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OCCURRENCE OF EYESPOT ON WINTER WHEAT IN THE CENTRAL-SOUTHERN REGION OF POLAND

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Abstract: Eyespot is one of the most important diseases of economical significance in wheat production in the regions of Śląskie and Opolskie voivodeships where it presents a permanent problem. The aim of performed experiment was to collect data on the occurrence and characteristics of *Oculimacula yallundae* and *O. acuformis* strains in these regions in the years 2003–2008, and to perform their taxonomic characterization. The studies originated as an attempt to find the explanation of processes related to winter wheat infection by casual agents of this disease in the Śląskie and Opolskie Provinces.

Key words: eyespot, *Oculimacula yallundae, O. acuformis,* infection index, PCR, laboratory analysis

INTRODUCTION

Eyespot is one of the most important diseases of economical significance in wheat production in the regions of moderate climate (Scott and Hollins 1974). It is a commonly occurring disease in winter wheat cultures in the regions of Śląskie and Opolskie voivodeships. Its intensity may by variable, however it presents a permanent problem in these regions (Głazek *et al.* 2008; Jaczewska-Kalicka 1998; Korbas 2008). It is caused by pathogenic fungi *Oculimacula yallundae* (type W) and *O. acuformis* (type R) (Nirenberg 1980; Nicholson *et al.* 1997). Epidemiological differences between these two species are not clear and the disease development is difficult to predict (Fitt and Goulds 1988). The aim of performed experiment was to collect data on the occurrence and characteristics of *O. yallundae* and *O. acuformis* strains in the regions of Śląskie and Opolskie voivodeships in 2003–2008 and to perform their taxonomic characterization. Numerous plants with both initial and advanced symptoms of eyespot occurring on winter wheat cultivars at growth stage of late milk BBCH 73–77 were collected (Fig. 1) (Wan *et al.* 2005) The studies originated as an attempt to find the explanation of processes related to winter wheat infection by casual agents of the disease in the Śląskie and Opolskie provinces.

Fig. 1. Initial and advanced symptoms of eyespot on winter wheat at BBCH 73–75

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MATERIALS AND METHODS

Each year (2003–2008) winter wheat plants were taken for analysis from 5–8 chosen plantations. Those were experimental plantations, small area plantations and large commercial plantations as well (Table 1).

Table 1. Derivation of cultures used for mycological characterization of eyespot. A list of analyzed plantations

Year	Location	Number of plantations	Date of sampling
2003	śląskie	7	75–77 BBCH
2004	śląskie	5	75–77 BBCH
2005	śląskie	7	75–77 BBCH
	opolskie	1	75–77 BBCH
2006	śląskie	4	75–77 BBCH
	opolskie	1	75–77 BBCH
2007	śląskie	6	75–77 BBCH
	opolskie	2	75–77 BBCH
2008	śląskie	4	75–77 BBCH
	opolskie	1	75–77 BBCH

Visual analysis of 100 plants randomly taken from each plantation non – treated with fungicides was performed at 75–77 BBCH according to EPPO standards PP 1/28 (3).

From plant fragments of diseased tissues mycelium was taken in the laboratory for culturing the fungus on artificial medium (PDA) (Bojarczuk 1970). After obtaining pure cultures discs of 5 mm diameter were cut out and transferred to PDA plates. Fungus cultures showing typical for *O.yallundae* (type W) or *O.acuformis* (type R) morphology and growth rate were chosen (Scott *et al.* 1975; Creighton *et al.* 1988, 1991; Creighton 1989) for the further study.

Culturing the fungus obtained from excised culture discs was performed at uniform conditions of light and temperature of 18–19°C. At fixed time intervals measurements of linear growth of cultures were done after 5, 10 and 15 days from the transferring date. The morphology of cultures was compared with literature data (Nicholson *et al.* 1991; Nirenberg 1981; Dyer *et al.* 1994). Fungi from these cultures were grown on water agar to obtain a sporulation (Bojarczuk 1970). In the years 2002, 2003 and 2007, 2008 PCR Multiscreen Cereal Stem Base Disease Analysis was conducted to confirm visual diagnosis. For this purpose plants showing disease symptoms were chosen and send to a specialized laboratory NeoAdgen in Scotland (Smith 1990; Collet *et al*. 1992; Turner 1999). In 2008 a study based on PCR technique was also done in the Department of Mycology Institute of Plant Protection – National Research Institute in Poznań to confirm laboratory results (Turner *et al*. 1999).

RESULTS AND DISCUSSION

In the recent years, differences were observed with respect to the development and level of infection of wheat at the late milk growth stage of BBCH 75–77.

Table 2 shows examples of the level of infection in 2002–2003 and 2007–2008. The percentage of plants with disease symptoms on stems ranged from 26 to 100. Harmfulness of the disease, however, is assessed based on the rate of plants with medium and high level of infection (Scott *et al.* 1974). In order to compare the *Oculimacula* spp. infection on different plantations, infection pressure index was determined according to the EPPO standard

Table. 2. Occurrence of eyespot in winter wheat selected plantations

Sośnicowice 2002

No.	Localization/ cultivar		Date of sampling	*Oculimacula* spp.				
				% infected plants				infection index*, according to EPPO P1/28(3)
				infection severity			sum	
				slight (1)	moderate (2)	severe (3)		
1	śląskie	Mikon	73 BBCH	22	8	0	30	0,12
2	śląskie	Mikon	77 BBCH	26.7	38.9	5.5	71.1	0.41
3	śląskie	Zyta	75 BBCH	10	90	0	100	0.70

Sośnicowice 2003

No.	Localization/ cultivar		Date of sampling	*Oculimacula* spp .				
				% infected plants				infection index*, according to EPPO P1/28(3)
				infection severity			sum	
				slight (1)	moderate (2)	severe (3)		
1	śląskie	Mikon	77 BBCH	61	36	3	100	0.45
2	śląskie	Mikon	77 BBCH	91	9	0	100	0.30
3	śląskie	Kobra	77 BBCH	65	24	11	100	0.45
4	śląskie	Kobra	77 BBCH	35	13	6	54	0.25
5	śląskie	Mikon	77 BBCH	22	13	1	36	0.16

continued table 2

Sośnicowice 2007

No.	Localization/ cultivar		Date of sampling	*Oculimacula* spp.				
				% infected plants				infection index*, according to EPPO P1/28(3)
				infection severity			sum	
				slight (1)	moderate (2)	severe (3)		
1	śląskie	Turnia	75–77 BBCH	11.25	57.75	9.75	78.75	0.56
2	śląskie	Nadobna	75–77 BBCH	13.25	62	8	83.25	0.58
3	śląskie	Finezja	75–77 BBCH	19.75	13	0.25	33	0.15
4	śląskie	Zyta	75–77 BBCH	28.50	30.50	1.75	53.00	0.32
5	opolskie	Buteo	75–77 BBCH	26	52	0	78	0.46
6	opolskie	Bogatka	75–77 BBCH	28	45	5	68	0.45
7	śląskie	Rubens	75–77 BBCH	19.00	18.50	2.25	39.75	0.21

Sośnicowice 2008

No.	Localization/ cultivar		Date of sampling	*Oculimacula* spp.				
				% infected plants				infection index*, according to EPPO P1 28(3)
				infection severity			sum	
				slight (1)	moderate (2)	severe (3)		
1	śląskie	Rubens	75–77 BBCH	26.5	30.25	6.5	63.25	0.36
2	śląskie	Nadobna	75–77 BBCH	25.75	39.75	11.25	54.75	0.48
3	opolskie	Nadobna	75–77 BBCH	9	11	79	99	0.90
4	opolskie	Bogatka	75–77 BBCH	20	25	53	98	0.77
5	opolskie	Finezja	75–77 BBCH	15	33	48	96	0.77
6	śląskie	Turnia	75–77 BBCH	29.25	9.50	0.75	39.50	0.15
7	śląskie	Zyta	75–77 BBCH	25	1	0	26	0.07

*) $x = \frac{(n(II) \times 0.25) + (n(III) \times 0.75) + n(IV)}{n(I + II + III + IV)}$

x – infection index
(I) – non-infected
(II) – slight
(III) – moderate
(IV) – severe
n – number of assessed plants

Table 3. Rate of linear growth of *O. yallundae* and *O. acuformis* cultures – means for 2003–2008

Year	Average growth of type W [mm]		
	5 days	10 days	15 days
2003	10.19	29.56	43.22
2004	12.00	26.81	46.91
2005	19.23	39.73	51.18
2006	12.03	24.15	35.73
2007	11.42	21.87	31.01
2008	9.71	22.32	32.99
Means for 2003–2008	12.43	27.41	40.17
Year	average growth of type R [mm]		
	5 days	10 days	15 days
2003	8.69	15.24	22.08
2004	7.41	14.06	22.06
2005	8.54	13.46	20.66
2006	7.75	12.38	23.25
2007	8.57	10.61	13.60
2008	6.75	10.68	13.27
Means for 2003–2008	7.95	12.74	19.15

P1/28 (3) (Table 2). The lowest infection index of 0.07 was found for Zyta cultivar grown in the Silesian Province and the highest of 0.90 for Nadobna in Opolskie in 2008. The average calculated infection pressure index was 0.41. It indicates that the pathogen regularly infects about half of wheat plants to the extent that significantly weakens their condition.

The most important element of performed research was to identify fungal species of causal disease agents, as it could be of significance in the infection process, and in a delayed appearance of disease symptoms.

In each year of investigation from chosen plantations of winter wheat on which the occurrence of eyespot was observed, plants for laboratory analysis were taken. From fragments of diseased tissues isolations were made, and from obtained cultures were chosen those exhibiting typical characteristics for *O. yallundae* or *O. acuformis*. Measurements of linear growth were made. In case of *O. yallundae* after 15 days it amounted to 31–51 mm, (type W) and for *O. acuformis* (type R) it was 13–22 mm (Table 3).

The difference in linear growth means for 6 years for *O. yallundae* and *O. acuformis* was 20 mm (Fig. 2). In each year much faster growth of *O. yallundae* was evident. On figure 3 a, b are shown 15 days old cultures of types W and R. The next figure 4a–e shows differences in morphology of cultures of W and R types evident after about 4 weeks from transferring. Cultures of *O. yallundae* were characterized by darker colour and even colony margins. Cultures of *O. acuformis* are lighter, margins were uneven and latter they might be "fethery". (Fig. 4f). Cultures of both strains had a button – like protrusion in their central part which is confirmed by other authors (Bojarczuk 1970; Nirenberg 1981; Bateman *et al.* 1990).

To confirm the correct classification of obtained fungi to the species further methods were applied. Among others, sporulation was observed on water agar and it was stated that sporulations of *O. yallundae* was more abundant, spores were shorter and slightly curved, and *O. acuformis* had longer spores and sometimes slightly curved at the end (Nirenberg 1981; Korbas 2008; Bojarczuk 1970) (Fig. 5a, b). In 2003–2008 it was stated that mean length of *O. yallundae* spores was 61,2 micrometers, and for *O. acuformis* it was 70 micrometers (Table 4). According to Nirenberg (1981) length of conidia of *yallundae* was from 35 to 80 micrometers and for *acuformis* 43–120 micrometers.

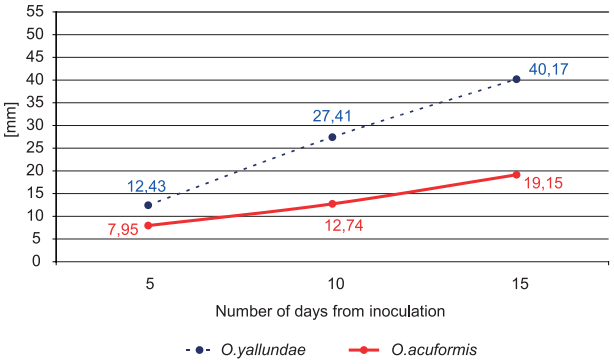
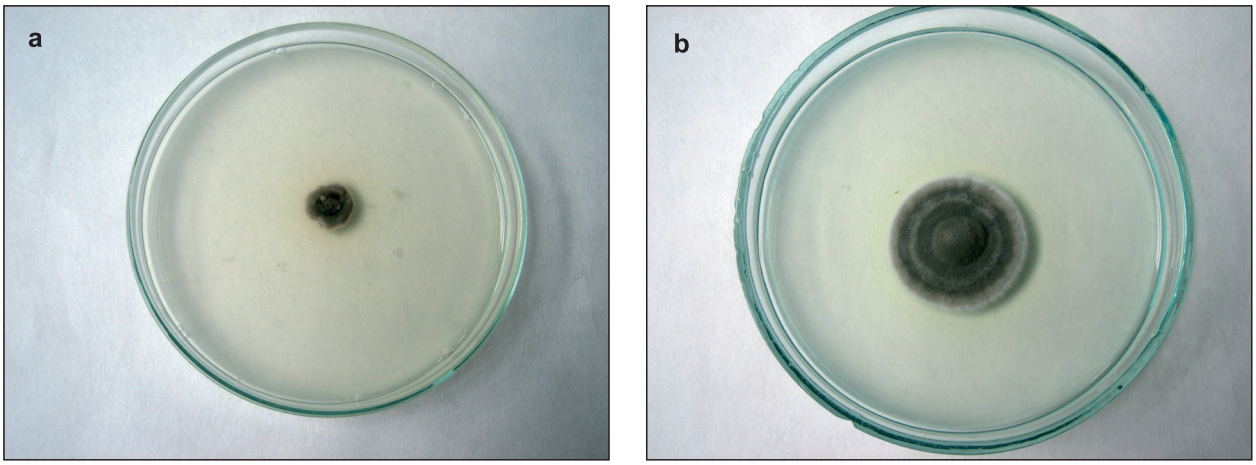


Fig. 2. Growth rate of *O. yallundae* and *O. acuformis* cultures on PDA medium. Average of 2003–2008

Table 4. Mean length of W and R types conidia from studied cultures of *O. yallundae* and *O. acuformis*

Year	Mean length of conidia [µm]	
	Type W *O. yallundae*	Type R *O. acuformis*
2003	57.2	64.1
2004	60.3	38.3
2005	59.0	85.5
2006	86.6	98.6
2007	53.9	60.4
2008	56.0	71.1
mean	61.2	70.0



O. acuformis ***O. yallundae***

Fig. 3. 15-days old cultures: a – *O. acuformis*, b – *O. yallundae*

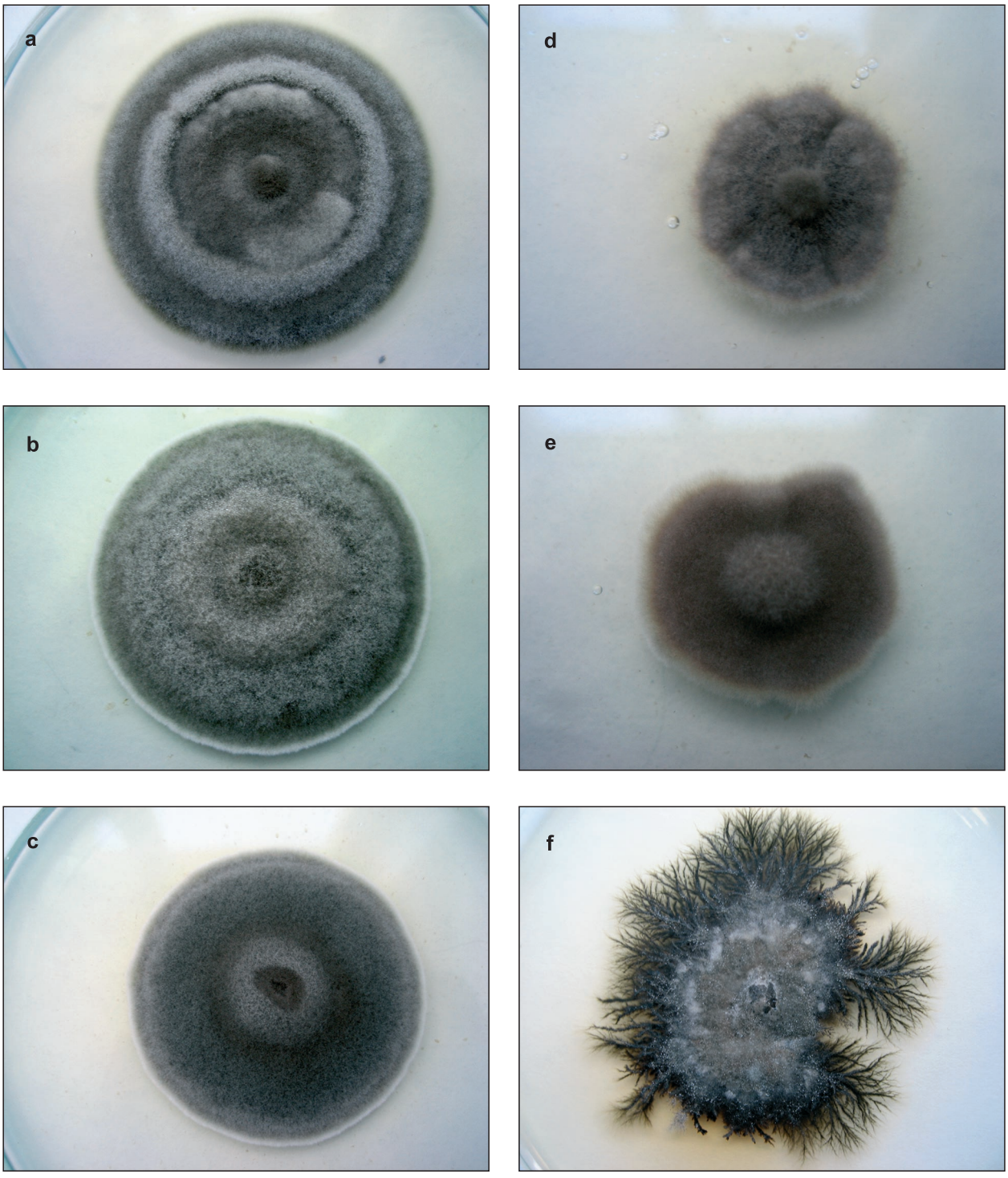


Fig. 4. Morphological differences of *Oculimacula* cultures 4 weeks after inoculation:
a–c *O. yallundae*, d–f *O. acuformis*

Further studies were performed using PCR technique. The use of specific for W or R types primers enabled the confirmation laboratory studies and their correct classification (Korbas 2008; Nicholson 1997). On figure 6 spectrum showing the results of PCR analysis with specific primers for *O. yallundae* and *O. acuformis* is presented.

A highly significant element for elucidation of differentiated wheat infection at the milky ripening stage BBCH 71–77 were PCR studies in which the participation of types W and R were determined. At this stage the species *O. acuformis* was clearly prevalent and comprised 70–100% as compared to *O. yallundae* occurring in 0–30% (Fig. 7). In investigated plants from 26 plantations during 6 years on 24 plantations type R was present. Type W was found on 4 plantations (Table 5).

Laboratory studies and PCR tests confirmed the participation of two species of *Oculimacula* in the infection process of winter wheat in the discussed regions.

The prevailing presence of *O. acuformis* can be explained by a late appearance of disease symptoms, as this

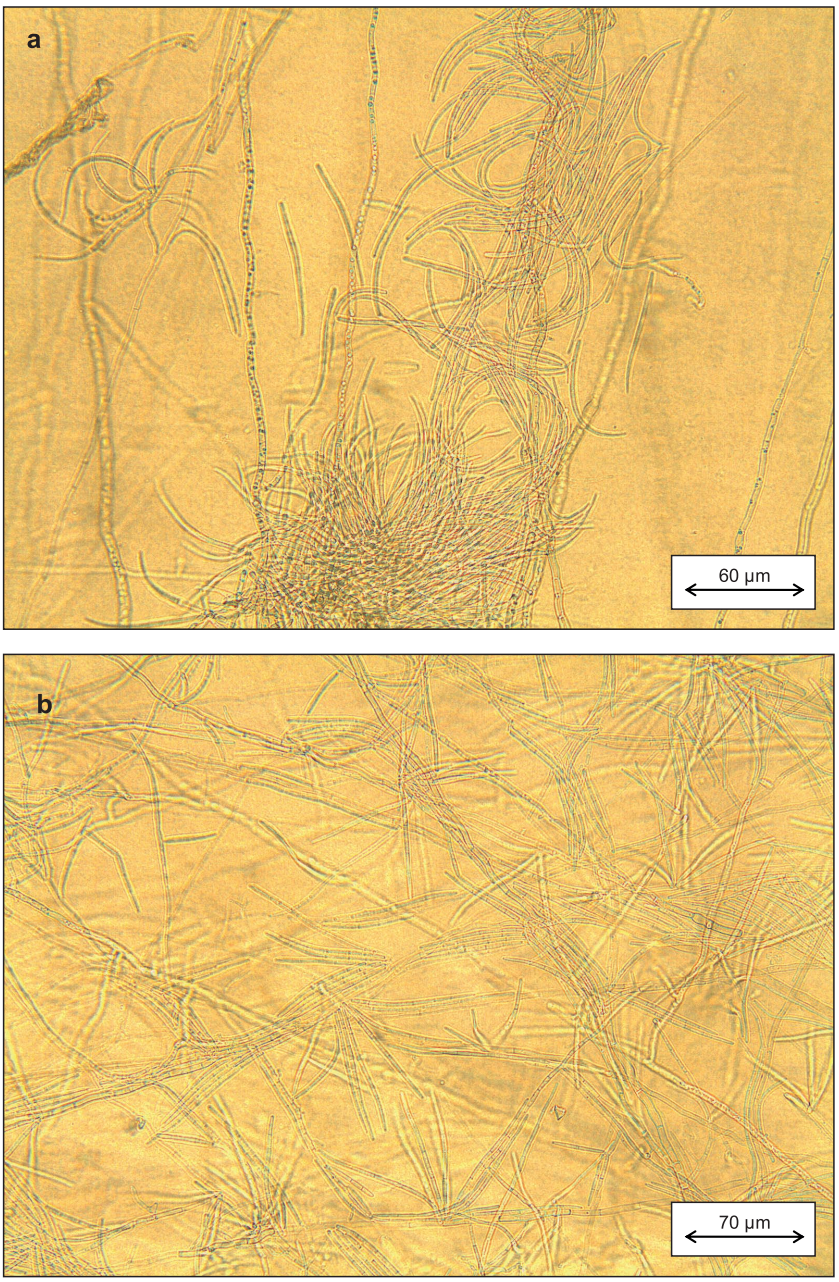
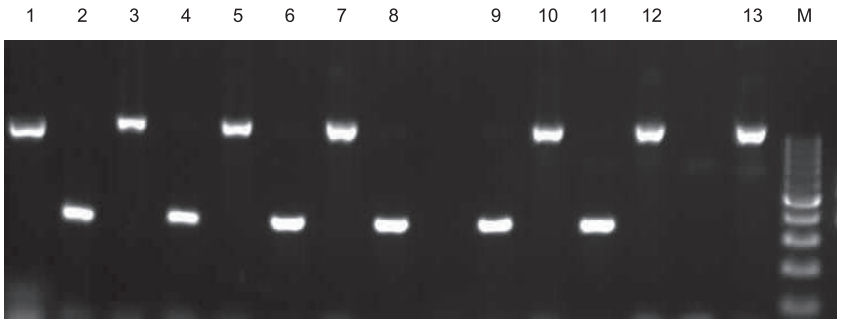


Fig. 5. Conidiospores of *O. yallundae* (a), *O. acuformis* (b)



M – ladder size marker, 1, 3, 5, 7, 10, 12, 13 – isolates of *O. yallunda* and 2, 4, 6, 8, 9, 11 – isolates of *O. acuformis*

Fig. 6. Spectrum showing the results of PCR analysis of *O.* cultures with specific primers for *O. yallundae* and *O. acuformis*

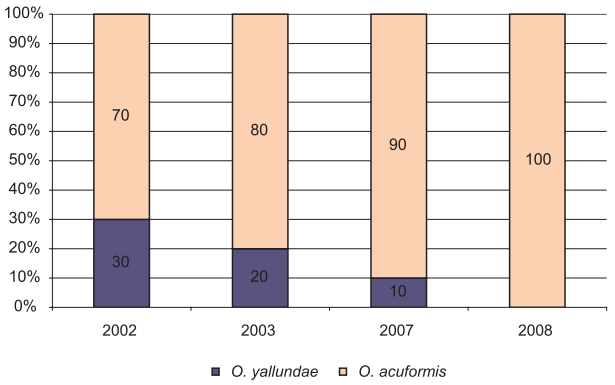


Fig. 7. Per cent share of *O. yallundae* and *O. acuformis* in fungal population of *Oculimacula* spp. in 2002, 2003 and 2007, 2008

Table 5. Results of PCR Multiscreen analysis of winter wheat plants from 22 different plantations

Year	Location	Type*		Development stage
		W *O. yallundae*	R *O. acuformis*	
2002	śląskie	0	2	71 BBCH
		0	5	77 BBCH
		5	5	75 BBCH
2003	śląskie	0	3	77 BBCH
		0	1	77 BBCH
		0	2	77 BBCH
		0	0	77 BBCH
		0	0	77 BBCH
2007	śląskie	0	5	75–77 BBCH
		0	5	75–77 BBCH
		0	2	75–77 BBCH
		0	4	75–77 BBCH
		0	2	75–77 BBCH
	opolskie	3	5	75–77 BBCH
		5	5	75–77 BBCH
2008	śląskie	0	1	75–77 BBCH
		2	1	75–77 BBCH
		0	1	75–77 BBCH
	opolskie	0	5	75–77 BBCH
		2	5	75–77 BBCH
	śląskie	0	0	75–77 BBCH
	opolskie	0	5	75–77 BBCH

* 0÷5 scale: 0 – disease not detected; 1–5 – increasing level of occurrence

species has a longer period of latent infection and develops more slowly than the species *O. yallundae* (Ray *et al*. 2004).

A prevalence of *O. acuformis* and restricted occurrence *O. yallundae* may be related to the common use of carbendazim for controlling eyespot (Bateman 2002; Bierman *et al*. 2002; Birchmore *et al*. 1992; Blein *et al*. 2009; Dayer *et al*. 1995; Głazek *et al*. 1993; Korbas *et al*. 1999; Smith *et al*. 1994).

Stating a regular occurrence in 2003–2008 of *O. acuformis* in the studied regions also permits to elucidate the problem of late appearance of disease symptoms at the beginning of ripening when the intensity of disease symptoms by this species considerably increases and endangers yielding of winter wheat.

Results of the presented above research point at the need of their continuation because this information presents a valuable indications for agricultural practice, permitting a proper application of wheat protection against eyespot.

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POLISH SUMMARY

WYSTĘPOWANIE ŁAMLIWOŚCI ŹDŹBŁA NA PSZENICY OZIMEJ W REJONIE POLSKI POŁUDNIOWO-ŚRODKOWEJ

Celem pracy było zebranie danych o występowaniu i cechach gatunków *Oculimacula yallundae* i *O. acuformis* w rejonie Śląska i województwa opolskiego w latach 2003–2008 oraz wykonanie charakterystyki taksonomicznej. Badania podjęto w celu wyjaśnienia równoczesnego występowania początkowych i zaawansowanych objawów łamliwości źdźbła w fazach dojrzewania ziarniaków pszenicy, BBCH 73–77. Miały one także na celu wyjaśnienie zagadnień związanych z procesem infekcji pszenicy ozimej przez patogeny powodujące łamliwość źdźbła w omawianym rejonie. Corocznie na 5–8 plantacjach prowadzono ocenę wzrokową porażenia, a wyliczony średni wskaźnik porażenia wynosił 0,41. Świadczy to o występowaniu choroby, gdy średnio połowa roślin jest zainfekowana w stopniu znacząco obniżającym ich kondycję. Pobierano porażone rośliny do badań laboratoryjnych. Izolowano patogeny. Uzyskiwano kultury wykazujące cechy morfologiczne charakterystyczne dla badanych gatunków. Pomiary wzrostu liniowego kultur badanych grzybów wykazały szybszy rozwój *O. yallundae*. Na wodnym agarze obserwowano ich zarodnikowanie i stwierdzono, że średnia długość zarodników *O. yallundae* wynosiła 61,2 μm, a *O. acuformis* 70 μm.

Analizy PCR kultur grzybów potwierdziły ich przynależność gatunkową. Analiza roślin przy użyciu metody PCR Multiscreen Stem Base Analysis wykazała, że na 19 plantacjach występował gatunek *O. acuformis*, a na pięciu – *O. yallundae*. Gatunek *O. acuformis* był w wyraźnej przewadze stanowiąc 70–100% w stosunku do *O. yallundae*, który występował w 0–30%. Stwierdzone w latach 2003–2008 regularne występowanie *O. acuformis* w badanym rejonie pozwala na wyjaśnienie zagadnienia późno pojawiających się symptomów chorobowych, a także daje wskazówki dla praktyki rolniczej, pozwalające na właściwy dobór fungicydów dla ochrony pszenicy ozimej.