



A FOUR YEAR STUDY OF THE PIGMENT CONTENT ON WILD-GROWING PLANTS AT MOUSSALA PEAK

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- Moussala Peak (2925 m a.s.l.) - the highest peak of Rila Mountain (Bulgaria) and Balkan Peninsula; characterized by specific microclimate conditions.
- At this altitude, growing plants are exposed to the impact of more than one environmental factor: prolonged UV irradiation, low temperatures, high wind velocity, etc.
- With the increase of the altitude alpine conditions extremely varied.











Aim

This study aims to assess whether alterations in the pigment content of wild-growing species at alpine conditions exist for four years.

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Materials and Methods

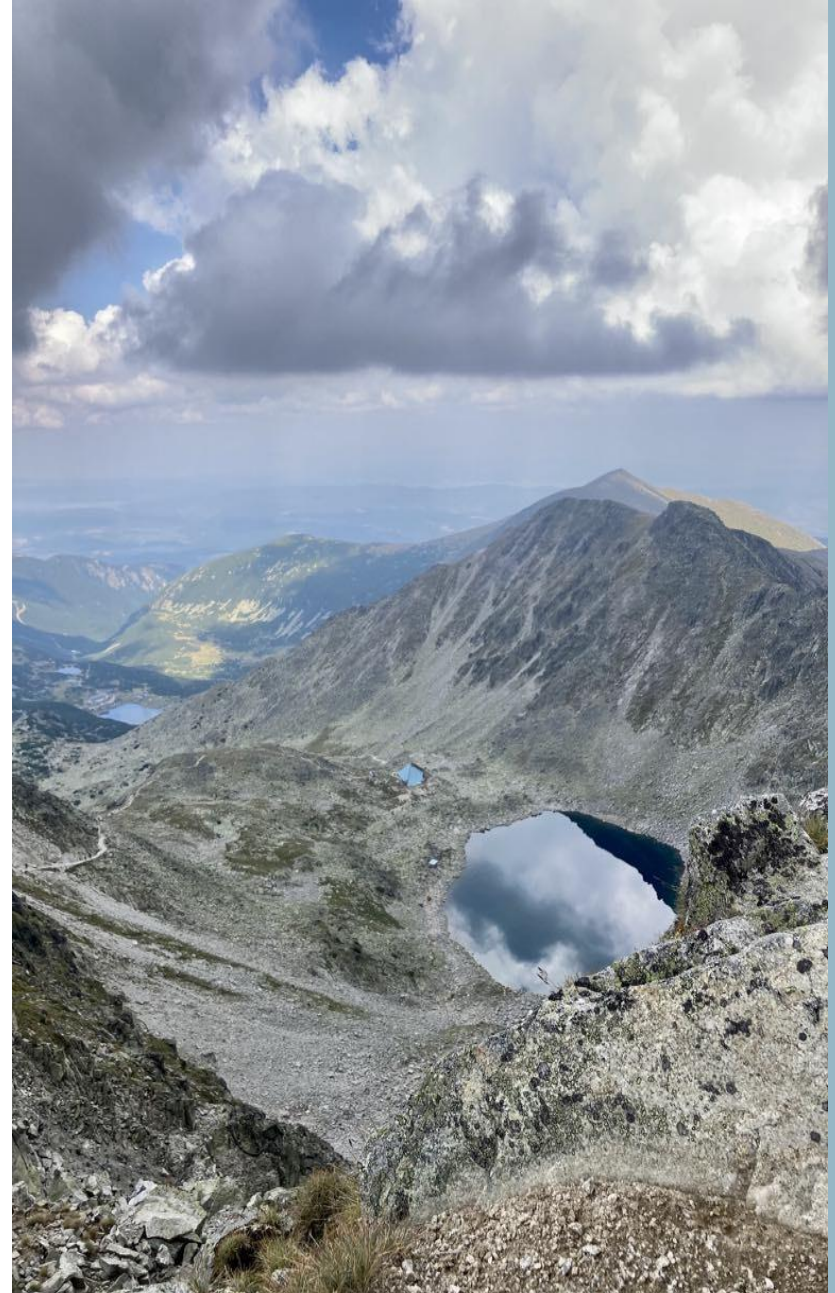
Plant material: wild-growing plants, collected in the growing seasons of 2020, 2021, 2022 and 2023 year, during July/August.

Species	Plant family	Foto
<i>Saxifraga cymosa</i> Waldst & Kit	Saxifragaceae	
<i>Anthemis carpatica</i> Waldst. & Kit. ex Willd.	Asteraceae	
<i>Geum repens</i> L.	Rosaceae	
<i>Doronicum columnae</i> Ten.	Asteraceae	
<i>Achillea clusiana</i> L.	Asteraceae	
<i>Armeria alpina</i> L.	Plumbaginaceae	
<i>Festuca valida</i> (R.Uechtr.) Péntzes	Poaceae	
<i>Sesleria coerulans</i> Friv.	Poaceae	
<i>Pedicularis ornantha</i> Griseb.	Scrophulariaceae	
<i>Campanula alpina</i> Jacq.	Campanulaceae	

Experimental site (alpine conditions):

Moussala Peak

- 2925 m a.s.l.;
- $42^{\circ}10'45.13''$ N; $23^{\circ}35'06.81''$ E coordinates;
- alpine type vegetation:
 - a suite of azonal scree and rock vegetation, calcareous and acid grasslands, sedge heaths, snowbeds, soligenous mires, and spring vegetation;
 - above the tree line, the most important habitats are grasslands (snow remains for more than 210 days);
 - wild-growing species here can survive in poor soil, lower temperature and short summer;
- mountain-meadow alpine soil type (pH around 4.5).



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Radiation conditions and UV measurements at the experimental site:

- Data for the average daily value of “Erythemat UV irradiance” response (UVE) for experimental site Moussala Peak is from 2020 to 2023 (kWh/m²).
- Average daily values for July/August (during the days when plants are collected) are calculated.
- The UVE response is measured by a UV sensor and defined by ISO/CIE 17166:2019.
- UVE is typically made up of about 17% UVA and 83% UVB for a clear sky around solar noon.
- The UVA/UVB sensor can typically measure the daily total of UVE irradiance with an uncertainty of 5%.

Endpoint: Photosynthetic pigment content (total chlorophylls, chlorophyll *a*, chlorophyll *b*, total carotenoids and chlorophyll *a/b* ratio) (Arnon, 1949).

Statistical data analysis: One-way ANOVA with Tukey multiple comparison tests (GraphPad Prism 6.04 software, San Diego, USA) were used to assess differences among samples.

Results

Daily UVE doses

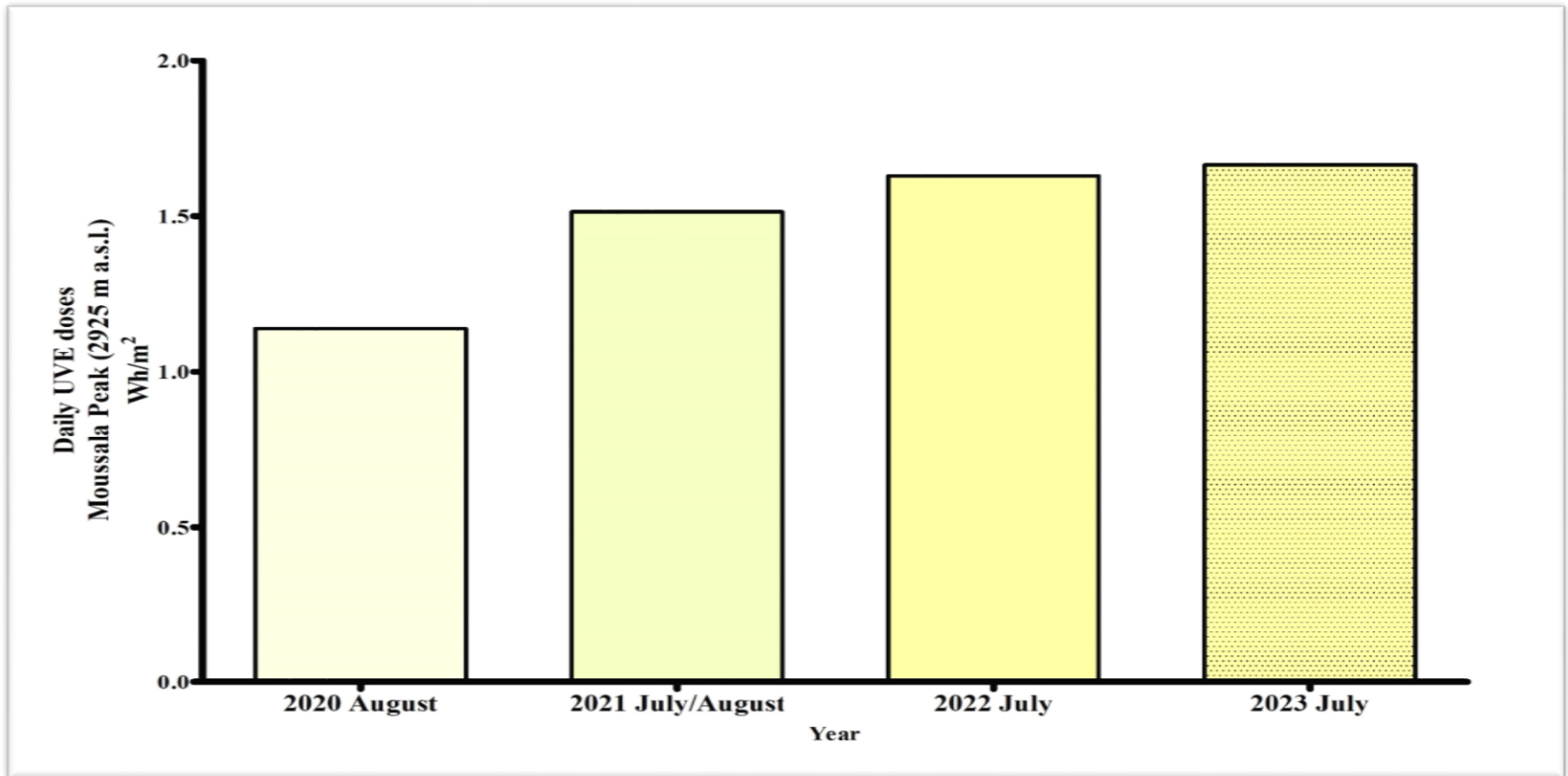


Figure 1. Daily UVE doses measured on Moussala Peak for days of July/August from 2020 to 2023, when plants were collected (Wh/m²).

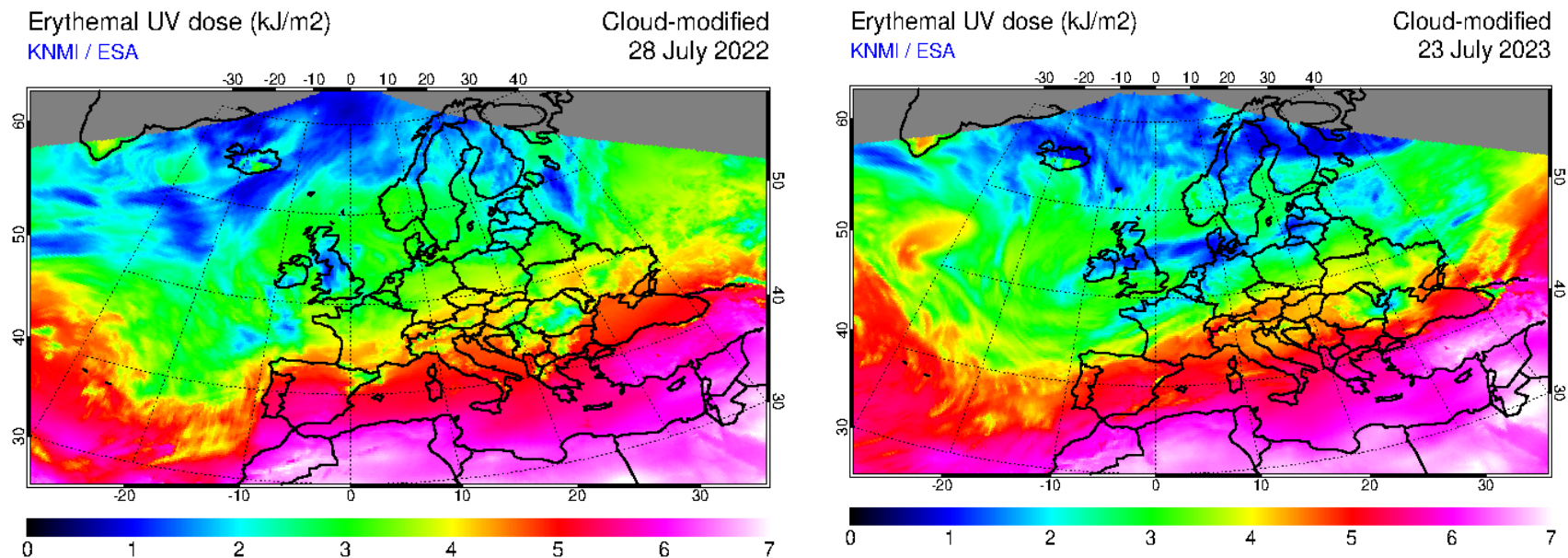


Figure 2. Erythemat UV dose measured on Moussala Peak for days of July from 2022 and 2023 years, when plants were collected (kJ/m²).

Chlorophyll *a* and chlorophyll *b* content

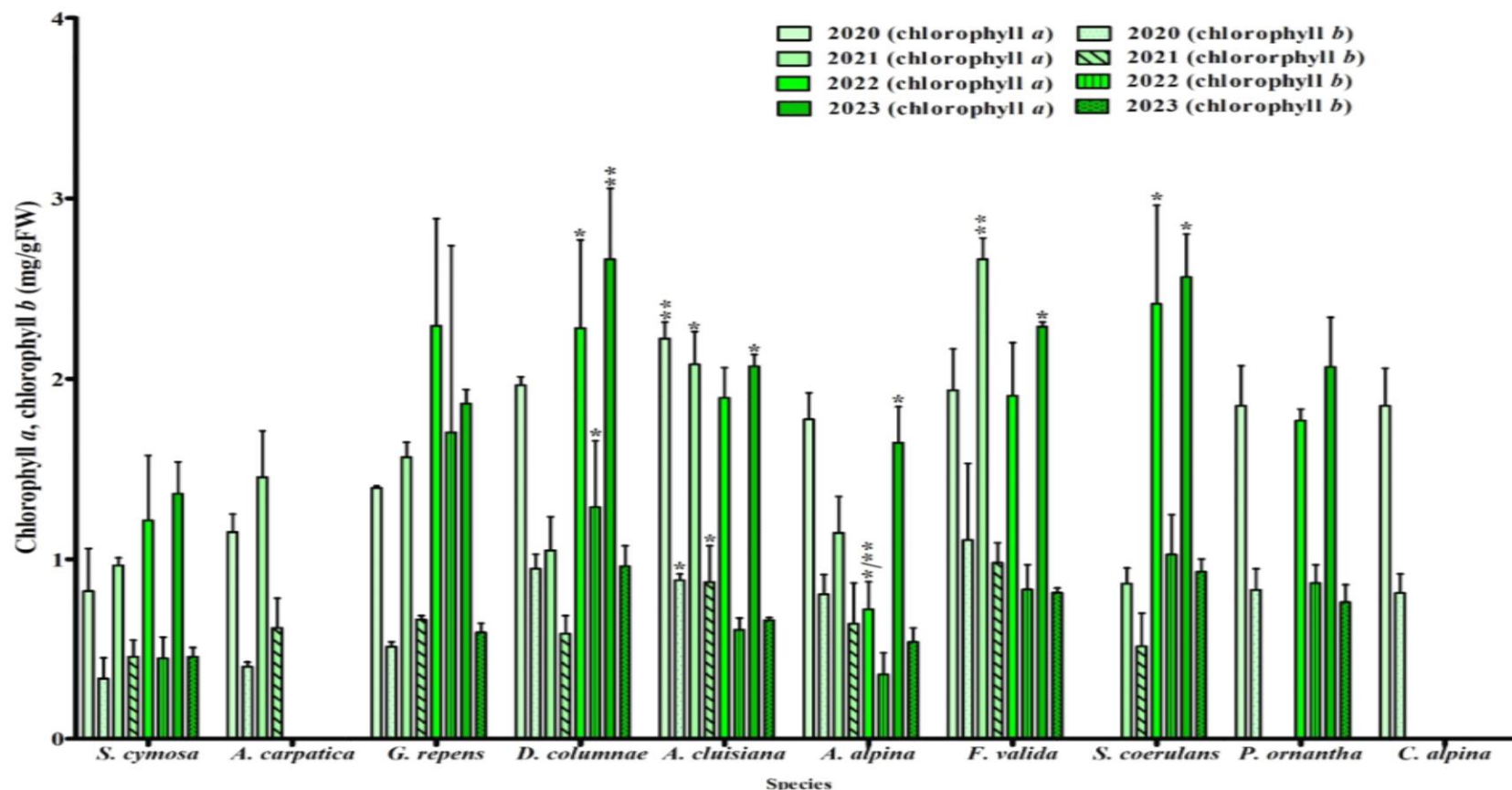


Figure 3. Chlorophyll *a* and chlorophyll *b* content. Statistically significant differences between: control vs. year (* $p < 0.05$, ** $p < 0.01$) for chl. *a* of *A. clusiana*, *A. alpina*, *F. valida* and for chl. *b* of *D. columnae*, *A. clusiana*; 2021 vs. 2022 (* $p < 0.05$), 2021 vs. 2023 (** $p < 0.01$)- chl. *a* of *D. columnae* (after the slash); 2020 vs. 2022 (** $p < 0.01$), 2022 vs. 2023 (* $p < 0.05$) – chl. *a* of *A. alpina* (after the slash); 2021 vs. 2022 (* $p < 0.05$), 2021 vs. 2023 (* $p < 0.05$) – chl. *a* of *S. coerulans*. Model plant *Hordeum vulgare* L. (Poaceae) (not presented in the figure), grown at laboratory conditions is used as a control.

Total chlorophylls content

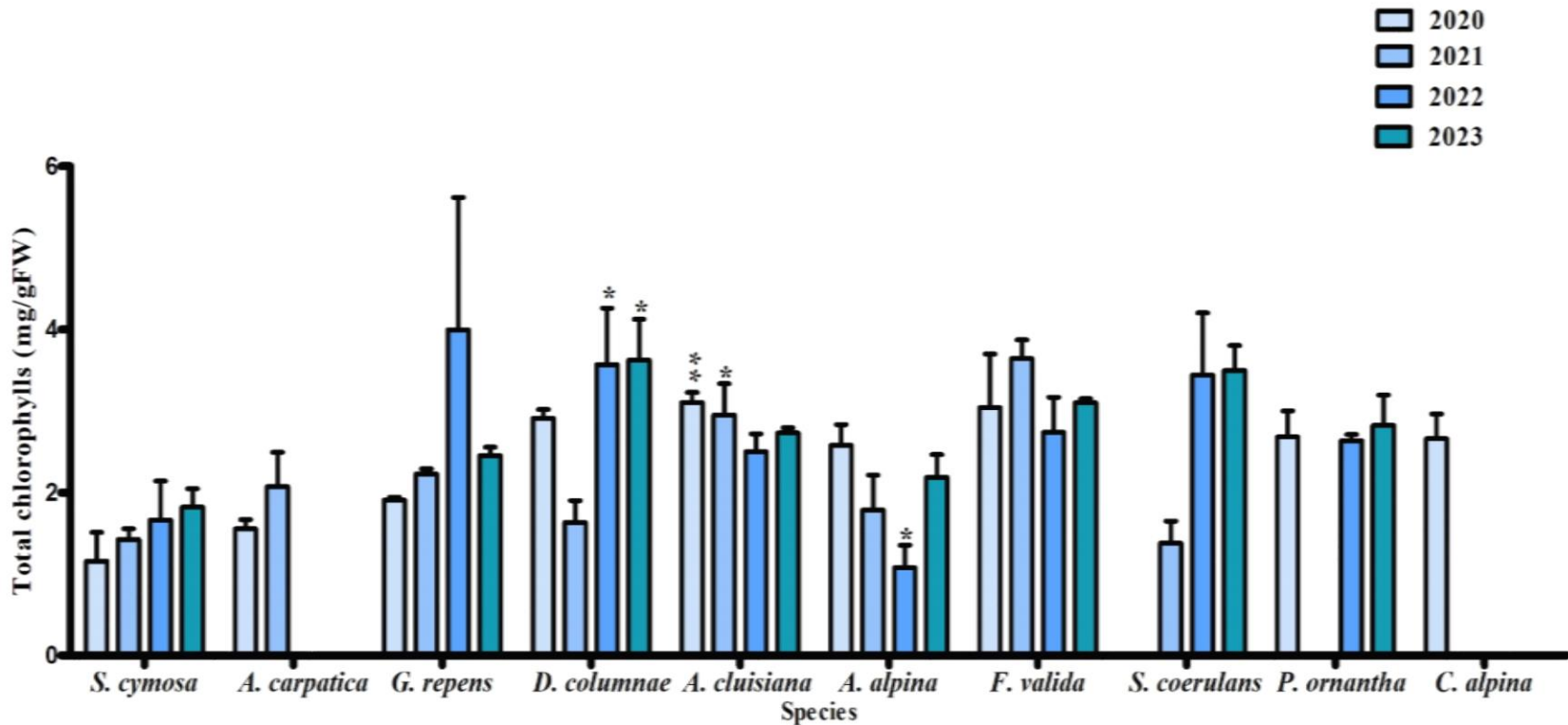


Figure 4. Total chlorophylls content. Statistically significant differences between: control vs. year (* $p < 0.05$, ** $p < 0.01$) for *A. clusiana*; 2021 vs. 2022 (* $p < 0.05$), 2021 vs. 2023 (* $p < 0.05$) for *D. columnae*; 2020 vs. 2022 (* $p < 0.05$) for *A. alpina*. *H. vulgare* L. (not presented in the figure), grown at laboratory conditions is used as a control.

Total carotenoids content

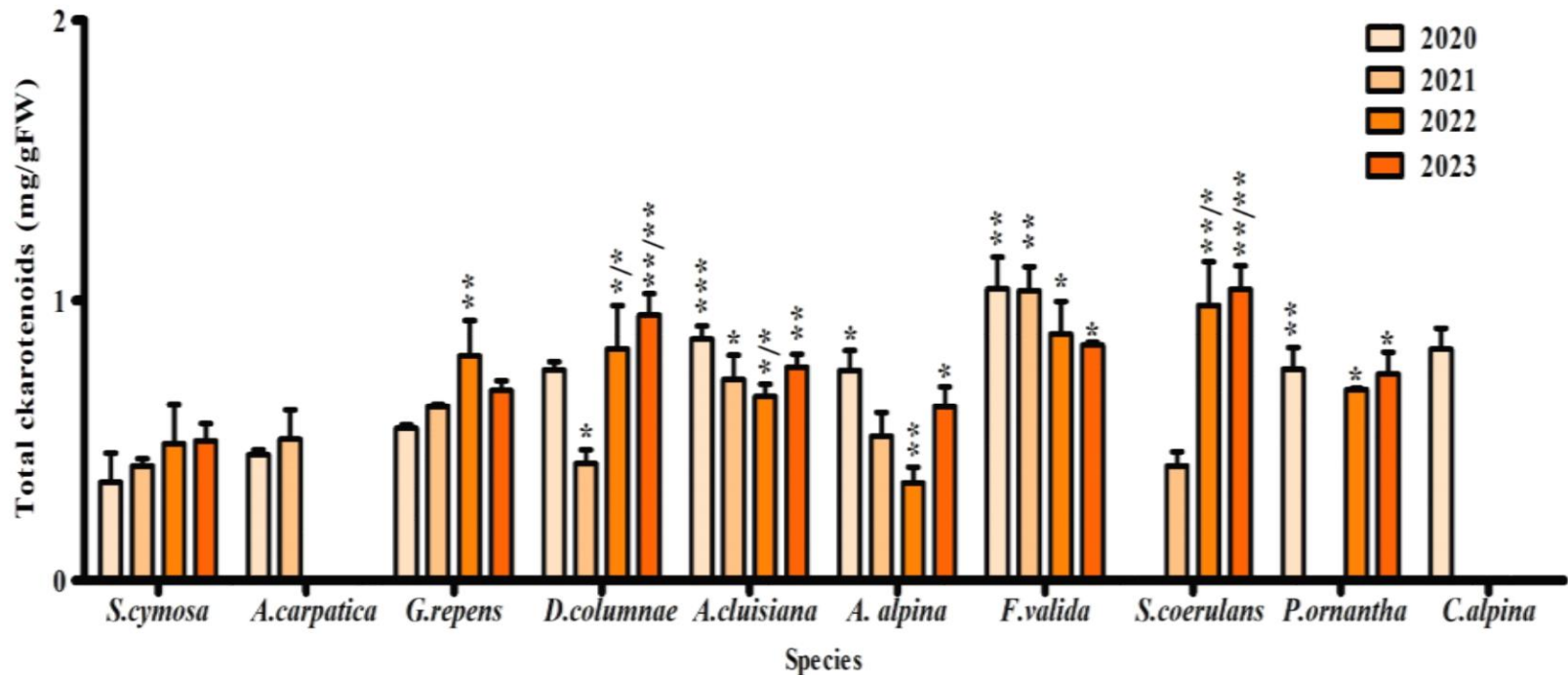
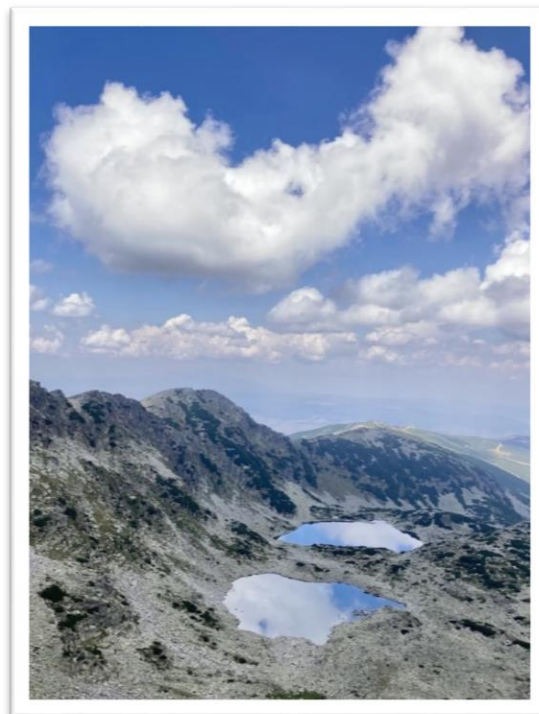
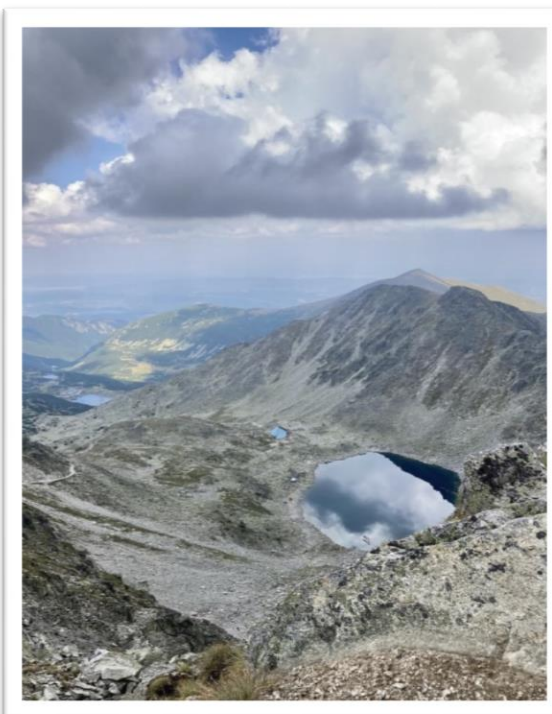
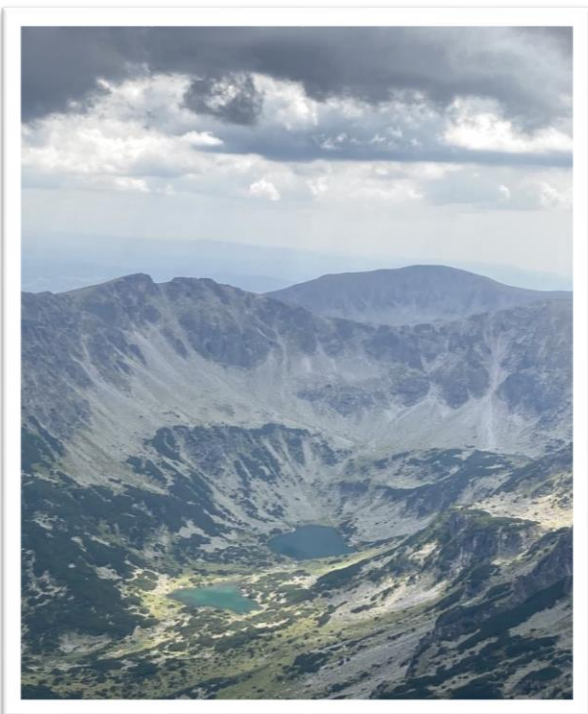


Figure 5. Total carotenoids content. Statistically significant differences between: control vs. year (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) for *G. repens*, *D. columnae*, *A. clusiana*, *A. alpina*, *F. valida*, *S. coerulans*, *P. ornantha*; 2020 vs. 2021 (* $p < 0.05$), 2021 vs. 2022 (* $p < 0.05$), 2021 vs. 2023 (** $p < 0.01$) for *D. columnae* (after the slash); 2020 vs. 2022 (* $p < 0.05$) for *A. clusiana* (after the slash); 2020 vs. 2022 (** $p < 0.01$), 2022 vs. 2023 (* $p < 0.05$) for *A. alpina*; 2021 vs. 2022 (* $p < 0.05$), 2021 vs. 2023 (** $p < 0.01$) for *S. coerulans* (after the slash). *H. vulgare* L. (not presented in the figure) grown at laboratory conditions, is used as a control.

- The pigment content was altered depending on the studied plant species and the alpine conditions for the respective studied years.
- Higher levels of total chlorophylls, chl. *a*, chl. *b* and total carotenoids were obtained in 2022-2023 years for most of the plant species than those obtained in 2020-2021.
- The biological data are in good correlation with the UV data. The four-year study (from 2020 to 2023) found an insignificant increase in the intensity of UV radiation with time. The highest UV intensity was measured for July 2023. On the other hand, statistical data showed that the effect of the studied species is significantly higher than the effect of the studied year.
- No change in the chlorophyll *a/b* ratio was obtained for all studied alpine plants. However, higher values of chlorophyll *a/b* ratio for all studied species were detected for 2023 than those from 2020 to 2022.

Conclusion

- Studied wild-growing species probably have different adaptive mechanisms to cope with the environmental stress at this altitude.
- To understand the mechanisms of interaction of factors and plant response in the long-term aspect of time further studies on the impact of extreme environmental factors at the Moussala Peak, on the pigment content of plants are needed.



Thank you for the attention!

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