JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 42, No. 3 (2002)

OCCURRENCE AND PATHOGENICITY OF FUNGI FROM *GAEUMANNOMYCES-PHIALOPHORA* COMPLEX ISOLATED FROM WINTER WHEAT SEEDLINGS

Agnieszka Mączyńska, Hanna Sikora, Barbara Krzyzińska

Institute of Plant Protection in Poznań, Sośnicowice Branch,
Gliwicka 29, 44-153 Sośnicowice, Poland
e-mail: a.maczynska@ior.gliwice.pl

Accepted: July 30, 2002

Abstract: Roots of winter wheat grown in the field were examined for the occurrence of the fungi *Gaeumannomyces graminis, Phialophora* and *Fusarium* spp. Plants were sampled and examined in the autumn of 2000 and 2001 and in the following spring. Root systems were visually assessed and a percentage of affected roots were determined on 100 plants per field. More and less virulent members of the *G. graminis-Phialophora* complex and other fungi were isolated from infected roots. Above 85% of isolated fungi were classified as *Gaeumannomyces-Phialophora* complex. Morphological characteristics of the fungi isolated from plant roots were analysed in laboratory tests. In pathogenicity tests were assessed: disease severity, height of plants, percentage of chlorotic or necrotic leaves and biomass of whole plants.

Key words: *Gaeumannomyces graminis, Phialophora* spp., *Fusarium* spp. winter wheat, take-all

INTRODUCION

Winter wheat is a major agricultural crop grown in Poland on the area of around 1.9 million ha (Rocznik Statystyczny Rzeczypospolitej Polskiej 2001). In Southern Poland which is the major region of wheat production, this crop plant is frequently cultivated in the proportion more than 40% in rotation. This creates a serious threat of disease, especially root and stem base diseases. In Poland, root diseases of winter wheat are mainly caused by *Fusarium* spp. and *Gaeumannomyces graminis var. tritici* (Ggt), both infecting stem base as well.

In the course of research work on winter wheat diseases occurring in Southern Silesia, we stated disease symptoms on roots of young winter wheat plants in the autumn. They usually appeared as browning of seminal roots, lesions were usually confined to small sections of a root, although they were sometimes more extensive. It is well known that visual diagnosis of symptoms caused by *G. graminis* and species

of *Fusarium* is difficult because of their similarity (Huber and McCay-Buis 1993; Hornby et al. 1998). Thus, the isolation of pathogens on agar media and microscopical examination is necessary.

Considerable research work on root and stem base diseases was performed in Poland in the 1980's. Martyniuk (1986) studied the occurrence and biology of *Gaeumannomyces-Phialophora* complex on wheat and other graminicolous hosts; he considered take-all disease as a serious threat to winter wheat in Poland. Recently it has been shown that the disease occurs on the whole territory of Poland (Korbas et al. 2001), although detailed data on its current effect on winter wheat yield are not available. Łacicowa et al. (1985) have shown that several species of *Fusarium* are common pathogens of seedlings and older wheat plants, which can be quite damaging on commercial fields. The health status of young plants in the autumn should not be ignored, because primary infection of roots may lead to more serious secondary infections, which can be more damaging, and negatively influence the quality of grain yield. According to a model presented by Meynard (1985) the infection of roots of winter wheat plants by *G. graminis* from sowing till beginning of tillering affected no plants/m^2, no ears/m^2 and the amount of grain yield. The latter parameter is also related to the decrease of 1000-grain weight. However, the grain yield of wheat plants is not always related to the infection at early growth stages. Green and Irvins (1984) reported that late infections recorded 3 weeks prior to anthesis in winter wheat crop in which no take-all was detected in March or early April lowered the total biomass and decreased grain yield.

The aim of the research work presented in this paper was to answer the questions which pathogens caused damage to roots of winter wheat in the autumn in Upper Silesia, and to define their pathogenicity, with a special reference to the fungi of *Gaeumannomyces-Phialophora* complex most commonly isolated from infected roots in the years 2000–2001.

MATERIALS AND METHODS

In the autumn of 2000 and 2001 (October) and in the early spring (April) of the following years samples of young winter wheat plants from 12 fields in the region of Upper Silesia were taken. One hundred plants were picked up at random, diagonally across each field and roots were visually assessed for the occurrence and severity of infection. Per cent of infected roots as related to the whole root system of individual plants was determined, and for statistical calculations average values for each field were used. Isolations from fresh plant material were made on acidified to pH 5,0–5,5 PDA plates. Before plating, segments of roots showing brown necroses were disinfected for 30 sec. in 10% sodium hypochlorite. Fungi growing out from infected root segments were transfered to PDA plates and identified according to techniques described by Hornby et al. (1998) on the basis of colony morphology, growth rate of mycelium, microscopic characteristics of fungal organs, and pathogenicity tests. A special attention was paid to the differentiation of cultures of *Gaeumannomyces* (Ggt) and *Phialophora* sp.

Patogenicity of isolated fungi to winter wheat seedlings cv. Kobra was tested in pot experiments performed in 4 replications. As resistance of *Triticum* germplasm to

Ggt has not been stated (Scott 1981), the choice of a test variety was optional. Perlite was used as a substrate. Discs f 1 cm of 7–10-old cultures of test fungi grown on PDA at the temperature 20°C were placed on the surface of perlite in pots, on each disc a pregerminated wheat seed previously disinfected for 30 min in 10% sodium hypochlorite was placed, and afterwards covered with 1.5 cm layer of perlite. Pots were watered as necessary. After incubation for 4 weeks in temperature 18–19°C the percentage of necrotic roots, yellowing leaves, height of plants and their biomass were recorded. Statistical analysis was performed at the level of probability 0.05%.

Afterwards, shoots of the plants from perlite experiment were cut off 2 cm above seminal roots, thoroughly rinsed with water and placed into test tubes while being still moist. Test tubes were plugged with cotton plugs, and incubated for 6 weeks in the cool room at the temperature 10–15°C and under fluorescent light with photoperiod 12:12. The roots were examined weekly for further development of disease symptoms, and the presence of fruiting bodies. Perithecia and ascospores of Ggt, which frequently appeared on diseased roots were subjected to microscopic examination, and their measurements were taken to confirm visual identification of the causal agent of disease symptoms.

RESULTS AND DISCUSSION

The infection of winter wheat roots in the autumn of 2000 and 2001 in the region of Upper Silesia was low and amounted to 10.4 and 10.2% of infected plants, respectively. Isolations made from those roots (Tab. 1) showed that in both years the main cause of infection were fungi of *Gaeumannomyces-Phialophora* complex. They accounted for 87.4% and 86.5% of isolations in the two successive years. However, the proportion of isolates of the complex identified as Ggt was relatively low: 16% in the first year of the study and 12% in the second year. We also isolated small numbers of *Fusarium* spp., mainly *F. oxysporum*, the species that is not pathogenic to wheat. Other species of Fusarium were rarely isolated in the autumn. *F. culmorum* and *F. nivale* were present in both years in proportion 1,5%, and in 2001 only *F. culmorum* was occasionally found.

The proportions of Ggt and *Phialophora* sp. in the spring were different from those in the autumn of previous years, especially in 2001. In April of 2001 fungi of *Gaeumannomyces-Phialophora* complex comprised 88.6% of total isolations, 52% of which were isolates of *Phialophora*. *Fusarium* spp. were present in the amount of 7.2%. In April of 2002 however, only 36.2% of isolates were identified as *Gaeumannomyces-Phialophora* complex, and dominant fungi were species of *Fusarium*. It is well known that the frequency of occurrence of pathogens on cultivated plants largely depends on weather and other enviromental conditions. For example, in 1997 in the region of Upper Silesia the epidemic occurrence of *Fusarium* spp. was recorded (Głazek et al. 1998), which was not the case in subsequent years.

As mentioned above, the occurrence of winter wheat seedlings with infected roots in the autumn of both years was rare. Numbers of plants with diseased roots were usually similar in the following spring, and that was not clearly related to the occurrence of Ggt. Although this may change in time, as indicated by Green and

Ivins (1984) who recorded late root infections 3 weeks prior to anthesis in winter wheat crop where no take-all was detected in March or early April.

Numerous authors observed high differentiation of infection level with Ggt in different years. This is usually explained by environmental conditions related to the weather and cultural practices (Cambell and Benson 1994; Asher and Shipton 1981; Cook et al. 1972; Hornby et al. 1998; Korbas et al. 2000; Slope et al. 1979).

Isolates of *Gaeumannomyces-Phialophora* complex were identified using traditional methods, which are still routinely used (Hornby et al. 1998).

On PDA most isolates of Ggt formed dark-grey and mostly flat colonies. Variation in culture colour was also observed, as described in literature (Hornby et al. 1998).

Colonies of *Phialophora* sp. grew faster than those of Ggt and usually formed abundant aerial mycelium reaching after 10 days Petri plate lid. In culture, tips of leading hyphae on colony edge of both species curled back in a characteristic manner. Both species also formed dark, nearly round swollen cells (ϕ18–25 mm), but only isolates of *Phialophora* sp. produced sclerotium-like, hirsute structures (ϕ200–225 mm), as described by other authors, and in Poland by Martyniuk (1986). Ggt seldom formed perithecia on PDA, but these fruiting bodies were frequently observed on roots of winter wheat seedlings in laboratory conditions, as was also reported by Amein (1998), Holden and Hornby (1981), Hornby et al. (1998) and Martyniuk (1986). Perithecia were round to oval, with cylindrical and usually curved neck (ϕ300–500 mm), containing asci and ascospores similar to those described by other authors.

Phialophora sp. did not form perithecia, and lobed hypopodia were not observed.

Daecon (1976; 1981) divided fungi from *Gaeumannomyces-Phialophora* complex into two groups. Members of the first group were highly pathogenic to wheat roots and were identified as *Gaeumannomyces graminis var. tritici* (Ggt), while members of the second group were weakly pathogenic and were recognized as isolates of *Phialophora*. We used the same classification on the basis of pathogenicity tests (Tab. 2). In laboratory tests isolates of Ggt strongly infected roots of winter wheat seedlings in 2000, mean infection of root system reached the value of 41.6%. 61.1% of leaves became chlorotic, height of seedlings and their biomass was reduced by 22.4 and 63.0%. Isolates obtained in the autumn of 2001 were less pathogenic. However, 10.9% of root system became infected, 9.0% of seedling leaves showed symptoms of chlorosis, height of seedlings and biomass was considerably reduced. Despite of differences between results obtained in 2000 and 2001 in both years root infection was statistically significant, as compared to control seedlings and seedlings infected by isolates of *Phialophora*. Isolates of the later fungus did not significantly affect the growth of seedlings, neither their appearance.

The growth of isolates of both species on seedlings roots showed characteristic differences. Isolates of Ggt developed on the surface of roots thick, dark runner hyphae, which were able to penetrate corex and vascular system (Deacon 1976; Hornby et al. 1998, Martyniuk 1986), this resulted in distinct disease symptoms. Isolates of *Phialophora* also developed thick, dark runner hyphae on root surface, but

Table 1. Percentage of different species of fungi isolated from infected roots of seedlings of winter wheat

Year	*G. graminis-Phialophora*		*F. oxysporum*		*F. culmorum*		*F. nivale*		*Epicoccum* spp.		*Trichoderma* spp.		Others	
	No. isolates	%	No. isolates	%	No. isolates	%	No. isolates	%	No. isolates	%	No. isolates	%	No. isolates	%
2000	118	87.4	5	3.7	2	1.5	2	1.5	3	2.2	-	-	5	3.7
2001	96	86.5	3	2.7	1	0.9	-	-	2	1.8	6	5.4	3	5.4

Table 2. Results of pathogenicity tests of fungi from *Gaeumannomyces-Phialophora complex*

Object	% of infected roots	% of yellowed leaves	Mean height of plants [cm]	Height of plants as related to control [%]	Weight of 10 plants [g]	Biomass of plants as related to control [%]
			Autumn 2000			
Untreated	0.0a	0.0a	4.9 a	100	4.6a	100
Phialophora spp.	2.4a	2.4a	4.9a	100	4.3a	93.48
G. graminis	41.6c	61.1b	3.8b	77.55	1.7b	36.96
LSD (0.05)	2.45	2.55	0.25		0.42	
			Autumn 2001			
Untreated	0.0a	0.0a	4.2a	100	5.1a	100
Phialophora spp.	0.8a	0.2a	4.3a	102.38	4.8a	94.12
G. graminis	10.9b	9.0b	4.0a	95.25	3.7b	72.55
LSD (0.05)	1.05	0.82	0.45		0.35	

they only penetrated epidermis and formed subepidermal vesicles, which could be usually seen on the main root.

Rare occurrence of take-all on winter wheat seedling roots in the autumn of the years 2000 and 2001 may be attributed to the abundant root colonisation by *Phialophora* sp. These fungi have been shown to suppress the onset of take-all disease since the 1960's and were regarded as competitors of Ggt (Hornby et al. 1998). Since then much work have been done on possibilities of using *Phialophora* sp. as biological control agents (Deacon and Henry 1981; Gutteridge and Slope 1978; Hornby et al. 1998; Hornby et al. 1990). Although the results usually confirmed suppressive abilities of *Phialophora* sp. against Ggt, and in particular of *Phialophora* sp. (lobed hyphopodia) the problem of their practical application still remains unsolved. In experiments conducted in Poland, Martyniuk and Myśków (1984) found, that the control of take-all with *Phialophora* sp. in the field was only partial.

On the basis of our results it can be concluded that the relationship between the occurrence and severity of take-all and the colonisation of roots by *Phialophora* sp. can be regarded as highly probable, as it was frequently suggested in scientific literature.

CONCUSIONS

1. *Gaeumannomyces graminis var. tritici* occurred in *Gaeumannomyces-Phialophora* complex, the isolates of the later fungus were present on roots in a considerable majority. This suggests that their presence may have contributed to a relatively low degree of infection of wheat seedling roots by Ggt.
2. Isolations made from diseased roots in the following spring suggested that the frequency of occurrence on Ggt on diseased roots in the autumn may not always be related to the frequency recorded in later stages of plant growth.
3. A common, although not frequent occurrence of *Gaeumannomyces graminis var. tritici* on winter wheat roots in the autumn, as stated in 2000 and 2001, creates a potential danger to winter wheat crop, especially under favourable conditions for the disease development.

REFERENCES

Amein T.A.M. 1988. Etiologia i ekologia zgorzeli podstawy źdźbła pszenicy. Praca doktorska, AR Poznań.

Asher M.J.C., Shipton P.J. 1981. Biology and control of take-all. Academic Press. London: 54–60.

Cambell C. L., Benson D. M. 1994. Epidemiology and Management of Root Diseases. Sprin Varley: 181–184.

Cook R. J., Papendrick R.W., Griffin D.M. 1972. Growth of two root-rot fungi as affected by osmotic and matric water potential. Proceedings of the Soil Science Society of America 36: 78–82.

Daecon J.W. 1976. Biology of the *Gaeumannomyces graminis* /Arx and Olivier/ – *Phialophora radicicola* Cain complex on roots of the Gramineae. EPPO Bull. 6: 349–363.

Daecon J.W. 1981. Ecological relationship with other fungi: competitors and hyperparasites. In Biology and control of take-all. Academic Press, London: 75–101.

Daecon J.W., Henry C.M. 1981. Death of the root corex of winter wheat in field conditions: effects of break crops and possible implications for the take all fungus and its biological control agent, *Phialophora radicicola var. graminicola*. Journal of Agricultural Research. Cambridge 96: 579–585.

Głazek M., Sikora H., Krzyzińska B., Mączyńska A. 1998. Healthiness of winter wheat seeds from flooded areas in the region of Upper Silesia. J. Plant Potection Res., 38 (2): 142–148.

Green C.F., Ivins J.D. 1984. Late infestations of take-all (*Gaeumannomyces graminis var. tritici*) on winter wheat (*Triticum aestivum cv. Virtutue*): yield, yield components and photosysthenic potential. Field Crops Research 8: 199–206.

Gutteridge R.J., Slope D.B. 1978. Effect of inoculating soils with *Phialophora radicicola var. graminicola* on take-all disease of wheat. Plant Pathology 27: 131–135.

Holden M., Hornby D,. 1981. Methods of producing perithecia of *Gaeumannomyces graminis* and their application to related fungi. Trans. Br. Mycol. Soc., 77:107–118.

Hornby D., Bateman G.L., Gutteridge R. J., Lucas P., Osburn A. E., Ward E., Yarham D.J. 1998. Take-all disease of cereals. CAB International: 212–232

Hornby D. Gutteridge R., Parsonage R. 1990. *Phialophora* spp. in relation to take-all disease of cereals. Institute of Arable Crop Research Report for 1990: 73–74

Huber D. M., McCay-Buis T.S. 1993. A multiple component analysis of the take-all disease of cereals. Plant Disease 77, 5: 437–447.

Korbas M., Ławecki T., Kubiak K., Różalski K. 2000. Porażenie korzeni pszenicy przez *Gaeumannomyces graminis* w zachodniej Polsce. Prog. Plant Protection/Post. Ochr. Roślin 40 (1): 201–205.

Korbas M., Martyniuk S., Rozbicki J., Beale R. 2001. Pszenica po pszenicy. Zgorzel podstawy źdźbła oraz inne choroby podsuszkowe zbóż. Fundacja Rozwój SGGW: 5–9.

Łacicowa B., Wagner A., Kiecana I. 1985. Fuzariozy pszenicy uprawianej na Lubelszczyźnie. Rocz. Nauk Roln. – Seria E – Ochrona Roślin 15 (1/2): 67–86.

Martyniuk S. 1986. Ekologia i właściwości fitopatogena korzeni zbóż *Gaeumannomyces graminis (Sacc.)* Arx i Olivier i grzybów pokrewnych z rodzaju *Phialophora*. Praca doktorska. IUNG Puławy.

Martyniuk S., Myśków W. 1984. Control of take-all fungus *Phialophora* sp. (lobed hyphopodia) in microplot experiments with wheat. Zentralblatt für Mikrobiologie 139: 575–579.

Meynard J.M. 1985. Construction d'ininéraires techniques pour la conduite du blé d'hiver. Docteur-Ingeniéur Thesis. INA-PQ, Paris: 125–130.

Rocznik Statystyczny Rzeczypospolitej Polskiej. 2001. Główny Urząd Statystyczny. Rok LXI. Warszawa, 350 pp.

Scott P.R. 1981. Variation in host susceptibility. p. 219–236. In: "Biology and control of take-all" (MJC Asher, PJ Shipton, eds). Academic Press. London.

Slope D.B., Prew R.D., Gutteridge R.J., Etheridge J. 1979. Take-all, *Gaeumannomyces graminis var. tritici* and yield of wheat grown after ley and arable rotations in relation to the occurrence of *Phialophora radicicola var. graminicola*. Journal of Agricultural Science, Cambridge 93: 377–389.

POLISH SUMMARY

WYSTĘPOWANIE I PATOGENICZNOŚĆ GRZYBÓW Z KOMPLEKSU *GAEUMANNOMYCES-PHIALOPHORA* WYIZOLOWANYCH Z SIEWEK PSZENICY OZIMEJ

W naszym kraju udział zbóż w strukturze zasiewów jest duży i w wielu rejonach sięga 70%. Powszechnie obserwowane w praktyce rolniczej rozszerzanie uprawy pszenicy ozimej zwiększa ryzyko porażenia roślin przez choroby grzybowe.

W badaniach prowadzonych jesienią, oceniano procent zainfekowanych korzeni przez choroby grzybowe. Porażenie to w obydwóch latach 2000 i 2001 było niskie i wynosiło nieco ponad 10% roślin z objawami chorobowymi. Głównymi sprawcami brunatnienia korzeni okazały się grzyby z grupy *G. graminis-Phialophora*: ponad 85% wyizolowanych kultur należało do tej grupy grzybów. Procentowy udział grzybów z rodzaju *Fusarium* nie przekroczył 7%. W dalszym etapie badań na podstawie tempa wzrostu i morfologii koloni przeprowadzono identyfikację wyizolowanych kultur *G. graminis* i *Phialophora*. Procentowy udział izolatów grzyba *G. graminis* w grupie *G. graminis-Phialophora* był stosunkowo niski i wyniósł 16% w roku 2000 i 12% w roku 2001. Sprawdzano chorobotwórczość wyizolowanych kultur *G. graminis* w stosunku do siewek pszenicy. Oznaczono: stopień zmian nekrotycznych na korzeniach i pochwach liściowych, procent zżółkniętych liści, wzrost roślin i ich biomasę. Testy patogeniczności wykazały, że istotne znaczenie w ograniczeniu zdrowotności siewek miały jedynie izolaty *G. graminis* występujące w kompleksie *Gaeumannomyces-Phialophora*.