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# BIODIVERSITY OF FUNGI INHABITING THE HIGHBUSH BLUEBERRY STEMS

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**Abstract**. The aim of the work was to examine the health status of 11 highbush blueberry (*Vaccinium corymbosum* L.) cultivars cropped on the plantations in a south-eastern region of Poland as well as to determine the composition of fungus species colonizing their stems. The performed observations of health status indicated that stems with disease symptoms were found on almost all bushes. Three types of symptoms were found: spots on stems, from which mainly *Topospora myrtilli* was isolated, widespread stem necrosis inhabited by *Phomopsis archeri* and necrosis of stem tops caused by *Botrytis cinerea*. Moreover, the following isolates of fungi were obtained *Cytospora*, *Phoma*, *Fusarium*, *Alternaria* and *Seimatosporium vaccinii*, *Myxothyrium leptideum* and *Sordaria fimicola*. It was confirmed that the most malignant pathogen of highbush blueberry in the studied region was *Topospora myrtilli.*

**Key words**: Vaccinium corymbosum, Topospora myrtilli, diseases, fungi

## INTRODUCTION

Among the microorganisms causing diseases of highbush blueberry (*Vaccinium corymbosum* L.) in all crop regions all over the world fungi play a particularly significant role. Taking into account perennial character of highbush blueberry crop the species causing diseases of stems should be recognized as considerably dangerous and harmful. *Topospora myrtilli* (Feltg.) Boerema, *Phomopsis vaccinii* Shear, *Botrytis cinerea* Pers. and *Monilinia vaccini-corymbosi* (Reade) Honey are often listed pathogenic species. The infections caused by these fungi are important factors that decrease the yield and quality of crops and that are responsible for significant losses during fruit storage [Weingartner and Klos 1975, Borecki and Pliszka 1978, Stromeng and Stensvand 2001, Szmagara and Machowicz-Stefaniak 2005, Szmagara 2008].

Taking into account the lack in literature of a complex mycological elaboration concerning highbush blueberry pathogens deteriorating crop health in Poland and baring in

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mind the high demand for the knowledge in this field, the studies aiming at determining species composition of fungi inhabiting highbush blueberry stems and at defining their morphology were undertaken.

## EXPERIMENTAL PROCEDURES

The investigations were carried out in 2001–2003 at plantations of highbush blueberry under different habitat conditions of the south-eastern region of Poland, i.e.: near Hrubieszów (A), near Lublin (B), and near Puławy (C). Eleven different cultivars were cropped in the plantations, but only Bluecrop occurred on each of them.

Twenty bushes of every cultivar were selected randomly for studies at each plantation. The estimation of heath status was performed directly in the field in two periods of a plant vegetation cycle, i.e. in spring during bud swelling and in autumn when leaves fall down. The percentage of bushes with the symptoms of stem canker and the share of stems with disease symptoms in a bush were determined. Then, ten stems with disease symptoms for every cultivar from each plantation in each vegetation period were collected for further macro- and microscopic laboratory studies.

The presence of fungi was determined on the base of etiological symptoms occurring on infected stems and confirmed by mycological analysis using artificial culture method described by Machowicz-Stefaniak and Zalewska [2000].

In 2001 all collected stems were cut onto three parts: upper, middle and bottom fragments as all of them had characteristic disease symptoms. Because *Topospora myrtilli* was isolated from each part of a stem, in 2002 and 2003 the collected samples also consisted of all three fragments of stems and they were further handled as one group.

The plant material was rinsed in water (30 min), then surfaced sterilized with 50% $C_2H_5OH$ (30 sec) and finally rinsed three times (for 3 min) with sterile distilled water. The 1–3 cm parts of stems were placed onto solid maltose medium in Petri dishes. The dishes were incubated at 22°C in dark for 8 days. The fungus colonies obtained were segregated and pure cultures were obtained [Raiłło 1950], which were identified to species level. The identification of fungi was performed on standard or specific media. For identification of the *Phoma* genus fungi the following media were used: oat (OA), cherry (CA) and maltose (MA) [Gruyter and Noordeloos 1992], for *Penicillium* Chapek-dox and maltose media [Ramirez 1982], and for *Fusarium* SNA and PDA media [Nelson et al. 1983]. For other fungus species the following procedures were employed: De Vries [1952]; Gilman [1957]; Seaver [1961]; Rifai [1969]; Ellis [1971]; Pidopličko [1978]; Sutton [1980]; Henlin [1992].

The results obtained were statistically analysed using the analysis of variance and Tukey's HSD test.

## RESULTS

Field observations of highbush blueberry health status indicated that nearly all bushes were infected. In general three types of disease symptoms were distinguished (tab. 1, 2): canker spots, widespread necrosis and necrosis of stem tops.

Table 1. Percentage of stems in bush with symptoms of canker of highbush blueberry stems
Tabela 1. Procent pędów w krzewie z objawami zrakowacenia pędów borówki wysokiej

| Year of studies Rok badań | Vegetation period Okres wegetacji | Plantation A Plantacja A | | | | | Plantation B Plantacja B | | | | | | Plantation C Plantacja C | | | | | Average % Średni % | | | Average of total results Średnia ogółem |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Bluecrop | Darrow | Duke | Earliblue | Jersey | Bluecrop | Bluejay | Darrow | Duke | Herbert | Jersey | Berkeley | Bluecrop | Blueray | Ivanhoe | Record | plantation A plantacja A | plantation B plantacja B | plantation C plantacja C | |
| 2001 | Autumn Jesień | 0.00 a | 0.00 a | 0.00 a | 4.13 ab | 2.66 ab | 0.00 a | 4.49 ab | 4.46 ab | 0.00 a | 2.28 ab | 3.73 ab | 0.00 a | 2.08 ab | 0.00 a | 0.00 a | 0.00 a | 1.35 ab | 2.49 ab | 0.41 a | 1.42 ab |
| 2002 | Spring Wiosna | 0.92 a | 0.00 a | 0.00 a | 3.32 ab | 2.85 ab | 0.00 a | 5.39 abcd | 2.42 ab | 0.00 a | 3.50 ab | 5.07 abc | 1.85 ab | 0.89 a | 3.31 ab | 3.91 ab | 0.96 a | 1.42 ab | 2.73 ab | 2.18 ab | 2.11 b |
| | Autumn Jesień | 0.00 a | 0.00 a | 0.00 a | 1.59 ab | 1.35 ab | 0.35 a | 1.17 ab | 0.25 a | 0.00 a | 0.40 a | 4.07 ab | 3.16 ab | 0.51 a | 1.78 ab | 0.41 a | 0.00 a | 0.59 a | 1.04 a | 1.17 ab | 0.93 ab |
| 2003 | Spring Wiosna | 0.00 a | 0.00 a | 0.00 a | 1.34 ab | 1.20 ab | 0.00 a | 0.32 a | 0.97 a | 0.00 a | 0.53 a | 2.69 ab | 0.00 a | 0.00 a | 0.00 a | 0.00 a | 0.00 a | 0.50 a | 0.75 a | 0.00 a | 0.42 a |
| | Autumn Jesień | 11.70 d | 0.00 a | 4.52 ab | 7.57 cd | 11.06 cd | 0.00 a | 1.59 ab | 1.07 a | 0.00 a | 1.97 ab | 21.36 e | 0.00 a | 0.00 a | 0.00 a | 0.00 a | 0.00 a | 6.97 c | 4.33 bc | 0.00 a | 3.76 c |
| $LSD_{0,05}$ | | | | | | | | | | 6.44 | | | | | | | | | 3.42 | | 1.60 |
| Average of total results Średnia ogółem | | 2.52 bcd | 0.00 a | 0.90 abc | 3.59 d | 3.82 d | 0.07 a | 2.59 cd | 1.83 abcd | 0.00 a | 1.74 abcd | 7.38 e | 1.00 abc | 0.69 abc | 1.01 abc | 0.86 abc | 0.19 ab | 2.17 b | 2.27 b | 0.75 a | |
| $LSD_{0.05}$ | | | | | | | | | | 2.33 | | | | | | | | | 1.05 | | |

The means marked with the same letter are not differ in a significant way
Wartości oznaczone tą samą literą nie różnią się istotnie

Table 2. Fungi isolated from particular type of disease symptoms occurred on stems of studied highbush blueberry cultivars in 2001

Tabela 2. Grzyby wyizolowane z poszczególnych typów objawów chorobowych występujących na pędach badanych odmian borówki wysokiej w 2001

| Fungi<br>Grzyby | Elipsoidal spots<br>Plamy elipsoidalne | Widespread spots<br>Plamy rozległe | Necrosis of stem tops<br>Nekroza wierzchołków pędów | Total<br>Ogółem |
|---|---|---|---|---|
| *Alternaria alternata* Keissler | 149 (24.47) | 212 (32.42) | 175 (22.91) | 536 (26.44) |
| *Alternaria raphani* Groves et Skolko. | 31 (5.09) | 27 (4.13) | 43 (5.63) | 101 (4.98) |
| *Botrytis cinerea* Pers. | 27 (4.43) | 9 (1.38) | 43 (5.63) | 79 (3.90) |
| *Cladosporium cladosporioides* (Fres.) de Vries | 1 (0.16) | 19 (2.91) | 3 (0.39) | 23 (1.13) |
| *Cytospora* sp. | 7 (1.15) | 14 (2.14) | 10 (1.31) | 31 (1.53) |
| *Epicoccum purpurascens* Ehrenberg | 68 (11.17) | 90 (13.76) | 166 (21.73) | 324 (15.98) |
| *Fusarium avenaceum* (Fr.) Sacc. | 34 (5.58) | 45 (6.88) | 67 (8.77) | 146 (7.20) |
| *Fusarium culmorum* (W.G. Smith) Sacc. | 4 (0.66) | 5 (0.76) | 31 (4.06) | 40 (1.97) |
| *Fusarium equiseti* (Corda) Sacc. | 12 (1.97) | | 1 (0.13) | 13 (0.64) |
| *Fusarium oxysporum* Schlecht. emend. Snyd et Hans. | 4 (0.66) | 7 (1.07) | 56 (7.33) | 67 (3.31) |
| *Fusarium sambucinum* Fuckel | 8 (1.31) | 1 (0.15) | 2 (0.26) | 11 (0.54) |
| *Fusarium sporotrichioides* Sherb. | 4 (0.66) | | 22 (2.88) | 26 (1.28) |
| *Myxothyrium leptideum* (Fr.) Bub. et Kabat | | | 16 (2.09) | 16 (0.79) |
| *Penicillium decumbens* Thom | 12 (1.97) | 24 (3.67) | | 36 (1.78) |
| *Phoma capitulum* Pawar, Mathur &Tchirumalachar | 49 (8.05) | 41 (6.27) | 7 (0.92) | 97 (4.79) |
| *Phoma dennisii* Boerema nom. nov. | 8 (1.31) | 8 (1.22) | 6 (0.79) | 22 (1.09) |
| *Phoma exigua* Desm. | 11 (1.81) | 28 (4.28) | 7 (0.92) | 46 (2.27) |
| *Phoma glomerata* (Cda) Wollenw. & Hochapf. | 1 (0.16) | 10 (1.53) | 8 (1.05) | 19 (0.94) |
| *Phoma medicaginis* Malbr. et Roum. | 6 (0.99) | 7 (1.07) | 2 (0.26) | 15 (0.74) |
| *Phoma pomorum* Thum. | | 6 (0.92) | 7 (0.92) | 13 (0.64) |
| *Phomopsis archeri* nom. nov. | 35 (5.75) | 50 (7.65) | 52 (6.81) | 137 (6.76) |
| *Phyllosticta* sp. | | 27 (4.13) | 9 (1.18) | 36 (1.78) |
| *Saccharomyces* spp. | 8 (1.31) | 2 (0.31) | 3 (0.39) | 13 (0.64) |
| *Topospora myrtilli* (Feltg.) Boerema | 102 (16.75) | 14 (2.14) | 27 (3.53) | 143 (7.05) |
| *Trichoderma harzianum* Rifai aggr. | 18 (2.96) | 8 (1.22) | 1 (0.13) | 27 (1.33) |
| *Trichoderma koningii* Oud. aggr. | 10 (1.64) | | | 10 (0.49) |
| Total – Ogółem | 609 (100) | 654 (100) | 764 (100) | 2027 (100) |

The numbers given in parentheses correspond to frequency of given species among total isolates obtained from places with a given disease symptom.

Liczby podane w nawiasach odpowiadają częstotliwości występowania poszczególnych gatunków wśród wszystkich izolatów uzyskanych z miejsc z danym objawem chorobowym

Canker spots, ellipsoidal, with brown margin, up to 40–50 mm long occurred mainly at the base of stems but they were also present in higher parts of stems, most often around leafstalk or bud marks. The share of bushes with such symptoms varied between plantations and cultivars and ranged from 0.5 to 53.3%. On the surface of spots pycnidia were often present in concentric circles. The plant tissues in central part had grey colour (fig. 1). The microscopic observations of pycnidia indicated the presence of many conidial spores typical for *Topospora* (fig. 2).

The average percentage of stems with canker symptoms in a bush was at the level from 0.25 to 21.36 (table 1). In the case of 'Jersey' cultivar cropped on plantations A and B the highest percentage of stems with such symptoms in a bush was observed in autumn 2003 and reached 21.36 and 11.06%, respectively. A relatively high average

Fig. 1. Canker spot and pycnidia of *T. myrtilli* (photo by M. Szmagara)
Ryc. 1. Plama zgorzelowa i piknidia *T. myrtilli* (wyk. M. Szmagara)

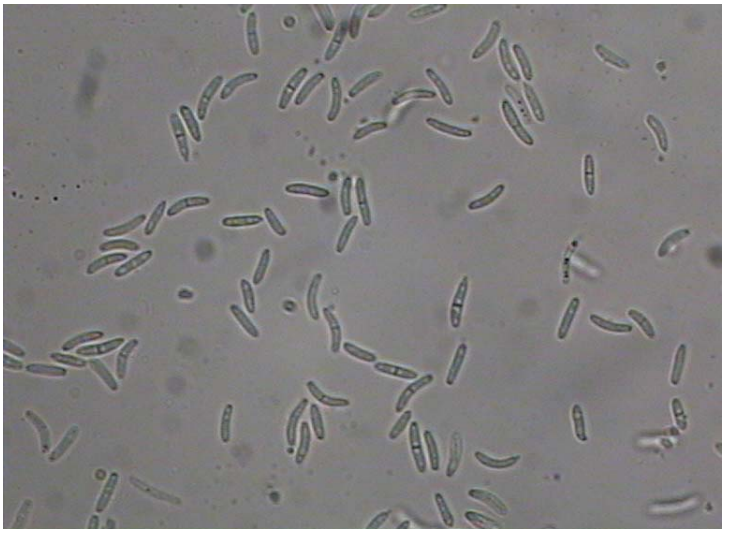

Fig. 2. Conidia of *T. myrtilli*, 750× (photo by M. Szmagara)
Ryc. 2. Konidia *T. myrtilli*, 750× (wyk. M. Szmagara)

percentage of stems with canker spots occurred on 'Earliblue' and 'Bluecrop' on plantations A 7.57% and 11.70%, respectively. The values were significantly higher than those obtained for the other cultivars (tab. 1). Similarly, the average percentage for all plantations and cultivars was the highest in autumn 2003 (3.76%) and was significantly different from other seasons. For particular cultivars the average percentage of stems with disease symptoms, independent of period of study, was the highest for 'Jersey' on plantation B (7.38%) and the difference was statistically significant. Similar values were observed for 'Jersey' (3.82%), 'Earliblue'(3.59%) and 'Bluecrop' (2.52%) cropped on plantation A and 'Bluejay'(2.59%) growing on plantation B (tab. 1). Of all plantations the lowest value of the average percentage of infected stems in a bush was observed for plantation C (0.75%). It differed significantly from the values characterizing other plantations of highbush blueberry (tab. 1).

The stems with widespread necrosis symptoms, cracking and peeling of epiderm, were observed during studies. The disease changes, white-grey or silver, with visible pycnidia had irregular shapes and took significant surfaces at different heights of a stem. The symptoms of disease were visible already in the first half of summer and its further intensification was observed in autumn (fig. 3).

The necrosis of stem tops occurred on stems of length up to 10 cm. The tops of stems were brown and fragile (fig. 4). The developing mycelium with conidial spores of *Botrytis cinerea* were noticed during damp weather on dead stem tops. Died parts of stems were colonized by other fungus species such as *Fusarium avenaceum*, which formed sporodochia with spores.

The mycological analysis of fungi inhabiting stems collected during 3 years of studies allowed to obtain 5553 isolates of fungi belonging to 32 species (tab. 3). *Topospora myrtilli* was isolated from stems of all the examined cultivars (fig. 1, 2). During investigations 410 isolates of this fungus were obtained (tab. 3). *T. myrtilli* was most often isolated from ellipsoidal spots occurring mainly on the lower parts of stems (tab. 2). The results of mycological analysis performed in 2001 indicated that this fungus amounted to 16.75% of fungi isolated from such spots (tab. 2). *T. myrtilli* was also isolated from widespread necrosis occurring at different heights of stems but the isolates constituted only 2.14% of fungi obtained from such places (tab. 2). This species was also obtained from stem tops with necrosis symptoms, with frequency of occurrence of 3.53% (tab. 2). The cultures of *T. myrtilli* obtained during the three years of studies amounted to 7.38% of all isolates (fig. 5).

Species of genera *Phomopsis*, *Cytospora* and *Phoma* (tab. 2, 3 and fig. 5) were isolated predominantly from stems with symptoms of widespread necrosis, crashing of bark and peeling of epiderm. Numerous isolates of *Phomopsis archeri* were obtained from almost all cultivars of highbush blueberry every year (tab. 2, 3). *Phomopsis archeri* was most often isolated from stems of 'Darrow' cultivars cropped on plantations A (10.20%) and B (14.32%) and from 'Berkeley' cultivars on plantation C (10.27%) (tab. 3).

Fungi of genus *Cytospora* also belonged to cultures frequently isolated from various parts of stems (tab. 3). They were at the level of 4.85% of all isolates obtained during the three years of studies (fig. 5). *Cytospora chrysosperma* was isolated from cultivars cropped on plantations A and B and from 'Record" cultivar on plantation C (tab. 3).

Fig. 3. Necrosis and cracks of the bark of highbush blueberry stems and pycnidia of *Phomopsis* sp. and *Phoma* sp. (photo by M. Szmagara)
Ryc. 3. Nekroza i pękanie kory pędów borówki wysokiej oraz piknidia *Phomopsis* sp. i *Phoma* sp. (wyk. M. Szmagara)

Fig. 4. Necrosis of stem tops caused by *B. cinerea* (photo by M. Szmagara)
Ryc. 4. Nekroza wierzchołków pędów powodowana przez *B. cinerea* (wyk. M. Szmagara)

Table 3. Fungi isolated from stems of studied highbush blueberry cultivars in 2001–2003
Tabela 3. Grzyby wyizolowane z pędów badanych odmian borówki wysokiej w latach 2001–2003

| Fungi – Grzyby | Number of isolates – Liczba izolatów | | | | | | | | | | | | | | | | Total Ogółem |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | plantation A – plantacja A | | | | | plantation B – plantacja B | | | | | | plantation C – plantacja C | | | | | |
| | Blue-crop | Dar-row | Duke | Earli-blue | Jersey | Blue-crop | Blue-jay | Dar-row | Duke | Her-bert | Jersey | Berke-ley | Blue-crop | Blue-ray | Ivan-hoe | Record | |
| *Alternaria alternata* Keissler | 148 | 97 | 152 | 97 | 107 | 99 | 42 | 21 | 115 | 108 | 57 | 85 | 60 | 116 | 92 | 99 | 1495 |
| *Alternaria raphani* Groves et Skolko. | 15 | 2 | 9 | 10 | 6 | 20 | 36 | 1 | | 4 | | 31 | 10 | | 12 | | 156 |
| *Botrytis cinerea* Pers. | 32 | 53 | 4 | 23 | 46 | 35 | 15 | 46 | 13 | 15 | 10 | 14 | 57 | 17 | 7 | 26 | 413 |
| *Cladosporium cladosporioides* (Fres.) de Vries | 1 | 2 | 3 | 1 | 3 | 15 | 3 | 4 | 6 | 5 | 5 | 3 | 1 | 1 | | | 53 |
| *Cytospora chrysosperma* (Pers.) Fr. | 11 | 16 | 21 | 8 | 7 | 19 | 3 | 1 | 4 | 6 | 4 | | | | | 7 | 107 |
| *Cytospora* sp. | 9 | | 2 | 6 | 11 | | | 3 | 4 | 4 | 2 | 10 | 14 | 37 | 20 | 40 | 162 |
| *Drechslera dematioidea* (Bubak & Wróblewski) Subram. & Jain | | | | | | | 2 | | | | | | | | | 7 | 9 |
| *Epiccocum purpurascens* Ehrenberg | 45 | 58 | 39 | 33 | 36 | 19 | 33 | 29 | 36 | 42 | 20 | 29 | 110 | 33 | 86 | 20 | 668 |
| *Fusarium avenaceum* (Fr.) Sacc. | 20 | 1 | 45 | 42 | 32 | 13 | 6 | 34 | 9 | 31 | 18 | 35 | 34 | 16 | 15 | 64 | 415 |
| *Fusarium culmorum* (W.G. Smith) Sacc. | 7 | | | 3 | 1 | | 1 | | 4 | | | 9 | 2 | 3 | 1 | 28 | 59 |
| *Fusarium equiseti* (Corda) Sacc. | | | | | | | | | | | | | | 12 | 1 | | 13 |
| *Fusarium oxysporum* Schlecht. emend. Snyd et Hans. | 1 | | 17 | 4 | 9 | 4 | 6 | 22 | 6 | 4 | 3 | 10 | 29 | 11 | 9 | 29 | 164 |
| *Fusarium sambucinum* Fuckel | | 4 | 1 | | | | | | 3 | | | 1 | | 1 | 1 | | 11 |
| *Fusarium sporotrichioides* Sherb. | 10 | 1 | 1 | 9 | 3 | 5 | 48 | 2 | 19 | 10 | 9 | 9 | | 6 | 2 | 3 | 137 |
| *Myxothyrium leptideum* (Fr.) Bub. et Kabat | | | 16 | | | | | | | | | | | | | | 16 |
| *Penicillium decumbens* Thom | 2 | 6 | 1 | 2 | 16 | 1 | 1 | 27 | 22 | | 5 | | | | | 1 | 84 |
| *Phoma capitulum* Pawar, Mathur &Tchirumalachar | 3 | 7 | 4 | 14 | 13 | | 9 | 9 | 2 | 8 | 9 | 10 | 31 | | 36 | 12 | 167 |
| *Phoma dennisii* Boerema nom. nov. | 1 | | 1 | 1 | | | | 1 | 6 | | | | | 8 | 3 | 1 | 22 |
| *Phoma exigua* Desm. | 3 | | 4 | 5 | 1 | 2 | 4 | 2 | 1 | | 15 | | 4 | 2 | 1 | 2 | 46 |
| *Phoma glomerata* (Cda) Wollenw. & Hochapf. | | | 2 | | | 1 | 6 | | | | 19 | | | 1 | | 1 | 30 |
| *Phoma medicaginis* Malbr. et Roum. | 1 | 5 | | 1 | 9 | 10 | 3 | 16 | 3 | 3 | 8 | 33 | 4 | 16 | 16 | 12 | 140 |
| *Phoma pomorum* Thum. | 3 | 7 | 2 | 5 | 7 | 8 | | | | 5 | 4 | 14 | 4 | 21 | 13 | 6 | 99 |
| *Phomopsis archeri* nom. nov. | 12 | 35 | | 9 | 5 | 7 | 19 | 48 | | 9 | 11 | 37 | 10 | 18 | 14 | 19 | 253 |
| *Phyllosticta* sp. | | | | 3 | 2 | | | 1 | 1 | 5 | 7 | 4 | 2 | 16 | 4 | 2 | 47 |
| *Saccharomyces* spp. | 3 | 9 | 2 | 8 | 3 | 2 | | 8 | 6 | 1 | 1 | 3 | 11 | 2 | 4 | 4 | 67 |
| *Sclerotinia* sp. | | | 2 | | | | 28 | 5 | 16 | 12 | | | 8 | | | | 71 |
| *Seimatosporium vaccinii* (Fekl) Ericsson | | 5 | | | | | | 4 | | | | 2 | | | | | 11 |
| *Sordaria fimicola* (Rob.) Ces. De & Not. | | 3 | | | | | | | | | | 5 | | | | | 8 |
| *Topospora myrtilli* (Feltg.) Boerema | 25 | | 2 | 65 | 37 | 49 | 49 | 22 | 6 | 13 | 123 | 10 | 1 | 3 | 4 | 1 | 410 |
| *Trichoderma hamatum* (Bon.) Bain. aggr. | | 1 | | | 4 | | | | | | | 6 | | | | | 11 |
| *Trichoderma harzianum* Rifai aggr. | 4 | 17 | 10 | 6 | 13 | | | 9 | 8 | 3 | 20 | | | 10 | | | 100 |
| *Trichoderma koningii* Oud. aggr. | 4 | 14 | | 17 | 38 | | 1 | 20 | 3 | 10 | 2 | | | | | | 109 |
| Total – Ogółem | 360 | 343 | 340 | 372 | 409 | 309 | 315 | 335 | 293 | 298 | 352 | 360 | 392 | 350 | 341 | 384 | 5553 |

Fungi of the *Phoma* genus were obtained from different parts of stems in amount of 9.08% of total isolates (tab. 3 and fig. 5). The widespread necrosis places were the sources of mainly *P. capitulum, P. medicaginis, P. pomorum*, the percentage of which was 33.13%, 27.77%, and 19.64% of all *Phoma* genera isolates, respectively (tab. 3).

Isolates of *Botrytis cinerea* were obtained mainly from died tops of stems of all cultivars every year. In total 413 isolates of this species was acquired which constituted 7.44% of all isolated fungi (tab. 2, 3, and fig. 5). This fungus was isolated most often from 'Bluecrop' and 'Darrow' cultivars stems, and most rarely from 'Duke' and 'Ivanhoe' stems (tab. 3).

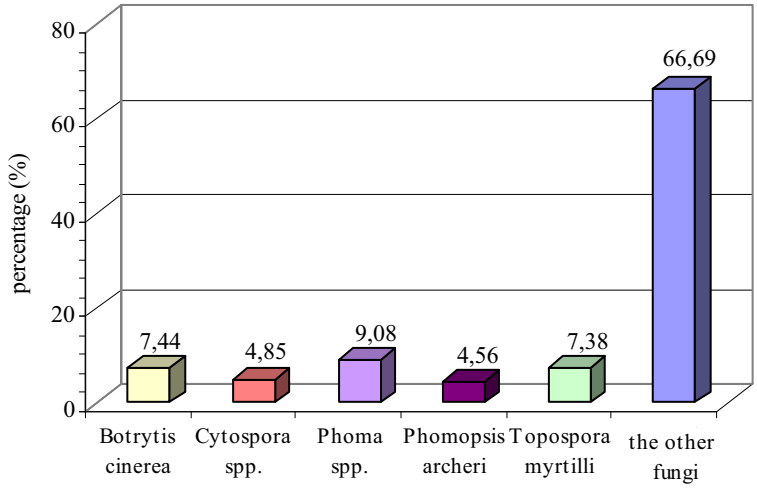


Fig. 5. The percentage of some fungal species isolated from blueberry stems in 2001–2003
Ryc. 5. Procentowy udział wybranych gatunków grzybów wyizolowanych z pędów borówki w latach 2001–2003

The single isolates of *Seimatosporium vaccinii* were obtained from "Darrow" highbush blueberry stems in 2002. In the next year mentioned species was also isolated from stems of this cultivar as well as from 'Berkeley' cultivated on other plantations (tab. 3).

Some cultures of *Phyllosticta* sp., 0.85% of all fungi, were isolated during the investigation period (tab. 2, 3). This fungus was most often obtained from widespread canker spots and died stem tops (tab. 3).

A part of all isolates (1.28%) producing only white, downy mycelium and black, flat sclerotia was determined as *Sclerotinia* sp. (tab. 3).

*Alternaria alternata*, *Alternaria raphani* and *Cladosporium cladosporioides* were isolated from stems in amount of 26.92%, 2.81% and 0.95% of all cultures, respectively (tab. 2, 3).

Moreover, a number of *Fusarium* spp., *Epicoccum purpurascens*, *Penicillium decumbens*, *Drechslera dematioidea*, *Sordaria fimicola* and *Myxothyrium leptideum* isolates were obtained from the plant material (tab. 2, 3).

Fungi of genus *Trichoderma*, i.e. *T. koningii*, *T. harzianum* and *T. hamatum* were acquired most often from the base of stems, more rarely from widespread spots and scarcely ever from the tops of stems with necrosis symptoms (tab. 2, 3).

## DISCUSSION

The performed studies confirmed the occurrence of infectious diseases on aboveground parts of highbush blueberry, which was reported previously by other researchers [Borecki and Pliszka 1978, Machowicz-Stefaniak and Zalewska 2001, Stromeng and Stensvand 2001]. The observed etiological and disease symptoms pointed out that the fungi were likely to cause the disease. They inhabited stems of all studied cultivars of highbush blueberry cropped in south-eastern region of Poland. Mycological analyses proved that stems were infected by various fungus species. It seems that among them *Topospora myrtilli*, fungus with well documented pathogenicity towards highbush blueberry stems and causing stem canker [Borecki and Pliszka 1978], should be pointed out as the most prominent and dangerous. This fungus is recognized as particularly harmful disease factor for highbush blueberry in all crop regions since it causes stem canker [Anderson 1956, Weingartner and Klos 1975, Milholland 1982, Rossman et al. 1987, Farr et al. 1995, Stromeng and Stensvand 2001]. The detection of this disease on a plantation is possible on the base of symptoms: characteristic ellipsoidal canker spots produced by the fungus and occurrence of conidial forms of sporulation [Weingartner and Klos 1975, Borecki and Pliszka 1978, Stromeng and Stensvand 2001, Szmagara 2008]. The ability of *Topospora myrtilli* to cause the necrosis on a whole circuit of a stem leading to its death above place of infection and withering of bushes was affirmed in our and many other studies [Stromeng and Stensvand 2001, Machowicz-Stefaniak et al. 2002, Szmagara and Machowicz-Stefaniak 2005, Szmagara 2008]. Despite repeated isolations of fungi from high blueberry stems in consecutive years, both before and after resting time of a bush, the perfect stage of *T. myrtilli* was not produced. It indicated that the teleomorph of this fungus did not play significant role in spreading of disease under the studied conditions, which is concurrent with other authors' findings [Borecki and Pliszka 1978, Stromeng and Stensvand 2001].

The frequent isolation of the dangerous pathogen *Phomopsis archeri* from stems of the studied cultivars of highbush blueberry implies its significant role in plant infections. Fungi of the genus *Phomopsis* are reported in literature as very dangerous and often occurring pathogens of this plant [Weingartner and Klos 1975, Milholland 1982, Milholland and Daykin 1983, Farr et al. 1995, Kačergius et al. 2004]. *Phomopsis vaccinii* is listed as a major cause of a disease complex induced by at least 32 fungi species responsible for generating the fruit rot [Stiles and Oudemans 1999]. Death of stems caused by *P. vaccinii* is a grave from economical point of view disease, it can menace the production of highbush blueberry and cranberry [Kačergius et al. 2004, Gabler et al. 2004].

Common infection of the stems by fungi from the genus *Cytospora* also deserves the attention. A number of species of this genera such as: *C. schulzeri*, *C. leucostoma*, *C. corylicolai* and *C. personata* are well-known to cause dangerous diseases of stems of fruit and ornamental trees and bushes [Sutton 1980, Machowicz-Stefaniak and Zalewska 2000, 2001].

The frequent isolation of *Botrytis cinerea* from parts of highbush blueberry confirms that this polyphagic species [Machowicz-Stefaniak 1998a] can also menace highbush blueberry cultivations [Machowicz-Stefaniak et al. 2002]. Particularly high harmfulness of this fungus for fruit of orchard crops, especially bilberry whortleberry, both during harvest and storage time is well known [Machowicz-Stefaniak 1998a]. In the light of presented studies the infection of stem tops of highbush blueberry by this pathogen should also be recognize as highly dangerous. The significant harmfulness of *B. cinerea* for young stems of highbush blueberry should be taken into account after late-spring ground frosts [Machowicz-Stefaniak 1998a]. Moreover, it can be supposed that *B. cinerea*, known for their high competitive abilities [Machowicz-Stefaniak 1998b], could colonize these parts of stems that were originally infected by other pathogens. In current studies the phenomenon was indeed observed, among others in the case of canker necrosis caused by *T. myrtilli* inhabited simultaneously by *B. cinerea*.

Confirmed inhabitation of stems by *Sclerotinia* sp. suggests that apart from *B. cinerea* also this fungus and its anamorpha, probably *Monilia* sp. can be the cause of diseases of higbush blueberry cropped in Poland. It implies polyphagic character of fungus and ability of *Monilia* sp. to infect the plants of *Ericaceae* genus [Zalewska 1999, Machowicz-Stefaniak and Zalewska 2001].

It seems that fungi of the *Phoma* genus relatively often isolated during studies can also contribute to infection of highbush blueberry organs. Such a conclusion is reinforced by a known fact of high harmfulness of *Phoma* spp. to stems, leaves and fruits of fruit trees. *P. idei* Oudem. is dangerous for raspberry stems, *P. prunicola* Opiz. ur. et Hochopf. for leaves of apple and pear trees, *P. vitis* Bonord. and *P. herbarum* Westd. for stems of grapevine and *P. uvicola* Berk et Curt. for grapes [Sutton 1980, Stojanovič 1986, Machowicz-Stefaniak and Kuropatwa 1991, Machowicz-Stefaniak and Zalewska 2001].

The isolation of several *Seimatosporium vaccinii* from highbush blueberry seems interesting. The occurrence of this fungus was earlier noted on plants from the genus *Vaccinium* in Switzerland, England, New Zealand and USA. It was isolated from stems of *Vaccinium myrtillus*, *Vaccinium* spp., *Rhododendron catawbiense*, *Rhododendron sp.*, *Staphylea trifolia* and *Crataegus oxyacantha* [Sutton 1980, Farr et al. 1995]. The ability of infecting of crop plant stems by other species of the *Seimatosporium* genus was also reported [Shoemaker 1964, Shoemaker and Müller 1964, Jaime et al. 1988]. It can be presupposed that *S. vaccinii* could be found in a complex of fungi infecting highbush blueberry stems in Poland. It is highly probable because its occurrence was detected on studied bush stems on plantations distant from each other.

The fungus *Phyllosticta* sp. isolated from highbush blueberry stems was also found on other plants of genera *Vaccinium*. *Phyllosticta vaccinii* and *Phyllosticta elongata* are pathogens causing rot of blueberry and cranberry fruit [Weideman and Boone 1983, Oudemans et al. 1998, Stiles and Oudemans 1999].

*Myxothyrium leptideum* sampled from stems of highbush blueberry, noted on plants of the genus *Vaccinium* in northern and central Europe, is known for causing spot of red bilberry (*V. vitis-idea*) [Sutton 1980].

The performed studies indicated the significant participation of *Alternaria alternata, Epicoccum purpurascens, Fusarium* spp., *Saccharomyces* spp., *Cladosporium* sp. and *Trichoderma* spp. among phyllospheric fungi of highbush blueberry. In the literature *Alternaria alternata* is mentioned as agent causing rot of fruit and spot of leaves, and *Fusarium* spp. as a cause of rot of roots and fruit of genus *Vaccinium* plants [Farr et al. 1995, Oudemans et al. 1998, Stiles and Oudemans 1999]. Moreover, in case of some species their pathogenicity toward *Ericaceae* and their part in causing fruit rot were not completely determined [Oudemans et al. 1998].

## CONCLUSIONS

1. *Topospora myrtilli*, *Phomopsis archeri*, *Botrytis cinerea* and *Cytospora* spp. belong to fungi causing necrotic changes on highbush blueberry stems.

2. The frequent occurrence and high harmfulness of *T. myrtilli* allow to recognize this fungus as the most abundant pathogen of highbush blueberry stems in the studied region.

3. The occurrence of *Phomopsis* spp., known for causing widespread necrosis on stems, should be recognized as malignant for highbush blueberry.

4. Detailed explanation of function and significance of the fungi of genera: *Cytospora*, *Phoma*, *Myxothyrium* and *Seimatosporium* for highbush blueberry requires further investigations.

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## BIORÓŻNORODNOŚĆ GRZYBÓW ZASIEDLAJĄCYCH PĘDY BORÓWKI WYSOKIEJ

**Streszczenie**. Celem pracy było określenie zdrowotności 11 odmian borówki wysokiej (*Vaccinium corymbosum* L.) uprawianych na plantacjach w południowo-wschodnim regionie Polski, jak również wyznaczenie składu gatunkowego grzybów kolonizujących ich pędy. Przeprowadzone obserwacje zdrowotności wskazały, że pędy z objawami chorobowymi były odnajdywane niemal we wszystkich krzewach. Wyróżniono trzy rodzaje objawów: plamy zgorzelowe, z których izolowano głównie *Topospora myrtilli*, rozległe plamy nekrotyczne zasiedlane głównie przez *Phomopsis archeri* oraz nekrozę wierzchołków pędów powodowaną przez *Botrytis cinerea*. Ponadto uzyskano izolaty następujących grzybów: *Cytospora*, *Phoma*, *Fusarium*, *Alternaria* oraz *Seimatosporium vaccinii*, *Myxothyrium leptideum* i *Sordaria fimicola*. Stwierdzono, że najgroźniejszym patogenem borówki wysokiej w badanym regionie był *Topospora myrtilli*.

**Słowa kluczowe**: *Vaccinium corymbosum, Topospora myrtilli,* choroby*,* grzyby

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