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PHOTOSYNTHETIC ACTIVITY AND PROTECTIVE REACTIONS OF MOSSES IN FOREST ECOSYSTEMS OF THE UKRAINIAN ROZTOCHIA UNDER CHANGING ECOLOGICAL CONDITIONS

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Background. The photosynthetic activity of mosses has not been studied sufficiently, in contrast to vascular plants. Taking into account the specifics of the moss gametophyte organization (poikilohydricity, absence of epidermis, stomata, cuticle), it is relevant to perform a comparative analysis of photosynthetic activity and flavonoid-based antioxidant systems in endohydric and ectohydric moss species in relation to microecological gradients of moisture and temperature in forest ecosystems of the Ukrainian Roztochia.

Materials and Methods. The objects of the research were forest endohydric mosses *Polytrichum formosum* Hedw., *Atrichum undulatum* (Hedw.) P. Beauv. and ruderal ectohydric moss *Ceratodon purpureus* (Hedw.) Brid. from experimental plots of forest coenoses that differed in environmental conditions. The content of photosynthetic pigments, Rubisco activity, photosynthesis intensity, antioxidant activity and flavonoids content were determined using standard methods.

Results. Significant plasticity of the mosses' photosynthetic apparatus (chlorophylls and carotenoids content, Chl *a*/*b* ratio) was noted, which indicates an adaptation of bryophytes to changes in water and temperature regimes as well as light intensity. It was shown that the carboxylase activity of Rubisco is an indicator of the photosynthesis intensity of mosses and differs in endohydric and ectohydric species. Increasing antioxidant activity in moss cells relative to the environmental conditions was studied. The content of flavonoids and their absorption spectra in the moss shoots were studied.

Conclusions. Under unfavorable environmental conditions, an increase in the content of carotenoids in moss shoots, a decrease in the proportion of chlorophyll *a* in the total pool of chlorophylls to 52–56 %, as well as an increase in the proportion of chlorophyll *b*, was established. The higher intensity of photosynthesis in endohydric mosses *Atrichum undulatum* and *Polytrichum formosum* was ensured by a 25–53 % higher and more stable Rubisco activity, compared to the ectohydric moss *Ceratodon purpureus*. Under conditions of moisture deficiency, an increase in antioxidant activity by 27–30 % and in flavonoids content by 22–48 % in moss shoots was noted. In *Atrichum undulatum* gametophores, a 3.5–4.0 times higher antiradical activity was observed, indicating prospects for its further research. Analysis of absorption spectra of flavonoids extracts from *P. formosum* and *C. purpureus* showed the presence of flavonols and anthocyanins.

Keywords: photosynthesis, Rubisco, antioxidant activity, flavonoids, mosses, forest ecosystems

INTRODUCTION

Characteristics of the mosses' photosynthetic apparatus. Based on biomass and productivity, bryophytes are often dominant in plant communities in forest ecosystems, where their ecological role is well studied (Lobachevska *et al*., 2023; Siwach *et al*., 2021). However, the photosynthetic activity of bryophytes, in particular, the intensity of CO_2 assimilation, Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity, and their dependence on microclimatic conditions have not been sufficiently studied, compared to vascular plants (Antala *et al*., 2024; Coe *et al*., 2019). It is also known that Rubisco is one of the main limiting factors of photosynthesis and productivity of mosses; its activity largely depends on the conditions of plant water supply (Perera-Castro *et al*., 2021). Mosses, as poikilohydric plants, can lose water to the level of 5–10 % of the dry weight and the water potential in the cells can decrease to –100 MPa (Glime, 2019). It was shown that Rubisco inactivation under low cellular water potential is caused by proteolytic degradation of Rubisco activase as well as Rubisco large and small subunits, lack of ATP, a decreased ribulose-1,5-bisphosphate supply and low chloroplast CO_2 concentration (Perera-Castro *et al*., 2022).

Morpho-physiological features of mosses. The specific organization of the moss gametophyte (poikilohydricity, small size, single-layered leaf blade, absence of epidermis, stomata, cuticle) is also important (Glime, 2019). That is, cells in which photosynthesis occurs are not protected from increased insolation, dehydration and other negative factors, therefore, their existence in changing conditions can be ensured, largely, by the plasticity of the photosynthetic apparatus and the activity of antioxidant defense systems (Kyyak, 2022).

The role of secondary metabolites in the antioxidant defense. An effective indicator of the adaptation of bryophytes to changing environmental conditions is the antioxidant activity in cells, which is caused by non-enzymatic antioxidants, in particular, phenolic compounds (flavonoids, terpenes) (Sabovljević & Sabovljević, 2020). Some of these secondary metabolites obtained from moss extracts have demonstrated effective antibacterial activity *in vitro* and high antioxidant potential, in comparison with the known natural antioxidants, such as ascorbic acid and α-tocopherol (Cianciullo *et al*., 2022; Smolińska-Kondla *et al*., 2022).

In this regard, the aim of this study was to perform a comparative analysis of photosynthetic activity and flavonoid-based antioxidant systems in endohydric and ectohydric moss species in relation to microecological gradients of moisture and temperature in forest ecosystems of the Ukrainian Roztochia.

MATERIALS AND METHODS

The objects of research were epigeic bryophytes of forest ecosystems: forest endohydric mosses *Polytrichum formosum* Hedw., *Atrichum undulatum* (Hedw.) P. Beauv. and ruderal ectohydric moss *Ceratodon purpureus* (Hedw.) Brid. The research was conducted during June–August 2022–2024. In the work, we used freshly collected plant material that was selected from three localities on the territory of the Roztochia Nature Reserve and the Yavoriv National Nature Park, which differed in water, temperature regimes, and light intensity (**Table 1**):

Plot 1 – the territory of a complete reserve in the old-growth beech forest of the Vereshchytskyi Nature Conservation Research Department. A characteristic feature of old-growth beech forests is high shading due to well-developed mixed-age undergrowth of *Fagus sylvatica* L. and *Pinus sylvestris* L., which creates favorable conditions for the development of forest bryophytes, therefore, indicators from this site were used as a control;

Plot 2 – stationary recreation area „Vereshchytsia" in the Yavoriv National Nature Park (pine plantation with beech-hornbeam undergrowth);

Plot 3 – 40-year-old logging area of the Stradch Educational and Production Forestry Plant.

In the recreational zone and in the logging area less favorable microclimatic conditions were noted: an increase in the level of insolation to 82.0–110.0 thousand lux, an increase in air temperature above moss turfs by 14–43 % and, accordingly, a significant decrease in air humidity on the soil surface by 33–40 %, compared with the indicators from the experimental site in the old-growth forest (**Table 1**).

Table 1. **Characteristics of microclimatic conditions in the study areas in the forest ecosystems of the Ukrainian Roztochia (June-August 2022–2024)***

Experimental site	Light intensity, thousand lux	Air temperature on the soil surface, °C	Temperature in moss turf, °C	Air humidity on the soil surface, %
Plot 1. (old-growth forest)	22.5–45.0	24.5–25.8	18.5–21.2	38.2–42.5
Plot 2. (stationary recreation area)	82.0–95.0	26.5–29.5	22.5–24.0	26.6– 28.5
Plot 3. (logging area)	95.0–110.0	31.5–37.0	25.5–26.0	24.0 – 25.5

Note: * – the table presents the range of measured values; the measurement error did not exceed 15 %

The temperature and humidity of the air on the substrate surface were determined using the GM1361 thermohygrometer (BENETECH, China). The light intensity in the experimental plots was measured with a Yu-116 luxmeter ("Standard-M" LLC, Ukraine).

Research methods. To determine the content of photosynthetic pigments, a sample of fresh plant material (100–200 mg) was homogenized with the addition of

100 % acetone under conditions of maximum shading, after which it was centrifuged at 5000 rpm for 10 min (Opn-12 centrifuge, Kazakhstan). The optical density of the supernatant was measured on a Specord 210 Plus spectrophotometer (Analytic Jena, Germany) at wavelengths corresponding to the maximum absorption of chlorophylls – 662 nm, 644 nm, and carotenoids – 440 nm. The concentration of pigments was determined by the methods of G. Holm (1954) and D. Wettstein (1957).

Rubisco extraction and carboxylase activity measurements were performed using the method described by R. Lilley and D. Walker (Lilley & Walker, 1974) (with minor modifications). Principle of the method: the reaction of carboxylation was initiated by adding ribose-1,5-bisphosphate to the incubation medium as a CO_2 acceptor. The carboxylation reaction provides the synthesis of two molecules of triphosphoglyceric acid, which in subsequent reactions are reduced to triphosphoglyceric aldehyde using the energy products of the light stage of photosynthesis (ATP and NADPH). To ensure this reaction, to the incubation medium was added NADPH as a reaction cofactor and ATP. The enzyme activity was estimated by the oxidation of NADPH, which was followed by changes in absorbance at 340 nm using a Specord 210 Plus spectrophotometer (Analytic Jena, Germany). To obtain a plant extract 100–200 mg of plant material were homogenized in 2 mL of medium containing 0.05 M Tris–HCl buffer (pH 8.0), 1 mM $MgCl_2$, 0.1 mM EDTA, and 5 mM mercaptoethanol. The solutions were centrifuged for 20 min at 12,000 rpm (Opn-12 centrifuge, Kazakhstan). The incubation medium for determining Rubisco activity contained 0.05 M Tris-HCl buffer (pH 8.2), 10 mM $MgCl_2$, 10 mM of dithiothreitol (DTT), 50 mM $NaHCO_3$, 0.15 mM NADPH, and 0.3 mL of plant extract. The tubes with the incubation medium were incubated in a thermostat at 30 °C for 2 min. The reaction was started by adding 0.2 mL of a mixture of 1 mM ATP and 1 mM ribose-1,5-bisphosphate to each tube. An equal volume of 30 % acetic acid was added to the control sample. Samples were incubated for 4 min, after which the reaction was stopped by adding an equal volume of 30 % acetic acid. The optical density was measured on a spectrophotometer Specord 210 Plus (Analytic Jena, Germany) at a wavelength of 340 nm. The enzyme activity was expressed in μmol NADPH /mg protein/min (the NADPH absorption coefficient was 6.22 mM/cm). The content of total soluble protein was determined by the method of M. Bradford (Bradford, 1976).

The intensity of photosynthesis was determined by a chamberless method based on the accumulation of organic carbon in leaves (Nikolaichuk *et al*., 2000). The experiment was carried out on plants in field conditions. A portion of freshly collected plant material (50 mg) was put into test tubes with 0.4 N potassium dichromate solution and then boiled in a water bath for 20 min until the samples dissolved (burned). After 2 hours, the experiment was repeated. After cooling the test tubes, the contents were analyzed spectrophotometrically at λ = 590 nm on a Specord 210 Plus spectrophotometer (Analytic Jena, Germany). The intensity of photosynthesis was expressed in mg CO_2/g dry weight/h.

The antioxidant activity was assessed in the reaction of the plant extract with a radical solution – 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method of V. Brand-Williams (1995). Plant material (100 mg) was homogenized with 1 mL of 50 mM Tris-HCl buffer (pH 8.0), centrifuged for 15 min (13000 rpm, +4 °C) (Opn-12 centrifuge, Kazakhstan) and the supernatant was used for analysis. The DPPH solution (Sigma, USA) was prepared in 96 % ethyl alcohol (radical concentration – $6 \cdot 10^{-6}$ M). After that 2.8 mL of the radical solution and 0.2 mL of the plant extract were added to

the test tubes. The optical density of the mixture was determined on a Specord 210 Plus spectrophotometer (λ = 517 nm) (Analytic Jena, Germany). Antioxidant activity was expressed as a percentage of DPPH inhibition and, based on the calculated percentages of inhibition, a graph of the dependence of the DPPH staining inhibition values on the extract concentration was constructed. The extract concentration that caused 50 % inhibition of free radical staining (EC_{50}) was determined from the graph. A lower EC_{50} value indicated a higher antioxidant activity of plants. An aqueous solution of ascorbic acid (Sigma, USA) in the concentration range of 0.025–1.000 mg/mL was used as a positive control.

To determine flavonoids, 50 mg of crushed plant material was extracted with the addition of 2 mL of methanol for 24 hours. The homogenate was centrifuged at 5000 rpm for 10 min, then 1 mL of supernatant was added to 0.5 mL of 2 % $AlCl_3$ solution (Sigma, USA), 0.5 mL of 1 mol/L sodium acetate and 0.5 mL of distilled water (Pękal & Pyrzynska, 2014). The mixture was analyzed spectrophotometrically on a Specord 210 Plus spectrophotometer (Analytic Jena, Germany) at a wavelength of 425 nm. The flavonoids content was determined according to a calibration curve constructed using quercetin (Sigma, USA) and expressed in mg/g of dry matter.

The absorption spectra of flavonoids complexes with aluminum chloride were evaluated on a Specord 210 Plus spectrophotometer in the range of 400–700 nm with a step of 0.1 nm and compared with the absorption spectra of standard flavonoid solutions – rutin and quercetin (Sigma, USA). The concentrations of standard flavonoids solutions were 100 μM (Pękal & Pyrzynska, 2014).

All studies were conducted in a 4-fold replication. The results were statistically analyzed; the mean value, median and standard deviation were determined. The selections were compared using two-way ANOVA with post-hoc HSD Tukey test, considering differences between the selections significant at the level of $p < 0.05$, 0.01, 0.001 and 0.0001. Correlation analysis was used to assess the relationship between data sets. All calculations and developments of diagrams were made using OriginPro 2018 software.

RESULTS AND DISCUSSION

Forest moss species (*Atrichum undulatum*, *Polytrichum formosum*) are more confined to shaded localities, while the cosmopolitan, ruderal moss *Ceratodon purpureus* occurs in open, dry habitats. The quantitative composition of photosynthetic pigments in the mosses' shoots was investigated and a higher total chlorophyll content (3.12–4.07 mg/g dry weight) was detected in the samples of forest mosses *Polytrichum formosum* and *Atrichum undulatum*, which occur in shaded habitats (**Table 2**). In addition, these are endohydric mosses characterized by internal water conductivity that provides a higher and more stable moisture content in the gametophyte, which is also favorable for the functioning of the photosynthetic apparatus. For samples of these mosses from the recreation area and logging territory in conditions of a decline in water and temperature regimes, a decrease in the chlorophyll content by 9.4–35.2 % was noted. In *Ceratodon purpureus* shoots, which is confined to open habitats, a lower amount of chlorophyll (1.72–2.36 mg/g dry weight) was observed. These plants are more often found in areas with a high light intensity, which changes the quantitative composition of the photosynthetic apparatus. In this case, an increase in the proportion of carotenoids in the total pool of pigments was detected, which indicates their photoprotective function. Two-way ANOVA analysis of variance data on the content of chlorophylls *a* and *b*

in moss shoots revealed statistically significant effects of moss species ($F = 893.82$, $p < 0.0001$), experimental plot ($F = 92.49$, $p < 0.0001$), and interaction for species × experimental plot ($F = 12.91$, $p < 0.0001$).

Table 2. **Content of photosynthetic pigments (mg/g dry weight) in moss shoots from experimental plots in forest ecosystems of the Ukrainian Roztochia (June–August 2022–2024) (M±m; n=4)**

Moss sampling site	Content of pigments (mg/g dry weight)					
	Chl *a*	Chl *b*	Chl *a* + *b*	Carotenoids	Chl/C*	Chl *a*/*b*
Plot 1 (old-growth forest)						
Atrichum undulatum	2.73±0.05^{bbbb}	1.32±0.04^{bbbb}	4.05±0.04^{bbbb}	1.14±0.02 ^{bbbb}	3.55	2.07
Polytrichum formosum	2.57±0.0.05^{bbbb}	1.35±0.05^{bbbb}	3,92±0.05^{bbbb}	1.27±0.02	3.09	1.90
Ceratodon purpureus	1.55±0.02	0.79±0.01	2.34±0.03	1.35±0.03	1.73	1.96
Plot 2 (stationary recreation area)						
Atrichum undulatum	2.33±0.04$^{aaaa, bbbb}$	1.37±0.02^{bbbb}	3.70±0.05$^{aaaa, bbbb}$	2.07±0.04$^{aa, bbbb}$	1.79	1.70
Polytrichum formosum	2.30±0.05^{bbbb}	1.46±0.03^{bbbb}	3.76±0.08^{bbbb}	1.83±0.04$^{aaaa, bbbb}$	2.05	1.58
Ceratodon purpureus	1.20±0.01^{aaaa}	0.95±0.01^{aaaa}	2.15±0.02^{aaa}	1.57±0.02^{aaaa}	1.37	1.26
Plot 3 (logging area)						
Atrichum undulatum	2.06±0.04$^{aaaa, bbbb}$	1.68±0.03$^{aaaa, bbbb}$	3.74±0.07$^{aa, bbbb}$	1.87±0.04$^{aa, bbb}$	1.87	1.08
Polytrichum formosum	1.67±0.02$^{aaaa, bbb}$	1.37±0.08^{bbb}	3.04±0.08$^{aaaa, bbbb}$	1.68±0.04^{aaaa}	1.81	1.22
Ceratodon purpureus	0.96±0.01$^{aaaa, bbbb}$	0.78±0.02	1.74±0.03^{aaaa}	1.61±0.02^{aaaa}	1.08	1.23

Notes: * – Chl/C – ratio of the sum of chlorophylls to carotenoids.
^{aa} – the difference between samples of the same species is statistically significant compared to the indexes from plot 1 (control) at $p<0.01$; ^{aaa} – at $p<0.001$; ^{aaaa} – at $p<0.0001$.
^{bbb} – the difference between samples of different moss species within one experimental plot is statistically significant compared to samples of *Ceratodon purpureus* at $p<0.001$; ^{bbbb} – at $p<0.0001$

The ratio of chlorophylls to carotenoids (Chl/C) in the shoots of *Ceratodon purpureus* was 1.08–1.73, whereas for *Polytrichum formosum* and *Atrichum undulatum* from the experimental plots in the territory of complete preservation of old-growth forests, these indexes were higher (3.09–3.55). However, in the logging area with a higher level of insolation they decreased to 1.81–1.87, which also indicated an increase in the content of carotenoids in chloroplasts and their protective role. Two-way ANOVA analysis of variance data on the content of carotenoids in moss shoots revealed statistically significant effects of moss species ($F = 27.30$, $p < 0.0001$), experimental plot ($F = 284.91$, $p < 0.0001$) and interaction for species × experimental plot ($F = 35.04$, $p < 0.0001$).

In shaded areas in the old-growth forest, the ratio of chlorophyll *a*/*b* in the pigment complex of the studied mosses was within 1.90–2.07, which is similar to the parameters

of shade plants. Under unfavorable conditions of water and temperature regimes and high insolation in the recreation and logging areas, a decrease in the ratio of chlorophyll *a*/*b* to 1.08–1.70 was noted. Two-way ANOVA analysis of variance data on the content of chlorophyll *a* in moss shoots revealed statistically significant effects of moss species ($F = 902.19$, $p < 0.0001$), experimental plot ($F = 315.66$, $p < 0.0001$), and interaction for species × experimental plot ($F = 10.35$, $p < 0.0001$).

Literature data also confirms the low values of the chlorophyll *a/b* ratio in moss chloroplasts. For example, in 39 moss species from various habitats in southwest England, the average chlorophyll *a*/*b* ratio was 2.39±0.51, for liverworts – 1.98±0.30 (Marschall & Proctor, 2004). The increase in the proportion of chlorophyll *b* under stress conditions may be a manifestation of a compensatory adaptive reaction of the pigment apparatus to changes in the microclimatic conditions, since it is known that chlorophyll *b* in moss chloroplasts is less subject to destruction due to higher hydration and stronger bonds in chlorophyll-protein complexes (Glime, 2019; Hanson & Rice 2014). Two-way ANOVA analysis of variance data on the content of chlorophyll *b* in moss shoots revealed statistically significant effects of moss species ($F = 288.64$, $p < 0.0001$), experimental plot ($F = 11.01$, $p < 0.0001$) and interaction for species × experimental plot ($F = 15.42$, $p < 0.0001$).

The water status of photosynthetic cells and the temperature under constant lighting conditions determine the intensity of CO_2 assimilation. The efficiency of photosynthesis decreases under the influence of water deficit and high temperature stress, which is a consequence of lower cell conductivity for CO_2, inhibition of key photosynthetic enzymes and ATP synthases, deactivation of Rubisco and damage photosystem II due to the formation of excessive amounts of reactive oxygen species (Kiriziy & Stasyk, 2022; Zandalinas *et al*., 2018). In this regard, we investigated the carboxylase activity of Ribulose-1,6-bisphosphate carboxylase/oxygenase and its relationship with photosynthetic intensity in mosses from experimental plots under different water and temperature regimes. The enzyme activity in the shoots of the studied mosses *Polytrichum formosum*, *Atrichum undulatum*, and *Ceratodon purpureus* was in a wide range of 19.9–60.1 μmol NADPH/mg protein/min, which depended on the local conditions and the moss species characteristics. In the old-growth beech forest under more favorable and stable water supply conditions, when the moisture content in the moss turf was 56.2–70.4 %, carboxylase activity was higher (43.8–60.1 μmol NADPH/mg protein/min), and *Atrichum undulatum* plants were distinguished by a greater enzymatic activity (**Fig. 1**). Significant differences were determined in mosses from the experimental plots in the recreation and logging areas, where an unfavorable hydrothermal regime was recorded in the summer months (see **Table 1**). Under such conditions, the turfs of endohydric mosses *Polytrichum formosum* and *Atrichum undulatum* lost less moisture (the relative moisture content in the turfs was 42.4–55.0 %), which led to a partial decrease in the carboxylase activity of Rubisco by 20–26 %. For the ectohydric species *Ceratodon purpureus*, a decrease in enzymatic activity was almost two-fold (to 19.9 μmol NADPH/mg protein/min) in the recreational zone and in the logging area, which could be due to a moisture deficit in the moss turfs (the relative moisture content in the plants was 24.7–28.4 %). Two-way ANOVA analysis of variance of data on Rubisco carboxylase activity in moss shoots revealed statistically significant effects of moss species ($F = 98.19$, $p < 0.0001$), experimental plot ($F = 99.77$, $p < 0.0001$) and interaction for species × experimental plot ($F = 11.20$, $p < 0.0001$).

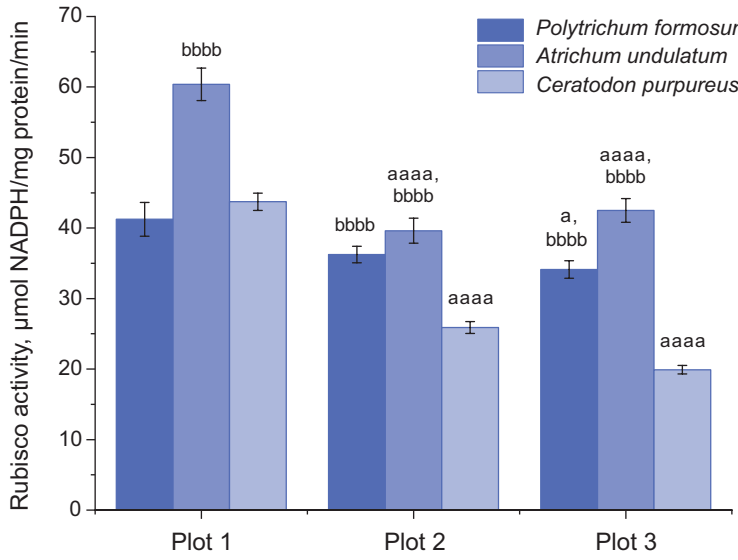

Fig. 1. Carboxylase activity of Rubisco in shoots of mosses *Polytrichum formosum*, *Atrichum undulatum*, and *Ceratodon purpureus* from experimental plots of forest ecosystems of the Ukrainian Roztochia: plot 1 – old-growth forest (control), plot 2 – recreation area; plot 3 – logging area (M±m; n = 4).

[a] – the difference between samples is statistically significant compared to the indexes from plot 1 (control) at p <0.05; [aaaa] – at p <0.0001.

[bbbb] – the difference between samples of different moss species within one experimental plot is statistically significant compared to samples of *Ceratodon purpureus* at p <0.0001

Differences in the Rubisco carboxylase activity in the mosses' gametophyte also affected the intensity of plant photosynthesis. The assimilation rates of CO_2 in moss shoots were the highest in the old-growth beech forest. For the endohydric mosses *Polytrichum formosum* and *Atrichum undulatum*, photosynthetic activity was noted at the level of 4.23–4.74 mg CO_2/g dry weight/h, for *Ceratodon purpureus* – 2.6±0.09 mg CO_2/g dry weight/h (**Fig. 2**).

In areas with unfavorable water regime, the photosynthetic activity of mosses decreased by almost 1.5–2.0 times. More significant changes were detected in samples of *Ceratodon purpureus*. Two-way ANOVA analysis of variance of data on photosynthesis intensity in moss shoots revealed statistically significant effects of moss species ($F = 209.68$, $p < 0.0001$), experimental plot ($F = 182.30$, $p<0.0001$) and interaction for species × experimental plot ($F = 8.42$, $p < 0.001$). A high correlation ($r = 0.88$) was noted between the Rubisco activity and the photosynthesis intensity in mosses (**Fig. 3**).

Therefore, Rubisco is an indicator of the photosynthetic intensity in mosses of forest ecosystems and depends on the characteristics of their water regime. The carboxylase activity of Rubisco in moss shoots was studied relative to microclimatic conditions in forest ecosystems. Differences between endohydric and ectohydric species were established: endohydric mosses *P. formosum* and *A. undulatum*, which better retain moisture in the gametophyte, were characterized by 25–53 % higher and more stable indexes of Rubisco activity compared to the ectohydric moss *C. purpureus*, especially in the logging area with the least favorable conditions of moisture and temperature.

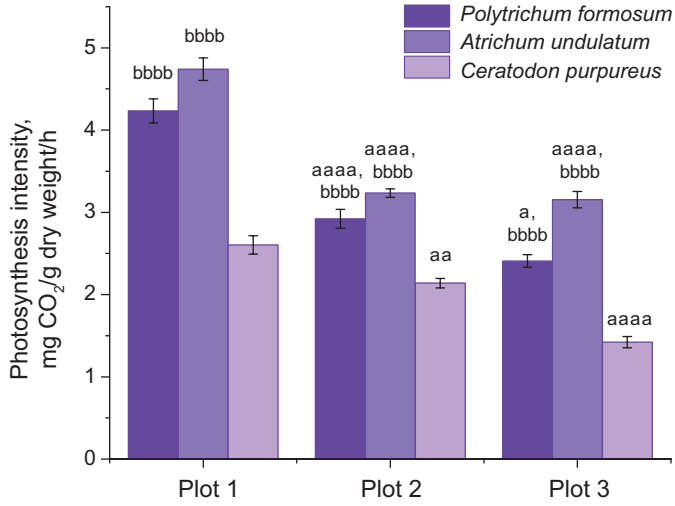

Fig. 2. Photosynthesis intensity in shoots of mosses *Polytrichum formosum*, *Atrichum undulatum*, and *Ceratodon purpureus* from experimental plots of forest ecosystems of the Ukrainian Roztochia: plot 1 – old-growth forest (control), plot 2 – recreation area; plot 3 – logging area (M±m; n = 4).

[a] – the difference between samples is statistically significant compared to the indexes from plot 1 (control) at p <0.05; [aa] – at p <0.01; [aaa] – at p <0.001; [aaaa] – at p <0.0001.

[bbbb] – the difference between samples of different moss species within one experimental plot is statistically significant compared to samples of *Ceratodon purpureus* at p <0.0001

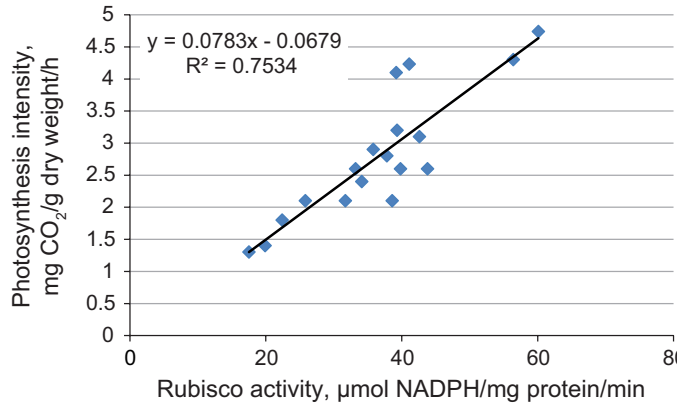

Fig. 3. Relationship between Rubisco activity and intensity of photosynthesis in shoots of mosses *Polytrichum formosum*, *Atrichum undulatum*, and *Ceratodon purpureus* from experimental plots of forest ecosystems of the Ukrainian Roztochia (r = 0.85)

A decrease in the intensity of photosynthesis under unfavorable microclimatic conditions is largely caused by the oxidative stress. Dehydration, high temperature, as well as high insolation enhance photooxidative stress and, accordingly, excessive production of reactive oxygen species in chloroplasts, thereby triggering the activation of antioxidant defense systems as a compensatory mechanism. Non-enzymatic antioxidants (phenolic compounds, ascorbate, glutathione) play an important role in protecting bryophyte cells from oxidative damage (Kyyak, 2022; Smolińska-Kondla *et al*., 2022). The main group of phenols in bryophyte cells is flavonoids (Davies *et al*., 2020).

It was found that the antioxidant activity (EC_{50}) in the studied mosses was in the range of 0.25–1.38 mg/mL (**Table 3**), which indicated a high antiradical activity in bryophyte cells (in comparison, the EC_{50} of ascorbic acid was 0.06 mg/mL). For *Polytrichum formosum* and *Ceratodon purpureus* plants, an increase in antioxidant activity by 27–30 % was recorded in plant samples from the logging area, where microclimatic conditions are the least favorable. It was determined that there is 3.5–4.0-fold higher antioxidant activity in the shoots of *Atrichum undulatum*, which indicates a significant antioxidant potential of this moss species. Two-way ANOVA analysis of variance of antioxidant activity data in moss shoots revealed statistically significant effects of moss species ($F = 1290.78$, $p < 0.0001$), experimental site ($F = 116.90$, $p < 0.0001$) and interaction for species × experimental site ($F = 39.55$, $p < 0.0001$).

Table. 3. **Antioxidant activity and flavonoid content in the shoots of mosses *Polytrichum formosum*, *Atrichum undulatum* and *Ceratodon purpureus* from the experimental plots in forest ecosystems of the Ukrainian Roztochia (**M±m; n = 4**)**

Moss sampling site	Antioxidant activity, EC_{50}, mg/mL	Flavonoids content, mg/g dry weight
Plot 1 (old-growth forest)		
Atrichum undulatum	0.28 ± 0.003^{bbbb}	2.12 ± 0.06^{bb}
Polytrichum formosum	1.38 ± 0.03^{bbbb}	1.74 ± 0.04^{bbbb}
Ceratodon purpureus	1.16±0.03	2.35±0.05
Plot 2 (stationary recreation area)		
Atrichum undulatum	0.29 ± 0.01^{bbbb}	2.62 ± 0.09^{aaa}
Polytrichum formosum	$1.22\pm0.03^{aa,\ b}$	$2.14\pm0.04^{aaaa,\ bbbb}$
Ceratodon purpureus	$1.32\pm0.03^{\ aa}$	2.73 ± 0.03^{aaaa}
Plot 3 (logging area)		
Atrichum undulatum	$0.25\pm0.003^{aaa,\ bbbb}$	$3.13\pm0.06^{aaaa,\ b}$
Polytrichum formosum	$1.02\pm0.03^{aaaa,\ bbbb}$	$2.44\pm0.06^{aaaa,\ bbbb}$
Ceratodon purpureus	0.76 ± 0.02^{aaaa}	2.88 ± 0.05^{aaaa}

Notes: ^{aa} – the difference between samples is statistically significant compared to the indexes from plot 1 (control) at $p < 0.01$; ^{aaa} – at $p < 0.001$; ^{aaaa} – at $p < 0.0001$.
^{b} – the difference between samples of different moss species within one experimental plot is statistically significant compared to samples of *Ceratodon purpureus* at $p < 0.05$; ^{bb} – at $p < 0.01$; ^{bbb} – at $p < 0.001$; ^{bbbb} – at $p < 0.0001$

The study of the flavonoids content in shoots of *Polytrichum formosum*, *Atrichum undulatum*, and *Ceratodon purpureus* also showed that unfavorable water and temperature regimes in the recreation area and in the logging territory initiated an increase in the content of flavonoids in moss samples by 1.2–1.5 times (**Table 3**). The highest concentrations of these metabolites were found in the gametophores of *Atrichum undulatum* and *Ceratodon purpureus*. Two-way ANOVA analysis of variance of flavonoids content data in moss shoots revealed statistically significant effects of moss species ($F = 94.86$, $p < 0.0001$), experimental site ($F = 140.71$, $p < 0.0001$), and interaction for species × experimental site ($F = 5.39$, $p < 0.001$).

To determine which flavonoids are involved in the protective responses of the studied mosses, the absorption spectra of flavonoids extracts complexed with aluminum chloride

were analyzed. The similarity of the spectra in the samples of *Ceratodon purpureus* and *Polytrichum formosum* was established (**Fig. 4**). In samples of these mosses, 2 absorption maxima were determined in the spectral region of 425–460 nm and a peak in the long-wave region (675 nm). It should be noted that the interaction of flavonoids with aluminum chloride leads to a bathochromic shift of the absorption maxima of the initial reagents by 66–67 nm (Pékal & Pyrzynska, 2014). Therefore, the first maximum of the absorption spectrum can obviously correspond to the flavonols rutin or quercetin, since their absorption peaks are in the spectral region of 356 nm and 370 nm (Pékal & Pyrzynska, 2014), respectively, and their complexes with $AlCl_3$ – 422 nm and 436 nm (**Fig. 4*A*,*B*,*D***). In the gametophyte of other moss species (*Entosthodon hungaricus* (Boros) Loeske, *Hennediella heimii* (Hedw.) R. H. Zander, *Physcomitrium patens* (Hedw.) Mitt.), a significant increase in the content of the flavonols rutin and quercetin under salt stress conditions was also found (Ćosić *et al*., 2023). It is known that these flavonols are the most effective superoxide radical reducers among flavonoids (Davies *et al*., 2020). Peaks in the long-wavelength region of the spectrum may correspond to anthocyanins, the antioxidant properties of which are conditioned by high donor activity and the ability to stabilize an unpaired electron, which stops chain-free radical reactions (Ahmad *et al*., 2010).

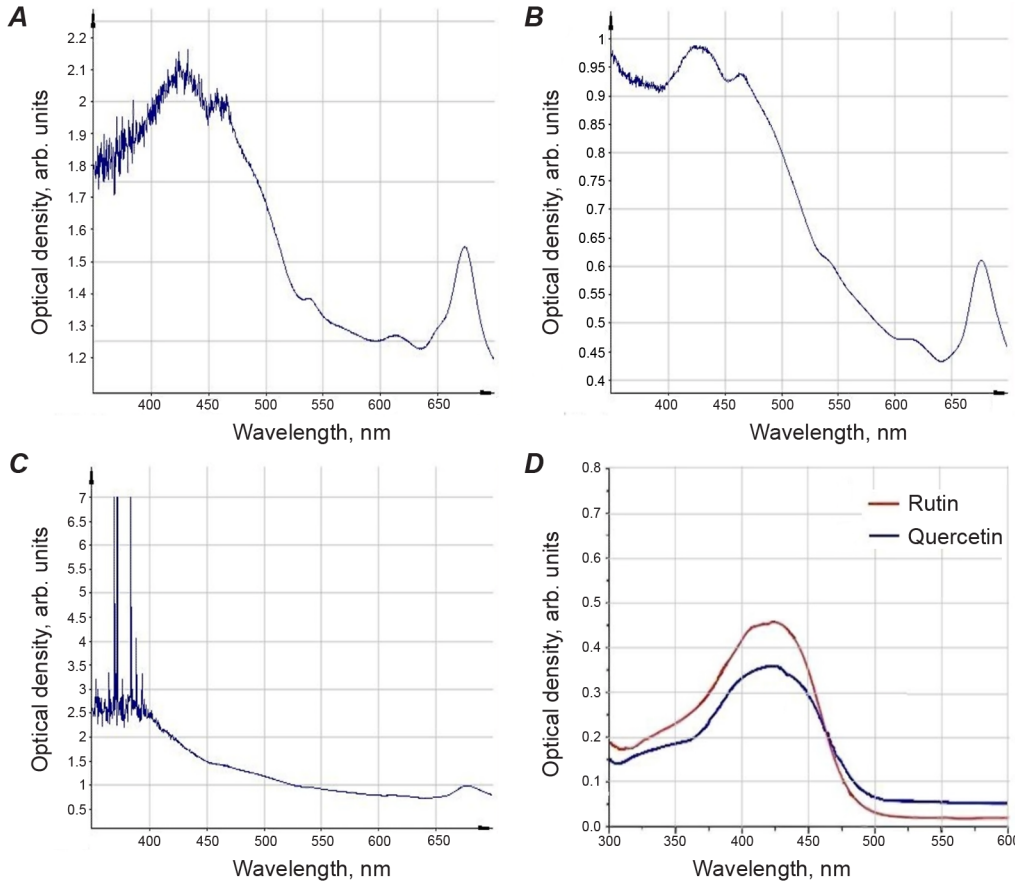

Fig. 4. Absorption spectra of flavonoid complexes with aluminum chloride from extracts of moss shoots of *Ceratodon purpureus* (***A***), *Polytrichum formosum* (***B***), *Atrichum undulatum* (***C***) from the experimental plot in the logging area; ***D*** – absorption spectra of rutin and quercetin in aluminum chloride solution

In *Atrichum undulatum* samples, the absorption spectra are more shifted to the UV region of the spectrum (**Fig. 4*C***), since the absorption maxima of flavonoids complexed with $AlCl_3$ were recorded in the region of 375–390 nm, which may correspond to flavones (Pékal & Pyrzynska, 2014) (these results still require further research). One peak in the long-wavelength region of the spectrum (650 nm) was also found, which may correspond to anthocyanins. The presence of flavones in the moss shoots of *Atrichum undulatum*, *Polytrichum formosum*, *Rhytidiadelphus squarrosus* (Hedw.) Warnst. has been confirmed by other authors (Wolski *et al*., 2021).

Thus, the spectral composition of flavonoids in bryophytes of forest ecosystems indicates their involvement in protecting cells from free radical processes caused by adverse microclimatic conditions.

CONCLUSIONS

A significant plasticity of the photosynthetic apparatus of mosses (chlorophylls and carotenoids content, Chl *a*/*b* ratio) was noted, which indicates the adaptation of bryophytes to changes in water and temperature regimes and light intensity. Forest endohydric mosses *Atrichum undulatum* and *Polytrichum formosum* were characterized by a higher chlorophyll content and a lower proportion of carotenoids in the total pigment pool (Chl/C ratio 3.05–3.57) compared with the ruderal moss *Ceratodon purpureus*. Under unfavorable conditions, an increase in the amount of carotenoids, a decrease in the proportion of chlorophyll *a* in the total chlorophyll pool to 52–56 % and an increase in the proportion of chlorophyll *b* were found.

The carboxylase activity of ribulose-1,6-bisphosphate carboxylase/oxygenase is an indicator of the intensity of photosynthesis in mosses and depends on the characteristics of their water regime. The higher activity of CO_2 assimilation in endohydric mosses *Atrichum undulatum* and *Polytrichum formosum* was ensured by a 25–53 % higher and more stable Rubisco activity, compared with the ectohydric moss *Ceratodon purpureus*.

In the experimental plots of the logging area with less favorable temperature and water regimes and high insolation, an increase in antioxidant activity in moss shoots was noted, which provided intracellular protection against free radical damage. A 3.5–4.0 times higher antiradical activity was detected in the shoots of moss *Atrichum undulatum*, which indicates a significant antioxidant potential of this moss species and prospects for its further research.

Under unfavorable ecological conditions, an increase in the content of flavonoids by 22–48 % was determined in moss shoots, compared with plants from the territory of the old-growth beech forest. Analysis of the absorption spectra of flavonoids in shoots of *Ceratodon purpureus* and *Polytrichum formosum* showed the presence of flavonols rutin and quercetin, and anthocyanins. In *Atrichum undulatum* samples, the absorption spectra of flavonoids are more shifted to the UV region of the spectrum, which, obviously, may correspond to flavones and requires more detailed studies.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Rights: this article does not include animal studies.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

AUTHOR CONTRIBUTIONS

Conceptualization, [N.K.; O.T.]; methodology [N.K.; O.B.]; formal analysis [N.K.; O.T.]; investigation [N.K., O.T., O.B., R.S.]; resources [N.K., O.T., O.B.]; data curation [N.K.; O.T.; R.S.] writing – original draft preparation [N.K.]; writing review and editing [N.K.; O.T.; O.B.; R.S.]; visualization [N.K.; R.S.]; supervision [N.K.]; project administration [N.K.; O.T.]; funding acquisition [-].

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ФОТОСИНТЕТИЧНА АКТИВНІСТЬ І ЗАХИСНІ РЕАКЦІЇ МОХІВ У ЛІСОВИХ ЕКОСИСТЕМАХ УКРАЇНСЬКОГО РОЗТОЧЧЯ В МІНЛИВИХ ЕКОЛОГІЧНИХ УМОВАХ

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Обґрунтування. Фотосинтетична активність мохів вивчена недостатньо, порівняно зі судинними рослинами. Беручи до уваги специфіку організації гаметофіту мохів (пойкілогідричність, відсутність епідермісу, продихів, кутикули), актуальним є порівняльний аналіз фотосинтетичної активності й участі неензиматичних антиоксидантів – флавоноїдів у захисних реакціях ектогідричних та ендогідричних мохів лісових екосистем Українського Розточчя в мінливих екологічних умовах.

Матеріали і методи. Об'єктами досліджень були лісові ендогідричні мохи *Polytrichum formosum* Hedw., *Atrichum undulatum* (Hedw.) P. Beauv. та рудеральний ектогідричний мох *Ceratodon purpureus* (Hedw.) Brid. із дослідних ділянок лісових ценозів, що відрізнялися за екологічними умовами: вміст фотосинтетичних пігментів, активність Рубіско, інтенсивність фотосинтезу, антиоксидантну активність і вміст флавоноїдів визначали за стандартними методиками.

Результати. Відзначено значну пластичність фотосинтетичного апарату мохів (вмісту хлорофілів і каротиноїдів, співвідношення Хл *a*/*b*), що свідчить про адаптацію бріофітів до зміни водного й температурного режиму та інтенсивності світла. З'ясовано, що карбоксилазна активність Рубіско є індикатором інтенсивності фотосинтезу мохів і відрізняється в ендогідричних і ектогідричних видів. Виявлено підвищення антиоксидантної активності у клітинах мохів залежно від екологічних умов. Досліджено вміст флавоноїдів і їхні спектри поглинання у пагонах мохів залежно від умов місцевиростань.

Висновки. У несприятливих екологічних умовах у пагонах мохів виявлено зростання вмісту каротиноїдів, зменшення частки хлорофілу a в сумарному пулі хлорофілів до 52–56 % та збільшення частки хлорофілу *b*. Вища інтенсивність фотосинтезу в ендогідричних мохів *Atrichum undulatum* і *Polytrichum formosum* забезпечувалася більшими на 25–53 % та стабільнішими показниками активності Рубіско, порівняно з ектогідричним мохом C*eratodon purpureus*.

В умовах дефіциту вологи у пагонах мохів відзначено зростання антиоксидантної активності на 27–30 % та збільшення вмісту флавоноїдів на 22–48 %. У гаметофорах *Atrichum undulatum* визначено в 3,5–4,0 рази вищі показники антирадикальної активності, що вказує на перспективність його подальших досліджень. Аналіз спектрів поглинання екстрактів флавоноїдів у *P. formosum* і *C. purpureus* показав наявність флавонолів і антоціанів.

Ключові слова: фотосинтез, Рубіско, антиоксидантна активність, флавоноїди, мохи, лісові екосистеми

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