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Rhizoctonia cerealis anastomosis group *GAG-1*, the common pathogen of wheat, barley and sugar beet

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Isolates of *Rhizoctonia cerealis* anastomosis group *GAG-1* were obtained from sharp eyespot lesions on wheat and on barley culms and from diseased sugar beet seedlings. Isolates of *R. cerealis* were collected from a fields with crop rotation experiments: sugar beet-spring wheat-winter barley. In pathogenicity tests isolates of *R. cerealis* from sugar beet seedlings and from sharp eyespot lesions on wheat and barley were pathogenic to these crops. Isolates of *R. cerealis* from sharp eyespot lesions on wheat and barley caused severe damping-off of sugar beet. Isolates of *R. cerealis* from sugar beet seedlings also caused symptoms of sharp eyespot on wheat and barley. None of the wheat and barley isolates of *R. cerealis* tested caused root-rot on wheat or barley seedlings. Isolates of *R. cerealis* obtained from diseased plants of wheat, barley and sugar beet were similar in morphology of cultures and anastomosed with *GAG-1* tester isolate.

The relatinoship between anastomosis, colony characters, growth rate, hyphal diameter and pathogenicity of *AG-4*, *AG-2-2* and *AG-5* isolates obtained together with *R. cerealis* from diseased plants were also investigated.

Key words: wheat, barley, sugar beet, *Rhizoctonia solani*, *R. cerealis*, *AG-4*, *AG-2-2*, *AG-5*, *GAG-1* anastomosis groups, pathogenicity.

INTRODUCTION

Rhizoctonia solani Kühn, teleomorph. *Thanatephorus cucumeris* (Frank) Donk, caused disease on different plant species. It has been widely recorded as causing damping-off in sugar beet (Ruppel 1972; Naito et al. 1975; Naito, Sugimoto, Yamaguchi 1978; Hecker, Ruppel 1977; Herr, Roberts 1980; Herr 1982, 1988, 1991; Allen et al. 1985; Carling, Summer 1992).

R. solani is a complex species and has been divided into subspecific groups based on hyphal anastomosis and colonies morphology. Twelve anastomosis groups of *R. solani* (AGs 1-11 and AG-BJ) are now recognized (S n e h, B u r p e e, O g o s h i 1991; C a r l i n g, S u m m e r 1992). *R. solani* AGs 1, 2-1, 2-2, 3, 4 and 5 have been isolated from diseased sugar beet seedlings in Japan (N a i t o et al. 1975) and the USA (H e c k e r, R u p p e l 1977; R u p p e l 1977; H e r r, R o b e r t s 1980; H e r r 1982, 1988, 1991). R u p p e l (1977) found a positive correlation between *R. solani* groups based on anastomosis and morphology of cultures while S h e r w o o d (1969) also reported some correlation between anastomosis, the morphology and physiology of isolates.

Binucleate *Rhizoctonia* spp., that form *Ceratobasidium* teleomorph, also cause diseases on several crops (B u r p e e et al. 1980; M o e n, H a r r i s 1985; O n i k i, O g o s h i, A r a k i 1986; K a t a r i a, H o f f m a n 1988; S n e h, B u r p e e, O g o s h i 1991; C a r l i n g, S u m m e r 1992). A binucleate *R. cerealis* (teleomorph *Ceratobasidium cereale* Murray et Burpee) has been reported as a pathogen of graminaceous hosts, causing sharp eyespot disease of cereals (B o e r e m a, V e r h o e v e n 1977) and yellow patch of turfgrass (B u r p e e 1980; B u r p e e, M a r t i n 1992). There have been also some reports of *R. cerealis* infection of non-graminaceous species (H o l l i n s, J e l l i s, S c o t t 1983; W o n g, B a r b e t t i, S i v a s i t h a m p a r a m 1985). Seven *Ceratobasidium* anastomosis groups (CAGs 1-7) have been identified by B u r p e e in Canada (B u r p e e et al. 1980). In Japan seventeen anastomosis groups (AG-A-AG-Q) have been identified by O g o s h i (O g o s h i 1975, 1985, 1987). In the Netherlands B o e r e m a and V e r h o e v e n (1977) reported that sharp eyespot disease of cereals was caused by *Rhizoctonia cerealis*. This species was subsequently confirmed to cause sharp eyespot disease of cereals in the USA (L i p p s, H e r r, 1982) and in Britain (C l a r k s o n, C o o k 1983). It also causes of yellow patch of turfgrass in Canada and the USA (B u r p e e 1980; M a r t i n, L u c a s 1984; B u r p e e, M a r t i n 1992). *Ceratobasidium cereale* was subsequently described as the teleomorph. of *R. cerealis* (M u r r a y, B u r p e e 1984; O n i k i, O g o s h i, A r a k i 1986).

B u r p e e et al. (1980) and K a t a r i a with H o f f m a n (1980) studied they biology of binucleate isolates of *Rhizoctonia* spp. from diverse sources and they found that all the CAG-1 isolates were collected from graminaceous hosts. B u r p e e (1980) and L i p p s with H e r r (1988) showed that CAG-1 anastomosed with the type culture of *R. cerealis*, thus equating CAG-1 with *R. cerealis*.

The purpose of this study was to identify the isolates obtained from sharp eyespot lesions on wheat and barley and from sugar beet seedlings and to determine their pathogenicity to these crops.

MATERIALS AND METHODS

Wheat, barley, sugar beet plants and also soils samples were collected from plots in Experimental Station Bałcyny in Olsztyn region (NE Poland) in the years 1994-1996. They were obtained under the crop rotation: sugar beet--spring wheat-winter barley amended with different organic manures (biological compost, straw, stable manure) and four levels of mineral nitrogen (0, 30, 60, 90, 120, N/hectare for wheat and barley and 0, 60, 100, 1400, 180 N/hectare for sugar beet). The crop rotation was established in 1994 year. Isolates of *Rhizoctonia* spp. were obtained from sharp eyespot lesions on wheat and barley culms and diseased seedlings of sugar beet, using standard procedure. Isolates of *Rhizoctonia* spp. were collected also from soil by indirect and direct method using technique of H e n i s et al. (1978) and C a s t r o (1982).

S e l e c t i v e m e d i a:

- Ko-Hora medium amended: benomyl-500 mg/l, prochloraz-500 mg/l (K o, K o r a 1971);
- water agar (WA) amended with ethanol-20 ml/l, $NaNO_3$ 5 g/l, KH_2PO_4 0.5 g/l (T r u j i l l o et al. 1987);
- 2% WA amended with fungicides: benomyl (Benlate 50 WP), penycurone (Monceren 25 WP), cyproconazole (Alto 25 WP), tolclofosmethyle (Rhizolex 10 WP) — each 500 mg/l (S u m m e r 1987; K a t a r i a, H o f f m a n 1988; K a t a r i a, G i s i 1989).

In comparative test (V i n c e l l i, B e a u p r e 1989) these media were rated equally effective in the selective recovery of *Rhizoctonia* spp. from soil and plant samples. Both media were dispensed at approximately 12 ml per Petri dish (PD) — 9 cm in diameter. The dishes with three selective media were used for plant and soil samples assay Tissue selections 1-1.5 cm long were cut from the margins of lesions, washed under tap water, rinsed with and antibiotic solution (10 mg chloramphenicol and 100 mg of neomycin per 11 of distilled water) and transferred aseptically to Ko-Hora medium and to two other selective media. After the tissues had been grown for 3 days at 21-22°C in the dark, cultures were examined at 400 × for mycelia of *R. solani.*

Cultures on potato-dextrose agar PDA were stained by a rapid technique with 0.5% aniline blue and by a HCl-Giemsa nuclear staining procedure, which to allowed count of nuclei in vegetative cells and width of hyphae (H e r r 1979).

Hyphal anastomosis were observed on agar-coated slides and 1.5% WA in PD (S n e h, B u r p e e, O g o s h i 1991; C a r l i n g, S u m m e r 1992). All the wheat and barley culm isolates and sugar-beet seedling isolates were paired with known *AGs 1, 2, 3, 4, 5, 6, 7, BJ* and *GAG-1* tester isolates to determine their affinities. Isolates of *R. cerealis* and *R. solani* were paired in all possible combinations with representative isolate from diseased culms of wheat and barley and seedlings of sugarbeet. Tester isolate (*GAG-1* of *R. cerealis*) was provided by B u r p e e (Canada) *AGs 1, 2, 3, 4, 5,* by C a r l i n g (USA) *AGs 5, 6, 7, 8, BJ* and by O g o s h i (Japan).

Agar discs (5-7 mm in diameter) cut from margin of actively growing colonies on PDA were transferred to PD containing 15 ml of PDA and OA media. Three dishes of each isolate were incubated at 22-23°C in the dark. Two measurements at right angles were taken and the increase in colony diameter between 24 and 48 hours of growth was recorded. The morphology and colour of colonies were compared during the first week of growth and after 24 days.

In pathogenicity assay only isolates of *Rhizoctonia cerealis* (*GAG-1*) were used. Inoculum for pathogenicity test on wheat, barley and sugar-beet was grown on PDA containing L-asparagine (2 g/l) in 9 cm PD a 20°C for 7 days. Cultures of each isolate then macerated in 200 ml of distilled water, mixed with sterile sand and placed in plastic pots (10 cm in diam). Seeds of wheat, barley and sugarbeet were surface sterilized in a 95% ethyl alcohol for 30 sec. then rinsed with sterile water and planted wet on sand. Four replicate pots were used per isolate. After 21 days, plants were washed free of adhering sand and rated for development of lesions. Twenty isolates of *R. cerealis* (*GAG-1*) for each crop were used. Noninoculated control also were sown.

In the second experiment pathogenicity of cultures was tested by an inoculum layer technique (L i p s, H e r r 1982). Plastic pots with sterile sand as described above were used. A completely colonized 2% WA layer inoculum from a PD culture was placed to cover the top of sand. A non-colonized agar layer was used for the control. Surface-sterlilzed wheat, barley and sugar beet seeds were arranged on top of the agar inoculum and then covered with 50 ml of the sand. Five replicate pots were randomized and plants were maintained at 10-25°C. Seedlings were washed free of adhering sand and both root lenght and fresh weight of tops were recorded.

RESULTS

Altogether 120 isolates belonging to *Rhizoctonia* spp. were isolated. Among these isolates 31% were multinucleate, grew relatively fast on PDA (18-26 mm/24 h) and developed a brown pigmentation with age. These isolates were assigned to *R. solani* Kühn; 69% isolates of *Rhizoctonia* spp. had binucleate hyphal cells and were assigned to *Rhizoctonia*-like isolates.

As many as 71.2% of *R. solani* isolates obtained from diseased sugar beet seedling anastomosed with the *AG-2-1*, *AG-2-2*, *AG-4* and *AG-5* tester isolates. Only 11.1% of multinucleate and binucleate osolates of *Rhizoctonia* spp. failed to anastomose with tester isolates from *AG-1* through *AG-9* and with the *GAG-1* tester isolates. Some of them failed to anastomose with one another. Isolates of *Rhizoctonia* spp. which did not anastomose with tester isolates were designated as a single unidentified and indigenous anastomosis groups.

Many of the 69.7% of *Rhizoctonia* spp. isolates obtained from wheat and barley culms and 17.7% sugar beet's isolates were assigned to *R. cerealis* (*GAG-1*). All these isolates were anastomosing with the *GAG-1* tester isolate. Relatively slow growth on PDA was observed for these isolates (9-14 mm in diam./24 hours at 20°C). These isolates were usually white-creamy or yellow-white to light-tan coloured.

Characteristic of colonies. Sugar beet's isolates of *Rhizoctonia solani* which belonging to anastomose group *AG-2-2* and *AG-5* were similar in appearance on OA and PDA. On the first medium all the isolates of both groups were light-brown with a central rusty brown or nearly black sclerotial crust which sometimes formed droplets of tan coloured exudates. Isolates of both groups on PDA were light -brown with spare aerial mycelium and dense aggregates of monilioid cells formed a central masses or at colony perimeters.

R. solani AG-4 isolates were morphologically distinct from the other *R. solani* anastomosis groups and *R. cerealis*. They were characteristic on OA by a central grey-green wrinkled sclerotial crust with small droplets of tan exudate. On PDA, colonies were white to creamy coloured granular in appearance because of aggregates of monilioid cells adpressed to the surface of the medium and covering it. Small sclerotia (<1 m in diam.) were dispersed over the surface of cultures.

Some isolates of *AG-4* obtained from culms of wheat and barley differed in colony morphology from sugarbeet's *AG-4* isolates. After 14 days of growth on PDA wheat and barley *AG-4* isolates were tan to chocolate-brown. Choclate--brown sclerotia (2-5 mm in diam) were dispersed over the surfaces of cultures (Table 1).

Multinucleate isolates of *R. solani* unassignable to any anastomosis group formed on OA samll sclerotia (<1,0 mm in diam.) on colony perimeters or were attached to the sides of PD. The absence of a central sclerotial crust characterized cultures of the unidentified anastomose groups of *R. solani*. Culture of this anastomose group of *R. solani* on PDA had abundant aerial mycelium yellow-tan to brown-tan or sometimes chocolate-brown with tufts of monilioid cells adhering to the PD lids.

Isolates of *Rhizoctonia cerealis* (*GAG-1*) obtained from wheat and barley culms formed white-creamy, yellow-white or light-tan coloured mycelium on PDA and produced a very few sclerotia usually darkly pigmented covering the agar surface. Isolates of *R. cerealis* (*GAG-1*) obtained from diseased sugar beet seedling differed in colony morphology from wheat and barley isolates of *GAG-1*. *R. cerealis* isolates of sugar beet on PDA were creamy coloured and usually remained of this colour after 24 days. *R. cerealis* isolates of sugar beet also differed by the pattern of sclerotia OA. On OA aerial mycelium was sparse, and creamy to brown, sclerotia often coalesced to form unbroken rings at the edges of the PD. Sclerotia usually were not formed on PDA.

Table 1
Characteristics of representative anastomose groups of *Rhizoctonia* spp.

Species and anastomosis group	Characteristics used for classification on PDA		On oat meal agar	Hyphal mean width µm	Number of nuclei per cell
	after 3-5 days growth	after 14 days of growth			
Rhizoctonia solani AG-4 (teleomorph. *Thanathoporus cucumeris*)	Mealy mycelial growth closely appressed to the medium surface; initial colony growth dense, spidery and white in colour; uneven colony borders; fast radial colony growth (15-21 mm/day); hyphae 5-8 µm in diameter.	Nonrinded, chocolate brown sclerotia (2-5 mm in diameter); tan to chocolate brown colony pigmentation.	Mycelia ligth brown with a central grey-green wrinkled sclerotial crust with small droplets of tan exudate; sclerotia small (< 1.0 mm).	5.2	4-7
Multinucleate *Rhizoctonia solani* (*T. cucumeris*) endogenous group that did not anastomose with tester isolates(*AG-1* to *AG-9*)	Isolates white to light tan. Mycelium floccose in early stages of growth; fast radial colony growth (14-20 mm/day); hyphae 5-7 µm in diameter.	Isolates from brown to dark brown. A few isolates yellowish pigmentation. Concentric rings of dark and light mycelium visible in most cultures and this zonation was apparent from early stages of development; mycelium became increasingly appressed to the agar surface as cultured aged; sclerotia generally ranged from few to many and to 2.0 mm in size; individual sclerotia often coalesced into large clumps; mature sclerotia were tan to light brown and scattered randomly over the agar surface.	Small sclerotia (< 1.0 mm in diam.) on colony perimeters or were attached to the PD. Absence of the central sclerotial crust.	7.2-8.1	5-7
Binucleate *Rhizoctonia* spp. *CAG-4* telemorph. *Ceratobasidium* spp.)	Fine mealy mycelial growth in colony centre becoming dense after *5-7 days; colonies usually form radial* tufts of mycelium; colonies tan to brown; slow radial colony growth (approximately 10 mm/day); hyphae < 5 µm in diameter.	Aerial mycelia with tan sclerotia (0.5-1.0 mm in diameter); colonies *cinnamon brown.*		4-5	2

Binucleate *Rhizoctonia* spp. *CAG-3* (*Ceratobasidium cornigerum*)	Some isolates form concentric growth rings; most of isolates form buff--colored clumps of monilioid cells closely appressed to the medium surface; buff-colored colonies; fast radial colony growth (12-18 mm/day); hyphae $<5\,\mu m$ in diameter.	Most isolates ecentually form tan to greyish brown sclerotia (2-4 mm in diameter) in the centre and at the border of colonies.		3-5	2
Binculeate *Rhizoctonia* spp. that did not anastomose with tester isolates (*GAG-1* to *GAG-7*). Groups I, II	Mycelial growth weakly to strongly; some isolates form concentric growth rings; even colony borders; radiate, white to yellowish mycelium, hyphae 3 μm in diameter; variable radial growth rate (8-15 mm/day).	Some isolates form aerial clumps of monilioid cells; on brown pigmentation formed, colonies remain white to yellow.		2-3	2
Group II	Even colony margines; colonies white; slow radial colony growth (8-10 mm/day); hyphae $<5\,\mu m$ in diameter.	Colonies white and velvetlike in appearance. Sclerotia absent or low.		2-4	2
Group III	Fine, nonpatterned growth in colony centre; radiate, brown-pigmented pattern visible from the underside of cultures; hyphae $<5\,\mu m$ in diameter.	Colonies dark brown. Sclerotia absent or low.		4-5	
Rhizoctonia zeae *WAG-Z* (teleomorph. *Waitera circinata*)	White mycelial growth; white to salmon-coloured sclerotia initials usually evident and embedded in the medium	In the medium sclerotia (approximately 1 mm in diameter) spherical, salmon-coloured; colonies salmon-coloured.		5-7	5-7
Rhizoctonia cerealis (teleomorph. *Ceratobasidium cereale*) Anastomosis group *GAG-1*	White mycelial growth; white to cream-coloured growth 10-14 mm/day very few sclerotia or absent covering the agar surface, hyphae $<5\,\mu m$ diameter.	Colonies cream coloured; in some cultures mycelial pigmentation increased with age resulting yellow-white, light-tan coloration; sclerotia absent or low darkly pigmented (2-4 mm in diameter) covering the surface of colony.	Aerial mycelium sparse and cream to brown, sclerotia often coalesced to form umbroken rings at the edges of the dishes.	4-5	2

The width of hyphae within each group of *R. solani* varied considerably (Table 1). Isolates of *AG-5* had on an average the widest hyphae, while the *AG-4* isolates were characterized by the narrowest one. The mean width of hyphae of *AG-4* was significantly lower than of *AG-2-2*, *AG-5* and the unidentified indigenous anastomose groups. The width of hyphae of *R. cerealis* (*GAG-1*) also differed considerably. The maximum width overlapped with the minimum width of hyphae of *R. solani* anastomose group of *AG-4* but the mean hyphal width of *R. cerealis* (*GAG-1*) was significantly smaller than all *R. solani* groups studied. The growth rates in a representative anastomosis group of *R. solani*, *R. cerealis* (*GAG-1*) and *Rhizoctonia* spp. are presented in Table 1.

P a t h o g e n i c i t y t e s t. All the binucleate *Rhizoctonia* spp. isolates caused sharp eyespot disease of wheat and barley, they also caused damping--off of sugar beet seedlings proving that they were *R. cerealis*. Under the fied conditions lesions on culms were sharply defined with dark-brown margins and creamy coloured central areas. They frequently coalesced into larger multiple lesions, extending up the culm for several centimetres, or girdling them.

R. cerealis (*GAG-1*) was isolated from all the diseased sugar beet seedlings. *R. cerealis* infected the seedlings at the cotyledon stage of shortly after the appearance of the first pair of true leaves. When seedlings became infected a few days after emergence, the entire hypocotyls became necrotic up to the bases of the cotyledons. Older collapsed seedlings had, necrotic hypocotyls below soil level.

The results obtained confirmed that *R. cerealis* (*GAG-1*) is able to be an agressive primary pathogen of sugar beet seedlings. Isolates of *R. cerealis* (*GAG-1*) which were weak pathogenes of sugar beet also proved to have lower pathogenicity to wheat and barley. Isolates of *R. cerealis* extremely virulent to sugar beet seedlings were obtained from plots where sugar beet had been grown after spring wheat and next year after winter barley.

DISCUSSION

Rhizoctonia cerealis was established in a present study as a primary pathogen of sugar beet seedlings. This is in contrary with earlier reports (B o e r e m a, V e r h o e v e n 1977; B u r p e e 1980; M a r t i n, L u c a s 1984; M o e n, H a r r i s 1985; O g o s h i, C o o k, B a s s e t t 1990; B u r p e e, M a r t i n 1992) in which *R. cerealis* was described as a pathogen causing diseases only in graminaceous hosts. More recently, however, there have been some reports of *R. cerealis* infecting non-graminaceous hosts. K a t a r i a and H o f f m a n (1988), H o l l i n s, J e l l i s and S c o t t (1983) found that *R. cerealis* infected potato stolons but the authors concluded that it was prinicipally a pathogen of cereals. In Australia W o n g,

Barbetti, Sivasithamparam (1985) isolated *R. cerealis* from disease roots of subterraneum clover (*Trifolium subterraneum*) and showed that some isolates caused lesions on tap roots of subterraneum clover seedlings. In Ireland O'Sullivan and Kavanagh (1990, 1991) reported that *R. cerealis* caused damping-off of sugar beet. They also isolated *R. cerealis* from diseased seedlings of sugar beet under the field conditions.

The results of study and recent reports of the other studies concern *R. cerealis* infection of non-graminaceous hosts suggest that the species is probably an unspecialized pathogen capable causing disease in a range of taxonomically unrelated hosts (Kataria, Hoffman 1988; Sneh, Burpee, Ogoshi 1991; Burpee, Martin 1992; Carling, Summer 1992). In this study indicated that *R. cerealis* was specialized pathogen of wheat and barley which causing primarily sharp eyespot lesion and may be adapted to sugar beet under specifity cropping system *R. cerealis* was able to infect sugar beet and caused damping off its when followed after wheat, or the monoculture of wheat. This statement is in agreement with Hollins, Jellis, Scott (1983); Kataria, Hoffman (1988); Sneh, Burpee, Ogoshi (1991); Carling, Summer (1992) who concluded that *R. cerealis* caused the disease of potatoes when sugar beet was cropping after potatoes or monoculture of potatoes, and was able to infect also pea under specifity cropping system.

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Patogeniczność dwujądrowych izolatów *Rhizoctonia cerealis* (z grupy zgodności wegetatywnej *GAG-1*) względem pszenicy, jęczmienia i buraków cukrowch

S t r e s z c z e n i e

Izolaty *Rhizoctonia cerealis* Van der Hoeven (teleomorfa *Ceratobasidium cereale* Murray et Burpee, grupa zgodności wegetatywnej *GAG-1*) u pszenicy i jęczmienia wywoływały głównie łamliwość źdźbeł.

Stwierdzono, że w płodozmianie burak cukrowy (pszenica jara) jęczmień ozimy izolaty *R. cerealis* (*GAG-1*) wywołują również zgorzel siewek buraków cukrowych. Z porażonych okazów buraków cukrowych wyosobniono również wielojądrowe izolaty *R. solani* Kühn (teleomorfa *Thanatheporus cucumeris* (Frank) Donk) należące głównie do grup zgodności wegetatywnej *AG-4*, *AG-2-2* i *AG-5*. Podano charakterystykę izolatów *R. cerealis* z grupy zgodności wegetatywnej *GAG-1* i *R. solani* z grup zgodności wegetatywnej *AG-4*, *AG-2-2* i *AG-5*.