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ORIGINAL PAPER

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INFLUENCE OF TEMPERATURE AND FUNGAL COMMUNITY ON GROWTH AND SPORULATION OF *Diaporthe* FROM FRUIT PLANTS

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ABSTRACT

The purpose of this experiment was to determine the influence of temperature and fungi colonizing fruit plants on *Diaporthe*, a pathogenic fungus in Poland. The biotic series method was used to test *in vitro* the effects of the fungi colonizing fruit trees on isolates of *Diaporthe*. Among the 13 fungal species tested, six inhibited the growth and development of *Diaporthe*; while another two species were neutral and the rest showed the lack of limiting impact in relation to the pathogen. Fungi present in the shoots of fruit plants was able to limit the growth and development of *Diaporthe* spp., both in chemically-protected and non-protected orchards. Studies on the effect of temperature indicated that the optimum temperature for vegetative growth of isolates of *Diaporthe* ranged from 20°C to 30°C, and for conidial sporulation from 25°C to 30°C. However, the ability of *Diaporthe* sp. to survive at extreme temperature (–20°C and +35°C) enables their vegetative growth in climatic conditions worldwide.

Key words: fungal community, interactions, orchard plants pathogen, temperature

INTRODUCTION

The fungal genus *Diaporthe* (synonym *Phomopsis*) includes important plant pathogens with diverse host associations and worldwide distribution [Yang et al. 2018, Marin-Felix et. al. 2019]. They cause diseases such as canker, die-back, pod blight, stem-end rot, storage rot and other disease on leaves, stems and petioles of many different plant species [Udayanga et al. 2011, Gomes et al. 2013, Guarnaccia and Crous 2018, Guarnaccia et al. 2018]. As a result of changes in the rules of fungal nomenclature the name *Diaporthe* was adopted over *Phomopsis*, a name that must now be regarded as a synonym of *Diaporthe* [Gomes et al. 2013, Udayanga et al. 2014, Rossman et al. 2015, Guarnaccia et al. 2018]. Recently, a number of *Diaporthe* isolates in the *Phomopsis* asexual morph were obtained from shoots of fruit trees from orchards in the south-eastern region of Poland.

A number of fungi are antagonistic to other fungi both in the soil as well as on above-ground plant parts [Mańka 1974, Król 2004a, 2004b, Król and Machowicz-Stefaniak 2008, Rhouma et al. 2008]. These antagonists may prevent colonization of the shoots by pathogenic fungi and thus prevent disease development. Disease development depends not only on the presence of the pathogen, but also on environmental conditions, such as the temperature and the fungal community near the host plant [Fokkema 1993, Mańka 1995, Król, 2004a, 2004b, Król and Machowicz-Stefaniak 2008, Zalewska et al. 2004, Rhouma et al. 2008, Machowicz-Stefaniak 2009].

Because of the increasing severity of diseases caused by *Diaporthe* spp. in the orchards worldwide and frequent isolation of these fungi from shoots of fruit plants in Poland, these studies were undertaken

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in order to investigate the influence of some fungal species inhabiting shoots of fruit plants on *Diaporthe* spp. This study was performed both in protected and non-protected orchards. Another aim of the present work was to determine the effect of temperature on the growth and morphological structures of the *Diaporthe* isolates. This study hypothesized that conditions would not be favorable for development of isolates of *Diaporthe* in south-eastern Poland due to the strong competition from fungi inhabiting shoots and higher thermal requirements.

MATERIALS AND METHODS

To determine the influence of fungal community on the pathogens, isolates of *Diaporthe* was obtained one each from shoots of apple (264J), pear (292G), cherry (322W) and plum (352S), as well as 13 species of fungi most frequently isolated from the same shoots of fruit plants from chemically protected and unprotected orchards located in Lublin and Świętokrzyskie provinces (south-eastern Poland) in 2010–2012. The *Diaporthe* isolates were genetically and morphologically characterized and the pathogenic character of the fungus was confirmed by pathogenicity tests according to Koch's postulates [Abramczyk and Król 2016, Abramczyk et al. 2018]. These 13 species of fungi constituted over 80% of the fungal community and belonged to the following species: *Alternaria alternata*, *Botrytis cinerea*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Clonostachys rosea*, *Colletotrichum gloeosporioides*, *Epicoccum nigrum*, *Fusarium fujikuroi*, *Trichoderma koningii*, *Trichothecium roseum*, *Phoma herbarum*, *Saccharomyces* sp. and *Truncatella truncata*.

The biotic series method was used for testing the effect of fungal community on a pathogen [Mańka 1974, 1995]. Inoculum as mycelial discs of 3 mm in diameter were derived from the peripheral part of a 10-day-old cultures of the each *Diaporthe* isolate and from each of the community fungus component. These were placed mycelium down at a distance of 2 cm from each other on PDA medium (Difco) in Petri dishes. Separately growing colonies of each fungal species constituted controls. For each experimental combination one *Diaporthe* isolate with one individual community component plus the control, five repetitions (Petri dishes) were prepared. The Petri dishes were incubated in an incubator at 21°C. After 10 days, the growth of the dual cultures was evaluated, according to the scale provided by Mańka [1995] (tab. 1).

The evaluation results in obtaining individual biotic effect (IBE) value which consists of the arithmetic sum of points for surrounding of colony, inhibition zone and reduction of colony size, and which denominates the effect of one of the species on the pathogen's growth. The IBE value multiplied by spe-

Table 1. Scale for evaluating the situation in dual fungi culture

Situation in dual culture	Estimation* (points)
i) Surrounding of colony	
– Both fungal colonies, i.e. of the pathogen (A) and of the fungal community component (B) meeting along a straight line	0
– Colony B meeting colony A along a slightly curved line surrounding less than 1/3 of it	+1
– Colony B meeting colony A along a curved line surrounding at least 1/3, but less than 1/2 of it	+2
– Colony B meeting colony A along a curved line surrounding at least ½ but less than 2/3 of it	+3
– Colony B meeting colony A along a curved line surrounding 2/3 or more of it	+4
ii) Inhibition zone	
– Each millimeter of inhibition zone between partner colonies in favour of colony B	+1
iii) Reduction of colony size	
– Colony A at least 1/3 but less than ½ smaller than its separately-grown control colony	+1
– Colony A at least 1/2 but less than 2/3 smaller than its separately-grown control colony	+2
– Colony A at least 2/3 smaller than its separately-grown control colony	+3
– Colony A completely undeveloped	+4

* In opponent situations in dual fungi cultures the estimates would be of the same absolute value but with minus signs

cies frequency results in general biotic effect (GBE), treated as the effect of all the component's isolates on the pathogen. After summarizing all the GBE's the summary biotic effect (SBE) was determined, which illustrated the effect of entire fungal community on the pathogen. Any of the biotic effects mentioned, may be positive (indicating suppressive effect on pathogen's growth), negative (indicating supporting effect on pathogen's growth), or neutral (0). The intensity of the supporting or suppressing effect was expressed by the absolute value of the biotic effect [Mańka 1974, 1995]. Biotic interaction among the tested fungi and *Diaporthe* spp. was evaluated separately for fungal communities obtained from protected and non-protected orchards.

The same isolates as in the previous studies (264J, 292G, 322W, 352S) were selected to study the effect of temperature on growth and morphological structures of *Diaporthe* spp. For each isolate, three replicated plates of PDA were inoculated in the center with a 3-mm of mycelial disc of each isolate of *Diaporthe* spp. and incubated at –20°C, 0°C, 5°C, 10°C, 15°C, 20°C, 25°C, 30°C and 35°C. Depending on the temperature range, Petri dishes were incubated in incubator, refrigerator or freezer for 14 days in the dark. Linear growth of each fungal species was determined by measuring the diameter of the colony every 2 days. At the same time the morphology of each colony, the rate of conidiomata formation and conidia secretion were evaluated.

To analyze accurately the formation of morphological structures, macro- and microscopic observations were carried out up on cultures for 40 days. Furthermore, the Petri dishes kept at –20°C for 14 days were transferred to room temperature (25 ±1°C) to check whether the studied isolates will be able to grow, or they are killed. Experimental results were statistically analyzed by analysis of variance followed by Tukey's confidence intervals [Oktaba 1987].

RESULTS

Because obtained results to the biotic effects were similar for all tested *Diaporthe* sp. isolates, the results are presented as mean values calculated for the 4 isolates together. Among 13 tested species of fungi, 6 inhibited the growth and development of *Diaporthe* spp. which was shown by positive values of

Table 2. Biotic effect of the fungal community isolated from shoots of fruit plants (apple, pear, cherry, plum) on the growth of *Diaporthe* isolates (the chemically protected orchards)

Species of fungi		2010		2011		2012	
	IBE*	frequency	GBE**	frequency	GBE**	frequency	GBE**
Alternaria alternata (Fr.) Keissl.	+2	718	+1436	549	+1098	479	+958
Botrytis cinerea Pers.	0	28	0	163	0	74	0
Chaetomium globosum Kunze	+2	16	+32	35	+70	3	+6
Cladosporium cladosporioides (Fres.) de Vries	–3	5	–15	57	–171	0	0
Colletotrichum gloeosporioides (Penz.) Sacc.	–1	38	–38	46	–46	0	0
Epicoccum nigrum Link	+2	12	+24	32	+64	22	+44
Fusarium fujikuroi Nirenberg	+1	333	+333	233	+233	168	+168
Clonostachys rosea (Link) Schroers, et al.	0	20	0	16	0	41	0
Phoma herbarum Westend.	–1	163	–163	369	–369	221	–221
Saccharomyces spp.	–1	32	–32	96	–96	0	0
Trichoderma koningii Oudem.	+6	0	0	134	+804	6	+36
Trichothecium roseum (Pers.) Link	+1	0	0	31	+31	0	0
Truncatella truncata (Lév.) Steyaert	–1	0	0	0	0	0	0
Number of isolates		1365		1761		1014	
SBE***			+1577		+1618		+991

* IBE – individual biotic effect, ** GBE – general biotic effect, *** SBE – summary biotic effect

the individual biotic effect (IBE) – Tables 2 and 3. The highest inhibitory effect on *Diaporthe* spp. was exerted by *Trichoderma koningii*. The fungi that slightly limited the growth of the pathogen were: *Alternaria alternata*, *Epicoccum nigrum*, *Chaetomium globosum* as the IBE was +2, and *Fusarium fujikuroi* and *Trichothecium roseum* reaching the value of +1 (Tabs 2, 3). *Clonostachys rosea* and *Botrytis cinerea* showed a neutral effect on *Diaporthe* spp. and after 10 days of dual growth their IBEs were 0 (Tabs 2, 3).

Cladosporium cladosporioides (–3), *Phoma herbarum* (–1), *Colletotrichum gloeosporioides* (–1), *Truncatella truncata* (–1) and *Saccharomyces* (–1) showed the negative values of the IBE's indicating the lack of limiting impact in relation to the pathogen (Tabs 2, 3). The summary biotic effect (SBE), illus-

Table 3. Biotic effect of the fungal community isolated from shoots of fruit plants (apple, pear, cherry, plum) on the growth of *Diaporthe* isolates (the unprotected orchards)

Species of fungi		2010		2011		2012	
	IBE*	frequency	GBE**	frequency	GBE**	frequency	GBE**
Alternaria alternata (Fr.) Keissl.	+2	100	+200	96	+192	104	+208
Botrytis cinerea Pers.	0	0	0	18	0	9	0
Chaetomium globosum Kunze	+2	11	+22	20	+40	18	+36
Cladosporium cladosporioides (Fres.) de Vries	–3	2	–6	9	–27	12	–36
Colletotrichum gloeosporioides (Penz.) Sacc.	–1	11	–11	8	–8	8	–8
Epicoccum nigrum Link	+2	8	+16	52	+104	53	+106
Fusarium fujikuroi Nirenberg	+1	111	+111	41	+41	76	+76
Clonostachys rosea (Link) Schroers et al.	0	22	0	13	0	0	0
Phoma herbarum Westend.	–1	130	–130	43	–43	119	–119
Saccharomyces spp.	–1	48	–48	16	–16	81	–81
Trichoderma koningii Oudem.	+6	0	0	35	+210	53	+318
Trichothecium roseum (Pers.) Link	+1	0	0	2	+2	0	0
Truncatella truncata (Lév.) Steyaert	–1	3	–3	0	0	0	0
Number of isolates		446		353		533	
SBE***			+151		+495		+500

* IBE – individual biotic effect, ** GBE – general biotic effect, *** SBE – summary biotic effect

Table 4. Influence of temperature on the diameter of the colony (mm) of *Diaporthe* after 14 days of growth on PDA medium

Temperature isolates	–20°C	0°C	5°C	10°C	15°C	20°C	25°C	30°C	35°C
264 J	0aA	0aA	23,6aB	39,3aC	70aD	90aE	90aE	90aE	0aA
292 G	0aA	0aA	27,6bB	40,6aC	75,3bD	90aE	90aE	90aE	0aA
322 W	0aA	0aA	29,3cB	35bC	73,6cD	90aE	90aE	90aE	0aA
352 Ś	0aA	0aA	27bB	34bC	70aD	90aE	90aE	90aE	0aA

Small letters – variations between the isolates of the fungus at a given emperature (NIR = 1,58); capitals – variations depending on the temperature for a given isolate of the fungus (NIR = 2,89); the values are not significantly different if they are marked with the same letter

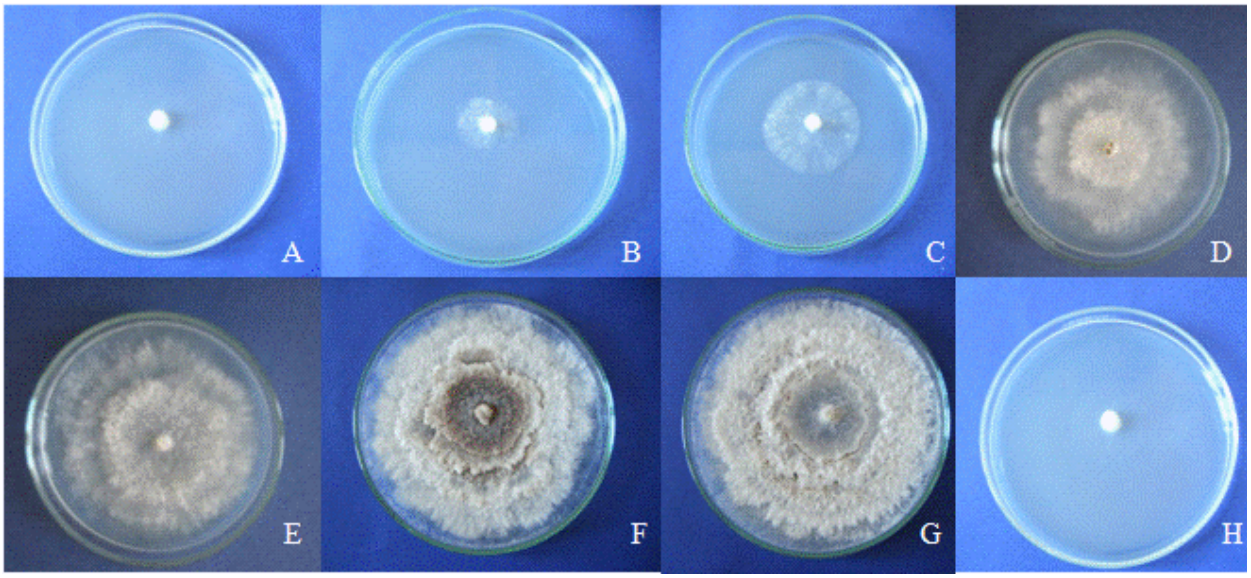

Fig. 1. Growth of *Diaporthe* isolate 264 J on PDA medium at different temperature: A – 0°C, B – 5°C, C – 10°C, D – 15°C, E – 20°C, F – 25°C, G – 30°C, H – 35°C after 14 days incubation

trating the influence of the whole fungal communities present on shoots of orchard plants in south-eastern Poland on *Diaporthe* spp. received positive values, in all three years of the study, both in chemically protected (Tab. 2) and non-protected orchards (Tab. 3).

The analyses of the effect of temperature on growth and morphological structures of *Diaporthe* spp. revealed substantial effect on the size of the colonies of the tested isolates. However, there was no apparent difference in reaction to a given temperature between the studied isolates (Tab. 4).

After 14 days of culture, the greatest diameters of the colonies were observed in the temperature range from 20°C to 30°C, because the culture of all the strains covered the entire surface of the Petri dish (Fig. 1, Tab. 4). Simultaneously under the same conditions the earliest mycelial growth was noted at 20°C to 30°C. After 8 days, the mycelium occupied almost the entire surface of the Petri dish and had a typical zonation growth. In these temperature ranges, single pycnidia were formed after 14–24 days of culture, depending on the isolate and temperature (Fig. 1, Tab. 5). Initially, they were few but at 25°C they covered approximately 2/3 of the surface of the medium. The diameter of the isolates tested ranged from 23.6–29.3 mm, 34–40.6 mm and 70–75.3 mm at 5°C, 10°C and 15°C, respectively (Tab. 4). The diameter of the colonies at high temperatures was significantly larger than that of the fungi incubated at of 5°C and 15°C. The isolates from cherry (322W) and pear (292G) grew faster than other isolates. At 10°C the fastest growing cultures were ones from apple (264J) and pear (292G) – Table 4. There was no mycelial growth for 5 days at 5°C. On the sixth day, more abundant hyphae developed from the inoculum disc and on the eighth day mycelium outgrown on medium. In the following days, colonies of individual isolates gradually increased in diameter. Subsequently, the fungus developed normally, although the mycelium was slightly looser at 5–15°C than at 20°C to 30°C.

Colonies of *Diaporthe* spp. developed a typical zonated growth after 40 days of incubation. Single conidiomata appeared only after 40 days of culture within the colonies of all tested isolates. In isolates growing at 10°C and 15°C, the first mycelial growth was observed after 4 days of incubation. At first, it was very delicate and developed only on the top of the inoculum. On the sixth day, mycelium was already abundant, but only grew upwards and not towards the surface of the agar medium. In the following days the mycelium grew from inoculum on agar surface, it had a distinctive zonation appearance. Only few conidia

Table 5. Influence of temperature on *Diaporthe* sporulation

	Beginning of conidiomata formation (days)	The number of conidiomata on the colony surface	Beginning of sporulation (days)
–20°C	–	–	–
0°C	–	–	–
5°C	40	very few	–
10°C	40	very few	–
15°C	24–26	few	30–40
20°C	10–25	few	40
25°C	12–25	on ¾ of the surface	28–40
30°C	12–18	few	26
35°C	40	very few	–

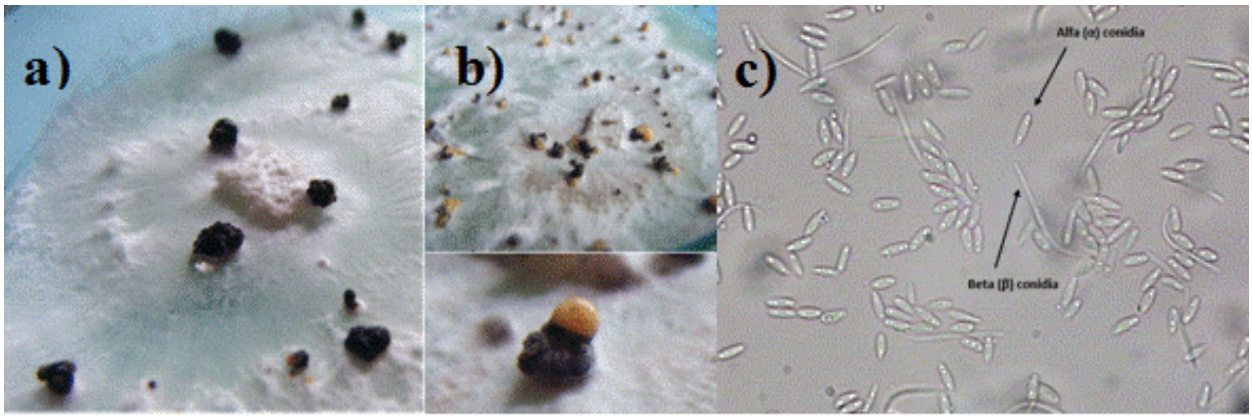

Fig. 2. Development of pycnidia of *Diaporthe* on PDA medium at 25°C; a) conidiomata formation, b) sporulation, c) alfa (α) and beta (β) conidia (zoom x600) of *Diaporthe* (264J) at 25°C [phot. B. Abramczyk]

appeared after 40 days of incubation at 10°C and after 24–26 days at 15°C, depending on the isolate (Tab. 5). At 0°C and 35°C, there was no mycelial growth for the entire duration of the experiment. Delicate, white coating of the fungal hyphae remained only on the top of the inoculum and 2–3 mm around it (Fig. 1, Tab. 4). At –20°C, the medium frozen and no fungal growth appeared. After moving plates from –20°C, 0°C and 35°C to room temperature the fungal colonies slowly began to grow around the inoculum. Initially the structure of the mycelium was slightly looser than in the case of the cultures growing at 20–30°C. But after a few days the colonies had a typical *Diaporthe* appearance and developed on their surface numerous pycnidia (Fig. 2a).

Sporulation of *Diaporthe* isolates observed only at 15°C to 30°C (Fig. 2b, Tab. 5). Sporulation started after 26 days of incubation at 30°C and after 28 days at 25°C, while at 20°C and 15°C, respectively after incubation of 40 and 30 days (Tab. 5). In microscopic preparations both types (α and β) of *Diaporthe* conidia were always observed (Fig. 2c). In other cases, no sporulation of the fungus was observed up to 40 days of culture, i.e. when the experiment was terminated.

DISCUSSION

The positive, negative and neutral IBE values obtained in the present study indicate that some fungal species may limit the growth of *Diaporthe* isolates while others are limited by them. However, the presence in the studied fungal community of numerous *A. alternata* and *F. fujikuroi* isolates which limited the pathogen growth, resulted in positive values of GBE and consequently SBE in all three years of the study.

In turn, the positive summary biotic effects (SBE) obtained suggest that the communities of fungi present in the shoots of fruit plants were able to limit the growth and development of *Diaporthe* spp., both in chemically protected and non-protected orchards. Presumably, such abilities are of particular importance in non-protected orchards, where shoots of fruit trees are inhabited by numerous species of fungi that continually affect each other. This is of great practical significance, as it suggests that the *Diaporthe* spp. did not find a favorable conditions for growth in the company of native common species inhabiting shoots of fruit plants.

In the literature, there is not much information on biotic interactions of fungal species and *Diaporthe* spp. Kuropatwa [1993] showed weak competitive abilities of *Phomopsis viticola* and Machowicz-Stefaniak [2009] proved weak competitive properties of *P. diachenii*, which was limited by most species of fungi representing the community of caraway, with the exception of *Colletotrichum gloeosporioides* and *Septoria carvi*.

Noteworthy is the isolation of *Trichoderma* and *Clonostachys* species that effectively limited many phytopathogens [Fokkema 1993, Król 2004a, 2004b, Zalewska et al. 2004, Zimowska 2004, Rhouma et al. 2008]. The parasitic abilities, rapid growth and competition for nutrients together with the production of antibiotics that inhibit the growth of other fungi determine the high efficiency of these antagonists [Papavizas 1985, Łacicowa 1989, Jacobsen and Backman 1993, Rhouma et al. 2008]. It is therefore a desirable phenomenon to occur in the phyllosphere of fruit plants.

Growth of *Diaporthe* colonies at temperatures from 0°C to 35°C and sporulation from 15°C to 30°C, regardless of the tested isolate, indicate the ability of this fungus to adapt to different environmental conditions, which was reported previously by other authors [Uecker 1988, Udayanga et al. 2011, Gomes et al. 2013].

However, the optimum temperature for vegetative growth of *Diaporthe* should be considered to be in the range from 20°C to 30°C, and for conidial sporulation from 25°C to 30°C. Requiring high temperature for sporulation suggests that these fungi may threaten the orchard plants, especially during hot periods of the growing season. This points to their thermophilic nature, which was also by Uddin et al. [1997], Kanematsu et al. [1999] and Gramaje et al. [2012]. The survival of *Diaporthe* isolates in under extreme temperature ranges such as –20°C and +35°C enables their vegetative growth and explains the presence of these fungi in diverse climatic conditions worldwide.

CONCLUSIONS

1. Native species of fungi colonizing the shoots of fruit trees are able to limit growth and development of *Diaporthe* spp. and should be considered a beneficial phenomenon.
2. Vegetative growth of *Diaporthe* colonies in a wide temperature range, regardless of the tested isolate, indicates the ability of this fungus to adapt to different environmental conditions.
3. Requiring high temperature for sporulation suggests that *Diaporthe* may threaten the orchard plants in Polish climatic conditions especially during hot periods of the growing season.

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