

SEASONAL DYNAMICS OF FUNCTIONAL PARAMETERS OF WINTER-GREEN STEPPE RELICT *GLOBULARIA PUNCTATA* LAPEYR.

Elena Bogdanova¹, Larissa Ivanova^{2,3}, Polina Yudina², Galina Semenova⁴, Viktor Nesterov¹ and Olga Rozentsvet¹

¹ Samara Federal Research Scientific Center, Russian Academy of Sciences, Institute of Ecology of Volga River Basin, Russian Academy of Sciences, Togliatti 445003, Russia, ²University of Tyumen, Tyumen 625003, Russia, ³ Institute Botanic Garden, Ural Branch, Russian Academy of Sciences, Ekaterinburg 620144, Russia, ⁴Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino 142290, Russia

INTRODUCTION



Globularia punctata Lapeyr. (Plantaginaceae) belongs to the ecological groups of pliocene relict plants. This plant species is characterized by a specific adaptability to particular habitat conditions (climatic, geomorphological, edaphic, and biological). *G. punctata* plants possess low ecological plasticity; plants grow mainly on stony slopes of mountain carbonate grass steppe. The phenological development of *G. punctata* proceeds according to the winter-green type. It is known that wintergreenness is a facultative biomorphological feature that depends on climate, ecological conditions of habitat, and the ontogenetic state of plants. The biological role of wintergreenness consists in maintaining the assimilation apparatus throughout winter, which enables plants to switch to photosynthesis in spring within maximum short terms. Due to a long vegetation period, winter-green plants are exposed to different abiotic factors (drop in temperature in the autumn-winter period, intensive insolation in the spring-summer period, different soil moisture, and others).

The study of the structure of leaves, tissues, and cells, as well as the parameters of chloroplasts and the organization of photosynthetic membranes is important for understanding the defense mechanisms underlying the winter hardiness of plants. However, the information on wintergreen plants is still very scarce. We assumed that, like evergreen conifers, wintergreen plant species implement defense strategy, which make it possible to protect the assimilation apparatus from destruction at low temperatures.

Considering the ecological specificity, a pliocene relict *G. punctata* is a valuable and unique object of investigations. Therefore, the goal of the work was to study the structural and functional features of this species and their seasonal variability. We hypothesized that the adjustment of a wintergreen plant to cold period is provided by structural changes of a leaf at different scales from whole-leaf to cell- and subcell-level. In turn, structural reorganization is a basis of physiological and biochemical adaptation of a wintergreen leaf.

MATERIALS AND METHODS



Fig. 1. Photos of *G. punctata* during the sampling period

G. punctata is a wintergreen plant and exhibits slow leaf development during the entire year. Plant materials were sampled in June, November 2019, and January 2020. The age of sampled leaves was different in different periods. In June, we analyzed approximately 1-2-months young leaves, whereas in November the leaves were 5 months older, and the oldest leaves were sampled in January. The average monthly daily air temperature during the observation period was +25/+14 °C (day/night) in June, 0/-4 °C in November, and -3/-5 °C in January. The height of the snow cover in January at the site of collection of plants was 60 cm. Plants grew on an open, weakly grassy rocky slope of Samarskaya Luka Natural Park (53° 23' N. latitude, 49° 37' E. longitude).

RESULTS

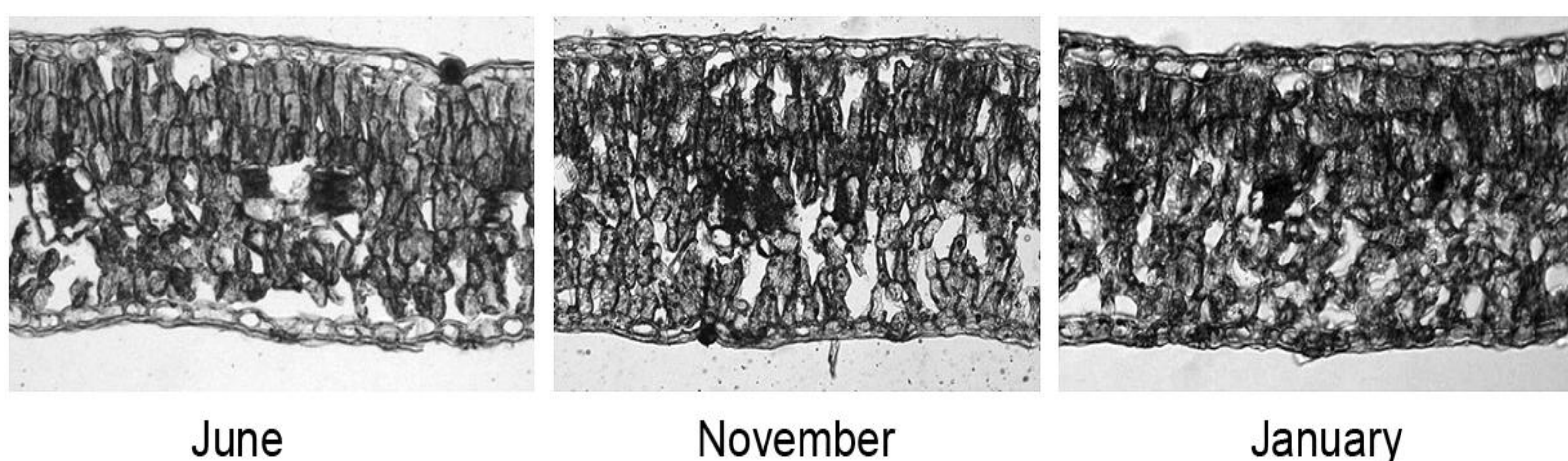


Fig. 3. Leaf cross-sections of *G. punctata* in different months of the year.

Table 1. Seasonal changes in the concentration of photosynthetic pigments in the leaves of *G. punctata*.

Pigments	Growing season		
	June	November	January
Chlorophyll a (mg g ⁻¹ DW)	3.2 ± 0.2a	2.2 ± 0.1c	2.8 ± 0.05b
Chlorophyll b (mg g ⁻¹ DW)	1.1 ± 0.1a	0.8 ± 0.1a	1.1 ± 0.03a
Carotenoids (mg g ⁻¹ DW)	1.2 ± 0.1a	1.0 ± 0.1a	1.2 ± 0.1a
LHC (%)	56.3	58.7	62.1

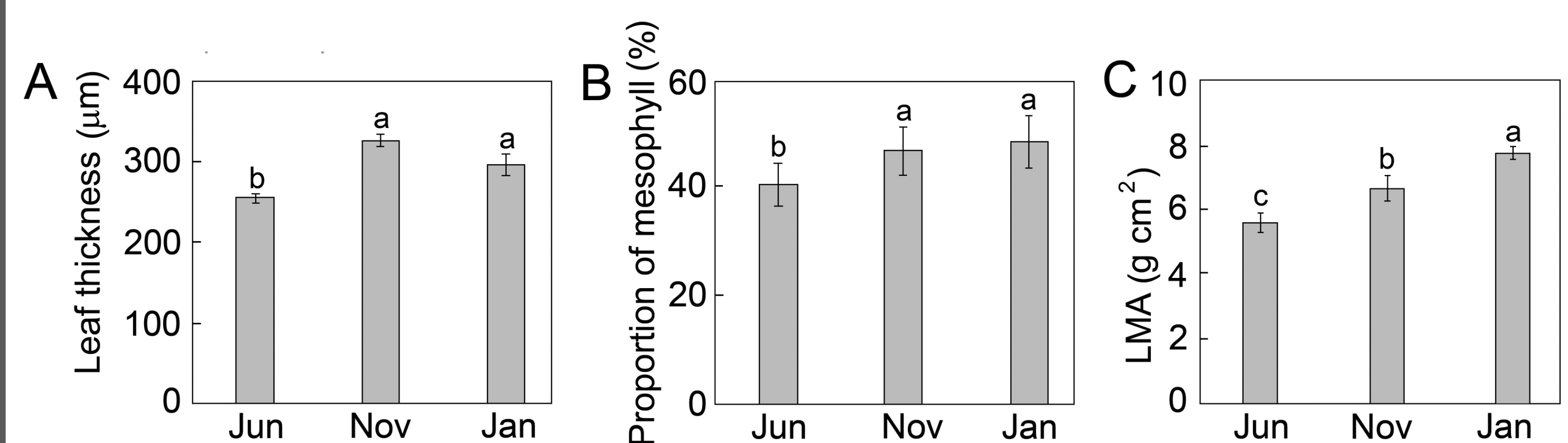


Fig. 4. Seasonal changes in leaf thickness (A), partial volume of mesophyll (B) and LMA (C) *G. punctata*. Each bar represents the mean ± SE (n = 3). Different letters indicate statistically significant differences (one-way ANOVA with the Tukey's test, $P < 0.05$).

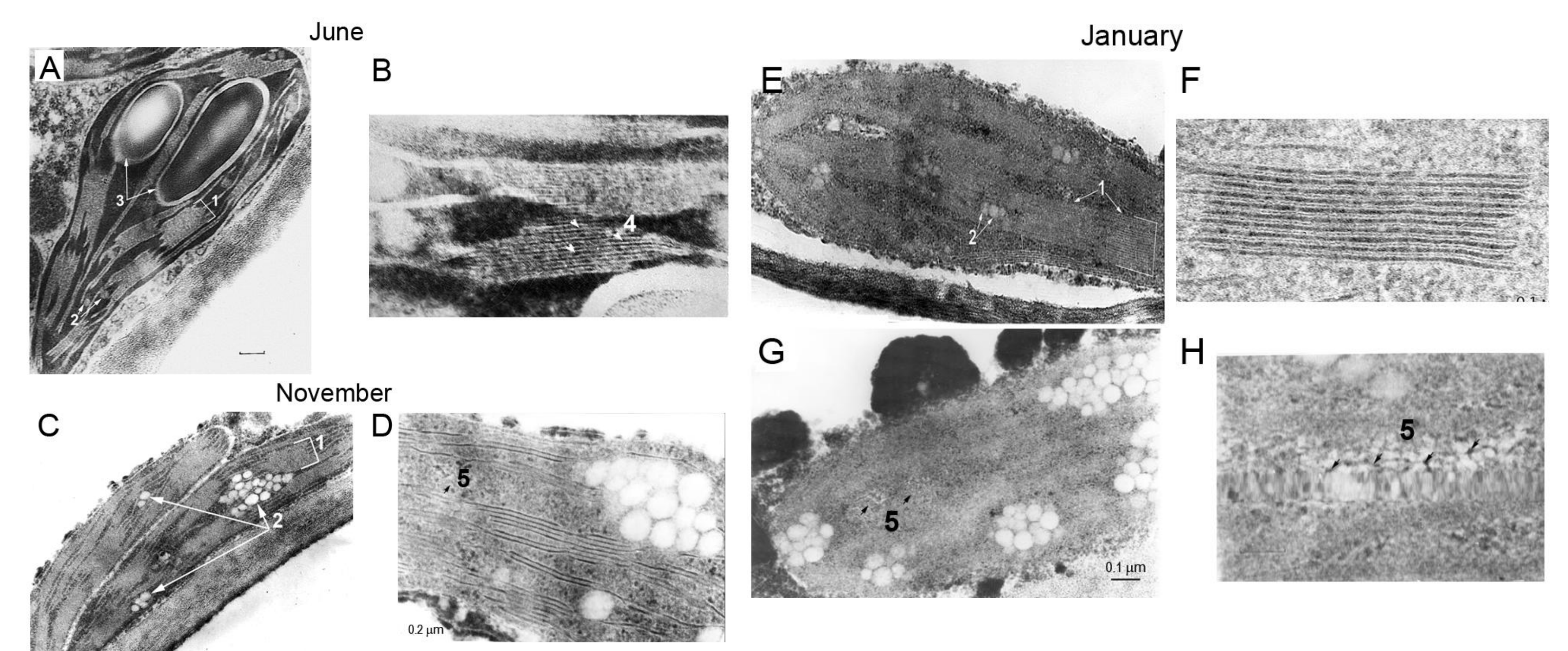


Fig. 6. Chloroplasts of *G. punctata* leaves in different months (scale bar – 0.2 μm) and accumulation of electron-dense matter inside chloroplast thylakoids (scale bar – 0.1 μm). 1 – grana, 2 – plastoglobules, 3 – starch, 4 – electron dense substance, 5 – DNA (scale bar 0.1 μm). A, B – June, B, C, D – November, E, F – January. G, H – Arrows indicate DNA strands in the chloroplasts of plants.

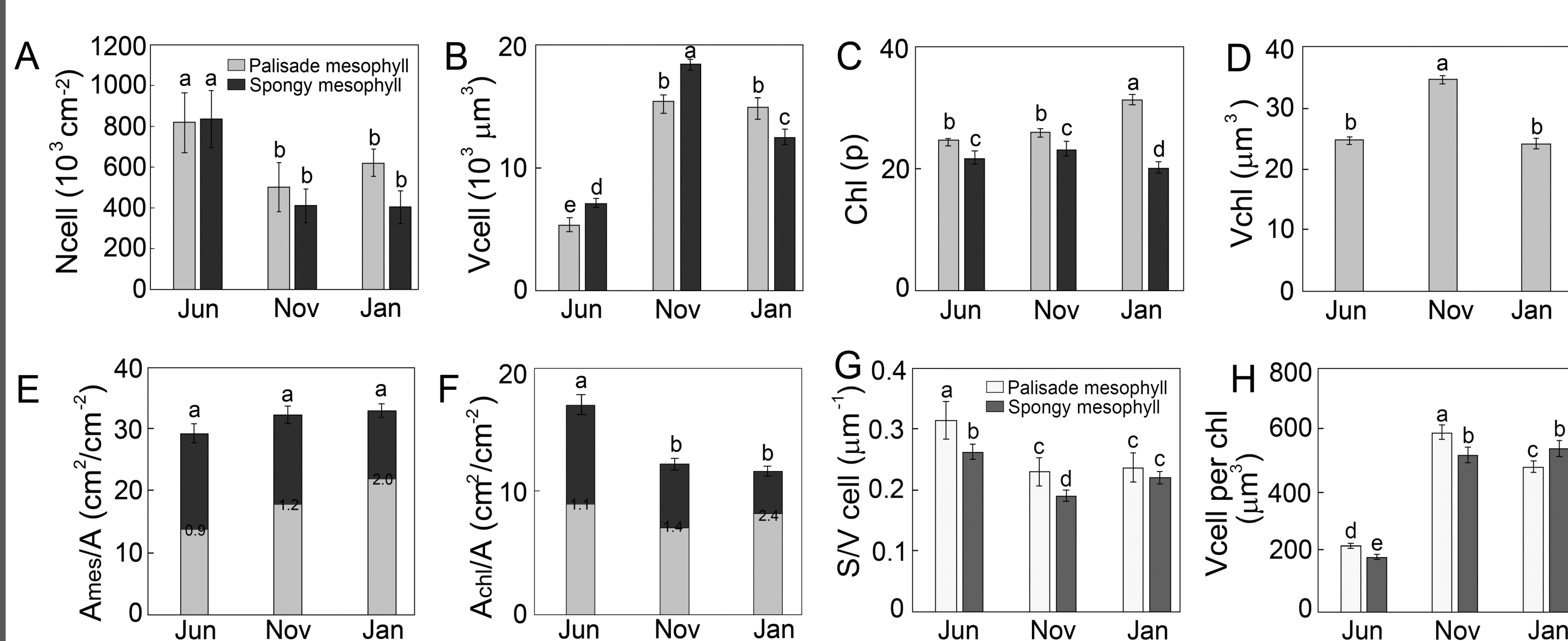


Fig. 5. Seasonal changes in the parameters of photosynthetic tissues of *G. punctata*. Ncell is the number of mesophyll cells (A), Vcell is the cell volume (B), Chl is the number of chloroplasts per cell (C), Vchl is chloroplast volume (D), Ames/A – mesophyll surface area per unit leaf area (E), Achl/A – chloroplast surface area per unit leaf area, S/V cells – the surface volume ratio of the cell (G), Vcell per chl – the cell volume per one chloroplast (H).

CONCLUSION

Thus, we carried out the comprehensive analysis of leaf functional traits at different scales from whole-leaf to cell- and subcell-level including photosynthetic membranes and the lipid composition of the perennial herb *G. punctata*. *G. punctata* assimilating organs retained the viability throughout the vegetation period due to a rearrangement of the entire tissue-cellular organization of the photosynthetic apparatus. The adaptive strategy of a wintergreen plant in terms of the leaf structure is similar to this of evergreen conifers. The functional integrity of the photosynthetic apparatus is maintained, among other things, by the specific composition of lipids and FA.

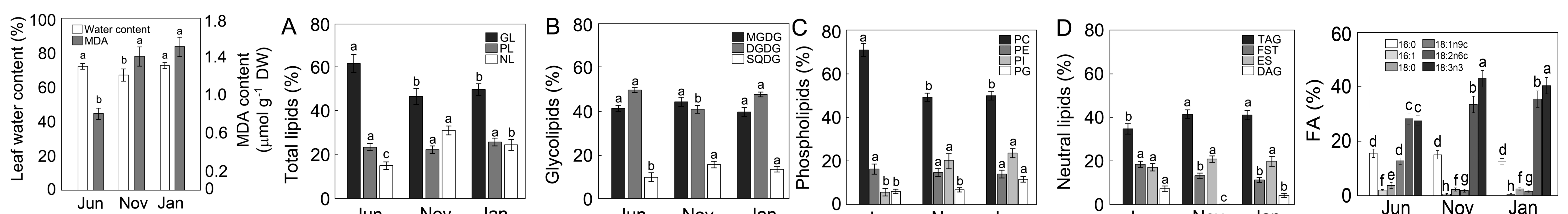


Fig. 7. Seasonal changes leaf water content and malondialdehyde (MDA), the lipid content and fatty acids (FA) in leaves of *G. punctata*.