PHYSIOLOGICAL CHARACTERISTICS OF THE SPECIES RUSCUS ACULEATUS L. ADAPTED TO THE CLIMATIC CONDITIONS OF THE SOUTH-WEST AREA OF THE OLTENIA REGION-ROMANIA

BUSE–DRAGOMIR LUMINITA[1], NICOLAE ION[2]
1University of Craiova, luminita25dragomir@yahoo.com
2University of Craiova, ionnicolaebio@yahoo.com

***Coresponding author:** ionnicolaebio@yahoo.com*

ABSTRACT

Ruscus aculeatus L. is a protected species represented by significant populations in the the southwest area of the Oltenia region.

The studies carried out in Mehedinti County, within the forests of Corcova locality, were conducted between July and September 2023, during a period characterised by minimal precipitation. In these conditions, Ruscus aculeatus plants were notably unaffected, with chlorophyll levels remaining relatively stable.

The values of photosynthesis intensity remained constant during the determinations and were not influenced by the water regime. While these values may be considered low compared to other species, they are correlated with stomatal conductance and transpiration intensity.

Despite the general intolerance of most shade plants to dry soil, Ruscus aculeatus demonstrates an exceptional ability to adapt to both shade conditions and severe drought.

Key words**: **photosynthesis, chlorophyll, water, drought

INTRODUCTION

Ruscus aculeatus is a rhizomatous geophyte and grows primarily in the temperate biome. Native to: Albania, Algeria, Azores, Baleares, Bulgaria, Canary Is., Corse, Cyprus, East Aegean Is., France, Great Britain, Greece, Hungary, Italy, Lebanon-Syria, Libya, Morocco, North Caucasus, Palestine, Portugal, Romania, Sardegna, Sicilia, Spain, Switzerland, Transcaucasus, Tunisia, Turkey, Serbia.
https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:540443-1

In Europe, *Ruscus aculeatus* L. is most widespread around the Mediterranean. Northwards it is found in southern and western Switzerland and northern France, across into the Azores, reaching its northern European limits between 50 and 55° N (Preston, 2007 cited by Peter A. , Tarek A. Mukassabi (2014)(figure 1).

In Romania, *Ruscus aculeatus* L. grows sporadically in the forest-steppe area, forests, thickets of forest and is a mesotrophic-eutrophic, xeromesophilic, mesophilic, thermophilic, subthermophilic, sciatophilic species, being identified in the counties of Bihor, Caraș Severin, Mehedinți, Dolj, Giurgiu, Constanța, Tulcea, Bacău (Ciocîrlan, V., 1990).

Butcher's broom, is an evergreen shrub in the *Asparagaceae* family. The plant grows well in partial or lightly shaded sites, but will tolerate full shade, in a range of soil types, and in average well-drained soil. It is hardy and tolerates drought, heat, and salt. Plants have a slowly creeping rootstock and will form large clumps. The flowers are followed by bright, waxy, red berries that last from late summer into the

winter.
https://plants.ces.ncsu.edu/plants/ruscus-aculeatus/
The English name - Butcher's Broom - derives from the use by the European butchers of the stems to clean their cutting board not only because of their stiffness and solidity, but also because of the essential oil which was credited with antibacterial properties. *Ruscus aculeatus* is a small, clump-forming shrub with erect shoots bearing stiff, ovate, leaf-like phylloclade. Tiny green flowers appear in late winter and spring on the phylloclade. Both root and stem are used in preparations. According European Pharmacopoeia VIII (2014), the herbal substance (Rusci rhizoma) consists of the dried, whole or fragmented underground parts of Ruscus aculeatus L containing not less than 1.0 per cent of total sapogenins expressed as ruscogenins (mixture of neoruscogenin and ruscogenin).
https://www.ema.europa.eu/en/documents/herbal-report/draft-assessment-report-ruscus-aculeatus-l-rhizome-revision-1_en.pdf

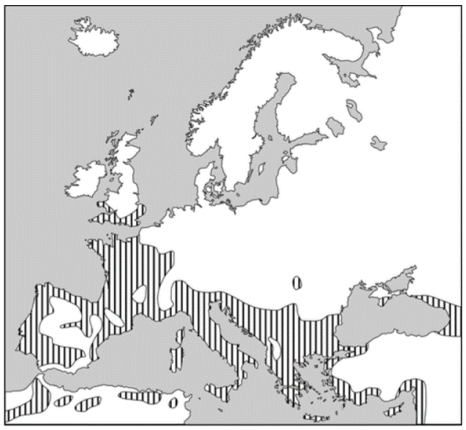

Figure 1. The European distribution of *Ruscus aculeatus*, modified from de Bolòs and Vigo (2001) cited by Peter A. Thomas, Tarek A. Mukassabi (2014)
https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/1365-2745.12265

The HPLC-MS/MS tests performed on plants from the hills of Lipova (Romania) showed that the highest concentration of sapogenin is present in the rhizome samples (0.11% ruscogenin and 0.17% neoruscogenin), and among the culture samples of tissue in vitro the highest concentration of sapogenin is present in shoots (0.017% ruscogenin and 0.075). % neoruscogenin). On the other hand, all samples contain more neoruscogenin than ruscogenin (G Balica et all 2007).
Ruscogenin was the main constituent in the extract of Ruscus aculeatus. The contents of ruscogenin in the underground and the aerial parts *of R.aculeatus* varied between 0.02-0.12 and 0.03-0.05 %, respectively. In general, ruscogenin contents of underground parts is higher than in the aerial parts (Tansi S. et all, 2009).
Despite its comparative rarity, *Ruscus aculeatus* has a long and diverse history of usage in Europe. Extensive usage, particularly medicinal, is putting pressure on populations, especially in eastern Europe due to excessive harvesting of the roots and rhizomes (Marossy 2006). In Turkey, Coşkun et al. (2006) reported an average annual export of 900 t of dried, cleaned roots (equivalent to 4500 t fresh mass). In sandy areas, where digging machinery could be used, they noted losses of whole populations, although in stony areas, where collecting was done using hand tools, the populations, although reduced, were still extant. But it should be noted that some uses have encouraged the planting and conservation of the species, especially as an ornamental plant across Europe (Banciu, Mitoi , Brezeanu, 2009).
Across Europe, *R. aculeatus* is given some protection as a rare and endangered species by the Habitats Directive, listed in Annex V (plant species of community interest whose taking in the wild and exploitation may be subject to management measures). A number of eastern European countries, where harvesting is more intense, have put specific conservation measures in place. In Bulgaria, harvesting in under legal

control and in Romania R. aculeatus is protected by law as a 'monument of nature' (Marossy 2006; Banciu , Aiftimie-Păunescu 2012). Climate change and invasive species may be detrimental to *R. aculeatus* (Vicente et al. 2011) in the future, but there is currently little threat to this species and it is listed as of 'least concern' by IUCN -2011 (Peter A. Thomas, Tarek A. Mukassabi (2014).

MATERIALS AND METHODS

The determinations were made in 2023 in the months of July, August and September, in a period characterized by insignificant amounts of precipitation and very high temperatures (figure 2-4).

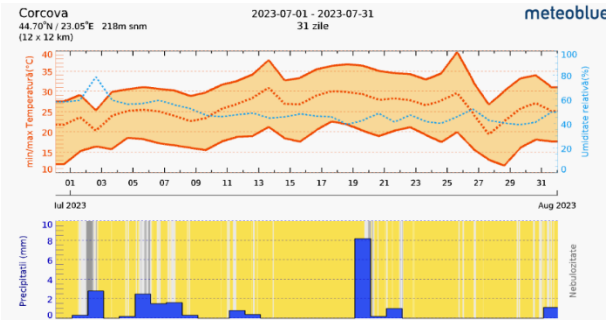

Figure 2. July 2023 - climatic conditions in Corcova

https://www.meteoblue.com/ro/vreme/historyclimate/weatherarchive/corcova_rom%c3%a2nia_680841?fcstlength=1m&year=2023&month=7

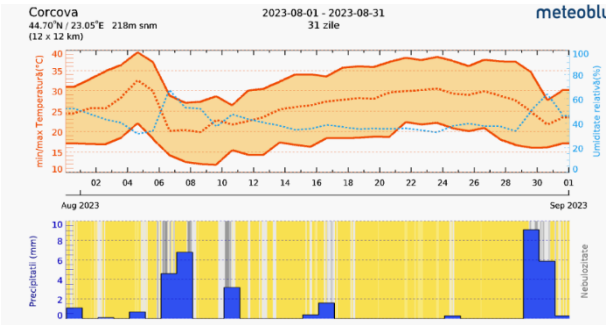

Figure 3. August 2023 - climatic conditions in Corcova

https://www.meteoblue.com/ro/vreme/historyclimate/weatherarchive/corcova_rom%c3%a2nia_680841?fcstlength=1m&year=2023&month=8

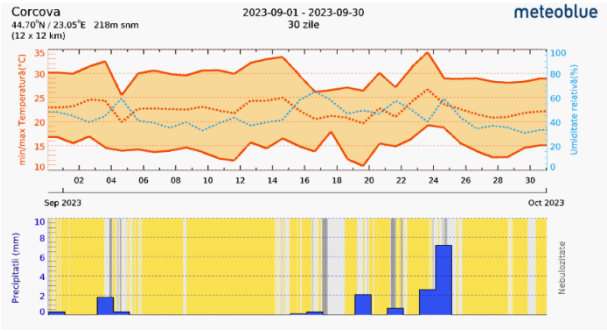

Figure 4. September 2023- climatic conditions in Corcova

The determinations were made on plants growing in the beech forest, on the border with the oak, on steep, eroded slopes, were the soil cannot retain water.

Ruscus aculeatus L. (Asparagaceae) is an evergreen, woody monocotyledonous shrub with modified photosynthetic stems (phylloclades) that grows in dry, shady forest areas. The combined drought and shade tolerance of *Ruscus* species has aroused the interest of scientists, as shade plants are normally very sensitive to drought.

Ruscus aculeatus L. is cited in the Oltenia region of Romania by Roman N. (1974), Beldie Al. (1979), Boșcaiu and others (1994), Ciocîrlan, V. (2000, 2009), Donita N. and others (2005), Costache, I. (2011).

Interesting studies have been carried out by Pivovaroff A. et all (2013). The authors elucidated the potential mechanisms that allow *Ruscus* species to survive in shaded environments prone to pronounced soil drought and studied form-function relationships based on a detailed study of traits for two species of *Ruscus aculeatus* L. and *Ruscus microglossum*, focusing on gas exchange, water regime, morphology, anatomy and nutrient and isotope composition. They then compared these trait values with published data for other species.

Mature plants were used and the measurements were made on the cladodes at the top of the branches.

The analyzed physiological indices have been the photosynthesis intensity, transpiration intensity, cuticular conductance, total water content, the

water types (bound and unbound water), the suction force and the content of pigments.

Photosynthesis, transpiration and stomatal conductance were determined with the portable Lci apparatus.

The total water content was determined gravimetrically by drying the plant material at the oven at 105 ^{0}C. The water forms (bound and unbound) were determined by the Artihovski method (Boldor O., 1983).

The suction force of the parenchyma was determined by immersing equal portions of leaves in solutions with different concentrations of sucrose, determining the isotonic solution and enforcing Avogadro's law (Boldor O., 1983).

The quantity of chlorophyll pigments from the cladodes has been determined with the Minolta chlorophyll Meter.

RESULTS AND DISCUSION

In the pedo-climatic conditions of the Corcova area, in the analyzed plants, the highest value of the intensity of photosynthesis (9.16 µmol/ m^2/s) was recorded in July at a photosynthetically active radiation value of 1003 µmol/ m^2/ s and a cladode temperature of 29^0 C

At approximately the same values of light intensity and temperature, in the months of August and September, the values of the intensity of photosynthesis were lower, but the differences were insignificant (8,91 µmol/ m^2/s, 8,56 µmol/ m^2/s (figure 5).

The diurnal variation of photosynthesis measured in July followed an upward curve until around 11 a.m., then it remained almost constant until 6 p.m., after which it began to decrease, and stopped at 8 p.m. In August, the highest value was recorded at 10 a.m. Diurnal variation of photosynthesis measured in the middle of September in a period characterized by severe drought indicated relatively constant values throughout the day, with a maximum of 8.23 µmol/ m^2/s at 10 a.m. at a photosynthetically active radiation value of 931 µmol/m^2/s (figure 6).

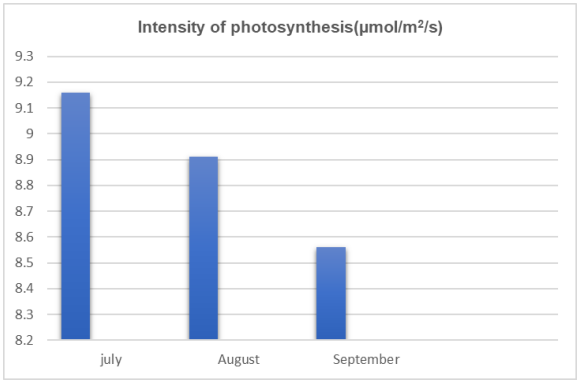

Figure 5. Maximum values of photosynthesis of cladodes of *Ruscus aculeatus* in the three months of determinations (µmol/ m^2/s)

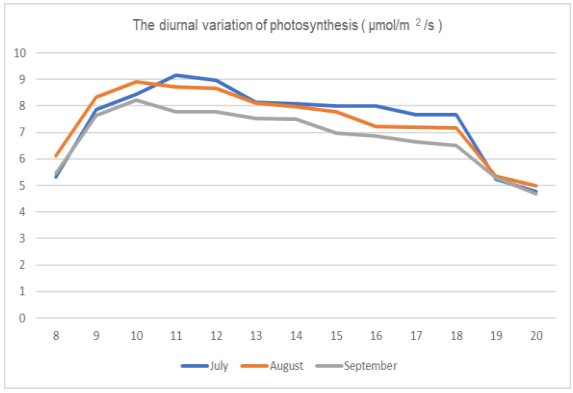

Figure 6. The diurnal variation of photosynthesis measured in three months of determinations(µmol/ m^2/s)

The diurnal variation of cuticular conductance follows approximately the same curve as that of photosynthesis, being reduced throughout the period of determinations (figure 7). Low values of cuticular conductance help to maintain water in plant but prevent the entry of carbon dioxide into the assimilating cells. This is how the low values of photosynthesis in this species can be explained.

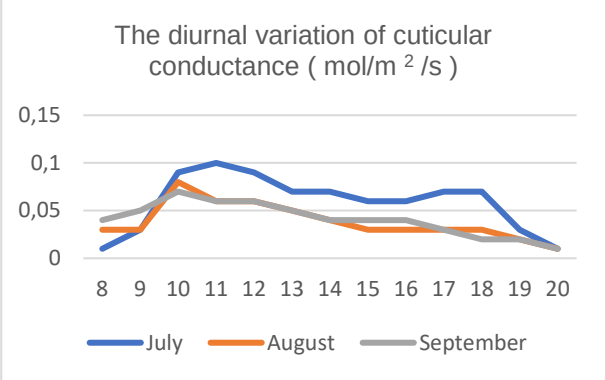

Figure 7. Diurnal variation of the cuticular conductance (mol/m^2/s) in cladodes of *Ruscus aculeatus* during the three months of determinations

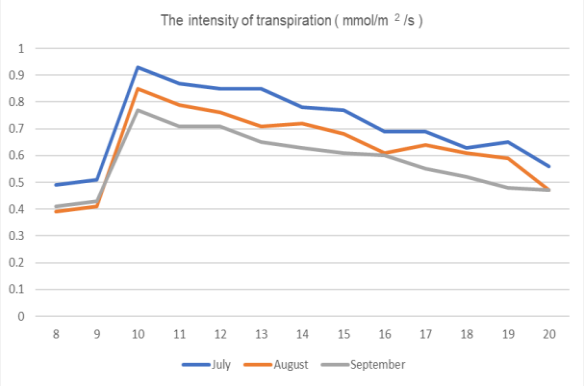

Figure 8. Diurnal variation of the transpiration intensity (mmol/m^2/s) in cladodes of *Ruscus aculeatus* during the three months of determinations

According to several authors (Roberts et al. 1984; Waring and Schlesinger 1985; Wherley and Sinclair 2009) cited by Gobin L. et all (2015), stomatal conductance decreases when the vapor pressure deficit increases.

The effect of drought on plants is complex and plants respond with many protective adaptations. During drought, plants suffer from dehydration of cells and tissues, as well as a considerable increase in body temperature. Drought does not only affect plants in different ways. Various ecological groups or even individual species have different types of drought responses (Henckel P.A, 1976).

Drought affects both tree hydraulics and C balance because trees, as with all vascular plants, respond to decreasing soil water availability with stomatal closure, thereby reducing C assimilation rates. Consequently, long-lived plants such as trees might be forced into a negative C balance, by mobilizing stored C to fulfill metabolic needs, until reserves are eventually depleted (Sala et al., 2010).

In correlation with the cuticular conductance, the intensity of transpiration has low values with small variations throughout the day (figure 8). It is interesting that in general shade plants have high cuticular transpiration, but in the case of the species *Ruscus aculeatus* this particularity does not exist.

The total water content of the cladodes remained constant during the determinations, being on average 65% for all terminal branches with variations of a maximum of one percent (figure 9).

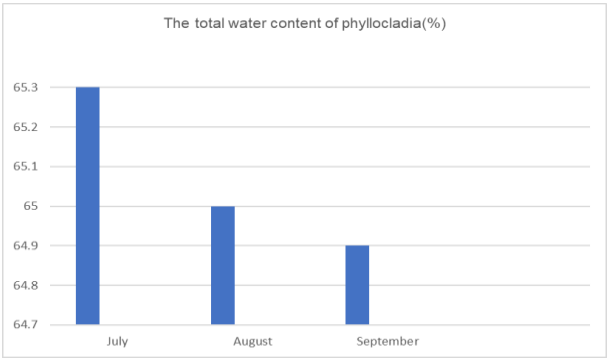

Figure 9. Total water content of cladodes (%) during the three months of determinations

The determination of the percentages of free and bound water in the cladodes indicates that *Ruscus aculeatus* presents high values of the percentage of bound water to ensure survival in conditions of water stress (figure 10).

This adaptation, correlated with reduced transpiration, allows plants to survive when the amount of water in the environment is minimal.

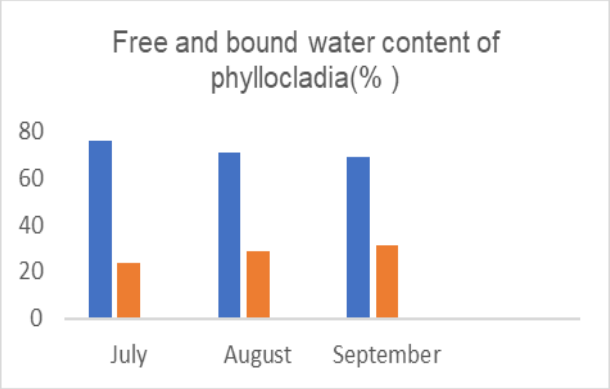

Figure 10. Free and bound water from cladodes (percentages of total water)

The suction force of *Ruscus aculeatus* rhizomes has high values, on average 23 atm (figure 11). This particularity, which is based on an increase in the concentration of cellular juice, is very important because it ensures the absorption and ascent of water in the plant's body in the conditions of a dry soil.

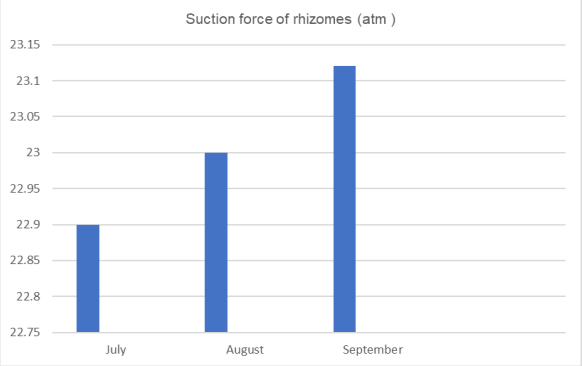

Figure 11. The suction force of Ruscus aculeatus rhizomes (atm)

The content of chlorophyll pigments does not show significant variations during the three months of determinations and has an average value of 48.5 SPAD in July, 48.5 in August and 51.3 in September for the cladodes at the top of the plants(figure 12).

The slightly higher value of the chlorophyll content in August and then in September is due to the maturation of the cladodes and the physiological accumulation of a larger amount of pigments.

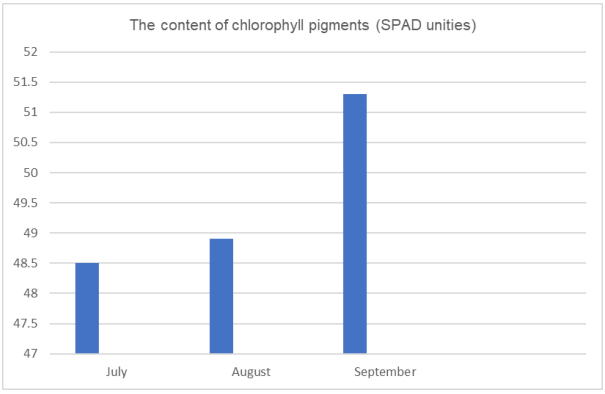

Figure 12. The content of chlorophyll pigments (SPAD unities)

CONCLUSIONS

In the climatic conditions of the S-W area of Romania, *Ruscus aculeatus* has adapted perfectly to soil drought and low atmospheric humidity. The mature specimens found in the Corcova area - Mehedinti county show small variations in the physiological parameters during the day, but also during the three months of determinations. The photosynthesis values are reduced, in correlation with the cuticular conductance values, but the reduced transpiration and high suction force, as well as the high percentage of bound water ensure the survival and growth of plants in these conditions.

REFERENCES

Balica, G., Vlase, L., Deliu, C., Tămaş, M.,Crişan, G. (2007), Comparative phytochemical study on rhizome and tissue culture of *Ruscus aculeatus* L. , *Planta Medica*, 73, P226

Banciu, C. , Aiftimie-Păunescu, A. (2012) *In vitro* propagation of rare species *Ruscus aculeatus* L. and histological peculiarities of the regenerants. *Analele Universităţii din Oradea–Fascicula Biologie*, 19, 67–73.

Banciu, C., Mitoi, M.E. & Brezeanu, A. (2009) Biochemical peculiarity of in vitro morphogenesis under conservation strategy of Ruscus aculeatus L. Annals of Forest Research, 52, 109–116.

Beldie, Al. (1977, 1979). Flora României – determinator ilustrat al plantelor

vasculare. Editura Academiei Române, București: I: 412 pp.; II: 406 pp.

Boldor, O. (1983), Fiziologia plantelor lucrari practice, Editura Didáctica si pedagocica Bucuresti

Boșcaiu, N., Coldea, Gh., Horeanu, C. (1994). Lista roșie a plantelor vasculare dispărute, periclitate, vulnerabile și rare din Flora României. *Rev. Ocrot. nat. med. înconj*. București, 38(1): 45-56.

Ciocîrlan, V. (2000, 2009). *Flora ilustrată a României. Pterydophyta et Spermatophyta.* Editura Ceres. București: I: 1038pp.; II: 1141pp.

Costache, I. (2011). *Flora și Vegetația bazinului hidrografic inferior al râului Motru.* Vol.I. Editura Universitaria Craiova, 215 pp.

Coşkun, M., Güvenç, A., Kiliç, C.S. & Arihan, O. (2006) Ruscus aculeatus trade in Turkey: is it sustainable? Planta Medica, 72, P-298.G. Scholar

Doniţă, N., Popescu, A., Paucă-Comănescu, M., Mihăilescu, S., Biriș, I. A. (2005). *Habitatele din România*. Editura Tehnică Silvică. Bucureşti, 496

Doniţă, N., Popescu, A., Paucă-Comănescu, M., Mihăilescu, S., Biriș, I. (2005). *Habitatele din România* - Modificări conform, amendamentelor propuse de România și Bulgaria la Directiva Habitate (92/43?EEC). Editura Tehnică Silvică. Bucureşti

Gobin, R., Korboulewsky, N.,Dumas, Y., Balandier, P. (2015), Transpiration of four common understorey plant species according to drought intensity in temperate forests, Annals of forest science.https://annforsci.biomedcentral.com/articles/10.1007/s13595-015-0510-9

Henckel, P.A. (1964), *Physiology of plants under drought*, K.A. Timiryazev Institute of Plant Physiology, USSR Academy of Science, Moscow
https://www.annualreviews.org/doi/abs/10.1146/annurev.pp.15.060164.002051?journalCode=arplant.1

Marossy, A. (2006) Wild plant species endangered through intensive harvest. *Nature Conservation* (eds D. Gafta & J. Akeroyd), pp. 65–67. Springer, Berlin, Germany.

Pivovaroff, A.,Sharifi, R., Scoffoni, C., Sack L.,Rundel, P.(2013), Making the best of the worst of times: traits underlying of *Ruscus aculeatus* and *Ruscus microglossum*(Asparagaceae), National Library of Medicine
https://pubmed.ncbi.nlm.nih.gov/32480962/

Roman, N. (1974). *Flora și vegetația din sudul podișului Mehedinți*. Editura Academiei Române, București: 222 pp.

Sala, A., Piper, F., Hoch, G. (2010) Physiological mechanisms of drought-induced tree mortality are far from being resolved. New Phytol. 186, 274–281.

Tansi, S., Karaman, S. , Toncer, O. (2009) Ecological and morphological variation in wild *Ruscus aculeatus* from Mediterranean region of southern Turkey. *Acta Horticulturae*, 862, 175–183.Google Schollar

Thomas, P.A.,Mukassabi, T.A.(2014) Biological Flora of the British Isles: *Ruscus aculeatus*, Journal of ecology
https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/1365-2745.1226

https://www.meteoblue.com/ro/vreme/historyclimate/weatherarchive/corcova_rom%c3%a2nia_680841?fcstlength=1m&year=2023&month=9
https://www.meteoblue.com/ro/vreme/historyclimate/weatherarchive/corcova_rom%c3%a2nia_680841?fcstlength=1m&year=2023&month=8
https://www.meteoblue.com/ro/vreme/historyclimate/weatherarchive/corcova_rom%c3%a2nia_680841?fcstlength=1m&year=2023&month=7
https://pubmed.ncbi.nlm.nih.gov/32480962/