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THE INFLUENCE OF DIRECT SOWING IN MONOCULTURE OF WINTER WHEAT ON COLONIZATION OF STEM BASES BY *FUSARIUM* SPP.

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Abstract

Effects of different cultivation systems and different doses of a nitrogen fertilizer on stem-base disease of winter wheat grown in monoculture were studied in three successive growing seasons 1998–2001. More stem-base disease occurred in wheat that was direct-sown and undersown with white clover than in wheat that was either direct-sown without undersowing or was cultivated traditionally by ploughing. Application of nitrogen increased disease, which was most severe at the higher dose (120 kg/ha). *Fusarium* species, particularly *F. avenaceum*, were most commonly associated with stem-base disease.

Key words: direct sowing, fertilizer, fungi, stem-base disease, undersown clover, wheat

Introduction

The high cost of ploughing means that other technologies for winter wheat cultivation which, at the same time, would guarantee high and stable crop yields while not restricting production, need to be found (Kuś and Jończyk 1997). An extreme simplification is direct sowing, where seeds are sown without prior tillage of the soil. The great advantage of this is that large areas can be sown in a short period of time with maximum economy of labour and energy costs, and avoidance of erosion by water and wind, which can be considerable with traditional cultivation (Radecki and Opic 1991, Weber et al. 1999).

Direct sowing may, however, increase infection of wheat by pathogens, including those that infect through the root system. This infection arises from crop debris left in the field after the previous harvest, which is a direct source of inoculum

Phytopathol. Pol. 43: 25–36
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ISSN 1230-0462

for the new crop. In Lower Silesia, fungi of the genus *Fusarium* have recently been a particular problem for wheat grown in monoculture. The warm climate of this region provides very favourable conditions for their growth. Undersowing with plants of *Fabaceae*, e.g. white clover (*Trifolium repens*), is one of methods used to increase the health of winter wheat grown in cereal rotations. It is a low cost method of cultivation that requires, however, the application of increased doses of the mineral fertilizers before growing the main crop.

The objective of these studies was to evaluate the effects of different methods of cultivation and different nitrogen fertilization on the degree of infection of winter wheat by pathogens responsible for root and stem-base disease. Direct sowing, with and without undersown white clover, was compared with traditional cultivation.

Materials and methods

Studies on the health of winter wheat (*Triticum aestivum*), cultivar 'Kobra', grown in monoculture were carried out at the Agriculture Experimental Station in Pawłowice in the vicinity of Wrocław (15°6'0"N, 17°2'0"E). Field experiments were established in 30 m^2 plots arranged in four randomized replicate blocks, in the successive cropping seasons 1998/99, 1999/2000 and 2000/01, on lessivê soil developed from silty clay. The same treatments were applied to the same plots in each year.

Three methods of cultivation were applied: 1) direct sowing of wheat, which was preceded only by spraying with herbicide (glyphosate, as Roundup 360 SL, 6 l/ha) after harvest of the previous wheat crop; 2) direct sowing of wheat, undersown with white clover, preceded by spraying with herbicide (glyphosate, as Roundup 360 SL, 6 l/ha) after harvest of the previous wheat crop, followed by the harvest of white clover; 3) traditional cultivation, which included a first ploughing followed by ploughing with harrowing, and seed-bed preparation by cultivator and roller before sowing the wheat.

Two methods of nitrogen fertilization were compared: 60 kg/ha in one dose (in March) or 120 kg/ha in two doses (in March and May). Nitrogen was applied in the form of ammonium applied in spring to all three methods of cultivation. No nitrogen fertilizer was applied to control plots.

The winter wheat was sampled at milk- and dough-ripe growth stages (GS 75-83) (Zadoks et al. 1974) for laboratory examination. 70 randomly chosen stems were collected from each plot. Stems were grouped into four categories: healthy stems, stems with stains (spot lesions), stems with stripes (elongated lesions), stems with blackened bases. The extent of necrotic symptoms on the stem base was scored using a 0–5 scale: 0 – healthy plants, 1 – stains, stripes and blackening on 1–5% of the stem-base area, 2 – 6–10%, 3 – 11–25%, 4 – 26–75%, 5 – lesions covering the entire stem base. A disease index was calculated for each replicate using the formula:

$$Wp = \frac{\sum(P \times W)}{n}$$

where Σ ($P \times W$) is a sum of the product of the number of plants in each score category (P) and the score value (W) and n is the number of plants assessed. The disease index combines all types of symptom, since they were dominated by those characteristic of *Fusarium* species (about 83% consisted of stripes). Conclusions are based on analysis of variance.

10 stems collected from each plot (six with stripes, three with stains, and one with blackened base) were used for mycological analysis. A piece 5 cm long was cut from each stem base and surface sterilized for 1 min in sodium hypochlorite (0.5% available chlorine). After removing of ends, each piece was cut into 5 mm pieces, which were placed on cereals medium (Rashid and Schlösser 1977). There were six pieces per Petri dish and 70 pieces per plot. After incubation for 7–14 days at 25°C, the plates were examined microscopically. Subcultures on potato dextrose agar (PDA) slants were made for preservation of cultures. Sporulating fungi were identified on the basis of their morphology according to the available literature.

Results

The weather patterns in the growing seasons of 1998/99, 1999/2000 and 2000/01 was compared (Table 1). In the years of the first two experiments, the March–July temperature was about 2°C higher than the long-term (40-year) aver-

Table 1

Weather conditions for the Agriculture Experimental Station Pawłowice

Factor	Mean	Months									
		X	XI	XII	I	II	III	IV	V	VI	VII
Temperature	for months										
	1998/99	8.5	0.0	–0.4	1.3	–0.1	5.8	10.3	15.5	17.9	20.6
	1999/2000	9.4	3.1	1.2	–0.4	4.6	4.8	11.9	16.6	18.6	16.9
	2000/01	12.7	6.5	2.4	0.4	1.2	3.8	8.5	14.0	14.2	18.8
	for years										
	1961–2000	9.0	3.8	0.1	–1.5	–0.2	3.4	8.3	13.6	16.8	18.3
Rainfall	for months										
	1998/99	82.4	24.4	31.5	15.5	31.1	53.2	45.9	21.6	53.7	91.7
	1999/2000	25.7	35.3	18.5	49.2	29.5	110.3	7.8	64.2	23.5	130.4
	2000/01	12.9	42.7	23.5	31.8	18.3	44.9	31.2	25.7	68.0	158.8
	for years										
	1961–2000	38.0	39.0	36.1	29.5	26.6	32.5	34.8	57.4	65.8	74.8

age, and in the third year had the warmest autumn-winter season, with the October–February temperature 1.4–3.7°C higher than the long-term average. February 2000 was particularly warm with the temperature 4.8°C higher than the average in 1961–2000. In the last year, the March–July temperature was similar to the long-term average, except for the cold June.

The rainfall distribution was very uneven in all three years of experiment (Table 1). In the first and the third year the total rainfall in October–July was similar to the long-term average, while in the second year the rainfall was 60 mm more than the average in 1961–2000. There was low rainfall in the autumn-winter 1998/99 and

Table 2

Disease index (0–5 scale) on stem bases of winter wheat grown in different cultivation systems and with different amounts of nitrogen fertilizer

Combination		Years			
cultivation system	nitrogen fertilization (kg/ha)	1998/99	1999/2000	2000/01	mean
Traditional cultivation	0	1.84	3.80	3.14	2.93
	60	2.29	4.06	3.17	3.17
	120	2.50	4.03	3.21	3.24
Direct sowing	0	1.81	2.08	2.17	2.02
	60	2.35	2.95	2.95	2.74
	120	2.64	3.31	2.90	2.95
Direct sowing undersown with white clover	0	3.12	3.93	3.36	3.47
	60	3.20	4.00	3.60	3.60
	120	3.66	4.12	3.28	3.69
$LSD_{p=0.05}$		n.s.	0.44	n.s.	n.s.
Means for combinations					
Cultivation system					
Traditional cultivation		2.21 a	3.96 a	3.18	3.12
Direct sowing		2.27 a	2.78	2.68	2.57
Direct sowing undersown with white clover		3.33	4.02 a	3.52	3.62
$LSD_{p=0.05}$		0.26	0.30	0.14	0.13
Nitrogen fertilization (kg/ha)					
0		2.56 a	3.27	2.89	2.81
60		2.61 a	3.67 a	3.24 a	3.17
120		2.94	3.82 a	3.24 a	3.33
$LSD_{p=0.05}$		0.19	0.23	0.26	0.13
Years		2.60 a	3.59 a	3.12 a	3.10
$LSD_{p=0.05}$			0.13		–

n.s. – not significant.
Values in columns followed by the same letter are not significantly different.

much rain in spring 1999. In the second year there was little rainfall until December and twice as much as the long-term average in January, March and July. Rainfall was several times less than in many recent years and drought conditions occurred in April and June 2000. In winter-spring of the third year the monthly rainfall totals were similar to the long-term average values. In May 2001, as in the first year, rainfall was less than half the average, while the July total was greatly increased and caused a delay in the wheat harvest.

Assessment of disease showed that the method of cultivation significantly affected the extent of symptoms on stem bases (Table 2). Averaged over all three years there was least disease after direct sowing and most disease after direct sowing with undersown white clover. There were similar amounts of disease after direct sowing and traditional cultivation only in 1998/99. There was most disease in wheat grown after direct sowing with undersown white clover in all years but a similar amount of disease occurred with traditional cultivation in 1999/2000.

The amount of nitrogen fertilizer significantly affected the amount of stem-base disease (Table 2). Averaged over all three years, there was most disease on plants from plots to which the higher amount of the nitrogen (120 kg/ha) had been applied. In the last two years, the single dose of nitrogen (60 kg/ha) led to increases in disease, compared with the control, that were similar to those of the higher dose.

Species of *Fusarium* were isolated from wheat stem bases in all three years of experiment (Table 3). The frequency of all *Fusarium* spp. was least in the first year and increased by 67% in the second year and 57% in the third year.

The frequency of all *Fusarium* spp. was increased by application of the higher dose of nitrogen fertilizer in first two years, averaged over all cultivation treatments. In the last year, however, *Fusarium* species were isolated most frequently from wheat grown with traditional cultivation and from the direct sown wheat fertilized with 60 kg N per 1 ha. In 1998/99 and 1999/2000, *Fusarium* species were isolated most frequently from wheat grown after direct sowing with undersown white clover and from wheat with traditional cultivation. In the first two years, *Fusarium* spp. were least frequent in wheat grown from direct sowing, where least stem-base disease also occurred in the second year. The genus *Fusarium* was represented most commonly by *F. avenaceum* (Table 3). *Fusarium graminearum*, *F. culmorum*, *F. equiseti* and *F. oxysporum* were the most common of the other species. In first two years, *F. avenaceum* was most frequent in the direct-sown wheat undersown with white clover, where it amounted to 92% and 59% of the total number of *Fusarium* isolates. In the third year, however, *F. avenaceum* was most frequent in the traditionally-grown wheat (92%). The lowest frequency of *F. avenaceum* was always recorded from stem bases of wheat grown with direct sowing. In the first two years, *F. graminearum* and *F. culmorum* were isolated more frequently from wheat grown traditionally and from the direct-sown wheat than from direct-sown wheat with undersown clover. They accounted for 60% of the total number of *Fusarium* isolates.

Other pathogenic fungi, *Oculimacula yallundae*, *Rhizoctonia cerealis* and *Bipolaris sorokiniana* were recorded less frequently (up to 9% of the total number of fungal isolates) (Table 3). In the first two years, they tended to be isolated most fre-

Table 3

Fungi isolated from stem bases of winter wheat grown in different cultivation systems and with different amounts of nitrogen fertilizer (number of isolates)

Species of fungi	1998/99									1999/2000									2000/01								
	traditional cultivation			direct sowing			direct sowing undersown with white clover			traditional cultivation			direct sowing			direct sowing undersown with white clover			traditional cultivation			direct sowing			direct sowing undersown with white clover		
	0	60	120	0	60	120	0	60	120	0	60	120	0	60	120	0	60	120	0	60	120	0	60	120	0	60	120
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Alternaria alternata	34	27	35	73	40	46	28	24	25	16	4	13	35	31	14	35	15	5				9	2	6	5	8	
Bipolaris sorokiniana	2	2		3	4		2	7	1										4								
Botrytis cinerea	7	4	14				2																				
Cladosporium cladosporioides		2	1	5		1																					
Epicoccum purpurascens	14	36	24	5	16	50	1	7						1													
Fusarium avenaceum	38	100	113	26	73	63	177	163	222	136	77	111	55	130	98	177	151	149	145	210	149	112	156	161	149	137	167
Fusarium culmorum	29	11	10	7	23	33	17	24	17	32	57	42	57	41	74	18	33	45	6		11	11					
Fusarium equiseti	5	1									9	13	6	3	3		2	5					5				
Fusarium graminearum	5		15	11	1	11		6		14	92	63	40	42	36	10	26	34	18	6	23	53	20	28	24	43	25
Fusarium oxysporum	2	2	7			5			2	3		2	5					2				10	7		4		
Fusarium poae										24		12	19		14	6	7	9									
Fusarium sambucinum																			5	13	12	14	35	24	1	2	15
Fusarium scirpi																			21		11		4		1	28	6
Fusarium semitectum																								6			
Fusarium solani										1																	
Fusarium sporotrichioides										7			12		3	12	18		5				5		4	12	10

Table 3 – cont.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Gliocladium catenulatum																					4				2		
Nigrospora oryzae				6																				4			
Oculimacula yallundae										12		7	4	8	6	17		9	18	3	18	1	3	6	10	9	16
Phoma herbarum																										10	
Phoma leveillei																				12		4					
Rhizoctonia cerealis	2	1	3	2			1	3			6		7	6	7	10	3	2							2		
Rhizoctonia solani	3		6	1	2	1	2	1	7				2														
Trichoderma hamatum																								1			
Trichoderma harzianum						1				2				1	6												
Non-sporulating fungi	82	55	31	104	101	61	35	33	17	4	6	3	36	15	7	8		6	22	4	17	46	10	27	43	5	37
Other fungi	8			3	3	1				4		4	10	2	6		2					1		1	1	9	1
Total	231	241	259	246	263	273	265	268	291	255	251	270	288	280	274	293	257	266	244	248	245	261	247	264	246	263	277

0, 60, 120 – kg N per 1 ha.

quently from stems of wheat grown from direct sowing with undersown white clover, and in the last year from wheat grown traditionally (Table 3).

Saprotrophic fungi were represented mostly by *Alternaria alternata* and *Epicoccum purpurascens* (Table 3). In the first year, both species were isolated frequently, while in the next two years only *A. alternata* was recorded. The latter was isolated most frequently from stem bases of wheat grown from direct sowing. Its frequency was highest in the first year and accounted for 30% of the total number of fungal isolates (Table 3).

Discussion

In the experiment reported here, winter wheat was grown in monoculture. Many authors emphasize that cultivation of wheat after wheat increases the density of wheat pathogens in the soil (Sturz and Bernier 1989, Nadolnik et al. 1998, Różalski et al. 1998 a, Cuilin et al. 2000, Dill-Macky and Jones 2000, Kurowski 2002). Most stem-base disease with symptoms typical of those caused by *Fusarium* species, was observed in the second year of cultivation of wheat in monoculture regardless of the cultivation treatment applied. The high disease was associated with a dry April and June (as reported in "Results"), which might also account for the high incidence of *F. culmorum* in the second year of cultivation (Tables 1, 3). Jaczewska-Kalicka (2002) confirms that such moisture conditions favour the infection of wheat by some *Fusarium* species.

The higher dose of the nitrogen fertilization sometimes favoured the growth of fungi. It increased disease more consistently. Nitrogen stimulates plant growth and can decrease resistance to infection by pathogens as suggested by the disease results and some of the results for frequency of *Fusarium* species isolated from diseased stem bases (Blecharczyk et al. 1999). In all three years, most disease usually occurred in plots to which the double dose of the nitrogen fertilization (120 kg/ha) had been applied. This phenomenon has been observed previously and confirmed experimentally (Remlein 1996, Różalski et al. 1998 b). In last two years of the present study, however, the single dose of nitrogen (60 kg/ha) tended to favour the infection of the lower wheat internodes by *Fusarium*, compared with control with no nitrogen fertilizer applied.

The method of cultivation affected the frequency of *Fusarium* and other fungi associated with disease. Most disease was recorded after direct sowing with undersown white clover. Cultivation that did not involve ploughing the stubble field could cause an accumulation of pathogenic fungi in the upper layers of the soil and create a reservoir of inoculum for the next crop. A similar observation was made by Weber et al. (2001).

The isolation of several species of *Fusarium* from the diseased stem bases suggests that they were the main cause of the stem-base disease of wheat. *Fusarium* species are common in the soil habitat. They are primary colonizers of the cereals' roots and are characterized by a high competitiveness with other microorganisms

(Pląskowska 1997 a, Weber et al. 2001, Narkiewicz-Jodko et al. 2005). Their accumulation in soil may have resulted from a shortage of their natural antagonists, e.g. *Trichoderma*, *Gliocladium* and *Penicillium* species (Pląskowska 1997 b).

In the first and second year of the study, *Fusarium* species were the most frequently isolated from wheat grown from direct sowing with undersown white clover. This may be partly a consequence of the accumulation of *Fusarium* species on roots of the white clover. The tendency of *Fusarium* species to accumulate on roots of plants of *Fabaceae* was reported by Pląskowska (1997 b).

In the last two years, greater incidence of stem-base disease associated with a greater frequency of *Fusarium* species was observed in wheat grown with traditional cultivation. This may be explained by the phenomenon described as the preservation effect of ploughing. Weber et al. (2001) showed that organic residues left on the surface after harvest are moved down in the soil by ploughing. The lower temperature and higher moisture in the deeper layers of the soil help the survival of the pathogens on the infested residues. The next ploughing, applied particularly in the cultivation of cereals, brings the infested residues (the inoculum source) up to the surface of the soil where the fungi find conditions favourable for their growth.

Weber et al. (2001) concluded that simplifying the procedures for the cultivation of cereals was not beneficial for the health of plants because it created conditions favourable for pathogenic fungi and infection of plants. Our results only partly support this. They suggest that only direct sowing of wheat undersown with white clover, in the first year of the study, created conditions that were adverse for the plants and beneficial for pathogens.

The species isolated most frequently from diseased stems was *F. avenaceum*, while *F. culmorum* and *F. graminearum* were somewhat less frequent. Pląskowska (1997 b) and Narkiewicz-Jodko et al. (2005) found that the first two species were the most common species colonizing stem bases of cereals. *Fusarium avenaceum* was most frequent in direct-sown wheat undersown with white clover in the first two years of study and also with traditional cultivation in the last year. *Fusarium culmorum*, which is generally recognized as one of the most important stem-base pathogens of wheat, occurred less frequently. This fungus was isolated most frequently in the first two years from direct sown wheat grown and in traditionally cultivated wheat. The growth of *F. culmorum* is usually favoured by warm and dry growing seasons with temperature around 25°C (Kurowski 2002, Matkowski and Pląskowska 2003). In colder growing seasons, which occurred during three years of study, the dominant species was *F. avenaceum*. According to Narkiewicz-Jodko et al. (2005), the population of *Fusarium* species is strongly modified, quantitatively and qualitatively, by environmental factors. Therefore, depending on the soil and weather conditions, either the first or the second species usually predominates.

The other pathogenic fungi, *O. yallundae*, *R. cerealis* and *B. sorokiniana*, were recorded less frequently. The first of them was isolated in the second and the third year mainly from diseased wheat grown from direct sowing with undersown white clover. An absence of *Gaeumannomyces graminis* in all three years seems to be due to its weak competitiveness with other microorganisms (Pląskowska 1997 a).

Among the saprotrophic fungi, *A. alternata* and *E. purpurascens* predominated in the first year. In the subsequent two years, only *A. alternata* was isolated. This fungus occurred most frequently in wheat grown from direct sowing, where the least disease was observed. Competition between *Fusarium* species and *A. alternata* was observed; a low incidence of colonization of the diseased stem tissues by *Fusarium* was associated with the frequent occurrence of *A. alternata*. Similar competition between these two fungi was observed also by Narkiewicz-Jodko et al. (2005). The growth of *A. alternata* is favoured by temperatures 21–27°C, which was recorded in June/July 1999.

Conclusions

1. In the present studies, *Fusarium* species, mostly *F. avenaceum*, were the main fungi associated with stem-base disease of winter wheat.
2. In monoculture, direct sowing tended to limit the infection of the winter wheat by pathogens associated with stem-base disease.
3. In monoculture, direct sowing of wheat undersown with white clover increased stem-base disease, which was associated with an accumulation of *Fusarium* pathogens.
4. The higher dose of nitrogen (120 kg/ha) tended to favour infection of winter wheat by stem-base pathogens.

Streszczenie

WPŁYW SIEWU BEZPOŚREDNIEGO
W MONOKULTURZE PSZENICY OZIMEJ
NA ZASIEDLENIE PODSTAWY ŹDŹBŁA PRZEZ *FUSARIUM* SPP.

Badania zdrowotności pszenicy ozimej w zależności od sposobu uprawy roli i dawki nawożenia azotem przeprowadzono w latach 1998–2001. Materiałem do badań była pszenica ozima odmiany 'Kobra' uprawiana w monokulturze. Celem pracy była ocena wpływu siewu bezpośredniego, siewu bezpośredniego z wsiewką koniczyny białej oraz uprawy tradycyjnej na stopień porażenia pszenicy ozimej przez patogeny powodujące zamieranie korzeni i podstawy źdźbła, przy zróżnicowanym nawożeniu azotem.

We wszystkich latach badań najsłabiej porażana przez patogeny powodujące choroby podsuszkowe, wśród których dominowały gatunki z rodzaju *Fusarium,* była pszenica uprawiana w siewie bezpośrednim. Tylko w sezonie wegetacyjnym 1998/99 stwierdzono, że rośliny w siewie bezpośrednim i w uprawie tradycyjnej chorowały na zbliżonym poziomie. Najwięcej zmian chorobowych zanotowano na źdźbłach pszenicy uprawianej w siewie bezpośrednim z wsiewką koniczyny białej. Zastosowanie największej dawki nawożenia azotem (120 kg/ha) przyczyniło się

do pogorszenia zdrowotności roślin. Największym zagrożeniem dla zdrowotności pszenicy były grzyby z rodzaju *Fusarium*, a szczególnie *F. avenaceum*.

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Accepted for publication: 20.03.2007