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Anatomical changes in the stems of highbush blueberry (Vaccinium australe Small), caused by the fungus Godronia cassandrae f. vaccinii (Peck) Groves

MAREK SZYNDEL[1] and JANINA WIŚNIEWSKA[2]

[1]Department of Plant Pathology, Agricultural University of Warsaw (SGGW-AR), Nowoursynowska 166, 02-766 Warszawa, Poland; [2]Research Institute of Pomology, Pomologiczna 18, 96-100 Skierniewice, Poland

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Abstract

Some histological changes were observed in blueberry stems infected by the fungus *G. cassandrae*. Dead cells of subepidermal collenchyma and cortical parenchyma filled with brown flocculent deposits were seen in the lesion areas. Pycnidia characteristic for the conidial stage of the fungus (*Topospora myrtilli*) were found in the collenchyma layer. The diseased tissues were found to be separated from the healthy ones by a layer of cork cells which was initiated under the epidermis and ended under the pericycle. Beneath this cork layer lamellar collenchyma and collenchyma-like phelloderma formed. Similar histopathological changes were observed in blueberry stems infected by seven other fungi.

INTRODUCTION

When studies on the highbush blueberry (*Vaccinium australe* Small) were started in Poland attention was devoted not only to the technique of its cultivation, but simultaneously to diseases attacking this plant. One of the most dangerous disease is fusicoccum canker caused by the fungus *Godronia cassandrae* f. *vaccinii* (Peck) Groves (G r o v e s, 1965), the conidial stage – *Topospora myrtilli* (Fetg) Boerma (B o e r m a and V e r h o e v e r y, 1972). After the isolation and identification of the pathogenic agent (B o r e c k i and P l i s z k a, 1978), the histological-anatomical changes in highbush blueberry shoots caused by the fungus *Godronia cassandrae* were investigated for diagnostic purposes and for establishing the damages caused by the pathogen. At the same time the histological-anatomical structure of one-, two- and many-year-old healthy shoots was described. This structure has been presented only fragmentarily in the available literature (E c k and C h i l d e r s, 1966; M e l z e r, 1978).

MATERIAL AND METHODS

The plant material for study was divided into four parts: A – healthy shoots, B – shoots artificially infected with *Godronia cassandrae*, C – shoots artificially infected with the fungi: *Botrytis cinerea, Diaporthe perniciosa, Pezicula alba, P. corticola, P. malicorticis, Phytophthora cinnamomi, P. drechsleri, Stereum purpureum, Trichothecium roseum*, D – shoots naturally infected by *Godronia cassandrae*.

The blueberry variety 'Jersey' was used for the experiments. Material was collected from plantations in the following localities: Albigowa, Kuryłówka, Sławoszyno, Pękanino, Radacz, Ursynów. The plants were artificially infected at the Experimental Station in Sinołęka in September 1976 and May-June 1977. The shoots were kept for 72 h in FAA (50% ethanol – 100 cm^3, 40% formaldehyd – 6.3 cm^3, glacial acetic acid – 2.5 cm^3). The shoots were then deaerated, kept for 24 h in 50 per cent ethanol and stored in 75 per cent ethanol. Three days before preparation the material was soaked in 50 per cent technically pure glycerin. Sections 10, 15, 20 and 30 μm thick were prepared on a microtome and stored in glycerin.

RESULTS

The anatomical structure of one-year-old shoots of the highbush blueberry is shown in Fig. 1. The epidermis cells are covered with a thick layer of cuticle and are very compact. The stomata are not numerous (Fig. 2). The cells closing the stomata are thin-walled and have sharp ends. The substomatal chamber is well developed. Under the epidermis there is a several-cell layer of parenchyma containing chloroplasts. Some cortex parenchyma cells undergo hypertrophy. Large spaces form in this tissue which may correspond to the air channels described by Weingartner and Klos (1975). It is possible that they arise owing to lysis of the walls of the above mentioned enlarged cells. The cortex parenchyma layer adheres directly to the compact fibres of the pericycle with a very narrow lumen. In young shoots the fibres are arranged in agglomerations of several cells. As the shoot grows they form a more and more compact layer interrupted only by core rays. Phloem tissue lies under the pericycle fibres, and further inwards a thin cambium layer and xylem tissue.

The core rays run from the pith to the cambium and are further prolonged in the phloem. Starch grains are visible in their cells. The pith consists of cells with thin cellulose walls and distinct straight pits. Starch grains are visible in these cells. In young shoots the pith is relatively large.

The anatomical structure of two- and many-year-old shoots is shown in Figs. 3 and 5. A thick cuticle layer covers the one-cell layer epidermis of two-year shoots. The lenticels forming under it are filled with cells with suberised walls.

Phellogen forming these cells produces a phelloderm layer on the inner side (Fig. 4). Under the epidermis layer, at the site where cork has not yet formed, collenchyma is present. The cortex parenchyma in older shoots is compressed so that the air channels disappear. The pericycle is formed of a compact layer of cells with a very narrow lumen (Fig. 5). Pits are visible in the cell walls. Deeper, secondary phloem, cambium and secondary xylem can be seen. There are slight differences between spring and summer xylem. In the cells of secondary rays starch grains can be seen. The pith cells are identical to those in one-year-old shoots and also contain starch, but the pith diameter is many times smaller in proportion to the whole diameter. Cork bark is formed on older shoots.

The anatomical structure of a two-year-old highbush blueberry infected with the fungus *G. cassandrae* is shown in Fig. 6.

Under the intact epidermis layer, close to the lenticel there are degenerated collenchyma and cortex parenchyma cells. The infected part is separated from the healthy one by a shallow arc of cork cells dark brown in colour (Fig. 9). This layer

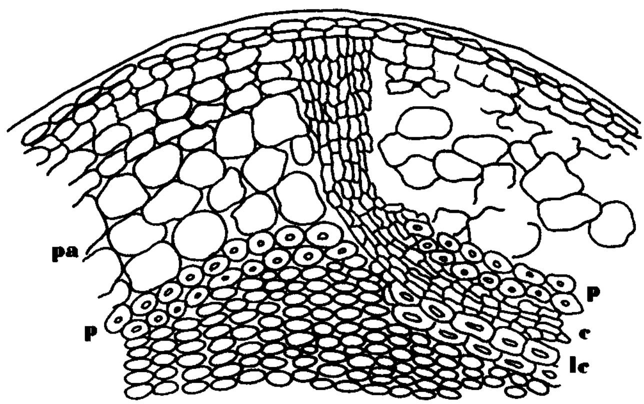


Fig. 9. Scheme of anatomical changes in blueberry stem induced by *G. cassandrae*: ac – air channels, pa – parenchyma, p – pericycle, lc – lamellar collenchyma, ph – phellogen, c – cork

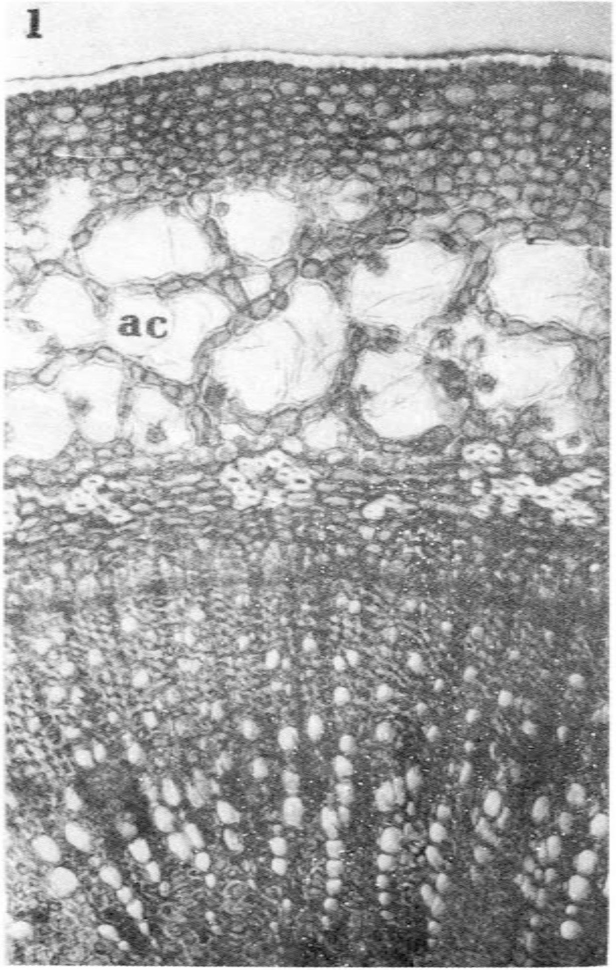
extends from the epidermis to the pericycle fibres. The infected part of the primary cortex cut off in this way undergoes partial destruction. The cut off cells gradually die, the chloroplasts become brown and frequently form in the cells opaque floccular sediments. In the part of the shoot infested with the fungus a great number of tyloses form in the conducting tissues. Hyphae were not detected either in the cells or the intercellular spaces, but pycnidia of the fungus *T. myrtilli* were found either under the epidermis or after its breaking on the surface of the shoot (Fig. 8). Pycnidia form in or on the collenchyma layer and as they mature they emerge to the surface of the shoot. Under the separating cork layer forming under the pericycle fibres lamellar collenchyma layer is formed differing from the subepidermal collenchyma by the appearance of thickenings mainly in tangential cell walls (Fig. 7). This was not observed in the structure of subepidermal collenchyma cells. Under the layer of lamellar collenchyma phloem is visible on the shoot cross section. The remaining parts of the shoot show no changes, and close to the point of lesion collenchyma formation is not observed (on the same preparation) under the pericycle fibres although a cork layer forms close to the dead tissues. It is possible that as the hyphae develop under the cork layer, lamellar collenchyma may also develop and in the vessels more numerous tyloses will appear. No differences were found between the anatomical structure of shoots artificially and naturally infected. At the base of strongly infected shoots a large amount of callus tissue forms, surrounding the shoot as a thick ring. "Wundkallus" was investigated by M e l z e r in 1978.

Anatomical structure of *Vaccinium australe* shoot artificially infected by other fungi

The anatomical changes in the shoot caused by the fungi *P. alba, P. corticola, B. cinerea* and *D. perniciosa* were very similar to those produced by *G. cassandrae.* The lesions on the shoots, were, however, less extensive. After infection with the fungus *P. drechsleri* and *P. malicorticis* the typical symptoms of gradual tissue disintegration did not appear, only tyloses were found in the vessels at the site where the shoot had been artificially infected. In infection with *P. cinnamomi* cork cutting off the infected tissues from the healthy ones did not form a shallow arc, only ran radially from the shoot axis, but is also formed under the pericycle fibres. Under it a layer of lamellar collenchyma is formed. The infected tissues separated by cork underwent complete destruction. Infection with the fungus *S. purpureum* caused inhibition of xylem increment and formation of lamellar collenchyma under the pericycle fibres. Owing to this there occur, at the site where infection symptoms did not appear, islets of xylem increment. On these islets deep situated collenchyma does not form. Owing to infection with the fungus *T. roseum* severe degeneration takes place – the phloem and partly xylem tissues crumble at the site of infection and the remaining hole is surrounded by a cork layer. The same is formed over the pericycle and deep situated lamellar collenchyma is absent. Changes are not noted in the cortex parenchyma although the collenchyma under the epidermis turns slightly brown.

DISCUSSION

Anatomical examination of healthy highbush blueberry shoots performed at present did not reveal any new elements as compared with earlier studies (E c k



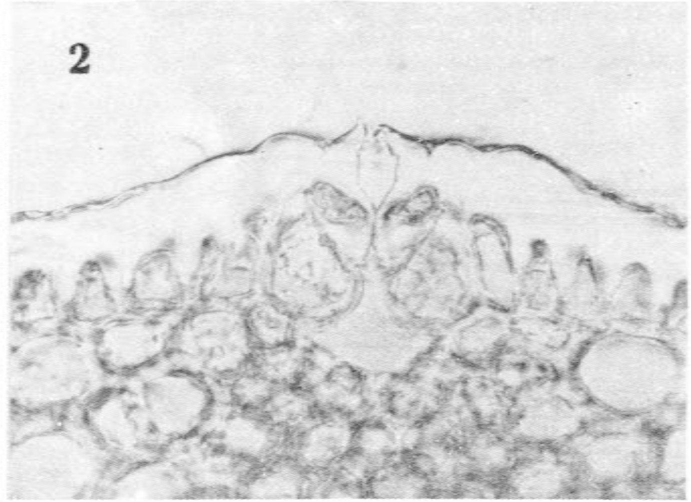
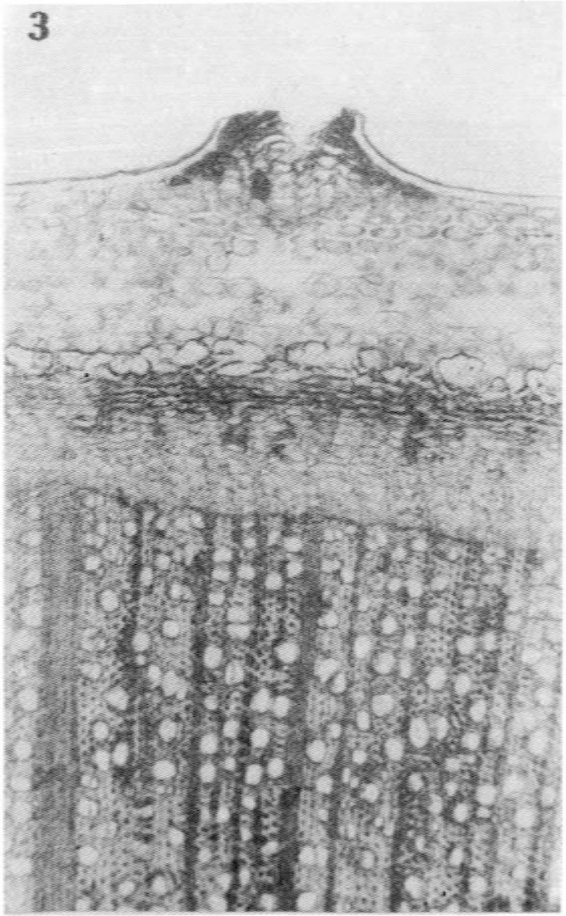


Fig. 1. Cross-section of current-year healthy blueberry stem (× 63). Explanations as in Fig. 9

Fig. 2. Stomata in the epidermis of current-year healthy blueberry stem (× 252)



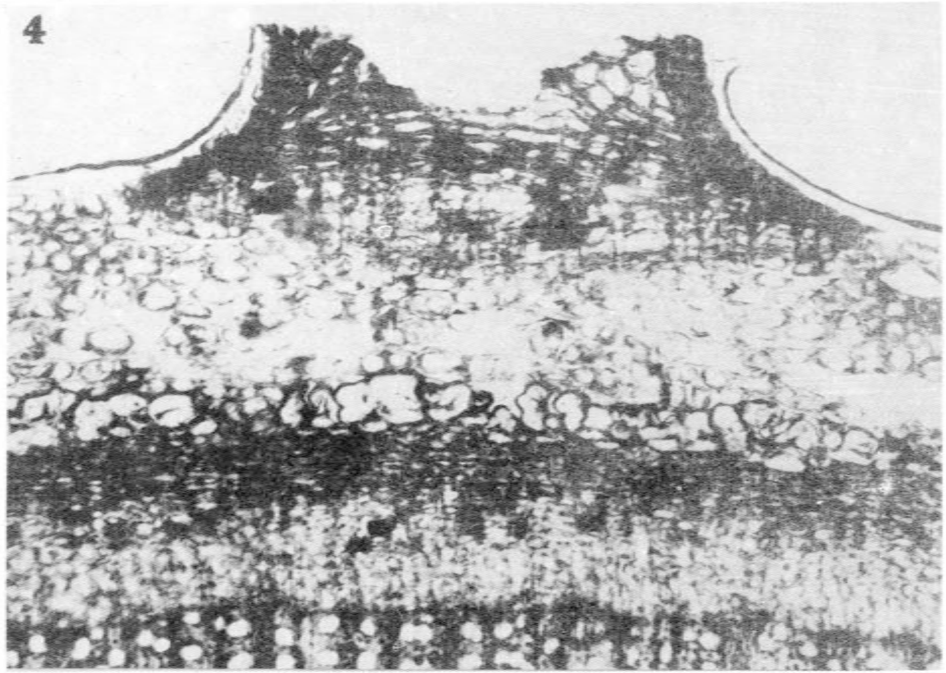
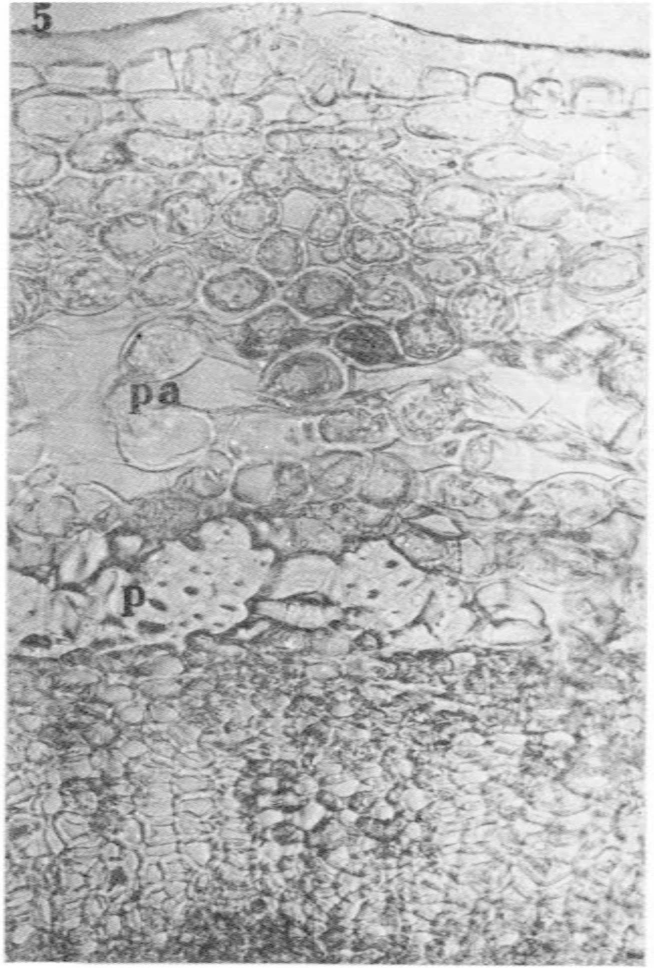


Fig. 3. Cross-section of the two-year-old healthy blueberry stem (× 63)

Fig. 4. Lenticel on two-year-old blueberry stem (× 80)



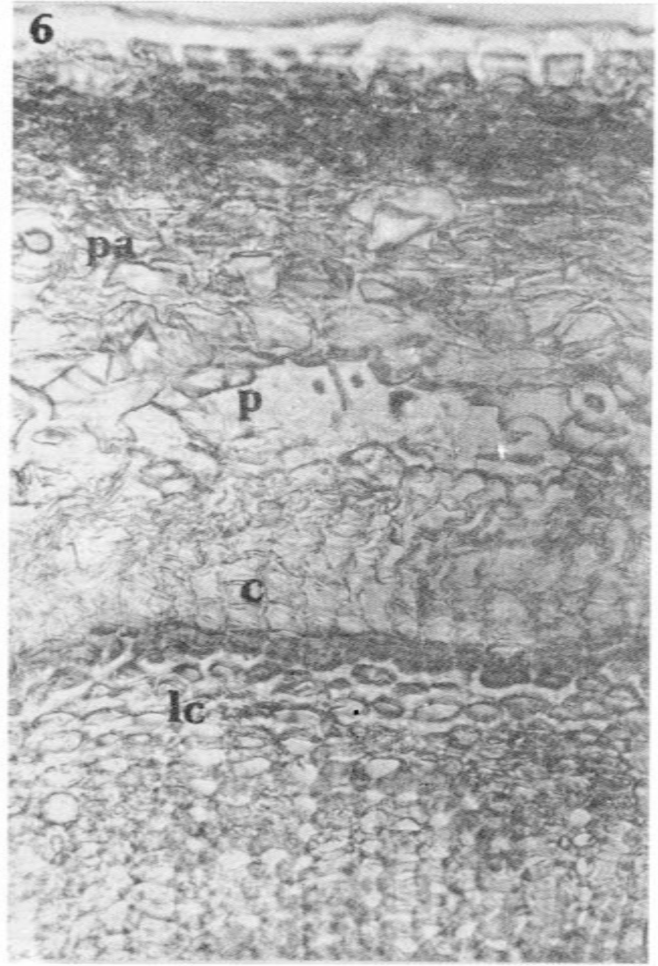
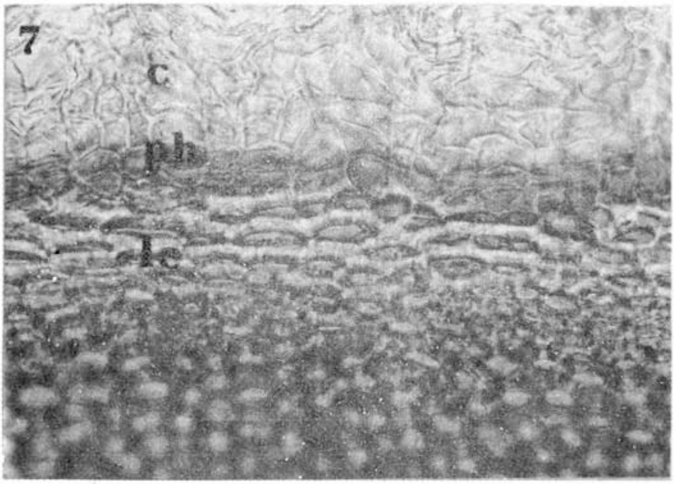


Fig. 5. Details of anatomy of a two-year-old healthy blueberry stem (× 175). Explanations as in Fig. 9

Fig. 6. Histopathological changes induced by *G. cassandrae* in two-year-old blueberry stem (× 175). Explanations as in Fig. 9



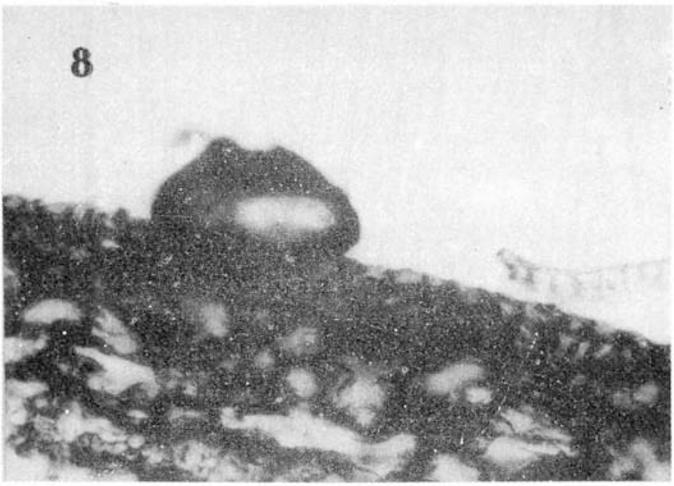


Fig. 7. Lamellar collenchyma or collenchyma-like phelloderm beneath the cork layer under the pericycle on cross-section of two-year-old blueberry stem infected by *G. cassandrae* (× 252)

Fig. 8. Pycnidium of fungus *T. myrtilli* on blueberry stem (× 82)

and Childers, 1966; Melzer, 1978). It was, however, found that, after infection of *V. australe* with *G. cassandrae* collenchyma gradually disintegrated under the epidermis and so did cortical parenchyma in two- and many-year-old shoots. Their cells, turned brown are filled with opaque floccular sediments or undergo complete destruction. The tyloses visible in the vessels probably cause earlier wilting of the plants. The infected tissues are separated from the healthy ones by a cork layer. Similar symptoms were described by Weingartner and Klos (1975) and by Melzer (1978). They also report the presence of fungal hyphae in the intercellular spaces which we at present did not observe. On the other hand, we found well developed pycnidia of the fungus *T. myrtilli*. A new cell layer not referred to in the literature was identified, forming on infected shoots under the pericycle fibres and in the cortex parenchyma the separating cork layer. This is deep situated lamellar collenchyma or a phelloderm layer similar to lamellar collenchyma. This layer markedly differs from the subepidermal collenchyma (see Results). Such pronounced histological changes cause rapid death of the plants, therefore, the pathogen should be considered as highly noxious. Since, as demonstrated here, infections by various fungi often give very similar symptoms of histological-anatomical changes as those due to *G. cassandrae*, the pathological changes are not sufficient for establishing the diagnosis and the pathogenic factor has to be precisely identified. The highbush blueberry is a plant very sensitive to climatic conditions, moisture, insolation and temperature and also to mechanical injury. Therefore the differences observed after infection with the fungi *S. purpureum* and *T. roseum* should be considered with caution. It is no excluded that these changes are not due to the activity of the fungi but they are a reaction of the plants to the above mentioned conditions.

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Zmiany w budowie anatomicznej pędów borówki wysokiej *Vaccinium australe* Small spowodowane przez grzyb *Godronia cassandrae* f. *vaccinii* (Peck) Groves

Streszczenie

W wyniku porażenia pędów *Vaccinium australe* przez grzyb *Godronia cassandrae* obserwuje się zamieranie kolenchymy pod epidermą i parenchymy korowej, których zbrązowiałe komórki wypełnione są kłaczkowatymi osadami. W naczyniach widoczne są zatyczki. Nie obserwowano strzępek grzybni, ale znaleziono pędy z piknidiami grzyba *Topospora myrtilli*, które tworzą się na warstwie kolenchymy. Tkanki porażone oddzielone są od tkanek zdrowych warstwą korka, który zakłada się łukiem od epidermy aż pod włókna perycyklu. Pod warstwą korka tworzy się głębinowa kolenchyma płatowa bądź jest to warstwa fellodermy, upodabniająca się do kolenchymy płatowej. Warstwa ta wyraźnie różni się od kolenchymy pod epidermą. W roślinach zdrowych głębinowej kolenchymy płatowej nie stwierdzono. Porównanie zmian wywołanych przez 7 innych grzybów wykazało, że zmiany powodowane przez *Godronia cassandrae* są niespecyficzne.