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ORIGINAL ARTICLE

Pathogenicity and Fungicide Sensitivity of *Rhizoctonia solani* and *R. cerealis* Isolates

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Abstract The *Rhizoctonia solani* species consists of multinucleate isolates that belong to anastomosis groups AG1–AG3 and differ in virulence and host affinity. *R. cerealis* is a binucleate species of anastomosis group AG-D which causes sharp eyespot, a common plant disease in Poland. *Rhizoctonia* spp. is a ubiquitous soil pathogen that poses a significant threat for global crop production due to the absence of effective crop protection products. The aim of this study was to determine the virulence of *R. solani* and *R. cerealis* isolates towards *Beta vulgaris, Zea mays, Triticum spelta* and *T. aestivum* seedlings, to confirm the presence of endopolygalacturonase genes *pg1* and *pg5* in the genomes of the tested isolates and to evaluate the tested isolates' sensitivity to triazole, strobilurin, imidazole and carboxamide fungicides. All tested isolates infected *B. vulgaris* seedlings. but none of them were virulent against *Z. mays* plants. *R. solani* isolates AG4 PL and AG2-2IIIB PL were characterized by the highest virulence (average infestation score of 2.37 and 2.53 points on a scale of 0–3 points) against sugar beet seedlings. The prevalence of infections caused by most of the analysed isolates (in particular *R. solani* AG4 J—11.8, and *R. cerealis* RC2—0.78) was higher in spelt than in bread wheat. The virulence of the analysed isolates was not correlated with the presence of *pg1* and *pg5* genes. The efficacy of the tested fungicides in controlling *Rhizoctonia* spp. infections was estimated at 100% (propiconazole + cyproconazole), 98.8% (penthiopyrad), 95.4% (tebuconazole) and 78.3% (azoxystrobin).

Keywords Sugar beet · *Pg1* and *pg5* genes · Maize · Spelt · Common wheat

Pathogenität und Sensibilität bei Anwendung von Fungiziden der Isolate *Rhizoctonia solani* und *R. cerealis*

Zusammenfassung Die Art *Rhizoctonia solani* ist eine Ansammlung mehrkerniger Isolate, die die anastomosen Gruppen AG1–AG13 bilden. Diese Gruppen unterscheiden sich voneinander in Bezug auf ihr Virulenzniveau und ihre Affinität im Hinblick auf Pflanzen. *R. cerealis* ist eine zweikernige Art der Gruppe AG-D und bewirkt eine ovale Ringfleckenbildung bei Getreide. Die Gefahr des Befalls von Pflanzen mit diesen Pathogenen ist groß, da die Pathogene allgemein in Böden auftreten und es an effektiven Schutzmethoden für Getreide und für Zuckerrüben mangelt. Ziel der Untersuchungen war es, die Virulenz der Isolate *R. solani* und *R. cerealis* in Bezug auf die Sämlinge *Beta vulgaris, Zea mays, Triticum spelta* und *T. aestivum* zu bewerten. Weiterhin sollte der Nachweis der Anwesenheit der Endo-Polygalacturonase-Gene *Pg1* und *Pg5* in den Genomen der getesteten Isolate geführt sowie ihre Sensibilität gegenüber Triazolen, Strobilurinen, Imidazolen und Carbonsäureamiden bewertet werden. Sämtliche getesteten Isolate infizier-

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ten den Sämling B. wulgaris, kein Isolat befiel Z. mays. Die Isolate R. solani AG4 PL i AG2-2IIIB PL unterschieden sich durch ihr Virulenzniveau (der durchschnittliche Befall lag in der vorgenannten Reihenfolge bei 2,37 und 2,53 auf einer Skala von 0 bis 3) in Bezug auf Sämlinge der Zuckerrübe. Die Sämlinge des Dinkels zeigten einen stärkeren Befall durch die meisten der Isolate beider Arten (insbesondere durch R. solani AG4 J – 1,18 und R. cerealis RC2 – 0,78) als die Sämlinge des Weichweizens. Die Anwesenheit der Gene *Pg1* und *Pg5* korrelierte nicht mit der Virulenz der Isolate. Die durchschnittliche Wirksamkeit der Fungizide bei der Entwicklungshemmung von Isolatkolonien *Rhizoctonia* spp. betrug in der nachstehenden Reihenfolge 100 % (Propikonasol + Cyprokonasol) 98,8 % (Penthiopyrad), 95,4 % (Prochloraz), 94,6 % (Tebuconazol) und 78,29 % (Azoxystrobin).

Schlüsselwörter Zuckerrübe · *Pg1* und *Pg5* Gene · Mais · Dinkel · Saatweizen

Introduction

Rhizoctonia solani Kühn (teleomorph *Thanatephorus cucumeris* A.B. Frank Donk) is a cosmopolitan fungal species that occurs mainly in soil where it develops filaments and sclerotia. The pathogen causes necrosis, damping off and rot, and it contributes to considerable losses in crop production (Melzer et al. 2016). *R. solani* consists of multinucleate anamorphs that belong to different anastomosis groups and differ in virulence, host affinity and colony structure (Sneh et al. 1996). At least 13 anastomosis groups (AG1–AG13) have been identified to date, some of which include subgroups with unique biochemical and molecular properties (Carling et al. 2002; Wibberg et al. 2016). Group AG2 consists of two subgroups, where subgroup AG2-2IIIB is more virulent towards sugar beet seedlings than subgroup AG2-2IV (Panella 2005). In a study by Moliszewska and Schneider (2002), group AG4 isolates were more virulent towards sugar beet seedlings than group AG5 isolates. In certain regions of Europe, the United States and Australia, wheat is also a host for *R. solani* AG1-IB, AG2, AG4, AG5 and AG-8 isolates. However, wheat infections caused by the above pathogen generally occur locally and are relatively mild (Bockus et al. 2010). *R. solani* AG1-IA infects maize seedlings and aerial plant parts, leading to tissue necrosis and dwarfing (Zhang et al. 2012; Gao et al. 2016).

The main reservoirs of *R. cerealis* van der Hoeven (teleomorph *Ceratobasidium cereale* D.I. Murray and Burpee) are soil and plant residues where the pathogen develops filaments and sclerotia. The pathogen colonizes various crop plants, including wheat, sugar beets, cotton and tomatoes (Ray et al. 2004). *R. cerealis* is a binucleate species that belongs to group AG-D (Woodhall et al. 2017) and causes sharp eyespot in cereals. The disease causes damage to the phloem and inhibits the transport of water and nutrients in plants (Cromey et al. 2002). The incidence of sharp spot in Central Europe varies and is generally on the rise (Mikołajska and Wachowska 1996; Lemańczyk 2010; Lemańczyk and Kwaśna 2013). *R. cerealis* generally does not cause significant losses in wheat production, but this pathogen could pose a growing threat due to (1) early sowing (Colbach et al. 1997), (2) growing fungicide use, in particular fungicides targeting *Fusarium* fungi, which destroy the natural antagonists of *R. cerealis* (van der Hoeven and Bollen 1980), and (3) cultivation of wheat varieties sensitive to *R. cerealis* (Cromey et al. 2005; Lemańczyk and Kwaśna 2013).

Pathogens of the genus *Rhizoctonia* are most widely controlled with fungicides. Potatoes are protected with penflufen, flutolanil, pencycuron and azoxystrobin which are characterized by various efficacy against different AG group isolates (Campion et al. 2003). Attempts have been made to protect sugar beets with QoI fungicides (azoxystrobin) applied with starter fertilizers (Noor and Khan 2014). The efficacy of commercially available fungicides in managing *R. solani* and *R. cerealis* has not been widely researched to date (Compant et al. 2005). The aim of this study was to compare the virulence of *R. solani* (of various origin) and *R. cerealis* isolates. as compared with *Fusarium oxysporum* isolates, against *Beta vulgaris, Zea mays, Triticum spelta* and *T. aestivum* seedlings. The presence of endopolygalacturonase genes *pg1* and *pg5* in the genomes of the tested isolates was determined. The sensitivity of *Rhizoctonia* spp. fungi to triazole, strobilurin, imidazole and carboxamide fungicides was also evaluated.

Table 1 Origin of *Rhizoctonia* spp. isolates

Isolates	Plant	Country of origin
R. cerealis RC2	Winter wheat	Poland
R. cerealis RC10	Winter wheat	Poland
R. solani AG4 PL	Sugar beet	Poland
R. solani AG4 NL	Sugar beet	Holland
R. solani AG4 J	Peanut	Japan
R. solani AG2-2IIIB PL	Sugar beet	Poland
R. solani AG2-2IIIB J	Sugar beet	Japan
F. oxysporum	Winter wheat	Poland

Materials and Methods

Origin of Isolates

Two isolates of the binucleate species *R. cerealis.* AG-D RC2 and RC10, from winter wheat culms with symptoms

Table 2 Description of plant protection products

Fungicide	Active substance content	Manufacturer	Fungicide dose field	Concentration in PDA (%)
Amistar 250 SC	Azoxystrobin – 250 g dm^{-3}	Syngenta Poland	3 dm^3/ha	0.75
Vertisan 200 EC	Penthiopyrad – 200 g dm^{-3}	Du Pont Poland	1 dm^3/ha	0.25
Artea 330 EC	Propiconazole – 250 g Cyproconazole – 80 g dm^{-3}	Syngenta Poland	0.5 dm^3/ha	0.12
Atak 450 EC	Prochloraz – 450 g dm^{-3}	Barclay Chemicals	1 dm^3/ha	0.25
Helicur 250 EW	Tebuconazole – 250 g dm^{-3}	Helm Poland	1 dm^3/ha	0.25

of sharp eyespot were analysed (Table 1). Six *R. solani* isolates, including three isolates from anastomosis group AG4 and three isolates from anastomosis group AG2-2IIIB, were also examined. The isolates were obtained from sugar beets displaying symptoms of disease, excluding *R. solani* AG4J which was isolated from peanuts. The isolates originated from Poland, the Netherlands and Japan. Isolates of the genus *Rhizoctonia* were identified based on the number of nuclei in filament cells which were stained, compared with reference isolates and subjected to molecular analyses (Mikołajska and Wachowska 1996; Moliszewska and Schneider 2002). The *F. oxysporum* isolate from horticultural soil was characterized by high pathogenicity towards non-crop plants and was used as reference material.

Analysis of Isolate Pathogenicity

The experiment was conducted in 4.5 × 4.5 cm pots filled with 36 g of horticultural soil combined 1:1 with sand, in six replications. Three seeds and 7-day-old isolates of *R. solani, R cerealis* and *F. oxysporum* cultured on discs of potato glucose medium (Merck, Poland) were placed in each pot (Table 1). Sensitivity to pathogens was analysed in seedlings of sugar beets cv. Toleranz KWS (increased resistance to powdery mildew), maize cv. Silvino (resistant to *Fusarium* spp.), spelt cv. Wirtas (resistant to stem-base diseases) and common wheat cv. Sumai (resistant to *Fusarium* spp.) was analysed. The severity of infection on seedlings was evaluated after 7 days of growth in a phytotron at a temperature of 23.5 °C during the day and 22.5 °C at night (12/12 h light/dark cycle). Seedlings were evaluated on a 4-point scale where: 0 points—healthy seedlings, 1 point—weakly infected seedlings, 2 points—strongly infected seedlings, 3 points—necrotized seedlings.

Pathogens' Sensitivity to Fungicides

Seven-day-old colonies of pathogen isolates with a diameter of 5 mm were placed on the PDA growth medium containing fungicide doses indicated in Table 2, corresponding to the recommended field doses. Colony growth was observed for 7 consecutive days. Colony size was determined in the ImageJ 1.49 program.

Identification of Endopolygalacturonase Genes *pg1* and *pg5*

Gene markers were amplified with endoF/endoR2 and PG2F/PG2R primer pairs (endoF: CCAGAGTGCCGATACCGATT, endoR2 GCTTAGYGAACAKGGAGTG, PG2F: AGATGCAAGGCCGATGATGT, PG2R: TCCATGTACTTCTCCTCACC). The reaction mixture with a final volume of 20 µl contained 10 ng of genomic DNA, 1 µM of each primer, 1 U Taq DNA polymerase (Sigma, Poland), 10X buffer with $MgCl_2$, and 200 µM of the dNTP mix (Sigma, Poland). The thermal cycler was programmed as follows: 94 °C (5 min), (50 cycles of 94 °C (1 min), 52 °C (1 min), 72 °C (2 min)), 72 °C (10 min). Amplification was conducted in the Mastercycler Ep Gradient thermal cycler (Eppendorf, Poland). Amplicons with the size of 1560 bp for the *pg1* gene and 1800 bp for the *pg*5 gene were visualized in a transiluminator (UVP, Poland).

Statistical Analysis

Data were processed by analysis of variance (ANOVA) in the Statistica 12 program (Statsoft Inc. 2014), and the significance of differences between means was evaluated by the SNK test ($p < 0.01$). Fungicide efficacy was calculated with the use of Abbott's formula:

Efficacy = 100% – [F : K × 100%], where:

- K—area of fungal colony on the control plate,
- F—area of fungal colony on a plate containing the tested fungicide.

Results

In the pathogenicity test, plant inoculation with *R. solani* isolates AG2-2IIIB and AG4, *R. cerealis* isolate AG-D and *F. oxysporum* had no significant influence on seedling emergence (Table 3 and 4). Seedling emergence was clearly inhibited only in *B. vulgaris* inoculated with *R. solani* iso-

Table 3 Two-way analysis of variance (ANOVA) of seedlings emergence, isolates pathogenicity and their sensitivity to fungicides

Seedlings emergence			Isolates pathogenicity			Pathogens sensitivity to fungicides		
Objects	df	F	Objects	df	F	Objects	df	F
Plants (P)	3	8.017	Plants (P)	3	109.66**	Isolates (I)	8	212.77**
Isolates (I)	9	0.974	Isolates (I)	9	6.69*	Fungicide (F)	5	2359.59**
P × I	27	2.224**	P × I	27	5.62*	I × F	40	92.48**

** statistically the high significant differences ($p<0.01$)
* statistically the significant differences ($p<0.05$)

Table 4 Emergence of seedlings infected by *R. solani* and *R. cerealis* after 7 days growth

Isolates	*Beta vulgaris*	*Zea mays*	*Triticum spelta*	*Triticum aestivum*	Mean
R. solani AG2-2IIIB PL	2.50^{ab}	1.50^{ab}	2.00^{ab}	2.17^{ab}	2.04
R. solani AG4 PL	0.50^{a}	1.33^{ab}	2.50^{ab}	2.17^{ab}	1.62
R. cerealis RC2	2.14^{ab}	1.28^{ab}	1.57^{ab}	1.43^{ab}	1.61
R. cerealis RC10	2.00^{ab}	1.50^{ab}	1.33^{ab}	1.17^{ab}	1.50
R. solani AG2-2IIIB NL	3.00^{b}	1.00^{ab}	1.17^{ab}	2.33^{ab}	1.87
R. solani AG4 NL	1.00^{ab}	1.43^{ab}	1.86^{ab}	1.43^{ab}	1.43
R. solani AG2-2IIIB J	2.17^{ab}	1.33^{ab}	1.83^{ab}	1.33^{ab}	1.67

Means with different superscripts are significantly different according to Newman-Keuls ($p < 0.01$) test
[a,b]For plant × isolate interaction

Table 5 Pathogenicity of *R. solani* and *R. cerealis* isolates (0–3 scale) and identification of genes *pg1* and *pg5*

Isolates	*Beta vulgaris*	*Zea mays*	*Triticum spelta*	*Triticum aestivum*	Mean	Presence of genetic markers	
						pg1	*pg5*
R. cerealis RC2	$1.44^{b–d}$	0^{h}	$0.78^{e–h}$	$0.37^{f–h}$	0.80^{AB}	No	Yes
R. cerealis RC10	$1.56^{b–d}$	0^{h}	0^{h}	$0.55^{f–h}$	0.64^{BC}	No	Yes
R. solani AG4 PL	2.37^{a}	0^{h}	$0.38^{f–h}$	0.15^{gh}	0.61^{BC}	No	No
R. solani AG4 NL	$0.50^{f–h}$	0^{h}	$0.50^{f–h}$	0^{h}	0.27^{CD}	Not Studied	Not studied
R. solani AG4 J	$0.83^{e–h}$	0^{h}	$1.18^{b–e}$	$0.80^{e–h}$	0.73^{AB}	Not Studied	Not studied
R. solani AG2-2IIIB PL	2.53^{a}	0^{h}	$0.81^{e–h}$	0^{h}	1.04^{A}	No	Yes
R. solani AG2-2IIIB NL	$1.94^{a–c}$	0^{h}	0.10^{gh}	0^{h}	0.64^{BC}	Not studied	Not studied
R. solani AG2-2IIIB J	$1.94^{a–c}$	0^{h}	0.07^{h}	0^{h}	0.67^{BC}	No	Yes
F. oxysporum	$1.06^{e–g}$	0^{h}	0^{h}	0^{h}	0.30^{BC}	Yes	Yes
Control	0.22^{gh}	0^{h}	0^{h}	0^{h}	0.05^{A}	–	–
Mean	1.40^{Z}	0^{X}	0.35^{Y}	0.15^{X}	–	–	–

Means with different superscripts are significantly different according to Newman-Keuls ($p < 0.01$) test
[X–Y]For plants
[A–D]For isolates
[a–h]For plant × isolate interaction

late AG4 PL from Poland (PL) (Table 4). None of the tested isolates were pathogenic towards maize (Table 5). *R. solani* AG2-2IIIB, in particular the Polish isolate, were most aggressive towards sugar beet seedlings. Most plants inoculated with this isolate were necrotized 7 days after inoculation, and the severity of infection was estimated at 2.53 points on a 4-point scale. The severity of infection reached 1.94 for the remaining group AG2-2IIIB isolates. Isolates belonging to this group sporadically infected *T. spelta* seedlings, *R. solani* AG4 isolates, despite belonging to the same anastomosis group, differed significantly in pathogenicity subject to the region of origin. *R. solani* isolate AG4 PL was pathogenic mainly towards sugar beet seedlings (2.37 points on average) and sporadically towards

Table 6 Sensitivity of *R. solani* and *R. cerealis* isolates to selected fungicides (area of colony cm^2. values in brackets (%) respond to fungicide efficiency)

Isolates	Azoxystrobin	Propiconazole Cyproconazole	Prochloraz	Penthiopyrad	Tebuconazole	Control	Mean
R. cerealis RC2	0^{o}(100)	0^{o}(100)	0^{o}(100)	0^{o}(100)	0^{o}(100)	22.22^{d}	3.70^{U}
R. cerealis RC10	0^{o}(100)	0^{o}(100)	0^{o}(100)	0^{o}(100)	0^{o}(100)	39.26^{c}	6.54^{W}
R. solani AG4 PL	9.80^{fg}(85)	0^{o}(100)	$3.89^{h–m}$(94)	0^{o}(100)	0^{o}(100)	63.58^{a}	12.88^{Y}
R. solani AG4 NL	$4.54^{h–k}$(47)	0^{o}(100)	$2.69^{h–n}$(69)	0^{o}(100)	0^{o}(100)	8.63^{g}	2.64^{U}
R. solani AG4 J	$4.71^{h–j}$(90)	0^{o}(100)	0^{o}(100)	0^{o}(100)	$4.12^{h–m}$(91)	46.89^{a}	9.28^{X}
R. solani AG2-2IIIB PL	$3.52^{h–m}$(91)	0^{o}(100)	$3.81^{h–m}$(91)	0^{o}(100)	5.08^{h}(88)	40.68^{b}	8.85^{X}
R. solani AG2-2IIIB NL	0^{o}(100)	0^{o}(100)	4.91^{h}(88)	$4.11^{h–m}$(90)	$3.51^{h–m}$(91)	40.40^{c}	19.42^{Z}
R. solani AG2-2IIIB J	5.39^{h}(81)	0^{o}(100)	0^{o}(100)	0^{o}(100)	5.23^{h}(82)	29.00^{c}	6.60^{W}
F. oxysporum	$3.92^{h–m}$(79)	0^{o}(100)	0^{o}(100)	0^{o}(100)	0^{o}(100)	19.22^{e}	3.85^{U}
Mean	0.80^{C}(78.3)	0^{A}(100)	1.69^{B}(95.4)	0.45^{A}(98.8)	0.19^{B}(94.6)	37.01^{D}	–

Means with different superscripts are significantly different according to Newman-Keuls ($p < 0.01$) test
$^{A–C}$For fungicides
$^{X–W}$For isolates
$^{a–m}$For fungicide × isolate interaction

T. spelta (0.38) and *T. aestivum* (0.15), *R. solani* isolate AG4 J from Japan produced symptoms of infection in the above plants and was most virulent towards *T. spelta* (1.18), *R. solani* isolate AG4 NL sporadically infected *B. vulgaris* and *T. spelta* seedlings. Binucleate *R. cerealis* isolates AG-D RC2 and RC10 infected *B. vulgaris, T. aestivum* and *T. spelta*. and their virulence towards *B. vulgaris* seedlings was relatively high (1.44 and 1.56 points on average). The *F. oxysporum* isolate was characterized by the lowest average virulence, and it produced symptoms of infection only in sugar beet seedlings after 7 days of growth. The presence of *pg1* and *pg5* gene markers was observed only in the above isolate (Table 5).

All of the tested fungicides limited or completely inhibited the growth of *R. solani* colonies (Table 6). *R. cerealis* was sensitive to all of the analysed fungicides (100% efficiency). The tested fungicides were characterized by the following average efficiency: 100% (propiconazole + cyproconazole), 98.8% (penthiopyrad), 95.4% (prochloraz), 94.6% (tebuconazole) and 78.29% (azoxystrobin). Propiconazole and cyproconazole completely inhibited the growth of all tested isolates in vitro, whereas penthiopyrad effectively inhibited all isolates except for *R. solani* AG2-2IIIB NL (89.82% efficiency). Prochloraz inhibited the growth of Polish and Dutch isolates of *R. solani* AG2-2IIIB and *R. solani* AG4 in 69.00% relative to control. The remaining isolates were completely inhibited by the above fungicide. Tebuconazole inhibited Polish and Dutch isolates of *R. solani* from anastomosis group AG4. The above fungicide inhibited the growth of the remaining isolates in 87% to 91%. The least efficient fungicide was azoxystrobin which completely inhibited the growth of only *R. cerealis* isolates RC2 and RC10 and *R. solani* isolate AG2-2IIIB NL. Its efficacy against the remaining isolates ranged from 47.30% in *R. solani* AG4 NL to 91.14% in *R. solani* AG2-2IIIB PL.

Discussion

In this study *R. solani* (anastomosis groups AG2-2IIIB and AG4), *R. cerealis* and *F. oxysporum* infected mostly *B. vulgaris* and less frequently, *T. spelta* and *T. aestivum*. According to the literature, *B. vulgaris* seedlings are frequently infected by *R. solani* (Ithurrart et al. 2004; Buhre et al. 2009), *R. cerealis* (O'Sullivan and Kavanagh 1990) and *F. oxysporum* (Hanson and Jacobsen 2006; Hill et al. 2011) soil pathogens. In earlier studies, maize was colonized by the destructive pathogen *R. solani* f. sp. Sasakii whose prevalence and strong virulence were confirmed mainly in tropical regions as well as in the United States and Germany (Singh and Shahi 2012). This pathogen was initially classified in anastomosis group AG11 (Ogoshi 1987). In successive years. other *R. solani* isolates from groups AG1-IA, AG4 and AG5 were identified in maize seedlings and aerial plant parts (Zhang et al. 2012; Gao et al. 2016). In our study, maize was not infected by *R. cerealis* or *F. oxysporum* and *R. solani* isolates can differ in host affinity and virulence, which suggests that the analysed *R. solani* isolates from groups AG2-2IIIB and AG4 did not infect maize seedlings because their respective anastomosis groups were characterized by low affinity for maize.

R. cerealis is a fungal species that colonizes mainly cereals, in particular wheat and triticale (Burpee 1980). The

fungus has never been isolated from maize, which indicates that the pathogen has a narrow and fixed host range. *F. oxysporum* is composed of many morphologically indistinguishable variants (Kistler 1997). This observation was used to identify *F. oxysporum* f. sp. *betae* which colonizes sugar beets (Michielse and Rep 2009). In our study, the analysed isolate of *F. oxysporum* was virulent only against sugar beets, and it did not infect other potential hosts—maize and two wheat species. The markers of both endopolygalacturonase genes *pg1* and *pg5*, which are virulence factors, were identified only in the *F. oxysporum* isolate. The infection process is facilitated by enzymes which enable pathogens to penetrate host tissues by degrading the cell wall (Yang et al. 2016). among them polygalacturonases (PG) that degrade pectin. The enzyme endopolygalacturonase is encoded by genes *pg1* and *pg5*. According to Hirano and Arie (2009), the *pg1* gene has the highest number of nucleotide sections encoding the amino acid sequence in PG protein molecules. Genes *pg1* and *pg5* are positioned in different loci. The *pg1* gene is located on chromosome 10, and the *pg5* gene—on chromosome 9 (Hirano and Arie 2009).

The analysed wheat species, *T. aestivum* and *T. spelta,* were most sensitive to *R. solani* isolate AG4 J. The virulence of *R. solani* AG4 against wheat has not been documented to date. Selected *R. solani* isolates have been found to exert varied effects on wheat. According to Tewoldemedhin et al. (2006), AG2-1 isolates are weakly virulent against wheat, whereas Roberts and Sivasithamparam (1986) found that AG2-1 isolates are highly virulent and cause significant losses in wheat yields. In our study, only Polish and Japanese *R. solani* isolates belonging to the AG2-2IIIB group harboured the pathogenicity gene *pg5*. Despite the above, these isolates did not infect wheat, but they were highly virulent against sugar beet seedlings. The above findings could suggest that the pathogenicity of *R. solani* isolates, including isolates representing the same anastomosis group and subgroup, against wheat is determined by other, unknown factors. According to recent research, isolates belonging to group AG8 are potentially most virulent against wheat and show a preference for plants of the genus *Poaceae* (Paulitz et al. 2002).

Propiconazole and cyproconazole (Artea 330 EC), which are triazole compounds, were most effective in inhibiting the growth of all tested isolates. The effectiveness of triazole compounds against *R. solani* in rice was confirmed by Johnson et al. (2013) and Gupta et al. (2015). In contrast, Bolton et al. (2010) reported that propiconazole was ineffective against *R. solani* in sugar beets. These discrepancies indicate that triazole compounds differ significantly in their efficacy. In our study, azoxystrobin exerted an inhibitory effect on the growth of *R. solani* and *R. cerealis*, which is consistent with the findings of Bolton et al. (2010). Limited efficacy of azoxystrobin against *R. solani* isolate AG4 NL has already been reported in the literature, *R. solani* isolates resistant to strobilurin were identified in rice (Olaya et al. 2012). Pieczul and Świerczyńska (2014) demonstrated that *R. cerealis* isolates from cereals were characterized by increased resistance to azoxystrobin.

Summary

R. solani and *R. cerealis* isolates were most virulent against sugar beets, followed by spelt and common wheat. The analysed *R. solani* isolates representing anastomosis group AG2-2IIIB were characterized by the highest virulence against the tested plants. The evaluated fungicides were more effective against isolates of *R. solani* AG2-2IIIB and *R. cerealis* than *R. solani* AG4. Triazole fungicides effectively inhibited the growth of all tested isolates, whereas the efficacy of azoxystrobin against *R. solani* AG4 was limited.

Conflict of interest K. Kucharska, B. Katulski, K. Goriewa, A. Duba and U. Wachowska declare that they have no competing interests.

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