**УДК 634.232, 635.89**

# EFFECT OF INOCULATION WITH SYMBIOTIC ENDO- AND ECTOMYCORRHIZAL FUNGI ON CONTENT OF BASIC MINERAL NUTRIENTS IN SWEET CHERRY LEAVES

**Gerasko Tatyana**
PhD of Agricultural Sciences, Associate Professor,
Dmytro Motornyi Tavria State
Agrotechnological University,
Melitopol, Ukraine
**Pyda Svitlana**
Dr. of Agricultural Sciences, Professor,
Volodymyr Hnatyuk Ternopil
National Pedagogical University,
Ternopil, Ukraine

**Abstract:** There is an increasing interest in organic production all over the globe due to the desire of mankind for sustainable development and preservation of agroecosystems for future generations. Mycorrhization fully meets organic standards and is a natural way to increase the efficiency of agricultural production. Our task was to determine the content of basic mineral nutrients in sweet cherry leaves by root inoculation with symbiotic endo- and ectomycorrhizal fungi. The results of our research show that in the absence of fertilizers and irrigation, inoculation with endomycorrhizal fungi significantly decreased leaf P and K uptake. Endo-ectomycorrhiza, like endomycorrhiza, reduced leaf P, but significantly increased leaf K uptake in the second year of the study.

**Keywords:** *Prunus avium L., Prunus mahaleb*, organic orchard, mycorrhizal fungi, leaf nutrient status.

Green agricultural production is a need in Ukraine, given the declining soil

fertility and low efficiency of agriculture [1]. Mycorrhization is a natural way to increase the efficiency of agricultural production, as it saves on mineral fertilizers and irrigation water [2]. The positive effect of mycorrhiza on plant productivity has been confirmed by many studies around the world on a variety of crops, including fruit trees [3]. But there are also reports of insignificant or negative impact of mycorrhiza on moisture and the content of basic mineral nutrients in plants [4-9]. Thus, the effect of mycorrhization on the physiological parameters of plants is insufficiently studied. Sweet cherry is the dominant fruit crop of Zaporizhia region and, especially, Melitopol district. Therefore, we are interested in developing an effective organic technology for growing sweet cherry at our region. The mycorrhization of sweet cherry trees can become one of the elements of this technology, given that sweet cherry (Prunus avium L.) and its rootstock (Prunus mahaleb) are able to form a symbiosis with mycorrhizal fungi [10,11]. But today little is known about the impact of mycorrhiza on the physiological parameters of sweet cherry trees in the Southern Steppe of Ukraine. The aim of this study was to investigate the effect of endo- and ectomycorrhizal root inoculation on the content of basic mineral nutrients in sweet cherry leaves.

The experiment was conducted in an organic orchard of sweet cherry (Prunus avium L.) cultivar Dilemma/Prunus mahaleb planted in 2011 at 7 × 5 m. The work was conducted in the southern steppe of Ukraine (Melitopol district, Zaporizhia region). The soil cover of the investigated area is the chestnut soils, which are very low-humus. The research site is located in the Steppe zone, in the second agro-climatic region, which is characterized as arid and very warm. The orchard floor was kept under "live mulch" (natural herbs, mowed 4 times during the growing season and the clippings were left on the ground for decomposition). The scheme of the experiment was as follows: 1) Control - without inoculation; 2) Inoculation of sweet cherry roots with MycoApplay Superconcentrate 10 (root inoculation with endomycorrhizal fungi); 3) Inoculation of sweet cherry roots with MycoApplay Micronized Endo / Ecto (root inoculation with endo-ectomycorrhizal fungi). Any other management was identical in each treatment. Synthetic mineral fertilizers and

chemical plantprotection products were not used. Leaves for analysis were collected in the first decade of August, with the full development of the leaf surface. The dried leaf tissues were ground and the content of basic nutrients (N, P and K) was analyzed by conventional methods [12,13]. Biochemical analysis was performed in three biological replicates. The results were compared by Student's t test, significant differences were determined at a level of $P < 0,05$. All data were analyzed using Microsoft Excel 2010 [14].

The results of our study show that the content of total nitrogen in sweet cherry leaves by inoculation of roots with endo- and ecto-mycorrhizal fungi did not differ significantly from control (without inoculation) and was 71-74% of the optimal supply of this nutrient. The content of phosphorus and potassium in the leaves of sweet cherry in all treatments of the experiment was also significantly lower than the optimal values - respectively, 34-48% and 41-55%. But the phosphorus content in the leaves of control trees was significantly higher than the level of insufficient supply of this element. In the variants with endo- and ecto-mycorrhizal root inoculation, the phosphorus content in the leaves was significantly lower than control (without inoculation) and corresponded to the indicator of insufficient supply of plants with these elements. The potassium content in the variant with root inoculation with endomycorrhizal fungi was significantly lower than the control variant and even lower than the level of insufficient supply of this element. During inoculation of roots by endo-ectomycorrhizal fungi, the potassium content in the leaves was significantly higher than control variant (without inoculation) and corresponded to the level of insufficient supply of plants with this element. The significant decrease in the content of basic nutrients in sweet cherry leaves in all variants of the experiment (compared to the optimal level of supply of sweet cherry trees in southern Ukraine) occurred, in our opinion, for several reasons: first, low nutrient content in the soil; secondly, the lack of mineral fertilizers; third, the lack of watering; fourth, keeping the soil under "living mulch". At the same time, the potassium content decreased the most, probably due to its consumption by competitive vegetation - natural herbs, which acted as "living mulch". Such patterns have long been known and well described in the

scientific literature: "living mulch" in the orchard leads to a deficiency of nutrients in the leaves of fruit trees [15,16]. The negative effect on the phosphorus content in sweet cherry leaves, which we observed when inoculating the roots with endo- and ecto-mycorrhizal fungi, seems interesting. There are numerous reports of a positive effect of mycorrhiza on the absorption of nutrients by plants [3]. But in unfavorable living conditions, mycorrhizal fungi are able to compete with plants for nutrients and become consumers instead of a source [4-9]. For example, fungi can reabsorb phosphorus released on the periarbuscular surface, controlling its entry into partner plants [17-19]. But with inoculation of roots by endo-ectomycorrhizal fungi, the potassium content in sweet cherry leaves increased compared to control (without inoculation). Which indicates a positive effect of endo-ectomycorrhizal inoculant on potassium intake by a symbiotic plant. A similar effect is described by K. Garcia and S.D. Zimmermann [20]. It remains an open question what species of fungi caused such a positive effect, because the inoculum includes 4 species of endomycorrhizal (Glomus intraradices, Glomus mosseae, Glomus agregatum, Glomus etunicatum) and 7 species of ectomycorrhizal (Rhizopogon villosulus, Rhizopoglepogon luteolus, Pisolithus tinctorius; Scleroderma cepa, Scleroderma citrinum) fungi.

We can recommend for farmers who grow sweet cherries using organic technology in the South of Ukraine to combine mycorrhization of trees with the use of organic and permitted by organic standards mineral fertilizers and drip irrigation of trees, which will provide optimal conditions for full functioning mycorrhizal symbiosis.

## LIST OF REFERENCES

1. Petrychenko VF, Korniychuk OV, Voronetska IS (2018) Biological farming in conditions of transformational changes in the agrarian production of Ukraine. Agricultural Science and Practice 5(2), 3-12. doi:10.15407/agrisp5.02.003.

2. Berruti A, Lumini E, Balestrini R and Bianciotto V (2016) Arbuscular Mycorrhizal Fungi as Natural Biofertilizers: Let's Benefit from Past Successes. Front. Microbiol. 6:1559. doi: 10.3389/fmicb.2015.01559

3. Rajesh Naik SM et al. (2018) Role of Arbuscular Mycorrhiza in Fruit Crops Production. Int. J. Pure App. Biosci. 6(5):1126-1133. doi:10.18782/2320-7051.7088

4. Graham JH, Syvertsen JP and Smith ML, Jr. (1971) Water relations of mycorrhizal and P-fertilized non mycorrhizal Citrus under drought stress. New Phytologist 15: 411-419. doi:10.1111/j.1469-8137.1987.tb00878.x

5. Kokkoris V, Hamel C, Hart MM (2019) Mycorrhizal response in crop versus wild plants. PLoS ONE 14(8): e0221037. doi:10.1371/journal.pone.0221037

6. Poulsen KH, Nagy R, Gao L-L, Smith SE, Bucher M, Smith FA, et al. (2005) Physiological and molecular evidence for Pi uptake via the symbiotic pathway in a reduced mycorrhizal colonization mutant in tomato associated with a compatible fungus. New Phytol. Blackwell Science Ltd 168: 445–454. pmid:16219083

7. Christophersen HM, Smith FA, Smith SE. (2009) Arbuscular mycorrhizal colonization reduces arsenate uptake in barley via downregulation of transporters in the direct epidermal phosphate uptake pathway. New Phytol. 184: 962–974. pmid:19754635

8. Grace EJ, Cotsaftis O, Tester M, Smith FA, Smith SE. (2009) Arbuscular mycorrhizal inhibition of growth in barley cannot be attributed to extent of colonization, fungal phosphorus uptake or effects on expression of plant phosphate transporter genes. New Phytol. 181: 938–949. pmid:19140934

9. Facelli E, Smith SE, Facelli JM, Christophersen HM, Andrew Smith F. (2010) Underground friends or enemies: Model plants help to unravel direct and indirect effects of arbuscular mycorrhizal fungi on plant competition. New Phytol. 185: 1050–1061. pmid:20356347

10. Mycorrhizal Status of Plant Species and Genera. URL: https://mycorrhizae.com/wp-content/uploads/2017/04/Status-of-Families-and-Genera-New-v1.3.pdf

11. Yilmaz N, Çetiner S, Ortaş İ (2020) The Effekt of Mycopphiza on Plant Growth during Acclimatization of Some in Vitro Grown Sweet Cherry Rootstocks. International Journal of Agricultural and Natural Sciences. 13(1): 10-19. URL:

http://www.ijans.org/index.php/ijans/article/view/489

12. Gorodnij MM, Mel'nichuk SD, Gonchar OM et al. (2006) Prikladna biohimiya ta upravlinnya yakistyu produkciï roslinnictva. Kiïv: Aristej (in Ukrainian).

13. Ermakov AI, Arasimovich VV, YArosh NP et al. (1987) Metody biohimicheskogo issledovaniya rastenij. Leningrad: Agropromizdat (in Russian).

14. Lakyn HF (1990) Biometryia. Vysshaia shkola, Moscow (in Russian).

15. Semenyuk GM (1983) Diagnostika mineral'nogo pitaniya plodovyh kul'tur. Kishinev: SHtiinca (in Russian).

16. Gospodarenko GM (2010) Agrohimiya: Pidruchnik. Kiev (in Ukrainian).

17. Fiorilli V, Lanfranco L, and Bonfante P (2013) The expression of GintPT, the phosphate transporter of *Rhizophagus irregularis*, depends on the symbiotic status and phosphate availability. Planta 237: 1267–1277. doi: 10.1007/s00425-013-1842-z

18. Balestrini R, Gómez-Ariza J, Lanfranco L and Bonfante P (2007) Laser microdissection reveals that transcripts for five plant and one fungal phosphate transporter genes are contemporaneously present in arbusculated cells. Mol. Plant Microbe Interact. 20:1055–1062. doi: 10.1094/MPMI-20-9-1055

19. Tisserant E, Kohler A, Dozolme-Seddas P, Balestrini R et al. (2012). The transcriptome of the arbuscular mycorrhizal fungus *Glomus intraradices* (DAOM 197198) reveals functional tradeoffs in an obligate symbiont. New Phytol. 193: 755–769. doi: 10.1111/j.1469-8137.2011.03948.x

20. Garcia K and Zimmermann SD (2014) The role of mycorrhizal associations in plant potassium nutrition. Front. Plant Sci. 5:337. doi: 10.3389/fpls.2014.00337

# EFFECT OF INOCULATION WITH SYMBIOTIC ENDO- AND ECTOMYCORRHIZAL FUNGI ON PHYSIOLOGICAL PARAMETERS OF SWEET CHERRY LEAVES

**Tatyana Gerasko**,
PhD of Agricultural Sciences, Associate Professor,
Department of Horticulture, Viticulture and Biochemistry,
Dmytro Motornyi Tavria State
Agrotechnological University,
Melitopol, Ukraine

**Svitlana Pyda**,
Dr. of Agricultural Sciences, Professor,
Department of Botany and Zoology,
Volodymyr Hnatyuk Ternopil
National Pedagogical University,
Ternopil, Ukraine

Sweet cherry (Prunus avium L.) is the dominant fruit crop of our region. The relevance of our research is due to the need to develop a sustainable, independent of external resources organic technology for growing sweet cherry. Abandonment of synthetic mineral fertilizers and chemical plant protection products, inoculation of fruit tree roots with endo- and ectomycorrhizal fungi should to promote agroecosystem sustenability. But little is known about the effects of inoculation with symbiotic endo- and ectomycorrhizal fungi on physiological parameters of sweet cherry leaves in organic orchards. The aim of this study was to investigate the effect of endo- and ectomycorrhizal root inoculation on the physiological parameters of sweet cherry trees, in particular, on the physiological parameters of leaves. Our task was to determine the total leaf area, specific leaf density, leaf total moisture content, water-retaining ability, chlorophyll content and ratio in sweet cherry leaves by root inoculation with symbiotic endo- and ectomycorrhizal fungi.

The work was conducted from 2018 to 2020 years in the Southern Steppe of Ukraine in Zelene village near the city of Melitopol (46°46'N, 35°17'E). The soil cover of the investigated area is the chestnut soils, which are very low-humus. Soils have a weakly alkaline reaction of soil solution (pH varies within 7.1-7.4). On the background of a light granulometric composition, the humus content in the upper humus horizon is 0.6%. The analysis of aqueous extraction revealed that the total content of water-soluble salts does not exceed 0.015-0.024%. Mineral nitrogen was not detected, the content of $P_2O_5$ is 5.4; $K_2O$ - 6.5 mg / kg of soil. Despite the lack of nutrients and low humus content, the soil is quite suitable for growing sweet cherries, which is confirmed

by more than a century of production experience. Soil conditions of the experimental garden are favorable for mycorrhization, as it is shown that the low level of soil nitrogen and phosphorus contributes to the colonization of plant roots by symbiotic fungi [1]. The plant material for the research was the sweet cherry cv. Dilema (Prunus avium L. / Prunus mahaleb), planted in 2011 at 7 × 5 m. The cv. Dilema is a medium-early sweet cherry obtained by crossing cv. Drogan Yellow and Valery Chkalov. The tree is vigorous, forms a spreading, slightly drooping, dense crown. The fruits are convex-heart-shaped, the skin and flesh are dark red, excellent sour-sweet, refreshing taste. It ripens in the conditions of Melitopol in the first decade of June. Orchard rows were oriented north to south. Trees were trained as central leader.

For our fungal treatments we chose MycoApplay Superconcentrate 10 (endomycorrhizal fungi) and MycoApplay Micronized Endo / Ecto (endo-ectomycorrhizal fungi) to inoculate sweet cherry roots. MycoApplay Superconcentrate 10 is a concentrated, thin, suspended material with a particle size of less than 300 μm, containing 10 million endomycorrhizal propagules per pound of 4 species of fungi: Glomus intraradices (modern name Rhizophagus intraradices), Glomus mosseae, Glomus agregatum, Glomus etunicatum [2]. MycoApplay Micronized Endo/Ecto is a concentrated, thin, suspended powder mycorrhizal inoculum with a particle size of less than 300 μm, containing mycorrhizal propagules of 4 species of endomycorrhizal fungi (1 million propagules per pound Glomus intraradices, Glomus mosseae, Glomus agregatum and 7 species of ectomycorrhizal fungi (5.5 million propagules per pound Rhizopogon villosulus, Rhizopogon luteolus, Rhizopogon amylopogon, Rhizopogon fulvigleba; 100 million propagules per pound Pisolithus tinctorius; 5 million propagules per pound Scleroderma cepa and Scleroderma citrinum [2]. The orchard floor was kept under "live mulch" (natural herbs, mowed 4 times during the growing season and the clippings were left on the ground for decomposition). The following treatments were applied: 1. Control - without inoculation; 2. Inoculation of sweet cherry roots with MycoApplay Superconcentrate 10 (root inoculation with endomycorrhizal fungi); 3. Inoculation of sweet cherry roots with MycoApplay Micronized Endo / Ecto (root inoculation with endo-ectomycorrhizal fungi). Any other management was identical in each treatment. Synthetic mineral fertilizers and chemical plantprotection products were not used. The experiment was designed as a randomized complete block with three treatments in four replicates. Each experimental plot contained 4 control trees surrounded by 14 " guard " trees. The physiological parameters of leaves were determined and statistically compared by conventional methods [3-5].

The results of our research show that inoculation with endomycorrhizal fungi increased leaf total moisture content and water-retaining ability than uninoculated trees. The total leaf area at the beginning of the study (1 year) was significantly reduced in endomycorrhizal-inoculated trees, while the specific leaf density was increased (compared to control trees). In the following year, the total leaf area was significantly increased in endomycorrhizal-inoculated trees, and the specific leaf density did not differ significantly from control trees.

Leaf chlorophyll content of mycorrhizal-inoculated trees was significantly decreased compared to control trees, both in 2019 and in 2020. The chlorophyll ratio

(a / b) in 2019 was significantly increased in endomycorrhizal-inoculated trees and significantly decreased in endo-ectomycorrhizal-inoculated trees compared to uninoculated trees. In 2020 chlorophyll ratio (a / b) was significantly lower both in endomycorrhizal-inoculated and endo-ectomycorrhizal-inoculated trees than uninoculated trees. Inoculation of fruit tree roots with endo-ectomycorrhizal fungi did not significantly affect the leaf total moisture content and the total leaf area, but significantly increased the water-retaining ability in the first year of research.

**List of references**

1. Martinez TN, Johnson NC (2010) Agricultural management influences propagule densities and functioning of arbuscular mycorrhizas in low- and high-input agroecosystems in arid environments. Applied Soil Ecology. 46(2): 300-306. doi: 10.1016/j.apsoil.2010.07.001
2. MycoApply Mycorrhizal Product Line: What is the Best Option for You? URL: https://mycorrhizae.com/mycoapply-mycorrhizal-product-line-what-is-the-best-option-for-you/
3. Karpenchuk GK, Melnyk AV (1987) Uchety, nabliudenyia, analyzy, obrabotka dannykh v opytakh s plodovymy i yahodnymy rastenyiamy: Metodycheskye rekomendatsyy. Uman. s.-kh. yn-t, Uman (in Russian).
4. Pochynok HN (1976) Metody biokhymycheskoho analyza rastenyi. Nauk. Dumka, Kiev (in Russian).
5. Lakyn HF (1990) Biometryia. Vysshaia shkola, Moscow (in Russian).