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THE RUST FUNGUS *UROMYCES GERANII* (DC.) LEV. LOCALIZATION AND IMPACT ON ANATOMY OF THE HOST PLANT *GERANIUM SANGUINEUM* L.

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Abstract. The investigations on the rust fungus *Uromyces geranii* (DC.) Lev. influence on the anatomy of the vegetative organs of the host plant *Geranium sanguineum* L. (Geraniaceae) were conducted. The local and endophytic arrangement of *U. geranii* in the tissues of the host plant were identified. The tissues hypertrophy and organs deformation in *G. sanguineum* were revealed as a consequences of the fungus *U. geranii* influence as well as increment of pericyclic ring sclerenchyma lignification in the stem. While the mature aecia and uredinia on the stem cause the epidermis tears and fill the primary cortex up to the stele.

Key words: *Uromyces geranii, Geranium sanguineum,* rust fungi, anatomy, vegetative organs

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Introduction

The harmfulness of rust fungi is revealed not only in the fact that fungi are parasites penetrating the tissues of the host plant and feeding on the host's expense, but also in the decrease of assimilation surface often inducing death of leaves and stems. The violation of the anatomical integrity of the plant tissues under the influence of rust fungi results in the change of water, mineral and organic substances' transportation, destabilizes the physiological condition of the plants making them more vulnerable to abiotic environmental factors (Rubin *et al.* 1975).

Accordingly to the literary sources (Kuprevich & Ulyanischev 1975, Dudka *et al.* 2004), the rust fungus *Uromyces geranii* (DC.) Lév. is a monoecious type with the full cycle (macrocyclic) of development: spermogonia (pycnia) (0), aecia (I), uredinia (II) and telia (III) developing on *Geranium sanguineum* L. (Geraniaceae).

The aim of our research was to reveal the localization of the rust fungus *U. geranii* and to investigate its influence on the anatomical structure of the host plant *G. sanguineum.*

Material and methods

The exploration was carried out in the vicinity of Nautsniy town in Bakhchisaray region of Crimea on the top of one of the summits of Sel-Buhra Mountain (658.2 m a.s.l.) on two homogeneous test areas 100 m^2 each. The natural vegetation of the limestone Mountains of Sel-Buhra is represented with pubescent oak and juniper sparse wood, where *G. sanguineum* forms rather large and multiple curtains. According to the schematic map of the Crimean zones, Nautsniy town is located in Mountainous Crimea (Didukh 1992). The identification of the samples of *U. geranii* on the leaves of the host plant was made using standard methods: according to the field guide (Kuprevich & Ulyanischev 1975), while the name of the host plant is represented by Jena (2012).

The plants of *G. sanguineum* infected by rust fungus *U. geranii* were the objects of the study. The variants of the experiment were as follows: healthy plant control test and the experiment with plants infected by fungus (Fig. 1). In order to detect mycelial hyphae in plant tissues the fixator was used (Barykina *et al.* 2004). In order to identify lignin the medications were treated with phloroglucin and hydrochloric acid.

Fig. 1. The pycnia and aecia of the rust fungus *Uromyces geranii* on the leaves of *Geranium sanguineum*.

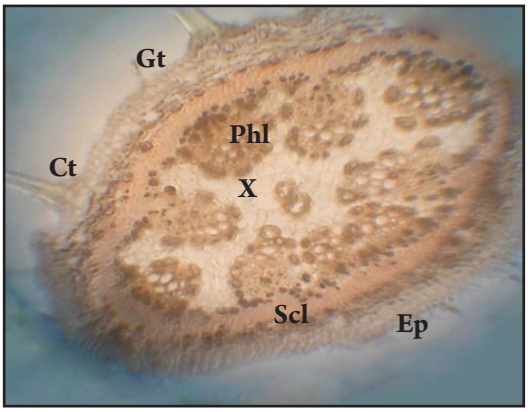

Fig. 4. Cross section through the petiole of *Geranium sanguineum* control plant (magn. 15×20): **Ct** – covering trichome, **Ep** – epidermis, **Gt** – glandular trichome, **Phl** – phloem, **Scl** – sclerenchyma ring, **X** – xylem.

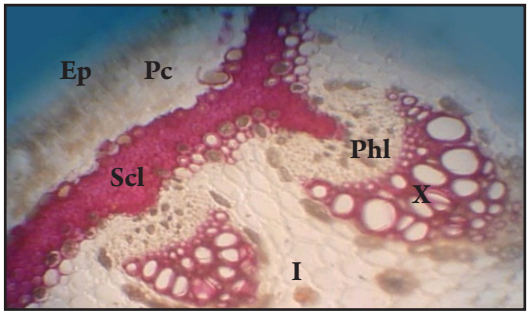

Fig. 2. Cross section through the stem of *Geranium sanguineum* control plant (magn. 15×20): **Ep** – epidermis, **I** – ideoblasts; **Pc** – primary cortex; **Phl** – phloem; **Scl** – sclerenchyma ring; **X** – xylem.

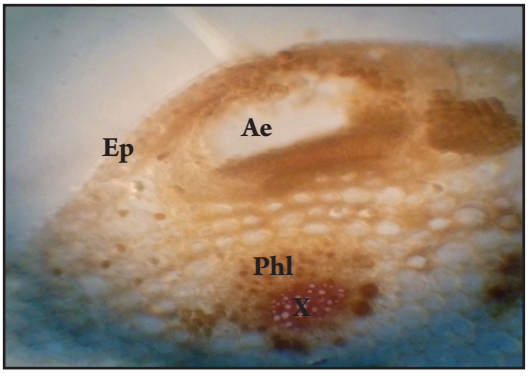

Fig. 5. Cross section through the petiole of *Geranium sanguineum* infected by *Uromyces geranii* aeciospores (magn. 15×20): **Ae** – aecia, **Ep** – epidermis, **Phl** – phloem, **X** – xylem.

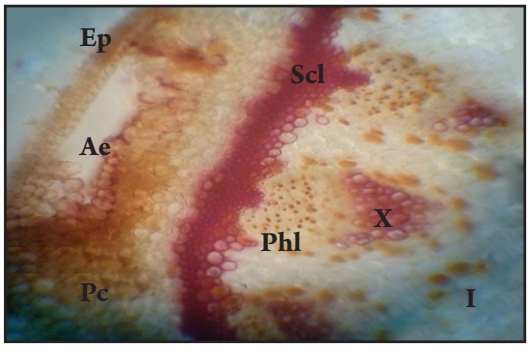

Fig. 3. Cross section through the stem of *Geranium sanguineum* infected by *Uromyces geranii* aeciospores (magn. 15×20): **Ae** – aecia; **Ep** – epidermis; **I** – ideoblasts; **Pc** – primary cortex; **Phl** – phloem; **Scl** – sclerenchyma ring; **X** – xylem.

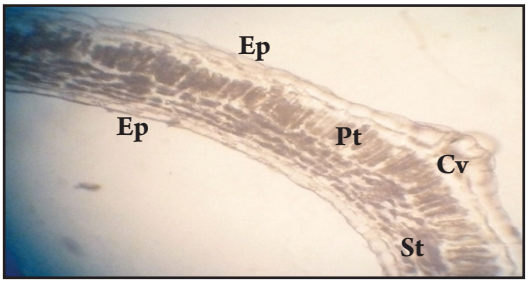

Fig. 6. Cross section through the leaf blade of *Geranium sanguineum* control plant (magn. 15×20): **Cv** – central vein, **Ep** – epidermis, **Pt** – palisade tissue, **St** – sponge tissue.

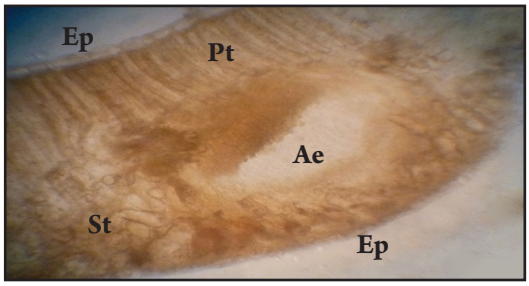

Fig. 7. Cross section through the leaf blade of *Geranium sanguineum* infected by *Uromyces geranii* aeciospores (magn. 15×20): **Ae** – aecia, **Ep** – epidermis, **Pt** – palisade tissue, **St** – sponge tissue.

Results and discussion

The main area of *G. sanquineum* distribution is European-Mediterranean. In Ukraine the species is widespread mainly in the Crimean Mountains. Its biomorphological characteristic: aeropedophyte, litophyte, polycarpous summer-winter green, semirosette, herbaceous, early-middle annual plant species with middle-rhizome, sympodial shoot branching and racemiferous roots. Its ecomorphological characteristic: xeromesophyte, skioheliophyte, glycophyte, melliferous, source of vitamins, technical, a rather frequently occurred plant (Golubev 1996).

The pycnia of *U. geranii* grow on both sides of the leaf blades in small groups of aecia, forming the yellow spots, spherical, small, 135-150 μm, sometimes orange or brown. The aecia are located on the underside of the leaf blades, as well as on the veins and petioles gathered into oblong loose groups on small rounded or elongated, thickened, yellow, or brown spots, sometimes arranged in circles, rounded and yellow, 0.2-0.3 mm in diameter. The peridium is calyciform with slightly dissected, upraised and contorted edge; the peridium cells are rounded or elongated, 28-40(45)×12-22 μm; external and internal walls are 4-5 μm width; the external wall is the cross-striated, the internal – barbellate; veruccae are arranged in short lines.

The uredinia located on the underside of the leaves or rarely on petioles, stems and pedicels have an appearance of yellow-reddish or brown spots. The uredinia are often sparse or arranged in small groups, round or oblong in shape, 1-2 mm long. The telia are similar to uredinia, but are darker-colored.

Anatomical structure of G. sanguineum stem

On the external side the stem is covered by monolayer epidermis with strongly thickened external and internal tangential walls. The cuticle on the epidermis, sparsely located trichomes and glandular hairs with three-celled pedicel and capitulum are formed. Under the epidermis the primary cortex follows where one or two cells-layered angled collenchyma is represented. Under the primary cortex stele is located. Its pericycle forms a ring of mechanical tissue consisting of 5-8 cell layers. The internal layers of sclerenchyma form the ring which covers outside the largest vascular bundles between which the smaller ones are located. The bundles are open collateral (Fig. 2). There is the marrow in the center of the stem, formed by large thin-

Table 1. The impact of the rust fungus *Uromyces geranii* on the size of parenchyma cells in *Geranium sanguineum*.

Vegetative organ	Variant	Length of the parenchyma cells, μm	Width of parenchyma cells, μm
Stem	Control	19.4±0.8	18.8±0.4
	Experiment	30.1±1.4	29.6±0.4
Petiole	Control	16.9±0.6	15.7±0.5
	Experiment	31.0±2.1	29.7±0.3
Leaf	Control (palisade parenchyma)	44.6±1.4	19.4±1.2
	Experiment (palisade parenchyma)	101.9±6.3	20.6±0.4
	Control (spongy parenchyma)	14.5±1.2	13.8±0.3
	Experiment (spongy parenchyma)	44.6±1.0	42.8±0.3

walled parenchymal cells. In the parenchymal tissue of the whole stem idioblasts with essential oils found mostly in the marrow.

The mature aecia and uredinia of rust fungus *U. geranii* on the stem of the infected plants cause the breaks in epidermis and fill in a primary cortex toward the stele. The subepidermal layer of cells is more clearly expressed in comparison to the control variant (Fig. 3). Visually the amount of covering glandular hairs on the epidermis increases. The cells of the primary cortex adjacent to aecia and uredinia are crumpled and between them the air cavities are formed. Apparently the parasite which penetrated into the plant contributes to the development of the air cavities in the primary cortex of the host plant's stem. The cavities are required for the aecia and uredinia laying and forming. Until maturation and distribution of aecia and uredinia the remaining cells of subepidermal layer protect the stem from unfavorable environmental factors. The idioblasts contain essential oils and may be abundantly found in all the tissues of a stem in all topographic zones.

There has been observed an increasing lignification of pericyclic sclerenchyma ring under the influence of *U. geranii* fungus. Moreover, there was identified the hypertrophy of the tissues of *G. sanguineum* infected by rust fungus, which probably promotes the excretion of the growth regulators by the plant cells.

Anatomy of G. sanguineum petiole

The leaf petiole is visually more pubescent with glandular and simple trichomes comparing to stem. The structure of leaf petiole epidermis is the same as of the stem. The pericyclic sclerenchyma ring is poorly lignificated. The number of vascular bundles is about 6-8 (Fig. 4). The idioblasts are also present.

Due the infection of the petiole by the fungus *U. geranii* a strong thickening of the external wall of the epidermis as well as the development of a monolayer lamellar collenchyma are observed. In the area of primary cortex the cells are stretched and large intercellular spaces are observed. The sclerenchyma ring is missing. The number of conducting bundles under the fungal infection reduces to 4-5 (Fig. 5).

Anatomy of G. sanguineum leaf blade

The leaf blades are pubescent, reniform in contour, palmately veined, deeply palmately dissected, with 5-7 lobes with 3-5 linear lacinias each. The type of the leaf is hypostomal. The upper epidermis of the leaf blader includes large cells with thick external walls without cuticle but with numerous trichomes – simple and glandular. The mesophyll of the leaf is represented by palisade tissue consisting of a single layer of strongly elongated cells with large chloroplasts, its longitudinal axes are perpendicular to the surface of the leaf blade. Between the palisade cells there are intercellular spaces. The loose tissue is formed by 5-4 lines of quite closely adjacent to each other cells with slightly expressed intercellular spaces (Fig. 6). The lower epidermis has smaller cells than the upper one, while on the lower side of the leaf there are only glandular trichomes. The veins are represented by closed collateral vascular bundles with well-developed xylem. However, its histological elements are poorly lignificated. Around the bundle there is a well expressed parenchymal sheath.

Under the influence of rust fungus *U. geranii* the hypertrophy of the mesophyll cells is observed: the palisade and spongy mesophyll cells show a significant proliferation (Fig. 7). As seen from Tab. 1, under the influence of parasitic fungus the tissues' hypertrophy in vegetative organs of *G. sanguineum* observes. As a result of the tissue deformation under the influence of rust fungus the average length of parenchyma cells in the stem of *G. sanguineum* increases up to 30.1 μm, which is 1.6 times higher compared to the control test (19.4 μm). Under the influence of the infection the hypertrophy of infected parts of the petiole's tissues is also observed. For example, in the control test the parenchyma cells' length was 16.9 μm, while in the experiment it was 31.0 μm, which is 1.8 times higher. The same tendency deduces when comparing data of the length of the palisade parenchyma's cells (in the experiment this indicator was 2.3 times higher than in control testing). For instance, after the leaves of *G. sanguineum* were infected by the

rust fungus it was observed that mesophyll cells' length was 53.2 μm which is 1.6 times more than in the control group – 32.4 μm (Tab. 1).

Conclusions

1. It was found out that mature aecia and uredinia of the rust fungus *Uromyces geranii* causing tear of the epidermis fill the primary cortex of the stem up to the stele.
2. As a result of local and endophytic development of the fungus in the tissues of *Geranium sanguineum* a cell hypertrophy as well as increased lignification of pericyclic sclerenchyma ring of the stem occurs in the place of intrusion of *U. geranii*.
3. In the case of petiole infected by the fungus *U. geranii*, a strong thickening of the external walls of the epidermal cells and the development of a monolayer lamellar collenchyma were observed. In the area of primary cortex the cells are elongated with large intercellular spaces; the sclerenchyma ring is missing.
4. The place of parasite penetration into the leaf is marked by the necrosis of epidermis and mesophyll to the extent that necrotized tissue falls out from the plant.

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