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EFFECT OF SULFUR FERTILIZATION ON THE SANITARY STATE OF PLANTS OF THE FAMILY BRASSICACEAE

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A b s t r a c t

The experiment was carried out in the years 2006-2008 in Bałcyny (N=53º35`49"; E=19º51`20"). The aim of this study was to determine the effect of sulfur fertilization on the sanitary state of spring oilseed rape, winter oilseed rape, white mustard and Chinese mustard as well as on the species composition of fungi colonizing their seeds. Sulfur fertilization had a beneficial effect on the health of Brassicaceae plants infested by Alternaria blight, grey mould, Sclerotinia stem rot, Phoma stem canker and Verticillium wilt, but it had a varying effect on the occurrence of powdery mildew. *Alternaria alternata* and *Penicillium* spp. were isolated most frequently from Brassicaceae seeds. In general, more fungi (including pathogenic to Brassicaceae) were isolated from the seeds of plants grown in non-sulfur fertilized plots. Pathogens occurred primarily on the seed surface, and their number decreased after surface disinfection of seeds.

Key words: spring oilseed rape; winter oilseed rape; white mustard; Chinese mustard, sulfur fertilization; diseases; fungi colonizing seeds

INTRODUCTION

The EU strategy to reduce the atmospheric emissions of sulfur dioxide has led to sulfur deficiency in soil, which has recently become a serious problem in Poland (P o d l e ś n a , 2002). Sulfur deficiency may reduce the utilization of other nutrients, in particular nitrogen, by plants, since nitrogen metabolism is closely related to sulfur metabolism (J a n z e n and B e t t a n y , 1984). Therefore, sulfur deficit can affect both the yield and quality of crops. Sulfur affects taste, flavor and appearance as well as processing suitability and nutritional value of agricultural products. Sulfur content also influences yield and quality of oil obtained (P o d l e ś n a , 2005). Fertilization, especially sulfur fertilization, has a significant effect on glucosinolate content of the tissues of cruciferous plants (K a c h l i c k i , 1990; O l e s z e k , 1995). The products of glucosinolate hydrolysis show toxicity against pathogenic fungi, bacteria, viruses, insects and higher plants (O l e s z e k , 1995; M a j c h r z a k et al. 2001; M a j c h r z a k et al. 2004).

The aim of the study was to determine the effect of sulfur fertilization on the sanitary state of spring oilseed rape, winter oilseed rape, white mustard and Chinese mustard as well as on species composition of the fungal communities colonizing seeds of the above Brassicaceae species.

MATERIALS AND METHODS

The experiment was carried out in the years 2006-2008 in trial plots located at the Production and Experimental Station in Bałcyny near Ostróda in Poland (N=53º35`49"; E=19º51`20"). A field experiment was performed in a randomized block design, in six replications. The experimental material comprised spring oilseed rape (*Brassica napus* var. *arvensis* f. *annua*) cv. Hunter, winter oilseed rape (*B. napus* var. *arvensis* f. *biennis*) cv. Californium, white mustard (*Sinapis alba*) cv. Borowska and Chinese mustard (*B. juncea*) cv. Małopolska. The experiment was established on typical grey-brown medium-silty podsolic soil of good wheat complex, developed from light loam. Brassicaceae plants were grown in accordance with the relevant cultivation and fertilization standards. Mineral NPK fertilizers were applied as follows ($kg \cdot ha^{-1}$): winter rapeseed – 240:70:220, spring rapeseed and white mustard – 100:40:120, Chinese mustard – 70:30:80. In sulfur-fertilized treatments, the following doses of S ($kg \cdot ha^{-1}$) were applied at the beginning of the growing season: winter rapeseed – 60, spring rapeseed and white mustard – 40, Chinese mustard – 25. Before sowing, rape seeds were dressed with Cruiser OSR 322 FS (thiamethoxam + metalaxyl-M + fludioxonil), and mustard seeds – with Zaprawa nasienna T (thiram). Crops were not protected against pathogens with fungicides throughout the growing season.

The health status of 25 plants collected randomly from each plot was evaluated three times during the growing season, using a four-point scale of Hillstrand and Auld (1982). The evaluation results which pointed to the highest severity of diseases were considered in the analysis. The injury index was calculated according to McKinney's formula (Łacicowa, 1970).

Fungi were isolated from seeds of the investigated plants after harvest. Every year 100 seeds were randomly sampled in each treatment. Half of the seeds were disinfected for 30 seconds in 50% ethyl alcohol and for another 30 seconds in 0.1% sodium hypochlorite. The seeds were rinsed three times with sterile water, then dried on sterile filter paper and placed in Petri dishes on potato-glucose agar. The remaining seeds were rinsed with sterile water, and the further procedure was the same as for disinfected seeds. The colonies that developed were transferred onto potato-glucose agar slants, and the fungi were identified to species using available keys, following spore formation.

RESULTS

Powdery mildew occurred on spring oilseed rape leaves, and the symptoms of Alternaria blight were observed on siliques in all years of the study, however, grey mould occurred on siliques only in 2006 (Table 1a). The severity of diseases was dependent on weather conditions. The highest infection rate was noted in 2007, and it was lower in the sulfur-fertilized plots. Also in 2006, the incidence of powdery mildew and grey mould was lower in the sulfur-fertilized plots. Only in 2008 the severity of powdery mildew and Alternaria blight was higher in the sulfur-fertilized plots than in the control treatments.

The symptoms of Alternaria blight were noted on siliques of winter oilseed rape over the entire experimental period. The other diseases occurred in one year only – grey mould and Verticillium wilt in 2006, Sclerotinia stem root and Phoma stem canker in 2008 (Table 1b). In all cases, the sanitary state of winter oilseed rape was positively affected by sulfur fertilization.

The symptoms of Alternaria blight were observed on both the leaves and siliques of white mustard (Table 1c). The highest infection rate was noted in 2007. Again, sulfur fertilization had a beneficial effect on the health status of plants.

Powdery mildew occurred on Chinese mustard leaves, while siliques were attacked by Alternaria blight in all years of the study (Table 1d). Sulfur fertilization had varied effects on the health status of plants. In 2006 the effect of sulfur fertilization was non-significant with respect to both diseases. In 2007 sulfur fertilization exerted a beneficial influence, and in 2008 the plots fertilized with sulfur were characterized by higher severity of powdery mildew and lower severity of Alternaria blight on siliques.

A total of 407 fungal cultures representing 11 taxa were isolated from spring rape seeds (Table 2a), including 179 from sulfur-fertilized plants and 228 from non-fertilized plants. Seed disinfection contributed to a decrease in the number of fungal colonies – 193 colonies were isolated from disinfected seeds and 214 from non-disinfected seeds. The predominant species on spring rape seeds was *Alternaria alternata*, pathogenic to Brassicaceae (44.7% of isolates from the seeds of sulfur-fertilized plants and 53.5% from the seeds of non-fertilized plants). Fungi of the genus *Penicillium* (18.4% and 21.1% respectively) and *Cladosporium cladosporioides* (24.6% and 10.5% respectively) also occurred in great abundance. Other pathogens of the Brassicaceae, isolated in the study, were *Alternaria brassicae*, *Phoma lingam*, *Fusarium oxysporum* and *F. sporotrichioides*. All of them dominated on seeds of the plants that were not fertilized with sulfur. Disinfection reduced the percentage share of potential pathogens of Brassicaceae among the obtained isolates. The only species whose proportion increased following seed disinfection was *Cladosporium cladosporioides.*

A total of 249 fungal colonies belonging to 15 taxa were isolated from winter rape seeds (Table 2b), including 98 from the seeds of sulfur-fertilized plants and 151 from the seeds of non-fertilized plants. Seed disinfection considerably decreased the number of fungal colonies – 95 colonies were isolated from disinfected seeds and 154 from non-disinfected seeds. The predominant species on winter rape seeds was *A. alternata* (50.0% of isolates from the seeds of sulfur-fertilized plants and 55.0% from the seeds of non-fertilized plants). Fungi of the genus *Penicillium* were also abundant (23.5% and 20.5%, respectively). Other pathogens of the Brassicaceae were *A. brassicae*, *F. oxysporum*, *F. poae* and *F. tricinctum*. They dominated on seeds of the plants that were not fertilized with sulfur. Disinfection reduced the percentage share of potential pathogens of Brassicaceae among the obtained isolates.

A total of 524 fungal colonies representing 12 taxa were isolated from white mustard seeds (Table 2c), including 250 from sulfur-fertilized plants and 274 from non-fertilized plants. Seed disinfection contributed to a decrease in the number of fungal colonies

– 253 colonies were isolated from disinfected seeds and 271 from non-disinfected seeds. Fungi of the genus *Penicillium* dominated among the obtained isolates (58.0% of isolates from the seeds of sulfur-fertilized plants and 49.3% from the seeds of non-fertilized plants). *A. alternata* also occurred in great abundance (34.8% and 39.0%, respectively). Other pathogens of the Brassicaceae were *A. brassicae*, *F. oxysporum*, *F. equiseti* and *Microdochium nivale*. They dominated on seeds of the plants that were fertilized with sulfur. Disinfection reduced the percentage share of potential pathogens of Brassicaceae among the obtained isolates, and *Fusarium* spp. were isolated only from non-disinfected seeds.

Table 1

Intensity of diseases on plants of the family Brassicaceae (injury index in %)

a/ spring oilseed rape

Disease (Pathogen)	Plant part	Year	Fertilization		LSD (p=0.05)
			with sulfur	without sulfur	
Powdery mildew (*Erysiphe cruciferarum*)	leaves	2006	38.7	40.7	1.41
		2007	68.0	79.6	1.06
		2008	24.6	13.9	1.56
Alternaria blight (*Alternaria alternata* et al.)	siliques	2006	6.9	5.4	n.s.
		2007	68.4	70.6	1.01
		2008	17.3	9.7	1.70
Grey mould (*Botrytis cinerea*)	siliques	2006	34.0	37.0	2.33

b/ winter oilseed rape

Disease (Pathogen)	Plant part	Year	with sulfur	without sulfur	LSD (p=0.05)
Alternaria blight (*Alternaria alternata* et al.)	siliques	2006	13.9	17.1	1.57
		2007	5.9	8.3	0.75
		2008	8.0	14.7	1.37
Grey mould (*Botrytis cinerea*)	siliques	2006	6.9	8.9	0.99
Verticillium wilt (*Verticilium dahlie*)	leaves	2006	4.9	6.1	0.78
Phoma stem canker (*Phoma lingam*)	stems	2008	13.2	22.3	2.37
Sclerotinia stem root (*Sclerotinia sclerotiorum*)	stems	2008	13.1	18.6	1.68

c/ white mustard

Disease (Pathogen)	Plant part	Year	with sulfur	without sulfur	LSD (p=0.05)
Alternaria blight (*Alternaria alternata* et al.)	leaves	2006	7.0	7.1	n.s.
		2007	57.7	69.4	1.01
		2008	3.1	7.7	1.14
Alternaria blight (*Alternaria alternata* et al.)	siliques	2007	6.7	18.9	0.82
		2008	0.3	1.7	0.50

d/ Chinese mustard

Disease (Pathogen)	Plant part	Year	with sulfur	without sulfur	LSD (p=0.05)
Powdery mildew (*Erysiphe cruciferarum*)	leaves	2006	77.2	73.9	n.s.
		2007	74.3	88.7	1.07
		2008	99.4	89.7	1.28
Alternaria blight (*Alternaria alternata* et al.)	siliques	2006	42.6	41.0	n.s.
		2007	53.4	81.3	0.94
		2008	5.2	5.8	0.47

n.s. – not significant differences

Table 2

Fungi isolated from seeds of Brassicaceae plants

a/ spring oilseed rape

Species of fungus	Fertilization				Sum	
	with sulfur		without sulfur			
	d	nd	d	nd	d	nd
Alternaria alternata (Fr.) Keissler	31	49	60	62	91	111
Alternaria brassicae (Berk.) Sacc.		2	4	6	4	8
Aspergillus niger van Tieghem	8				8	
Cladosporium cladosporioides (Fresen.) de Vries	28	16	9	15	37	31
Epicoccum purpurascens Ehrenb. ex Schlecht.	1			2	1	2
Fusarium oxysporum Schlecht.		2	3	2	3	4
Fusarium sporotrichioides Sherb.			1	3	1	3
Mucor circinelloides van Tieghem	1				1	
Penicillium spp.	11	22	25	23	36	45
Phoma lingam (Tode) Desm.				3		3
Non-sporulating fungi	5	3	6	4	11	7
Total	85	94	108	120	193	214

b/ winter oilseed rape

Species of fungus	Fertilization				Sum	
	with sulfur		without sulfur			
	d	nd	d	nd	d	nd
Acremonium murorum W. Gams			1		1	
Acremonium strictum W. Gams	6	1		1	6	2
Alternaria alternata (Fr.) Keissler	16	33	29	54	45	87
Alternaria brassicae (Berk.) Sacc.	1	3	4	4	5	7
Aspergillus niger van Tieghem	1				1	
Cladosporium cladosporioides (Fresen.) de Vries	1	4	2	1	3	5
Epicoccum purpurascens Ehrenb. ex Schlecht.		1	2	1	2	2
Fusarium oxysporum Schlecht.		1	1	2	1	3
Fusarium poae (Peck.) Wollenw.				1		1
Fusarium tricinctum (Corda) Sacc.	1				1	
Mucor hiemalis Wehmer				2		2
Penicillium spp.	5	18	14	17	19	35
Non-sporulating fungi	4	2	7	7	11	9
Yeast-like fungi				1		1
Total	35	63	60	91	95	154

Table 2 cont.

c/ white mustard

Species of fungus	Fertilization				Sum	
	with sulfur		without sulfur			
	d	nd	d	nd	d	nd
Alternaria alternata (Fr.) Keissler	47	40	54	53	121	93
Alternaria brassicae (Berk.) Sacc.	2	1	6	9	8	10
Aspergillus sp.	2				2	
Cladosporium cladosporioides (Fresen.) de Vries	1				1	
Epicoccum purpurascens Ehrenb. ex Schlecht.			1		1	
Fusarium equiseti (Corda) Sacc.		5		3		8
Fusarium oxysporum Schlecht.		3				3
Microdochium nivale (Fr.) Samuels et Hallett				1		1
Penicillium spp.	67	78	71	64	138	162
Rhizopus nigricans Ehr.		4		6		10
Non-sporulating fungi			2		2	
Yeast-like fungi				4		4
Total	119	131	134	140	273	291

d/ Chinese mustard

Species of fungus	Fertilization				Sum	
	with sulfur		without sulfur			
	d	nd	d	nd	d	nd
Alternaria alternata (Fr.) Keissler	57	59	63	70	120	129
Alternaria brassicae (Berk.) Sacc.	3	1	2	7	5	8
Cladosporium cladosporioides (Fresen.) de Vries				3		3
Epicoccum purpurascens Ehrenb. ex Schlecht.		3	1	2	1	5
Fusarium oxysporum Schlecht.		1		5		6
Monodictys levis (Wiltshire) Hughes				1		1
Mucor circinelloides van Tieghem	5	10			5	10
Mucor hiemalis Wehmer				3		3
Penicillium spp.	24	19	29	28	53	47
Phoma lingam (Tode) Desm.			2		2	
Rhizopus nigricans Ehr.		5		2		7
Non-sporulating fungi	2		4	1	6	1
Yeast-like fungi		1	4		4	1
Total	91	99	105	122	196	221

d – disinfected seeds

nd – non-disinfected seeds

A total of 417 fungal colonies belonging to 13 taxa were isolated from Chinese mustard seeds (Table 2d), including 190 from sulfur-fertilized plants and 227 from non-fertilized plants. Seed disinfection decreased the number of fungal colonies – 196 colonies were isolated from disinfected seeds and 221 from non-disinfected seeds. The predominant species on Chinese mustard seeds was *A. alternata* (61.1% of isolates from the seeds of sulfur-fertilized plants and 58.6% from the seeds of non-fertilized plants). Fungi of the genus *Penicillium* were also abundant (22.6% and 25.1%, respectively). Other pathogens of the Brassicaceae were *A. brassicae*, *F. oxysporum* and *Phoma lingam* which occurred in higher abundance on seeds of the plants that were not fertilized with sulfur.

DISCUSSION

The symptoms of Alternaria blight were observed throughout the entire experimental period on all four species of Brassicaceae. The disease incidence was determined primarily by weather conditions during the growing season. The year 2007, in which June was dry and total precipitation in July was twice higher than the long-term average, provided the most favorable conditions for the development of Alternaria blight on spring crucifers. Winter rapeseed, which reaches maturity at the end of June and at the beginning of July, was not significantly infected in 2007. The considerable effect of weather conditions on the severity of diseases attacking cruciferous plants, including the most severe one - Alternaria blight, has been described by numerous authors (Plachka, 1996 ; Jędryczka et al. 2002; Kurowski and Budzyński, 2003; Kurowski and Jankowski, 2003).

In each year of the study, the disease infection rates were affected by sulfur fertilization. The application of sulfur fertilizers was found to alleviate the severity of infection caused by *Alternaria* spp. on winter rapeseed, white mustard and Chinese mustard. As regards spring rapeseed, the effect of sulfur fertilization varied throughout the experimental period. Powdery mildew, which occurred in all years of the study but only on spring rapeseed and Chinese mustard, attacked to a higher degree non-fertilized plants during the first two years, and fertilized plants in the last year. The symptoms of other diseases, i.e. grey mould, Sclerotinia stem rot, Phoma stem canker and Verticillium wilt, were noted in one year only and were stronger in the non-fertilized treatments. It may be concluded that in this experiment sulfur fertilization had a beneficial influence on the health status of Brassicaceae plants, and the best results were reported for winter rapeseed fertilized with the highest doses of nitrogen and sulfur.

Brassicaceae have a high sulfur demand. Sulfur availability plays an important role in plant protection against stress factors such as frost and drought. The frost resistance of crops is dependent on carbohydrate content and protein hydration, determined by sulfur-containing compounds – sulfhydryls (Levitt et al. 1961). According to Podleśna et al. (2003), these compounds are also capable of protecting plants against drought. Plants living under stress conditions are more exposed to attack by various pathogens, in particular incidental pathogens, including those noted in the present study (except for *Erysiphe cruciferarum*). Cruciferous plants produce glucosinolates which contain sulfur and contribute to protection against pathogens. The products of glucosinolate hydrolysis inhibit *in vitro* growth of *Phoma lingam* and *Sclerotinia sclerotiorum* (Schnug and Ceynowa, 1990). A lower incidence of fungal diseases on the Brassicaceae fertilized with sulfur was also demonstrated by Pałosz (1995), Jędryczka et al. (2002) and Sadowski et al. (2002). The protective effect of sulfur was reported with respect to, among others, Alternaria blight, powdery mildew, downy mildew, light leaf spot and Phoma stem canker.

Alternaria alternata was isolated most frequently from the seeds of Brassicaceae plants, followed by *Penicillium* spp. Other fungal species occurred sporadically. Apart from *A. alternata*, the following pathogens were also isolated from Brassicaceae seeds: *A. brassicae*, *Fusarium equiseti*, *F. oxysporum*, *F. sporotrichioides* and *F. tricinctum*. *Alternaria alternata* is a typical cosmopolitan species which dominates among the isolates obtained from many plant species. It is considered potentially dangerous due to the production of toxins (Chełkowski and Grabarkiewicz-Szczęsna, 1991). Fungi of the genus *Penicillium* also occur commonly and are characterized by strong enzymatic properties. *Alternaria alternata* and members of the genus *Penicillium* are considered by many authors to infest most frequently cruciferous seeds (Richardson, 1996; Majchrzak et al. 2002).

In general, more fungi (including those pathogenic to Brassicaceae) were isolated from the seeds of plants grown in the control plots – without sulfur fertilization. The application of sulfur fertilizers caused substantial reductions in the abundance of fungi colonizing Brassicaceae seeds. The greatest differences were observed with regard to winter rapeseed. Only *C. cladosporioides* and, to a much lower degree, *Penicillium* spp., found better conditions for development on seeds of the sulfur-fertilized plants (7.0% and 3.4%, and 34.0% and 30.8% of all isolates from the Brassicaceae, respectively). These results are consistent with field trial findings which showed that sulfur-fertilized plants were healthier and that glucosinolates, whose concentration is highest in seeds, inhibited the growth of many fungi on seeds (Schnug and Ceynowa, 1990; Wielebski and Wójtowicz, 2003).

The total number of fungal colonies isolated from disinfected seeds was lower, as compared with non-disinfected seeds. Pathogens occurred primarily on the seed surface, and their numbers decreased after surface disinfection of seeds. Similar results were reported by Musiał (1996) for grass seeds, and by Majchrzak et al. (2002) for Brassicaceae seeds.

CONCLUSIONS

1. The most common disease, whose symptoms were observed most frequently on the aboveground parts of Brassicaceae, was Alternaria blight. Infection severity was determined primarily by weather conditions towards the end of the growing season.
2. Sulfur fertilization had a beneficial effect on the health status of Brassicaceae.
3. *Alternaria alternata* and fungi of the genus *Penicillium* dominated on the seeds of cruciferous plants.
4. The application of sulfur fertilizers substantially reduced the abundance of fungi, including pathogens, colonizing Brassicaceae seeds.
5. Winter oilseed rape fertilized with high doses of nitrogen showed the strongest response to sulfur fertilization.

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Wpływ nawożenia siarką na zdrowotność roślin kapustnych

Streszczenie

Badania prowadzono w latach 2006-2008 na poletkach doświadczalnych zlokalizowanych w Zakładzie Produkcyjno-Doświadczalnym w Bałcynach k. Ostródy (N=53°35`49"; E=19°51`20"). Oceniano wpływ nawożenia siarką na zdrowotność rzepaku jarego, rzepaku ozimego, gorczycy białej i gorczycy sarepskiej oraz na skład gatunkowy grzybów zasiedlających nasiona tych roślin kapustnych.

Generalnie nawożenie siarką wpłynęło korzystnie na zdrowotność roślin kapustnych zmniejszając nasilenie czerni krzyżowych, szarej pleśni, zgnilizny twardzikowej, suchej plamistości i werticiliozy, natomiast wpływ nawożenia siarką na rozwój mączniaka prawdziwego był niejednoznaczny i zależał przede wszystkim od przebiegu pogody.

Z nasion roślin kapustnych izolowano głównie *Alternaria alternata*, a w mniejszym nasileniu grzyby rodzaju *Penicillium*. Z nasion roślin nie nawożonych siarką izolowano więcej grzybów, w tym patogenicznych dla roślin kapustnych. Patogeny występowały głównie na powierzchni nasion, a ich liczba malała po powierzchniowym odkażeniu nasion.