SCIENTIFIC HORIZONS
Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 25(6), 75-88

UDC 581.45:634.23:631.872
DOI: 10.48077/scihor.25(6).2022.75-88

Biochemical Composition of Sweet Cherry Leaves Depending on the Method of Soil Maintenance in an Organic Garden

Tetyana Gerasko[1], Svitlana Pyda[2], Yulia Pashchenko[1], Lyubov Pokopceva[1], Tetiana Tymoshchuk[3*]

[1]Dmytro Motornyi Tavria State Agrotechnological University
72312, 18 B. Khmelnytskyi Ave., Melitopol, Ukraine

[2]Volodymyr Hnatyuk Ternopil National Pedagogical University
46024, 2 Maksym Kryvonos Str., Ternopil, Ukraine

[3]Polissia National University
10008, 7 Staryi Blvd., Zhytomyr, Ukraine

Article's History:
Received: 11.07.2022
Revised: 12.08.2022
Accepted: 11.09.2022

Suggested Citation:
Gerasko, G., Pyda, S., Pashchenko, Yu., Pokopceva, L., & Tymoshchuk, T. (2022). Biochemical composition of sweet cherry leaves depending on the method of soil maintenance in an organic garden. *Scientific Horizons*, 25(6), 75-88.

Abstract. Conducting sustainable agriculture involves not only increasing the productivity of crops and increasing the volume of crop production, but also preserving ecosystems. Mulching the soil in orchards is one of the ways to preserve the natural balance of agricultural landscapes. But the effect of competition with grasses on the biochemical composition of fruit tree tissues has not yet been definitively elucidated. The purpose of this study was to determine the influence of soil retention under mulching (compared to pure steam) on the biochemical composition of cherry leaf tissues. The research was conducted in an organic cherry orchard (*Prunus avium* L. / *Prunus mahaleb*) during 2017-2019 in the conditions of the Southern Steppe of Ukraine. A significant decrease in the content of ascorbate, glutathione, sugars, total reducing activity and activity of antioxidant enzymes was established under conditions of prolonged drought and an increase in the content of titrated acids. The total reducing activity under mulching conditions tended to increase. A gradual increase in the content of titrated acids, ascorbate, sugars, phenolic substances, and glutathione was recorded in the leaves of cherry trees from the flowering phase to November. In the autumn phase, a significant increase in sugars and phenolic substances was established in cherry leaves under the conditions of mulching in 2017, and in 2018 – phenolic substances; in 2019 – phenolic substances and ascorbate. An increase in the content of malondialdehyde (MDA) and the activity of antioxidant enzymes was found during the growing season of cherries in both variants of the experiment. In the November phase only in 2019, the MDA content was significantly higher by 14% under the condition of mulching. Under mulching conditions, a significant increase in ascorbate peroxidase (by 28-30%) and polyphenol oxidase (by 45-46%) was determined. In 2018 and 2019, a 2.4-fold increase in peroxidase activity in cherry leaves was determined. Research results help to understand the mechanisms of adaptation of fruit plants to stress factors (drought, competition with natural grasses) and can be used as an argument in favour of mulching in organic cherry orchards

Keywords: mulching, vitamin C, glutathione, titrated acids, catalase, ascorbate peroxidase, polyphenol oxidase

*Corresponding author

INTRODUCTION

The development of sustainable agricultural production requires the creation of a suitable scientific base to have a clear idea of the consequences of innovative and "green" technologies for plant physiology. Maintaining garden soil under mulching has numerous positive ecological effects: it contributes to the preservation of agrocenotic relationships (Yao *et al*., 2005), ensures optimal soil temperature and humidity (Gerasko *et al*., 2021), attracts beneficial insects-entomophages and pollinators (Mateos-Fierro *et al*., 2021), promotes the development of symbiotic mycorrhizae (Balestrini *et al*., 2018) and beneficial microorganisms in the soil, the number of which increases due to the well-developed rhizosphere of grasses (Yao *et al*., 2005). However, at present, the question of the effect of soil mulching of fruit plantations on biochemical processes in tree tissues has not been fully investigated.

One of the most popular fruit crops is the sweet cherry (*Prunus avium* L.), which is valued by consumers for its attractive appearance, high fruit taste, and nutritional value (Szpadzik *et al*., 2019; Pereira *et al*., 2020; Ivanova *et al*., 2021). Many scientists investigate the biochemical composition of sweet cherry fruits depending on the genetic characteristics of varieties, weather factors and conditions of the growing region (Picariello *et al*., 2016; Ivanova *et al*., 2020; Ivanova *et al*., 2022). Recently, there has been increasing attention to the content of biologically active substances (BAS) in the vegetative organs of fruit trees, namely sweet cherries (Prvulović *et al*., 2011; Bastos *et al*., 2015; Jesus *et al*., 2019), by-products of fruit growing (Yüksekkaya *et al*., 2009; Djilas *et al*., 2011; Afonso *et al*., 2019), and in the leaves of fruit trees (Dziadek *et al*., 2018; Dziadek *et al*., 2019; Uysal *et al*., 2020). This is explained by the fact that, firstly, leaves and by-products of fruit growing can be used for therapeutic and illness prevention purposes in the human diet, and, secondly, the content of these substances can indicate the physiological state of fruit trees (Polonskaya *et al*., 2007; Grebennikova *et al*., 2011; Bonyanpour *et al*., 2020). The course of oxidation-reduction processes in the leaves of fruit trees during the growing season also deserves attention.

In the conditions of the Southern Steppe of Ukraine, plants face various stresses, including drought, extreme temperatures, and excessive light intensity. Mulching can create additional stressful conditions for fruit trees as a result of competition with them for the most important ecological factors, such as nutrients and light (Atucha *et al*., 2011). In response to stressful conditions, reactive oxygen species (ROS) are produced in excess, which cause oxidative damage to cell membranes (lipid peroxidation, LP), proteins, and nucleic acids (Sharma *et al*., 2012). One of the products of lipid peroxidation is malondialdehyde, the accumulation of which can be used to judge the intensity of LP in plant tissues (Gill *et al*., 2013). To normalize the level of ROS in plants, the antioxidant defence system (ADS), which contains non-enzymatic and enzymatic antioxidants, works (Atucha *et al*., 2011; Sharma *et al*., 2012; Gill *et al*., 2013). Information on the content of biologically active substances, malondialdehyde and the activity of antioxidant enzymes in sweet cherry leaves will help find out how mulching affects the level of stress in the tissues of sweet cherry leaves in the conditions of the Southern Steppe of Ukraine.

The purpose of this study was to determine the content of biologically active substances, malondialdehyde, and the activity of antioxidant enzymes in sweet cherry leaves under mulching conditions in an organic garden in the South of Ukraine.

MATERIALS AND METHODS

The research was carried out during 2017-2019 in the conditions of the Scientific Research Garden of the Tavria State Agricultural Technological University (Zelene village, Melitopol district, Zaporizhzhia Oblast: 46°46'N, 35°17'E). The soil of the experimental plots is chestnut, with a sandy granulometric composition. Despite the insufficient provision of nutrients, low humus content, the soils of the study region are suitable for growing cherries. The weather conditions of the research years were warmer in terms of the average annual air temperature by 1.2-1.6°C than the long-term indicators, but in 2017 and 2019 they were inferior in terms of the average annual amount of precipitation.

Thus, the amount of precipitation in these years was 11 and 8% less, respectively, than the average multi-year data. In 2017, April was cold (the average monthly air temperature was below 10°C) and rich in precipitation (76% more than the long-term norm). May 2017 was cool and very dry. The drought continued in June 2017 as well. July 2017 was relatively satisfactory in terms of precipitation and less hot, compared to long-term data. The amount of precipitation in August-September 2017 was considerably higher than the long-term average, but the average monthly temperature in August was 3.2°C higher than the long-term norm. September and October 2017 were relatively satisfactory in terms of moisture supply. In 2018, the drought lasted from April to the end of June. July 2018 was satisfactory in terms of precipitation, but August this year was the driest in all the years of the study (precipitation was 82% below the multi-year average). Abundant precipitation in September 2018 was replaced by a drought in October (the amount of precipitation was 44% less than the average long-term norm). April and May 2019 were satisfactory in terms of moisture supply (the amount of precipitation was, respectively, 44% and 107% more than the average multi-year norm). But June 2019 was unusually hot and dry. The drought continued in July 2019 as well. August 2019 was relatively satisfactory in terms of average monthly temperature and amount

of precipitation. In September 2019, there was a severe drought that continued into October this year. As a summary regarding the weather conditions for the development of sweet cherry leaves, it can be stated that in the months of April and May, which are most important for the formation of the leaf plate, in 2017 the conditions were satisfactory in terms of moisture supply, but cold in April and cold and dry in May; in 2018, both April and May are dry and hot; in 2019, it is warm and satisfactory in terms of moisture supply in April and May.

The research was conducted in sweet cherry (*Prunus avium* L.) fruit plantations of Dilema variety on Antipka (Prunus mahaleb) rootstock. Tree planting scheme in 2011 – 7×5 m. The mid-early variety Dilemma was obtained as a result of crossing two varieties, Drohana Zhovta and Valerii Chkalov. Strong-growing trees of the variety form sprawling, slightly drooping, dense crowns. The shape of the fruits is convex-heart-shaped with a dark red skin and pulp. The fruits are characterized by an excellent sweet-sour, refreshing taste. Fruit ripening in the conditions of the region is observed in the first decade of June. Fruits are mainly used fresh.

The experiment was laid out as a randomized complete block with three replications. There are 10 control trees in each repetition. The scheme of soil retention in the experimental areas is as follows:

Option 1. Pure steam (weeding was carried out to a depth of 15 cm, manual weeding);

Option 2. Covering the soil with natural grasses or "living mulch" (natural grasses were mowed, biomass was left on the soil surface).

Plant care measures were identical in each version of the experiment. Synthetic pesticides and mineral fertilizers were not used in the sweet cherry growing technology. During the growing season (from April to November), leaves were collected annually for analysis. Leaf collection was carried out 4 times per growing season in the phases of flowering (BBCH 61-65), ripening of fruits (BBCH 87-89), completion of shoot growth (BBCH 91) and November (BBCH 93-97). For biochemical analyses, 100 intact leaves were selected in three replicates from each variant of the experiment.

The main elements of records and observations: the content of malondialdehyde (MDA, nmol/g), the activity of antioxidant enzymes – catalase (CAT, μmol H_2O_2/g·min), ascorbate peroxidase (APO, mg oxidized ascorbic acid/g), polyphenol oxidase (PPO, c.u./g·min), peroxidases (PO, mcat/g); the content of sugars (S, %), titrated acids (TA, %), phenolic substances (Phen, mg GA/100 g), ascorbic acid (As, mg/100 g), glutathione (Glu, mg/100 g) in sweet cherry leaves.

The intensity of lipid peroxidation (LP) was determined by the accumulation of the secondary LP product – MDA. The method is based on the fact that at 95°C in an acidic environment, MDA reacts with TBA, forming a pink trimethyl complex with an absorption maximum at 535 nm (Costa *et al.*, 2002). For the analysis, 250 mg of plant material was taken, homogenized with 4 ml of 20% trichloroacetic acid (TCA). 4 ml of 0.5% TBA dissolved in 20% TCA was added to 1 ml of filtrate and boiled at 95°C in a water bath for 30 minutes, followed by cooling. Optical density was measured at wavelengths of 532 nm and 600 nm. Then the MDA content was calculated according to formula 1:

$$C = \frac{(E635 - E600)}{E}, \quad (1)$$

where C is the concentration of MDA; E532 and E600 are the optical density at wavelengths of 532 nm and 600 nm, respectively; E is the molar extinction coefficient of the trimethyl complex for a beam path length of 1 cm ($E=1.55\times10^5$ M^{-1} cm^{-1}). The result of MDA content calculations was displayed in nM per gram of plant material weight.

The activity of catalase (CAT, EC 1.11.1.6) was determined by the spectrophotometric method according to the degree of decomposition of hydrogen peroxide by catalase, the residue of which was determined by reaction with ammonium molybdate, expressed in μmol H_2O_2/g·min.

The activity of ascorbate peroxidase (APO, EC 1.11.1.11) was determined by titrating the remaining unoxidized ascorbic acid with 0.001 solution of Tillmans reagent (2,6-dichlorophenolindophenol) until a light pink colouration that did not disappear within 30 s (Gorodniy *et al.*, 2006). In the control, APO was deactivated with metaphosphoric acid. APO activity was determined according to formula 2:

$$X = \frac{(a - b) \times T \times V_1}{H \times V_2}, \quad (2)$$

where X is APO activity (mg of oxidized ascorbic acid per 1 g of raw mass); a is the amount of 0.001 Tillmans reagent used for the titration of the control sample, ml; b is the amount of 0.001 Tillmans reagent used for titration of the test sample, ml; T is the reagent titre (amount of ascorbic acid corresponding to 1 ml of paint), mg; V_1 is the total volume of the extract, ml; H is the weight of plant material, g; V_2 is the volume of extract taken for titration, ml.

Determination of the activity of polyphenol oxidase (PPO, EC 1.10.3.1) was carried out by the spectrophotometric method, which is based on the measurement of the optical density of the reaction products formed during the oxidation of pyrocatechin over a certain period. The optical density was measured at 420 nm. The activity of polyphenol oxidase was expressed in conventional units per 1 g of raw tissue in 1 min.

Determination of peroxidase activity (PO, EC 1.11.1.7) was carried out by oxidation of indigo carmine with oxygen released during the decomposition of hydrogen peroxide under the influence of peroxidase (Frew

et al., 1983): upon oxidation, indigo carmine changes its colour from blue-green to yellow-pink. 1 g of plant tissue was homogenized with 6 ml of acetate buffer with pH=4.9. 2 ml of indigo carmine was added. 1 ml of homogenate was taken, and 0.5 ml of 0.03 M hydrogen peroxide solution was added. After 2 minutes, 20% sulphuric acid was added to stop the reaction. Photometry was performed at a wavelength of 610 nm against distilled water in a cuvette with a working length of 1 cm. In parallel, a control sample was prepared, where instead of hydrogen peroxide, 0.5 ml of distilled water was added. PO activity was expressed in mcat/g of plant material weight.

The content of sugars (%) in plant tissues was determined by the photometric method based on the ability of monosaccharides to reduce picric acid (2, 4, 6-trinitrophenol) to picramic acid. The final product of the reaction acquires an intense red colour. The content of titrated acids in the studied samples was determined by the generally accepted method (Methods of ...). The total content of phenolic substances was determined photometrically using the Folin-Chocalteau reagent and calculated in mg of gallic acid (GA) per 100 g of raw material (Waterhouse, 2002). The content of ascorbic acid, glutathione and total reducing activity of plant tissues was determined according to well-known methods (Gorodniy *et al*., 2006).

All studied analyses were performed in triplicate. The obtained experimental data were compared by Tukey's mean separation test at a significance level of $P \leq 0.05$ and were processed using the Pearson correlation analysis method using Minitab 19 software (Minitab Inc., State College, PA).

RESULTS AND DISCUSSION

As a result of the research, it was established that the total reducing activity in sweet cherry leaves varied between 8.5-18.7 ml of KJO_3/100 g of raw material for keeping the soil in the garden under pure steam and from 9.3 to 20.2 ml of KJO_3/100 g of raw material when covering the soil with natural herbs (Tables 1-3). Total reducing activity tended to increase under mulching conditions, but this indicator was statistically significantly greater under mulching conditions only in the flowering phase in 2018 (9% more, compared to pure steam conditions).

Table 1. *Phytochemical composition of sweet cherry leaves of the Dilema variety depending on soil maintenance in the garden (2017), $\bar{M}\pm m$*

Variant	Sugars, %	Titrated acids, %	Phenolic substances, mg GA/100 g	Ascorbate, mg/100 g	Glutathione, mg/100 g	Total reducing activity, ml of KJO_3/100 g
Flowering						
Pure steam	3.2±0.23	1.1±0.06	7.9±0.60	8.4±0.61	30.7±2.09	10.1±0.63
Mulching	3.8±0.24*	1.4±0.08*	10.2±0.65*	9.7±0.64	32.2±2.11	10.5±0.69
Fruit ripening						
Pure steam	3.4±0.25	1.1±0.08	43.2±3.04[a]	9.1±0.73	33.8±2.25	11.2±0.68
Mulching	4.1±0.27*	1.5±0.08*	50.4±3.76*[a]	10.6±0.75	36.8±2.29	12.1±0.76
Completion of shoot growth						
Pure steam	3.8±0.29	1.3±0.09[a]	136.5±10.25[a]	10.7±0.79	38.4±2.59	12.5±0.78
Mulching	4.5±0.32*	1.5±0.10*	175.1±10.69*[a]	12.4±0.83	39.9±2.66	13.3±0.83
Defoliation						
Pure steam	4.2±0.34	1.6±0.10[a]	155.0±13.61[a]	11.1±0.85	41.4±2.84	13.5±0.88
Mulching	4.8±0.28*	1.8±0.12[a]	228.5±15.65*[a]	12.3±0.89	42.9±2.99	14.6±0.93

Note: **the difference between options is significant at $P \leq 0.05$; and the difference compared to the previous stage of organogenesis is significant at $P \leq 0.05$*

Table 2. *Phytochemical composition of sweet cherry leaves of the Dilema variety depending on soil maintenance in the garden (2018), $\bar{M}\pm m$*

Variant	Sugars, %	Titrated acids, %	Phenolic substances, mg GA/100 g	Ascorbate, mg/100 g	Glutathione, mg/100 g	Total reducing activity, ml of KJO_3/100 g
Flowering						
Pure steam	2.8±0.20	0.9±0.06	8.8±0.56	7.0±0.29	26.1±1.58	8.5±0.49
Mulching	3.4±0.21*	1.1±0.06	13.8±0.61*	7.5±0.31	27.9±1.71	9.3±0.52*

Table 1, Continued

Variant	Sugars, %	Titrated acids, %	Phenolic substances, mg GA/100 g	Ascorbate, mg/100 g	Glutathione, mg/100 g	Total reducing activity, ml of KJO_3/100 g
Fruit ripening						
Pure steam	3.0±0.22	1.0±0.07	48.6±2.62a	7.9±0.33a	29.2±1.85	9.5±0.59a
Mulching	3.7±0.22*	1.2±0.07	68.7±2.81*a	8.4±0.35a	30.7±1.89	10.1±0.63
Completion of shoot growth						
Pure steam	3.4±0.25	1.1±0.08	153.5±10.90a	8.8±0.41a	32.2±1.91	10.5±0.69
Mulching	4.1±0.26*	1.3±0.09	197.2±11.82*a	9.2±0.53a	35.3±2.19	11.5±0.74
Defoliation						
Pure steam	3.8±0.27	1.4±0.09[a]	174.5±13.32[a]	9.7±0.61[a]	36.8±2.54[a]	12.1±0.91[a]
Mulching	4.3±0.28	1.6±0.10[a]	257.1±15.11*[a]	10.1±0.70[a]	39.9±2.72[a]	13.4±0.89[a]

Note: **the difference between options is significant at P≤0.05; and the difference compared to the previous stage of organogenesis is significant at P≤0.05*

Table 3*. Phytochemical composition of sweet cherry leaves of the Dilema variety depending on soil maintenance in the garden (2019), $\bar{M}$±m*

Variant	Sugars, %	Titrated acids, %	Phenolic substances, mg GA/100 g	Ascorbate, mg/100 g	Glutathione, mg/100 g	Total reducing activity, ml of KJO_3/100 g
Flowering						
Pure steam	2.9±0.11	1.0±0.06	11.2±0.75	9.5±0.27	46.1±2.14	14.8±0.51
Mulching	3.5±0.18*	1.2±0.07	17.5±0.80*	10.6±0.39*	47.6±2.19	15.5±0.57
Fruit ripening						
Pure steam	3.1±0.21	1.1±0.07	61.2±2.62a	11.4±0.51a	49.1±2.29	15.9±0.65
Mulching	3.6±0.22*	1.3±0.07*	87.2±2.82*a	13.2±0.67*a	52.1±3.20	17.1±0.49
Completion of shoot growth						
Pure steam	3.5±0.20	1.2±0.08	195.5±12.16a	12.3±0.65	55.3±3.51	17.8±0.87a
Mulching	4.2±0.22*	1.4±0.09*	250.4±15.28*a	14.1±0.67	56.8±3.49	18.5±0.83
Defoliation						
Pure steam	3.9±0.24	1.3±0.09	242.3±15.65a	13.2±0.71	58.3±3.91	18.7±0.95
Mulching	4.5±0.26	1.5±0.09	298.1±16.98*a	15.5±0.79*	61.4±3.99	20.2±0.99

Note: **the difference between options is significant at P≤0.05; and the difference compared to the previous stage of organogenesis is significant at P≤0.05*

The content of ascorbate in sweet cherry leaves varied between 7.0-13.2 mg/100 g of raw material under conditions of pure steam and 7.5-15.5 mg/100 g of raw material when the soil was covered with natural grasses. In 2017 and 2018, there was no significant difference in the content of ascorbate in the leaves. In the experiment, the highest accumulation of ascorbate in leaves was noted in the November phase in the year 2019, which was relatively satisfactory in terms of moisture supply. Moreover, under the conditions of mulching, ascorbate accumulated substantially more in the leaves, compared to the conditions of pure steam: in the flowering and leaf phases, respectively, by 12 and 17%. Ascorbate is a widespread and effective antioxidant associated with photosynthetic reactions, which accumulates in high concentrations in both photosynthetic and non-photosynthetic organs and tissues and has many functions in plants, the main ones being the protection of the glutathione pool and the proper functioning of some enzymes (Gest *et al.*, 2012).

Under the conditions of keeping the soil under black steam, the content of glutathione in sweet cherry leaves was in the range of 26.1-58.3 mg/100 g of raw material. When the soil was covered with natural grasses,

the studied indicator varied from 27.9 to 61.4 mg/100 g of raw material. It should be noted that over the years of research, no significant difference between the variants of the experiment has been established. The tripeptide glutathione (GSH, γ-glutamyl-cysteinyl-glycine) is one of the main components of the ascorbate-glutathione (AsA-GSH) pathway and plays a significant role in protecting cells from oxidative stress and the accumulation of harmful LP products in plant tissues (Anjum *et al*., 2010; Walker *et al*., 2018).

Notably, in 2018, when the weather conditions were unfavourable, the total reducing activity in sweet cherry leaves tended to decrease in both versions of the experiment. Thus, the studied indicator decreased by 14% when the soil was kept under black steam and by 12% when covered with natural grasses. This was probably due to a decrease in the content of ascorbate and glutathione in leaves in 2018 compared to 2017. Thus, the content of ascorbate in sweet cherry leaves in 2018 decreased by 15% when the soil was kept under clean steam and by 22% when mulched compared to since 2017. The content of glutathione in sweet cherry leaves decreased in 2018 by 14% when the soil was kept under clean steam and by 12% when mulched compared to 2017. Similar trends have already been described in the scientific literature: under the stress of progressive drought in apple trees the initial response was a slight oxidation of glutathione with a subsequent increase in glutathione concentration, but as drought stress continued to increase, glutathione concentration decreased substantially (Polonskaya *et al*., 2007). In 2019 (with sufficient moisture supply), the total reducing activity significantly increased, compared to 2018, in both variants of the experiment: under conditions of pure steam by 66%, under conditions of mulching – by 62%. The content of ascorbate in leaves in 2019 under conditions of pure steam increased by 41% compared to 2018, under conditions of mulching – by 51%. The same trend was observed regarding the content of glutathione in leaves: in 2019, under the conditions of mulching, this indicator was 64% more, compared to 2018, under the conditions of pure steam – by 69%. If one compares the total reducing activity in sweet cherry leaves in the favourable year of 2019 with the average year in terms of climatic conditions in 2017, then this indicator was 42% higher in both versions of the experiment. Likewise, the content of ascorbate in 2019 was higher compared to 2017: under the conditions of pure steam by 20%, under the conditions of mulching – by 18%. The content of glutathione in the leaves also increased in 2019, compared to 2017: under the conditions of mulching – by 44%, under the conditions of pure steam – by 45%. From the above, it can be concluded that with sufficient moisture supply, the content of ascorbate, glutathione, and general reducing activity in sweet cherry leaves increases. Ascorbate consumption occurred under drought conditions, while under mulching conditions it was consumed more, but also produced more. This is confirmed by the absence of a significant difference in the investigated indicator between the variants of the experiment. Glutathione content and total reducing activity under drought conditions decreased in the same way, both under mulching conditions and under clean steam conditions, no substantial difference in these parameters between the experimental variants was noted during all three years of research. The content of ascorbate, glutathione, and total reducing activity in sweet cherry leaves gradually increased from the flowering phase to November and had maximum values in the November phase. A decrease found in the content of ascorbate, glutathione, and total reducing activity in leaves under drought conditions indicates stress exhaustion of sweet cherry trees, when a high level of stress leads to a decrease in the production of protective antioxidants (Anjum *et al*., 2010).

In the flowering phase (BBCH 61-65), the sugar content in the leaves was 2.8-3.2% when the soil was kept under pure steam and 3.4-3.8% when covered with natural grasses. In the fruit ripening phase (BBCH 87-89), the sugar content in the leaves increased. Thus, on pure steam, the sugar content in leaves was 3.0-3.4%, and on mulching – 3.6-4.1%. In the phase of completion of shoot growth, a difference between the experimental variants was also observed. The content of sugars in the leaves was higher under the condition of soil mulching (4.1-4.5%) compared to pure steam (3.4-3.8%). However, in the defoliation phase (BBCH 91), the difference between the test options was statistically significant (by 14%) only in 2017. In general, the highest content of sugars in the leaves was accumulated before the defoliation phase and was 4.3–4.8% in the conditions of mulching and 3.8-4.2% in the conditions of pure steam. The content of sugars decreased under unfavourable weather conditions in 2018 almost equally in both versions of the experiment – by 10-11%.

Organic acids in plant tissues perform several essential functions, e.g., they help to change the turgor pressure in the stomata, which provokes their movement (Richter, 2021). During the growing season, the content of titrated acids in sweet cherry leaves increased from 0.9 to 1.6% under the condition of pure steam and from 1.1 to 1.8% under the condition of mulching. The content of titrated acids in sweet cherry leaves under the condition of being covered with natural grasses was significantly higher compared to pure steam: in 2017, by 27, 36 and 15% in the phases of flowering, ripening of fruits and completion of shoot growth, respectively; in 2019, by 18 and 17% in the phases of fruit ripening and the completion of shoot growth, respectively. In 2018, there was no substantial difference between the experimental variants in the content of titrated acids in the leaves. Under unfavourable weather conditions in 2018 (compared to 2017), the content of titrated acids in the leaves of sweet cherry trees decreased: under clean

steam conditions by 14%, under mulching conditions by 16%. However, in the year 2019, which was more favourable in terms of moisture supply, the acid content increased insubstantially compared to 2018 (only by 3% under clean steam conditions and by 2% under mulching conditions). Thus, in 2017, the content of titrated acids in sweet cherry leaves was the highest in three years of research. This can be explained by the cold conditions of April and May 2017, which contributed to the shift of leaf metabolism towards the accumulation of acids.

Total phenolic concentration in plant leaves is a major component of stress tolerance (Cheynier *et al*., 2013). The total content of phenolic substances in sweet cherry leaves was 7.9-242.3 mg of GA/100 g of raw material when the soil was kept under pure steam. Under the condition of covering the soil with natural grasses, the studied indicator increased significantly and ranged from 10.2 to 298.1 mg GA/100 g of raw material. There was a substantial difference between the variants of the experiment in terms of the content of phenolic substances throughout all years of research. Thus, the content of phenolic substances in sweet cherry leaves was 16-56% higher compared to pure steam. Under adverse weather conditions in 2018, the content of phenolic substances (in contrast to ascorbate, glutathione, sugars and titrated acids) increased by 12% when the soil was kept under pure steam and by 24% when covered with natural grasses. In 2019, the content of phenols increased by 30% compared to 2018 when kept under pure steam and by 24% when covered with natural grasses. The total content of phenolic substances in sweet cherry leaves, established by our research in the fruit ripening phase, is consistent with the data of Turkish scientists (Uysal *et al*., 2020). As a result of the research, a gradual increase in the content of the following biologically active substances was recorded in sweet cherry leaves from the flowering phase (BBCH 61-65) to the defoliation phase (BBCH 91): ascorbate, glutathione, sugars, titrated acids and phenolic substances. In the defoliation phase, sugars and phenolic substances were substantially more in the leaves of sweet cherry trees under mulching conditions in 2017, phenolic substances in 2018, and phenolic substances and ascorbate in 2019 compared to the conditions of pure steam.

Correlation analysis confirms a strong direct positive correlation between BAS content and total reducing activity (Table 4). At the same time, the total reducing activity in sweet cherry leaves was most closely correlated with the content of glutathione and phenolic substances. The accumulation of biologically active substances occurred in a close correlation (Table 5). The closest correlation was noted between the content of phenolic substances and glutathione, sugars and titrated acids, ascorbate, and glutathione.

Table 4. *Correlation coefficients (r^2) of general reducing activity (GRA) with the content of biologically active substances (BAS) in sweet cherry leaves*

Year of research	Sugars	Titrated acids	Phenolic substances	Ascorbate	Glutathione
2017	0.81	0.71	0.93	0.85	0.98
2018	0.82	0.94	0.92	0.96	0.99
2019	0.85	0.82	0.95	0.91	0.99

Table 5. *Correlation coefficients (r^2) between the content of biologically active substances (BAS) in sweet cherry leaves*

Year	Sugars and				Titrated acids and			Ascorbate and		Phenolic substances and glutathione
	Titrated acids	Ascorbate	Phenolic substances	Glutathione	Ascorbate	Phenolic substances	Glutathione	Phenolic substances	Glutathione	
2017	0.90	0.92	0.74	0.78	0.75	0.60	0.69	0.82	0.84	0.93
2018	0.89	0.77	0.76	0.81	0.90	0.76	0.91	0.93	0.98	0.94
2019	0.96	0.86	0.77	0.81	0.89	0.69	0.76	0.80	0.86	0.97

Similar trends have already been noted in the studies of A.K. Polonskaya, V.N. Yezhov, and O.A. Grebennikova (Polonskaya *et al*., 2007; Grebennikova *et al*., 2011). The content of biologically active substances in plum leaves increased during the growing season and was the largest in the defoliation phase, and there was a close correlation between antioxidant activity and the content of biologically active substances. Comparative chemical analysis of fruits and leaves of apricot, plum, nectarine showed that the leaves of these crops are a valuable source of biologically active substances: the ascorbate content in the leaves of these crops was 12.4-21.3 g/100 g of raw material, organic acids – 1.0-2.1 g/100 g; carbohydrates – 8.0-14.8 g/100 g. Dry apricot leaves collected during defoliation contained up to 56.8 mg/100 g of ascorbate (Polonskaya *et al*., 2007).

Malondialdehyde (MDA) is one of the end products of the peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage (Sharma *et al.*, 2012). Under the conditions of mulching, the content of MDA in the tissues of sweet cherry leaves in 2017 was substantially higher in the phases of flowering (by 58%) and the completion of shoot growth (by 36%), compared to the leaves of sweet cherry trees on pure steam (Table 6).

***Table 6**. The content of malondialdehyde (MDA) and the activity of ADS enzymes in the sweet cherry leaves of Dilema variety depending on the content of the soil (2017), $\bar{M}\pm m$*

Variant	MDA, nmol/g	CAT, μmol H_2O_2/g·min	APO, mg of oxidized ascorbic acid/g	PPO, c.u./g·min	PO, mcat/g
Flowering					
Pure steam	35.1± 1.44	5.5±0.39	5.4±0.44	7.4±0.45	3.5±0.29
Mulching	55.5±5.25*	5.5±0.43	6.3±0.54	10.7±0.49*	5.4±0.33*
Fruit ripening					
Pure steam	104.3±9.81[a]	6.54±0.53[a]	7.2±0.69[a]	14.2±0.57[a]	5.9±0.45[a]
Mulching	115.6±10.43[a]	7.62±0.69[a]	8.3±0.71[a]	16.9±0.55*[a]	15.3±0.47*[a]
Completion of shoot growth					
Pure steam	219.7±17.91[a]	10.3±0.55	15.9±0.99[a]	30.9±0.79[a]	25.6±0.85[a]
Mulching	297.8±20.93*[a]	12.4±0.92*[a]	17.3±0.75[a]	35.6±0.81*[a]	33.1±0.88*[a]
Defoliation					
Pure steam	265.5±25.34	9.1± 0.73[a]	20.1±0.85[a]	35.4±0.88[a]	43.5±0.89[a]
Mulching	288.5±16.55	9.3± 0.82[a]	25.8±0.79*[a]	45.8±0.85*[a]	45.9±0.96[a]

***Note:** *the difference between options is significant at P≤0.05; and the difference compared to the previous stage of organogenesis is significant at P≤0.05*

In the phases of fruit ripening and defoliation, there was a tendency to increase the content of MDA in leaves under the conditions of mulching, but the difference between the variants of the experiment was statistically insignificant. In 2018, the content of MDA in leaves was substantially lower under the conditions of mulching in the phases of flowering (by 34%), ripening of fruits (by 40%) and the completion of shoot growth – by 28% (Table 7).

***Table 7**. The content of malondialdehyde (MDA) and the activity of ADS enzymes in the sweet cherry leaves of Dilema variety depending on the content of the soil (2018), $\bar{M}\pm m$*

Variant	MDA, nmol/g	CAT, μmol H_2O_2/ g·min	APO, mg of oxidized ascorbic acid/g	PPO, c.u./g·min	PO, mcat/g
Flowering					
Pure steam	15.1±3.35	3.8±0.23	4.1±0.34	5.6±0.35	2.7±0.19
Mulching	10.0±1.15*	4.5±0.20*	4.9±0.41	8.2±0.39*	4.1±0.24*
Fruit ripening					
Pure steam	59.5± 14.57	7.2±0.25a	5.5±0.43[a]	10.7±0.44[a]	4.4±0.35[a]
Mulching	35.8±8.35*	7.6±0.19a	6.3±0.51[a]	13.0±0.46*[a]	11.6±0.49*[a]
Completion of shoot growth					
Pure steam	191.3±18.47[a]	12.2±0.32[a]	12.1±0.76[a]	23.4±0.74[a]	11.8±0.54[a]
Mulching	138.5±12.53*a	17.3±0.34*[a]	13.3±0.79[a]	27.4±0.75*[a]	25.4±0.75*[a]
Defoliation					
Pure steam	255.4±20.15[a]	10.0± 0.30[a]	15.2±0.99[a]	26.9±0.81[a]	10.2±0.78
Mulching	223.8±20.17[a]	9.4± 0.32[a]	19.8±0.95*[a]	35.2±0.87*[a]	35.3±0.99*[a]

***Note:** *the difference between options is significant at P≤0.05; and the difference compared to the previous stage of organogenesis is significant at P≤0.05*

The tendency to decrease the content of MDA in the leaves on the mulching remained in the defoliation phase, but the difference between the variants was insignificant here. In general, the content of MDA in the sweet cherry leaves on the mulching decreased in 2018 by 57% compared to 2017. In 2019, a tendency to increase the content of MDA in the leaves on the mulching was noted, and in the phases of flowering, fruit ripening and defoliation, the MDA content was substantially larger in the leaves of sweet cherry trees grown on mulching (Table 8). The general trend for both variants of the experiment was a gradual increase in the content of MDA in leaf tissues during the growing season and the maximum accumulation of MDA in the defoliation phase.

***Table 8**. The content of malondialdehyde (MDA) and the activity of ADS enzymes in the sweet cherry leaves of Dilema variety depending on the content of the soil (2019), $\bar{M}\pm m$*

Variant	MDA, nmol/g	CAT, μmol H_2O_2/g·min	APO, mg of oxidized ascorbic acid/g	PPO, c.u./g·min	PO mcat/g
Flowering					
Pure steam	13.5±2.67	12.2±0.35	6.0±0.43	8.3±0.23	3.9±0.25
Mulching	35.4±2.75*	17.9±0.30*	7.1±0.45*	12.1±0.29*	6.1±0.29*
Fruit ripening					
Pure steam	138.0±10.44[a]	19.0±0.25[a]	8.1±0.57[a]	15.9±0.35[a]	6.6±0.33[a]
Mulching	155.5±13.45*a	19.1±0.29[a]	9.4±0.60*[a]	19.7±0.37*[a]	17.2±0.37*[a]
Completion of shoot growth					
Pure steam	236.2±21.18[a]	19.3±0.63[a]	17.8±0.88[a]	34.6±0.67[a]	17.4±0.74[a]
Mulching	234.5±19.25[a]	26.6±0.54*[a]	19.5±0.85[a]	40.2±0.75*[a]	37.4±9.78*[a]
Defoliation					
Pure steam	255.6±11.28	27.2± 0.33[a]	22.5±0.90[a]	39.8±0.99[a]	15.1±0.89
Mulching	290.5±11.55*a	27.3± 0.25[a]	29.2±0.93*[a]	51.7±0.84*[a]	51.8±0.85*[a]

***Note:** *the difference between options is significant at P≤0.05; and the difference compared to the previous stage of organogenesis is significant at P≤0.05*

Considering the extremely dry conditions of the formation of the leaf plate in 2018, sweet cherry trees suffered less from oxidative stress in the conditions of mulching. However, during the 2018 growing season, the MDA content also decreased in the leaves of sweet cherry trees under pure steam conditions (by 29% on average) compared to 2017. An increase in the MDA content in sweet cherry leaves under the conditions of mulching indicates a higher level of lipid peroxidation and is an indicator of oxidative damage to cell membranes. The presented data are fully consistent with the previously described general trends inherent in the non-specific response of plants to stress: a decrease in the activity of metabolic processes, which is accompanied by the induction of the formation of compounds necessary for the preservation of the integral structure of cells and the life potential of the plant as a whole (Polonskaya *et al*., 2007). In 2019, the content of MDA in sweet cherry leaves increased substantially, compared to 2018, especially in the phase of fruit ripening: under conditions of pure steam by 2.3 times, under conditions of mulching – by 4.3 times. In general, the content of MDA in sweet cherry leaves from both variants of the experiment in 2019 was more, compared to the similar phases of 2018, by an average of 2 times.

In 2017, the content of MDA in sweet cherry leaves was strongly correlated with the activity of antioxidant enzymes and the content of biologically active substances (Table 9). The correlation of MDA content with PPO activity and the content of phenolic substances, glutathione, and total reducing activity was the strongest in 2017. In 2018, the correlations of MDA content with the activity of antioxidant enzymes and the content of biologically active substances were much weaker, compared to 2017. The highest correlation of MDA content in leaves in 2018 was noted with the activity of APO, PPO, and the content of ascorbate and phenolic substances. In 2019 (under conditions of satisfactory moisture supply during the formation of the leaf blade), the content of MDA was strongly correlated with the activity of antioxidant enzymes and the content of BAS in sweet cherry leaves, most strongly with the activity of PPO and the content of glutathione, phenolic substances and general reducing activity.

***Table 9**. Correlation coefficients (r^2) of MDA content with general reducing activity (GRA), ADS enzyme activity and the content of BAS in sweet cherry leaves*

Year of research	Correlation coefficients of MDA content with									
	CAT	APO	PPO	PO	Sugars	Titrated acids	Phenolic substances	Ascorbate	Glutathione	GRA
2017	0.83	0.88	0.95	0.90	0.68	0.50	0.95	0.90	0.91	0.91
2018	0.37	0.86	0.83	0.36	0.41	0.57	0.80	0.82	0.79	0.79
2019	0.76	0.82	0.90	0.58	0.63	0.62	0.91	0.81	0.93	0.93

The activity of catalase in both versions of the experiment in 2017 and 2018 increased until the phase of shoot growth completion and further decreased until the defoliation phase. In 2019, catalase activity continued to increase until the defoliation phase. Under the conditions of mulching during all three years of research, a tendency to increase catalase activity in the tissues of sweet cherry leaves was observed, but a statistically significant difference between the experimental variants was only in the phase of the end of shoot growth in 2017 (by 20%), in the phases of flowering and end of shoot growth in 2018 (by 18% and 42%, respectively) and in the phases of flowering and the end of shoot growth in 2019 (by 47% and 38%, respectively). Catalase activity in sweet cherry leaves in 2018 in the flowering phase was significantly lower, compared to the same period in 2017: under mulching conditions by 18%, under pure steam conditions – by 31%. But in the future, the activity of catalase in sweet cherry leaves increased, and in the phase of fruit ripening it was 40% more in the conditions of mulching, and 10% more in the conditions of pure steam, compared to the same period in 2017. In general, it can be stated that during the entire vegetation period of 2018, the activity of catalase did not significantly differ from the level of 2017, showing an increase of 4%. In 2019, catalase activity also increased insignificantly compared to 2018 – by an average of 2.6% in both variants of the experiment. But, if we compare with 2017, in 2019, the activity of catalase in leaves increased by 171% under the conditions of mulching, and under the conditions of pure steam – by 150%. Stresses have been reported to cause either enhancement or depletion of CAT activity in plants, depending on the intensity, duration, and type of stress. Depletion of CAT activity is observed when the intensity of protein turnover decreases under stress (Sharma *et al*., 2012; Gill *et al*., 2013; Cai *et al*., 2019).

The activity of ascorbate peroxidase in leaves increased throughout the growing season and reached maximum values in the defoliation phase. Moreover, under the conditions of mulching, APO activity in sweet cherry leaves was significantly higher, compared to the conditions of pure steam: in the defoliation phase of 2017 by 28%, in the defoliation phase of 2018 by 30%, in the flowering, fruit ripening and defoliation phases of 2019, respectively, by 10%, 16%, and 29%. In 2018, APO activity in leaves decreased by 23% under mulching conditions and by 24% under clean steam conditions compared to 2017. In 2019, APO activity in sweet cherry leaves increased by 47% under mulching conditions and by 50% under clean steam conditions couple compared to 2018. But, if one compares the data of 2019 with the data of 2017, the activity of APO showed a tendency to decrease (on average, by 6% for both variants of the experiment).

The activity of polyphenol oxidase in sweet cherry leaves increased continuously during the growing season, reaching a maximum in the defoliation phase. At the same time, under the conditions of mulching, the activity of polyphenol oxidase in sweet cherry leaves was consistently higher, compared to the conditions of pure steam: in the flowering phase by 45%, 46%, and 45% (in 2017, 2018, and 2019, respectively). In the fruit ripening phase – by 19.21% and 24%; in the phase of completion of shoot growth – by 15%, 17%, and 16%; in the defoliation phase – by 29%, 30%, and 29%. In 2018, the activity of PPO in the leaves of both variants of the experiment decreased, on average, by 24%. In 2019, compared to 2018, PPO activity increased, on average, by 48% for both variants of the experiment. But, if compared with 2017, PPO activity increased in sweet cherry leaves in 2019 by only 12% under pure steam conditions and by 14% under mulching conditions.

Notably, PPO activity in sweet cherry leaves was strongly and stably correlated with the content of phenolic substances during all years of research (r^2=0.99, p=0.0001). APO activity in leaves was highly correlated with ascorbate content (r^2=0.96 in 2017 and 2018 and r^2=0.76 in 2019, p=0.0001).

The activity of peroxidase in leaves was significantly higher under the conditions of mulching, compared to the conditions of pure steam: in 2017, in the flowering phase by 54%, in the fruit ripening phase by 159%, in the phase of the completion of shoot growth – by 29%; in 2018, in the flowering phase by 51%, in the fruit ripening phase by 164%, in the phase of shoot completion by 115%, in the defoliation phase by 246%; in 2019, in the flowering phase by 56%, in

the fruit ripening phase by 160%, in the phase of shoot completion by 114%, in the defoliation phase by 243%. But the dynamics of peroxidase activity differed over the years of research. Thus, in 2017, peroxidase activity increased throughout the growing season in both variants of the experiment, reaching a maximum in the defoliation phase. In 2018 and 2019, an increase in peroxidase activity was observed in both variants before the phase of shoot growth completion. Later, until the defoliation phase, peroxidase activity in the leaves of sweet cherry trees in pure steam slightly decreased (statistically insubstantial), and under mulching conditions it continued to increase, reaching a maximum in the defoliation phase.

In 2018, peroxidase activity decreased in leaves by 24% under mulching conditions and by 45% under pure steam conditions compared to 2017. In 2019, PO activity in leaves increased by 48% under mulching conditions and by 47% under pure steam conditions compared to 2018. If we compare the data of 2019 with the data of 2017, the activity of PO in sweet cherry leaves under the conditions of pure steam decreased by 19%, and under the conditions of mulching, it increased by 13%.

The dynamics of APO and PPO activity in sweet cherry leaves did not coincide with the dynamics of CAT activity after the phase of shoot growth completion in 2017 and 2018 and with the dynamics of PO activity after the phase of shoot growth completion in 2018 and 2019: APO and PPO activity after the completion of shoot growth continued to increase to the defoliation phase, while CAT activity significantly decreased and PO activity showed a decreasing trend. Similar trends have already been described by B. Cai: opposite trends in the activity of CAT, PO, and APO were noted in the flower buds of cherries when the resting period was disturbed. Regarding the range of fluctuations in enzymatic activity, our data are similar to fluctuations in the activity of antioxidant enzymes under the action of exogenous treatment of sweet cherry trees with gibberellins (Cai *et al*., 2019). This suggests that phytohormones, which are produced in leaf tissues under mulching conditions, are involved in increasing the activity of antioxidant enzymes in leaves under mulching conditions. We can assume that the reason here is the activity of soil microorganisms that exist in the rhizosphere of trees and natural grasses, primarily plant growth-stimulating rhizobacteria and mycorrhizal fungi, which can provide trees with moisture, nutrients, hormones, enzymes and increase the tolerance of fruit trees to abiotic stresses. (Esitken, 2011; Turrini *et al*., 2017). Mulching provides better conditions for the existence of soil microorganisms (Yao *et al*., 2005, Balestrini *et al*., 2018). Therefore, the greater number and activity of soil microorganisms may be the reason for the increase in the activity of antioxidant enzymes in sweet cherry leaves under mulching conditions (compared to standard mechanical treatment).

The BAR content in the leaves of fruit crops is often higher than in the fruits (Tabart *et al*., 2006; Teleszko *et al*., 2015, Oszmiański *et al*., 2016). Organic practices in horticulture contribute to the accumulation of biologically active substances and antioxidants not only in fruits, but also in plant leaves (Hallmann & Sabała, 2020). In the study presented here, both variants of sweet cherry trees were grown using organic technology, so they contained a sufficiently large amount of biologically active substances and antioxidants in the leaf tissues. But trees under mulching conditions experienced added stress due to competition with grasses, which led to increased accumulation of protective anti-stress substances. Thus, it can be stated that the leaves of sweet cherry trees grown under mulching conditions are a powerful source of biologically active substances and antioxidants and can be used as a dietary supplement and medicinal raw material.

CONCLUSIONS

1. Prolonged drought contributed to a substantial decrease in the content of ascorbate, glutathione, sugars, total reducing activity and activity of antioxidant enzymes in the tissues of sweet cherry leaves, while the content of titrated acids increased substantially in both variants of the experiment.

2. The content of biologically active substances (ascorbate, glutathione, sugars, titrated acids and phenolic substances) in the leaves of sweet cherry trees gradually increased from the flowering phase to the defoliation phase. In the defoliation phase, the leaves of sweet cherry trees under mulching conditions had substantially more sugars (by 14%) in 2017 and ascorbate (by 17%) in 2019 compared to pure steam conditions. Over the years of research, under the condition of mulching, the total content of phenolic substances in sweet cherry leaves was 16-56% higher compared to the conditions of pure steam.

3. The content of malondialdehyde tended to increase under the conditions of mulching, and substantially increased in both variants of the experiment during the growing season. In the defoliation phase, a statistically substantial difference between the options was noted only in 2019, when the MDA content under mulching conditions was 14% higher compared to keeping the soil under pure steam.

4. The activity of antioxidant enzymes increased during the growing season in both versions of the experiment. Mulching increased the activity of antioxidant enzymes, but statistically significant differences compared to the conditions of pure steam were only for ascorbate peroxidase (by 28-30%) and polyphenol oxidase (by 45-46%) during all three years of research. During 2018 and 2019, the activity of peroxidase in sweet cherry leaves was substantially 2.4 times higher under the conditions of mulching with natural grasses compared to the conditions of pure steam.

5. The established regularities can be explained by the formation of stressful conditions of competition with natural herbs that activate the synthesis of anti-stress BASs.

6. The leaves of sweet cherry trees under the conditions of mulching are a powerful source of biologically active substances and antioxidants and can be used as a dietary supplement and medicinal raw material.

REFERENCES

[1] Afonso, S., Oliveira, I.V., Meyer, A.S., Aires, A., Saavedra, M.J., & Gonçalves, B. (2020). Phenolic profile and bioactive potential of stems and seed kernels of sweet cherry fruit. *Antioxidants*, 9(12), article number 1295. doi: 10.3390/antiox9121295.

[2] Anjum, N.A., Umar, S., & Chan, M.T. (2010). Ascorbate-glutathione pathway and stress tolerance in plants. Dordrecht: Science + Business Media B.V.

[3] Atucha, A., Merwin, I.A., & Brown, M.G. (2011). Long-term effects of four groundcover management systems in an apple orchard. *Horticulture Science*, 46(8), 1176-1183. doi: 10.21273/HORTSCI.46.8.1176.

[4] Balestrini, R. Chitarra, W., Antoniou, C., Ruocco, M., & Fotopoulos, V. (2018). Improvement of plant performance under water deficit with the employment of biological and chemical priming agents. *Journal of Agricultural Science*, 156, 680-688. doi: 10.1017/S0021859618000126.

[5] Bastos, C., Barros, L., Dueñas, M., Calhelha, R.C., Queiroz, M.J.R.P., Santos-Buelga, C. & Ferreira, I.C.F.R. (2015). Chemical characterisation and bioactive properties of *Prunus avium* L.: The widely studied fruits and the unexplored stems. *Food Chemistry*, 173, 1045-1053. doi: 10.1016/j.foodchem.2014.10.145.

[6] Bonyanpour, A.R., & Jamali, B. (2020). Seasonal enzymatic and nonenzymatic antioxidant responses in seven Iranian pomegranate cultivars. *Advances in Horticultural Science*, 34(3), 265-276. doi: 10.13128/ahsc8283.

[7] Cai, B., Wang, H., Liu, T., Zhuang, W., Wang, Z., Qu, S.C., & Qin, Y.L. (2019). Effects of gibberellins A4 on budbreak, antioxidant enzymes' activity and proline content of flower buds in sweet cherry (*Prunus avium*). *Acta Physiologiae Plantarum*, 41, article number 88. doi: 10.1007/s11738-019-2876-z.

[8] Cheynier, V., Comte, G., Davies, K.M., Lattanzio, V., & Martens, S. (2013). Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiology and Biochemistry*, 72, 1-20. doi: 10.1016/j.plaphy.2013.05.009.

[9] Costa, H., Gallego, S.M., & Tomaro, M.L. (2002). Effect of UV-B radiation on antioxidant defense system in sunflower cotyledons. *Plant Science*, 162(6), 939-945. doi: 10.1016/S0168-9452(02)00051-1.

[10] Djilas, S., Čanadanović-Brunet, J., & Ćetković, G. (2009). By-products of fruits processing as a source of phytochemicals. *Chemical Industry & Chemical Engineering Quarterly*, 15, 191-202. doi: 10.2298/CICEQ0904191D.

[11] Dziadek, K., Kopeć, A., & Czaplicki, S. (2018). The petioles and leaves of sweet cherry (*Prunus avium* L.) as a potential source of natural bioactive compounds. *European Food Research Technology*, 244(8), 1415-1426. doi: 10.1007/s00217-018-3055-y.

[12] Dziadek, K., Kopeć, A., & Tabaszewska, M. (2019). Potential of sweet cherry (*Prunus avium* L.) by-products: bioactive compounds and antioxidant activity of leaves and petioles. *European Food Research Technology*, 245, 763-772. doi: 10.1007/s00217-018-3198-x.

[13] Esitken, A. (2011). Use of plant growth promoting rhizobacteria in horticultural crops. In D.K. Maheshwari (Ed.), *Bacteria in agrobiology: Crop ecosystems* (pp.189-235). doi: 10.1007/978-3-642-18357-7_8.

[14] Frew, J.E., Jones, P., & Sholes, G. (1983). Spectrophotometric determination of hydrogen peroxide and organic hydropheroxides at low concentrations in aqueous solution. *Analytica Chimica Acta*, 155, 139-146. doi: 10.1016/S0003-2670(00)85587-7.

[15] Gerasko, T., Pyda, S., & Ivanova, I. (2021). Effect of living mulch on soil conditions and morphometrical indices of sweet cherry trees. *International Journal of Applied Agricultural Sciences*, 7(1), 50-56. doi: 10.11648/j.ijaas.20210701.14.

[16] Gest, N., Gautier, H., & Stevens, R. (2012). Ascorbate as seen through plant evolution: The rise of a successful molecule. *Journal of Experimental Botany*, 2012, 64(1), 33-53. doi: 10.1093/jxb/ers297.

[17] Gill, S.S., Anjum, N.A., Hasanuzzaman, M., Gill, R., Trivedi, D.K., Ahmad, I., Pereira, E., & Tuteja, N. (2013). Glutathione and glutathione reductase: A boon in disguise for plant abiotic stress defense operations. *Plant Physiology and Biochemistry*, 70, 204-212. doi: 10.1016/j.plaphy.2013.05.032.

[18] Gorodniy, M.M. (Ed). (2006). Applied biochemistry and quality management of crop products: Textbook, Kyiv: Aristei.

[19] Grebennikova, O.A. (2011). *Biologically active speech in fruits, leaves and processing products of promising varieties in alichi*. (Candidate thesis, Odesa I.I. Mechnikov National University, Odesa, Ukraine).

[20] Hallmann, E., & Sabała, P. (2020). Organic and conventional herbs quality reflected by their antioxidant compounds concentration. *Applied Science*, 10, article number 3468. doi: 10.3390/app10103468.

[21] Ivanova, I., Serdyuk, M., Kryvonos, I., Yeremenko, O., & Tymoshuk, T. (2020). Formation of flavoring qualities of sweet cherry fruits under the influence of weather factors. *Scientific Horizons*, 4(89), 72-81.

[22] Ivanova, I., Serdyuk, M., Malkina, V., Priss, O., Herasko, T., & Tymoshchuk, T. (2021). Investigation into sugars accumulation in sweet cherry fruits under abiotic factors effects. *Agronomy Research,* 19(2), 44-457. doi: 10.15159/ar.21.004.
[23] Ivanova, I., Serdyuk, M., Malkina, V., Tymoshchuk, T., Vorovka, M., Mrynskyi, I., & Adamovych, A. (2022). Studies of the impact of environmental conditions and varietal features of sweet cherry on the accumulation of vitamin C in fruits by using the regression analysis method. *Acta Agriculturae Slovenica*, 118(2), 1-12. doi: 10.14720/aas.2022.118.2.2404.
[24] Jesus, F., Gonçalves, A.C., Alves, G., & Silva, L.R. (2019). Exploring the phenolic profile, antioxidant, antidiabetic and anti-hemolytic potential of Prunus avium vegetal parts. *Food Research International*, 116, 600-610. doi: 10.1016/j.foodres.2018.08.079.
[25] Mateos-Fierro, Z., Fountain, M.T., Garratt, M.P.D., & Ashbrook, K. (2021). Active management of wildflower strips in commercial sweet cherry orchards enhances natural enemies and pest regulation services. *Agriculture, Ecosystems & Environment*, 317, article number 107485. doi: 10.1016/j.agee.2021.107485.
[26] Methods of qualification examination of plant varieties for suitability for distribution in Ukraine. Methods for determining the quality of crop products. (n.d.). Retrieved from http://www.minagro.gov.ua/.
[27] Oszmiański, J., Wojdyło, A., Lachowicz, S., Gorzelany, J., & Matłok, N. (2016). Comparison of bioactive potential of cranberry fruit and fruit-based products versus leaves. *Journal of Functional Foods*, 22, 232-242. doi: 10.1016/j.jff.2016.01.015.
[28] Pereira, S., Silva, V., Bacelar, E., Guedes, F., Silva, A.P., Ribeiro, C., & Gonçalves, B. (2020). Cracking in sweet cherry cultivars early bigi and lapins: Correlation with quality attributes. *Plants*, 9, article number 1557. doi: 10.3390/plants9111557.
[29] Picariello, G., De Vito, V., Ferranti, P., Paolucci, M., & Volpe, M.G. (2016). Species- and cultivardependent traits of Prunus avium and Prunus cerasus polyphenols. *Journal Food Composition and Analysis*, 40, 50-57.
[30] Polonskaya, A.K., Ezhov, V.N., Korniliev, G.V., & Grebennikova, O.A. (2007). Biologically active substances of the leaves of some fruit crops in connection with the prospect of their use in food. *Scientific Notes of the Tavrichesky National University named after Vernadsky. Series "Biology, Chemistry"*, 20(59), 122-127.
[31] Prvulović, D., Popović., M, Malenčić, Đ., & Ljubojevic, M. (2011). Phenolic compounds in sweet cherry (*Prunus avium* L.) petioles and their antioxidant properties. *Research Journal of Agricultural Science*, 43, 198-202
[32] Richter, A.A. (2001). *Improving the quality of fruits of southern cultures*. Simferopol: Tavria.
[33] Sharma, P., Jha, A., Dubey, R.S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, 2012, 1-26. doi: 10.1155/2012/217037.
[34] Szpadzik, E., Krupa, T., Wojciech, N., & Jadczuk-Tobjasz, E. (2019). Yielding and fruit quality of selected sweet cherry (Prunus avium) cultivars in the conditions of central Poland. *Acta Scientiarum Polonorum Hortorum Cultus*, 18(3), 117-126. doi: 10.24326/asphc.2019.3.11.
[35] Tabart, J., Kevers, C, Pincemail, J., Defraigne, J.-O., & Dommes, J. (2006). Antioxidant capacity of black currant varies with organs, season and cultivars. *Journal Agricultural Food Chemistry*, 54, 6271-6276. doi: 10.1021/jf061112y.
[36] Teleszko, M., & Wojdyło, A. (2015). Comparison of phenolic compounds and antioxidant potential between selected edible fruits and their leaves. *Journal of Functional Foods*, 14, 736-746. doi: 10.1016/j.jff.2015.02.041.
[37] Turrini, A., Caruso, G., Avio, L., & Gennai, C. (2017). Protective green cover enhances soil respiration and native mycorrhizal potential compared with soil tillage in a high-density olive orchard in a long term study. *Applied Soil Ecology*, 116, 70-78. doi: 10.1016/j.apsoil.2017.04.001.
[38] Uysal, S. (2020). Comparative antioxidant capacity and enzyme inhibitory effect of extracts from *Prunus avium* leaves. *Kastamonu University Journal of Forestry Faculty*, 20(3), 234-242. doi: 10.17475/kastorman.849538.
[39] Walker, R.P., Benincasa, P., Battistelli, A., Moscatello, S., Técsi, L., Leegood, R.C., & Famiani, F. (2018). Gluconeogenesis and nitrogen metabolism in maize. *Plant Physiology Biochemistry*, 130, 324-333. doi: 10.1016/j.plaphy.2018.07.009.
[40] Waterhouse, A.L. (2002). Polyphenolics: Determinaiton of total phenolics. In R.E. Wrolstad (Ed.), *Current protocols in food analytical chemistry* (pp. 1111-1118). New York: John Wiley & Sons.
[41] Yao, S.R., Merwin, I.A., Bird, G.W., Abawi, G.S., & Thies, J.E. (2005). Orchard floor management practices that maintain vegetative or biomass groundcover stimulate soil microbial activity and alter soil microbial community composition. *Plant Soil*, 271(1/2), 377-389. doi: 10.1007/s11104-004-3610-0.
[42] Yüksekkaya, Ş., Bülent, B., Sağlam, H., Pekmez, H., Cansu, Ü., Karaaslan, A., & Karaaslan, M. (2021). Valorization of fruit processing by-products: Free, esterified and insoluble bound phytochemical extraction from cherry (*Prunus avium*) tissues and their biological activities. *Journal of Food Measurement & Characterization*, 15(2), 1092-1107. doi: 10.1007/s11694-020-00698-5.

Біохімічний склад листків черешні залежно від способу утримання ґрунту в органічному саду

Тетяна Володимирівна Гераська[1], Світлана Василівна Пида[2], Юлія Петрівна Пащенко[1], Любов Анатоліївна Покопцева[1], Тетяна Миколаївна Тимощук[3]

[1]Таврійський державний агротехнологічний університет імені Дмитра Моторного
72312, просп. Б. Хмельницького, 18, м. Мелітополь, Україна

[2]Тернопільський національний педагогічний університет імені Володимира Гнатюка
46024, вул. Максима Кривоноса, 2, м. Тернопіль, Україна

[3]Поліський національний університет
10008, б-р Старий, 7, м. Житомир, Україна

Анотація. Ведення сталого сільського господарства передбачає не лише підвищення продуктивності культур і збільшення обсягів виробництва рослинницької продукції, але й збереження екосистем. Задерніння ґрунту у плодових садах є одним із шляхів збереження природної рівноваги агроландшафтів. Але вплив конкуренції з травами на біохімічний склад тканин плодових дерев наразі остаточно не з'ясований. Метою досліджень було встановити вплив утримання ґрунту під задерніннням (порівняно з чистим паром) на біохімічний склад тканин листків черешні. Дослідження проводили у органічному саду черешні (*Prunus avium* L./*Prunus mahaleb*) впродовж 2017–2019 рр. в умовах Південного Степу України. Встановлено істотне зменшення умісту аскорбату, глутатіону, цукрів, загальної редукуючої активності та активності антиоксидантних ферментів за умов тривалої посухи та збільшення умісту титрованих кислот. Загальна редукуюча активність за умов задерніння мала тенденцію до збільшення. У листках дерев черешні від фази цвітіння до листопаду зафіксовано поступове збільшення умісту титрованих кислот, аскорбату, цукрів, фенольних речовини і глутатіону. У фазі листопаду у листках черешні за умов задерніння встановлено достовірне збільшення у 2017 р. цукрів і фенольних речовин, у 2018 р. – фенольних речовин; у 2019 р. – фенольних речовин і аскорбату. Упродовж вегетації черешні на обох варіантах досліду встановлено збільшення умісту малонового діальдегіду (МДА) та активності антиоксидантних ферментів. У фазі листопаду лише у 2019 р. вміст МДА був достовірно більшим на 14 % за умови задерніння. За умов задерніння визначено достовірне збільшення аскорбатпероксидази (на 28–30 %) і поліфенолоксидази (на 45–46 %) У 2018 і 2019 р. встановлено збільшення у 2,4 рази активності пероксидази у листках черешні. Результати досліджень допомагають зрозуміти механізми адаптації плодових рослин до стресових факторів (посухи, конкуренції з природними травами) і можуть бути використані як аргумент на користь задерніння у органічних садах черешні

Ключові слова: задерніння, вітамін С, глутатіон, титровані кислоти, каталаза, аскорбатпероксидаза, поліфенолоксидази