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Observations on the biomorphology of the Sicilian endemic *Trifolium bivonae* (*Fabaceae*)

Abstract

Colombo, P.: Observations on the biomorphology of the Sicilian endemic *Trifolium bivonae* (*Fabaceae*). — Fl. Medit. 12: 275-283. 2002. — ISSN 1120-4052.

Anatomical observations at the S.E.M. have been carried out on *Trifolium bivonae* Guss., an endemic species occurring in northern Sicily only on flyschoid substrate. In order to clarify possible signs of the plant response to ecological factors, the research has been conducted both by analysing the foliar epidermis and the morphology of the root. The latter seems to play an important role in determining the plant adaptation to the environment.

Introduction

Trifolium is one of the most important genera of the *Fabacea*e family not only for its agricultural importance, but also for the great number of species included there (Zohary 1972). Its distribution concerns the temperate regions of both hemispheres, however, the Mediterranean is the largest area where it occurs and probably its centre of origin (Gillet 1952). On the grounds of morphological, cytological and geographical similarities, Melchior (1964) established the tribe *Trifolieae* and included there 5 genera. Among these *Trifolium* is the most advanced and its distribution covers extensive areas ranging from the sub-arctic to the sub-tropical, tropical and equatorial regions of Africa and South America. The species, however, only grow in specific natural habitats: al least 2/3 of them occur in restricted habitats such as the top of the mountains, rocky slopes, forests, logged-off lands, meadows. In Sicily, many species are found on the mountains and preferably on siliceous or calcareous substrata. Other species occur on clayey substrata (*T. congestum*), while others grow in forests (*T. pratense*) or in grazing, in humid sites (*T. repens*, *T. resupinatum*) while no species is found along sandy littoral.

Trifolium bivonae Guss. is endemic to northern Sicily where it occurs only on Flyshoid substrata. It was included in the *Section* Lotoidea (Coombe 1961). It is a scapose hemicryptophyte with ephemeral stems, leaves and shoots owing to the particular seasonal trend.

Material and methods

The plant material collected from the wild, after preliminary staining-fresh observations, was fixed in F.A.A. and later dehydrated, coloured with safranin and dipped in paraffin.

10-15? thin sections were obtained using a revolving type microtome and were later mounted on Canada balsam. Additional materials were later mounted on JB-4 resin, sectioned in 5-7? sheets and coloured with toluidine blue following Feder and O'Brien.

Replications were obtained from the leaves in order to highlight the morphological aspects. The observations consisted in determining both the number and size of the epidermal cells, the number and position of the stomata, the presence of hairs, the thickness and number of strata forming the upper and lower epidermis as well as the thickness of the leaf on the whole and beside of the mesophyll, of the palisade and of the spongy parenchyma.

In order to highlight the fine pattern of veins in the leaves, the Fuchs method (1963) was applied. The terminology employed for the pattern of veins follows Hickey (1973), whereas the anatomical terms are those employed by Esaù (1965). The samples were later brought to critical point, then metal sprayed and finally observed at the S.E.M.

Results

Anatomy of the aerial portion.
The leaf, the stalk, the stem and the inflorescence were sectioned.

- Leaf.
The leaves on the stem are all amphystomatic and thin (179 ?), while the protruding central ribbing is 342 ? thick. The edge is round and facing upwards, with bundles of sclereids and fibres permeating the mesophyll under the epidermis. The upper surface of the lamina consists of round and convex cells covered with a grid-like texture. Fig. 1-3 show the general aspect of the epidermal cells and the arrangement of the ribbing consisting of pairs of elongated and hollow cells. As for the quantitative aspect of the epidermal parameters the table in fig. 7 shows the mean values of the superior and inferior caulinar portions as well as of the basal ones. Tiny and hollow stomata are spread in both upper and lower blades (Fig. 4). Their number and dimension, according to the different groups of leaves, are also given in the above mentioned table (Fig. 7).
The lower blade is quite different. The sheath is striped (Fig. 2-5), with elongated cells that are larger and less convex. The stomata, arranged more superficially, are different, larger, bullate and mixed with articulate vesicular hairs, with fans of cuticle threads branching off their basis. On the margin, teeth are provided with the hydathodes at the apex (Fig. 6). The transversal section shows collateral vascular bundles provided with a sclerenchymatous covering round the liberian portion. The leaf architecture, according to Hichey (1973, 1979) is craspedodromous (Fig. 8), with secondary veins not reaching the margin indentation. The veins are cladodromous, being bifurcate at the margin.
In general, the discoloured leaf shows a pattern of central veins and 12 pairs of symmetrical secondary veins which bifurcate repeatedly as they diverge from the central pattern (Fig. 8). The panels are regular, the areolas large and irregular, and there are loose branches with dichotomous branchings. The most evident characteristic of the leaves is the dif-

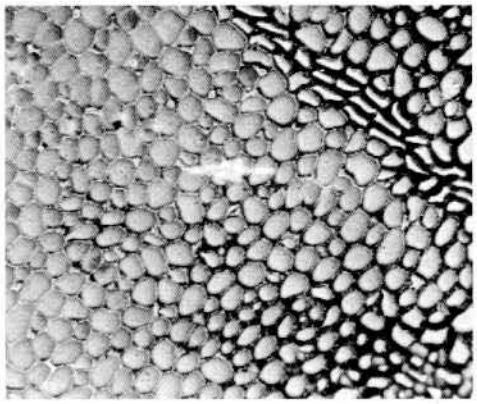


Fig. 1. Epidermal replication, upper leaf blade (optical microscope).

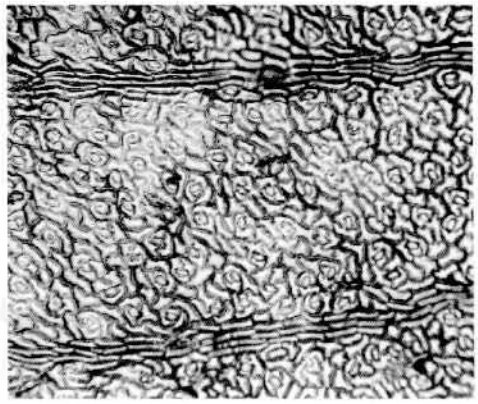


Fig. 2. Epidermal replication, lower blade (optical microscope).

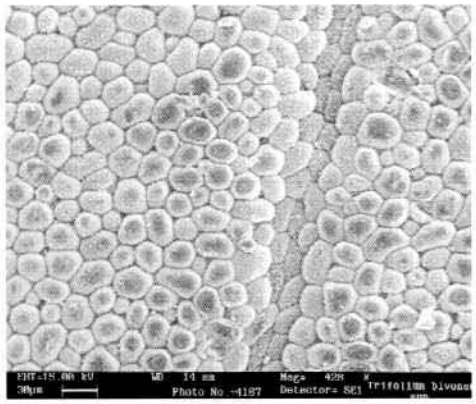


Fig. 3. Epidermis of upper blade (S.E.M.).

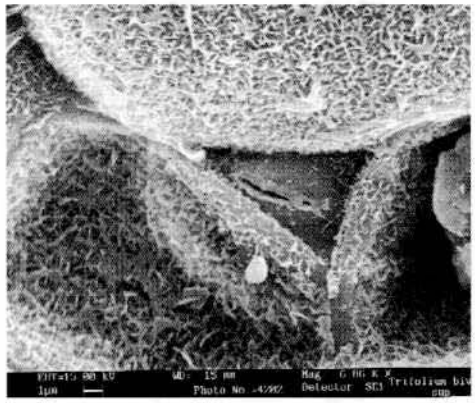


Fig. 4. Stomata in upper blade.

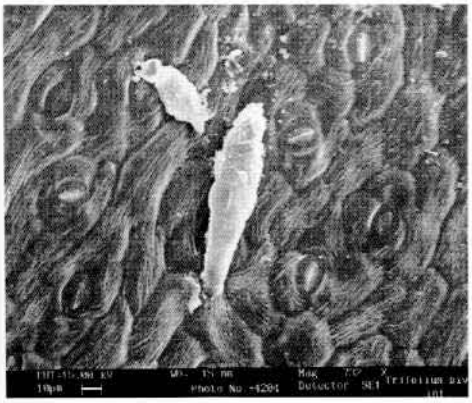


Fig. 5. Cuticle threads and hairs (S.E.M.).

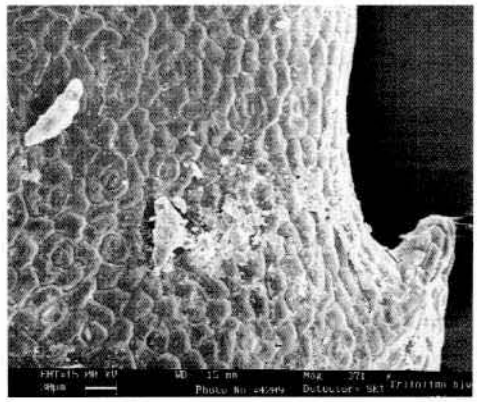
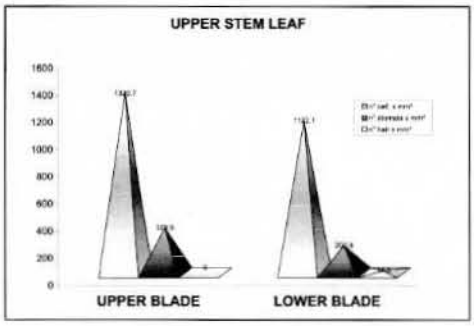
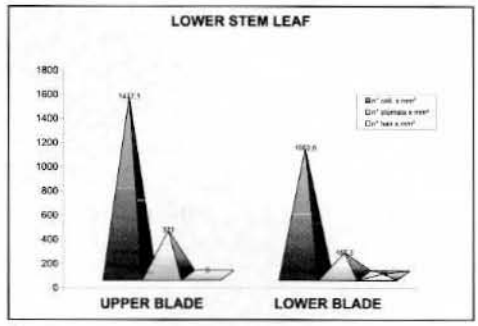
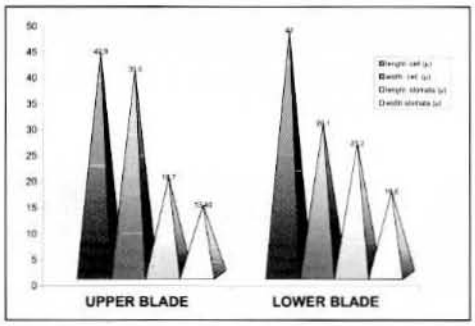
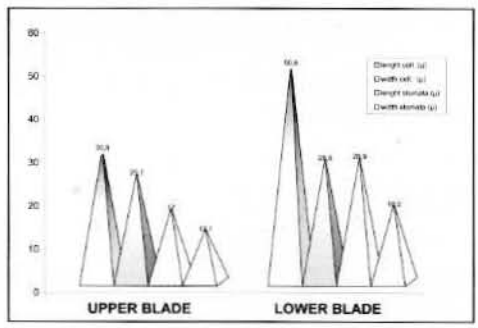


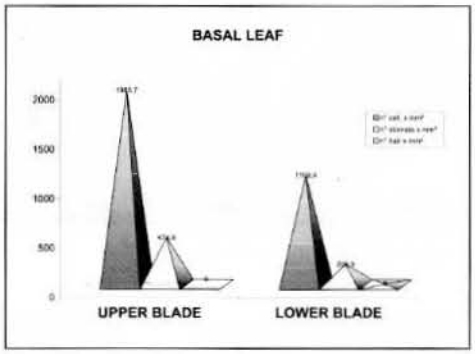
Fig. 6. Hydathodes (S.E.M.).

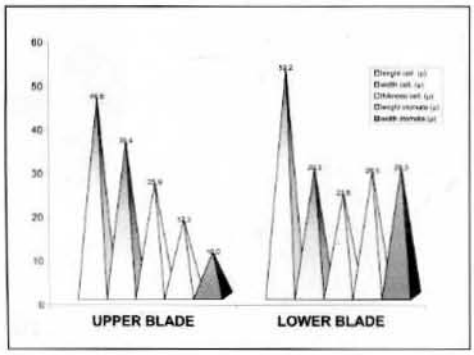












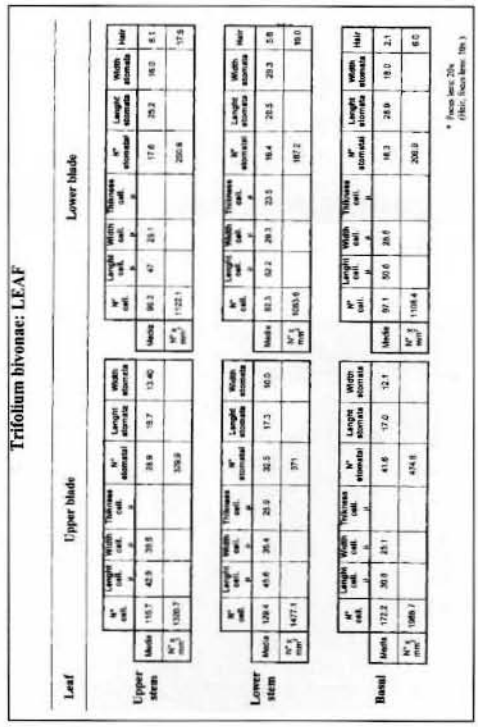


Fig. 7. Epidermal parameters.

ference in size between the primary and the secondary veins, the latter being very thick, strongly lignified and consisting of numerous sclereid bundles running across the bundles (Fig. 11). Additionally, an external secreting apparatus is often present to which the lateral secondary veins are attached together with an internal secreting apparatus in the mesophyll, with mucilaginous cells often including crystals (Fig. 9-10). Very often the mucilaginous cells are closely connected to the vascular tissue. By colouring the leaf with ruthenium red (Catalano, 1925), the mucilage becomes deep red quite visible under the microscope.

- Stem

The transversal section shows a thin monostratified epidermis with chlorophyllian cortical parenchyma and about ten open collateral bundles showing a thick covering of sclerenchimatic fibres protecting the liber (Fig. 12). The xylem is made up of endarch protoxylem and exarch metaxylem, both digitate towards the pith, which in its turn is made up of large elongated cells with thin walls. Among the vascular bundles there lies a slight deuteroxylem ring, wich does not generate a complete secondary apparatus since the prostrate-ascending stems die at beginning of winter.

- Flowers

The head was sectioned and single flowers were observed in their different stages of growth and ripening that always proceed in a centrifugal direction (Fig.13).

Trifolium bivonae has typical cleistogamic flowers not suitable for pollination by bees or other insects that has never been recorded. This aspect agrees with the fact that autogamous species are quite frequent in the *Section* Lotoideae.

Anatomy of the hypogeous apparatus

- Root

The root is thick and penetrates deeply into the soil, reaching 90 cm or more. It has a diameter of about 4.5 cm near the collar decreasing down to 0.25 cm at the apex (Fig. 14). The root develops perpendicularly to the surface. Seconndary plagiotrop roots are absent. It is to be noted that this species is exclusive to deep soil where the roots can penetrate without coming across any barriers.

The transversal section of the root shows a multi-layered suberous exoderm isolating the root from the surrounding environment. Beneath that there is a parenchymal cortex with large funnel-shaped pith rays due to large dilated rays starting at the furthest areas of the cortex and becoming thinner towards the central pith passing through the phloem and the xylem. This is undoubtedly a secondary, perennial structure where the meta and the deuteroxylem elements mix with parenchyma and fibres at regular intervals.

The seasonal interval is quite evident: at least 10-15 seasonal rings can be counted although a greater number could be confirmed in the older portions of the root (Fig. 15). The number of the seasonal xylem rings is related to the liberian structure where the secondary elements overlap at regular intervals, that is a layer of fibres a layer of phloem with annexed cells alternate. Considering that there are two layers of cells forming during the vegetative periods, the counting of the seasonal rings in the xylem is confirmed.

Fig. 8. Leaf architecture.

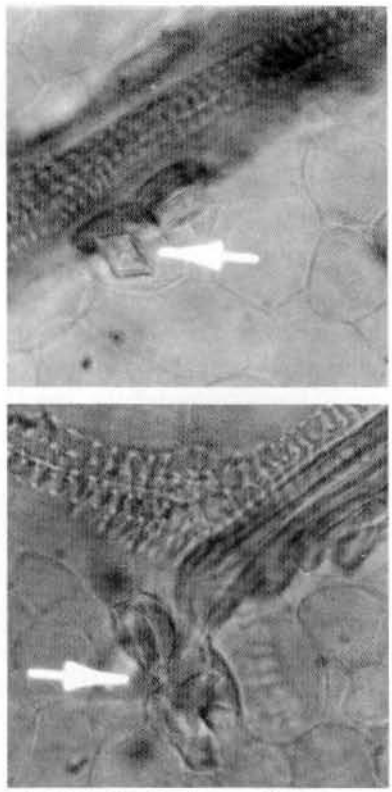


Fig 9-10. Mucilaginous cells and crystals.

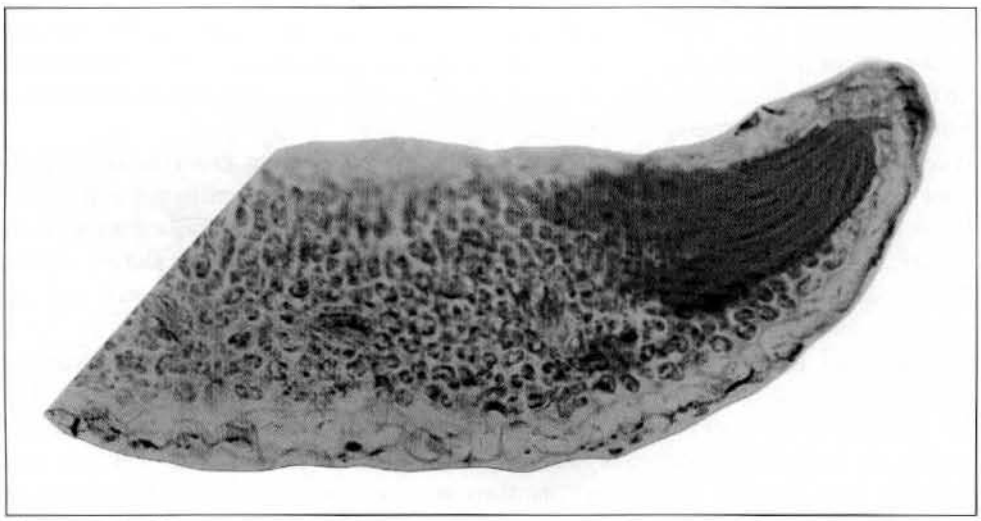


Fig. 11. Sclereids.

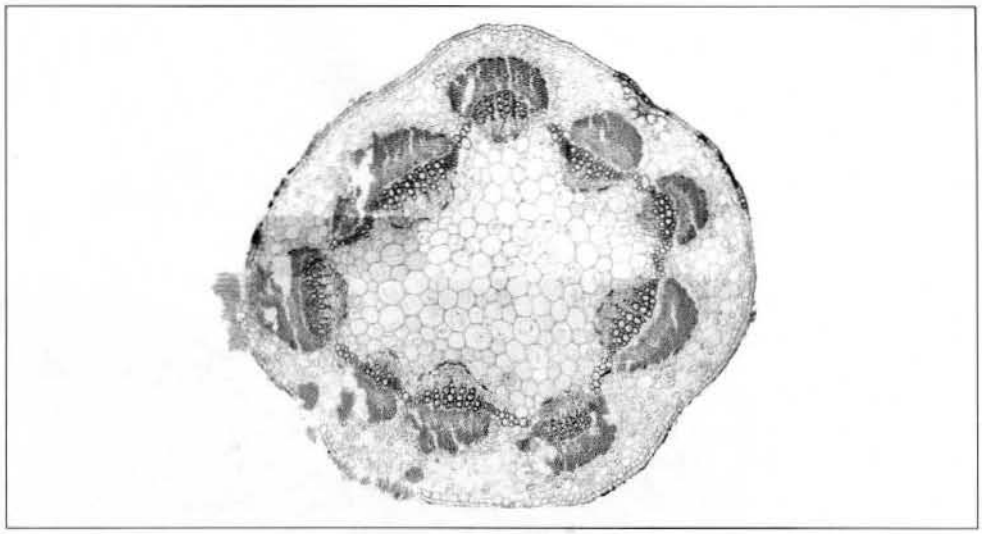


Fig. 12. Stem.

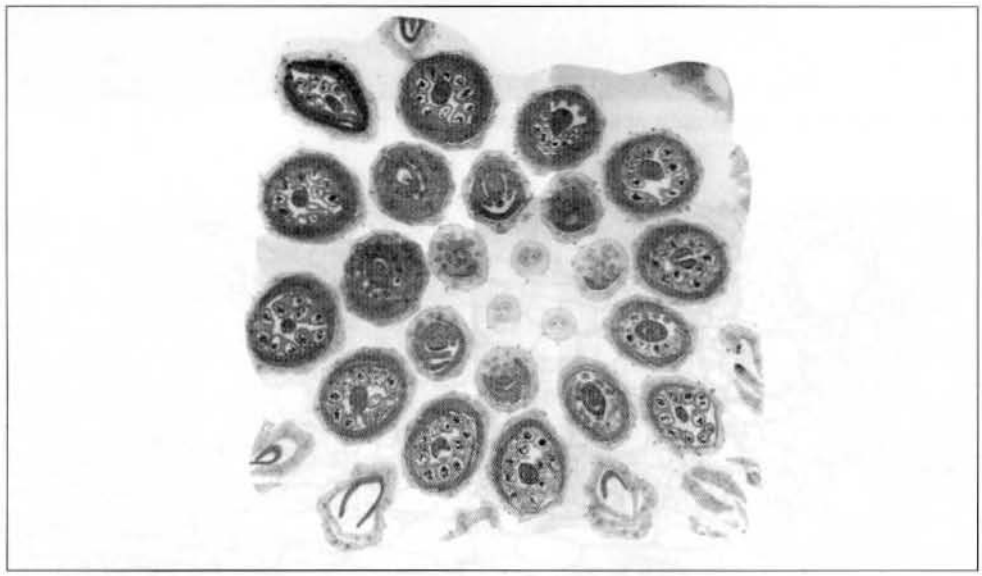


Fig. 13. Flower head.

Discussion and conclusions

The anatomical observations show that the foliar epidermal parameters vary more or less deeply depending on the position of the leaves in the rosette. This is evidentn in the basal part of the rosette, where an increase number of stomata can be are found, whose dimension does not vary and does not depend on the level of the rosette. The same happens with the

Fig. 14. *Trifolium bivonae*: the root neck.

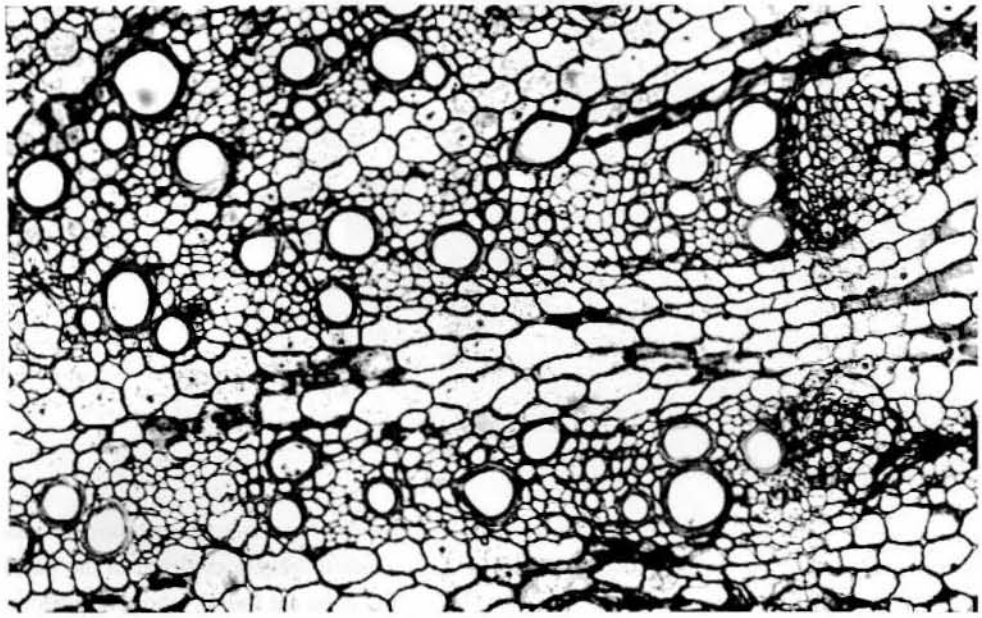


Fig. 15. Transverse section of the root coloured by Toluidina Blue.

epidermal cells: their number by mm^2 does increase noticeably when the basal leaves of the head are examined. A significant decrease in dimension is observed in the basal rosette. The upper epidermis of the leaves is generally glabrous, whereas on the lower one glandular hairs are found more abundant in the apical stem leaves and less abundant near the base. These data show that the leaves of the more superficial part of the rosetta react to the external and environmental factors by modifying the position and the number of the stomata and by adapting their epidermal cells. The radical leaves, on the contrary, show mesophyll characteristics because they grow in a more protected environment. The most interesting characteristics of *Trifolium bivonae* are clearly the signs of the seasonal cycles found in the root, despite its length and its depth. It is also very sensitive to mechanical stresses as it can only penetrate the soil if there are no significant obstacles. The Flysch is therefore the ideal substratum for the plant to put down roots. As a matter of fact, *T. bivonae* only occurs on Flysch substrate and its on limestone vicarious, *T. physodes* Steven, growing on limestone, has a quite different root apparatus. Its roots are indeed shorter and fibrous and the plant has scapose characteristics whereas *T. bivonae* has semi-scapose characteristics. Therefore *T. bivonae* is strongly influenced by the ecology of its root.

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