

**Research on the development of *Mycosphaerella graminicola*
(Fuckel) Schroeter teleomorph on wheat leaves from Poland
and Czech Republic**

**EWA MIRZWA-MRÓZ¹, LUDVIK TVARŮ•EK²,
CZESŁAW ZAMORSKI¹, BOGDAN NOWICKI¹**

¹Warsaw Agricultural University, Department of Plant Pathology, Nowoursynowska 159,
02 776 Warszawa, Poland

²Agricultural Research Institute Kroměříž• Ltd., Department of Integrated Plant Protection,
Havlíčková 2787, 767 01 Kroměříž•, Czech Republic

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Summary

The aim of the work was to study the development of pseudothecia of *M. graminicola*. The experiment was carried out in 2002/2003. Wheat leaves collected at Kończewice (near Toruń), Słupia Wielka (near Poznań) and Kroměříž• (Czech Republic) were kept on an experimental field in Warsaw. The pseudothecia were observed under the light and transmission electron microscopy.

The first pseudothecia were observed on the dead leaves at the end of July. The largest number of fruiting bodies were noted in October.

During the whole period of observation fruiting bodies contained asci in several stages of development. Most of them were immature. In spring the pseudothecia were not found.

Key words: *Mycosphaerella graminicola*, pseudothecia, teleomorph

INTRODUCTION

Septoria tritici blotch caused by *Mycosphaerella graminicola* (Fuckel) Schroeter (anamorph: *Septoria tritici* Rob. ex Desm.) is one of the main wheat leaf necrotic spot diseases. The pathogen infects mainly *Triticum aestivum* L. and *Triticum durum* L. and occasionally *X Triticosecale* Witt., *Secale cereale* L. and some grass species (Weber, 1922; Williams and Jones, 1973; Brokenshire, 1975; Zamorski and Schollenberger, 1995; Zamorski et al., 1996b).

Under favourable conditions the yield losses in wheat culture range from 30 up to 50% (Kema, 1996). The intensity of the disease depends on the cultivar susceptibility, the availability of inoculum, crop management and predisposing environmental

conditions (Simon and Cordo, 1998). Heavily infected leaves die prematurely (Eyal et al., 1987; Zamorski and Nowicki, 1997).

Until the end of 70th *M. graminicola* and *Septoria tritici* were considered to be the separate species. As late as in 1972 Sanderson connected the perfect stage *M. graminicola* with imperfect stage *S. tritici* (Sanderson, 1972, 1976).

So far the sexual stage (teleomorph) of *M. graminicola* has been identified in many countries of the world, namely Australia (Brown et al., 1978), Chile (Madruga, 1986), USA (Sanderson et al., 1986), Brazil (Metha, 1986), United Kingdom (Scott et al., 1988), Germany (Verreet, 1995), Netherlands (Kema, 1996), France (Halama, 1996) and Poland (Zamorski et al., 1996a).

M. graminicola teleomorph develops on straw debris and stubble (mainly on leaf sheaths) in the form of dark-brown pseudothecia (Brown et al., 1978; Kema, 1996; Sanderson and Hampton, 1978; Scott et al., 1988). On the same leaf besides the pseudothecia of *M. graminicola* the pseudothecia of *Phaeosphaeria* sp. and *Didymella exitialis* were found very often (Sanderson, 1972; Sanderson and Hampton, 1978; Eyal et al., 1987; Halama, 1996; Zamorski et al., 1996a). Because the ascospores can be carried by the wind for many kilometres (Sanderson and Hampton, 1978) it is suggested that they can be the efficient source of primary infection. (Brown et al., 1978; Sanderson and Hampton, 1978; Eyal et al., 1987; Scott et al., 1988; Verreet, 1995; Halama, 1996; Shaw, 1999). The occurrence of teleomorph in Poland was reported in 1996 (Zamorski et al., 1996a) but there is a lack of information concerning the development of this stage in our country.

MATERIALS AND METHODS

The experiment was carried out in 2002/2003. Wheat leaves collected at Kończewice (near Toruń), Słupia Wielka (near Poznań) and Kroměříž (Czech Republic) were kept on the experimental field in Warsaw. To determine seasonal occurrence of pseudothecia and the stage of their development samples of the leaves (from Kończewice and Słupia Wielka) were taken once a week in summer and autumn and twice a month in winter and spring. A hundred of pseudothecia, asci and ascospores have been measured to determine their size. The pseudothecia were investigated under the light (Olympus BX 50) and transmission electron microscope (JEOL type JEM 1220) in the Laboratory of Electron Microscopy of the Warsaw Agricultural University.

Leaves bearing fruiting bodies were cut into 5 x 2 mm pieces and fixed for 2 h in 4% paraformaldehyde and 5% glutaraldehyde (pH 7,2-7,3). Material was rinsed in 0,2 M cacodylic buffer (4 x 15 min.) and post-fixed with 2% buffered osmium tetroxide for 2 h. Materials were dehydrated in ethanol and acetone series and embedded in resin. Polymerisation lasted 24 h. at 60°C. Ultra-thin sections were cut with Diatome diamond knife by the Reichert Ultracut E Microtome, mounted on copper grids, stained for 20 min. in uranyl acetate and Reynold's citrate for 30 min. Thick sections for light microscope observations were cut by the same ultramicrotome but with glass knife and stained with methylene blue 2% and azure B 1%.

On the leaves from Kroměříž occurrence of pseudothecia and their development were observed using light microscope only.

RESULTS

The first pseudothecia with developed asci of *M. graminicola* were observed on the dead leaves at the end of July. The largest number of fruiting bodies were noted in October.

During the whole period of observation pseudothecia contained asci in several stages of development (asci with granulated cytoplasm without ascospores, asci with immature ascospores, asci with mature ascospores) were noted (Fig. 1-4). Most of asci was immature. The biggest percentage of asci in pseudothecia with mature ascospores was observed in September (average 18%) and October (average 22%) (Fig 5). The courses of air temperatures and rainfalls are represented Fig. 6.

Pseudothecia of *M. graminicola* were dark brown, globose, embedded in the host tissue with raised ostioles. Pseudothecia measured 68-99 x 70-110 μm . Bitunicate, obpyriform 30-46 μm long and 10-13 μm wide asci were formed in pseudothecia. Each ascus contained eight irregularly arranged ascospores which were two-celled, hyaline and measured 11-15 x 2,2-4,4 μm (one cell was slightly longer and broader than the other). No pseudoparaphyses were present.

The empty pseudothecia, after total ascospores release were observed as well. In spring the pseudothecia on the winter wheat leaves were not found.

The development of pseudothecia of *M. graminicola* on the leaves from the Czech Republic was similar.

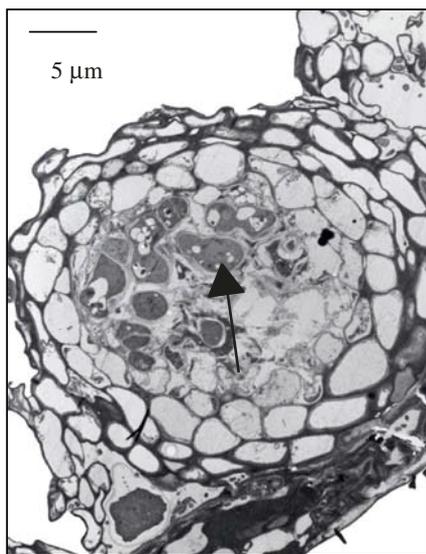


Fig. 1. The first phase of asci development

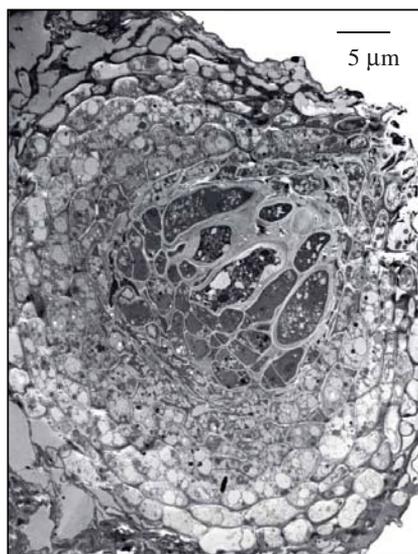


Fig. 2. Pseudothecium with immature asci with granulated cytoplasm

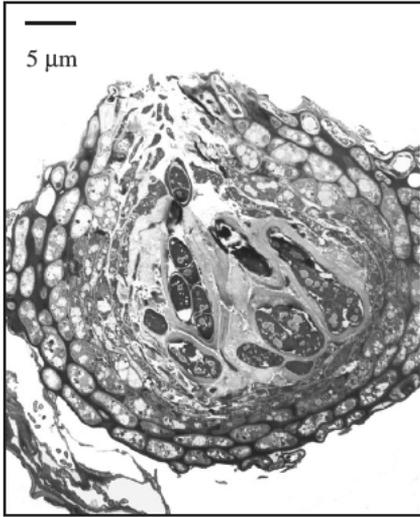


Fig. 3. Pseudothecium with asci containing single mature ascospores



Fig. 4. Asci of *M. graminicola* with mature ascospores

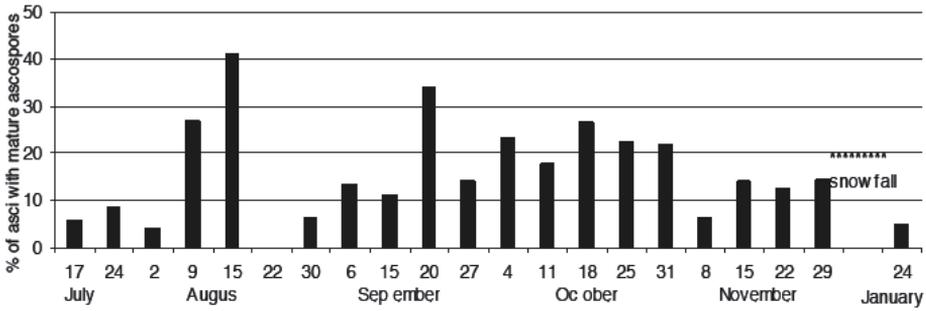


Fig. 5. Percentage of asci with mature ascospores in pseudothecia of *M. graminicola*

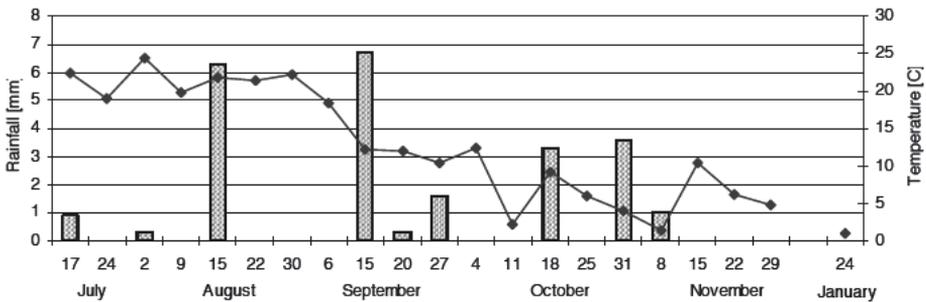


Fig. 6. Courses of air temperatures and rainfalls

DISCUSSION

In Poland like in many countries mentioned above the occurrence of pseudothecia of *M. graminicola* was observed. Our investigations have shown the presence of first pseudothecia on dead winter wheat leaves before harvest already in July. In other countries pseudothecia of this fungus appeared after harvest in autumn (Sanderson, 1972; Brown et al., 1978; Sanderson and Hampton, 1978; Scott et al., 1988; Halama 1996).

In Poland like in Australia (Brown et al., 1978) and Argentina (Cordo et al., 1999) the biggest number of pseudothecia with mature ascospores was observed in autumn (September, October), whereas in New Zealand in spring (April, May). This phenomenon can be probably explained by different weather conditions.

In France Halama (1996) observed the fruiting bodies mainly on the leaves. However in United Kingdom pseudothecia were found on leaf sheaths more readily than on leaf blades possibly because the leaf sheaths decompose later than leaves blades (Scott et al., 1988). In Poland the pseudothecia were observed on leaves like in France.

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Badania rozwoju teleomorfy grzyba *Mycosphaerella graminicola* (Fuckel) Schroeter na liściach pszenicy z Polski i Czech

Streszczenie

Celem pracy były badania nad rozwojem pseudotecjów grzyba *Mycosphaerella graminicola*. Obserwacje prowadzono przez cały sezon wegetacyjny w latach 2002/ 2003 na polu doświadczalnym w Warszawie (Ursynów, SGGW).

Materiałem roślinnym były martwe, porażone przez patogena liście pszenicy ozimej pochodzące z ZD IHAR Kończewice (woj. kujawsko-pomorskie), Stacji Hodowli Roślin w Słupi Wielkiej (woj. wielkopolskie) i z Agricultural Research Institute Kroměříž• Ltd. (Czechy). Liście były przechowywane na specjalnej konstrukcji, wykonanej z drewnianej ramy, na której umocowano gęstą, plastikową siatkę. Wykładano na nią liście z objawami septoriozy paskowanej liści pszenicy i otoczniami grzyba *M. graminicola*, następnie przykrywano drugą siatką.

Przebieg rozwoju otoczni ustalano na podstawie okresowych obserwacji makro- i mikroskopowych (raz w tygodniu latem i jesienią oraz 2 razy w miesiącu zimą i wiosną). Celem ich było określenie czasu pojawiania się kolejnych struktur grzyba, tj. ukształtowanych pseudotecjów, worków i zarodników workowych. Sporządzono dokumentację fotograficzną ilustrującą poszczególne etapy rozwoju otoczni grzyba *M. graminicola* z użyciem mikroskopu świetlnego i transmisyjnego.

Pierwsze pseudotecja grzyba obserwowano na obumarłych liściach pod koniec lipca. Największą liczbę otoczni notowano w październiku. W ciągu całego okresu obserwacji pseudotecja zawierały worki w różnych stadiach swego rozwoju. Większość z nich była niedojrzała. Wiosną pseudotecja patogena nie były już znajdowane.

