination M1RON (on the second measurement date it was significantly different from the intensity found in the combinations M0RON, M0REG and M0PNR) (Table 3).

The vines of the cultivars 'Regent' and 'Rondo' showed the highest intensity of transpiration. The cultivar 'Pinot Noir' conducted transpiration at a level similar to that of the cultivar 'Regent', but only on the second measurement date

of the first year of the study. In both years of the study, the highest intensity of transpiration was exhibited by vines of the cultivars 'Rondo' and 'Regent' subjected to mycorrhization (Table 3). The inoculation with mycorrhizal fungi increased the intensity of transpiration in the vines. Similar results had also been obtained by Wu & Xia (2006) and Zhu *et al.* (2012) in studies on the impact of mycorrhization with fungi of the genus *Glomus* on the intensity of transpiration in species such as *Citrus tangerina* and maize, respectively.

The plants subjected to mycorrhization were characterized by lower photosynthetic water-use efficiency – this correlation was proved on the second test date of the first year and on both test dates of the second year of the study. Comparing the tested grapevine cultivars with respect to this trait, it was found that the highest value of this index on one of the measurement dates of both years of the study was shown by the cultivar 'Pinot Noir'. On the second test date of the first year of the study, this was shown by the cultivars 'Regent' and 'Rondo', while on the first test date of the second year by the cultivars 'Pinot Noir' and 'Rondo'. The interaction of the experimental factors exerted a multi-directional effect, depending on the measurement date, on the efficiency of water use in photosynthesis in the tested grapevine cultivars (Table 3).

The study proved that mycorrhization of grapevine plants increased stomatal conductance for water – this correlation was recorded on both measurement dates in the first year of the study and on the first test date in the second year. The grapevine cultivars under comparison did not differ in terms of this physiological characteristic. After analyzing the interaction of the experimental factors, it was found that the inoculation with mycorrhizal fungi increased the stomatal conductance for water, determined in the two consecutive years of the study, on the first measurement date, in the grapevine cultivars 'Pinot Noir' and 'Rondo', and 'Regent' and 'Rondo', respectively. On the second test date, this relationship was demonstrated in both years of the study, for all the cultivars tested (Table 3). Similar results had been obtained by Wu & Xia (2006), Nicolás *et al.* (2015) and Zhu *et al.* (2012), according to which mycorrhization with fungi of the genus *Glomus* increased the stomatal conductance for water in the leaves of *Citrus tangerine*, the grapevine cultivar 'Crimson', and maize plants growing in drought conditions (no such relationship was found for maize grown under optimal conditions). On the first measurement date of testing, the concentration of CO₂ in the intercellular spaces of leaves was demonstrated to be higher in the grapevines subjected to inoculation with mycorrhizal fungi than in the control. Comparing the tested grapevine cultivars, it was found that the highest concentration of intercellular CO₂ was shown by the vine leaves of the cultivars 'Regent' and 'Rondo' (on the first test date of the first year of the study) and the cultivar 'Regent' (on the second test date of the first year and in the second year of the study). The smallest value of this trait, however, was shown by the grapevine cultivar 'Pinot Noir' on the first test date of the first year of the study and on both test dates of the second year. After analyzing the interaction of the experimental factors, it was found that the highest concentration of CO₂ in the intercellular spaces of leaves in the first year of the study was shown by

TABLE 3

Effect of mycorrhization on the intensity of CO_2 assimilation (A) and transpiration (E), photosynthetic water-use efficiency (ω_w), stomatal conductance for water (g_s), and CO_2 concentration in the intercellular spaces (c_i) in grapevines.

Factor I/ Factor II	M0	M1	mean	M0	M1	mean						
	2013			2014								
A – CO_2 assimilation intensity [$\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$]												
1st test date												
PNR	5.90 a*	7.88 b	6.89**	16.80 a	18.58 b	17.69						
REG	6.63 ab	7.18 ab	6.36	15.35 a	15.72 a	15.53						
RON	5.75 a	6.97 ab	6.91	18.52 b	20.27 c	19.39						
Mean	6.09	7.34		16.89	18.19							
2nd test date												
PNR	7.48 a	11.52 b	9.50	16.19 a	19.80 cd	17.99						
REG	18.05 c	18.99 c	18.52	18.10 b	19.88 cd	18.99						
RON	19.15 c	19.47 c	19.31	19.37 bc	21.28 d	20.32						
Mean	14.89	16.66		17.88	20.32							
E – transpiration intensity [$\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$]												
1st test date												
PNR	1.15 b	0.61 a	0.88	1.56 a	2.10 ab	1.83						
REG	1.01 ab	2.21 d	1.61	2.24 b	3.18 c	2.71						
RON	1.64 c	2.46 d	2.05	2.20 b	2.97 c	2.59						
Mean	1.27	1.76		2.00	2.75							
2nd test date												
PNR	1.31 a	2.33 b	1.82	1.41 a	2.65 bc	2.03						
REG	1.87 b	2.94 c	2.41	2.39 b	3.23 d	2.81						
RON	2.10 b	3.53 d	2.81	2.35 b	3.01 cd	2.68						
Mean	1.76	2.93		2.05	2.96							
ω_w – photosynthetic water-use efficiency [$\text{mmol} \cdot \text{mol}^{-1}$]												
1st test date												
PNR	5.13 b	12.9 c	9.01	10.76 c	8.85bc	9.80						
REG	6.56 b	3.25 a	4.90	6.85 ab	4.94 a	5.89						
RON	3.51 a	2.83 a	3.17	8.42 ab	6.83 ab	7.62						
Mean	5.06	6.33		8.68	6.87							
2nd test date												
PNR	5.70 a	4.94 a	5.30	11.48 c	7.47 a	9.47						
REG	9.65 b	6.46 a	8.05	7.57 a	6.15 a	6.86						
RON	9.12 b	5.52 a	7.32	8.24 b	7.08 a	7.66						
Mean	8.16	5.64		9.10	6.90							
g_s – stomatal conductance for water [$\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$]												
1st test date												
PNR	0.151 a	0.325 c	0.238	0.195 a	0.259 ab	0.227						
REG	0.141 a	0.183 ab	0.162	0.202 a	0.307 b	0.254						
RON	0.134 a	0.237 b	0.186	0.213 a	0.320 b	0.267						
Mean	0.142	0.248		0.203	0.295							
2nd test date												
PNR	0.228 a	0.420 d	0.324	0.300 c	0.347 d	0.323						
REG	0.297 b	0.403 cd	0.350	0.179 a	0.395 e	0.287						
RON	0.218 a	0.348 bc	0.283	0.221 b	0.337 cd	0.279						
Mean	0.248	0.390		0.233	0.359							

TABLE 3 (CONTINUED)

Factor I/ Factor II	M0 2013	M1	mean	M0 2014	M1	mean
$c_i - CO_2$ concentration in the intercellular spaces [$\mu\text{mol}\cdot\text{mol}^{-1}$]						
1st test date						
PNR	232.17 a	233.83 a	233.00	257.84 ab	242.70 a	250.27
REG	349.07 b	352.13 b	350.60	344.17 c	358.83 c	351.50
RON	330.50 b	341.33 b	335.92	286.67 b	348.67 c	317.67
Mean	303.91	309.10		296.22	316.73	
2nd test date						
PNR	274.18 b	316.72 c	295.45	255.17 a	324.50 bc	289.83
REG	357.33 de	384.67 e	371.00	386.00 d	354.00 cd	370.00
RON	228.17 a	332.33 cd	280.25	292.17 b	361.00 d	326.58
Mean	286.56	344.57		311.11	346.50	

* means for the interaction of experimental factors marked with the same letters do not differ significantly at the significance level of $\alpha = 0.05$,

** main effects were only compared where the interactions were not statistically significantly different

M0 – without mycorrhiza, M1 – with mycorrhiza,

PNR – ‘Pinot Noir’ on SO4, REG – ‘Regent’ on 5BB, RON – ‘Rondo’ on 125AA

the combinations M1REG, M0REG, M1RON and M0RON (1st date), and M1REG (2nd date). In the second year of the study, however, it was the combinations M1REG, M1RON and M0REG (1st date), and M0REG and M1RON (2nd date) – Table 3. According to Zhu *et al.* (2012), inoculation with the mycelium of *Claroideoglomus etunicatum* reduced the CO_2 concentration in the intercellular spaces of leaves of maize plants growing both under the conditions of optimal soil moisture content and in drought conditions.

The study presented here did not find a significant effect of mycorrhization on the concentration of the estimated assimilation pigments, i.e. chlorophyll ‘a’, ‘b’, total chlorophyll and carotenoids (Table 4). In the first year of the study, no differences were found in chlorophyll ‘a’ content and total chlorophyll in the leaves of the grapevine cultivars under comparison. On the second test date, a difference in chlorophyll ‘b’ content was found between the cultivar ‘Regent’, in which it was the highest, and the cultivar ‘Pinot Noir’. The tested grapevine cultivars were characterized by a varied concentration of carotenoids in the leaves, which depended on the test date and year of the study. On the first test date in the first year of the study, the lowest concentration of these pigments was shown by the cultivar ‘Regent’, and the highest by ‘Rondo’. On the second test date in the second year of the study, the cultivars ‘Regent’ and ‘Pinot Noir’ surpassed the cultivar ‘Rondo’ in terms of the carotenoid content. In the first year of the study, there was no significant effect of the interaction of the experimental factors on the amounts of chlorophyll ‘a’, ‘b’ and total chlorophyll in grapevine leaves. On the first test date in the second year of the study, the effect on the amount of chlorophyll ‘a’ and total chlorophyll was significant, but ambiguous. On the second test date of that year, the highest chlorophyll ‘a’ content was recorded in the mycorrhized and control plants of the cultivars ‘Pinot Noir’ and ‘Regent’. In the case of total chlorophyll, there was a proven difference between the amount of this pigment in plant leaves from

the combinations M0PNR, M1PNR, M1REG and M0PNR, where it was the largest, and the combinations M0RON and M1RON. On that test date, however, the highest amounts of carotenoids were found in the combinations M1PNR, M0REG and M0PNR. Different results had been obtained by Aslanpour *et al.* (2016), according to which the inoculation of white grapevines with the fungus *Glomus mosseae* increased the total chlorophyll content in the leaves. However, the authors did not demonstrate any differences in the chlorophyll content in the leaves of the tested grapevine cultivar depending on the species of fungi (*Glomus mosseae*, *Glomus fasciculatum*, *Glomus intraradies*) used for mycorrhization. Increased amounts of chlorophyll ‘a’, ‘b’ and total chlorophyll as a result of mycorrhization with the species *Claroideoglomus etunicatum* had also been found by Zhu *et al.* (2012) in the leaves of maize plants growing under the conditions of drought stress – such correlations were not observed in plants growing in a soil with an optimal moisture content. According to Smith & Read (2008), the increase in the chlorophyll content of the leaves of plants inoculated with mycorrhizal fungi may be caused by the increased uptake of phosphorus.

On both test dates, there was no effect of the mycorrhizal treatment on the size of the F_0 parameter (initial fluorescence after dark adaptation) in the leaves of the vines, and also the studied grapevine cultivars did not differ in respect of this trait. On the second test date, the smallest F_0 value was shown by the cultivar ‘Regent’ not subjected to mycorrhization, which may indicate that its photosynthetic apparatus, in comparison with plants from other combinations, was characterized by a higher efficiency of excitation energy transfer between chlorophyll molecules (Baker & Risenquist, 2004). The inoculation with mycorrhizal fungi did not affect the value of the F_M parameter, i.e. the maximum fluorescence after dark adaptation. After comparing the tested grapevine cultivars in respect of this trait, it was found that the highest F_M value was shown by the leaves of the cultivar ‘Rondo’,

TABLE 4

Effect of mycorrhization on the concentration of assimilation pigments in grapevine leaves.

Factor I/ Factor II	M0 2013	M1 2013	mean	M0 2014	M1 2014	mean
Chlorophyll 'a' [mg·g ⁻¹ FW]						
1st test date						
PNR	1.600 a*	1.585 a	1.593 A**	1.523 a	1.543 ab	1.533
REG	1.608 a	1.642 a	1.625 A	1.570 abc	1.498 a	1.534
RON	1.613 a	1.595 a	1.604 A	1.722 c	1.690 bc	1.706
Mean	1.607 A	1.607 A		1.605	1.577	
2nd test date						
PNR	1.653 a	1.665 a	1.659 A	1.658 b	1.688 b	1.672
REG	1.638 a	1.680 a	1.659 A	1.620 b	1.633 b	1.626
RON	1.705 a	1.718 a	1.711 A	1.485 a	1.498 a	1.491
Mean	1.665 A	1.688 A		1.587	1.606	
Chlorophyll 'b' [mg·g ⁻¹ FW]						
1st test date						
PNR	0.647 a	0.625 a	0.636 A	0.655 a	0.665 a	0.660 A
REG	0.583 a	0.585 a	0.584 A	0.585 a	0.657 a	0.621 A
RON	0.623 a	0.630 a	0.626 A	0.658 a	0.588 a	0.623 A
Mean	0.617 A	0.613 A		0.633 A	0.637 A	
2nd test date						
PNR	0.585 a	0.593 a	0.589 A	0.630 a	0.588 a	0.609 A
REG	0.642 a	0.670 a	0.656 B	0.597 a	0.637 a	0.618 A
RON	0.633 a	0.593 a	0.613 AB	0.600 a	0.585 a	0.593 A
Mean	0.620 A	0.618 A		0.609 A	0.603 A	
Total chlorophyll [mg·g ⁻¹ FW]						
1st test date						
PNR	2.248 a	2.210 a	2.229 A	2.178 a	2.208 ab	2.193
REG	2.190 a	2.228 a	2.209 A	2.155 a	2.155 a	2.155
RON	2.235 a	2.225 a	2.230 A	2.380 b	2.277 ab	2.329
Mean	2.224 A	2.221 A		2.238	2.213	
2nd test date						
PNR	2.237 a	2.258 a	2.248 A	2.288 b	2.275 b	2.281
REG	2.280 a	2.350 a	2.315 A	2.217 ab	2.270 b	2.244
RON	2.338 a	2.310 a	2.324 A	2.085 a	2.083 a	2.084
Mean	2.285 A	2.306 A		2.197	2.209	
Carotenoids [mg·g ⁻¹ FW]						
1st test date						
PNR	0.863 bc	0.895 c	0.879	0.805 a	0.840 a	0.823 A
REG	0.718 a	0.775 ab	0.746	0.830 a	0.825 a	0.827 A
RON	0.945 c	0.950 c	0.948	0.858 a	0.890 a	0.874 A
Mean	0.842	0.873		0.831 A	0.852 A	
2nd test date						
PNR	0.795 a	0.833 a	0.814 AB	0.973 b	0.995 b	0.984
REG	0.823 a	0.865 a	0.844 B	0.975 b	0.927 ab	0.951
RON	0.732 a	0.755 a	0.744 A	0.815 a	0.893 ab	0.854
Mean	0.783 A	0.817 A		0.921	0.938	

* means for the interaction of experimental factors marked with the same letters do not differ significantly at the significance level of $\alpha = 0.05$,

** main effects were only compared where the interactions were not statistically significantly different

M0 – without mycorrhiza, M1 – with mycorrhiza

PNR – 'Pinot Noir' on SO4, REG – 'Regent' on 5BB, RON – 'Rondo' on 125AA

TABLE 5

Effect of mycorrhization on the fluorescence parameters of chlorophyll 'a' in grapevine leaves.

Factor I/ Factor II	M0	M1	mean	M0	M1	mean
F_0 – initial fluorescence (zero)						
	1st test date			2nd test date		
PNR	567.5 a*	584.5 a	576.0 A**	565.7 b	589.0 b	577.3
REG	582.0 a	597.0 a	589.5 A	451.7 a	581.0 b	516.3
RON	586.5 a	579.0 a	582.8 A	584.7 b	545.7 b	565.2
Mean	578.7 A	586.8 A		534.0	571.9	
F_M – maximum fluorescence						
	1st test date			2nd test date		
PNR	290.0 a	290.0 a	290.0	2786.7 ab	3029.3 bc	2908.0
REG	290.0 a	295.0 ab	292.5	2819.7 ab	2629.3 a	2724.5
RON	300.0 b	300.0 b	300.0	3257.0 c	3659.0 d	3458.0
Mean	293.3	295		2954.4	3105.9	
F_v/F_M – maximum potential photochemical reaction efficiency in PS II						
	1st test date			2nd test date		
PNR	0.788 ab	0.801 b	0.794	0.797 ab	0.803 ab	0.800
REG	0.784 ab	0.761 a	0.773	0.791 ab	0.879 b	0.835
RON	0.816 b	0.809 b	0.812	0.801 ab	0.703 a	0.752
Mean	0.796	0.790		0.796	0.795	
T_{FM} – increase in time of chlorophyll fluorescence from the beginning of measurement to the maximum (F_M)						
	1st test date			2nd test date		
PNR	490.0 a	490.0 a	490.0	491.7 ab	486.7 a	489.2
REG	490.0 a	495.0 ab	492.5	486.7 a	491.7 ab	489.2
RON	500.0 b	500.0 b	500.0	500.0 b	498.3 b	499.2
Mean	493.3	495.0		492.8	492.2	
Area – surface area above the chlorophyll fluorescence curve and between F_0 and F_M points						
	1st test date			2nd test date		
PNR	43093.5 a	45982.5 a	44538.0	46024.3 a	45526.3 a	45775.3
REG	45090.0 a	43226.5 a	44158.3	44405.0 a	41180.7 a	42792.8
RON	62898.0 b	53966.5ab	58432.3	62290.0 b	61428.3 b	61859.2
Mean	50360.5	47725.2		50906.4	49378.4	
P I - PS II vitality index						
	1st test date			2nd test date		
PNR	0.633 a	0.737 ab	0.685	0.559 a	0.703 b	0.631
REG	0.610 a	0.476 a	0.543	0.572 a	0.718 b	0.645
RON	1.210 c	0.974 bc	1.092	0.942 c	1.095 d	1.018
Mean	0.818	0.729		0.691	0.838	

* means for the interaction of experimental factors marked with the same letters do not differ significantly at the significance level of $\alpha = 0.05$,
 ** main effects were only compared where the interactions were not statistically significantly different

M0 – without mycorrhiza, M1 – with mycorrhiza

PNR – 'Pinot Noir' on SO4, REG – 'Regent' on 5BB, RON – 'Rondo' on 125AA

which may indicate that in the other cultivars, due to stress, not all electron acceptors in PS II had been completely reduced (Kalaji & Łoboda, 2010). On the first measurement date, the highest F_M values (significantly different from all the combinations, except M1REG) were characterized by the vines of the cultivar 'Rondo', both the mycorrhized and control ones. On the second test date, however, it was the cultivar 'Rondo' subjected to fungal inoculation (Table 5). The maximum potential efficiency of photochemical reaction in PS II determined after dark adaptation (F_V/F_M) is a parameter considered to be a measure of the photochemical activity of the photosynthetic apparatus of plants and describes the efficiency with which PS II absorbs light energy. Under optimal growth conditions, it takes on values of approximately 0.85 relative units, according to Angelini *et al.* (2001) and Bjorkman & Demmig (1987). The tested grapevine cultivars, both those under mycorrhization and the untreated control, were characterized, on both measurement dates, by lower than optimal values of this index, which may have been due to the excessive rainfall that was recorded in that year of the study (Table 5). Lowering of the F_V/F_M value under the influence of excessive soil water content and root flooding had been demonstrated by Bertolde *et al.* (2012) and Yu *et al.* (2015) in one of the cultivars of the cocoa tree (*Theobroma cacao*) and the Euphrates poplar (*Populus euphratica*), respectively. On the first measurement date, higher values of the F_V/F_M parameter were shown by the cultivars 'Rondo' and 'Pinot Noir'. In the case of all the cultivars tested, no significant effect of mycorrhization on the maximum potential efficiency of the photochemical reaction in PS II was demonstrated. Comparing the values of this index determined on the first measurement date for the individual grapevine cultivars subjected to mycorrhization, it was found that they were the smallest for the cultivar 'Regent' (Table 5).

During the veraison stage in the grapevine cultivars tested, a higher value of the T_{FM} index was found in the plants under mycorrhization, which may indicate that they were in a state of stress for longer in comparison with the control. Similarly, when comparing the cultivars, a longer time to reach the maximum chlorophyll fluorescence was recorded on both measurement dates in the cultivar 'Rondo'. Analyzing the influence of the interaction of the experimental factors on the TFM parameter, its highest values, significantly different from all the experimental combinations except M1REG (in the first measurement date) and M0PNR, M1REG (in the second measurement date), were found in combinations M0RON and M1RON (Table 5).

The mycorrhizal treatment did not affect the value of the Area index, i.e. the size of the pool of reduced electron acceptors in PS II, which is, according to Kalaji & Łoboda (2007), one of the best performance indicators of the photosynthetic apparatus of plants; nor on the PS II (P I) vitality index describing the overall vitality of this photosystem. The highest values of these parameters of chlorophyll fluorescence, on both measurement dates, were shown by the cultivar 'Rondo', which, together with the T_{FM} values determined for this cultivar, may indicate a high efficiency of its repair mechanisms under stressful conditions. On the first measurement date, the highest value

of Area, significantly different from all the combinations except M1RON, was found in the leaves of the vines from the combination M0RON. On the second test day, however, it was the combinations M0RON and M1RON. According to Krause & Weiss (1991), the Area parameter informs about the number of acceptors available in PS II. On the first measurement date, the highest value of the P I index (significantly different from all the combinations except M1RON) was found in the cultivar 'Rondo' not subjected to mycorrhization. On the second measurement date, however, it was the mycorrhized cultivar 'Rondo'. On that date, the measurements also showed higher values, for all the cultivars, of the PS II vitality index after inoculation with mycorrhizal fungi (Table 5).

CONCLUSIONS

The number of isolated microorganisms in the soil depended mainly on the weather conditions prevailing during the growing season. The considerably greater amount of rainfall in the second year of the study limited the numbers of all the soil microorganisms determined in both growing seasons. In the individual years of the study, the changes in soil microbiology were also influenced by the mycorrhizal treatment, the type of rootstock and the cultivar grafted onto it. The applied inoculation had a stimulating effect on the colonization of roots by AM fungi, as evidenced by higher mycorrhizal frequency and intensity in the mycorrhized plants. The influence of the applied rootstock and the cultivar grafted onto it on mycorrhizal frequency was apparent only in the first year of the study.

The inoculation of the roots of the tested grapevine cultivars with mycorrhizal fungi increased the intensity of CO_2 assimilation (on one of the measurement dates, in each year of testing), transpiration and stomatal conductance for water in leaves. Mycorrhization reduced the efficiency of water use in photosynthesis and did not affect the concentration of assimilation pigments and such chlorophyll fluorescence parameters as F_o , F_M , F_V/F_M and PI in grapevine leaves. The highest values of the Area index and the PI index were shown by the cultivar 'Rondo', which, together with T_{FM} values (increase in time to achieve the maximum chlorophyll fluorescence) determined for this cultivar, may indicate a high efficiency of its repair mechanisms within its photosynthetic apparatus under stressful conditions. The obtained research results indicate that, among the tested cultivars, 'Rondo' is the most adapted to growing in the coldest zone (A) of viticulture. Beneficial effects of the mycorrhization on some physiological features of the examined grapevine varieties, including, *inter alia*, intensity of CO_2 assimilation, which determine plant productivity, may indicate usefulness of mycorrhizal inoculation in viticulture.

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