Rhizoctonia solani AG5 and its offspring – morphology and sensitivity to fungicides

Patrycja Hendel* 0000-0002-4346-9728[1], Ewa Moliszewska 0000-0003-4919-5139[1], Małgorzata Nabrdalik 0000-0002-2565-9034[1], Paweł Kudrys 0000-0003-1776-4894[1], Natalia Knap[1]

[1] University of Opole, Faculty of Natural Sciences and Technology, Institute of Environmental Engineering and Biotechnology, Opole, Poland
* To whom correspondence should be addressed: patrycja.hendel@uni.opole.pl

Abstract

The objective of these studies was to identify differences and similarities within the progeny of *Rhizoctonia solani* AG5, which arose from basidiospores produced by the maternal strain ID23. The following characteristics were analyzed: appearance of the mycelium (color, structure, zonation, and presence of sclerotia), growth rate (at 10°C, 20°C, and 30°C), fungicide sensitivity, and hyphal structure.

The mycelial color of *R. solani* AG5 ranged from white/cream to light and dark brown. The structure of the mycelium may be compacted or flattened with visible zoning or fluffy with dark brown sclerotia on the colony surface. Homokaryons and heterokaryons derived from homokaryons were analyzed by constructing a phylogenetic tree using morphological data. Single basidiospore-grown isolates formed a separate subclade, the majority of which were grouped with a maternal isolate; however, heterokaryons derived from them created a separate subclade. In addition, isolates grown in basidiospores germinated at low temperatures created their own group, but with some exceptions. This shows a divergence in the morphological parameters of the subsequent generations and within generations. The optimal temperature for growth was found to be between 20°C and 30°C. The exceptions were strains obtained from basidiospores that germinated at refrigerated temperatures. For these samples, 10°C was found to be the optimal growth temperature. The hyphae of homokaryons were characterized by the presence of branching at an almost right angle and the presence of a septum at the site of constriction of the branch itself. The mean diameter of hyphae ranged 2.93–15.60 µm, depending on the age of hyphae. The fungicidal compounds at a concentration of 10 ppm had no significant effect on the activity of the tested strains, whereas a tenfold increase in the dose reduced the growth ability of the tested isolates. The activity of fungicides containing azoxystrobin, thiuram, or thiophanate-methyl on *R. solani* resulted in a reduction in the mycelial growth rate only in the case of azoxystrobin and thiuram, and in some cases, it was completely inhibited (thiophanate-methyl).

Keywords: *Rhizoctonia solani*, basidiospore, progeny, morphology, hyphae, fungicides, azoxystrobin, thiuram, thiophanate-methyl.

Introduction

Rhizoctonia solani belongs to phylum Basidiomycota. This pathogen is widely distributed worldwide. It has a wide host range and attacks more than 250 plant species. This fungus attacks the root systems, stems, seedlings, fruits, and leaves. In the natural environment, they occur in the form of mycelial hyphae. *R. solani* can survive in the form of sclerotia for up to several years in soil or plant debris (Nagrodzka et al., 2016).

The mycelium produced by *R. solani* is colorless in the initial stage of development and ranges from light to dark brown in later stages. It consists of cells with an elongated shape and numerous branches. Each cell is tapered at the end and has a transverse septum located near the junction with the neighboring cell (Moliszewska et al., 2002; Stępniewska-Jarosz, 2012).

To date, 13 anastomosis groups (AG) have been identified within *R. solani*. These groups were further subdivided into subgroups. The reason for this subdivision is the great heterogeneity of *R. solani* and the ability of hyphae to join. AG2 and AG4 are the most frequently described groups because of their high pathogenicity in various plant species (Stępniewska-Jarosz, 2006).

This study focuses on the little-studied group AG5, which is considered to have low plant pathogenicity. It can be found on sugar beet seedlings with symptoms of seedling damping-off, among others. This group has not yet been fully characterized and described, and progeny production has not been developed under laboratory conditions. The aim of this study was to describe the morphological profile of the progeny obtained from basidiospores of one AG5 field strain.

Materials and Methods

Isolates. The isolate *R. solani* ID23 was used in this study, as the maternal strain was isolated in 1997 from diseased sugar beet seedlings with symptoms of damping-off. Seedlings were collected from a field area close to Opole, Poland. All fungi (Table 1) used in this study belonged to the *R. solani* collection created by E. Moliszewska at the University of Opole, Institute of Environmental Engineering and Biotechnology, and are routinely stored on PDA slants in a refrigerator (for usual use) and on barley grains overgrown by mycelium in a deep freezer for long-term storage.

Species determination for ID23 was performed using classical anastomosis tests, which showed that this strain belongs to AG5. This was confirmed by isolation and sequencing of the ITS1-ITS2 fragment of ribosomal DNA. The sequences were compared to those in the GenBank database (Moliszewska, 2009). Recently, this sequence was confirmed using the BLAST tool in the NCBI database.

Table 1. List of tested *R. solani* strains (various colors indicate SBIs obtained in separate cycles of sexual fruiting)

No.	Single Basidiospore Isolates (SBI)			Heterokaryons (H)
1	**SBI17A**	**ID23 I**	**13**	**H23 - II - II**
2	**SBI17B**	**ID23 II**	**15**	**H23 - II - III - 1**
3	**SBI25**	**ID23 III**	**19**	**H23 - II - IV**
4	**ID23 – 2015 A**	**ID23 V**	**25**	**H23 - II - IV - 1**
5	**ID23 – 2015 B**	**ID23 VI**	**27**	**H23 - II - VII**
6	**ID23 – 2015 C**	**ID23 VIII**	**28**	**H23 - II - VIII**
7	**ID23 – 2017 - I**	**ID23 IX**	**30**	**H23 - III - IX - 1**
8		**ID23 X**	**33**	**H23 - III - IX - 2**
9			**17L**	**H23 - V - X - 1**
10			**18L**	**H23 - V - X - 2**
11			**24L**	
12			**31L**	
13			**34L**	

Perfect stage culturing - basidiospore collecting. This study was based on a group of isolates obtained by culturing *R. solani* AG5 basidiospores under laboratory conditions. All cultured basidiospores were collected from *R. solani* AG5-labeled ID23 during several cycles of sexual fruiting. Initially, basidiospore production and collection were performed for a few isolates of the AG5 group using the soil-over method described by Toda and Hyakumachi (2006) (Photo 1). Finally, fruiting was obtained for only one isolate, which was coded as ID23.

Basidiospores were trapped on water agar (WA) and cultured at approximately 20°C. Each mycelium developed from a basidiospore was transferred to fresh PDA medium. WA plates with no germinating basidiospores were placed in a refrigerator and incubated at approximately 10°C for the next two months, and then newly developed mycelia were transferred to fresh PDA medium. The letter 'L' has been added in the lab code for the mycelia developed in 10°C.

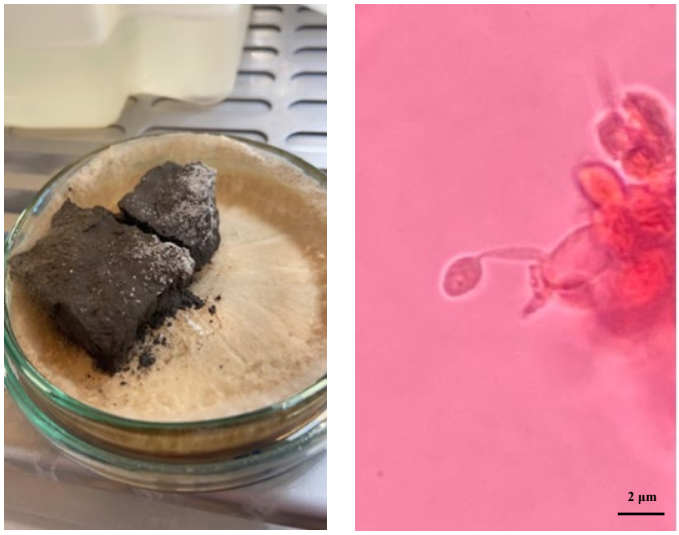


Photo 1. Perfect stages of *Rhizoctonia solani*. Left - fruiting visible on the soil surface. Right - basidium and basidiospore of *Rhizoctonia solani* - dyed with safranine 0; (×400)

Heterokaryon formation. Single basidiospore isolates (SBIs) served as progenies and parental strains to produce potential heterokaryons (Hs). Potential Hs were obtained through cultural pairing of SBIs (parental strains) and their cleavage from the contact sites of the two paired strains. Strains were inoculated on the opposite sides of the Petri dish on PDA medium with 2% activated carbon. After the distinctive tuft was created, potential Hs were transplanted onto a new PDA medium.

Morphology. The progenies of two generations (SBIs and Hs) were analyzed in terms of their morphological diversity. For the purpose of this study, all strains were inoculated onto PDA medium and incubated at 10°C, 20°C, and 30°C in the dark. The daily mycelial growth rate was observed for each strain. After two weeks of incubation, mycelia were morphologically characterized (color, concentric zonation, mycelial height and fluffiness, compactness, and presence of sclerotia). The experiment was performed with four replicates.

Morphological features of the analyzed fungi were compared by creating a similarity/dissimilarity tree using NTSYSpc 2.21w software and the UPGMA method.

Fungicides. In the next phase of the research, PDA supplemented with fungicides, azoxystrobin, thiophanate-methyl, and thiuram, used at concentrations of 10 and 100 ppm, allowed for evaluation of the resistance of the mycelium to these fungicides. Plates with PDA without supplements served as controls. In both experiments, the growth of mycelia [mm] was measured every 24 h along two vertical lines on the bottom of the Petri dishes for all tested

samples until the mycelium covered the entire dish (ϕ 90 mm). The fungicide test was performed at 21 ± 2°C. All experiments were performed in triplicate.

The results of measuring mycelial growth are expressed as the range of mycelial increases per day (mm/d).

Based on the data obtained from the test with fungicides, the coefficient of growth inhibition was calculated according to Abbot's formula (1925):

$$\Delta H = \frac{K_0 - F}{K_0} \cdot 100\%$$

where ΔH is the coefficient of linear growth inhibition of the fungus [%], K_0 is the diameter of the colony on the control dish [mm], and F is the colony diameter on the dish with fungicide.

In the final stage of the study, microscopic measurements of the samples incubated at 20°C were performed. The average hyphae diameter, as well as the minimum and maximum diameters [µm], were measured at a magnification of ×400.

Data analysis. The standard deviations were calculated for the growth parameters obtained in the tests using Excel. Statistical analysis was performed using the ANOVA method (p = 0.05) using the Statistica program, and significant differences among groups were compared by Tukey's test.

Results

Isolates – culturing, identification, heterokaryons, and monokaryons. The basic classification of the ID23 isolate and DNA isolation was performed in a previous study by Moliszewska (2009). Maternal isolate ID23 was confirmed to be *R. solani* AG5. The sequence showed 99.86% identity with the *R. solani* AG5 strain (accession No. JQ946291) and 100% identity with *Thanathephorus cucumeris* strain GZ-5 was determined to be AG5 (accession No. KR006028), both of which were deposited in GenBank. The ID23 sequence was submitted to the NCBI GenBank database under accession No. OP601638.

Strains were incubated at room temperature for two weeks and after which morphological characteristics were macroscopically evaluated, starting with the parental isolate. Based on this evaluation, progeny were further evaluated. Maternal *R. solani* ID23 was characterized by a light brown fluffy structure. Irregularly shaped and brown sclerotia were visible on the surface of the mycelium. Zonation of mycelial growth was not observed on the surface of the maternal ID23 isolate.

The color of the tested strains ranged from light beige to light or dark brown. Individual isolates also differed in the mycelial fluffiness, height, and hyphae density. In a few cases, concentric zonation and sclerotia have also been observed. Unfortunately, distinction of the

progenies by color to distinguish individual basidiospore-born isolates and Hs is not possible. However, they were grouped separately in a dendrogram of similarity (Figure 1).

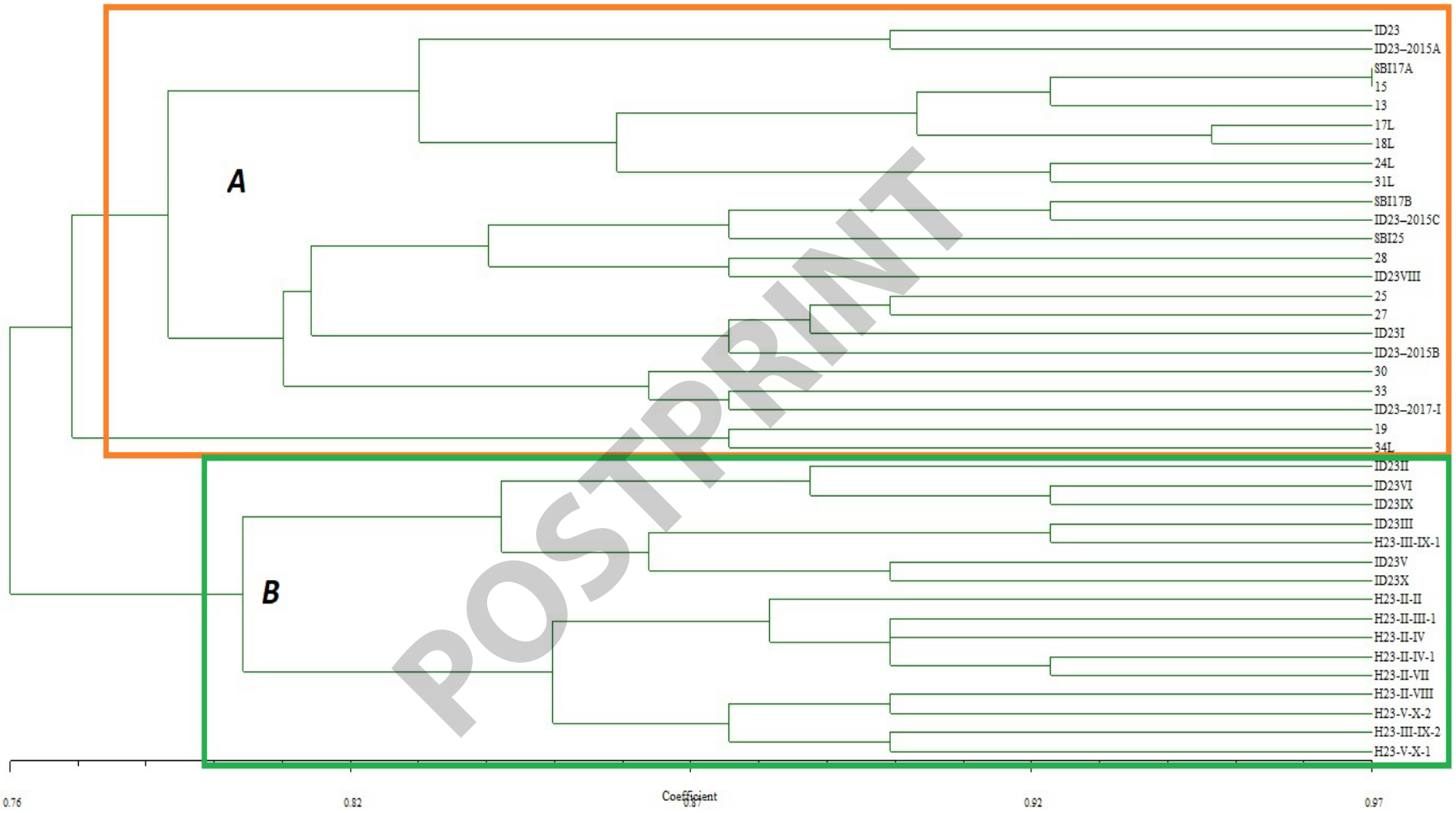


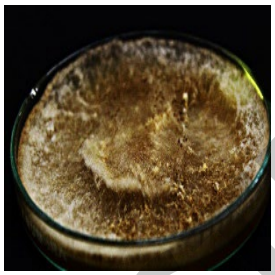
Figure 1. Similarity tree based on morphological features of maternal strain ID23 and its progenies. Strains with letter "H" in a code are heterokaryons

In each offspring group, mycelia took on different hues. Some strains developed a light beige to almost white mycelium, whereas others had a dark brown color. However, most isolates were light brown in color. Concentric zonation within the cultures was observed in strains whose mycelium grew flat and did not form sclerotia on their surfaces. In the observed isolates, all tested Hs were found to have the ability to form sclerotia as compact mycelial structures. They appear in the form of brown- and creamy-colored densely packed structures. These structures were also characterized by irregular or almost spherical shapes (Table 2).

R. solani AG5 isolates are characterized by their very diverse mycelial appearance. Depending on the strain, they can vary in terms of fluffiness and height. The study showed that high and fluffy mycelia were formed by all potentially heterokaryotic isolates and a few single basidiospore isolates. The remaining *R. solani* strains obtained from single basidiospores grew flat and formed compact structures (Table 2).

Table 2. Morphological characteristics of *R. solani* AG5 isolates

Characteristics		**Model picture**	**SBI**	**Heterokaryons**
Color	**White/ beige**		18L, 17L, SBI 17A, SBI 17B, 27, 25, SBI25, 15, 13, 28, 19, ID23-2017-I, ID23-2015A, ID23 II, ID23-2015B, ID23-2015C	H23-II-II, H23-II-III-1
	Light brown		31L, 30, 33, ID23 VI, ID23 X, ID23 IX, ID23 VIII, ID23 III, ID23 I	H23-V-X-1, H23-V-X-2, H23-III-IX-1, H23-III-IX-2, H23-II-VIII
	Dark brown		ID23 V, 24L, 34L	H23-II-IV, H23-II-IV-1, H23-II-VII,

Structure	**flat**		18L, 17L, SBI 17A, SBI 17B, 27, 25, SBI25, 15, 13, 33, 30, 28, 19, ID23 VI, ID23 X, ID23 IX, ID23 VIII, ID23 V, ID23 III, ID23 I, ID23-2017-I, ID23-2015A, ID23 II, ID23-2015B, ID23-2015C	-
	Fluffy		24L, 31L, 34L	H23-II-III-1, H23-V-X-1, H23-V-X-2, H23-III-IX-1, H23-III-IX-2, H23-II-VIII, H23-II-VII, H23-II-IV, H23-II-IV-1, H23-II-II
Sclerotia	**current**		ID23 IX, ID23 X, 19, 34L, ID23 VI, ID23 V, 33, ID23 III, ID23 VIII	H23-II-III-1, H23-V-X-1, H23-V-X-2, H23-III-IX-1, H23-III-IX-2, H23-II-VIII, H23-II-VII, H23-II-IV, H23-II-IV-1, H23-II-II
	absent		18L, 17L, SBI17A, SBI17B, SBI25, 31L, 24L, 27, 25, 15, 13, 30, 28, ID23-2017-I, ID23-2015A, ID23-2015B, ID23-2015C, ID23 II, ID23 I	-
Zoning	**current**		18L, 17L, SBI 17A, SBI 17B, 27, 25, SBI25, 15, 13, 33, 30, 28, 19, ID23 VI, ID23 X, ID23 IX, ID23 VIII, ID23 V, ID23 III, ID23 I	-
	absent		24L, 31L, 34L,ID23-2017-I, ID23-2015A,	H23-II-III-1, H23-V-X-1, H23-V-X-2, H23-III-IX-1, H23-III-IX-2, H23-

			ID23 II, ID23-2015B, ID23-2015C	II-VIII, H23-II-VII, H23-II-IV, H23-II-IV-1, H23-II-II

Based on the calculations, the daily growth at 10°C was determined to range from 2.5 to 15.2 mm/d, with the maternal strain being 4.6 mm/d (Figure 2). All other isolates, except a few strains signed with the letter "L," had a similar daily growth, oscillating between the values of 4.5–6.5 mm/d. The SBI isolates labeled "L" were characterized by the following growth values: 18L - 13.5, 17L - 14.8, 34L - 12.5, 31L - 15.2, and 24L - 14.7 mm/d, indicating that these samples grew most favorably under these temperature conditions and their growth rate was much higher than that of the maternal strain ID23. When Hs were compared with their ID23 parent isolates, their growth rate was found to be inconsiderable different from that of the parent, ranging 3.2–6.4 mm/d.

At a temperature of 20°C, the daily growth rate for the mother strain ID23 was 14.2 mm/d and the maximum value for the isolates studied was 21.4 mm/d (isolate ID23 II), and the minimum value was 5.7 mm/d (isolate 15) (Figure 2).

Increasing the incubation temperature to 30°C did not have as drastic effect on the modification of mycelial growth rates as when the temperature was increased from 10°C to 20°C. The daily growth of the maternal strain ID23 under these conditions was 14.1 mm/d. The highest recorded value was 21 mm/d (isolate H23-V-X-2), whereas the lowest was 6 mm/d (isolate 18L), which clearly confirmed the tendency of the isolates to have different optimal growth temperatures. At higher temperatures, the growth rate did not change over a wide range, and in most of the tested strains, it was similar. The lowest values of daily growth at 30°C were observed for "L" labeled-isolates. Hs at this temperature showed slightly higher growth rates than the parental strains. Only *R. solani* H23-II-VIII grew like one of its parent strains ID23 VIII (17.8 mm/d). For the other Hs, the growth rates were 0.2–2.9 mm/d higher at 30°C than at lower temperatures (Figure 2).

The graph in Figure 2 shows comparison of the strains tested at all incubation temperatures. Based on our observations, the most favorable temperature for rapid mycelium growth was concluded to be 30°C. However, the values observed in the tests conducted at 20°C suggest that a temperature range of 20°C–30°C may be optimal for the growth of *R. solani*. At a low temperature (10°C), the growth rates of the strains tested were less effective, as these isolates required a much longer incubation period. However, "L" labeled-isolates (obtained

after the basidiospore incubation in the refrigerator) were the only ones that showed more favorable growth at 10°C. This confirms that these isolates did not spontaneously activate their growth at room temperature, which was too high for them, and transferring the isolation WA plates to the cold room allowed them to grow at the beginning of this study.

Statistical analysis ($p=0.05$) showed that the growth of the tested groups of fungi (maternal isolate, SBI, and Hs) did not differ inconsiderable at 10°C, although considerable differences were observed between SBI and Hs at 20°C and 30°C. Maternal isolates did not differ notable in either group of progeny.

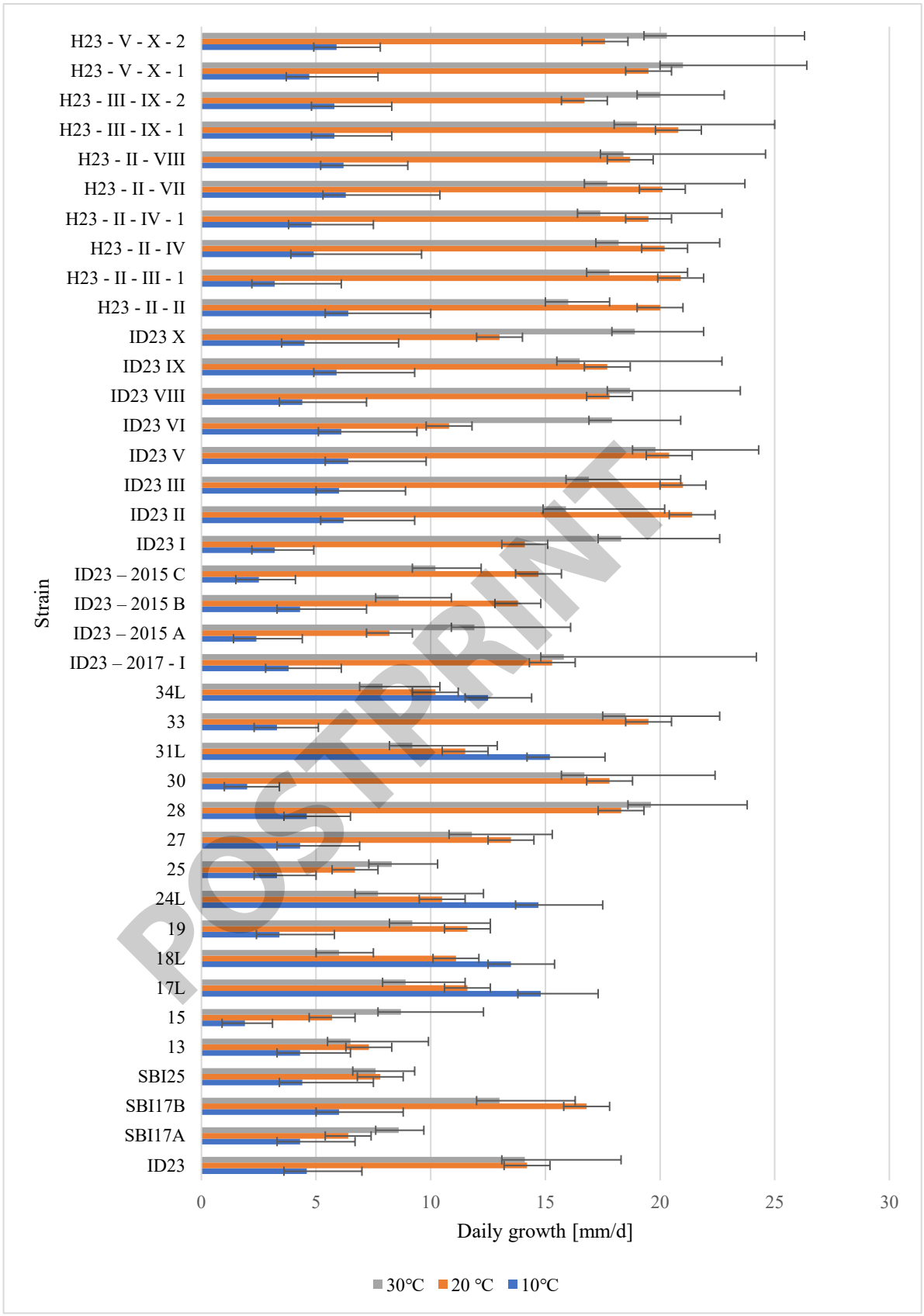


Figure 2. Influence of temperature on the average daily growth (mm/d) of tested mycelia AG5 group of *R. solani*

Microscopic evaluation of the examined strains of *R. solani* AG5. Microscopic evaluation of the mycelia was performed to determine the hyphal diameter (μm), considering the minimum, maximum, and average values. Maternal strain ID23, five exemplary potential Hs, and 12 single basidiospore isolates were considered in this test. In terms of anatomical structure, the hyphae of the progeny of *R. solani* AG5 were found to have diameters ranging 2.93 μm to 15.60 μm. The mean size of the hyphae in potential Hs ranged 3.90–13.65 μm. In the parent strain ID23, the mean value was 6.14 μm, the minimum was 3.90 μm, and the maximum was 7.80 μm. For SBI, these values were 2.93–15.60 μm. This could mean that single basidiospore isolates present greater differences in the diameter of the hyphae (Figure 3).

Analysis of the similarity between the Hs and their parent strains revealed that the maximum size of hyphae of isolate H23-II-III was the same as of its parent strains (9.75 μm); however, the mean value of the measurements was higher than that of isolate ID23 II, but identical to that of ID23 III. Isolate H23-II-VIII had a larger diameter than its parent strain ID23 II; however, it was similar as that of the second parental strain ID23 VIII (Figure 3). Another potential heterokaryon, H23-III-IX-2, resembled its parental isolate ID23 IX in the minimum and maximum diameter of hyphae, whereas the overall average size, in this case, was smaller than that of both parental strains. The maximum diameter of the *R. solani* H23-V-X-1 hyphae was identical to that of ID23 V and ID23 X, and the minimum diameter was the same as that of ID23 V, whereas a greater similarity was observed in the mean value of this parameter with *R. solani* ID23 X. General analysis of the relationship based on the diameter of the young hyphae of *R. solani* AG5 showed that the Hs did differ inconsiderablefrom the parental strains in their morphological structure. In the analysis, in most cases, the progeny was observed to adopt the characteristics of one of the parents or have the features of both, on average.

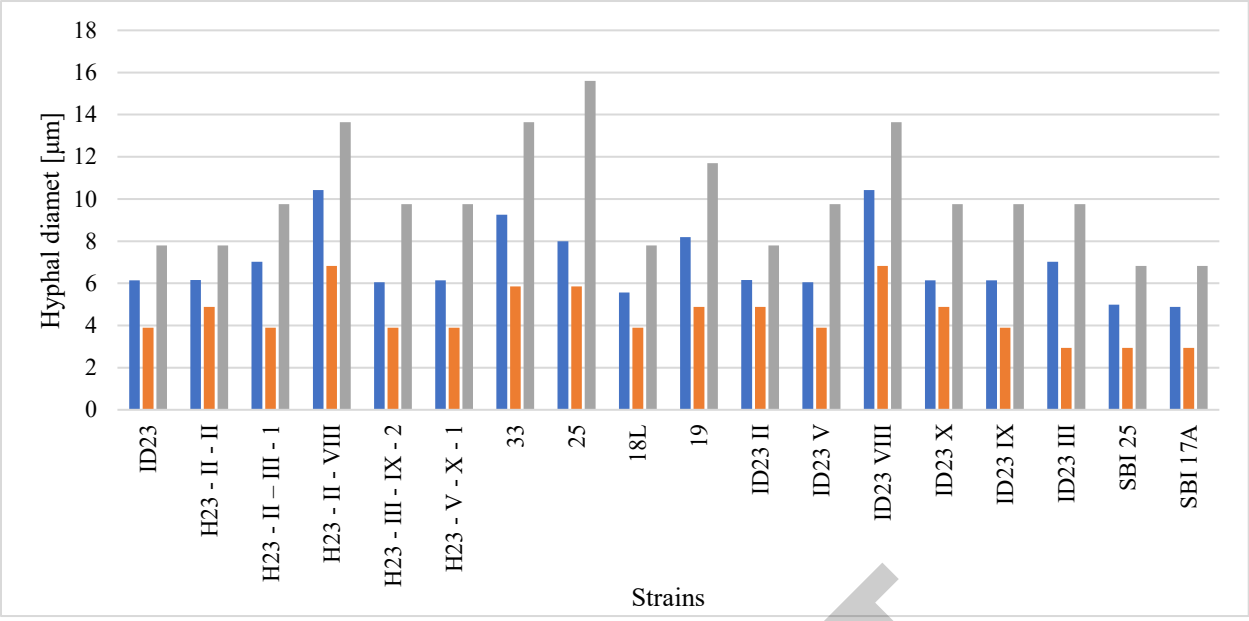


Figure 3. Hyphal diameter of selected monokaryons and heterokaryons of *R. solani* AG5 (µm)

Fungicides. Two fungicide concentrations were used in this study: 10 ppm of active substance, which refers to the possible field concentration of the preparations, and 100 ppm of active substance, which is extremely high; therefore, sensitive isolates should be extracted. On the medium containing 10 ppm of azoxystrobin (Amistar), the growth of the mycelium was found to be inhibited by 29% (isolate ID23 VI); however, in the case of the maternal strain ID23 and isolates SBI 17A, 24L, and ID23 VIII, it did not contribute to the slowing down of mycelial growth (Figure 4).

On the 10 ppm medium with thiuram, some isolates grew unchanged, which means that the fungicide had no effect on mycelium growth (isolates ID23, SBI17A, and ID23 2017-I), and the remaining strains slowed down their growth to a greater or lesser extent. The calculated growth inhibition coefficient (ΔH) varied between 0%–35%, and in one strain, the value was as high as 89% (ID23 I) (Figure 4).

A slight slowdown in mycelial growth was observed on PDA medium containing 10 ppm thiophanate-methyl. The calculated growth inhibition coefficients (ΔH) varied between 1%–43%, with low values for isolates ID23, ID23 II, and ID23 2017-I and the highest value for *R. solani* isolate 28 (Figure 4).

Increasing the concentration of the active substance to 100 ppm resulted in increased growth inhibition of *R. solani* AG5 isolates (Figure 5). On the PDA medium with azoxystrobin, the growth rate of the tested fungi was found to be inhibited by 0%–66%, therefore, it was inhibited, in most cases, almost twice as effectively as at a lower fungicide concentration. The lowest value of growth inhibition was obtained for the single basidiospore isolate 24L and the

highest value for a potential Hs isolate H23-II-VIII (Figure 4). The presence of thiuram contributed to an increase in growth inhibition compared to previous formulations, which in this case was 23%–80%. Thiuram had the least effect on the single basidiospore isolate SBI17A, while the greatest effect was observed on potential Hs, both strains H23-III–IX-1 and H23-III–IX-2 (Figure 5).

Thiophanate-methyl (100 ppm) affected tested strains the most effectively. In this case, the inhibition of the growth rate varied between 3.5 mm/d (isolate SBI 17A) and 19.0 mm/d (isolate ID23 V) (Figure 4). For "cool" strains, 17L, 18L, 24L, 31L, 34L, and all potential Hs, 100% growth inhibition by thiophanate-methyl was observed (Figure 4). Statistical analysis ($p = 0.05$) showed that significant differences among the tested groups of fungi (maternal isolate, SBI, and Hs) did not exist with the use of 10 ppm fungicides. In the case of the 100 ppm fungicide concentration, differences were significant between SBI and Hs, and the maternal isolate did not differ significantly from either group of strains.

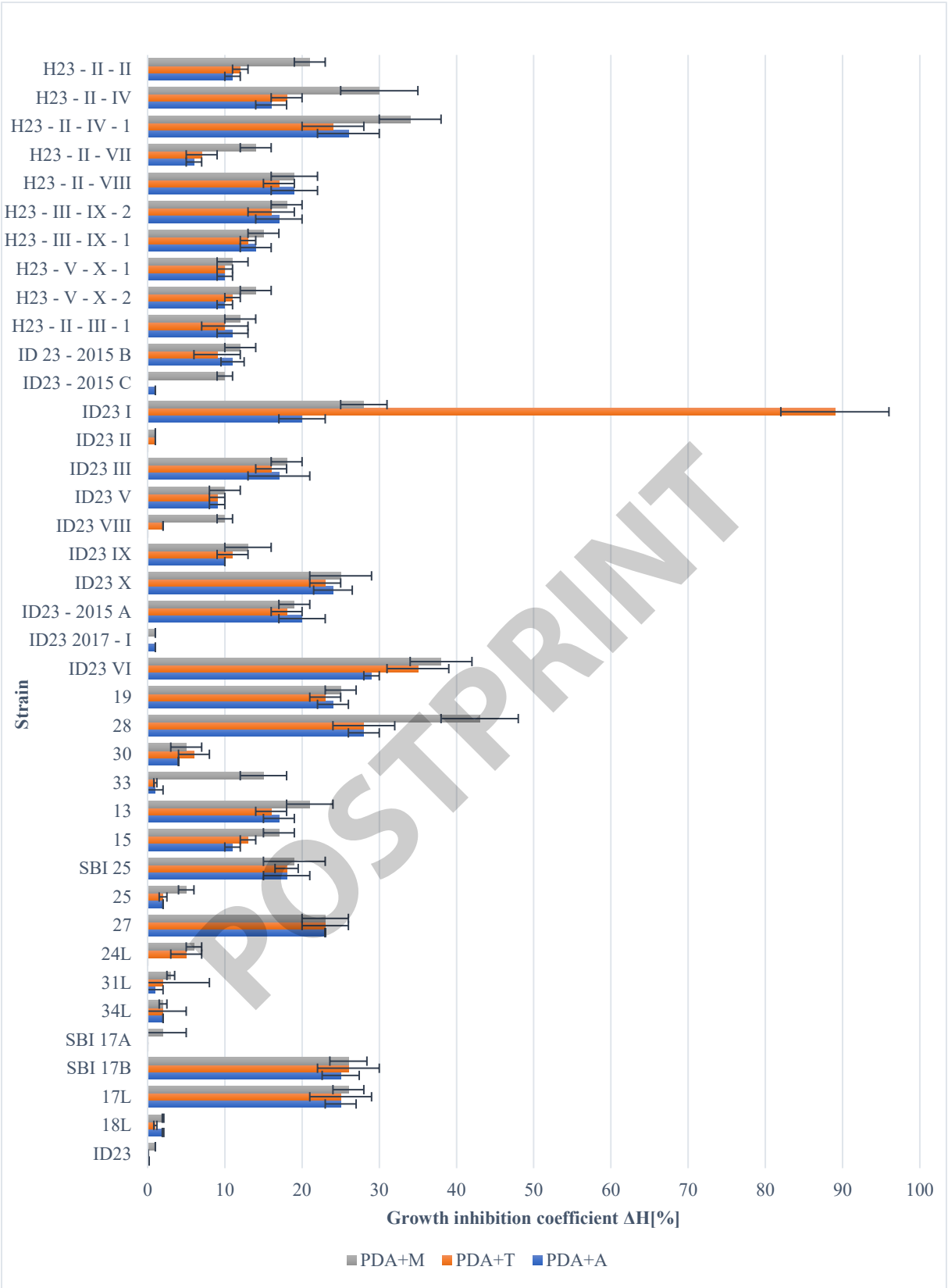


Figure 4. Influence of selected fungicides (10 ppm) on the growth of individual isolates of *R. solani* AG5 showed as an inhibition coefficient (ΔH%) (PDA+A - azoxystrobin, PDA+M - thiophanate-methyl, and PDA+T - thiuram)

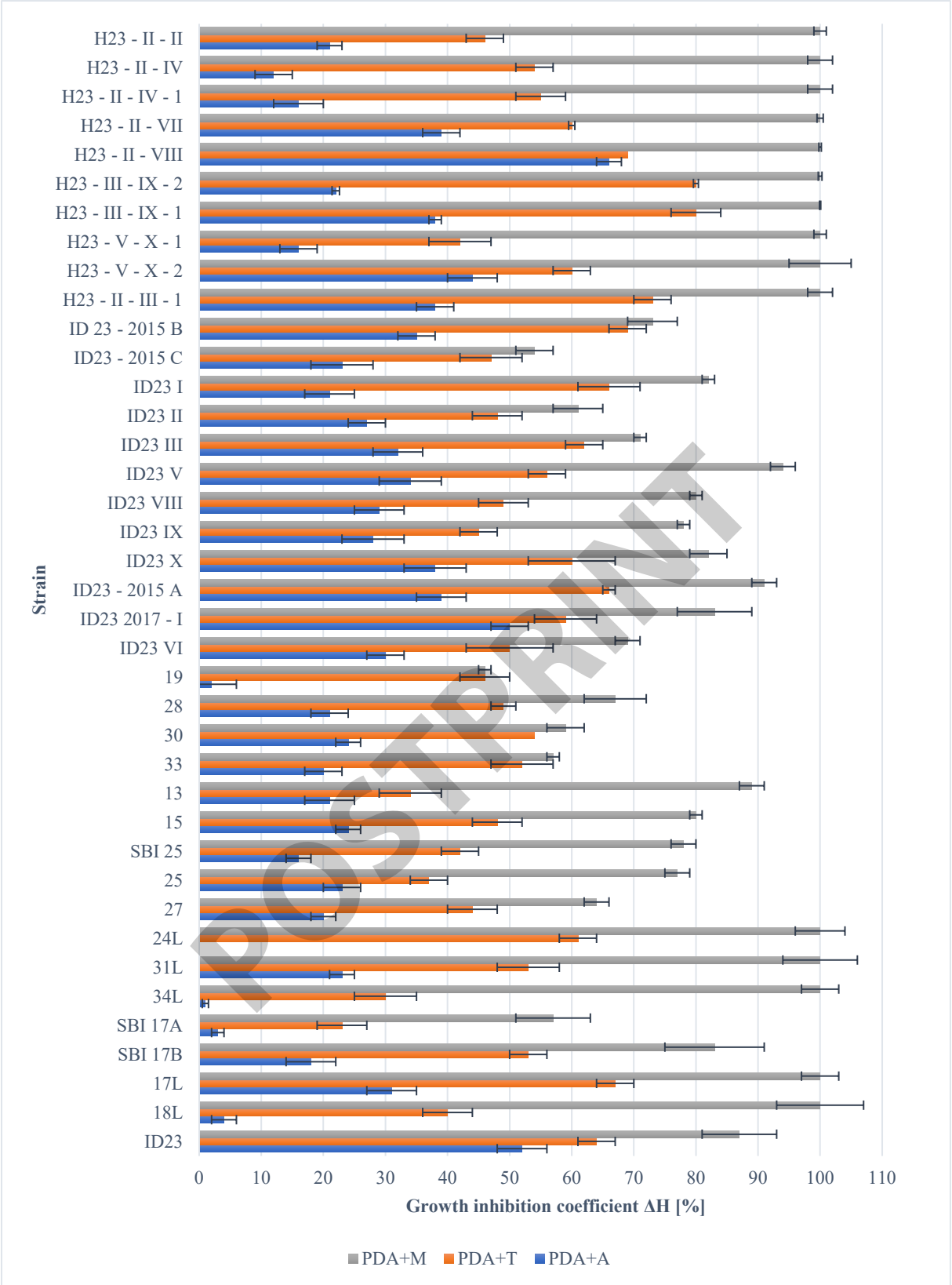


Figure 5. Influence of selected fungicides (100 ppm) on the growth of individual isolates of *R. solani* AG5 showed as an inhibition coefficient (ΔH%) (PDA+A- azoxystrobin, PDA+M- thiophanate-methyl, and PDA+T- thiuram).

Discussion

Isolates of *R. solani* AG 5 derived from one maternal strain, ID23, presented very different morphological features. The color of the mycelia ranged from almost white to creamy and light to dark brown. The mycelial structure varied from compact, with visible zonation, to fluffy, with distinct sclerotia on the colony surface. They appeared only on the edge of Petri dishes during the initial phase of growth or developed on the surface of the entire colony. Carling and Sumner (1995) did not provide a morphological description of AG5; however, they detailed basic information on its pathogenicity, concluding that AG5 is far less virulent than AG3 and that it is thiamine auxotrophic.

The morphology of *Rhizoctonia* mycelia is usually given in general descriptions of subsequent anastomosis groups. For example, Carling et al. (2002) showed that mycelia of *Rhizoctonia solani* AG13 were characterized by a light to dark brown color. After a few days of incubation, they noticed zonation in all incubated samples, which disappeared over time. Initially, dark brown sclerotia were observed only near the edge of the Petri dishes, but after a longer cultivation period, they also appeared randomly scattered over the entire surface of the mycelium. Moliszewska et al. (2020) described two AG11 isolates that formed white-beige- to creamy-colored mycelia with wide concentric zonation, and one of them formed light-colored sclerotia. This confirmed the morphological differences in the anastomosis group. Morphological differences among AG5 isolates isolated from carrots in Sweden were also observed by Marcou et al. (2021). Singh et al. (2018) observed the characteristics of *R. solani* isolated from seven agroecological zones that were adapted to maize cultivation in 2018. All 62 strains that were incubated were light to dark brown in color, and their structures varied from fluffy to compact. The studies on the growth of *R. solani* mycelia presented in this paper have shown that all isolates may grow more or less effectively depending on the ambient temperature. At 10°C, the average daily growth rate of mycelia was the lowest, ranging 1.9–15.2 mm. At 20°C, the growth increase for the tested isolates varied between 6.4–21.4 mm/d. Increasing the temperature to 30°C did not radically affect the growth activity of the fungal isolates compared to that at 20°C; in this case, the average growth of the mycelia per day varied between 6.0–21.0 mm. Moliszewska et al. (2020) showed that for two Polish AG11 isolates, the average daily rate of hyphal growth on PDA at 21°C was 22.8 mm and 22.6 mm, respectively.

The analysis of optimal growth temperature showed that *R. solani* AG5 grew the most favorably at both temperatures (20°C and 30°C), except the group of isolates, which showed

the highest growth activity at 10°C. This group of isolates was derived from single basidiospores incubated on WA in a refrigerator, which shows how great possibilities are coded in the *R. solani* AG5 genome in terms of optimal growth temperatures. This flexibility proves that it is always possible for some offspring to survive, independent of the environmental conditions.

Bełka and Mańka (2014) conducted tests with different strains of *R. solani* (including AG5) and showed that AG5 isolates reached the fastest growth among other anastomosis groups (13.9 mm/d). Furthermore, Carling et al. (2002) showed that the average daily mycelial growth for *R. solani* (AG13) was approximately 28.3 mm at the temperature of 25°C. This was much higher than the growth rates of our tested AG5 isolates and those observed by Bełka and Mańka (2014) and Moliszewska et al. (2020). Tomaso-Peterson (2007) identified pathogenic fungi in agronomic crops and turfgrasses, among which, 12 *R. solani* isolates were identified. One of them belonged to AG5, and its optimal growth temperature was found to be 21°C–29°C.

Harikrishnan et al. (2004) showed a relationship between temperature and the growth rate of *R. solani*. Mycelial growth was observed in different anastomosis groups, including 15 isolates of AG5. Although there were differences in growth rates among the tested isolates, in most cases, the fungi showed more effective growth with increasing temperature. None of the tested isolates showed the ability to grow below 5°C. The most rapid growth was recorded at temperatures of 20°C, 25°C, and 30°C, which agrees with our observations.

Papavizas and Davey (1961) found that the optimum temperature that affects the saprotrophic activity of *R. solani* depends on the soil type. For example, in a greenhouse where loamy sand was used, the optimum temperature for mycelial activity was 20°C, and at 30°C, the efficiency of the saprotrophic mycelial development decreased. They also demonstrated that *R. solani* showed the highest saprobic activity in fine sand in the temperature range of 26°C–30°C. Similar observations have been reported by Martinson (1963). Their research showed that the activity of *R. solani* mycelia increased with increasing temperature. They also determined the optimal growth temperature in the range of 15°C–25°C and found no differences between 25°C and 30°C. The last observation was similar to that observed for our tested isolates. Our group of "cold-grown" isolates refer to their lowest growth temperature (15°C), however, they were seen to grow the most intensively at 10°C. The growth of *R. solani* depends not only on temperature but also on the natural features of the isolates; the medium or soil type is also very important. *R. solani* is commonly known as a fast growing species. The importance of preferred growth temperature for *R. solani* showed by Minier and Hanson (2021) reported that below 15°C, no risk of Rhizoctonia damping-off was observed on sugar beets.

Other factors that may influence the *R. solani* growth rate in agricultural soils are fungicides. In this study, the following groups were investigated: strobilurin derivative — azoxystrobin, dithiocarbamate — thiuram, and benzimidazole — thiophanate-methyl. These compounds were found to inhibit the growth of *R. solani* mycelia, with thiophanate-methyl showing the highest antifungal activity. Thiophanate-methyl at a dose of 100 ppm contributed to almost total growth inhibition in many of the tested isolates. Two concentrations of fungicides were investigated in the study: lower (10 ppm) as an appropriate dose adequate for field applications to the soil, and higher (100 ppm), which was expected to completely inhibit the growth of fungi. Kucharska et al. (2018) tested such compounds as propiconazole + cyproconazole, penthiopyrad, prochloraz, tebuconazole, and azoxystrobin against *R. solani* AG2-2B and AG4. All of these were able to notable reduce or inhibit the growth of *R. solani*. The least effective fungicide was azoxystrobin (efficacy of 47.30%–91.14% inhibition), whereas the most effective compounds were propiconazole and cyproconazole. Moliszewska et al. (2020) showed that hymexazole, which is specific to Oomycetes, could increase the growth of both tested fungi from 13.5% to 28%. However, thiuram inhibited them by 35.8%–74.7% depending on the dose of the preparation. They concluded that both tested strains differed in response to both fungicides, which is similar to our observations for AG5 isolates. The differences observed for our isolates may be a result of the individual features inherited from the maternal isolate ID23. This also shows that some level of potential resistance to fungicides could be acquired in the field by the maternal isolate or by natural genetic changes that may occur during long-term storage.

Our research shows that *R. solani* AG5 is represented by isolates differing in morphological features as well as some physiological and genetic properties. A more detailed study of this group is currently needed because *R. solani* AG5 has been isolated from different hosts. Isolates have been found among other AGs in soil, cucumbers, potatoes, tomatoes, peppers, red cabbage, wheat, turfgrasses, cereals, beans, soybeans, sugar beets, pine seedlings, and apple plants (Ajayi- Oyetunde and Bradley ,2017; Amaradasa et al. 2013; Bełka and Mańka, 2017, El-Sharouny 2015; Erper et al., 2021, Fiers et al.,2011; Melzer et al., 2016; Shazad Gondal and Rauf, 2017; Woodhall et al., 2012; Yildrim and Erper, 2017). Recently, Hassan and Chang (2021) described the first case of damping-off in ovate-leaf *Atractylodes ovata* caused by AG5. They may exist as single pathogens, as in the Japanese monkshood (*Aconitum japonicum* subsp. *subcuneatum*) (Mori et al.,2020), wheat in the United Kingdom or Turkey, as well as barley and potato, or in mixed groups of different *Rhizoctonia*-complexes in which they usually play a minor role (Erper et al.,2011; Gonzalez et al.,2001; Moliszewska,

2009; Woodhall et al., 2012; Yildirim and Erper, 2017). According to the results of Moliszewska (1999), *R. solani* AG5 may comprise approximately 60% of the total amount of *R. solani* strains isolated from sugar beet seedlings in Poland (Moliszewska, 2009). *R. solani* AG5 can cause seedling damping-off and root rot in sugar beets and decrease plant size by slowing and stunting development (Moliszewska, 2002); however, these observations require a more detailed inspection. Recently, similar results were reported by Erper et al. (2021) for red cabbage. Some data suggest that members of AG5 may also act as severe pathogens, for example, on chickpeas in Turkey (Basbagci and Dolar, 2021), hence, this strain seems to have become more dangerous than previously thought. They also should not be considered a homogenous group; as Lübeck (2004) suggested, there are three possible groups based on RFLP patterns. This was partially confirmed by the groups formed in the phylogenetic tree (Figure 1). Therefore, they should be studied to understand their evolution in nature and how they use plants as nutrient sources. Knowledge gained from this type of research will allow the prevention of their potentially negative influence on crops in the future.

Conclusions

Research has shown that sexual reproduction leads to the segregation of features among progenies of the maternal isolate. In the tested isolates, several types of morphological features were observed, which were divided into two groups, one of the heterokaryons and the second of the homokaryons derived from basidiospores grouped together with their maternal isolate ID23. This study is the first to show that SBI progenies mostly carry a morphology similar to the maternal isolate, but after crossing them, the next generation of heterokaryotic progenies creates a separate group of morphological characteristics (Fig. 1). Other attributes of new generations, such as the optimal temperature of growth or sensitivity to fungicides, also provide distinct information. A group of cool-preferring isolates that were sensitive to some fungicides were identified. Higher sensitivity to fungicides has also been observed in heterokaryons than in mono/homokaryons.

This study shows the changes in the next generation of *R. solani*. Our observations of changes in progenies may contribute to our understanding of how *R. solani* AG5 modifies its aggressiveness to plants and resistance to fungicides. In our opinion, further research is needed to obtain fully described changes in the group AG5, to understand the details of its sexual reproduction, and to evaluate its notable in crops.

References

Abbott, W. S. (1925). A Method of Computing the Effectiveness of an Insecticide. *Journal of Economic Entomology*, *18*(2), 265–267. https://doi.org/10.1093/jee/18.2.265a

Ajayi-Oyetunde, O. O., & Bradley, C. A. (2017). Identification and Characterization of *Rhizoctonia* Species Associated with Soybean Seedling Disease. *Plant Disease*, *101*(4), 520–533. https://doi.org/10.1094/PDIS-06-16-0810-RE

Amaradasa, B. S., Horvath, B. J., Lakshman, D. K., & Warnke, S. E. (2013). DNA fingerprinting and anastomosis grouping reveal similar genetic diversity in *Rhizoctonia species* infecting turfgrasses in the transition zone of USA. *Mycologia*, *105*(5), 1190–1201. https://doi.org/10.3852/12-368

Basbagci, G., & Dolar, F. S. (2022). Characterization and pathogenicity of *Rhizoctonia solani* AG-5 isolates obtained from chickpea in Turkey. *Journal of Phytopathology*, *170*(3), 141–147. https://doi.org/10.1111/jph.13066

Bełka, M., & Mańka, M. (2014). Characteristics and diversity of Rhizoctonia spp. Population in soil of selected forest bare-root nurseries in Poland. *Acta Mycologica*, *1*(1), 279–290. https://doi.org/10.5586/am.2014.025

Carling, D. E., Baird, R. E., Gitaitis, R. D., Brainard, K. A., & Kuninaga, S. (2002). Characterization of AG-13, a Newly Reported Anastomosis Group of *Rhizoctonia solani*. *Phytopathology®*, *92*(8), 893–899. https://doi.org/10.1094/PHYTO.2002.92.8.893

Department of Forest Phytopathology, Poznań University of Life Sciences, Poland, Bełka, M., & Mańka, M. (2017). Diversity of multinucleate Rhizoctonia spp. In soil of two forest nurseries Garncarskibród and Lipka. *Acta Scientiarum Polonorum Silvarum Colendarum Ratio et Industria Lignaria*, *16*(4), 233–242. https://doi.org/10.17306/J.AFW.2017.4.24

El-Sharouny, E. E. (2015). Effect of different soil amendments on the microbial count correlated with resistance of apple plants towards pathogenic *Rhizoctonia solani* AG-5. *Biotechnology & Biotechnological Equipment*, *29*(3), 463–469. https://doi.org/10.1080/13102818.2014.1002285

Erper, I., Ozkoc, I., & Karaca, G. H. (2011). Identification and pathogenicity of *Rhizoctonia* species isolated from bean and soybean plants in Samsun, Turkey. *Archives Of Phytopathology And Plant Protection*, *44*(1), 78–84. https://doi.org/10.1080/03235400903395427

Erper, I., & Yildirim, E. (2017). Characterization and pathogenicity of Rhizoctonia SPP. isolated from vegetable crops grown in greenhouses in Samsun province, Turkey. *Bioscience Journal*, 257–267. https://doi.org/10.14393/BJ-v33n2-34580

Fiers, M., Edel-Hermann, V., Héraud, C., Gautheron, N., Chatot, C., Le Hingrat, Y., Bouchek-Mechiche, K., & Steinberg, C. (2011). Genetic diversity of *Rhizoctonia solani* associated with potato tubers in France. *Mycologia*, *103*(6), 1230–1244. https://doi.org/10.3852/10-231

Gondal, A. S., Rauf, A., & Naz, F. (2019). Anastomosis Groups of Rhizoctonia solani associated with tomato foot rot in Pothohar Region of Pakistan. *Scientific Reports*, *9*(1), 3910. https://doi.org/10.1038/s41598-019-40043-5

Gonzalez, D., Carling, D. E., Kuninaga, S., Vilgalys, R., & Cubeta, M. A. (2001). Ribosomal DNA systematics of *Ceratobasidium* and *Thanatephorus* with *Rhizoctonia* anamorphs. *Mycologia*, *93*(6), 1138–1150. https://doi.org/10.1080/00275514.2001.12063247

Harikrishnan, R., & Yang, X. B. (2004). Recovery of Anastomosis Groups of *Rhizoctonia solani* from Different Latitudinal Positions and Influence of Temperatures on Their Growth and Survival. *Plant Disease*, *88*(8), 817–823. https://doi.org/10.1094/PDIS.2004.88.8.817

Hassan, O., & Chang, T. (2021). First Report of Damping-Off of Ovate-Leaf Atractylodes Caused by *Rhizoctonia solani* AG-5 in South Korea. *Mycobiology*, *49*(2), 196–200. https://doi.org/10.1080/12298093.2021.1873900

Khachatourians, G. G., & Arora, D. K. (2001). *Applied mycology and biotechnology* (1st ed). Elsevier.

Kucharska, K., Katulski, B., Goriewa, K., Duba, A., & Wachowska, U. (2018). Pathogenicity and Fungicide Sensitivity of Rhizoctonia solani and R. cerealis Isolates. *Gesunde Pflanzen*, *70*(1), 13–19. https://doi.org/10.1007/s10343-017-0408-6

Marcou, S., Wikström, M., Ragnarsson, S., Persson, L., & Höfte, M. (2021). Occurrence and Anastomosis Grouping of Rhizoctonia spp. Inducing Black Scurf and Greyish-White Felt-Like Mycelium on Carrot in Sweden. *Journal of Fungi*, *7*(5), 396. https://doi.org/10.3390/jof7050396

Martinson, C. A. (1963). Inoculum potential relationships of *Rhizoctonia solani* measured with soil microbiological sampling tubes. *Phytopathology*, 53: 634-638.

Melzer, M. S., Yu, H., Labun, T., Dickson, A., & Boland, G. J. (2016). Characterization and pathogenicity of *Rhizoctonia* spp. From field crops in Canada. *Canadian Journal of Plant Pathology*, *38*(3), 367–374. https://doi.org/10.1080/07060661.2016.1199596

Minier, D. H., & Hanson, L. E. (2021). Effect of Low Temperature on the Aggressiveness of *Rhizoctonia solani* AG 2-2 Isolates on Sugar Beet (*Beta vulgaris*) Seedlings. *Plant Disease*, *105*(10), 3111–3117. https://doi.org/10.1094/PDIS-09-20-1990-RE

Moliszewska, E. B. (2009). *Etiologia wybranych chorób korzeni buraka cukrowego*. Wydawnictwo Uniw. Opolskiego.

Moliszewska, E. B., & Schneider, J. H. M. (2017). Some pathogenic properties of Rhizoctonia solani to sugar beet seedlings. *Plant Protection Science*, *38*(SI 2-6th Conf EFPP 2002), 322–324. https://doi.org/10.17221/10480-PPS

Moliszewska, E., Nabrdalik, M., & Ziembik, Z. (2020). Rhizoctonia solani AG 11 isolated for the first time from sugar beet in Poland. *Saudi Journal of Biological Sciences*, *27*(7), 1863–1870. https://doi.org/10.1016/j.sjbs.2020.05.026

Moliszewska, E. (2002). Preliminary anastomosis grouping by modified technique for *Rhizoctonia solani* isolated from sugarbeet seedlings, Phytopathologia Polonica, 26: 85-90.

Mori, M., Misawa, T., & Sato, T. (2020). First report of stem and root rot of monkshood caused by Rhizoctonia spp. In Japan. *Journal of General Plant Pathology*, *86*(3), 224–226. https://doi.org/10.1007/s10327-020-00906-6

Nagrodzka, K., Moliszewska, E., Grata, K., & Nabrdalik, M. (2016). Biologiczna kontrola Rhizoctonia solani AG 2-2IIIB przez metabolity *Bacillus subtilis. Proceedings of ECOpole*, 10(2): 741-748. DOI: 10.2429/proc.2016.10(1)081

Papavizas G. C., Davey C. B. (1961). Saprophytic behaviour of Rhizoctonia solani in soil. *Phytopathology,* 51: 693-699.

Singh, V., Amaradasa, B. S., Karjagi, C. G., Lakshman, D. K., Hooda, K. S., & Kumar, A. (2018). Morphological and molecular variability among Indian isolates of Rhizoctonia solani causing banded leaf and sheath blight in maize. *European Journal of Plant Pathology*, *152*(1), 45–60. https://doi.org/10.1007/s10658-018-1447-2

Singleton, L. L., Mihail, J. D., & Rush, C. M. (Red.). (1992). *Methods for research on soilborne phytopathogenic fungi*. APS Press.

Stępniewska-Jarosz, S. (2006). Grupy anastomozowe grzyba *Rhizoctonia solani*. *Postępy Nauk Rolniczych* 5: 125-134 (in Polish).

Toda, T., & Hyakumachi, M. (2006). Heterokaryon formation in *Thanatephorus cucumeris* anastomosis group 2-2 IV. *Mycologia*, *98*(5), 726–736. https://doi.org/10.1080/15572536.2006.11832644

Tomaso-Peterson, M., & Trevathan, L. E. (2007). Characterization of *Rhizoctonia* -Like Fungi Isolated from Agronomic Crops and Turfgrasses in Mississippi. *Plant Disease*, *91*(3), 260–265. https://doi.org/10.1094/PDIS-91-3-0260

Woodhall, J. W., Laurenson, L., & Peters, J. C. (2012). First report of *Rhizoctonia solani* anastomosis group 5 (AG5) in wheat in the UK. *New Disease Reports*, *26*(1), 9–9. https://doi.org/10.5197/j.2044-0588.2012.026.009