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MORPHO-ANATOMICAL CHANGES OF PLANT VEGETATIVE ORGANS OF OLIVE (*Oleea europaea*) TREE CULTURE TREATED WITH PESTICIDES

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Abstract. The objectives of this study were to assess and identify structural changes caused by pesticide treatment in *Olea europaea* olive tree cultures and *Avena fatua* plants from olive groves. For this purpose morpho-anatomical characteristics were assessed on shoots, roots and leaves to correlate the effect of pesticide treatment with anatomical and morphological aspects of leaves. This study concluded that the leaves treated with pesticides have less stomata and more numerous tector hairs as an adaptation to toxic treatment, compared with organic leaves from untreated cultures. These leaf abnormalities can seriously affect the efficiency of respiration, photosynthesis and the hydric control of the plants. Large intercellular spaces were observed in the foliar mesophyll of *Olea europaea* treated with pesticides. Differences in vegetative aerial organs were observed between treated and un-treated *Avena fatua* samples, namely the central parenchyma of stem was not reabsorbed and there were more layers of hypodermic sclerenchyma in treated leaves.

The results of the study should be heeded as a warning for all olive producers who use pesticides excessively and in an uncontrolled manner, in addition to encouraging the wider implementation of organic farming methods.

Keywords: *Olea europaea*, *Avena*, morpho-anatomy, pesticides.

INTRODUCTION

The olive plant *Olea europaea* is a member of the family *Oleaceae*. It is an evergreen tree or shrub that grows up to 12 m in height with a spread of about 8 m, but the tree can be maintained at a height of approximately 5 m with regular pruning. Many different subspecies within the genus *Olea* are found around the world with at least six subspecies of *Olea europea* L. identified, each with a specific geographical distribution, of which *Olea europaea* subsp. *europaea* corresponds to the Mediterranean or European olive [12]. The knowledge of the medicinal properties of the tree (*Olea europaea*), date back to the early 1800's, where it was used as a treatment for malarial infections. The doctors at that time stated that the properties of the tree, *Olea europaea*, deserved more extensive investigation [13]. Now, is knows that olive oil are hypoglycaemic [5], antioxidant [4], or arterial hypotensive [3, 7] effect. Olive leaf extract may have a role in regulating the composition of the human gastric flora by selectively reducing levels of H. pylori and C. jejuni. [9]. According to Arvanitoyannis and Kassaveti, 97% of the world's olive oil production is concentrated in the Mediterranean basin countries: Spain, Portugal, Italy, Greece, Turkey, Tunisia and Morocco [2]. Spain, Greece and Italy are the main three producers within the European Union, accounting also for approximately 80% of olive oil production world-wide [7].

Avena fatua (common wild oat) is a paniculate gramineae. *A. fatua* look similar to cultivated oats, preferring heavy soils, rich in calcium, moisture, clay and argillaceous minerals, and an optimum germination depth up to 20 cm *A. fatua* has linear leaves, the youngest being rolled and, dark green in colour, the first leaf without a stipule; the foliar limb is characterised by tector hair, without cross venation [11]. *Avena fatua* is frequently encountered in olive groves often being managed by various types of herbicides, or by mechanical methods.

Unfortunately, excessive accumulations of herbicides and pesticides have been observed in various agricultural products used for human consumption, and can have both dangerous and detrimental effects on the human body, including cutanate reactions, allergies, changes in melanin synthesis [6], or abnormal development in children [8]. The accumulation of several herbicides and pesticides has also been discovered to have potential carcinogenic effects [10]. These health warnings coupled with repeated food safety scares, animal welfare concerns and reservations regarding the impact of industrial agriculture on the environment [12] have led to an increase in consumer demand for environmentally friendly, chemical-free organic food. As a result, agriculture has shifted towards more organic methods in recent years, with the number of farmers taking an active interest in organic management continuously increasing [12]. Organic farming can also be implemented to enhance biodiversity through effective landscape management and crop regimes, whilst also improving the quality and abundance of crops themselves.

Increased pesticide use can have effects on plant species at a morpho-anatomical level, potentially indicative of high levels of accumulation. Morpho-anatomical analyses can therefore be used to determine the effects of pesticide application on different plant species, and is used here to indicate the effects of pesticides on both *Olea europaea* and *Avena fatua*.

MATERIALS AND METHODS

Organic untreated and pesticide-treated *Olea europaea* samples were collected from the South-West of Samos Island in Greece (samples indicated in Table 1). The organic sample was not treated with pesticides and was tested as a control (Fig. 1), whilst the treated sample had been exposed to pesticide 'Roundup'.

Imprinting of superior and inferior epidermis of foliar limbs was used to observe the stoma on the leaf surfaces by applying a colloidal draw on the leaflets [1]. The number of stomas/microscopic field and their dimensions (length/width) were determined using an optical microscope (Leitz brand, Webster M), using a 10X object lens and 7X ocular. Osteol aperture was measured using a 40X object lens and 7X ocular. The micrometric index was calculated using the method described by Andrei and Paraschivoiu [1]. Photos were captured using a digital camera integrated into the microscope.

Table 1. Vegetal material studied in this experiment.

Species	Type of examined organ	Type of study	Type of olive culture
Olea europaea	- foliar limbs - petioles	- morphologic - anatomic	- ecological - treated with pesticide
Avena fatua (from olive cultures)	- roots, - stems, - foliar limbs	- morphologic - anatomic	- ecological - treated with pesticide

Figure 1. The difference between two neighbouring olive groves (*Olea europaea*), on the Samos Island, Greece. The foreground indicates the culture treated with herbicides, whereas the background section is an organic culture (*Ormos - Marathokampos, May, 2009*).

Sections of plant material were created manually using shaving blades in a transversal plan, and were preserved in 70% alcohol [1]. Plant material was wetted throughout, and sections were placed in tap water at laboratory temperature using the blade. Colouration was done in a Petri dish using 'Congo Red' colouring and green iodine to indicate unlignified cellular walls. These sections were kept in tap water before being immersed in 'Congo Red' for 30 seconds, and were then washed and coloured with green iodine. Sections were then placed on a mount in a drop of water and covered with a slide. The handling of sections was carried out with a lot of care using a spatulate needle. After colouring, the 10 clearest coloured sections were chosen per sample. These were immediately analyzed using an optical microscope and the most representative images were taken with the adapted digital camera.

RESULTS

Olea europea

By morphological point of view, at leaf epidermis of *Olea europea* tree cultures the stomata were observed to be deeper in the epidermis, preventing excessive evapo-perspiration, and the imprinting method here was not identified. Tector hairs were observed to have a stellate hat on a foot (Fig. 2A - D) and were numerous, covering all of the epidermis, especially on inferior epidermis, for protection from water loss.

In cross section, the leaf of the organic untreated *Olea europea* culture shows the median nervure is right on adaxial side (Fig. 3A) and indicates slight protuberance in the leaves compared to the culture treated with pesticides because of the mechanic tissue cells in the upper of conductive tissue (Fig. 3B), and to the abaxial side, unlike the untreated culture (Fig. 3D). In addition, the untreated culture has more obvious leaf protuberance (but not very advanced) than the pesticide treated samples, due to the presence of sclerenchyma arc (Fig. 3E) on the periphery of phloem. *Olea europaea* foliar limb presents both epidermises with slightly oblong cells, perpendicular to the foliar mesophyll cells (Fig. 3F & G). Foliar mesophyll (with bifacial and dorsiventral structure) is differentiated into two or three stratified palisade tissue at the upper side (Fig. 3G) and spongy tissue on the bottom, showing cells with large intercellular spaces on leaves on samples treated with pesticides (Fig. 3C), compared with the untreated control.

No differences were observed between untreated and pesticide treated *Olea europea* leaves at petiol level, even in terms of periphloemic mechanic ring. Although the sclerenchyma tissue around the phloem vessels was not observed at petiol level in the control samples, it is present (Fig. 3 H & I). Petiol conductive tissues formed beams, very close between them, arranged in a circle. Pithy rays and the moderate sclerefied and lignified tissue come in contact with the mechanic ring consist of sclerefied fibre. In the centre there is central fundamental parenchyma.

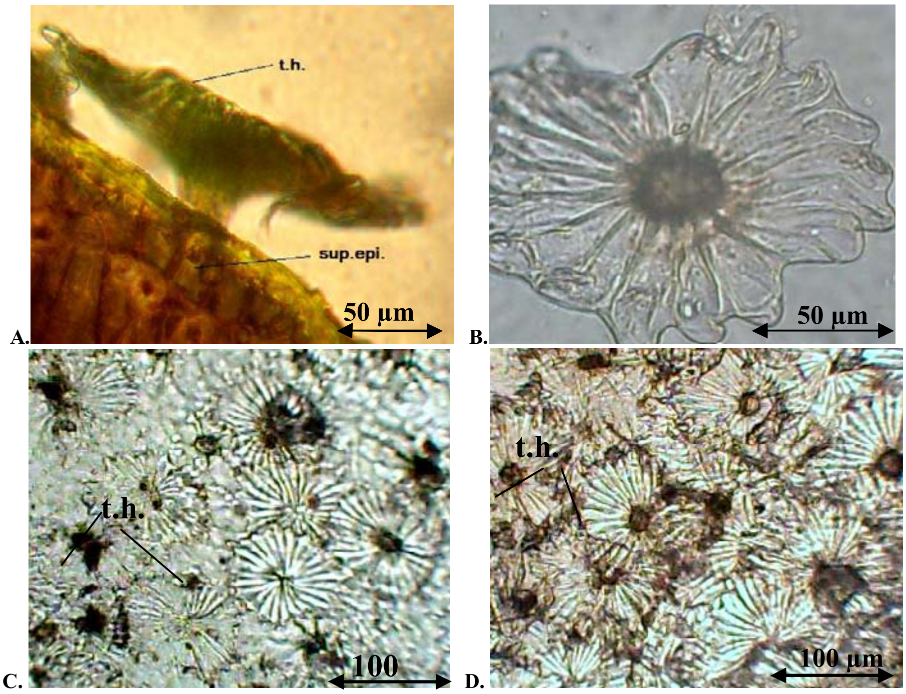

Figure 2. Leaf epidermis morphological aspects of *Olea europea* culture treated with pesticides: A - tector hair (profile); B – A - tector hair (upper) C - Superior epidermis; D – Numerous tector hairs on inferior epidermis (epi.sup. – superior epidermis; t.h. – tector hairs).

Avena fatua

The stomata of the *A. fatua* leaves were observed to be of barr-bell form, with nervure in parallel disposition (Fig. 4A & B). The leaves are amphy-stomatic. 10 stomatas/microscopic fields were identified on the superior epidermis (Fig. 4A) on both pesticide-treated and untreated samples. On pesticide-treated *A. fatua* samples, the number of stomata observed on the inferior epidermis was 25% less than the stomata observed in the untreated control sample (Fig. 4B). The untreated control had a stomata media frequency of 12 stomata/microscopic field on the foliar limb compared to 9 stomata/microscopic field on the pesticide-treated leaves at the same microscopic field.

The tector hairs on *A. fatua* leaves were unramified and numerous, especially on inferior epidermis; superior and inferior epidermis hair numbers are increased on treated leaves compared to the untreated control, probably a protection effect caused by pesticide-treatment solution.

No differences were observed between anatomical structures in transverse sections through the roots of *Avena fatua* between the untreated control and pesticide treated samples. Stem structure is primary, with an unistratified epidermis followed by the central cylinder, and a sclerified cortex. Conductive tissue was distributed to the periphery and the centre of organs only in untreated control samples (Fig. 5C). In the untreated samples the hypodermic sclerenchyma layer was observed to be less well represented having 3-4 rows of cells, compared to plant samples treated with pesticides, having 6-8 rows of sclerified cells on the stem periphery under the epidermis (Fig. 5D), and the centre of stem was occupied by a consistent pithy parenchyma.

An adaxial proeminence was observed in transverse sections of untreated *Avena* leaves (Fig. 5E) which was not present on treated samples (Fig. 5F). However, a number of layers of fundamental parenchyma and mechanic tissue (7-8 layers) were identified as being identical between treated and untreated samples of the foliar limb above the conductive tissue of the principal nervure, so that conductive tissue is located at the bottom of nervure. 3-4 layers of fundamental parenchyma cells were identified under the phloem, but they are not very long, because foliar mesophyll is well below under conductive tissue, forming true bays in fundamental tissue mass. Under the epidermis, 3-4 layers of tissue mechanic were observed at the abaxial side, which was also present at petiole level, but their numbers were equivalent between treated and untreated samples. A possible explanation for the number would be the supporting role of the petiol, in the leaf, which was increased in pesticide-treated leaves. The mesophyll consists of non-radiate chlorenchyma and adaxial without palisades in both treated and untreated samples. Bulliforms is present in discrete, regular adaxial groups.

DISCUSSIONS

O. europea leaf samples treated with pesticides cultures have numerous tector hairs (as an adaptation to toxic treatment), compared to the organic untreated control. The foliar mesophyll of treated *O. europea* leaves was observed to have larger intercellular spaces compared to untreated leaf samples.

Pesticide-treated *Avena fatua* samples were observed to have an adaptation system to against pesticide exposure on the inferior epidermis; stomata number was observed to decrease whilst tector hairs frequency increased on pesticide-treated samples. From an anatomical perspective, the differences were observed between untreated and treated plants were only related to the level of air, namely: the centre of

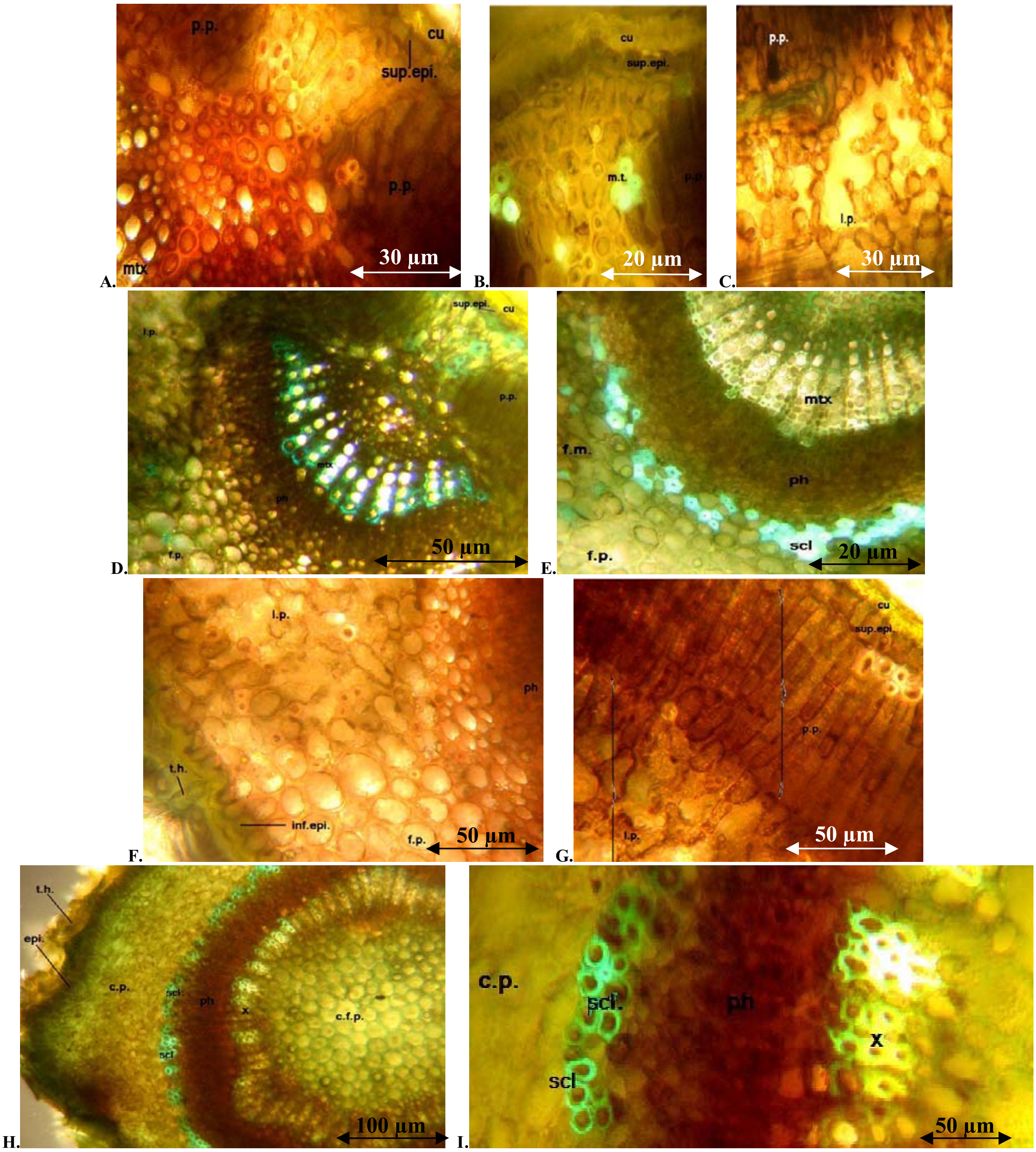

Figure 3. Anatomical aspects of foliar limbs (A – G) and petiol (H –I) f olive untreated leaves (A, D, F and H) or treated with pesticides leaves (B, C, E, G and I) (cu – cuticle; c.p. – cortical parenchyma; c.p.f. – fundamental central parenchyma (medullar parenchyma); epi – epidermis; inf.epi. – inferior epidermis; f.p. - fundamental parenchyma; f.m. – foliar mesophyll; l.p. – lacunose parenchyma; m.t. – mechanic tissue; p.p. – palisade parenchyma; ph – phloem; scl – sclerenchyma; sup.epi. – superior epidermis; t.h. – tector hair; x – xylem).

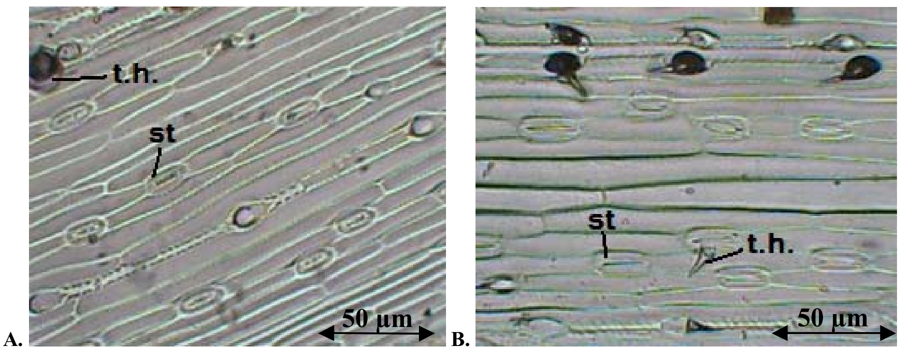

Figure 4. Epidermis aspect of *Avena fatua* leaves: superior epidermis of untreated leaves (A) and inferior epidermis of treated with pesticides leaves (st – stomata; t.h. – tector hairs).

stem is resolve (straw type) and hypodermic sclerenchyma layer was observed to be thinner in the untreated plants (control) compared to those treated. Adaxial protuberance was present on untreated leaves not due to layers of mechanic tissue, but was not identified on treated samples which had a plate on the upper.

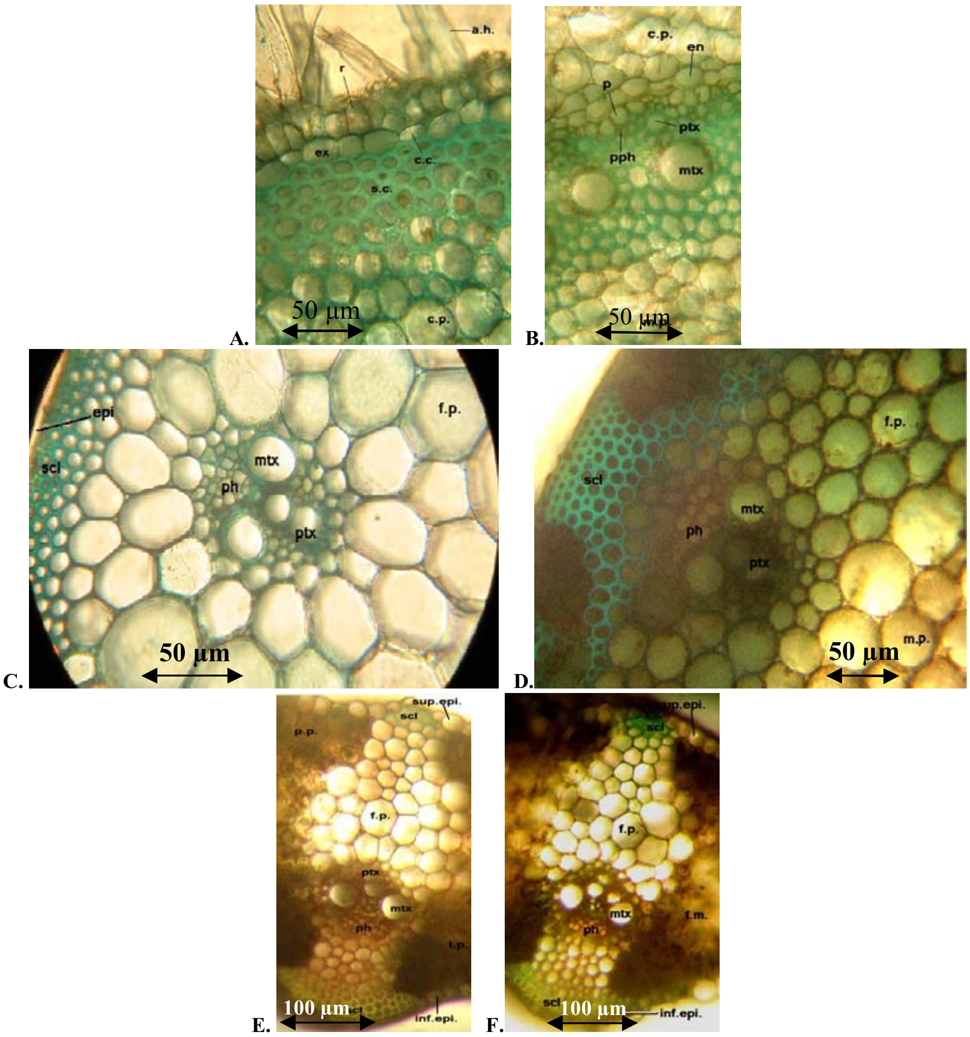

Figure 5. Anatomical structure of root (A and B), of stem (C and D) and of leaf (E and F) of *Avena fatua* provided from untreated plants (control) (A, C and E) or treated with pesticide (B, D and F) (a.h. – absorbent hairs; c.c.- communication cell; c.p. – cortical parenchyma; epi – epidermis; en – endodermis; ex – exodermis; inf.epi. – inferior epidermis; f.p. – fundamental parenchyma; mtx – metaxylem; m.p. – medullar parenchyma; p – pericicle; pph – protophloem; ptx – protoxylem; r – rhyzordermis; s.c. – sclerefied cell; scl – sclerenchyma; sup.epi. – superior epidermis).

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