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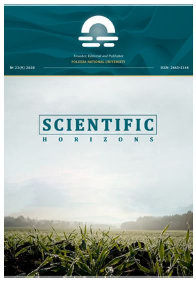

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Investigation of the response of sweet cherries to root mycorrhisation with biologics for sustainable horticulture development

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Abstract. Sustainable horticulture development is a priority area considering climate change, especially in the context of reduced water supply. The search for ways to regulate the resistance of fruit trees to stressors is an urgent issue for the world community. The use of beneficial microorganisms to inoculate plant roots helps reduce the use of synthetic substances and is an alternative for sustainable horticulture. The purpose of the study is to identify the specific features of the effect of root inoculation by mycorrhizal fungi on the reaction of sweet cherries to develop strategies for managing the production of fruit products. The study was conducted during 2018-2020 in the sweet sweet cherry orchard of the southern steppe subzone of Ukraine. Such research methods as field, laboratory, biochemical, physiological, and statistical were used. The regularities of the influence of mycorrhizal fungi on the total moisture content and water-holding

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capacity of sweet cherry leaves were examined. In the first year of studies on endomycorrhizal root inoculation, these indicators were higher compared to the control group. The positive effect of endo-ectomycorrhiza on the water-holding capacity of sweet cherry leaves was elucidated. The total leaf surface and the content of green pigments in the leaves were determined as one of the main indicators of the photosynthetic potential of a fruit crop. The effect of inoculation of sweet cherry roots by mycorrhizal fungi on the ratio of chlorophylls (a/b) in leaves was established. The parameters of the content of total nitrogen, phosphorus, and potassium in sweet cherry leaves were determined. The use of mycorrhizal fungi increases the adaptive properties of sweet cherry trees to stressful factors, namely, arid conditions in the south of Ukraine. The research results can be used by fruit producers to improve climate-optimised technologies, which will substantially reduce risks and possible economic losses, and preserve biodiversity

Keywords: endomycorrhiza; ectomycorrhiza; leaf area; *Prunus avium*; water-holding capacity; chlorophyll; basic elements of nutrition

INTRODUCTION

Sustainable fruit production plays a key role in ensuring food security, the well-being of the population, and the competitiveness of the country. Among the manifestations of climate change in the context of global warming, the main negative impacts on the production of fruit should be distinguished: a substantial increase in air temperature, changes in the thermal regime, fluctuations in the distribution of precipitation, increased flow of natural meteorological phenomena and extreme weather conditions. This affects the sustainable development of horticulture in most regions and requires the adaptation of cultivation technologies to these changes to reduce risks and possible economic losses. Therefore, it is now necessary to change approaches to conducting agribusiness, considering the principles of the European green course towards switching to innovative climate-optimised technologies.

Sweet cherries are among the most popular fruit crops, which consumers highly appreciate due to their pleasant taste, attractive appearance, and valuable nutritional and biochemical properties (Ivanova *et al.,* 2021). Sweet cherries can be grown in a wide range of climatic conditions (Serdyuk *et al.,* 2020; Ivanova *et al.*, 2022). Due to climate change, fruit producers are now facing challenges that pose a threat to increasing the volume of sweet cherries. The economic value of sweet cherries encourages improving the elements of its cultivation technology to enhance the conditions for plant growth and development, increase fruit production and increase quality (Gerasko *et al.,* 2022a). The use of beneficial microorganisms for mycorrhisation of plant roots helps to reduce the use of synthetic substances and is an alternative for sustainable horticulture (Nasif *et al.,* 2022). Inoculation of plant roots with beneficial microorganisms such as arbuscular mycorrhiza (AM) and rhizobacteria to stimulate plant growth and development is considered an alternative to the use of synthetic drugs (Cobb *et al.,* 2021). Arbuscular mycorrhiza, as a form of mutualistic symbiotic association, improves the supply of nutrients to the plant, increases tolerance to abiotic stresses and resistance to various pathogens and pests (Jain & Pundir, 2019; Lin *et al.,* 2021). According to researchers (Chen *et al.,* 2017; Brito *et al.,* 2021), the use of arbuscular mycorrhizal fungi (AMF) improves vegetative growth, the content of secondary metabolites, the assimilation of nutrients by plants, soil conditions for host plants by improving soil structure and promoting ecosystem sustainability.

In the study, M. Vosnjak *et al.* (2022) highlighted the physiological and biochemical reactions of the leaves of three-year-old sweet cherry trees under the influence of low temperatures in vivo 36 days after full flowering. The change in the examined physiological and biochemical parameters of leaves under the influence of low temperatures was established. A uniform and substantial decrease in gas exchange parameters, chlorophyll fluorescence, and an increase in the content of xanthophyll cycle pigments, especially seaxanthin and anteraxanthin, were observed. Despite the increase in seaxanthin, researchers noted a decrease in the content of chlorophylls.

The positive effect of mycorrhizal fungi has also been observed in studies with other types of fruit trees, crops, and wild plants. The effect of AM is particularly substantial when growing plants under unfavourable conditions (Neidhardt, 2021). The study by Zhang *et al.* (2018) found an increase in the tolerance of *Lolium perenne* plants to cadmium (Cd) under the influence of root inoculation with arbuscular mycorrhiza, as well as a decrease in the toxicity of Cd for host plants. According to researchers, root inoculation of *Glomus mosseae* can enhance the photosynthetic ability of leaves to assimilate carbon by improving the absorption of phosphorus by roots from the soil.

X. Cai *et al.* (2021) determined that complex inoculation by mycorrhizal fungus *Glomus mosseae* and bacteria *Bacillus subtilis* increased the content of nutrients in plants, total soluble protein, total soluble sugar, total content of free amino acids and reduced the damage to the root system by fusarium pathogens. In the paper of S. Gluszczek *et al.* (2020), the positive effect of organic fertilisers and mycorrhisation on the growth indicators of the root system, its colonisation with arbuscular mycorrhizal fungi, and the yield of sweet cherry trees was highlighted. Researchers noted a tendency for an increase in the raw and dry mass of roots, their diameter, the length of roots and their surface area under the influence of inoculation with a mycorrhizal substrate compared to control trees.

Anandakumar & Kalaiselvi (2022) recorded an increase in the length of shoots and roots, leaf area, leaf surface index, shoot and root biomass, and chlorophyll content in *Vigna mungo*. According to the authors, the number of spores of AM fungi is one of the key factors affecting the percentage of mycorrhizal colonisation of the root system of plants, which impacts the growth and productivity of host plants. Jumrani *et al.* (2022) found a reduction in the negative effect of high-temperature stress on plants when soy is inoculated with arbuscular mycorrhizal fungi. Inoculated AMF soybean plants showed an increase in leaf area, stem height, root and shoot length, and dry root biomass. Researchers also noted an increase in the content of chlorophyll, the number of stomata, the rate of photosynthesis, the conductivity of stomata, the rate of transpiration, and the efficiency of water use by plants under the influence of mycorrhisation of AM roots. The results of this study confirm the high efficiency of using mycorrhizal inoculants as a biofertiliser to increase soybean productivity under high-temperature stress.

The purpose of this study is to evaluate the effect of root inoculation by mycorrhizal fungi on physiological and biochemical composition of sweet cherry leaves. The objectives of the study are to determine the total area of leaves, the specific density of leaves, total moisture content, water-holding capacity, content and the ratio of chlorophylls, the content of the main elements of mineral nutrition in sweet cherry leaves by inoculation of roots by mycorrhizal fungi.

MATERIALS AND METHODS

The study was conducted during 2018-2020 on chestnut sandy soils in the conditions of the southern steppe subzone of Ukraine. The experimental plots are located in the research orchard of the Dmytro Motornyi Tavria State Agrotechnological University (46°46'N, 35°17'e). The nitrogen content in the horizon of 0-20 cm was 5.5 mg/100 g, the content of P_2O_5 – 5.4 mg/100 g and K_2O – 6.5 mg/g of soil. The reaction of the soil solution was slightly alkaline (pH 7.1-7.4). The humus content in the upper soil layer was 0.6%. The total content of water-soluble salts did not exceed 0.015-0.024%. The soil conditions of the experimental garden are favourable for mycorrhisation since the low level of soil supply with nitrogen and phosphorus contributes to the colonisation of plant roots by symbiotic fungi.

Over the years of research, the average long-term air temperature during the growing season was favourable for the growth and development of sweet cherries (Table 1).

Table 1. *Deviations of air temperature and precipitation compared to long-term averages*

Years	Months												Mid-year
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Average monthly air temperature, °C													
2018	+1.2	+1.1	-1.5	+3.0	+3.0	+2.2	+1.1	+2.6	+1.5	+2.9	-1.1	+0.6	+1.3
2019	+1.6	+2.4	+0.7	+1.0	+1.5	+4.1	-0.6	+0.4	+0.7	+1.4	+2.3	+3.8	+1.6
2020	+2.8	+3.6	+4.7	-1.0	-1.7	+1.6	+1.3	+0.2	+3.3	+4.6	+0.7	+5.3	+2.1
Precipitation, (%)													
2018	+21	+39	+96	-84	-52	-40	+81	-82	+104	-44	-3	+74	+10
2019	+19	-68	+6	+44	+107	-73	-5	+61	-67	-42	-48	-26	-8
2020	-74	+195	-82	-71	+69	+2	+10	-39	-51	-36	-57	-91	-18

Source: *developed by the authors based on the Melitopol meteorological station of Zaporizhia region (n.d.)*

Analysis of weather conditions over the years of research indicates a warming climate – the average annual temperature was higher by 1.3-2.1°C relative to long-term indicators. Overwintering conditions for sweet cherries have become more favourable. The air temperature of the coldest month – January was higher by 1.2-2.8°C compared to long-term indicators. The average annual precipitation did not differ much from the long-term norm. Notably, precipitation was distributed unevenly over the years of the study. In September 2018 and May 2020, waterlogging was observed. There were droughts in June 2018 and 2019, and in August 2018 and 2020. This is confirmed by the calculated hydrothermal coefficient (Table 2).

Table 2. *Hydrothermal coefficient*

Years	Months						
	IV	V	VI	VII	VIII	IX	X
2018	0.1	0.4	0.5	1.1.	0.1	1.4	0.5
2019	1.4	1.8	0.2	0.6	0.8	0.2	0.5

Table 2, Continued

Years	Months						
	IV	V	VI	VII	VIII	IX	X
2020	nm	0.9	0.8	0.6	0.5	0.8	1.0
Long-term norm	1.1.	0.9	0.9	0.6	0.5	0.8	1.0

Note: *nm denotes 'not measured': the hydrothermal coefficient is not determined if the average monthly air temperature is less than 10°C*
Source: *compiled by the authors*

The hottest month of August was very dry during all the years of research, with the exception of 2019, when 56 mm of precipitation fell and the hydrothermal coefficient was higher relative to the long-term norm. June and September 2019 were exceptionally dry when the hydrothermal coefficient was substantially lower than the long-term norm. The air temperature during the active growth of sweet cherries (from April to August) in 2019 was favourable in April, May, and July, while in April and May 44-107% more precipitation fell (compared to the long-term average norm). In June 2019, there was a severe drought (the air temperature is 4.3°C above the monthly average and 73% less precipitation than normal), which continued in July. The growing conditions of sweet cherries in 2020 were extremely unfavourable in April (the average monthly air temperature was 1°C lower than the average annual norm, while precipitation decreased by 71%). May was satisfactory in terms of precipitation (69% more than the long-term norm), but unfavourable in terms of air temperature (1.7°C below the long-term norm). Frosts in May 2020 substantially damaged sweet cherry blossoms, which led to the loss of most of the crop. June and July 2020 were more favourable for sweet cherry trees compared to 2019. In August 2020, there was a typical drought for this zone.

Mid-early sweet cherry of Dilema variety (*Prunus avium* L./*Prunus mahaleb*) was planted in 2011 according to the 7×5 m scheme. The Dilema variety was bred by crossing two varieties of Drogan yellow and Valery Chkalov. In trees of the variety, the crown is slightly drooping, dense, and spreading. The fruits of the Dilema variety are convex-heart-shaped with a dark red skin and pulp. According to sensory assessment, the fruits of the variety are characterised by an excellent sweet cherryand sour refreshing taste.

The study of the effectiveness of mycorrhizal fungi was conducted according to the scheme: 1. Control (without inoculation); 2. Inoculation of sweet cherry roots with MycoApply SuperConcentrate 10 (endomycorrhiza); 3. Inoculation of sweet cherry roots with MycoApply Micronised Endo/Ecto (endo-ecticorrhiza).

The composition of Mycoappplay SuperConcentrate 10 includes spores of 4 species of arbuscular-mycorrhizal (AM) fungi – *Glomus intraradices* (*Rhizophagus intraradices*), *Glomus agregatum, Glomus mosseae*, *Glomus etunicatum*. 1.13 g of the biological product contains 0.3 million fungal spores. MycoApply Micronised Endo/Ecto consists of 4 types of *endomycorrhizae* fungi (*Glomus mosseae*, *Glomus agregatum*, *Glomus intraradices*, and *Glomus etunicatum*) and 7 types of ectomycorrhizal (*Ectomycorrhizae*) fungi *Rhizopogon villosulus*, *Rhizopogon amylopogon*, *Rhizopogon luteolus*, *Pisolithus tinctorius*, *Rhizopogon fulvigleba*, *Scleroderma citrinum* and *Scleroderma cepa*). The repetition rate in the experiment is fourfold. In each variant, there were 4 accounting trees surrounded by 14 protective trees. Mycorrhisation of the roots of sweet cherry trees with biologics was conducted in September 2018. In the trunk circle of the tree in a radius less than the crown projection, 5 punctures of the soil were made to a depth of 10 cm at an angle of 45 degrees to do this. An aqueous suspension of mycorrhizal fungal spores was poured into the holes. Mineral fertilisers and pesticides were not used at the experimental sites. Rows and near-stem strips in the garden in the experimental plots were kept under turf with natural grasses, which were mowed and left on the soil surface. The vegetation cover in the experimental areas was represented by the following varieties: shepherd's purse (*Capsella bursa-pastoris* L.), hairy vetch (*Vicia villosa*), field chamomile (*Anthemis arvensis* L.), couch grass (*Elytrigia repens* L.), and Bermuda grass (*Cynodon dactylon* L.). In the summer, the wild oat (*Avena fatua*) prevailed in the grass. In various grasses, there were small groups of medicinal plants, in particular: yarrow (*Achillea millefolium* L.), viper's-bugloss (*Echium vulgare* L.), forking larkspur (*Delphinium consolida*), and orange mullein (*Verbascum phlomoides*). In the first decade of August, when the leaf surface was fully developed on sweet cherrytrees, leaf samples were taken for analysis.

The leaf surface area was determined by die-cutting. For this, ten leaves were taken from each tree from the middle of one-year shoots on the southern side of the crown. The selected leaves were weighed and corkscrew-punched through. The area of the cut fragment was 1 cm^2. The cut-out leaf fragments were weighed. The total area of leaves (S) in the sample was determined by the formula (1):

$$S = \frac{M \times S_1}{M_1}, \quad (1)$$

where M is the mass of leaves in the sample, g; S_1 – area of one die-cut, cm^2; n – number of die-cuts, pcs; M_1 – mass of die-cuts, g.

Further, the average area of leaves from one tree was calculated.

The parameters of the water regime of the leaves (total water content and water-holding capacity) were determined by weight. The content of the total amount of water in the tissues was determined by drying 10 sheets in metal buckets in a thermostat at 105°C to a constant mass. The repetition was threefold. The total water content (V) as a percentage of the crude weight of the suspension was determined by the formula (2):

$$W = \frac{b-c}{b-a} \times 100\ , \qquad (2)$$

where a is the mass of an empty weighing bottle, g; b is the mass of a weighing bottle with a raw suspension, g; c is the mass of a weighing bottle with a dry suspension, g

To determine the water-holding capacity of the leaves, the wilting method was used, which involves determining the loss of water during their drying. 10 leaves were selected (three times repeated) and weighed. The leaves were then placed in Petri dishes and placed in a thermostat at 23°C. Repeated weighings were performed after 2, 4, and 6 hours and water loss was determined. The lower the water loss, the greater the water-holding capacity. The water-holding capacity (WHC) was determined by the formula (3):

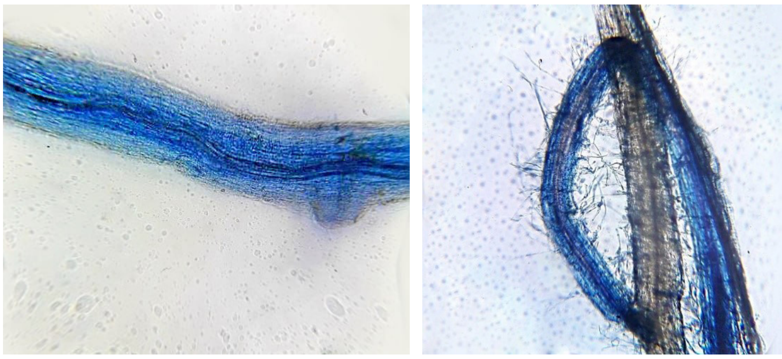
$$WHC = \frac{WL}{WC} \times 100\ , \qquad (3)$$

where WC is the water content in the leaves before drying, g; WL is the water loss per unit time, g.

The quantitative content of chlorophylls a and b in sweet cherry leaves was determined spectrophotometrically in a biochemical laboratory at the appropriate wavelength. Biochemical analysis of the leaves was performed three times in accordance with generally accepted methods. Sweet cherry leaves were selected for analysis after harvesting the fruit from each repeat separately. Immediately after harvesting, the leaves were dried at 65-70°C in a drying cabinet. For analysis, dried sweet cherryleaves were crushed and weighed. Suspension of one sample of plant material – 20 g of dry matter. The content of total nitrogen in plant material was determined by the Kjeldahl method, total phosphorus – colorimetrically on FEK LMF 74M, total potassium – by the flame-photometric method after ashing of the sediment in accordance with MVV 31-497058-019-2005 (Skrylnyk & Rozumna, 2005). The analytical repeatability of measurements is threefold. Statistical processing of experimental data was conducted by the variance method using Microsoft Excel software. Mean values and standard deviations were calculated for all data series. The substantiality level was set to $p<0.05$.

RESEARCH RESULTS

Symbiotic relationships of mycorrhizal fungi with sweet cherry roots can be traced as a result of the analysis of the examined plants. The detection rate of mycorrhizal plants in the variant with root mycorrhisation was 100%. Thus, all the trees where the mycorrhizal preparation was introduced were successfully mycorrhised. During the development of mycelium of mycorrhizal fungi, their hyphae are clearly visible on the treated roots of sweet cherries. On the processed roots of sweet cherries, under a 100-fold magnification, intensive overgrowth of the roots of the 1st, 2nd and subsequent orders with root hairs was observed (Fig. 1).



A B

Figure 1. *Sweet cherry root: A – before mycorrhisation by mycorrhizal fungi; B – after mycorrhisation by mycorizal fungi (×100 light microscopy)*

Source*: compiled by the authors*

In Fig. 1 B, a substantial increase in the number of sucking hairs on the roots of the 5th order can be seen as a result of symbiosis with mycorrhizal fungi and hyphae of mycorrhizal fungi inside the roots. The penetration of mycorrhizal fungal hyphae into the roots and root hairs of the examined sweet cherry trees was also recorded, which is evidence of the development of a symbiotic association. The results of the studies show that the total moisture content and water-holding capacity of tree leaves the roots of which were inoculated with endomycorrhizal biologics MycoApply SuperConcentrate 10 were substantially higher (56.9±0.49 and 93.5±0.98, respectively) compared to control trees in 2019 (Table 3).

__Table 3.__ Physiological parameters of sweet cherry leaves of the Dileta variety for inoculation of roots by mycorrhizal fungi

Variant	Total moisture content, %	water-holding capacity, %	Total area of leaves, m^2/ tree	Specific surface density of leaves, g/m^2
		2019		
Control (without inoculation)	53.5±0.98	84.6±0.84	60.1±5.23	69.1±4.35
MycoApply SuperConcentrate 10	56.9±0.49*	93.5±0.98*	50.4±4.25*	87.5±5.32*
MycoApply Micronised Endo/Ecto	52.7±0.95	96.4±0.97*	55.3±4.75	63.9±5.31
		2020		
Control (without inoculation)	57.0±0.95	95.4±0.63	44.3±3.77	93.0±5.75
MycoApply SuperConcentrate 10	55.0±0.87	95.1±0.99	55.5±4.95*	89.3±4.98
MycoApply Micronised Endo/Ecto	56.0±0.75	96.7±0.85	42.0±3.94	95.5±6.74

Note: ** – the difference is substantial at $p \leq 0.05$*
Source*: compiled by the authors*

In 2020, mycorrhization of roots by by endomycorrhiza did not affect the total moisture content and water-holding capacity of sweet cherry leaves. Root inoculation with Micronised Endo/Ecto did not substantially affect the total moisture content in sweet cherry leaves. In the first year of research, under the influence of endo-ectomycorrhiza, the water-holding capacity of leaves increased by up to 96.4%. The water-holding ability of leaf tissues characterises the content of free moisture in them. The increased water-holding capacity of the leaves indicates the absence of drought conditions or plant tolerance to such conditions. However, there was a severe drought in June 2019 (Table 1 and 2). Therefore, it can be stated that the inoculation of roots with endomycorrhiza and endo-ectomycorrhiza contributed to the tolerance of cherry trees to drought conditions. In the first year of the study, the total area of leaves on trees where root mycorrhization was performed with MycoApply Micronized Endo/Ecto and MycoApply SuperConcentrate 10 biological preparations was smaller by 4.7 and 9.7 m^2/tree, respectively, compared to the control (Table 3). In the second year, in inoculated trees with endomycorrhiza, the leaf area increased by 11.2 m^2/tree.

In inoculated trees with endo-ectomycorrhiza, on the contrary, the leaf area decreased by 2.3 m^2/tree. Notably, in 2020, there was a substantial decrease in the total area of leaves (13.3-15.8 m^2/ tree) on all variants compared to 2019. The specific surface density of the leaves in the first year of research after root mycorrhisation with MycoApply SuperConcentrate 10 biological preparation was considerably higher by 18.4 g/m^2 compared to the variant without inoculation. In the second year, the specific surface density of leaves in inoculated endomycorrhizal trees did not substantially differ from the control trees. The increase in leaf area under the action of MycoApply SuperConcentrate 10 in the second year of research without significant loss of specific surface density confirms the positive effect of endomycorrhiza on leaf morphology. Root inoculation with MycoApply Micronised Endo/Ecto BioLogics did not substantially affect the total leaf area and specific surface density of sweet cherry leaves. The accumulation of chlorophylls in sweet cherry leaves inoculated with endomycorrhiza and endo-ectomycorrhiza was substantially lower compared to control trees (Table 4).

__Table 4.__ Content and ratio of chlorophylls in sweet cherry leaves by inoculation of roots by mycorrhizal fungi

Variant	Sum of chlorophylls a and b (a + b), mg/m2 of leaf area		Chlorophyll ratio a/b	
	2019	2020	2019	2020
Control (no inoculation)	304.3±11.75	303.1±12.59	1.7±0.02	2.7±0.02
MycoApply SuperConcentrate 10	235.9±17.56*	277.7±15.82*	2.5±0.08*	2.3±0.05*
MycoApply Micronised Endo/Ecto	263.3±19.48*	244.5±13.73*	1.6±0.05*	2.0±0.02*

Note: * – the difference is substantial at $p \leq 0.05$
Source: compiled by the authors

Over the years of research, a decrease in the content of chlorophylls *a* and *b* has been established for roots mycorrhised with MycoApply SuperConcentrate 10 at 25.4-68.4 mg/m^2 compared to the non-inoculation variant. The decrease in the chlorophyll content in sweet cherry leaves during 2019 under the effects of endomycorrhiza can be explained by a significant decrease in their leaf area compared to non-inoculated trees. Although the inoculation of sweet cherry roots with endo-ectomycorrhiza did not notably affect the leaf area, the amount of chlorophyll was also lower than the control (without inoculation). Inoculation of roots with MycoApply Micronised Endo/Ecto reduced the content of chlorophylls *a* and *b* at 41-58. 6 mg/m^2 of leaf area compared to the control. The ratio of chlorophylls (a/b) in 2019 was substantially higher in trees inoculated with endomycorrhiza and substantially lower in trees inoculated with endomycorrhiza compared to the control. In 2020, this figure was substantially lower in sweet cherry leaves that were inoculated with endomycorrhiza and endo-ectomycorrhiza compared to trees without inoculation.

Consequently, inoculation of sweet cherry roots by both endomycorrhizae and endo-ectomycorrhizae contributed to the adaptive restructuring of the photosynthetic apparatus of leaves to overcome adverse living conditions. As for the increase in the ratio of chlorophylls (a/b) in 2019 due to the effects of endomycorrhizae, it can be assumed that it occurred due to the hormonal stimulation of trees by endomycorrhizae, which required many products of photosynthesis for its development. The total nitrogen content in sweet cherry leaves after root inoculation with endo- and ectomycorrhiza was not significantly different from the control (Table 5).

Table 5. *The content of nutrients in cherry leaves after root inoculation with mycorrhizal fungi, 2020*

Variant	Content, %		
	total nitrogen	total phosphorus	total potassium
Control (without inoculation)	1.79±0.062^{b}	0.24±0.007^{b,c}	0.65±0.024^{b,c}
MycoApply SuperConcentrate 10	1.70±0.067^{b}	0.19±0.007^{a,b}	0.58±0.022^{a,b,c}
MycoApply Micronised Endo/Ecto	1.70±0.065^{b}	0.20±0.008^{a,b}	0.77±0.029^{a,b}
* Insufficient supply	1.80±0.020	0.20±0.010	0.80±0.090
* Optimal supply	2.40±0.040	0.36±0.010	1.30±0.140

Note: ** the content of the main elements of nutrition in the leaves of fruit-bearing sweet cherrytrees in the south of Ukraine, defined as insufficient and optimal security for growth and fruiting*
[a] - the difference with the control (without inoculation) is substantial at P⩽0.05
[b] - the difference with the content of the element at optimal supply is reliable at P⩽0.05
[c] - the difference with the content of the element in case of insufficient supply is reliable at P⩽0.05
Source*: compiled by the authors*

After root mycorrhization with MycoApply SuperConcentrate 10 and MycoApply Micronized Endo/Ecto biological preparations, the content of total nitrogen in cherry leaves was 71-74% of the optimal supply of this nutrient for trees. In the leaves of sweet cherries of all the examined variants, the content of total phosphorus and potassium was substantially lower by 34-48% and 41-55%, respectively, from the optimal values. Notably, the phosphorus content in the leaves of non-inoculated trees was notably higher than the level of insufficient supply of this element. When the roots were inoculated with MycoApply SuperConcentrate 10 and MycoApply Micronised Endo/Ecto, the phosphorus content in the leaves was substantially lower than the control and corresponded to the indicator of insufficient supply of plants to these elements. In the leaves of trees that were inoculated with endomycorrhiza, the content of potassium was substantially lower than that of non-inoculated trees and the level of insufficient supply of this element. Inoculation of sweet cherry roots with MycoApply Micronised Endo/Ecto substantially increases the potassium content in the leaves compared to the control. In this variant, the potassium content in the leaves corresponds to the level of insufficient supply for plant growth and development with this element.

DISCUSSION

There are many reports in the scientific literature that mycorrhizal plants absorb more moisture and suffer less from drought (Rajesh *et al.,* 2018; Li *et al.,* 2019; Chandrasekaran, 2022). Basically, the positive effect of mycorrhizae on the water supply of symbiotic plants is explained by the fact that hyphae of mycorrhizal fungi come into association with the roots of symbiotic plants. This symbiosis substantially increases the absorption surface area of the roots due to branching, since mycorrhizal plants can have more root hairs to increase their resistance to drought stress (Zhang *et al.,* 2018).

The obtained results are consistent with the data of other authors regarding the formation of strong root branching as a result of their colonisation by fungal mycorrhiza (Chen *et al.,* 2020). Therefore, fungal mycorrhiza can mitigate plant growth restrictions caused by insufficient nutrient and moisture supply. In the conducted studies during 2020, the effect of mycorrhization of the roots of sweet cherry trees on the total moisture content and water-holding capacity of leaves was not identified. Similar studies have been conducted on other fruit crops (Rajesh Naik *et al.,* 2018). The water-holding ability of leaf tissues characterises the content of free moisture in them. One of the known physiological responses of plants to drought is the accumulation of osmolytes, the thickening and lignification of cell walls (Sharma *et al.,* 2019). Moreover, the free moisture content decreases. That is, an increased water holding capacity indicates the absence of drought conditions or plant tolerance to such conditions. The obtained experimental data are consistent with the justifications of other researchers regarding the increase of plant tolerance in an environment with a limited amount of water due to the association of arbuscular mycorrhizal fungi with host plants (Abdel-Salam *et al.,* 2018; Madouh & Quoreshi, 2023). For the sustainable development of horticulture in conditions of insufficient moisture, it is important that mycorrhiza provides fruit crops with moisture.

It is known that symbiotic mycorrhizal fungi synthesise biologically active compounds and thereby affect the hormonal regulation of plants, activating their growth (Li *et al.,* 2019; Shao *et al.,* 2023). Yet such a positive effect of mycorrhiza on growth processes takes time to develop the mycorrhizal network. Naturally, under optimal growing conditions, the process of mycorrhizal network development is faster. However, as can be seen from the results obtained in current studies, in the arid conditions of southern Ukraine, a positive effect of endomycorrhiza on the growth of leaf area is observed in the second year after root inoculation. Moreover, in the first year after root inoculation, mycorrhiza negatively affected leaf growth. Similar effects have already been described (Jin *et al.,* 2019; Kokkoris *et al.,* 2019) previously by researchers. It was established that mycorrhiza can lead to depression of growth processes in inoculated plants. The published papers based on the results of field and greenhouse tests highlight the influence of various agricultural techniques on the colonisation of plants by mycorrhiza, and the consequences of this colonisation on the yield, biomass, and assimilation of phosphorus by plants (Zhu *et al.,* 2019). Various studies confirm the positive effect of mycorrhizal fungi in the rhizospheric soil of different plants on the growth parameters of grasses (*Cenchrus ciliaris*). The maximum increase in the height of the plant, the number of leaves on the plant, the area of leaves, and the content of chlorophyll in the *Cenchrus ciliaris* under the influence of spores from rhizospheric soil was found (Thin *et al.*, 2022).

The decrease in leaf area in the second year of research cannot be explained by the influence of weather conditions in 2020. As after losing most of the crop due to spring frosts, trees that have lost fruit could grow a larger area of leaves. The absence of fruits, which are the main consumers of nutrients and water (Ayala & Lang, 2018), should contribute to an increase in the vegetative growth of trees. According to K. Rutkowski and G.P. Lysiak (2023), when growing fruit crops, it is important to maintain a balance between vegetative growth and tree fruiting. It can be concluded that the decrease in the leaf surface area in 2020 was caused by the extreme conditions of June 2019. During the period of intensive growth of shoots, extreme droughts (HTC – 0.2) and heat (the air temperature reached 36.4°C) were observed. Thus, in the arid conditions of the southern steppe of Ukraine, both negative (2019) and positive (2020) effects of endomycorrhiza on the total area of sweet cherry leaves were observed. Endo-ectomycorrhiza did not affect the total leaf area of inoculated trees. It is possible to protect sweet cherry trees from the negative impact of mycorrhiza on growth processes and accelerate the development of the mycorrhizal network by using irrigation. According to researchers, the most promising and resource-saving in the conditions of southern Ukraine is drip irrigation (Maliuk *et al.,* 2021).

The specific surface density of leaves in the first year of studies for root inoculation by endomycorrhiza was substantially higher, and the following year it almost did not differ from the control trees. It is known that the specific surface density of leaves is usually lower the larger the leaf area (Bondarenko, 2019).

The decrease in the content of chlorophyll in sweet cherry leaves under the influence of inoculation with endomycorrhiza and endo-ectomycorrhiza can be explained by the competition of natural grasses for the right to mycorrhiza with trees. Mycorrhizal fungi offer a selective advantage in supplying water, nutrients, vitamins, hormones, and enzymes to their host over competing non-host species (Zou *et al.,* 2023). It is known that natural herbs are better at establishing symbiosis with mycorrhizal fungi than cultivated plants (Trinchera *et al.,* 2019), which is probably manifested in the loss of nutrients by sweet cherry trees. In turn, less nutrient intake affects the chlorophyll content in sweet cherry leaves. Studies have established that the content of chlorophyll depends on the light, intensity of tree growth, type, variety, rootstock, and stress factors (Baslam *et al.,* 2020; Wojdyło *et al.,* 2021). A decrease in the ratio of chlorophylls (a/b) in leaves during root mycorrhisation indicates a restructuring of the photosynthetic apparatus of leaves towards the accumulation of chlorophyll b and carotenoids. This rearrangement of the photosynthetic apparatus is a well-known adaptive response of trees, which allows them to survive conditions of drought and excessive lighting (Markulj Kulundžić *et al.,* 2016).

The main reasons for a substantial decrease in the content of total nitrogen, phosphorus, and potassium in the experiment sweet cherry leaves compared to the optimal level of supply of sweet cherry trees in the south of Ukraine include: low content of basic nutrients in the soil of experimental plots; lack of mineral fertilisers; lack of irrigation; turf with natural grasses. Notably, the total potassium content in sweet cherry leaves decreased the most due to its consumption by competitive vegetation, i.e. natural grasses. They served as live mulch in the garden. Similar patterns regarding the decrease in the content of basic nutrients are well-covered in the scientific literature (Trinchera *et al.,* 2021). Turf with natural grasses in orchards causes a lack of nutrients in the leaves of fruit trees (Gerasko *et al.,* 2022b). In the conducted studies, a negative effect of root inoculation with endo- and ectomycorrhizae on the phosphorus content in sweet cherry leaves was observed. Researchers have established the positive effect of mycorrhiza on the assimilation of nutrients by plants (Ferrol *et al.,* 2019; Chauhan *et al.,* 2022) and determined that arbuscular mycorrhizal fungi are well-known symbiotic microorganisms that improve the growth of the host plant by mobilising fixed nutrients, mainly phosphorus, from the soil (Etesami *et al.,* 2021; Neidhardt, 2021). However, under unfavourable conditions, mycorrhizal fungi compete with plants for food elements and become consumers instead of a source (Kokkoris *et al.,* 2019). Thus, mycorrhizal fungi can reabsorb phosphorus released on the periarbuscular surface and control its supply to the partner plant (Kokkoris *et al.,* 2019; Zhang *et al.,* 2023).

In the conducted studies, an increase in the potassium content in sweet cherry leaves was recorded when the roots were inoculated with endo-ectomycorrhiza compared to the control (without inoculation). This confirms the positive effect of endo-ectomycorrhizal inoculant on potassium intake by a symbiotic plant. A similar effect has been described by C. Guerrero-Galán *et al*. (2018). It remains not fully understood what type of fungi caused such a positive effect since the biological product includes four types of endomycorrhizal and seven types of ectomycorrhizal fungi. Further research may be aimed at elucidating the role of arbuscular mycorrhizal fungi in improving sweet cherry growth and yield under multiple abiotic stresses. Therefore, understanding the symbiotic relationships of mycorrhizal fungi with various plants, including fruit crops, and their response to abiotic stresses can contribute to the development and implementation of climate-optimised technologies to ensure sustainable food production and biodiversity conservation.

CONCLUSIONS

Mycorrhization of cherry roots with MycoApply SuperConcentrate 10 in the conditions of southern Ukraine contributes to the adaptive restructuring of the photosynthetic apparatus of the leaves, the improvement of the water regime of the leaves in the first year after inoculation, and the increase of the leaf surface area in the second year after inoculation.

Inoculation of the roots by endo-ectomycorrhiza did not substantially affect the total moisture content of the leaves and the total area of the sweet cherry leaves. Inoculated trees with MycoApply Micronised Endo/Ecto increased the water-holding capacity of leaves by 1.3-11.8% compared to the control. Mycorrhization of endomycorrhizal and endo-ectomycorrhizal roots contributed to the tolerance of cherry trees to arid environmental conditions.

If the soil is not sufficiently provided with the basic elements of nutrition and moisture, the negative effect of endomycorrhiza on the content of phosphorus, potassium and the amount of chlorophylls *a* and *b* in sweet cherry leaves is manifested. The inoculation of roots with endo-ectomycorrhiza reduces the phosphorus content and the amount of chlorophylls *a* and *b* in sweet cherry leaves. An increase in the potassium content in sweet cherry leaves was determined in the second year of research under the influence of root inoculation with MycoApply Micronised Endo/Ecto.

Agricultural producers who grow sweet cherries using organic technology in the south of Ukraine can be recommended to combine inoculation of sweet cherry roots with mycorrhizal fungi and application of organic fertilisers and drip irrigation. This will provide optimal conditions for the full functioning of mycorrhizal symbiosis. Further research should assess the quality of sweet cherry fruits by biochemical parameters under the influence of mycorrhisation of roots with biologics for the sustainable development of horticulture.

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CONFLICT OF INTEREST

None.

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Дослідження реакції черешні на мікоризацію коренів біопрепаратами для стійкого розвитку садівництва

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Анотація. Стійкий розвиток садівництва є пріоритетним напрямом за зміни клімату, особливо в умовах зменшення вологозабезпечення. Пошук шляхів регуляції стійкості плодових дерев до стресорів є актуальним питання для світової спільноти. Використання корисних мікроорганізмів для інокуляції коренів рослин сприяє зменшенню застосування синтетичних речовин і є альтернативою для стійкого садівництва. Метою досліджень було з'ясувати особливості впливу інокуляції коренів мікоризними грибами на реакцію черешні для розробки стратегій управління виробництвом плодової продукції. Дослідження проводили протягом 2018-2020 рр. у черешневому саду Південної степової підзони України. Використані такі методи досліджень, як польовий, лабораторний, біохімічний, фізіологічний і статистичний. Досліджено закономірності впливу мікоризних грибів на загальний уміст вологи та водоутримувальну здатність листків черешні. У перший рік досліджень за інокуляції коренів ендомікоризою ці показники були вищими порівняно з контролем. З'ясовано позитивний вплив ендо-ектомікоризи на водоутримувальну здатність листків черешні. Визначено загальну листкову поверхню і уміст зелених пігментів у листі, як одних із основних показників фотосинтетичного потенціалу плодової культури. Встановлено наслідки інокуляції коренів черешні мікоризними грибами на співвідношення хлорофілів (a/b) у листках. Визначено параметри умісту загального азоту, фосфору і калію у листках черешні. Використання мікоризних грибів забезпечує підвищення адаптаційних властивостей дерев черешні до стресових чинників, а саме посушливих умов Півдня України. Результати досліджень можуть бути використані виробниками плодової продукції для удосконалення кліматично оптимізованих технологій, що забезпечить суттєве зменшення ризиків, можливих економічних втрат і збереження біорізноманіття

Ключові слова: ендомікориза; ектомікориза; площа листя; *Prunus avium*; водоутримувальна здатність; хлорофіл; основні елементи живлення