

THE OCCURRENCE OF ALTERNARIA BLIGHT (*ALTERNARIA* SPP.) AND PHOMA STEM CANKER (*PHOMA LINGAM*) ON OILSEED RAPE IN CENTRAL LITHUANIA AND PATHOGENIC FUNGI ON HARVESTED SEED

Irena Brazauskiene, Egle Petraitiene

Lithuanian Institute of Agriculture, Plant Protection Department
Instituto al. 1, Akademija, Dotnuva, 58344 Kedainiai distr., Lithuania
brazausk@lzi.lt; egle@lzi.lt

Accepted: September 30, 2006

Abstract: Field surveys were performed in winter and spring rape crops of agricultural companies and individual farmers during the period of 2001–2003. A questionnaire was completed for each experimental field, giving a description of the crop. The incidence and severity of fungal diseases were estimated annually in 14–18 winter and spring rape crops. Seed samples were taken from each field and composition fungal communities on harvested seed was estimated. *Alternaria* blight and *Phoma* stem canker were present on stems of all investigated winter and spring rape cultivars and *Alternaria* blight occurred on siliques in all experimental years. In 2001, most favourable year for spread of diseases, *Alternaria* blight damaged 87.2–100% of winter rape and 100% of spring rape siliques, maximum disease severity was 6.66 and 7.24%, respectively. All cultivars of winter and spring rape were susceptible to *Alternaria* blight. *Phoma* stem canker was more often found on stems of spring oilseed rape – up to 98% of stems with symptoms of *Phoma* stem canker. Seed fungal infection level was 10.0–100% in winter rape and 16.0–93.6% in spring rape seed samples. The most frequent fungi on seeds of winter and spring oilseed rape were *Alternaria* spp. and *Cladosporium* spp.

Key words: oilseed rape, *Alternaria* blight, *Alternaria* spp. *Phoma* stem canker, *Phoma lingam*, seed infection, *Cladosporium* spp.

INTRODUCTION

Oilseed rape is the main oil crop in Lithuania, and presently one of the most promising crops whose production area in 1990–2005 increased from 11 to 110 thousand hectares. This situation has resulted from the rapidly increasing demand for vegetable oil in food and other industries. The largest area sown with oilseed rape and the highest yields are produced in the regions of Central Lithuania. In order to secure abundant and high-quality rapeseed yield, several crop cultivation and management

issues, among which diseases caused by pathogens are the most relevant ones, have to be solved.

Various literature sources provide information on the incidence and severity of *Alternaria* blight (*Alternaria brassicae*) in Canada (Tewari 1991), India (Kolte et al. 1987), and Bangladesh (Meah et al. 1988). In European countries involved in oilseed rape production this disease is also very common – such evidence was obtained in Poland (Frencel et al. 1991; Mączyńska et al. 2001; Sadowski et al. 2003), France (Brun et al. 1987), Great Britain (Fitt et al. 1999). It is noteworthy that *Alternaria* blight is a serious problem in Denmark and Switzerland, however, it is not relevant in Sweden, Germany or the Netherlands (Bromand 1990). Weather conditions, especially air temperature and precipitation, have a great effect on *Alternaria* blight severity in different years. When the air temperature varies within optimal range (15–23°C), precipitation rate and relative air humidity during the silique ripening period have a decisive effect (Awasthi and Kolte 1989; Hong et al. 1996).

Phoma stem canker (*Phoma lingam*, anamorph of *Leptosphaeria maculans*) is often found in oilseed rape crops (McGee and Petrie 1978; Hall et al. 1993). Many researchers have reported that *Phoma lingam* comprises at least two groups differing in virulence – A – very virulent or “aggressive”, or Tox⁺ and B – weakly virulent, or “non-aggressive”, or Tox⁰. Recently these groups have been named as *Leptosphaeria maculans* (A-group) and *Leptosphaeria biglobosa* (B-group), on the basis of length of neck of pseudothecia (Shoemaker and Brun 2001). The disease is more widespread in Australia, Canada, England, France, and Germany – large oilseed rape producers, where weather conditions are conducive to the occurrence of this disease (West et al. 2001). In our neighbouring countries – Poland, Denmark, Sweden, this disease is widespread but is not very destructive (Jedryczka et al. 1999; Jensen 1994; Kuusk et al. 2002). In the last years the fungus population gradually changes to more aggressive pathotype (Karolewski et al. 2002). The disease spreads during the entire oilseed rape growing period, however, lesions on the crown develop when winter rape plants are infected in the autumn of the sowing year. Long, warm and wet autumns and mild winters are necessary for this.

One of the ways to control plant diseases is to cultivate resistant varieties. Some researchers' findings revealed the differences in disease resistance among oilseed rape varieties (Tewari and Skoropad 1976; Garbe 1993), they have also reported that *Alternaria* blight is more likely to occur on early-ripening varieties (Sweet and Beale 1991). However, in many authors' research it is noted that all the tested varieties of winter and spring rape and turnip rape were susceptible to *Alternaria* blight (Ramsbottom and Thomas 1999).

Alternaria blight is especially dangerous if it spreads early and affects young siliques and seed. The disease-affected seed is generally small, wrinkled and infested not only with *Alternaria*, but also with the fungi of other genera (Howliger et al. 1991). Seventy species of fungi assigned to thirty-six genera have been found to spread with rapeseed (Clear and Patrick 1995).

Based on research conducted at the Lithuanian Institute of Agriculture some data have been already published on spread and harmfulness of fungal diseases and efficacy of their control measures (Brazauskiene 1998; Brazauskiene and Petraitiene 2003; 2004). This research was designed to identify the main fungal diseases in winter and spring rape crops grown in Central Lithuania, to estimate their incidence and severity on stems and siliques and to identify fungi on harvested rape seeds.

MATERIALS AND METHODS

Incidence and severity of fungal diseases

During the period 2001–2003 surveys were conducted in the crops of winter and spring rape in the fields of agricultural companies and individual farmers. The experiments covered four Central Lithuania's districts: Kėdainiai, Jonava, Panevėžys, and Raseiniai. During the experimental period, stem and silique samples were collected from the total winter rape crop area of 1011 ha and spring rape crop area of 927 ha. The samples were taken at the end of ripening stage (BBCH 85–87) of rape crops. Growth stages were determined according to the scale described by Lancashire et al. (1991). During sampling the following data were entered into the questionnaire: district, farm, variety, previous-crop, sowing date, seed rate, plant protection products used, application timing, etc. The main data on winter rape crops are provided in Table 1, on spring rape crops in Table 2.

Table 1. The origin of winter oilseed rape crops assessed

Field code	District	Cultivar	Year	Previous crop
KKA011W	Kėdainiai	Kasimir	2001	black fallow
KKA012W	Kėdainiai	Kasimir	2001	black fallow
KAC013W	Kėdainiai	Accord	2001	black fallow
JAC011W	Jonava	Accord	2001	winter rye
JAC012W	Jonava	Accord	2001	winter wheat
KCA014W	Kėdainiai	Casino	2001	black fallow
PKA011W*	Panevėžys	Kasimir	2001	winter barely
KKA021W	Kėdainiai	Kasimir	2002	winter wheat
KKA022W	Kėdainiai	Kasimir	2002	black fallow
KAC023W	Kėdainiai	Accord	2002	black fallow
KAC024W	Kėdainiai	Accord	2002	black fallow
JAL021W	Jonava	Alaska	2002	winter barely
PPA021W	Panevėžys	Pantera	2002	winter barely
PAL022W*	Panevėžys	Alaska	2002	potatoes
KAL025W*	Kėdainiai	Alaska	2002	black fallow
KAL026W*	Kėdainiai	Alaska	2002	winter wheat
KKA031W	Kėdainiai	Kasimir	2003	black fallow
KAC032W	Kėdainiai	Accord	2003	black fallow
PCA031W	Panevėžys	Casino	2003	black fallow
PAL032W	Panevėžys	Alaska	2003	black fallow
PAT033W	Panevėžys	Attila	2003	oats
PKR034W	Panevėžys	Krokus	2003	black fallow
PMA035W	Panevėžys	Mazur	2003	black fallow
RCA031W*	Raseiniai	Casino	2003	grasses

* fungicide applied at the end of flowering stage

Table 2. The origin of spring oilseed rape crops assessed

Field code	District	Cultivar	Year	Previous crop
KSP011S	Kedainiai	Sponsor	2001	winter wheat
KSP012S	Kedainiai	Sponsor	2001	spring barely
JST011S	Jonava	Star	2001	winter wheat
JFO012S	Jonava	Forte	2001	spring barely
RAN011S	Raseiniai	Anita	2001	winter rye
PSP011S*	Panevezys	Sponsor	2001	winter wheat
KST013S*	Kedainiai	Star	2001	fallow
KSP021S	Kedainiai	Sponsor	2002	winter rye
KSP022S	Kedainiai	Sponsor	2002	buckwheat
KST023S	Kedainiai	Star	2002	spring rape
JOL021S	Jonava	Olga	2002	spring barely
JOL022S	Jonava	Olga	2002	spring barely
PFO021S	Panevezys	Forte	2002	spring barely
KSP031S	Kedainiai	Sponsor	2003	spring barely
KSP032S	Kedainiai	Sponsor	2003	spring barely
KSP033S	Kedainiai	Sponsor	2003	spring barely
KSP034S	Kedainiai	Sponsor	2003	spring barely
JSP031S	Jonava	Sponsor	2003	spring barely
KSP035S	Kedainiai	Sponsor	2003	spring barely
KST036S	Kedainiai	Star	2003	spring barely
JSE032S	Jonava	Senator	2003	spring barely
RST031S*	Raseiniai	Star	2003	spring barely

* fungicide applied at the end of flowering stage

To estimate the incidence and severity of fungal diseases stem samples were pulled at representative places of a field (10 stems per place, in total 100 stems per field). Silique samples were also picked – 5 earliest-formed (lower) siliques from the main stem of 50 plants (in total 250 siliques per field). All stem and silique samples were analysed in the laboratory. Fungal diseases were diagnosed according to external symptoms using descriptors. The number of fungal disease-affected stems and siliques was determined. *Alternaria* blight severity on stems and siliques was estimated according to the scale described by Conn et al. (1990) (0, 1, 5, 10, 20, 30 and 50% of stem or silique surface area is covered by *Alternaria* blight spots).

The severity of *Phoma* stem canker was estimated according to 0–4 score scale, where 0 – stem without visible *Phoma* stem canker symptoms, 1 – *Phoma* dry rot covers less than half of the root neck perimeter, 2 – the rot covers more than half of the perimeter, but root neck is incompletely girdled by *Phoma* rot, 3 – root neck is completely girdled by *Phoma* rot but the plant is not broken, 4 – root neck is completely girdled by *Phoma* rot, the plant is broken at *Phoma* stem canker lesion place, and is precociously mature (Chigogora and Hall 1995).

Average incidence of *Alternaria* blight and Phoma stem canker (the number of stems or siliques with *Alternaria* blight or Phoma stem canker symptoms expressed in per cent from the total number of stems and siliques tested) was calculated for each sample. Average severity of *Alternaria* blight was calculated according to the formula: $R_{jd} = \sum(nxb)/N$, where R_{jd} - average *Alternaria* blight severity per cent, (nxb) - number of stems or siliques multiplied by *Alternaria* blight severity per cent, N - total number of stems or siliques per sample. Average Phoma stem canker severity score was calculated according to the following formula: $R_f = (nx1 + nx2 + nx3 + nx4)/N$, where R_f - average Phoma stem canker severity score, n - number of stems according to Phoma stem canker severity score, N - total number of stems estimated per sample.

The data from agrometeorological bulletins were used to describe weather conditions of the experimental years. Figures 1 and 2 provide the total amount of precipitation in mm and days with precipitation of 1 mm and more during the period of intensive *Alternaria* blight spread on winter and spring oilseed rape stems and siliques. (Tabs. 1, 2).

Fungi occurring on harvested seed

Silique samples were threshed and seed samples were prepared for the determination of fungi. Abundance and diversity of fungi on seed (internal infection) were determined on PDA (Fluka, Switzerland) in Petri dishes. The seeds were surface-sterilised by soaking in 70% ethyl alcohol for 30 sec, rinsed in sterile water, drained on sterile filter paper and placed in a sterile box on the medium, 250 seeds per sample were sown (10 plates/replications \times 25 seeds). The plates were incubated for 7 days at 20°C in a thermostat Binder (Germany), with a 12 h light/dark cycle. After 7 days colonies of fungi were identified using a microscopy method and genera of fungi were discriminated using descriptors (Malone and Muskett 1997).

The experimental data were compared using the analysis of variance (ANOVA). Duncan's Multiple Range Test was used to determine significant differences between means and calculated for $p = 0.05$. The experimental findings were processed by the correlation regression analysis method.

RESULTS AND DISCUSSION

Incidence and severity of fungal diseases

***Alternaria* blight** (*Alternaria* spp.) incidence and severity on stems and siliques in winter and spring rape was diverse in different years, varied considerably between crops within the same experimental year and between the crops of the same variety within the same experimental year. The development of the fungus *Alternaria brassicae*, which is the major causal agent of *Alternaria* blight, is equally influenced by the air temperature and moisture. The optimal temperature for penetration of *Alternaria* spp. and lesion development is 15–25°C (Mridha and Wheeler 1993). Since mean daily air temperature during the period of *Alternaria* blight occurrence on winter rape stems and siliques (in July) and on spring rape stems and siliques (in August) ranged between optimal limits (17–21°C) during the experimental years, we gave a greater attention to the analysis of the amount of precipitation and the number of days with a precipitation rate of 1 mm and more.

Table 3. Incidence and severity of *Alternaria* blight and *Phoma* stem canker in various fields of winter oilseed rape in 2001–2003

Field code	Alternaria blight				Phoma stem canker	
	DI-S [%]	DS-S [%]	DI-P [%]	DS-P [%]	DI-S [%]	DS-S (b)
2001						
KKA011W	100	7.54	100	6.66	8.0	0.08
KKA012W	100	5.70	97.2	3.67	0	0
KAC013W	100	5.16	100	5.67	6.0	0.06
JAC011W	100	5.72	100	4.84	6.0	0.06
JAC012W	54.0	0.94	97.2	2.59	14.0	0.14
KCA014W	100	2.52	100	2.94	0.8	0.08
PKA011W*	56.0	0.62	87.2	1.02	4.0	0.04
Min–Max	54.0–100	0.62–7.54	87.2–100	1.02–6.66	0–14.0	0–0.14
2002						
KKA021W	34.0	0.34	12.0	0.12	4.0	0.04
KKA022W	38.0	0.54	34.4	0.38	8.4	0.08
KAC023W	48.0	0.48	61.2	0.64	12.0	0.12
KAC024W	62.0	0.94	34.8	0.35	8.0	0.08
JAL021W	80.0	1.20	90.8	1.83	6.0	0.06
PPA021W	52.0	0.60	35.2	0.38	6.0	0.06
PAL022W*	42.0	0.42	34.4	0.38	2.0	0.02
KAL025W*	22.0	0.22	38.8	0.42	6.0	0.06
KAL026W*	38.0	0.38	17.2	0.17	10.0	0.10
Min–Max	22.0–80.0	0.22–1.20	12.0–90.8	0.12–1.83	2.0–12.0	0.02–0.12
2003						
KKA031W	100	13.30	96.8	3.22	0	0
KAC032W	100	13.80	74.8	0.75	0	0
PCA031W	82.0	0.82	82.4	1.42	0	0
PAL032W	98.0	1.78	84.0	1.16	6.0	0.12
PAT033W	96.0	1.46	98.4	2.96	0	0
PKR034W	60.0	0.60	49.6	0.50	0	0
PMA035W	40.0	0.64	44.8	0.45	0	0
RCA031W*	72.0	0.80	11.2	0.11	6.0	0.12
Min–Max	40.0–100	0.60–13.8	11.2–96.8	0.11–3.22	0–6.0	0–0.12

* fungicide applied at the end of flowering stage

DI – disease incidence, DS – disease severity, S – stems, P – pods; (b) – score

In 2001 the incidence and severity of *Alternaria* blight were higher than in the other two experimental years (Tabs. 3, 4). The higher disease incidence and severity in the year 2001 were determined by the amount of rainfall in July – for winter rape and in August – at spring rape silique ripening period. The weather in July was rainy, the monthly precipitation rate in Central Lithuania's districts amounted to 91–145 mm, there were 12–16 days with precipitation 1 mm and more (Fig. 1). The highest daily precipitation rate in districts was 32–71 mm. The mean relative air humidity of July was 75–82%. In such weather conditions *Alternaria* blight spread on 54.0–100% of winter rape stems and on 87.2–100% of siliques; the maximum disease severity on stems amounted to 7.54, on siliques to 6.66% (Table 3).

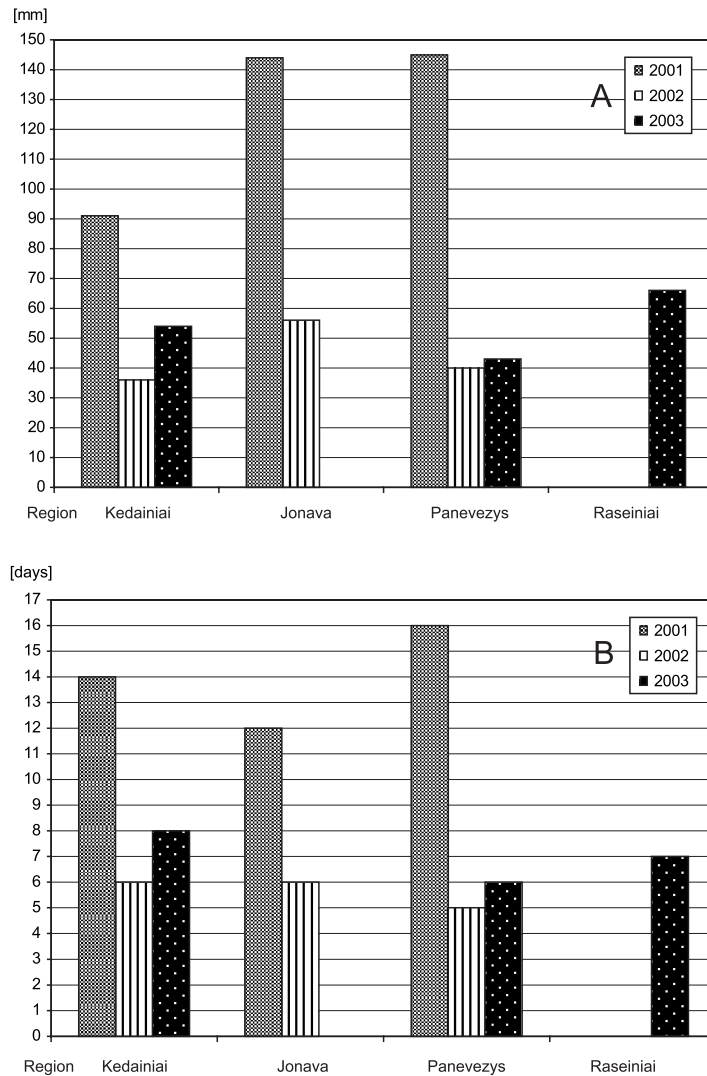


Fig. 1. The total sum of precipitation [mm] (A) and number of days with precipitation 1 mm and more (B) during the period of intensive *Alternaria* blight spread on winter oilseed rape (July)

Warm and rainy weather prevailed in August, too. The monthly precipitation rate was 55–72 mm and there were 8–10 days with a rainfall of 1 mm and more, and the largest daily precipitation rate amounted to 30–44 mm in districts (Fig. 2). Relative air humidity above 80% prevailed. When conditions were favourable, *Alternaria* blight affected 94.0–100% of spring rape stems and all siliques in all crops assessed. The maximum disease severity on stems was 18.6%, on siliques 7.24% (Table 4).

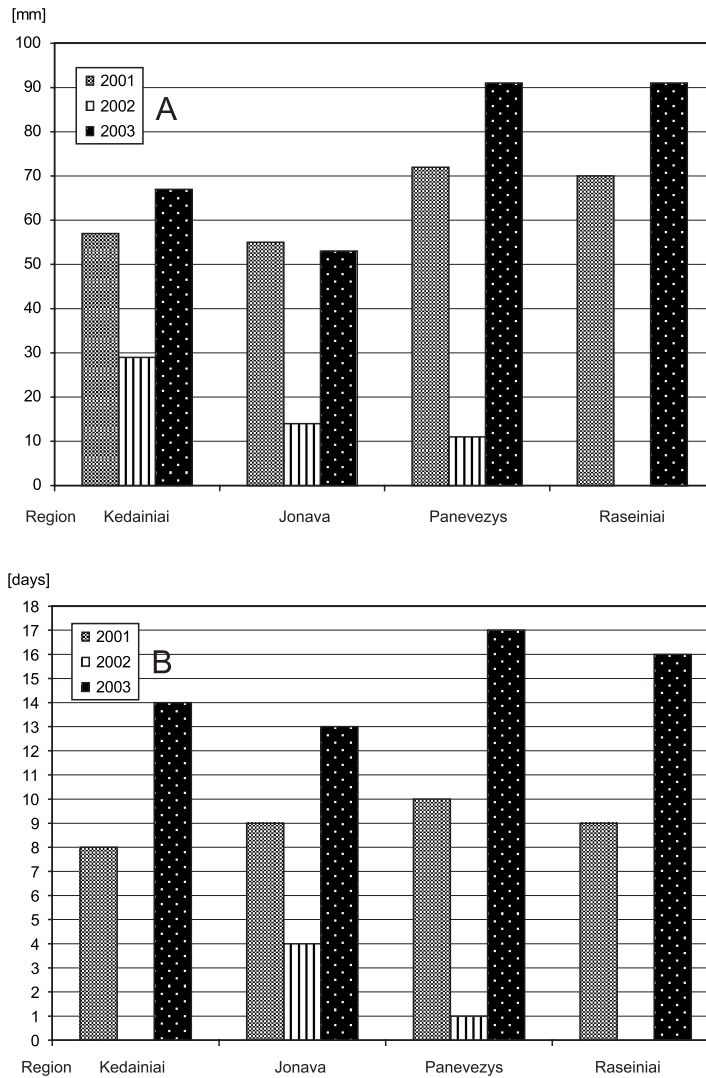


Fig. 2. The sum of precipitation [mm] (A) and number of days with precipitation 1mm and more (B) during the period of intensive *Alternaria* blight spread on spring oilseed rape (August)

In 2002 dry weather prevailed during more than half of July. In Kėdainiai, Panevėžys and Jonava districts the mean precipitation rate in July amounted to 36–56 mm, there were 5–6 days with a precipitation rate of 1 mm and more (Fig. 1). *Alternaria* blight in

seven of the nine winter rape assessed crops spread on 22.0–52.0% of stems and on 12.0–38.8% of siliques. The maximum disease severity on stems was 1.2%, on siliques – 1.83% (Table 3). Dry weather continued during August. The rate of precipitation was even lower than in July, as low as 11–29 mm and there were only 2–3 days with precipitation of 1 mm and more. Shortage of rainfall resulted in a very low relative air humidity 55–67%, which is uncommon for August. Due to a greater difference between the day-night temperatures, dew formed on plants at night, as a result, the disease spread on 63.2–98.8% of spring rape siliques, however its severity in most crops was very low and did not reach 2% of the silique surface area (Table 4).

Table 4. The incidence and severity of *Alternaria blight* and *Phoma stem canker* in various fields of spring oilseed rape in 2001–2003

Field code	<i>Alternaria blight</i>				<i>Phoma stem canker</i>	
	DI-S [%]	DS-S [%]	DI-P [%]	DS-P [%]	DI-S [%]	DS-S (b)
2001						
KSP011S	94.0	2.72	100	3.86	4.0	0.04
KSP012S	94.0	3.46	100	1.60	4.0	0.04
JST011S	100	17.70	100	5.62	48.0	0.50
JFO012S	100	14.50	100	3.68	88.0	1.04
RAN011S	100	18.20	100	7.21	98.0	1.40
PSP011S*	100	18.60	100	7.24	5.2	0.05
KST013S*	100	7.80	100	4.37	4.0	0.04
Min–Max	94.0–100	2.72–18.60	100	1.60–7.24	4.0–98.0	0.04–1.40
2002						
KSP021S	54.0	0.78	98.8	2.62	2.0	0.02
KSP022S	78.0	1.16	78.8	1.53	8.0	0.08
KST023S	30.0	0.30	63.2	0.71	0.8	0.01
JOL021S	70.0	0.70	67.6	1.12	2.0	0.02
JOL022S	26.0	0.26	74.4	0.85	2.0	0.02
PFO021S	30.0	0.30	98.4	3.90	2.0	0.02
Min–Max	26.0–78.0	0.26–1.16	63.2–98.8	0.71–3.90	0.8–8.0	0.01–0.08
2003						
KSP031S	40.0	0.40	34.8	0.35	0	0
KSP032S	100	3.90	27.6	0.35	0	0
KSP033S	24.0	0.24	14.0	0.14	34.0	0.52
KSP034S	72.0	1.36	26.0	0.26	22.0	0.46
JSP031S	34.0	0.42	67.6	0.71	0	0
KSP035S	64.0	0.64	42.4	0.42	8.0	0.16
KST036S	60.0	0.76	28.0	0.30	6.0	0.10
JSE032S	54.0	0.54	64.4	0.66	0	0
RST031S*	32.0	0.32	16.0	0.16	0	0
Min–Max	24.0–100	0.24–3.90	14.0–67.6	0.14–0.71	0–34.0	0–0.52

* fungicide applied at the end of flowering stage

DI – disease incidence, DS – disease severity, S – stems, P – pods; (b) – score

In 2003 during silique formation period the precipitation rate in Central zone was 43–66 mm, relative air humidity 73–82%. Such weather promoted more intensive spread and development of *Alternaria* blight on winter rape stems. The disease affected more than 80% of stems in five of the eight winter rape crops assessed. An especially high incidence of the disease was recorded in Kėdainiai district in the crops of Kazimir and Accord varieties where all stems were affected by a disease and the disease severity on stems was 13.3–13.8% (Table 3). Maximum disease severity on stems that year was by 11.5 times higher and on siliques by 1.8 times higher, compared with that in 2002. In August due to the cooler weather (mean monthly air temperature 16.7–17.3°C), and due to very uneven distribution of precipitation, the spread of *Alternaria* blight was significantly slowed down and differed in particular spring rape crops. The disease spread on 24.0–100% of stems and on 14.0–67.6% of siliques. The maximum disease severity on stems in spring rape crops in 2003 was 4.8 times lower than that in 2001 and 3.4 times higher than that in 2002, on siliques – about 10.2 times lower than that in 2001 and 5.5 times lower than in 2002. The incidence of *Alternaria* blight on stems that year was 1.5 times, and disease severity up to 5.5 times higher than on siliques.

The incidence and severity of **Phoma stem canker** on stems and siliques in winter and spring rape crops varied in different years. In winter rape Phoma stem canker spread inappreciably, in 2001 in separate crops this disease affected up to 14.0%, in 2002 up to 12.0% of stems. In 2003 the incidence of Phoma stem canker was even lower, the stems affected by the disease were identified only in two of the eight winter rape crops assessed, and the disease did not occur on siliques at all (Table 3).

In 2001 in Jonava district in spring rape crops of Star and Forte varieties the stems with Phoma stem canker symptoms accounted for 48.0–88.0%. In Raseiniai district in the crops of Anita variety 98.0% of plants were found to be affected with Phoma stem canker. In 2003 in Kėdainiai district in two crops of Sponsor variety the percentage of plants affected by the disease accounted for 22.0–34.0%. In the other spring rape crops the disease was identified on less than 8.0% of the plants during 2001–2003 (Table 4). The high disease incidence in some fields suggests that the cause of infection could be *L. biglobosa*, not *L. maculans* and more comprehensive research on the population structure of *Phoma lingam* in Lithuania is required.

In all experimental years other fungal pathogens such as *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Peronospora parasitica* did not occur in winter and spring rape crops.

Our experimental findings suggest that both winter and spring rape crops were equally susceptible to *Alternaria* blight. No significant differences in susceptibility to this disease were identified between different varieties. Black fallow and winter cereals prevailed among preceding crops for winter rape, and spring cereals for spring rape. All these crops are not host-plants for the pathogens common on rape, therefore they could not affect the occurrence of rape diseases. The information collected during the trials on winter and spring rape crops suggests that disease control products were rather rarely used in rape crops. During the three experimental years only 5 winter rape crops out of the total 24 winter rape crops observed (about 20% of the crops) were sprayed with tebuconazole at the end of flowering. Disease control products were applied only in 3 spring rape crops out of the 22 spring rape crops observed (13.6%).

Fungi on harvested seeds

In 2001, the year conducive to *Alternaria* blight development on siliques, **winter rape** seed was heavily infested with various fungi (Table 5). The total content of affected seed (with internal infection) in the samples taken from winter rape crops ranged from 82.8 to 87.2%. The fungi of *Alternaria* genus accounted for the largest share (82.4–86.4%) among the fungi identified on winter rape seed.

Table 5. Composition of fungal communities on harvested seed of winter oilseed rape in 2001–2003

Field code ¹	Infected seed [%]	Infected seed (internal infection) [%]					
		<i>Alternaria</i> spp.	<i>Cladosporium</i> spp.	<i>Botrytis</i> spp.	<i>Penicillium</i> spp.	<i>Mucor</i> spp.	others
2001							
KKA011W	84.8	83.6 ab ²	21.2 a	0 a	7.6 bc	0	4.8 ab
KKA012W	83.2	82.4 a	21.6 a	0 a	3.6 a	0	3.2 a
KAC013W	87.2	86.4 b	20.0 a	2.0 ab	9.2 c	0	4.8 ab
JAC011W	85.6	84.8 ab	21.6 a	0.4 a	4.4 ab	0	6.0 b
JAC012W	83.6	82.8 ab	28.0 a	2.8 b	4.8 ab	0	4.8 ab
KCA014W	86.8	86.0 ab	20.0 a	0 a	4.0 ab	0	3.6 ab
PKA011W*	82.8	82.8 ab	20.0 a	0 a	4.8 ab	0	4.8 ab
2002							
KKA021W	28.8	27.6 c	13.6 ab	0	0.8 a	0.4 ab	1.6 a
KKA022W	36.8	24.4 bc	9.6 ab	0	2.8 abc	1.2 ab	2.4 a
KAC023W	34.2	34.0 c	17.2 b	0	4.4 bcd	0.8 ab	1.2 a
KAC024W	28.0	26.4 c	11.2 ab	0	7.2 de	1.6 b	2.8 a
JAL021W	27.6	24.0 bc	12.0 ab	0	1.2 ab	0 a	0.8 a
PPA021W	30.4	29.6 c	4.4 a	0	3.2 abc	1.2 ab	1.6 a
PAL022W*	10.0	8.4 a	3.6 a	0	8.8 ef	1.6 b	0.8 a
KAL025W*	31.6	31.6 c	12.4 ab	0	9.2 ef	0.8 ab	6.4 b
KAL026W*	20.4	10.0 ab	6.8 a	0	11.6 f	1.6 b	3.2 a
2003							
KKA031W	100	75.6 e	24.0 de	0 a	5.2 abc	0 a	18.0 d
KAC032W	40.0	17.2 a	9.6 ab	0 a	9.2 c	0.4 a	12.0 bc
PCA031W	45.2	20.4 ab	16.0 bc	0 a	6.8 bc	5.2 b	14.8 cd
PAL032W	53.6	26.0 b	26.8 de	0.4 ab	4.0 ab	0 a	18.4 d
PAT033W	90.0	61.6 d	26.8 de	0 a	4.2 ab	0.4 a	31.2 e
PKR034W	98.4	15.2 a	7.6 a	0 a	86.8 e	0 a	4.4 a
PMA035W	100	49.6 c	29.6 e	1.6 c	36.0 d	0 a	4.0 a
RCA031W*	32.8	14.0 a	20.0 cd	0.4 ab	2.4 a	0 a	7.6 ab

* fungicide applied at the end of flowering stage

¹ detailed description of the crop is provided in Table 1

² means in columns for each year followed by the same letter are not significantly different according to Duncan's multiple range test ($p = 0.05$)

In 2002, when *Alternaria* blight severity on siliques was very low, winter rape seed infested with various fungi made up 10.0–36.8%. The fungi of *Alternaria* genus affected 8.4–34.0% of winter rape seed. In 2003 seed samples from the two winter rape crops 100% of seed were infected, in another two samples the affected seed accounted for 90–98.4%. That year the greatest differences in the contents of affected and healthy seed between separate winter rape crops were determined (the content of healthy seed between separate crops ranged from 0 to 67.2%, the content of seed affected by various fungi – from 32.8 to 100%). The fungi of *Alternaria* genus affected from 14.0 to 75.6% of winter rape seed.

The highest infestation with the fungi of *Cladosporium* genus was identified on winter rape seed in 2001 and 2003, 20.0–28.0%, and 7.6–29.6%, respectively. The fungi of *Botrytis* genus were found only in sporadic cases, and in 2002 were not identified at all.

Mould fungi (*Penicillium* spp., *Mucor* spp.) cause seed rot in storages. When rape-seed ripens in rainy weather or when underdried seed is stored, storage fungi become more active. The heaviest infestation of *Penicillium* and *Mucor* fungi on winter rape seed was identified in 2003, in separate cases as much as 86.8% of seed was infested with *Penicillium* spp.

Both the seed of spring and winter rape was especially heavily infested with the fungi of *Alternaria* genus in 2001. The highest disease severity on **spring rape** siliques was identified the same year. The total content of spring rape seed affected by fungi in the samples amounted to 82.4–93.6%, seed contaminated by *Alternaria* spp. accounted for 78.0–90.4% (Table 6). In 2002, the year unfavourable for the occurrence of *Alternaria* blight on siliques, the spring rape seed were slightly infected with the fungi of *Alternaria* genus (affected seed accounted for 5.6–30.8%). In 2003 the seed of spring rape contaminated by various fungi amounted to 37.6–95.2% and the seed contaminated by *Alternaria* spp. amounted to 16.0–62.4%. Fungicide application in the field at the end of flowering reduced the occurrence of *Alternaria* spp. on harvested seed of winter and spring rape, but not in all cases. In 2001 and 2003 spring rape seed was more heavily infested with the fungi of *Cladosporium*, *Penicillium* and other genera. The level of *Botrytis* and *Mucor* fungal infestation on both spring and winter rape seed was low during all experimental years.

The data obtained suggest that infection of winter and spring rape seed with the fungi of *Alternaria* spp. may vary considerably and in most cases it depends on *Alternaria* blight incidence and severity on siliques. Similar findings were also obtained by other researchers (Verma and Saharan 1994). Infestation by storage fungi and other pathogens is likely to be more severe on rape seed in the years unfavourable for *Alternaria* blight development on siliques (Portenko 1997).

It is known that rape seed affected by *Alternaria* spp. is a rather important source of *Alternaria* blight infection (Humpherson-Jones 1989). Our research findings on fungal diversity and abundance on harvested rape seed also suggest that causal agents of *Alternaria* blight, the fungi of *Alternaria* spp., spread abundantly with rape seed. Various fungi occurring on rape seed are a great concern for vegetable oil producers. The fungi of *Alternaria* and *Penicillium* genus are mentioned in many literature sources as producers of mycotoxins, therefore there exists a risk that vegetable oil can be contaminated with mycotoxins (McKenzie et al. 1988; Pedras et al. 1999).

Correlation – regression analysis of data suggests that with increasing *Alternaria* blight severity on winter and spring rape siliques the content of seed affected by the

Table 6. Composition of fungal communities on harvested seed of spring oilseed rape in 2001–2003

Field code ¹	Infected seed [%]	Infected seed (internal infection) [%]					
		<i>Alternaria</i> spp.	<i>Cladosporium</i> spp.	<i>Botrytis</i> spp.	<i>Penicillium</i> spp.	<i>Mucor</i> spp.	other
2001							
KSP011S	86.8	85.2 ab ²	28.4 a	0.8 a	2.0 a	0 a	4.8 bc
KSP012S	86.0	85.2 ab	20.4 a	0.4 a	3.2 ab	0 a	1.2 a
JST011S	82.4	78.0 a	24.8 a	0 a	1.6 a	0 a	5.2 bcd
JFO012S	91.6	90.4 b	25.6 a	1.2 a	1.2 a	2.4 b	6.8 bcd
RAN011S	90.4	90.4 b	21.6 a	0.8 a	2.4 a	0 a	3.6 ab
PSP011S*	92.4	88.4 b	21.2 a	0.8 a	2.0 a	0 a	7.6 cd
KST013S*	93.6	89.6 b	24.4 a	0.4 a	5.2 b	0 a	8.4 d
2002							
KSP021S	56.0	21.2 b	15.2 b	2.4 b	15.2 b	0.4 a	4.0 a
KSP022S	20.0	6.8 a	4.0 a	0 a	1.6 a	3.6 abc	6.8 a
KST023S	24.0	8.4 a	7.2 a	0 a	4.0 a	2.0 ab	6.8 a
JOL021S	16.0	7.2 a	5.2 a	0 a	3.6 a	0 a	4.0 a
JOL022S	24.0	5.6 a	3.6 a	0 a	6.0 a	6.4 bc	5.6 a
PFO021S	72.0	30.8 c	24.8 c	0 a	17.2 b	0 a	6.0 a
2003							
KSP031S	54.0	36.4 c	27.6 cde	0 a	7.6 bc	0 a	12.8 ab
KSP032S	95.2	61.6 e	20.4 bcd	0 a	4.4 ab	4.4 b	20.0 de
KSP033S	93.2	23.2 b	15.6 ab	0 a	5.2 abc	0.4 a	8.8 a
KSP034S	88.4	62.4 e	26.0 cde	0 a	17.2 e	0 a	15.6 bc
JSP031S	71.2	52.0 d	28.4 de	0 a	11.6 d	0 a	14.4 b
KSP035S	54.8	31.2 c	25.2 bcde	0 a	6.0 abc	0 a	16.4 bcd
KST036S	49.2	21.6 ab	18.0 abc	0 a	3.2 a	0 a	18.8 cde
JSE032S	92.4	60.0 e	30.8 e	0.8 b	3.2 a	0.8 a	14.8 bc
RST031S*	37.6	16.0 a	10.4 a	0 a	6.0 abc	0 a	14.0 b

* fungicide applied at the end of flowering stage

¹ detailed description of the crop is provided in Table 1

² means in columns for each year followed by the same letter are not significantly different according to Duncan's multiple range test ($p = 0.05$)

fungi of *Alternaria* spp. increases. The variation of *Alternaria*-affected seed content in winter (A) and spring (B) rape in relation to *Alternaria* blight severity on siliques was mathematically described by linear regression equations (Fig. 3). The calculations showed a strong correlation ($R = 0.769^{**}$ and $R = 0.723^{**}$) between *Alternaria* blight severity on siliques and the content of seed affected by *Alternaria* fungi for winter and spring rape. Both correlations were significant.

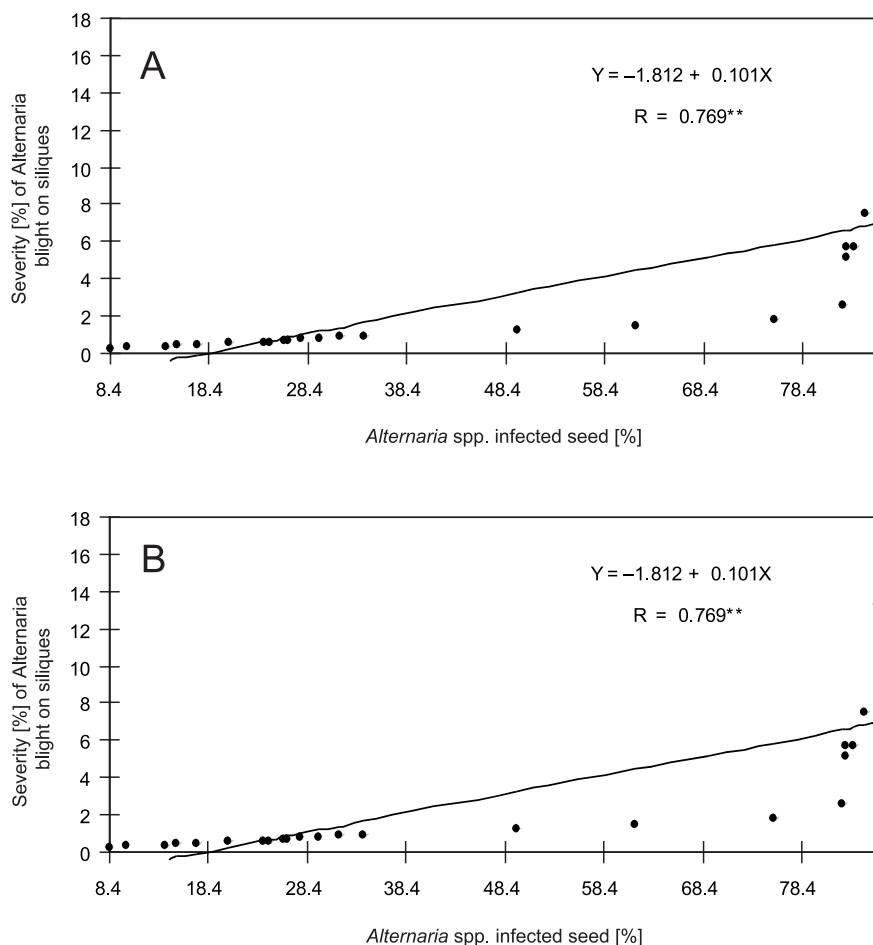


Fig. 3. The relationship between severity [%] of Alternaria blight on siliques (Y) and *Alternaria* spp. infected seed [%] (X) of winter (A) and spring (B) rape

CONCLUSIONS

1. The analysis of phytosanitary state of winter and spring rape crops during ripening stage over the period 2001–2003 suggests that two fungal diseases were widespread – Alternaria blight (*Alternaria brassicae*) and Phoma stem canker (*Phoma lingam*). Winter and spring rape were found to be equally susceptible to Alternaria blight. The year 2001 was the most conducive to the occurrence of Alternaria blight due to more abundant precipitation than in the other two years during winter and spring rape silique ripening stage.
2. Alternaria blight was identified on stems and siliques in all winter and spring rape crops in all experimental years. Alternaria blight severity on winter rape stems during the three experimental years was up to 13.80%, on siliques up to 6.66%, on spring rape stems up to 18.60%, on siliques up to 7.24%.

3. The incidence of Phoma stem canker during the three experimental years was higher on spring rape than on winter rape: the number of Phoma stem canker-affected spring rape stems ranged from 0 to 98.0%, and that of winter rape stems from 0 to 14.0%. The maximum disease severity on spring rape stems was estimated to amount to 1.4 severity score, and that on winter rape stems – to severity score 0.14.
4. During the three experimental years the total content of fungi-affected winter rape seed in the samples composed of the seed from farm-scale trials ranged from 10.0 to 100%, and that of spring rape from 16.0 to 93.6%. *Alternaria* spp. prevailed among the fungi identified on winter and spring rape seed: the content of winter rape seed affected by *Alternaria* spp. was 8.4–86.4%, and that of spring rape seed 5.6–90.4%. The fungi of *Cladosporium* genus affected up to 29.6% of winter and spring rape seed.

ACKNOWLEDGEMENTS

The present study was supported by the Lithuanian State Science and Studies Foundation, project MIKOTOKSINAS and project „Investigation of air-borne spore concentration of *Phoma*, *Alternaria* and *Drechslera* fungi using a Burkard spore suction trap” Registration No. T-05182.

REFERENCES

- Awasthi R.P., Kolte S.J. 1989. Variability in *Alternaria brassicae* affecting rapeseed and mustard. Indian Phytopath. Abstr. 42, p. 275.
- Brazauskiene I. 1998. Fungal diseases of winter oilseed rape and the possibilities to reduce their harmfulness. Agric. Sci. Articles 62: 132–141.
- Brazauskiene I., Petraitiene E. 2003. Dynamics of *Alternaria* blight (*Alternaria* spp.) spread on spring rape leaves and siliques and variation of the disease parameters under the effect of prochloraz and tebuconazole in relation to application time. J. Plant Protection Res. 43: 313–323.
- Brazauskiene I., Petraitiene E. 2004. Disease incidence and severity of phoma stem canker (*Phoma lingam*) on winter oilseed rape (*Brassica napus* L.) in Lithuania as affected by different prochloraz and tebuconazole application times. Z. PflKrankh. PflSchutz. 111: 439–450.
- Bromand B. 1990. Diversities in oilseed rape growing within the western palaeartic regional section. IOBC/WPRS Bull. 13 (4): 7–31.
- Brun H., Pleassis J., Renard M. 1987. Resistance of some crucifers to *Alternaria brassicae* (Berk.) Sacc. 7th International Rapeseed Congr., 11–16 May, Poznań, Poland: 1222–1227.
- Chigogora J.L., Hall R. 1995. Relationships among measures of blackleg in winter oilseed rape and infection of harvested seed by *Leptosphaeria maculans*. Can. J. Plant Pathol. 17: 25–30.
- Clear R.M., Patrick S.K. 1995. Frequency and distribution of seed borne fungi infecting canola seed from Ontario and Western Canada 1989 to 1993. Can. Plant Dis. Sur. 75: 9–17.
- Conn K.L., Tewari J.P., Awasthi R.P. 1990. A disease assessment key for *Alternaria* black spot in rape seed and mustard. Can. Plant Dis. Sur. 70: 19–22.
- Fitt B.D.L., Gladders P., Sutherland K.G., Turner J.A., Welham S.J. 1999. Epidemiology, forecasting and management of winter oilseed rape diseases in the UK. Proc. 10th Intern. Rapeseed Congr., September 26–29. Canberra, Australia. Available from <http://www.regional.org.au/au/gcirc/index.htm>
- Frencel I., Lewartowska E., Jedryczka M. 1991. The spectrum and severity of fungal diseases in field infections of winter oilseed rape in Poland. A review of the 1980s. IOBC/WPRS Bull. 14: 137–140.

- Garbe V. 1993. Effects of fungicide treatments in different varieties of winter rape. IOBC/WPRS Bull. 16 (9): 116–123.
- Hall R., Peters R.D., Assabgui R.A. 1993. Occurrence and impact of blackleg on oilseed rape in Ontario. Can. J. Plant Path. 15: 305–313.
- Hong C.X., Fitt B.D.L., Welhalm S.J. 1996. Effects of wetness period and temperature on development of dark pod spot (*Alternaria brassicae*) on oilseed rape (*Brassica napus*). Plant Pathol. 45: 1077–1089.
- Howlider M.A.R., Mean A.B., Jalaludin M., Rahman M.A. 1991. Effect of fungicides on *Alternaria* blight, yield and seed quality of mustard. Bangladesh J. Agri. 18: 127–132.
- Humpherson-Jones F.M. 1989. Survival of *Alternaria brassicae* and *Alternaria brassicicola* on crop debris of oilseed rape and cabbage. Ann. Appl. Biol. 115: 45–50.
- Jedryczka M., Fitt B.D.L., Kachlicki P., Lewartowska E., Rouxel T., Balesdent M.H. 1999. Comparison between Polish and UK populations of *Leptosphaeria maculans*, cause of stem canker of winter oilseed rape. Z. PflKrankh. PflSchutz. 106: 608–617.
- Jensen L.B. 1994. First record of ascomata of *Leptosphaeria maculans* on winter – grown oilseed rape in Denmark. Plant Pathol. 43: 759–761.
- Karolewski Z., Kosiada T., Hylak-Nowosad B., Nowacka K. 2002. Changes in population of *Leptosphaeria maculans* in Poland. Phytopathol. Pol. 25: 27–34.
- Kolte S.J., Awasthi R.P., Vishwanath. 1987. Assessment of yield losses due to *Alternaria* blight in rape-seed and mustard. Indian Phytopath. 40: 209–211.
- Kuus A.K., Happstadius I., Zhou I.A., Giese H., Dixelius C. 2002. Presence of *Leptosphaeria maculans* Group A and Group B Isolates in Sweden. J. Phytopath. 150: 349–356.
- Lancashire P.D., Bleiholder H., Van dem Boom T., Langeluddecke P., Staugs R., Weer E., Witzember A. 1991. A uniform decimal code for growth stages of crops and weeds. Ann. Appl. Biol. 119: 561–601.
- Malone J.P., Muskett A.E. 1997. Seed-Borne Fungi. Description of 77 Fungus Species. Ed. Sheppard J.W. ISTA, Zurich, Switzerland, 191 pp.
- Mączyńska A., Krzyżińska B., Drzewiecki S. 2001. Wpływ różnych terminów stosowania fungicydów na zdrowotność liuszczyń rzepaku ozimego. Prog. Plant Protection/Post. Ochr. Roślin 41: 638–642.
- McGee D.C., Petrie G.A. 1978. Variability of *Leptosphaeria maculans* in relation to blackleg of oilseed rape. Phytopathology 68: 625–630.
- McKenzie K.J., Robb J., Lennard J. 1988. Toxin production by *Alternaria* pathogens of oilseed rape (*Brassica napus*). Crop Res. 28: 67–81.
- Meah M.B., Howlider A.R., Alam M.K. 1988. Effect of fungicide spray at different time and frequencies on *Alternaria* blight of mustard. J. Agric. Sci. 21: 101–107.
- Mridha M.A., Wheeler B.E. 1993. In vitro effects of temperature and wet periods on infection of oilseed rape by *Alternaria brassicae*. Plant Pathol. 42: 671–675.
- Pedras M.S.C., Zaharia I.L., Smith K.C., Gai Y., Ward D.E. 1999. *Alternaria* blackspot phytotoxins: new strategies for determining specific disease resistance traits. Proc. 10th intern. Rapeseed congr., September 26–29. Canberra, Australia. Available from <http://www.regional.org.au/au/gcirc/index.htm>
- Portenko L.G. 1997. Vidovoy sostav vzbuditeley chernoy pjatnistosti rapsa i jego sorodichey v Tsentralnom Chernozemie. Proc. Conference “Nauchnoje nasledie P.P. Semenova-Tjan-Shanskogo i jego rol v razvitii sovremennoi nauki”. Lipetsk 2: 80–81.
- Ramsbottom J.E., Thomas J.E. 1999. Latest findings on variety and fungicide interreaction. Proc. 10th Intern. Rapeseed Congr., September 26–29. Canberra. Australia. Available from <http://www.regional.org.au/au/gcirc/index.htm>

- Sadowski C., Lukanowski A., Lenc L., Trzcinski J. 2003. Occurrence of dark leaf and pod spot on spring oilseed rape and fungi composition on harvested seeds depending on differentiated fertilization with sulphur. In 11th Intern. Rapeseed Congress (Denmark) 9 (18): 1103–1105.
- Shoemaker R.A., Brun H. 2001. The teleomorph of the weakly aggressive segregate of *Leptosphaeria maculans*. Can. J. Bot. 79: 412–419.
- Sweet J.B., Beale R.E. 1991. Disease resistance and fungicide response in oilseed rape cultivars. IOBC/WPRS Bull. 14 (6): 256–267.
- West J.S., Kharbanda P.D., Barbetti M.J., Fitt B.D.L. 2001. Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. Plant Pathol. 50: 10–27.
- Tewari J.P. 1991. Structural and biochemical bases of the blackspot disease of crucifers. Adv. Struct. Biol. 1: 325–349.
- Tewari J.P., Skoropad W.P. 1976. Relationship between epicuticular wax and blackspot caused by *Alternaria brassicae* in three lines of rapeseed. Can. J. Plant Sci. 56: 781–785.
- Verma P.R., Saharan G.S. 1994. Monograph on *Alternaria* Diseases of Crucifers. Agric. Canada Res. Branch Techn. Bull. 1994–6E, 162 pp.

POLISH SUMMARY

WYSTĘPOWANIE CZERNI KRZYŻOWYCH (*ALTERNARIA* SPP.) I SUCHEJ ZGNILIZNY KAPUSTNYCH (*PHOMA* LINGAM) NA RZEPAKU W CENTRALNEJ LITWIE I GRZYBY PASOŻYTNICZE NA ZEBRANYCH NASIONACH

W latach 2001–2003 przeprowadzono lustracje pól w przedsiębiorstwach rolniczych oraz gospodarstwach indywidualnych. Dla każdego pola sporządzono kwestionariusz opisujący stan uprawy. Występowanie i nasilenie chorób na rzepaku jarym i ozimym oceniano corocznie w 14–18 uprawach. Z każdego pola pobierano próby nasion, na których określano grzyby pasożytnicze. Czerń krzyżowych (*Alternaria brassicae*) na łuszczynach i sucha zgnilizna łodyg (*Phoma lingam*) występowały na wszystkich badanych polach oraz na wszystkich odmianach rzepaku jarego i ozimego. W 2001 roku najkorzystniejszym dla rozwoju chorób, czerń krzyżowych opanowała 87,2–100% łuszczyn rzepaku ozimego i 100% łuszczyn rzepaku jarego, a nasilenie choroby wynosiło odpowiednio 6,66 i 7,42%. Wszystkie odmiany rzepaku jarego i ozimego były wrażliwe na czerń krzyżowych.

Sucha zgnilizna łodyg wywoływana przez *Phoma lingam* była częściej spotykana na rzepaku jarym niż ozimym, a liczba roślin z objawami choroby dochodziła do 98%. Wszystkie badane próby nasion rzepaku były porażone. Zakażenie nasion rzepaku ozimego wynosiło 10–100%, natomiast zakażenie nasion rzepaku jarego wahało się w granicach 16,0–93,6%. Na nasionach najczęściej stwierdzano grzyby *Alternaria* spp. i *Cladosporium* spp.

