PHYSIOLOGICAL STRATEGIES FOR ADAPTATION TO ARIDITY IN XEROPHILIC ECOSYSTEMS FROM "CRACU GAIOARA" BOTANICAL RESERVE (MEHEDINȚI COUNTY - ROMANIA)

Luminița BUȘE-DRAGOMIR, Ion NICOLAE

University of Craiova, luminita25dragomir@yahoo.com
ionnicolaebio@yahoo.com

Coresponding author: ionnicolaebio@yahoo.com

Abstract

The Cracu Găioara Botanical Reserve is located on the left bank of the Danube, upstream from Drobeta-Turnu-Severin, within the administrative boundaries of the Dudașu Schelei commune, Mehedinți County. Although relatively small, the reserve hosts several rare species of European interest. The calcareous substrate, south-western exposure, and particular microclimatic conditions have facilitated the establishment of species with a sub-Mediterranean character. Ephedra distachia, a thermophilic relic, finds suitable habitat conditions here and is physiologically well adapted to aridity. Centaurea atropurpurea, another rarely taxon, exhibits physiological features characteristic of calciphilous and xerophilous plants. The accumulation of osmoprotective compounds-primarily carbohydrates-help stabilize proteins and maintain cellular turgor during prolonged periods of drought. Additionally, the low stomatal conductance values confer protection by reducing water loss under conditions of elevated evaporative demand.

Key words: drought, photosynthesis, stomatal conductance

INTRODUCTION

Cracu Găioara Reserve is a protected area of national interest, included in IUCN category IV (Botanical Reserve), located in Mehedinți County, on the administrative territory of Drobeta-Turnu-Severin, in the southeastern extremity of the Iron Gates Natural Park. The reserve is located on the left bank of the Danube, in the Getic Plateau (interference area of the Almaju and Locvei Mountains), near the village of Dudașu Schelei and DN6. Geographical data (coord.): approximately 44°38′48″N, 22°34′33″E. The reserve is very small, with an area of approximately 5 hectares.
https://legislatie.just.ro/Public/DetaliiDocument/21860 .

The relief is characterized by rocks and limestone/steppe meadows specific to the Danube margin. Geomorphology determines limestone microhabitats. The dominant soils are thin soils on limestone, with pedological influence that favors steppe elements and calciphilic species (according to the floristic character of the reservation). The climate has a temperate-continental character with local Mediterranean accents (descent/ascent of biogeographic elements), typical of the Danube Gorge / Iron Gates.

The reservation has a strictly botanical role and protects communities of calciphilic meadows and tertiary relict species. Among the species reported as protected or of interest in Cracu Găioara are *Ephedra distachya* (tertiary relict element), *Ceterach officinarum, Dianthus banaticus, Dianthus varciorovensis, Cephalaria uralensis (var. multifida), Scorzonera lanata, Stipa danubialis* - steppe-specific element, Centaurea atropurpurea.

Many of these species are considered relics or rarities on a regional scale and some also appear on national / regional conservation lists. The main reason for protection is the preservation of these unique floristic elements.

Given that the reserve is part of the Iron Gates Natural Park area, the local fauna is influenced by the regional biodiversity of the park, which includes numerous birds (over 200 species reported in the park), mammals, reptiles and amphibian's characteristic of limestone areas and the banks of the Danube. Therefore, the fauna in the immediate vicinity of the reserve is represented by species of rupicola's / steppe birds, pollinating insects associated with meadows and small mammals typical of meadow areas.

Xerophilous calciphilic species are plants adapted to calcareous substrate (rich in calcium carbonate $CaCO_3$), aridity conditions (dry soils, intense solar radiation, low rainfall), shallow, skeletal soils, with low water retention. They are typical of calcareous meadows, rocky foothills, sunny slopes and eroded slopes (Lux, A.et al, 2021).

Xerophilous calciphilic species present specific adaptations to life in dry conditions and calcareous soil. Adaptations to drought are reduced leaves, often acicular or tomentose, which reduce transpiration, thick cuticle and waxy layer, very deep or laterally extended roots to access residual water and efficient metabolism under water stress conditions.

Deep or laterally highly branched roots can exploit cracks in limestone rock; some species develop calcifuge/encapsulated roots that store Ca (Chai, S. et al, 2024).

The accumulation of calcium oxalate crystals in leaves and tissues is a frequently reported structural mechanism that allows Ca storage and limiting ionic toxicity (Chai, S. et al, 2024).

Adaptations to calcareous substrate are increased capacity to absorb nutrients in alkaline pH, tolerance to high Ca concentrations, development in rock cracks or on extremely thin soils.

Drought-adapted leaf anatomy consists of thickened cuticle, epidermis with suberized cells or deep stomata, positioned below the epidermis. All of these contribute to water conservation (Lux A., 2021).

Adaptations to intense light are the additional pigments represented by anthocyanins for tissue protection and leaf orientation to reduce the surface exposed to solar radiation

The physiological characteristics and biochemical mechanisms of calciphilic plants are numerous.

Ca^{2+} management is achieved by sequestering Ca in the form of oxalates/crystals or storing it in vacuoles, reducing absorption at the root level through changes in permeability; formation of calcified tissues in the roots/epidermis. These mechanisms reduce ionic stress (Chai S., 2024).

Alkaline pH decreases the solubility of Fe and P. Calcicoli present adaptations for the extraction of these elements: induction of organic root exudates (organic acids), increased activity of Fe transporters (formation of chemical combinations called chelates) or mutualistic relationships with mycorrhizae that mobilize P/Fe (Vélez-Bermúdez, I. C., Schmidt, W., 2023).

Exposure to high Ca levels is associated with intracellular calcium signaling (Ca^{2+}-dependent) and activation of antioxidant pathways (ABA accumulation, controlled ROS), demonstrated experimentally in lithophytic species and plants from karst systems (Meng, W. et al, 2023).

Mycorrhizae and soil microorganisms can facilitate nutrient mobilization (especially P) in calcareous soils. Recent reviews highlight the crucial role of root microbiota in tolerance to alkaline soils (Msimbira L.A., Smith, D.L., 2020).

Recent literature shows that the effects of drought, temperature and CO_2 can modify specific plant interactions with calcareous soils; however, the combined effects are incompletely understood — research in 2023–2024 recommends multifactorial experimental studies to predict the future distribution of calcicole plants (Bonpart T et al, 2024).

Recent experimental studies (Chai et al., 2024) show that calcic plants respond to high Ca environments through a coherent set of adjustments: oxalate synthesis, root modifications and adaptive photosynthetic settings (e.g. chlorophyll preservation and photosynthetic efficiency adjustments) (Chai S. et al, 2024).

From an ecological point of view, xerophilic calciphilic species contribute to the stabilization of unstable calcareous soils, create microhabitats for insects, reptiles and specialized microorganisms, are indicators of calcareous soils and dry steppe/meadow habitats.

These plants are among the most vulnerable to climate change and the invasion of neophyte species, many of which are rare, endemic and relict species.

Due to their extreme specialization, these plants are priority habitat indicators, being essential for maintaining the biodiversity of calcareous grasslands.

Restoration strategies must take into account nutritional strategies (calcicole are not only “Ca tolerant” — many are specialized in extracting Fe/P) and, therefore, reintroduction on an unsuitable substrate or using the wrong species is doomed to failure. Recent studies position restoration strategies according to functional ecology (calcicole vs. calcifuge) (Cross, A., Lambers, H., 2021).

MATERIAL AND METHODS

The studies were carried out in 2025 for three months: June, July and August in the Cracu Gaioara Botanical Reserve and consisted of physiological determinations carried out on the species *Ephedra distachia* and *Centaurea atropurpurea.*

Ephedra distachya is a short shrub, adapted to xeric conditions and sandy soils (fixed dunes, littoral-marine areas and steppe). It has thin, brownish-green branches, with very small (scaly) leaves arranged in opposite pairs at the nodes; the female fruits appear as fleshy, red bracts.

The leaves are very small, non-photosynthetic; photosynthesis is mainly carried out in the succulent, translucent green stems (branches) containing chlorenchyma. This is a common xerophytic adaptation in Ephedraceae (Gonzales et al, 2020).

The stem has an epidermis, a cortex formed by parenchyma (in which the chlorenchyma is found) and a central cylinder with xylem (formed by tracheids) and phloem (without true vessels, as in other gymnosperms). The cell wall of the conducting elements has typical gymnosperm characters (tracheids) (https://www.conifers.org/ep/Ephedraceae.php).

The leaf anatomy is reduced to a thin layer of sheath/scales that protects the nodes; In most cases, Ephedra leaves do not participate significantly in photosynthesis (Gonzales et al, 2020).

Stomata are found on stems and, in reduced leaves, are often recessed/less exposed. Thus, they reduce water loss. Plants have relatively thick cuticles and structural adaptations to reduce transpiration (e.g. reduced leaf sheaths, stem orientation) (Hollander J.L, 2010).

Ephedra generally functions as C3 plants with morphological-reductive adaptations (there is no generalized conclusive evidence that *E. distachya* practices CAM), and the photosynthetic capacity per unit area is supported by large masses of photosynthetic rods (Gonzales et al, 2020).

All parts of the plant contain alkaloids typical of the genus (ephedrines and derivatives), with concentrations being notable in stems and branches. These compounds play a role in chemical defense and have pharmaceutical importance (Ni S et al, 2013). The species is dioecious. The reproductive organs are small, cone-like (microsporophylls and megasporophylls/bractes), organized in groups; in females, the bracts form fleshy structures that surround the seeds (apparently fructoids), favoring dispersal (https://www.conifers.org/ep/Ephedraceae.php).

The pollen of *Ephedra* can be transported over long distances (by wind) however, some species retain traits that support entomophilous pollination. Cone anatomy suggests variability in pollination between species (Gonzales et al, 2020).

Centaurea atropurpurea is a perennial herb with a woody rhizome and woody bases; erect, robust, tall stems (usually 80–180 cm, in some descriptions 1–2 m). It forms a basal rosette of leaves and branched flowering stems. Leaves: deeply divided (pinnatisect/pinnatifid), the basal ones petiolate, the upper ones sessile; silvery gray-green color due to a tomentose pubescence at the base. The indumentum (hair) can be dense at the base of the plant, giving better tolerance to drought and reflection of solar radiation. Typical Asteraceae chapters, of a "thistle-like" appearance, with very dark red-purple flowers (sometimes ruby; rarely other shades). The involucre has bronze-brown scarious/scaly bracts; the flowers are attractive to pollinators (bees, butterflies). Observed flowering period: generally, June–August (varies locally) (https://www.gardenia.net/plant/centaurea-atropurpurea).

Involucres with rigid/scaly bracts, achenes (seeds) typical of the genus Centaurea; presence of tomentose hairs on the stem and base (tomentum) adaptation to sunny/dry environments. There are morphological variations between populations (different morphological groups) that may reflect adaptations to the substrate and microhabitat.

It prefers calcareous, rocky habitats or meadows with southern exposure and well-drained soils; it is considered a xeromesophytic species (tolerant to relative drought, but not strictly xeric). Due to the pubescence and morphology of the leaves, it reduces water loss and survives in conditions of strong radiation. In some areas (e.g. Transylvania, southwest) it is increasingly rare. The inflorescences contain essential oils and terpene compounds; studies on the composition of the essential oil have shown a dominance of sesquiterpenes, which has implications in ecology (plant-insect interactions) and in taxonomy/phylogeny (Vonica D.et al, 2012).

To know the physiological particularities of the two plant species, the following were determined: stomatal conductance, transpiration intensity, photosynthesis intensity, total water content, free and bound water and soluble sugar content in the branches.

The stomatal conductance, the intensity of the photosynthesis and transpiration processes were determined with the Lci portable device that measures the respective parameter with great precision and also has the advantage that the leaves of the analyzed plants can be kept on the plant, so that at intervals of time, new determinations can be made on the same leaves. In this way, graphs can be made regarding the diurnal dynamics, but also the seasonal dynamics of this physiological process. In addition, the device also measures the temperature in the assimilation chamber, as well as the amount of water vapor and light intensity, factors that influence all the vital processes of the plants.

The content of chlorophyll pigments was determined directly on plant leaves with the Minolta portable apparatus, which measures and expresses this parameter in SPAD units.

The water content of the leaves was determined by the gravimetric method, after drying the plant material in an oven at 105 degrees Celsius.

The soluble sugar content was determined by the refractometric method.

RESULTS AND DISCUSSIONS

The stomatal conductance

Stomatal conductance is the main factor controlling transpiration at the leaf level, directly influencing the flow of water vapor and, implicitly, the water status of the plant. The regulation of this parameter has major ecological and physiological significance, as it allows plants to optimize water use, prevent water stress and adapt their metabolic functioning to environmental conditions.

In plants belonging to the *Ephedra distycha* species, stomatal conductance values remained very low and relatively during the day with a maximum of 0.02 mol/m^2/s at 8:00 a.m (figure 1).

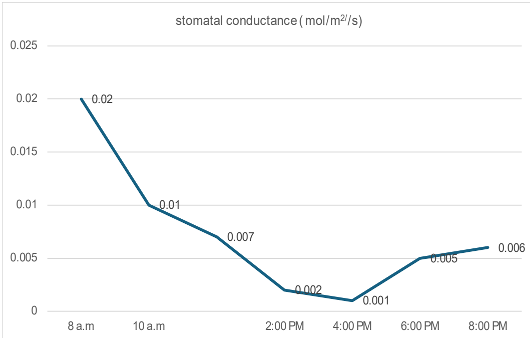

Figure 1. Diurnal variation of stomatal conductance (mol/m^2/s) in *Ephedra distachya*

The reduced diurnal variability of stomatal conductance observed in *Ephedra distachya* indicates strict control of stomatal opening, characteristic of plants with cam (crassulacean acid metabolism) photosynthetic metabolism. The low values of stomatal conductance during the day reflect a physiological adaptation to water stress conditions, by limiting water loss through transpiration. In this context, gas exchanges are temporally decoupled from the photochemical processes of photosynthesis, with co_2 fixation occurring predominantly during the night, when evaporation is minimal.

In *Centaurea atropurpurea*, stomatal conductance had higher values but also remained low under the climatic conditions of the analyzed period (figure 2).

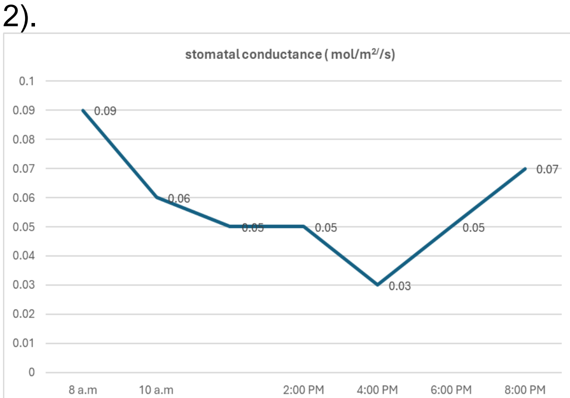

Figure 2. Diurnal variation of stomatal conductance (mol/m^2/s) in *Centaurea atropurpurea*

By controlling stomatal conductance, plants in the research area maintain a balance between root water absorption and transpiration losses. A low stomatal conductance prevents tissue dehydration and hydraulic collapse of the xylem, reducing the risk of cavitation.

The intensity of transpiration

In both species studied, the process of transpiration was maintained at very low values during the day.

After 10 a.m. in *Ephedra distachya,* the determinations were undetectable, indicating complete suppression of the process (figure 3).

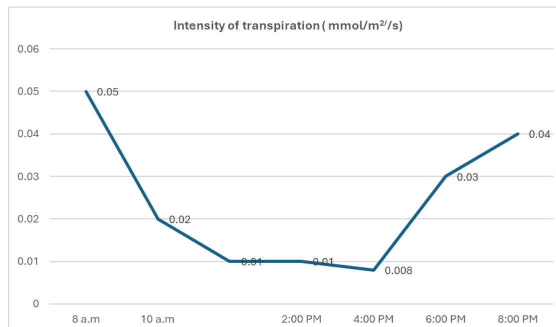

Figure 3. Diurnal variation of transpiration intensity (mmol/m^2/s) in *Ephedra distachya*

In *Centaurea atropurpurea*, the intensity of transpiration reached a maximum value around 10:00, then gradually decreased, reaching a minimum value at 16:00 (figure 4).

In the climatic conditions of the period and area studied, the day is characterized by high temperatures, intense solar radiation and a large vapor pressure deficit between the leaf and the atmosphere. These conditions favor massive water loss through transpiration. To prevent dehydration, plants reduce stomatal conductance (g_s) by partially or completely closing the stomata. The decrease in stomatal conductance limits the diffusion of water vapor from the intercellular spaces to the atmosphere, causing a significant reduction in the transpiration rate, even in the presence of high vapor pressure.

Limiting transpiration contributes to maintaining the leaf water potential (Ψ_l), ensuring the turgor necessary for cell growth and the functioning of metabolic enzymes.

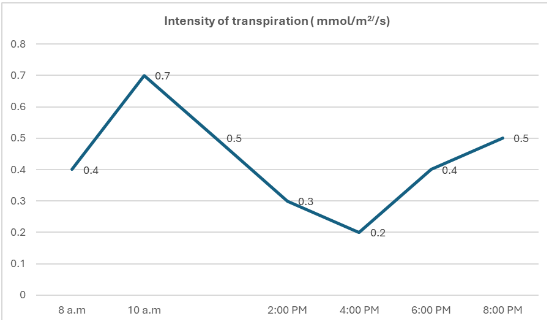

Figure 4. Diurnal variation of transpiration intensity (mmol/m^2/s) in *Centaurea atropurpurea*

The intensity of photosynthesis

In xerophilic plants in the analyzed area, stomatal conductance, transpiration and photosynthesis are interrelated through an adaptive mechanism that maximizes water conservation at the expense of diurnal photosynthesis. This strategy has major ecological significance, allowing survival in arid environments, but implies a physiological trade-off: reduced photosynthesis and growth rate. In *Ephedra distachya*, a CAM plant, the negative impact is reduced by temporal decoupling of carbon fixation and water loss processes. Stomata are also the main pathway for CO_2 to enter the leaf. Reducing stomatal conductance limits CO_2 entry and reduces the rate of photosynthesis leading to a trade-off between water conservation and carbon sequestration (figure 5).

Centaurea atropurpurea înregistreaza in decursul zilei un maxim al fotosintezei in jurul orei 10 a.m și o valoare minima la ora 4 p.m. (figure 6).

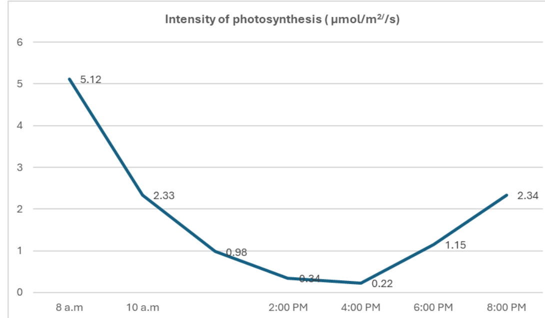

Figure 5. Diurnal variation of photosynthesis intensity (µmol/m^2/s) in *Ephedra distachya*

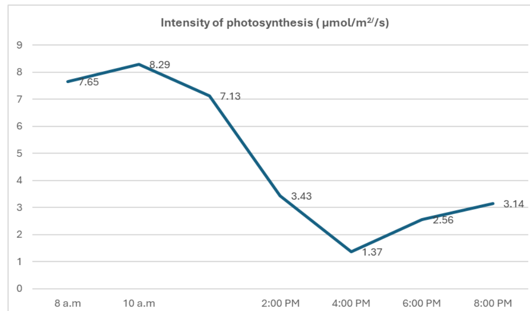

Figure 6. Diurnal variation of photosynthesis intensity (µmol/m^2/s) in *Centaurea atropurpurea*

The total water content of the leaves

The green branches of the *Ephedra distachya* species are the main photosynthetic organ, taking the place of the leaves, which are reduced and membranous to limit water loss.

The tissue of these branches stores enough water to support metabolism, but it is not as large as in succulent species. *Ephedra* does not accumulate extreme amounts of water but rather optimizes losses and flows. Thus, the maximum value of water content was recorded in June and was 57% (figure 7).

Compared to Ephedra, *Centaurea atropurpurea* had a higher water content throughout the determination period, with a maximum in June (73%) and a minimum in August of 65.7% (figure 7).

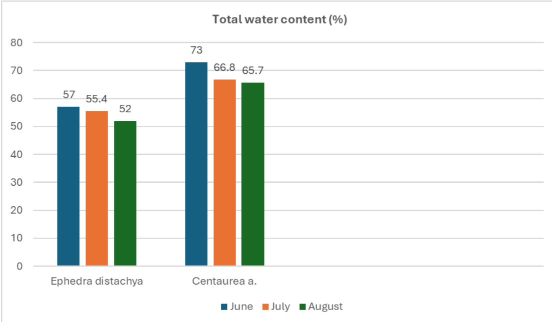

Figure 7. The total water content of the leaves during the three months of determinations (%)

The chlorophyll content of the leaves

In *Ephedra distachya* the chlorophyll content is kept relatively stable compared to drought sensitive plants, due to tolerance mechanisms. However, a slight decrease is observed starting in July, due to the onset of severe drought. *Centaurea atropurpurea* records a significant reduction in leaf chlorophyll content, with a minimum of 23 SPAD in August (figure 8).

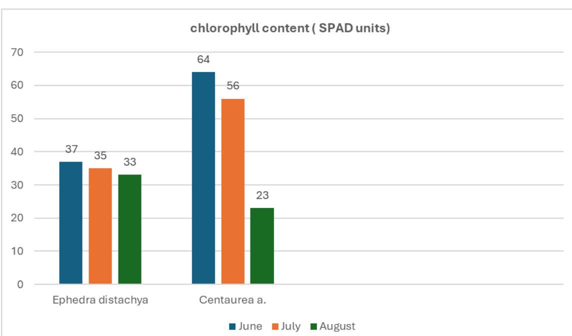

Figure 8. The chlorophyll content of the leaves in the three months of determinations (SPAD units)

CONCLUSIONS

The two species studied exhibit efficient water-use strategies.

The low transpiration rates and reduced stomatal conductance indicate that both species adopt a conservative water-use strategy. By limiting stomatal opening, they minimize water loss to the atmosphere, which is a key adaptation for survival under drought conditions.

The maintenance of low stomatal conductance suggests strong stomatal control in response to water deficit. This regulation allows the plants to balance carbon assimilation with water conservation, preventing excessive dehydration during prolonged dry periods.

The ability of these species to maintain relatively constant water content over a wide range of external moisture conditions indicates effective internal water homeostasis. This reflects isohydric behavior and highly efficient osmotic and structural adaptations that protect cell hydration.

The lack of major changes in tissue water content despite drought suggests that these plants possess mechanisms such as thick cuticles, reduced leaf area, or specialized tissues that reduce evaporative demand and enhance water retention.

These physiological characteristics explain the ecological success of *Ephedra distachya* and *Centaurea atropurpurea* in dry habitats. Their drought tolerance allows them to occupy niches where water availability is limiting and competition from less adapted species is reduced.

Although taxonomically distinct, both species exhibit similar drought-adaptive physiological responses. This suggests convergent evolution driven by selective pressure in water-limited environments.

Overall, the combination of low transpiration, strict stomatal control, and stable water content demonstrates that *Ephedra distachya* and *Centaurea atropurpurea* are well-adapted to drought through integrated physiological mechanisms that enhance survival under water stress.

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