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## **Mycorrhization as a tool for regulating the stress tolerance of sweet cherry in sustainable agriculture**

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**Abstract.** Encouraging the implementation of sustainable agriculture practices is a crucial strategy for plant health, biodiversity conservation, and addressing food security challenges. One of the key sustainable solutions involves the use of arbuscular mycorrhizal fungi to establish mycorrhizal associations. This study aimed to determine the impact of mycorrhization on the intensity of root colonisation by endomycorrhiza and the response of sweet cherry to the adoption of sustainable practices. The study investigated the effectiveness of root inoculation in sweet cherry using a formulation

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containing propagules of four fungal species: *Glomus intraradices*, *Glomus mosseae*, *Glomus aggregatum*, and *Glomus etunicatum*. The research was conducted in the Southern Steppe of Ukraine. The experiment employed field, microscopic, biochemical, and statistical methods. The degree of root colonisation by arbuscular mycorrhizal fungi under mycorrhization was assessed. The ability of *Glomus* fungi to establish an effective mycorrhizal symbiosis with sweet cherry roots has been confirmed, as indicated by an increase in both the frequency of detection and the intensity of mycorrhizal infection. The impact of endomycorrhizal root inoculation on tree growth parameters was investigated. A tendency towards an increase in the annual stem diameter increment, total annual shoot growth, and leaf area was observed. Leaves of inoculated trees with AMF contained higher moisture levels and exhibited greater water retention capacity. The total chlorophyll ( $a + b$ ) content and their ratio in leaf tissues of inoculated trees were significantly higher than in the control. A substantial increase in ascorbate and phenolic compounds was recorded in the leaves of mycorrhized trees. Root inoculation with arbuscular mycorrhizal fungi enhanced the activity of antioxidant enzymes while reducing the content of malondialdehyde. These findings may support the promotion and implementation of organic sweet cherry cultivation technologies in line with the Sustainable Development Goals and the European Green Deal initiatives

**Keywords:** *Prunus avium* L.; arbuscular mycorrhizal fungi; trees; symbiosis; sustainable practices; adaptability; biopreparation

## INTRODUCTION

Sustainable fruit production plays a pivotal role in addressing a nation's food security challenges, as fruits are an essential component of a healthy diet. Supporting agriculture in achieving sustainable development goals is paramount, as the health of a population directly influences both their well-being and the resilience of ecosystems. Strategic goals in transforming food systems involve finding solutions that embody the core principles of sustainable agriculture and introduce innovative approaches to balanced nutrition. The role of fruits within food systems is interconnected with numerous United Nations Sustainable Development Goals. Among the most valued fruits and a leading crop in many countries is the sweet cherry (*Prunus avium* L.). The current surge in sweet cherry consumption has driven increased production. It is imperative to reassess agricultural practices, aligning them with the principles of the European Green Deal. This necessitates a search for best practices in transitioning to organic cultivation methods.

Consumers worldwide appreciate sweet cherries for their delicious taste and numerous health benefits, including both preventive and therapeutic properties. According to research by M. Faienza *et al.* (2020), sweet cherries are rich in natural antioxidants, which have a positive impact on human health by neutralising harmful radicals and reducing the risk of certain diseases. These fruits are characterised by high levels of monosaccharides and low caloric content (Gerasko *et al.*, 2022a). As noted by A. Nunes *et al.* (2021), the nutritional and dietary value of sweet cherries is enhanced by the presence of vitamins, organic acids, minerals, and biologically active substances.

The quality of fruit, particularly sweet cherries, is a subject of extensive scientific discussion. Organic sweet cherries are considered to be the most beneficial for human health. However, a common challenge in organic horticulture is reduced yields due to the exclusion

of mineral fertilisers, chemical pesticides, and genetically modified organisms, as reported by F. Giampieri *et al.* (2022). Mycorrhization presents a promising avenue for developing new environmentally friendly innovations in agriculture. This could facilitate the broader application of biotechnology in agriculture, a key priority of the European Green Deal to ensure environmental and economic sustainability. As R. Kalamulla *et al.* (2022) highlight, arbuscular mycorrhizal fungi (AMF) play a crucial role in plant vitality, stimulating growth and development, and increasing yields by mitigating various plant stresses. Mycorrhizal symbiosis can alleviate both biotic (e.g., plant pathogens, weeds) and abiotic (e.g., salinity, drought, extreme temperatures, soil pH, heavy metals) stresses by altering a plant's physiological state.

Mycorrhization of fruit tree roots offers a sustainable solution for improving organic sweet cherry cultivation. As reported by G. Dar and P. Dunge (2020), mycorrhizal fungi can stimulate photosynthesis in fruit trees. Their research in Bulgaria highlighted the positive impact on the growth of sweet cherry trees, with increased trunk diameter and average annual shoot length. Mycorrhizal colonisation enhances the resistance of many plant species to diseases caused by soil pathogens. According to T. Gerasko *et al.* (2023), mycorrhizal associations can strengthen a plant's defence mechanisms or induce resistance by creating a less favourable environment for various pathogens. Thus, the use of AMF offers an alternative approach to disease control through induced resistance. M. Delaeter *et al.* (2024) suggest that inoculating plants with mycorrhizal fungi can become a sustainable agricultural practice to reduce the use of synthetic pesticides, considering AMF as an ecosystem service.

Research by R. Dhalaria *et al.* (2020) corroborates the ability of AMF to stimulate plant defence responses against various stressors. Scientists emphasise that

the utilisation of arbuscular mycorrhiza is a promising strategy for mitigating the negative impacts of heavy metals on plants. Leveraging the symbiotic properties of AMF is an effective approach to enhancing the resilience of fruit crops to adverse environmental conditions. As noted by B. Dowarah *et al.* (2022), this effect is attributable to changes in plant physiology. AMF facilitate improved uptake of essential nutrients, particularly phosphorus, from the soil, especially under drought stress conditions. However, as cautioned by M. Wang *et al.* (2023), under conditions of insufficient mineral nutrition, drought, and heat, negative effects can occur where mycorrhizal fungi, instead of being symbiotic, become parasitic. Thus, it can be concluded that the influence of root mycorrhization on the physiological parameters of fruit trees, particularly sweet cherry trees, remains incompletely understood, as mycorrhization is rarely applied to this specific fruit crop.

A review of the literature highlights the need to explore ways to reduce agriculture's environmental impact through the use of mycorrhization. This practice promotes more sustainable agricultural technologies, enhancing crop resilience to stress and reducing the reliance on synthetic fertilisers and pesticides. In the

context of the European Green Deal, mycorrhization exemplifies how agriculture can become more environmentally sustainable. The application of AMF for plant inoculation helps to reduce the use of chemical fertilisers, improve soil health, enhance plant resilience to climate change, and even contribute to biodiversity conservation. Consequently, mycorrhization is a crucial tool for achieving the Green Deal's objectives, from climate neutrality to the sustainable use of natural resources, assisting the agricultural sector in adapting to new demands and challenges in the face of climate change and environmental transformation.

This study aimed to investigate the impact of inoculating sweet cherry tree roots with mycorrhizal fungi on tree growth, leaf physiological parameters, and the enhancement of the crop's tolerance to drought conditions in the Southern Steppe of Ukraine.

## MATERIALS AND METHODS

Experiments were conducted in Heorhiivske Village, Vilyansk District, Zaporizhzhia Region (47°59'13" N, 35°16'28" E), located in the Southern Steppe zone of Ukraine. The agrochemical characteristics of the soil at the experimental site are presented in Table 1.

**Table 1.** Agrochemical characteristics of the soil at the experimental plot

Indicator		Method of determination	Actual value	Reference value
pH (saline)		–	6.5	–
Bulk density, g/cm³		–	1.1	–
Humus content, %		Tyurin method	3.7	6.0
Nutrient content, mg/ kg soil	N	Kornfield method	84	225
	P <sub>2</sub> O <sub>5</sub>	Chirikov method	103	200
	K <sub>2</sub> O	Chirikov method	121	200

**Source:** compiled by the authors

Analysis of the soil's agrochemical characteristics revealed that the soil solution had a near-neutral pH, and the soil density was optimal. The experimental plots were characterised by insufficient levels of humus and essential nutrients (N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O). However, the soil in the experimental plots was entirely suitable for sweet cherry cultivation. The climate of the region where the research was conducted is very warm and very dry, with the sum of temperatures above 10°C ranging from 4,150 to 4,239°C (according to the Meteo Farm weather service). The amount of precipitation during the growing season is 210–230 mm, and the annual average precipitation is 443 mm, with uneven distribution and low relative humidity. It can be concluded that the climatic conditions are favourable for sweet cherry cultivation provided that irrigation is applied.

The experiment was conducted using a sweet cherry orchard (*Prunus avium* L.) of the Kazka cultivar grafted onto *Prunus mahaleb* rootstock. The trees were

planted in 2015 according to a 7×5 m spacing pattern. The cold-hardy Kazka cultivar, characterised by early ripening, was created by crossing the cultivars Valeriy Chkalov and Drogana Zhovta. The Kazka cultivar has a pyramidal crown, pomegranate-red spherical slightly elongated fruits weighing up to 12 g with a small stone. The fruits are characterised by firm and uniform flesh and a sweet taste with a hint of honey. Active fruiting of this cultivar begins at the age of five, with up to 5 kg of fruit harvested from one tree. The average yield in the southern regions of Ukraine is about 30 kg. The experiment was designed as follows: 1. Control (no mycorrhization); 2. Mycorrhization of sweet cherry tree roots with the endomycorrhizal biopreparation MycoApply SuperConcentrate 10. This biopreparation consists of propagules of four species (AMF) of the genus *Glomus* – *G. intraradices* (*Rhizophagus intraradices*), *G. mosseae*, *G. etunicatum*, and *G. aggregatum*. One gram of the preparation contains 22,000 propagules of *Glomus* fungi.

The total area of the experimental plot was 2 hectares. There were four replicates, and each variant included four model trees. The experimental variants were separated by buffer zones consisting of three rows of trees.

In autumn 2020, sweet cherry tree roots were mycorrhized with AMF according to the manufacturer's instructions. An aqueous suspension of the biopreparation was applied to five points around the base of each tree, within a radius smaller than the tree's canopy projection. The solution was introduced into pre-drilled holes at a depth of 10 cm at a 45-degree angle. The sweet cherry trees were cultivated using organic methods, which involved the use of only organic fertilisers and biopreparations. The soil in the inter-rows and tree circles was maintained under a cover crop of natural grasses (living mulch), which was mowed at a height of 10 cm and left on the soil surface as mulch. Throughout the growing season, the sweet cherry trees were irrigated 6-8 times, with 40 litres of water applied to each tree.

The intensity of mycorrhization of sweet cherry tree roots was determined in early June 2021 using a microscopic method. The first step of the study involved the collection and preparation of sweet cherry roots. Fourth and fifth-order roots with a total length of 15 cm were excavated from each tree and washed free of soil. Following the experimental period, roots were prepared for microscopic analysis. The roots were boiled in test tubes containing a 15% KOH solution for one hour in a water bath. Subsequently, they were rinsed with tap water and treated with a 1% HCl solution. In the third step, the roots were stained with 0.05% trypan blue in a lactic acidglycerinwater mixture (14:1:1). The stained roots were divided into 1 cm fragments and placed on a microscope slide, sequentially immersed in drops of glycerin (3 groups of 5 fragments). Microscopic examination was conducted at a magnification of 150 times. Five fields of view were examined on each 1 cm root segment, totalling 75 fields of view per slide. The frequency of mycorrhizal infection was calculated as a percentage, determined by the ratio of infected to uninfected root system sections. The intensity of root mycorrhization was visually assessed on a scale of 0 to 3: 0 – no infection; 1 – sporadic infections; 2 – moderately infected roots; 3 – heavily infected roots.

Standard methods were used to measure the biometric parameters of the sweet cherry trees. To calculate annual trunk increment, the diameter was measured at a height of 0.3 m above the graft union using callipers in October 2020 and 2021. The total annual shoot growth was estimated by multiplying the number of new shoots on a tree by their average length in October 2021. For phytochemical analysis, sweet cherry leaves were collected in June 2021 during the fruit ripening stage. Ten leaves were collected from each tree. These leaves were sampled from the middle of one-year-old shoots on the south side of the crown. A 1 cm<sup>2</sup> section

was punched out of each leaf and weighed. Leaf area was calculated using the following formula (1):

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

where  $M$  is the mass of leaves in the sample, g;  $n$  is the number of punched fragments;  $S_1$  is the area of one punched fragment, cm<sup>2</sup>;  $M_1$  is the mass of punched fragments in the sample, g.

Specific leaf area was determined by dividing leaf mass by leaf area. The total moisture content in the leaves was determined by weighing before and after drying at 105°C to a constant mass. Water-holding capacity was calculated as the ratio of water lost (after 24 hours of wilting) to total moisture content (Gerasko *et al.*, 2023). The content of chlorophylls  $a$  and  $b$  (mg m<sup>-2</sup>) in sweet cherry leaves was determined spectrophotometrically based on the light absorption of an acetone extract of the leaf tissue. The intensity of lipid peroxidation was determined by the content of malondialdehyde (MDA), which at 95°C in an acidic environment reacts with thiobarbituric acid to form a pink-coloured trimethyl complex with a maximum absorption at 535 nm. The results were expressed as nmol MDA per gram of plant material (Gerasko *et al.*, 2022b). Catalase activity (CAT, EC 1.11.1.6) was determined spectrophotometrically by the remaining undecomposed hydrogen peroxide. The results were expressed as μmol H<sub>2</sub>O<sub>2</sub>/g·min. Ascorbate peroxidase activity (APX, EC 1.11.1.11) was determined by the remaining unoxidised ascorbic acid by titration with a 0.001 N solution of Tillmans reagent (2,6-dichlorophenolindophenol). The results were expressed as mg of oxidised ascorbate per gram of fresh weight. Polyphenol oxidase activity (PPO, EC 1.10.3.1) was determined by measuring the optical density (at 420 nm) of the products of pyrogallol oxidation. The results were expressed in arbitrary units per gram of fresh tissue per minute.

Peroxidase activity (PO, EC 1.11.1.7) was determined spectrophotometrically by measuring the oxidation of indigo carmine (at a wavelength of 610 nm against distilled water). The decomposition of hydrogen peroxide by peroxidase releases oxygen, which oxidises indigo carmine, resulting in a colour change from blue-green to yellow-pink. The results were expressed as mkat/g of plant material. Sugar content (%) in leaves was determined spectrophotometrically by the reduction of picric acid (2,4,6-trinitrophenol) to picramic acid. Titratable acidity (%) was determined by titration with NaOH. Phenolic compound content was determined using the FolinCiocalteu reagent spectrophotometrically at 765 nm, corresponding to the concentration of phenolic substances expressed as gallic acid (GA) and expressed as mg GA per 100 g of fresh weight (Yaman, 2022). The content of ascorbic acid and glutathione was determined based on their reducing properties and expressed as mg/100 g of fresh weight.

Laboratory analyses were performed in three biological replicates. The results were statistically analysed using analysis of variance. Significant differences were determined at  $P < 0.05$ : a) using the least significant difference for biometric parameters of sweet cherry trees; b) using Student's t-test for the frequency and intensity of mycorrhizal infection, and for physiological and phytochemical parameters of leaves. All data were analysed using Microsoft Excel 2010. The authors adhered

to the standards of the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979).

## RESULTS

Evaluation of the treated plants revealed a positive impact of the biopreparation on the development of a symbiotic association between mycorrhizal fungi and sweet cherry tree roots (Table 2).

**Table 2.** Frequency of detection and intensity of mycorrhizal infection in sweet cherry tree roots,  $\bar{M} \pm m$

Variant	Frequency of detection, %	Intensity, score
Control (without mycorrhization)	14.0 $\pm$ 0.49	1.3 $\pm$ 0.02
Mycorrhization with AMF	98.5 $\pm$ 0.06*	2.5 $\pm$ 0.02*

**Note:** \* – difference is significant at  $P \leq 0.05$

**Source:** developed by the authors

On the control variant (without root mycorrhization), native mycorrhizal fungi were detected. Inoculation of roots with the endomycorrhizal biopreparation MycoApply Superconcentrate 10 increased the frequency of mycorrhizal infection by 7 times. This led to almost complete colonisation of the roots by endomycorrhizal fungi. The frequency of mycorrhizal infection was as high as 98.5%. AMF colonisation of sweet cherry tree roots using MycoApply Superconcentrate 10 increased the intensity of mycorrhizal infection by 1.9 times compared to the control trees (without inoculation). This indicates successful mycorrhization of all trees in the experimental variant using AMF inoculation. The use of AMF positively influenced the growth and development of the aboveground system of sweet cherry trees inoculated with the MycoApply Superconcentrate 10 biopreparation (Table 3). Mycorrhization of sweet cherry

tree roots led to a trend towards increased annual diameter increment of the trunk by 9% compared to control trees (without inoculation). Similarly, the use of the endomycorrhizal biopreparation MycoApply Superconcentrate 10 promoted a 10% increase in total annual shoot growth compared to the control. However, these differences were not statistically significant. Mycorrhization of sweet cherry tree roots with AMF resulted in the maximum leaf area growth, reaching 67.8 m<sup>2</sup>/tree. This value is significantly higher by 8% compared to the control trees. This confirms the positive effect of endomycorrhiza on the growth parameters of sweet cherry trees. Research has shown a positive impact of mycorrhization of sweet cherry tree roots with the endomycorrhizal biopreparation MycoApply Superconcentrate 10 on the total moisture content and water-holding capacity of leaves (Table 4).

**Table 3.** Growth parameters of sweet cherry trees with root mycorrhization with AMF

Variant	Annual trunk diameter growth, cm	Total annual shoot growth, m/tree	Total leaf area, m <sup>2</sup> /tree
Control (without mycorrhization)	1.1	45.4	62.5
Mycorrhization with AMF	1.2	50.1	67.8
LSD <sub>05</sub>	0.52	4.91	5.29

**Source:** developed by the authors

**Table 4.** Physiological parameters of sweet cherry leaves under root mycorrhization with AMF,  $\bar{M} \pm m$

Variant	Total moisture content, %	Water-holding capacity, %	Specific leaf mass, g/m <sup>2</sup>
Control (without mycorrhization)	54.9 $\pm$ 0.46	87.2 $\pm$ 0.75	82.4 $\pm$ 4.25
Mycorrhization with AMF	57.9 $\pm$ 0.49*	94.5 $\pm$ 0.81*	75.2 $\pm$ 4.43

**Note:** \* – difference is significant at  $P \leq 0.05$

**Source:** developed by the authors

Inoculation of sweet cherry tree roots with AMF resulted in a significantly higher total moisture content

(by 5%) compared to control trees without endomycorrhizal inoculation. Under the influence of the



endomycorrhizal biopreparation MycoApply Superconcentrate 10, the waterholding capacity of leaves increased significantly to 94.5%. This is 8% higher compared to the control trees. The water-holding capacity of sweet cherry leaf tissues indicates the content of free water within them. The increased water-holding capacity of leaves following AMF inoculation contributes to the adaptation of sweet cherry trees to drought

stress. Specific leaf area in the experimental trees with root mycorrhization using the MycoApply Superconcentrate 10 biopreparation decreased significantly by 9% compared to the control variant. In sweet cherry leaves, an increase in the accumulation of chlorophylls *a* and *b* was observed following root mycorrhization with the MycoApply Superconcentrate 10 biopreparation (Table 5).

**Table 5.** Chlorophyll content and ratio in sweet cherry leaves under root mycorrhization with AMF,  $\bar{M} \pm m$

Variant	Chlorophyll content ( <i>a</i> + <i>b</i> ) per leaf area, mg/m <sup>2</sup>	Chlorophyll <i>a/b</i> ratio
Control (without mycorrhization)	278.5 ± 19.52	1.7 ± 0.04
Mycorrhization with AMF	325.2 ± 22.13*	1.8 ± 0.04*

**Note:** \* – difference is significant at  $P \leq 0.05$

**Source:** developed by the authors

Mycorrhization of sweet cherry tree roots led to a 17% increase in the content of chlorophylls *a* and *b* in sweet cherry leaves compared to the non-inoculated control. Inoculation with endomycorrhizal fungi also resulted in a 6% increase in the chlorophyll *a/b* ratio in sweet cherry leaves compared to the control. Based on these findings, it can be concluded that inoculating sweet cherry tree roots with AMF induced an

adaptive reorganisation of the photosynthetic apparatus to mitigate the effects of stress factors. The increase in the chlorophyll *a/b* ratio as a result of mycorrhization can be attributed to hormonal stimulation by AMF. Mycorrhizal fungi stimulate the efficiency of the photosynthesis process. Mycorrhization of sweet cherry tree roots had a significant impact on the phytochemical composition of sweet cherry leaves (Table 6).

**Table 6.** Phytochemical composition of sweet cherry leaves under root mycorrhization with AMF,  $\bar{M} \pm m$

Variant	Sugars, %	Titrated acids, %	Phenolic compounds, mg GA/100 g	Ascorbate, mg/100 g	Glutathione, mg/100 g
Control (without mycorrhization)	2.8 ± 0.22	1.3 ± 0.08	45.1 ± 3.55	10.2 ± 0.77	34.6 ± 2.29
Mycorrhization with AMF	3.0 ± 0.23	1.4 ± 0.08	52.5 ± 3.80*	12.5 ± 0.63*	36.5 ± 2.34

**Note:** \* – difference is significant at  $P \leq 0.05$

**Source:** developed by the authors

In sweet cherry leaves treated with the endomycorrhizal biopreparation MycoApply Superconcentrate 10, the content of phenolic compounds and ascorbic acid increased significantly by 16% and 23%, respectively. Mycorrhization of sweet cherry roots also showed a trend towards increased content of glutathione, sugars,

and titratable acids (by 6%, 7%, and 8%, respectively). However, this increase was not statistically significant. Analysis of the obtained data revealed that mycorrhization of roots with endomycorrhiza significantly reduced the damage from lipid peroxidation in sweet cherry leaves, as the MDA content decreased by 29% (Table 7).

**Table 7.** MDA content and antioxidant enzyme activity in sweet cherry leaves under root mycorrhization with AMF,  $\bar{M} \pm m$

Variant	MDA, nmol/g	CAT, $\mu\text{mol H}_2\text{O}_2/\text{g} \cdot \text{min}$	APX, mg oxidised ascorbate/g	PPO, a.u./g·min	PO, mkat/g
Control (without mycorrhization)	64.5 ± 4.65	7.3 ± 0.15	5.8 ± 0.18	11.3 ± 0.45	6.9 ± 0.43
Mycorrhization with AMF	45.9 ± 4.29*	7.7 ± 0.17*	6.6 ± 0.25*	15.1 ± 0.53*	12.3 ± 0.37*

**Note:** \* – difference is significant at  $P \leq 0.05$

**Source:** developed by the authors

In sweet cherry leaves treated with the endomycorrhizal biopreparation MycoApply Superconcentrate 10, the activity of antioxidant enzymes increased significantly. The most significant increase was observed in

peroxidase activity, which was 78% higher compared to the control. Mycorrhization of tree roots with endomycorrhiza resulted in increased activity of catalase, ascorbate peroxidase, and polyphenol oxidase by 5%,

14%, and 34%, respectively, compared to the control variants. Thus, the use of root mycorrhization significantly increases the adaptability and tolerance of sweet cherry trees to abiotic stress conditions. Therefore, mycorrhization can become an important tool for achieving the goals of the European Green Deal, contributing to the preservation of ecosystems, reducing the impact of agriculture on the environment, and increasing the resilience of crops to climate change.

## DISCUSSION

In this study, sweet cherry trees were grown in association with natural vegetation, as the orchard soil was maintained under grassing down (living mulch). As reported by A. Trinchera *et al.* (2019), mycorrhizal fungi more readily establish symbioses with wild grasses than with cultivated plants. This co-existence with a living mulch and mycorrhizal fungi brings both positive outcomes (the roots of “living mulch” always maintain a “reserve” of mycorrhizal fungi and other beneficial microbes) and risks. Under stressful conditions (drought, nutrient deficiency), mycorrhizal fungi may colonise grasses at the expense of trees, reducing nutrient content and chlorophyll levels in leaves. However, under optimal moisture and fertilisation conditions (irrigation, organic fertilisation), mycorrhization of sweet cherry tree roots with *Glomus* species can have a positive impact on both tree growth and leaf physiology. This is supported by the results obtained in the study by J. Ilic (2023).

The significant increase in the frequency and intensity of mycorrhizal infection in sweet cherry tree roots indicates the viability of the mycorrhizal fungi strains within the MycoApply Superconcentrate 10 preparation, their adaptability to the arid conditions of the Southern Steppe of Ukraine (including overwintering), and their competitiveness against the local mycoflora (14% of roots were already infected). This also confirms the ability of sweet cherry trees to establish symbiosis with endomycorrhizal fungi, as supported by the research of scientists S. Afonso *et al.* (2022). The study observed a trend towards improved growth parameters in sweet cherry trees as a result of the mycorrhizal symbiosis, confirming previously published data on the mycorrhization of fruit trees. Scientists V. Petrova and S. Krumov (2023) have investigated that the application of AMF positively influenced the growth characteristics of 16 new sweet cherry cultivars. Treated trees showed an increase in trunk diameter and average annual shoot length. Research by Y. Shao *et al.* (2023) established an increase (by 24%) in the cross-sectional area of the trunk of three apple cultivars (Topaz, Odra, and Chopin) and a promising clone (U 8869) after four years of mycorrhization in combination with growthstimulating bacteria.

According to the researchers in the current study, the stimulatory effect of mycorrhizal fungi on their plant partners is linked to the synthesis and supply of

biologically active compounds to the plant via the mycorrhizal network. The observed increase in leaf moisture content and waterholding capacity following root mycorrhization aligns with the findings of other researchers. In the studies of T.A. Madouh and A.M. Quoreshi (2023), AMF colonisation enhanced plant tolerance to drought stress through complex physiological changes, including increased photosynthesis, antioxidant enzyme activity, and reduced malondialdehyde levels. The improved water supply to plants following root mycorrhization is attributed to the increased absorptive surface area of the roots of mycorrhizal plants, as described in the scientific article of M. Chandrasekaran (2022).

The observed decrease in specific leaf area following root mycorrhization suggests that mycorrhizal plants were less affected by abiotic stresses (heat, drought). It has been reported that an increase in specific leaf area is an adaptive response of plants to heat, drought, and excessive insolation, as leaves with a higher specific mass have lower concentrations of cytoplasmic compounds and higher concentrations of cell wall compounds (especially lignin). Furthermore, it is a known physiological phenomenon in sweet cherry leaves that as their area increases, their specific mass decreases (Bondarenko, 2019). The significant increase in chlorophyll ( $a + b$ ) content and the chlorophyll  $a/b$  ratio in the leaves of mycorrhizal trees indicates the stimulation of photosynthetic activity by mycorrhizal fungi. Similar effects have been reported in the studies of other scientists (Bi *et al.*, 2021). The increase in chlorophyll content and ratio also indicates the successful overcoming of abiotic stresses by plants (Sharma *et al.*, 2023). Significant positive correlations between AMF colonisation and chlorophyll ( $a$  and  $b$ ) and carotenoids were found in research by W. Chen *et al.* (2020). This confirms the beneficial role of mycorrhizal fungi in the formation of photosynthetic pigments. The increase in chlorophyll content may be related to increased P and Mg uptake under the influence of mycorrhizal fungi. The elevated chlorophyll parameters indicate that AMF increase photosynthetic C fixation, which contributes to the progression of photosynthesis.

In studies conducted by A. Villani *et al.* (2021) and A. Wahab *et al.* (2023), increases in ascorbic acid and phenolic compound content, significant decreases in malondialdehyde content, and increases in antioxidant enzyme activity were observed in sweet cherry leaf tissues following root mycorrhization. This supports the hypothesis that mycorrhizal fungi play a protective role against various abiotic stresses by stimulating the production of tissue antioxidants in plants and increasing the activity of antioxidant enzymes. Based on the quantitative data obtained in these studies regarding phenolic compound and ascorbic acid content, sweet cherry leaves may compete with the fruit in terms of antioxidant capacity, as previously highlighted in research by K. Dziadek *et al.* (2019). Thanks to a better

understanding of the applications and benefits of AMF, there is a real possibility of using them to promote sustainable agricultural production. Therefore, the results of this research indicate a significant positive impact of mycorrhizal fungi on the growth, physiological state, and resistance of sweet cherry trees to abiotic and biotic stresses. Due to the ability of mycorrhiza to improve nutrient uptake, enhance photosynthetic activity, and activate antioxidant defence in plants, its application can become an important ecological tool for increasing the efficiency of organic horticulture.

### CONCLUSIONS

The roots of the control trees in this study were partially colonised by native mycorrhizal fungi (14%). Inoculation with the biopreparation MycoApply Superconcentrate 10 significantly increased both the frequency and intensity of mycorrhizal infection in sweet cherry tree roots. This confirms the successful mycorrhization of tree roots using AMF. Inoculated trees exhibited an increased annual trunk diameter increment (by 9%) and total annual shoot growth (by 10%) compared to the control (without AMF inoculation). Root mycorrhization resulted in a significant increase in the leaf area of sweet cherry trees (by 8%). Moreover, the mycorrhization of roots with AMF led to a significant increase in total leaf moisture content (by 5%) and water-holding capacity (by 8%) compared to the control trees. This, in turn, enhances the plant's adaptation to drought conditions when soil moisture is limited. A significant decrease in specific leaf area (by 9%) was observed in mycorrhizal trees.

Inoculation of sweet cherry tree roots with endomycorrhizal fungi resulted in a significant increase in both chlorophyll *a* and *b* content (by 17%) and their ratio (by 6%) in leaves. This confirms the stimulatory effect of mycorrhizal fungi on photosynthetic processes

in sweet cherry leaves. Furthermore, a significant increase in phenolic compounds (by 16%) and ascorbic acid (by 23%) was observed following root inoculation with MycoApply Superconcentrate 10. While there was a trend towards increased levels of glutathione, sugars, and titratable acids in sweet cherry leaves inoculated with AMF, these differences were not statistically significant. A notable decrease in MDA content (by 29%) was observed in sweet cherry leaves following root inoculation with endomycorrhiza, indicating reduced lipid peroxidation damage. Mycorrhization significantly increased the content of phenolic compounds and ascorbic acid in leaves, suggesting enhanced antioxidant capacity. Although the content of sugars, titratable acids, and glutathione showed a trend towards increase, these differences were not statistically significant. The activity of antioxidant enzymes, including catalase (increased by 5%), ascorbate peroxidase (increased by 14%), and polyphenol oxidase (increased by 34%), was significantly higher in sweet cherry leaves inoculated with MycoApply Superconcentrate 10. The most significant increase was observed in peroxidase activity (by 78%), which likely contributed to the decreased MDA content in leaf tissues. These findings demonstrate the potential of root inoculation with AMF to establish a robust mycorrhizal symbiosis and enhance sweet cherry tree adaptation to drought stress. This supports the promising future study of AMF efficacy for forming effective mycorrhizal associations on sweet cherry tree roots and enhancing plant stress resistance to abiotic and biotic factors in sustainable agriculture.

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### CONFLICT OF INTEREST

None.

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## Мікоризація як інструмент регуляції стресостійкості черешні у зеленому сільському господарстві

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**Анотація.** Стимулювання імплементації сталих практик в сільському господарстві є важливим рішенням для здоров'я рослин, збереження біорізноманіття і вирішення проблем продовольчої безпеки. Одним із важливих сталих рішень є використання арбускулярних мікоризних грибів для функціонування мікоризних асоціацій. Метою роботи було з'ясувати вплив мікоризації на інтенсивність заселення коренів ендомікоризою та реакцію черешні для впровадження сталих практик. У роботі вивчали ефективність інокуляції коренів черешні препаратом, що містить пропагули 4-х видів грибів *Glomus intraradices*, *Glomus mosseae*, *Glomus aggregatum* і *Glomus etunicatum*. Дослідження проводили в умовах Південного Степу України. У процесі експерименту було використано такі наукові методи – польовий, мікроскопічний, біохімічний і статистичний. Оцінено ступінь заселення коренів черешні АМГ за дії мікоризації. Доведено здатність грибів роду *Glomus* розвивати ефективний мікоризний симбіоз з коренями черешні, оскільки збільшилася частота виявлення і інтенсивність мікоризної інфекції. Досліджено вплив інокуляції коренів черешні ендомікоризою на ростові показники дерев. Спостерігали тенденцію до збільшення річного приросту діаметру штамбу, сумарного річного приросту пагонів і площі листків. Листки інокульованих дерев АМГ містили більше вологи і мали більшу водоутримувальну здатність. Сума хлорофілів *a* і *b* та їх співвідношення у тканинах листків інокульованих дерев були істотно вищими порівняно з контролем. У листках мікоризованих дерев істотно збільшився вміст аскорбату і фенольних речовин. Інокуляція коренів АМГ спричинила збільшення активності антиоксидантних ферментів і зменшення вмісту малонового діальдегіду. Ці результати можуть сприяти просуванню і впровадженню органічних технологій вирощування черешні для досягнення Цілей сталого розвитку та ініціатив Європейського зеленого курсу

**Ключові слова:** *Prunus avium* L.; арбускулярні мікоризні гриби; дерева; симбіоз; сталі практики; адаптивність; біопрепарат