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ORIGINAL PAPER

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FUNGI INHABITING FRUIT TREE SHOOTS WITH SPECIAL REFERENCE TO THE *Diaporthe* (*Phomopsis*) GENUS

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ABSTRACT

The purpose of the study was to determine which fungal species colonize the shoots of apple, pear, cherry, plum, apricot and peach in south-eastern Poland and whether *Diaporthe* (*Phomopsis*) isolates there are among them. The study was conducted in 2010–2012 and the plant material was collected from five orchards. In three of these chemical controls were carried out, while there was no protection applied in the other two. The results showed that species composition of the fungi was very similar regardless of host plant and year of study. *Alternaria* spp., *Fusarium* spp. and *Phoma* spp. were isolated most frequently from the studied shoots. Fungi cultures known for pathogenicity towards fruit trees such as *Botrytis cinerea, Colletotrichum gloeosporioides, Leucostoma* spp., *Monilinia* spp. and *Neofabraea* spp. were also isolated. Moreover, there were isolates of *Diaporthe* (*Phomopsis*) among the fungi present in the shoots. Cultures of these fungi were obtained both from shoots with disease symptoms and from visually healthy ones but mainly from shoots originating from unprotected orchards, what indicates their greater threat to fruit trees grown without chemical protection. This is the first documented report of the occurrence of *Diaporthe* (*Phomopsis*) on fruit trees in Polish conditions.

Key words: orchard plants, biodiversity of fungi, occurrence

INTRODUCTION

The shoots of fruit trees are inhabited by numerous fungi species including bark and wood pathogens. Among them, both worldwide and in Poland, the most frequently mentioned are: *Neonectria galligena* (Bres.) Rosman et Samuels, *Leucostoma* spp., *Neofabraea* spp., *Botryosphaeria* spp. causing cankers and *Chondrostereum purpurem* causing "silver leaf". [Regner et al. 1990, Spotts et al. 1990, Grabowski 1993, Grabowski and Leś 1996, Adams et al. 2002, Grabowski 2002, Latorre et al. 2002, Valiuškaite and Raudonis 2008, Gramaje et al. 2012, Romanazzi et al. 2012].

Recently, diseases caused by *Diaporthe* (*Phomopsis*) have become an increasing problem in the orchard regions of the world. These fungi are common in different climatic zones and colonize many plant species. *Diaporthe* (*Phomopsis*) species are associated with bark necrosis, shoot blight and cancer, decay, wilting, fruit rot and mummification [Farr et al. 1999, Kanematsu et al. 2000, Farr et al. 2002, Karaoglanidis and Bardas 2006, Živkovič et al. 2007, Król and Kowalik 2010, 2011, Udayanga et al. 2011, Gomes et al. 2013]. Among the species most dangerous for fruit plants are *Phomopsis viticola* Sacc., *P. perniciosa* Grove, *P. ambigua* (Sacc.) Trav., *P. amygdali* (Del.) Tuset et Portilla comb. nov., *P. mali* Roberts, *P. juglandina* (Sacc.) Höhn,

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P. ampelina (Berk. et Curt.) Grove., *P. oblonga* (Desm.) Traverso and *P. vaccinii* Shear. [Machowicz-Stefaniak 1993, Farr et al. 1999, Mostert et al. 2001, Król 2002, Arsenijević and Gavrilović 2005, Karaoglanidis and Bardas 2006, Michailides and Thomidis 2006, Król and Kowalik 2010, 2011, Udayanga et al. 2011, Mirabolfathy et al. 2013].

Though only some of the *Phomopsis* species produce their sexual stage *Diaporthe,* because of changes in the taxonomy of these fungi, the name *Diaporthe* has been conserved over *Phomopsis* and *Diaporthe eres* has been recognized as the main pathogen of woody plants from different botanical families including the family *Rosaceae*, *Juglandaceae*, *Vitaceae* and *Ericaceae* [Rossman et al. 2014, Udayanga et al. 2014].

The purpose of this study was to determine which fungi species inhabit the shoots of fruit trees in south-eastern Poland and to examine whether *Diaporthe* isolates are also present among the other species.

MATERIALS AND METHODS

The research materials were shoots of apple (*Malus domestica* Borkh.), pear (*Pyrus communis* L.), cherry (*Prunus cerasus* Mill.) and plum (*Prunus domestica* L) originating from three orchards located in the Lublin province (Lublin, Bełżyce, Wojciechów) and shoots of apple, pear, cherry, plum, apricot (*Prunus armeniaca* L.) and peach (*Prunus persica* (L.) Batsch) from two orchards in the Świętokrzyskie province (Winiary and Suliszów near Sandomierz).

In three orchards (Lublin, Winiary and Suliszów) chemical controls were performed in accordance with the recommendations of plant protection. In the other two domestic orchards (Bełżyce and Wojciechów), no chemical protection was applied. The studies included shoots of different cultivars of the studied plants showing disease symptoms in the form of necrosis or canker. Moreover, for comparative purposes, healthy shoots with no visible disease symptoms were sampled).

The plant material was collected twice during the growing season, i.e. the spring and autumn in 2010–2012. Twenty diseased shoots of each species of fruit trees were randomly chosen from the protected orchards while ten shoots were taken from the unprotected ones. In addition, five visually healthy shoots were also collected from each orchard and fruit tree (tab. 1).

Mycological analysis

The fungi were isolated using the artificial culture method [Król 2006]. First, shoot fragments were cut into pieces the size of a match, and the external tissue (cortex) was separated from the internal tissue (wood). Next, the plant material was washed under running tap water, surface-disinfected for 30 s in a 10% sodium hypochlorite solution and washed three times for 3 min in sterile distilled water. Each shoot was treated as a separate object from which 20 small pieces, 10 of

Table 1. Number of shoots collected for testing, depending on the host plant and the orchard location (one-time trial)

Host plant	Protected orchards			Unprotected orchards	
	Lublin	Suliszów	Winiary	Bełżyce	Wojciechów
Apple	20d + 5vh	20d + 5vh	–	10d + 5vh	10d + 5vh
Pear	20d + 5vh	–	20d + 5vh	10d + 5vh	10d + 5vh
Cherry	20d + 5vh	20d + 5vh	–	10d + 5vh	10d + 5vh
Plum	20d + 5vh	–	20d + 5vh	10d + 5vh	10d + 5vh
Apricot		20d + 5vh	20d + 5vh		
Peach		20d + 5vh	20d + 5vh		
Total	80d + 20vh	80d + 20vh	80d + 20vh	40d + 20vh	40d + 20vh

d – diseased shoots; vh – visually healthy shoots

Table 2. Fungi isolated from diseased shoots of fruit plants in the years 2010–2012 (protected orchards)

Fungus	Number of shoots	Percentage (%)	Number of isolates	Percentage (%)
Acremonium charticola (Lindau) W. Gams	32	2.22	128	1.32
Alternaria alternata (Fr.) Keissl.	788	54.72	3758	38.79
Aspergillus niger Tiegh.	9	0.62	39	0.40
Aureobasidium pullulans (de Bary et Löwenthal) G. Arnaud	21	1.49	36	0.37
Botrytis cinerea Pers.	129	8.96	368	3.80
Chaetomium globosum Kunze	43	2.97	126	1.30
Cladosporium carpophilum Thüm.	48	3.33	72	0.74
Cladosporium cladosporioides (Fres.) de Vries	40	2.78	120	1.24
Cladosporium herbarum (Pers.) Link	8	0.56	24	0.25
Cladosporium sphaerospermum Penz.	3	0.21	16	0.17
Clonostachys rosea (Link) Schroers, Samuels, Seifert et W. Gams	70	4.86	143	1.48
Colletotrichum gloeosporioides (Penz.) Sacc.	126	8.75	268	2.77
Diaporthe spp. (*Phomopsis* spp.)	134	9.31	295	3.05
Epicoccum nigrum Link	78	9.42	193	1.99
Fusarium oxysporum Schltd.	99	6.88	174	1.80
Fusarium poae (Peck.) Wollenw.	6	0.42	17	0.18
Fusarium sporotrichioides Sherb.	125	8.68	308	3.18
Gibberella baccata (Wallr.) Sacc.	9	0.62	30	0.31
Gibberella fujikuroi (Sawada) Wollenw.	283	19.65	887	9.16
Haematonectria haematococca (Berk. et Broom) Samuels et Rossman	58	4.03	149	1.54
Leucostoma personii (Nitschke) Höhn.	41	2.85	76	0.78
Monilinia sp.	19	1.32	42	0.43
Neofabraea alba (E.J. Guthric) Verkley	32	2.22	68	0.70
Neofabraea perennans Kienholz	19	1.32	39	0.40
Paraconiothyrium fuckelii (Sacc.) Verkley et Gruyter	16	1.11	33	0.34
Penicillium expansum Link	111	7.71	246	2.54
Peyronellaea pomorum (Thüm) Aveskamp, Gruyter et Verkley	76	5.28	257	2.65
Phoma glomerata (Corda) Wollenw. et Hochapfel	10	0.69	26	0.26
Phoma herbarum Westend.	370	25.69	1178	12.16
Seimatosporium spp.	1	0.07	5	0.05
Trichoderma koningii Oudem.	71	4.93	220	2.27
Trichoderma viride Pers.	1	0.07	3	0.03
Trichothecium roseum (Pers.) Link	24	1.67	64	0.66
Truncatella truncata (Lév.) Steyaert	2	0.14	6	0.06
Saccharomyces spp.	75	5.21	246	2,54
White no sporulating mycelium	2	0.14	5	0.05
Black no sporulating mycelium	9	0.62	22	0.23
Total	1440	100	9687	100

Table 3. Fungi isolated from diseased shoots of fruit plants in the years 2010–2012 (unprotected orchards)

Fungus	Number of shoots	Percentage (%)	Number of isolares	Percentage (%)
Acremonium charticola (Lindau) W. Gams	3	0.63	10	0.39
Alternaria alternata (Fr.) Keissl.	118	24.58	300	11.70
Aspergillus niger Tiegh.	10	2.08	33	1.29
Botrytis cinerea Pers.	14	2.92	27	1.05
Chaetomium globosum Kunze	13	2.71	49	1.91
Cladosporium cladosporioides (Fres.) de Vries	12	2.5	23	0.90
Cladosporium herbarum (Pers.) Link	6	1.25	11	0.43
Cladosporium sphaerospermum Penz.	2	0.42	3	0.12
Clonostachys rosea (Link) Schroers, Samuels, Seifert et W. Gams	12	2.5	35	1.37
Colletotrichum gloeosporioides (Penz.) Sacc.	7	1.46	27	1.05
Cylindrocarpon obtusisporum (Cook et Harkn.) Wollenw.	1	0.21	1	0.04
Diaporthe spp. (*Phomopsis* spp.)	145	30.21	550	21.45
Elsinoë ampelina Shear	1	0.21	1	0.04
Epicoccum nigrum Link	49	10.21	113	4.41
Fusarium oxysporum Schltd.	6	1.25	29	1.13
Fusarium poae (Peck.) Wollenw.	5	1.04	18	0.70
Fusarium sporotrichioides Sherb.	12	2.50	55	2.14
Gibberella avenacea (R.J Cook)	1	0.21	3	0.12
Gibberella baccata (Wallr.) Sacc.	3	0.63	9	0.35
Gibberella fujikuroi (Sawada) Wollenw.	65	13.54	228	8.89
Gibberella pulicaris (Kunze) Sacc.	1	0.21	1	0.04
Haematonectria haematococca (Berk. et Broom) Samuels et Rossman	87	18.13	296	11.54
Leucostoma personii (Nitschke) Höhn.	3	0.63	3	0.12
Neofabraea alba (E.J.Guthric) Verkley	5	1.04	15	0.58
Neofabraea perennans Kienholz	2	0.42	11	0.43
Paraconiothyrium fuckelii (Sacc.) Verkley et Gruyter	4	0.83	11	0.43
Penicillium expansum Link	37	7.71	78	3.04
Pestalotiopsis spp.	1	0.21	3	0.12
Peyronella pomorum (Thüm.) Aveskamp, Gruyter et Verkley	20	4.17	71	2.77
Phoma glomerata (Corda) Wollenw. Et Hochapfel	1	0.21	2	0.08
Phoma herbarum Westend.	104	21.70	292	11.38
Sordaria spp.	1	0.21	3	0.12
Stachybotrys chartarum (Ehrenb.) S. Hughes	1	0.21	3	0.12
Trichoderma harzianum Rifai	1	0.21	1	0.04
Trichoderma koningii Oudem.	34	7.08	88	3.43
Trichoderma viride Pers.	1	0.21	5	0.2
Trichothecium roseum (Pers.) Link	1	0.21	2	0.08
Truncatella truncata (Lév.) Steyaert	2	0.42	4	0.15
Saccharomyces spp.	36	7.50	145	5.65
Black no sporulating mycelium	1	0.21	5	0.20
Total	480	100	2564	100

Table 4. Fungi isolated from visually healthy shoots of fruit plants in the years 2010–2012 (protected and unprotected orchards)

Fungus	Number of shoots	Percentage (%)	Number of isolates	Percentage (%)
Acremonium charticola (Lindau) W. Gams	13	2.17	79	4.23
Alternaria alternata (Fr.) Keissl.	205	34.17	911	48.79
Alternaria tenuissima (Kunze) Wiltshire	1	0.17	5	0.27
Aspergillus niger Tiegh.	3	0.50	8	0.43
Aureobasidium pullulans (de Bary et Löwenthal) G. Arnaud	3	0.50	13	0.70
Botrytis cinerea Pers.	28	4.67	74	3.96
Chaetomium globosum Kunze	3	0.50	7	0.38
Cladosporium cladosporioides (Fres.) de Vries	6	1.00	17	0.91
Cladosporium sphaerospermum Penz.	5	0.83	31	1.66
Clonostachys rosea (Link) Schroers, Samuels, Seifert et W. Gams	10	1.67	51	2.73
Colletotrichum gloeosporioides (Penz.) Sacc.	8	1.33	33	1.77
Diaporthe spp. (*Phomopsis* spp.)	34	5.67	95	5.09
Epicoccum nigrum Link	9	1.50	17	0.91
Fusarium sporotrichioides Sherb	8	1.33	23	1.23
Gibberella fujikuroi (Sawada) Wollenw.	5	0.83	20	1.07
Haematonectria haematococca (Berk. et Broom) Samuels et Rossman	8	1.33	39	2.09
Leucostoma personii (Nitschke) Höhn.	9	1.50	11	0.59
Neofabraea alba (E.J.Guthric) Verkley	5	0.83	16	0.86
Penicillium expansum Link	6	1.00	21	1.12
Peyronella pomorum (Thüm.) Aveskamp, Gruyter et Verkley	12	2.00	50	2.68
Phoma herbarum Westend.	28	4.67	131	7.02
Sordaria spp.	4	0.67	10	0.53
Trichoderma koningii Oudem.	13	2.17	42	2.25
Saccharomyces spp.	33	5.50	163	8.73
Total	600	100	1867	100

the outer and inner tissues, were placed in Petri dishes with Czapek-Dox agar (BD Difco). Plates were incubated at 24°C in the dark for 12 days. Growing fungal colonies were transferred to 2% potato-dextrose agar (PDA, BD Difco) slants for further identification. In the case of *Diaporthe* isolates, which sporulate with difficulty, they were cultured in a poor PDA medium containing carnation leaf to stimulate sporulation [Castillo-Pando 1997]. Single spore colonies were prepared for morphological identification using the serial dilution method.

The system by Kirk et. al. [2008] was adopted as a basis for fungi classification and the names of fungi were given according to the current taxonomic status of the species in the Index Fungorum database.

RESULTS

Mycological analysis of fruit trees resulted in 14.118 fungal isolates belonging to more than 30 species within three years of research. Among them, 9.687 isolates originated from shoots showing various disease symptoms and harvested from chemically protected orchard, 2.564 from unprotected shoots and 1.867 cultures from apparently healthy ones (tabs 2, 3 and 4). The species composition of the fungi colonizing shoots of examined host plants was similar in the years of the study. *Alternaria alternata* and *Fusarium* spp. were isolated most frequently, regardless of the orchard and the host plant.

Table 5. Fungi most frequently colonizing shoots of fruit plants in protected orchards in the years 2010–2012

Fungus	Percentage of fungi					
	2010		2011		2012	
	et	it	et	it	et	it
Alternaria alternata	50.82	37.04	44.43	29.10	49.75	18.42
Botrytis cinerea	2.05	0.59	3.64	7.15	3.44	3.90
Cladosporium spp.	1.43	0.59	5.9	1.04	1.64	1.52
Clonostachys rosea	0.62	1.42	0.7	2.42	0.45	3.58
Diaporthe spp./*Phomopsis* spp.	0.96	0.24	3.52	3.41	1.97	6.76
Fusarium spp. + *Gibberella* spp. + *Haematonectria haematococca*	20.96	20.36	12.72	10.29	17.94	19.36
Phoma spp. + *Peyronella pomorum*	10.62	15.50	8.76	20.68	11.22	26.51
Trichoderma spp.	0.21	0.24	4.95	4.29	0.50	1.19
Other species of fungi	12.33	24.02	15.38	21.62	13.09	18.76

et – external tissue, it – internal tissue

Table 6. Fungi most frequently colonizing shoots of fruit plants in unprotected orchards in the years 2010–2012

Fungus	Percentage of fungi					
	2010		2011		2012	
	et	it	et	it	et	it
Alternaria alternata	17.70	4.49	12.57	7.88	11.65	11.37
Botrytis cinerea	0	0	1.77	2.22	1.61	0
Cladosporium spp.	0.4	0	2.16	2.96	1.79	0.58
Clonostachys rosea	1.19	6.53	0.79	2.22	0	0
Diaporthe spp./*Phomopsis* spp.	1.79	39.18	25.15	54.93	6.63	16.62
Fusarium spp. + *Gibberella* spp. + *Haematonectria haematococca*	44.93	13.47	17.29	6.89	32.44	24.20
Phoma spp. + *Peyronella pomorum*	19.68	24.49	10.02	6.89	13.26	15.45
Trichoderma spp.	0.99	0	4.91	2.46	6.10	5.83
Other species of fungi	13.32	11.84	25.34	13.55	26.52	25.95

et – external tissue, it – internal tissue

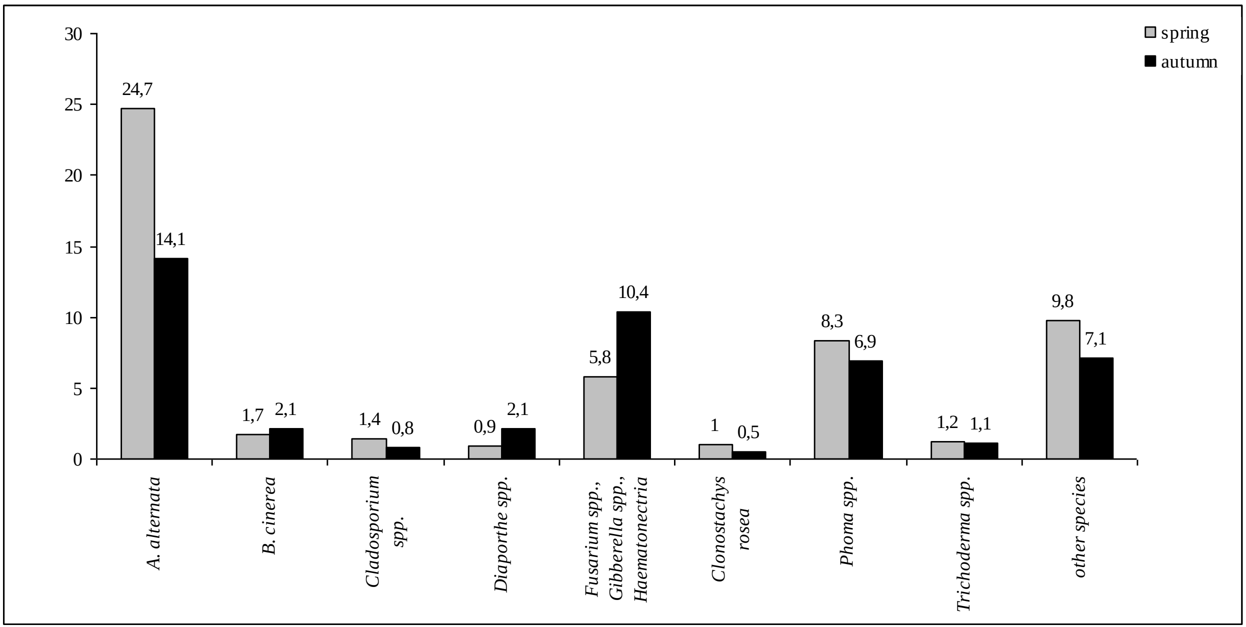

Fig. 1. Percentage of selected species of fungi inhabiting the shoots of fruit plants in 2010–2012 depending on term of studies (protected orchards)

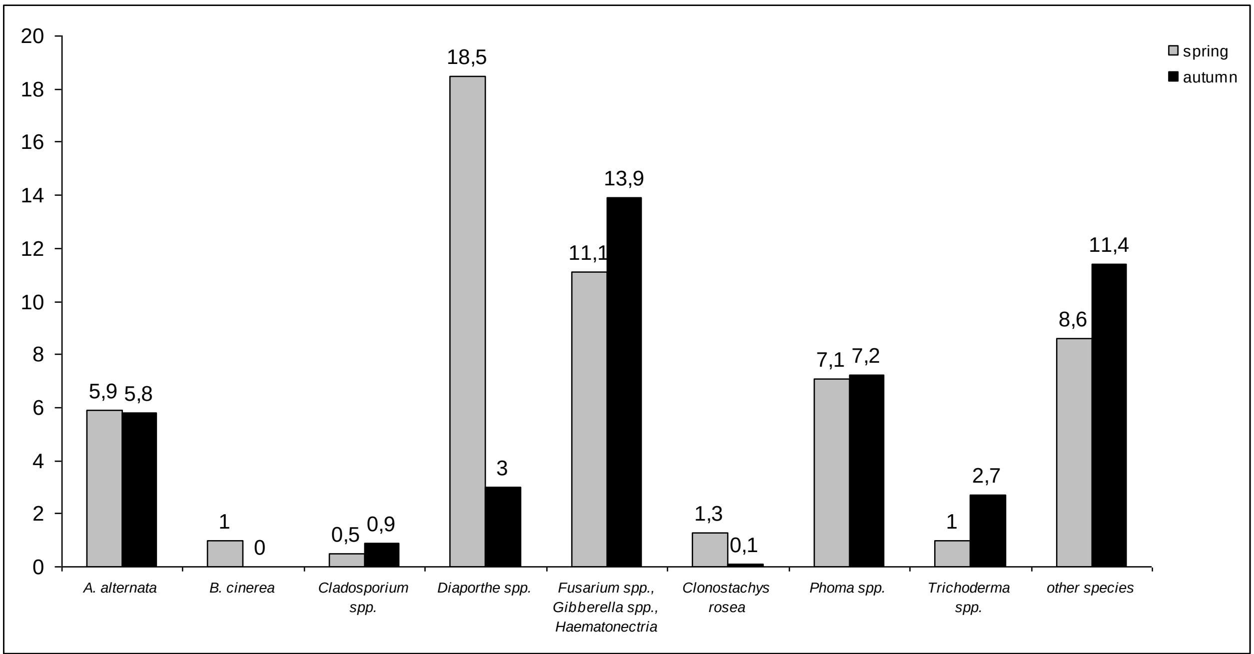

Fig. 2. Percentage of selected species of fungi inhabiting the shoots of fruit plants in 2010–2012 depending on term of studies (unprotected orchards)

Every year of the study *Alternaria alternata* was isolated from diseased shoots originating from both protected and unprotected orchards. The cultures of this fungus were isolated from 788 (54.72%) and 118 (24.58%) of the studied shoots and 3.758 (38.79%) and 300 (11.70%) isolates of this fungus were obtained from them, respectively (tabs 2 and 3). *Alternaria alternata* was isolated most frequently in 2010 from the protected orchards (tab. 5). This fungus species mostly inhabited the external tissue of the shoots collected in the spring season (tabs 5 and 6, figs 1 and 2).

Among the fungi often isolated from diseased shoots were also species of the genus *Fusarium*. This genus had been previously described within the class *Deuteromycotina* because the perfect stage of *Fusarium* spp. was not known. Currently, according to the latest taxonomy, the genus *Fusarium* is classified within the division of *Ascomycota*. The species in which the perfect stage is not known constantly belong to *Fusarium*, while species which produce the teleomorph stage belong to the genus *Gibberella* or *Haematonectria* [Kirk et al. 2008, http://www.indexfungorum.org/names/names.asp].

The above mentioned fungi were obtained from 580 shoots (40.28%) originating from protected orchards and from 180 (37.51%) shoots collected from unprotected ones. From these shoots, 1.565 (16.17%) and 639 (24.91%) fungal isolates were obtained, respectively (tabs 2 and 3). In the case of diseased shoots from chemically protected orchards the percentage of these fungi was the highest in 2010, both from external and internal tissue (tab. 5). Moreover, in the years of the study, *Fusarium* spp. were isolated more frequently in the autumn than in the spring (fig. 1). *Gibberella fujikuroi* (anamorph *F. verticillioides*) and *F. sporotrichioides* were the most often isolated. These fungi species were isolated respectively from 283 (19.65%) and 125 (8.68%) shoots, respectively, to give a total 9.16% and 3.18% of all fungal isolates (tab. 2). In unprotected orchards, *Fusarium* spp. were isolated most frequently in 2010 and 2012, mainly in the period of autumn and from the external tissue of shoots (tab. 6, fig. 2). *Haematonectria haematococca* (anamorph *F. solani*) was isolated most frequently and cultures of this fungus were isolated from 87 (18.13%) shoots, resulting in 11.54% of all fungal isolates. *Gibberella fujikuroi* (anamorph *F. verticilioides*) was also isolated quite often. The fungus inhabited 65 (13.54%) shoots and gave a total 8.89% of isolates (tab. 3).

Cultures of *Phoma* spp. (including *Peyronella pomorum*) were isolated each year from all of the studied orchards and the percentage of these fungi constituted 15.07% and 14.23% from chemically protected and unprotected orchards, respectively (tabs 2 and 3, figs 1 and 2). In the first orchard *Phoma* spp. were isolated most frequently in 2012 and mainly from internal tissue, while in the second ones the highest percentage of *Phoma* isolates were obtained in 2010 and they originated mainly from internal tissue, too (tabs 5 and 6). *Phoma herbarum* was isolated most frequently and its isolates constituted 12.16% and 11.38% of the total number of isolates in protected and unprotected orchards, respectively (tabs 2 and 3). Moreover, *Phoma* spp. were isolated in similar numbers in the spring and autumn seasons (figs 1 and 2).

The percentage of *Botrytis cinerea* cultures isolated from shoots originating from protected orchards was higher than the percentage of isolates from unprotected ones (tabs 2 and 3). From the protected shoots *B. cinerea* was isolated most frequently from internal tissue in 2011 and the number of isolates of the fungus predominated slightly in the autumn (tab. 5, fig. 1). In the unprotected orchards, *B. cinerea* was isolated only in the last two years of research and only in the spring season. This fungus species inhabited both external and internal tissue (tab. 6, fig. 2).

Species isolated in almost all years of the study also included *Clonostachys rosea* (syn. *Gliocladium roseum*, *G. catenulatum*), *Cladosporium* spp. and *Trichoderma* spp. (tabs 2 and 3). *Clonostachys rosea* inhabited the shoots of fruit trees with similar frequency in protected and unprotected orchards because its cultures were respectively 1.48% and 1.37% of the total number of isolates (tabs 2 and 3). *Clonostachys rosea* more frequently inhabited the internal tissue of the examined shoots but in protected orchards the fungus was isolated especially in the autumn season while in the unprotected orchards it was found mainly during the spring season (tabs 5 and 6, figs 1 and 2).

Among fungi of the genus *Cladosporium* the most isolates were represented by species *C. cladosporioides*, in all of the tested orchards (tabs 2 and 3). The majority of *Cladosporium* cultures were obtained in 2011 both from shoots originating from protected and unprotected orchards, especially from the external tissue (tabs 5 and 6, figs 1 and 2).

Cultures of *Trichoderma* spp. were also isolated in every year of study, which were obtained most frequently in 2011 from chemically protected orchards and in 2012 from unprotected ones, both from the external and internal tissues (tabs 5 and 6, figs 1 and 2).

Particularly noteworthy is the fact that *Diaporthe* cultures were isolated every year of the study. These fungi were obtained respectively from 134 (9.31%) and 145 (30.21%) shoots originating from protected and unprotected orchards, obtaining 3.05% and 21.45% isolates, respectively (tabs 2 and 3). *Diaporthe* isolates were obtained mainly in 2011 and 2012 from the protected orchards, while the isolates were obtained in 2011 from unprotected ones. These fungi usually inhabited the internal tissue of the shoots and more often were isolated in the spring than in the autumn (tabs 5 and 6, figs 1 and 2). Moreover, *Diaporthe* cultures were obtained especially from shoots of apple and cherry from the protected orchards and from shoots of pear and plum from unprotected ones (figs 3 and 4).

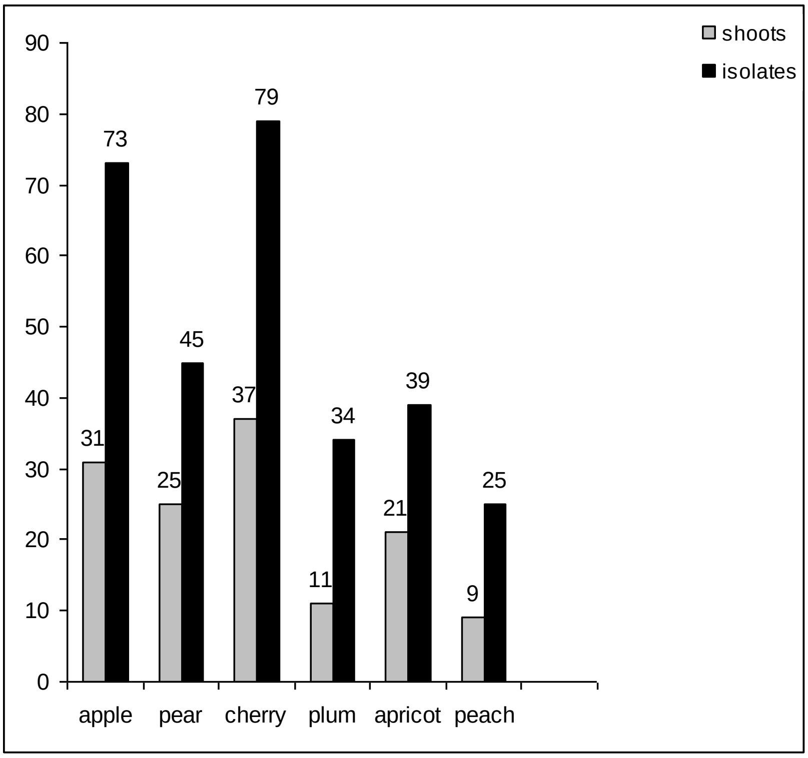

Fig. 3. The number of shoots and isolated from them cultures of *Diaporthe* (*Phomopsis*) in 2010–2012 depending on the plant host (protected orchards)

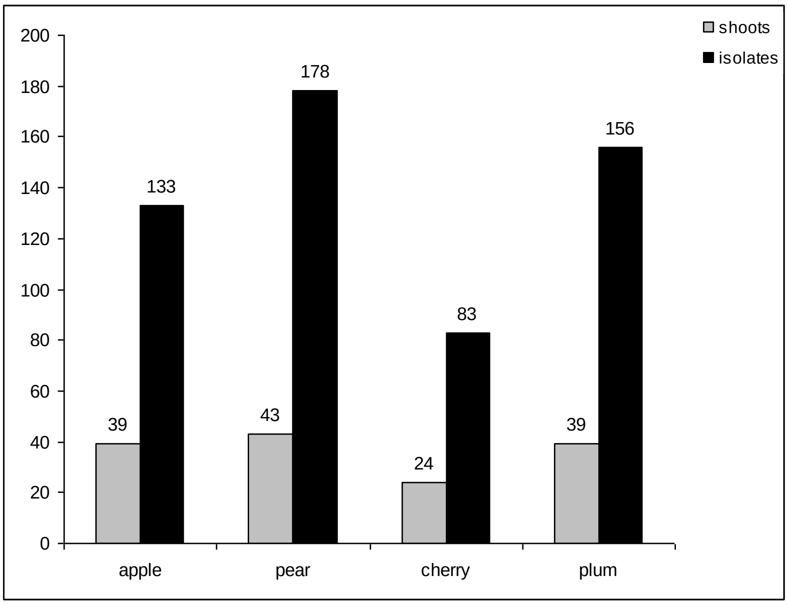

Fig. 4. The number of shoots and isolated from them cultures of *Diaporthe* (*Phomopsis*) in 2010–2012 depending on the plant host (unprotected orchards)

Other species of fungi were rare but among them were species known for their pathogenic abilities towards fruit trees, such as *Colletotrichum gloeosporioides*, *Leucostoma personii*, *Monilinia* spp. and *Neofabraea* spp. (tabs 2 and 3).

Among fungi isolated from apparently healthy shoots were also *Diaporthe* isolates, which were obtained from 34 (5.67%) shoots, giving 5.09% isolates (tab. 4). In addition, *Alternaria alternata* was isolated most frequently because it inhabited 34.17% of the studied shoots and its isolates constituted 48.79%. Numerous cultures of yeast as well as *Acremonim charticola*, *Botrytis cinerea* and *Phoma herbarum* were also obtained, whose isolates were 4.23%, 3.96% and 7.02%, respectively (tab. 4).

DISSCUSION

The results of the mycological analysis of apple, pear, cherry, plum, apricot and peach shoots confirm previous reports of the colonization of fruit tree shoots by numerous species of fungi, among which there are saprotrophic species, potential plant pathogens and antagonistic ones [Grabowski 1993, 1996, Grabowski and Leś 1996, Grabowski 2002]. The small variation in the species composition of fungi found on different host plants shows their ability to colonize different species of fruit trees growing in the orchard.

In the present study, obtaining the greater percentage of most fungi from shoots showing different disease symptoms than from apparently healthy ones indicates the preferences of saprotrophic species as well as some of the pathogens to colonize decaying wood, which is treated as a source of nutrients. According to Grabowski [2002], undamaged shoots are less attractive substrates due to the presence of tannins and phenols, which are unfavourable for fungi development.

The evident dominance of *Alternaria alternata* on shoots originating from protected orchards is probably due to the common occurrence [Tylkowska et al.

2003, Stuart et al. 2009] and resistance of this fungus on some of the preparations used in plant control against a variety of pathogens, as previously reported by other researchers [Timmer and Zitko 1997, Osowski 2004, Pres and Timmer 2006, Stuart et al. 2009].

These authors pointed out a complete lack of benzimidazole efficiency (thiophanate-methyl) in protection against *A. alternata* [Timmer and Zitko 1997, Stuart et al. 2009] explaining this phenomenon as the ability of the fungus to metabolize the ingredients of the listed fungicides. Furthermore, Timmer and Zitko [1997] proved the resistance of *A. alternata* to strobilurin. In turn, Pres and Timmer [2006] have shown little effect of mancozeb on reducing the development of *A. alternata*, although Osowski [2004] reported that the efficacy of fungicides containing mancozeb was better in controlling of potato antracnose when it was used in a mixture with another active ingredient.

It seems, that *A. alternata* had favourable conditions for development on the fruit tree shoots in protected orchards despite the intensive chemical control, especially since at the same time phyllosphere decreased the number of the other species of fungi in the shoots, that could compete with it. This hypothesis seems to be confirmed by the fact that at the same time a smaller number of *A. alternata* cultures were isolated from the shoots originating from unprotected orchards, where a greater diversity of fungi species was obtained.

Obtaining numerous isolates from the widely understood genus *Fusarium*, both from the shoots originating from the protected and unprotected orchards, indicates their importance in the shoots' environment, especially because among them there are numerous pathogens of many plant species [Kawchuk et al. 2002, Frużyńska-Jóźwiak and Andrzejczak 2010, Kiecana and Mielniczuk 2010, Kiecana et al. 2012, Danielewicz et al. 2013, Walkowiak and Krzyśko-Łupicka 2014].

The occurrence of *Fusarium* spp. on the shoots of fruit trees is probably due to their common presence in nature, their fast growth, which makes it easier to substrate colonize, competition with the other species of fungi as well as frequently appearing resistance of these fungi to fungicides, which was observed by Frużyńska-Jóźwiak and Andrzejczak [2010], Danielewicz et al. [2013] and Walkowiak and Krzyśko-Łupicka [2014], among others. They found that the proper selection of plant protection products affects the effectiveness of reducing the occurrence of *Fusarium* spp., because some of them are very effective while others, such as thiophanate-methyl and azoxystrobin, only slightly inhibit the growth of these fungi. On the other hand, Kawchuk et al. [2002] suggest that the resistance of isolates results from their origin from intensively protected plants.

The isolation of fungi species known for pathogenic abilities to fruit trees, i.e., *Botrytis cinerea, Coniothyrium fuckeli*, *Colletotrichum gloeosporioides, Neofabraea* spp., *Fusarium* spp. and *Cytospora leucostoma* and numerous saprotrophic species as: *Epicoccum nigrum*, *Chaetomium globosum*, *Phoma herbarum*, *Trichoderma koningii* and *Clonostachys rosea* both from shoots originating from protected and unprotected orchards, shows a large role of shoots in the transmission of pathogenic factors. Very important is the fact that some of these fungi cause not only bark and wood diseases but also threaten the fruit during growth and storage. *Alternaria* spp., *Fusarium avenaceum* and *Botrytis cinerea* cause, among others, leaf blotch and fruit spot, mouldy core, core browning and core rot, *Neofabraea* spp. bitter rot of apples and *Glomerella cingulata* (*C. gloeosporioides*) bitter rot of cherry [Gao et al. 2013, Wenneker et. al. 2016].

Particularly noteworthy is the isolation of fungi of the genus *Diaporthe*, the importance of which systematically increases [Machowicz-Stefaniak 1993, Uddin et al. 1997, 1998, Farr et al. 1999, 2002, Kačergius et al. 2004, Karaoglanidis and Bardas 2006, Król and Kowalik 2010, Mirabolfathy 2013].

The isolation of these fungi from shoots of apple, pear, cherry, plum, apricot and peach trees indicates the presence of these fungi in orchards in the provinces of Lublin and Świętokrzyskie, which is the first documented announcement of their occurrence in the conditions of our country. Thus, there is an increased number of fungi from the genus *Diaporthe* on fruit plants in Polish conditions, because previously their existence had been documented only on grapevine [Machowicz- Stefaniak 1993, Król 2002, 2005, 2006, 2007, Król and Kowalik 2010] and blueberry [Szmagara and Machowicz-Stefaniak 2005, Szmagara 2009].

However, non-specific symptoms caused by these fungi creates problems with their detection in fruit crops, because often *Diaporthe* isolates were obtained from shoots showing various blotch symptoms, necrosis or shoot damping, which were similar to the symptoms caused by the other bark and wood pathogens. Fungus conidiomata formation on diseased shoots could be useful in its identification, but their occurrence in natural conditions was not observed in the years of the study. In addition, colonization by *Diaporthe* of also visually healthy shoots, as demonstrated during the research, indicates the ability of these fungi for asymptomatic growth in the shoots of fruit trees, which creates the danger of unknowingly carrying them with the nursery material on long distances, as reported earlier by Mostert et al. [2000], Halleen et al. [2003] and Król [2006].

CONCLUSIONS

1. The shoots of fruit trees are inhabited by many species of fungi among which are both saprotrophic ones as well as species known for pathogenic abilities to bark and wood and to fruit during the vegetation period and storage.

2. The isolation of *Diaporthe* cultures from the shoots of apple, pear, cherry, plum, peach and apricot indicates the presence of these fungi in the investigated orchards of the Lubelskie and Świętokrzyskie provinces, which is the first information about the occurrence of *Diaporthe* on fruit trees in Poland.

3. The more frequent isolation of *Diaporthe* from shoots originating from unprotected orchards than protected ones indicates their greater threat to fruit trees grown without chemical protection.

4. Non-specific disease symptoms caused by *Diaporthe* hinder the identification of these fungi on the shoots of fruit trees.

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REFERENCES

Adams, G.C., Surve-Iyver, R.S., Iezzoni, A.F. (2002). Ribosomal DNA sequence divergence and group I introns within the *Leucostoma* species *L. cinctum*, *L. persoonii*, and *L. parapersoonii* sp. nov., ascomycetes that cause *Cytospora* canker of fruit trees. Mycologia, 94(6), 947–967.

Arsenijevič, M., Gavrilovič V. (2005). *Phomopsis perniciosa* Grove – uzročnik truleži uskladištenih plodova jabuke. Pestic. Fitomed., 20(3), 189–194.

Castillo-Pando, M.S., Nair, N.G., Emmett, R.W., Wicks, T.J. (1997). Inhibition in pycnidial viability of *Phomopsis viticola* on canes in situ as an aid to reducing inoculum potential of cane and leaf blight disease of grapevines. Austral. Plant Pathol., 26, 21–25.

Danielewicz, B., Gwiazdowski, R., Bednarek-Bartsch, A. (2013). Wpływ wybranych fungicydów na ograniczanie wzrostu kultur rodzaju *Fusarium*. Prog. Plant Prot. / Post. Ochr. Roślin, 53, 4, 759–61.

Farr, D.F., Castlebury, L.A., Pardo-Schultheiss, R.A. (1999). *Phomopsis amygdali* causes peach shoot blight of cultivated peach trees in the southeastern United States. Mycologia, 91, 1008–1015.

Farr, D.F., Castlebury, L.A., Rossman, A.Y. (2002). Morphological and molecular characterization of *Phomopsis vaccinii* and additional isolates of *Phomopsis* from blueberry and cranberry in the eastern United States. Mycologia, 94, 494–504.

Frużyńska-Jóźwiak, D., Andrzejczak, R. (2010). Wpływ wybranych preparatów na zdrowotność ozdobnych drzew i krzewów iglastych. Prog. Plant Prot./ Post. Ochr. Roślin, 50(4), 2067–2071.

Gao, L.L., Hang, Q., Sun, X.Y., Jiang, L., Hang, R., Sun, G.Y., Zha, Y.L., Bigos, A.R. (2013). Etiology of moldy core, core browning and core rot of Fuji apple in China. Plant Dis., 97, 4, 510–516.

Grabowski, M. (1993). Study on occurrence of *Nectria galligena* mycelium in wood of affected shoots of apple-trees at various points situated above and below the place of infection. Phytopathol. Pol., 5, 23–29.

Grabowski, M. (1996). Grzyby kolonizujące zrakowacenia na pędach jabłoni w sadach chemicznie chronionych oraz ich wpływ na rozwój procesu chorobowego wywołanego przez *Nectria galligena* Bres. Acta Agrar. Silv., Ser. Agrar., 34, 35–41.

Grabowski, M. (2002). Badania nad grzybami zasiedlającymi rozdrobnione pędy jabłoni pozostawione w sadzie po cięciu. Acta Agrobot., 55, 1, 79–87.

Grabowski, M., Leś, B. (1996). Fungi inhabiting cancerations on apple shoots and their application against *Nectria galligena* Bres. Phytopathol. Pol., 11, 15–22.

Gramaje, D., Agustí-Brisach, C., Pérez-Sierra, A., Moralejo, E., Olmo, D., Mostert, L., Damm, U., Armengol, J.(2012). Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain). Persoonia, 28, 1–13.

Gomes, R.R., Glienke, C., Videira, S.I.R., Lombard, L., Groenewald, J.Z., Crous, P.W. (2013). *Diaporthe*: a genus of endophitic, saprobic and plant pathogenic fungi. Persoonia, 31, 1–41.

Halleen, F., Crous, P.W., Petrini, O. (2003). Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. Australas. Plant Pathol., 32, 47–52.

Kačergius, A., Gabler, J., Jovaišienè, Z. (2004). Determination of *Phomopsis* canker and dieback of highbush blueberries and cranberries in Lithuania. Agron. Vēstis/ Latv. J. Agron. 7, 71–78.

Kanematsu, S., Minaka, N., Kobayashi, T., Kudo, A., Ohtsu, Y. (2000). Molecular phylogenetic analysis of ribosomal DNA internal transcribed spacer regions and comparison of fertility in *Phomopsis* isolates from fruit trees. J. Gen. Plant Pathol., 66, 191–201.

Karaoglanidis, G.S., Bardas, G. (2006). First report of *Phomopsis* fruit decay on apple caused by *Phomopsis mali* in Greece. Plant Dis., 90(3), 375.

Kawchuk, L.M., Hutchison, L.J., Verhaeghe, C.A., Lynch, D.R., Bains, P.S., Holey, J.D. (2002). Isolation of β-tubulin gene and characterization of thiabendazole resistance in *Giberella pulicaris*. Can. J. Plant Pathol., 24(2), 233–238.

Kiecana, I., Mielniczuk, E. (2010). *Fusarium* head blight of winter rye. Acta Agrobot., 63(1), 129–135.

Kiecana, I., Cegiełko, M., Mielniczuk, E., Perkowski, J. (2012). The occurrence of *Fusarium poae* (Peck) Wollenw. on oat (*Avena sativa* L.) panicles and its harmfulness. Acta Agrobot., 65(4), 169–178.

Kirk, P.M., Cannon, P.M., Minter, D.W., Stalpens, J.A. (2008). Ainsworth & Bisby's Dictionary of Fungi. 10th ed. CAB International, Wallingford, UK, 640 pp.

Król, E. (2002). Determination of genetic variability within *Phomosis* spp. using RAPD method. Phytopathol. Pol., 25, 35–46.

Król, E. (2005). Identification and differentiation of *Phomopsis* spp. isolates from grapevine and some other plant species. Phytopathol. Pol., 35, 151–156.

Król, E. (2006). Grzyby zasiedlające zdrowe łozy winorośli (*Vitis* spp.) w wybranych szkółkach. Acta Agrobot., 59(2), 163–173.

Król, E. (2007). *Phomopsis viticola* Sacc. jako patogen winorośli na świecie i w Polsce. Post. Nauk Roln., 4, 85–96.

Król, E., Kowalik, B. (2010). Charakterystyka grzybów rodzaju *Phomopsis* wyizolowanych z roślin sadowniczych. Prog. Plant Prot./ Post. Ochr. Roślin, 554, 53–59.

Król, E., Kowalik, B. (2011). Grzyby z rodzaju *Phomopsis* występujące na pędach roślin sadowniczych. Post. Nauk Rol., 2, 31–42.

Latorre, B.A., Rioja, M.E., Lillo, C., Muñoz, M. (2002). The effect of temperature and wetness duration on infection and a warning system for European canker (*Nectria galligena*) of apple in Chile. Crop Prot., 21(4), 285–291.

Machowicz-Stefaniak, Z. (1993). *Phomopsis viticola* Sacc. (*Sphaeropsidales Deuteromycotina*) nowy w Polsce patogen pędów winorośli. Acta Mycol., 28, 157–160.

Michailides, T.J., Thomidis, T. (2006). First report of *Phomopsis amygdali* causing fruit rot on peaches in Greece. Plant Dis., 90(12), 1551.

Mirabolfathy, M., Hoseinian, L., and Mirhoseini Moghadam, A. (2013). First report of *Phomopsis amygdali* (Del.) Tuset & Portilla causing galls on common hazel (*Corylus avellana*) twigs in Iran. Iran. J. Plant Pathol., 49(1), 43–45.

Mostert, L., Denman, S., Crous, P.W. (2000). In vitro screening of fungicides against *Phomopsis viticola* and *Diaporthe perjuncta*. S. Afr. J. Enol. Vitic., 21, 62–65.

Mostert, L, Crous, P.W., Kang, C.J., Phillips, A.J.L. (2001). Species of *Phomopsis* and a *Libertella* sp. occurring on grapevines with specific reference to South Africa: morphological, cultural, molecular and pathological characterization. Mycologia, 93, 145–166.

Osowski, J. (2004). Skuteczność różnych fungicydów zawierających mankozeb w ograniczaniu rozwoju alternariozy ziemniaka w badaniach polowych i laboratoryjnych. Biul. Inst. Hod. Aklimat. Rośl., 233, 295–302.

Pres, N.A., Timmer, L.W. (2006). Evaluation of the Alter-Rater model for spray timing for control of *Alternaria* brown spot on Murcott tangor in Brazil. Crop Prot., 25, 454–460.

Regner, K.M., Johnson, D.A., Gross, D.C. (1990). Etiology of canker and dieback of sweet cherry trees in Washington State. Plant Dis., 74, 430–433.

Romanazzi, G., Mancini, V., Murolo, S. (2012). First report of *Leucostoma cinctum* on sweet cherry and European plum in Italy. Phytopathol. Mediterr., 51(2), 365−368.

Rossman, A.Y., Udayanga, D., Castlebury, L.A., Hyde, K.D. (2014). Proposal to conserve the name *Diaporthe eres*, with a conserved type, against all other competing names (*Ascomycota*, *Diaporthales*, *Diaporthaceae*). Taxon 63, 934–935.

Stuart, M.R., Bastianel, F.A.A., Machado, M.A. (2009). Alternaria brown spot. Laranja, 30, 1–2, 29–44.

Spotts, R.A., Facteau, T.J., Cervantes, L.A., Chestnut, N.E. (1990). Incidence and control of *Cytospora* canker and bacterial canker in a young sweet cherry orchard in Oregon. Plant Dis., 74, 577–580.

Szmagara, M. (2009). Biodiversity of fungi inhabiting the highbush blueberry stems. Acta Sci. Pol. Hortorum Cultus, 8(1), 37–50.

Szmagara, M., Machowicz-Stefaniak, Z. (2005). Grzyby porażające pędy borówki wysokiej (*Vaccinium corymbosum* L.). Prog. Plant Prot./ Post. Ochr. Roślin, 45(2), 1130–1133.

Timmer, L.W., Zitko, S.E. (1997). Evaluation of fungicides for control of *Alternaria* brown spot and citrus scab. Proc. Fla. State Hort. Soc., 110, 71–76.

Tylkowska, K., Grabarkiewicz-Szczesna, J., Iwanowska, H. (2003). Production of toxins by *Alternaria alternata* and *A. radicina* and their effects on germination of carrot seeds. Seed Sci. Technol., 31, 309–316.

Udayanga, D., Liu, X.X., McKenzie, E.H.C., Chukeatirote, E., Bahkali, A.H., Hyde, K.D. (2011). The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. Fungal Divers., 50, 189–225.

Udayanga, D., Castlebury, L.A., Rossman, A. Y., Chukeatirote, E., Hyde, K.D. (2014). Insights into the genus *Diaporthe*: phylogenetic species delimitation in the *D. eres* species complex. Fungal Divers., 67, 203–229.

Uddin, W., Stevenson, K.L., Pardo-Schultheiss, R.A. (1997). Pathogenicity of a species of *Phomopsis* causing a shoot blight on peach in Georgia and evaluation of possible infection courts. Plant Dis., 81, 983–989.

Uddin, W., Stevenson, K.L., Pardo-Schultheiss, R.A., Rehner, S.A. (1998). Pathogenic and molecular characterization of three *Phomopsis* isolates from peach, plum and Asian pear. Plant Dis., 82, 732–737.

Valiuškaitê, A., Raudonis, L. (2008). Epidemiology of bark diseases of apple tree in Lithuania. Sodinink. Daržinink., 27(4), 51–57.

Walkowiak, W., Krzyśko-Łupicka, T. (2014). Nowe rozwiązania w ochronie zbóż przed fuzariozami. Prog. Plant Prot./Post. Ochr. Rośl., 54(2), 127–134.

Wenneker, M., Pham, K.T.K., Lemmers, M.E.C., de Boer, F.A., van der Lans, A.M., van Leeuwen, P.J., Hollinger, T.C. (2016). First report of *Fusarium avenaceum* causing wet care rot of "Elstar" apples in the Netherlands. Plant Dis., 100(7), 1501.

Živković, S.T., Stojanović, S.D., Balaź, J., Gavrilović, V.P. (2007). Characteristics of *Phomopsis* sp. isolates of plum trees origin. Proc. Nat. Sci., Matica Srpska Novi Sad, 113, 83–91.