DYNAMICS OF *ALTERNARIA* BLIGHT (*ALTERNARIA* SPP.) SPREAD ON SPRING OILSEED RAPE LEAVES AND SILIQUES AND VARIATION OF THE DISEASE PARAMETERS UNDER THE EFFECT OF PROCHLORAZ AND TEBUCONAZOLE IN RELATION TO APPLICATION TIME

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Abstract: The objective of the present study was to identify the dynamics of *Alternaria* blight spread on spring oilseed rape lower, middle and upper leaves and siliques, to determine the disease incidence (DI) and severity (DS) on leaves, stems, siliques and seeds under the effect of prochloraz and tebuconazole. Efficiency of the fungicides was compared in relation to their application time. Field experiments with the spring oilseed rape cv. 'Star' were conducted at the Lithuanian Institute of Agriculture during 1997–1999.

Key words: oilseed rape, *Alternaria* blight, dynamics of disease spread, fungicides, application time, disease incidence and severity

INTRODUCTION

Alternaria blight caused by Alternaria spp. is a widespread and economically important disease on oilseed rape (Brassica napus) in many countries where this crop plays an important role in arable crop production (Daebeler and Amelung 1988; Frencel et al. 1991; Sutherland et al. 1990; Clear and Patrick 1995). Alternaria blight may affect siliques ripen earlier what will result in smaller and wrinkled seeds (Hardwick et al. 1989). Sometimes this disease can become a serious problem to both winter and spring oilseed rape. In Lithuania the area sown with rape currently accounts for 50–70 thousand ha, of which 90% is sown with spring oilseed rape. For this reason studies on Alternaria blight, which is one of the most important diseases of rape in this region, are of special relevance. Since all rape varieties are susceptible to this disease and the differences between the varieties are inappreciable

(Hardwick et al. 1991), studies on *Alternaria* blight control on spring oilseed rape using fungicidal treatments are essential.

This paper describes dynamics of *Alternaria* blight spread on spring oilseed rape lower, middle or upper leaves, disease incidence and severity on leaves, stems, siliques and seeds, and the efficacy of different fungicide regimes against *Alternaria* blight.

MATERIALS AND METHODS

Three field experiments were established in the rotation at the Plant Protection Department, Dotnuva in the Lithuanian Institute of Agriculture. Previous crop in 1997 were perennial grasses and winter wheat in 1998–1999. Soil was ploughed in the autumn. In the spring pre-sowing practices included harrowing, and fertilizing $(P_2O_5 - 70, K_2O - 100, N - 120 \text{ kg/ha})$. Spring oilseed rape (cv. Star) was sown on April 30th in 1997, on May 1st in 1998, on April 26th in 1999 at a rate of 7 kg/ha (150 seeds/m²). Seeds were treated with Rapcol FDL 323 CS (furathiocarb + metalaxyl + fludioxonil) using 0.97 kg a.i./100 kg of seeds. The herbicide 'Butisan 400' (metazachlor) was applied at the seedling stage at a rate of 0.8 kg a.i./ha. In 1999 herbicide Bladex 50 SC (cyanazine) at a rate of 0.2 kg a.i./ha was applied one week after the use of Butisan 400 to control *Sinapis arvensis* in the field. Insects (mainly *Meligethes aeneus*) were controlled using the following insecticides in 1997 – twice Fastac (alpha-cypermethrin) at a rate of 13 g a.i./ha and Karate 5 EC (lambda-cihalothrin) at a rate of 7.5 g a.i/ha, in 1998 and 1999 Karate 5 EC was used only once.

Efficiency of two fungicides: Sportak 45 EC (prochloraz) 0.675 kg a.i./ha and Folicur 250 EW (tebuconazole) 0.25 kg a.i./ha against *Alternaria* blight on the spring rape cv. 'Star' was investigated. Each year experimental plots were randomly designed, with 4 replications, plot size – 10×3 m, record plot – 10×2.2 m. The spray applications were made with a knapsack sprayer, using 400 l/ha of water. The time of spray applications against *Alternaria* blight was determined according to the disease incidence on spring rape leaves and siliques. The time of the first application – when the first spots appeared on lower leaves, the time of the second application- when the spots appeared on middle leaves, and the third application – when the spots on siliques. The date of the later treatment did not depend on the disease incidence, but was chosen according to the growth stage of rape plants – at the end of flowering. Oilseed rape plants on control plots were not treated with fungicides

The severity of *Alternaria* blight was identified according to the scale described by Conn et al., 1990. Assessments were performed weekly on 10 marked plants per plot on 2 lower, middle and upper leaves. Disease incidence (DI) and severity (DS) on pods were estimated by assessing 5 siliques on the primary stem of each marked plant (50 siliques per plot). Before harvesting a 30-silique sample was taken per plot to assess a number of seeds per silique and disease occurrence on seed per silique. Visually diseased, shrivelled, small seeds were regarded as affected by *Alternaria* spp. To evaluate the DI and the DS on stems (BBCH 85-87) we assessed the affected area of stems of marked plants.

1000-seed weight and seed yield were determined. Experimental data were subjected to the statistical analysis by using software ANOVA.

RESULTS

Experimental results show that *Alternaria* blight occurred on leaves, stems, siliques, and seeds of the spring rape cv. 'Star' in all experimental years. On diseased oilseed rape plants *Alternaria brassicae* was a dominant species. The DI and the DS varied considerably in different years, changed throughout the season and were highly dependent on the weather conditions.

Under Lithuanian climatic conditions spring oilseed rape starts flowering in the third decade of June - first decade of July. In all experimental years the first spots of Alternaria blight on the lower leaves were identified at the beginning of flowering stage (BBCH 63). In the middle of the flowering stage (BBCH 64-65) the first spots of disease appeared on middle leaves. In 1997 only four days elapsed from the appearance of the first spots on lower leaves to their appearance on middle leaves, whereas in 1998 this period lasted 17 days. In 1998 slow development of rape plants as well as that of Alternaria blight on lower leaves was affected by the weather conditions as June was characterized by extremely low precipitation level. However, heavy rainfall in July (2.8 times higher than long-term average) created favourable conditions for the spread of the disease also in later development stages of rape plants. The first symptoms of disease on the upper leaves of spring rape appeared at different times in different years i.e. in 1997 and 1998 at the beginning of the third decade of July, and in 1999 in the first decade of July. However, in all experimental years the symptoms appeared at the same growth stage BBCH 70-71 (Tab. 1).

In 1997, the year conducive to the spread of *Alternaria* blight on leaves, one week after appearance of the disease on lower leaves, the DI was as high as 90%, and the DS amounted to 6%. At the same time, as much as 42.5% of middle leaves were found to be disease-affected. Two weeks after the appearance of disease symptoms on lower leaves, all lower leaves had *Alternaria* blight spots, the DS was 8%, and the DI on middle leaves was 76.9%, while disease spots covered 2.7% of middle leaves surface. Two weeks after the appearance of first spots on middle leaves the first symptoms of the disease appeared on the upper leaves, and a week later the disease symptoms were present on 90% of upper leaves, and the DS was 5.4% (Tabs. 2, 3).

Year	Appearance			
	on lower leaves growth stage/date	on middle leaves growth stage/date	on upper leaves growth stage/date	Duration in days
1997	63/July 3	64/July 7	70/July 21	18
1998	63/June 22	65/July 9	70/July 23	31
1999	63/June 23	65/July 3	71/July 8	15

Table 1. Spread of Alternaria blight on spring oilseed rape leaves in the control

*period from the appearance of the first spots of *Alternaria* blight on lower leaves to the appearance of the spots on the upper leaves

Year 1997	Treatment*	Disease incidence in %								
		on lowe	r leaves	on	on middle leaves			on upper leaves		
		July 9	July 15	July 9	July 15		July 29			
	С	90.0	100	42.5	76.9		90.0			
	1 P	100	100	40.0	65.0		63.4			
	2 T	84.5	100	40.0	62.5		70.5			
	3 P	-	-		65.0		78.0			
	4 T	-	-	-	85.0		56.7			
1998		June 30	July 7	July 13	July 23		July 23	July 30	August 5	
	С	32.5	82.5	75.0	100		55.0	100	100	
	1 P	30.0	87.5	30.0	90.0		42.5	95.0	100	
	2 T	20.0	72.5	47.5	80.0		30.0	97.5	100	
	3 P	-	-	40.0	82.5		27.5	97.5	100	
	4 T	-		30.0	100		17.5	75.0	100	
1999		July 1	July 8	July 1	July 8	July 12	July 8	July 12		
	С	95.0	100	57.5	100	100	80.0	80.0		
	1 P	92.5	100	47.5	97.5	100	50.0	65.0		
	2 T	87.5	100	35.0	95.0	100	40.0	60.0		
	3 P	-	-	-	100	100	52.0	60.0		
	4 T	-	-	-	100	100	45.0	55.0		

Table 2. Dynamics of *Alternaria* blight spread on spring oilseed rape lower, middle and upper leaves as related to prochloraz and tebuconazole application time

*C – control, P – prochloraz, T – tebuconazole, 1, 2 – fungicides applied at the time of appearance of first disease spots on lower leaves (July 3, 1997/BBCH 63; June 22, 1998/BBCH 63; June 23, 1999/BBCH 63), 3, 4 – fungicides applied at the time of appearance of first disease spots on middle leaves (July 7, 1997/BBCH 64; July 9, 1998/BBCH 65; July 1, 1999/BBCH 65)

In 1998, although the first spots of *Alternaria* blight on lower leaves were identified 10 days earlier than in 1997, due to relatively unfavourable weather conditions the disease spread more slowly and the DS on lower and middle leaves was considerably lower than in 1997. Seventeen days after the first disease symptoms were identified on lower leaves, the first disease symptoms appeared on middle leaves, and two weeks later the disease spots were identified on upper leaves. However, at the end of silique development stage *Alternaria* blight was present on all upper leaves, and the DS was the highest (10.28%) of all experimental years (Tabs. 2, 3).

In 1999 the spread of *Alternaria* blight on spring oilseed rape leaves was the most rapid and most intensive. Within 2 weeks the disease spread from the lower leaves onto the middle and upper leaves. The highest DS on the lower leaves amounted to 25%, and on middle leaves the disease covered 13.6% of the leaf surface. However, in 1999 the DS on the upper leaves was the lowest (0.90%) of all experimental years (Tab. 3).

At the time of identification of the first disease spots on the lower leaves prochloraz and tebuconazole application did not have any significant effect on the spread of *Alternaria* blight on those leaves. However, the spray application reduced the DI on middle and upper leaves as well as the DS on the lower, middle and upper leaves. Prochloraz reduced *Alternaria* blight incidence on the lower leaves by 4.6%–50%, on middle leaves by 17.2%–72.2%, and on upper leaves by

V	Treatment*	Disease severity %								
Year		on lowe	r leaves	on	middle lea	ives	on	upper lea	ves	
1997		July 9	July 15	July 9	July 15		July 29			
	С	6.0	8.0	2.3	2.7		5.4			
	1 P	5.4	6.2	1.9	1.7		1.7			
	2 T	4.3	6.0	1.8	1.3		1.8			
	3 P	-	-	-	1.4		3.0			
	4 T	_	-	-	1.3		2.2			
1998		June 30	July 7	July 13	July 23		July 23	July 30	August 5	
	С	0.52	3.25	1.08	2.60		0.55	4.50	10.28	
	1 P	0.30	3.10	0.30	1.22		0.42	3.45	6.88	
	2 T	0.20	1.62	0.57	1.30		0.30	2.53	6.10	
	3 P	-	-	0.60	1.32		0.28	2.60	6.00	
	4 T	-	-	0.30	1.20		0.18	2.15	5.75	
1999		July 1	July 8	July 1	July 8	July 12	July 8	July 12		
	С	2.15	25.0	0.58	10.38	13.63	0.82	0.90		
	1 P	1.52	12.5	0.48	5.62	7.83	0.48	0.65		
	2 T	1.48	10.3	0.35	4.82	6.93	0.42	0.60		
	3 P	-	-	_	8.25	7.78	0.52	0.60		
	4 T	-	-	-	7.62	7.13	0.40	0.55		

Table 3. Severity of *Alternaria* blight on spring oilseed rape lower, middle and upper leaves in relation to prochloraz and tebuconazole application time.

* note - see table 2

23.3%–68.5%, tebuconazole – by 25.0%–61.5%, 21.7%–53.6% and 33.3%–66.7%, respectively. However, the fungicides did not prolong the life of spring rape lower leaves. At the end of flowering (1998) or middle of silique development stage (1997 and 1999) spring rape shed the lower leaves in all treated plots.

When the symptoms of *Alternaria* blight were identified on middle leaves, a later application of the fungicides in some cases resulted in the decrease of the DI on middle and upper leaves and in all cases – the decrease of the DS, compared with the control (Tabs. 2, 3). However, in many cases biological efficacy of prochloraz and tebuconazole was lower than 50%. In individual years at the end of silique development stage or beginning of ripening stage, spring rape shed middle leaves irrespectively of *Alternaria* blight severity.

The upper leaves of spring rape were shed at the same time on all plots. The time of leaf persistence on plants differed in separate years and was more dependent on the meteorological conditions rather than the DS on spring rape leaves.

The first symptoms of *Alternaria* blight on siliques in control plots were spotted at growth stage 79. The earliest appearance of disease on siliques was recorded in 1999 (July 12), while the latest – in 1998 (July 30). In all experimental years the spread of *Alternaria* blight on siliques (from the appearance of the first spots on siliques to full ripeness) lasted from 37 to 40 days. The year of 1998 was distinguished by a long period from the appearance of the first spots on the lower leaves to full ripeness, while in 1997 and 1999 this period was similar (Tab. 4).

The DI on spring rape siliques was above 90% in all experimental years. Neither early application of fungicides (after disease symptoms appeared on the lower

Year	Appearance of the first spots on siliques		P 11 (1999)	Duration in days		
	date	growth stage	Full ripeness (date)	А	В	
1997	July 26	79	September 1	37	60	
1998	July 30	79	September 7	40	77	
1999	July 12	79	August 18	37	56	

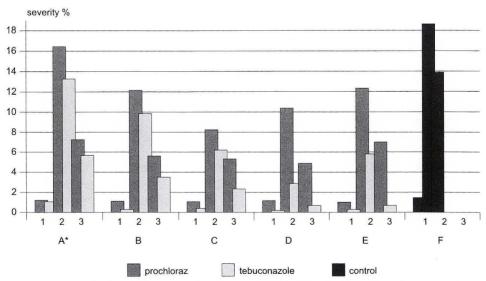
Table 4. Length of the period of *Alternaria* blight spread on spring oilseed rape siliques in the control plots

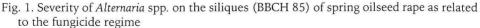
A – period from the appearance of the first spots of disease on siliques to full ripeness

B - period from the appearance of the first spots of disease on lower leaves to full ripeness

leaves) nor prochloraz application terms inhibited *Alternaria* blight spread on siliques. The latest terms of tebuconazole application had a slight suppressive effect on the DI on siliques.

The DS on spring rape siliques in the control plots was very diverse in different experimental years. In 1997, although the DS on the lower and upper leaves was medium and the disease spread on 92.5% of pods, the DS on siliques was very low, the lowest of all experimental years. In 1998, on the contrary, although the DS on lower and middle leaves was twice lower than in 1997, the DS on pods was the highest – 18.65%. Tebuconazole decreased *Alternaria* blight severity more efficiently than prochloraz, irrespectively of application time. Tebuconazole was the most effective in reducing the DS on siliques when applied at the end of flowering or even later – after appearance of the first spots of *Alternaria* blight on siliques (Fig. 1).





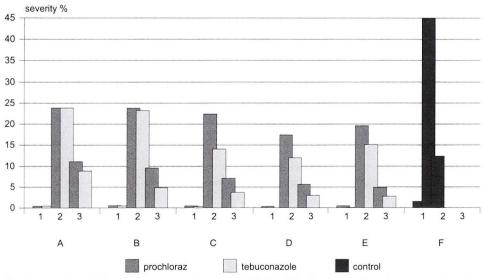
1 - 1997; 2 - 1998; 3 - 1999

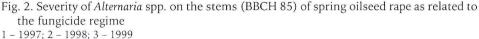
*Treatment performed at the time of appearance of first spots of *Alternaria* spp.: A – on lower leaves, B – on middle leaves, C – on upper leaves, D – on siliques, E – at the end of flowering, F – control (untreated)

In 1997 the DI on stems was low as only 40% of stems were affected on the control plots, while in 1998 and 1999 all stems of spring rape plants were affected. In 1997 the DS on rape stems as well as on siliques was the lowest. In 1998, when the climatic conditions were highly conducive to the spread of *Alternaria* blight during the silique ripening stage, the DS on rape stems was as high as 45.0% on the control plots, 30 times higher than in 1997. Prochloraz and tebuconazole sprayed at all application dates decreased the DS on rape stems, however, the best effect was achieved with later applications – at the end of flowering or after the appearance of *Alternaria* blight symptoms on siliques (Fig. 2).

In all experimental years the DI on seeds, i.e. the number of *Alternaria* blight-affected seeds per silique, was identified. No other diseases were identified on siliques, therefore all seeds with external disease symptoms were attributed to those affected by *Alternaria* spp. The DI on seeds in the control plots was on average 5.3%–13.9% of disease-affected seeds per silique. The lowest DI on seeds was identified in 1997, while the highest in 1998, when the DS on siliques was the highest (Tab. 5). Fungicides decreased the number of *Alternaria* blight-affected seeds per silique. The highest fungicide efficacy was stated when the fungicides were applied after the appearance of *Alternaria* blight symptoms on siliques.

In 1997 fungicide application did not have any effect on 1000 seed weight. In 1998 Folicur 250 EW applied at the end of flowering, and in 1999 Folicur 250 EW and Sportak 45 EC applied at all dates gave a significant increase in 1000 seed weight. In the years conducive to *Alternaria* blight occurrence fungicides significantly increased spring rape seed yield. The highest efficacy was given by





*note – see figure 1

Fungicide application time -	-	Number of Alternaria blight affected seeds per silique in %						
occurrence of first spots of <i>Alternaria</i> blight	Treatment*	1997	1998	1999				
On lower leaves	Р	3.9	1.5	3.8				
On lower leaves	Т	1.8	5.2	4.0				
On middle leaves	Р	4.0	1.5	6.1				
On middle leaves	Т	0.3	4.8	1.2				
On upper leaves	Р	3.5	4.0	6.0				
On upper leaves	Т	2.3	4.4	6.6				
On siliques	Р	0.7	0.7	1.4				
On siliques	Т	0.3	1.8	1.0				
End of flowering	Р	3.2	2.4	7.6				
End of flowering	Т	1.2	0.7	1.9				
-	С	5.3	13.9	8.5				
LSD (0.5)		0.57	0.88	0.90				

Table 5. Disease incidence on the seeds of spring rape cv. 'Star' as related to the fungicide application time

* P – prochloraz, T – tebuconazole, C – control

Table 6. 1000 seed weight and seed yield of spring oilseed rape cv. 'Star' in relation to the fungicide application time

Fungicide application time –	Treatment*	1000 seed weight in g			Seed yield, kg/ha		
occurrence of first spots of <i>Alternaria</i> blight		1997	1998	1999	1997	1998	1999
On lower leaves	Р	3.7	3.7	3.6	2,382	2,201	2,371
On lower leaves	Т	3.5	3.9	3.4	2,626	2,633	2,458
On middle leaves	Р	3.7	3.8	3.6	2,334	2,758	2,470
On middle leaves	Т	3.8	3.7	3.6	2,598	2,719	2,585
On upper leaves	Р	3.5	3.9	3.6	2,240	2,759	2,375
On upper leaves	Т	3.6	4.0	3.7	2,322	3,020	2,466
On siliques	Р	3.6	3.9	3.6	2,249	2,877	2,556
On siliques	Т	3.6	3.9	3.7	2,382	2,721	2,528
End of flowering	Р	3.6	3.9	3.6	2,510	2,861	2,482
End of flowering	Т	3.6	4.0	3.7	2,580	3,012	2,556
_	С	3.7	3.8	3.5	2,282	2,331	2,320
LSD (0.5)		0.12	0.13	0.04	312	258	140

* P – prochloraz, T – tebuconazole, C – control

tebuconazole applied at the latest dates – when *Alternaria* blight appeared on the upper leaves or siliques, or at the end of flowering (Tab. 6).

DISCUSSION

Our experimental findings confirmed that *Alternaria* blight is a very important fungal disease of spring oilseed rape in Lithuania. It occurred on spring oilseed rape leaves, stems, siliques and seeds in all experimental years. In each year the experimental field was situated close to the spring oilseed rape field from the previous year and winter oilseed rape field from that year, so inoculum of *Alternaria* spp. was the same or very similar every year. The DI and DS varied in different years and were much more dependent on the weather conditions.

Alternaria blight is a late disease of spring oilseed rape. The first spots of disease on spring rape lower leaves appeared at the beginning of flowering stage (BBCH 63), and on the upper leaves – at the beginning of silique development stage (BBCH 70–71). With the senescence of leaves the DS increased, since older leaves are more susceptible to this disease (Conn and Tewari 1989; Mridha and Wheeler 1993). It is believed that the leaf senescence effect might have been caused by the fact that the wax layer declines with the age of leaves, which creates better conditions for persistence of *Alternaria* spp. and germination on leaves (Conn and Tewari 1989).

The spread of *Alternaria* blight is related to the growth stages of oilseed rape plants. If conditions are conducive to rapid development of oilseed rape, the plants are subjected to accelerated senescence and more rapid spread of *Alternaria* blight. However, the DS is more related to the meteorological conditions (precipitation, temperature and relative air humidity) of the period. Various combinations of these factors can have a positive effect on the intensity of *Alternaria* blight spread (Hong et al. 1996).

The DI and DS on oilseed rape leaves did not have any direct effect on the DI and DS on siliques. Experimental findings suggest that in 1997 the DI and DS on lower and middle leaves were moderate, however, the DS on pods was very low – 1.46%, the lowest of all experimental years. In 1998, on the contrary, the spread of *Alternaria* blight was weak on leaves, but on siliques the DS was the highest. Other researchers did not find any relationship between DS on leaves and siliques too. According to Mean et al., poor relationship between disease severity on leaves of mustard and DI of silique can be argued by the fact that defoliation and silique formation occur simultaneously, thus leaving less surfaces as source of secondary infection to silique. However, the whole plant of mustard or oilseed rape, every part of those plants takes part in photosynthesis. Silique formation may continue under reduced leaf surface area and also infection of silique can spread from plant parts other than the leaves (Mean et al. 2002).

Factors influencing different development of *Alternaria* blight on leaves and siliques are still incompletely ascertained and understood (Verma and Saharan 1994). Hong and Fitt (1995) concluded that the difference in DS on rape leaves and siliques results from the difference in their surfaces. Larger and more spots formed on the flat leaf surfaces compared with siliques whose surfaces are arched.

Alternaria blight enhances defoliation (Howlider et al. 1985). However, our experimental findings show that although prochloraz and tebuconazole used after the appearance of the first spots on lower leaves suppressed DS on lower, middle and upper leaves, the fungicides did not help preserve the leaves on rape plants for a longer period than in the control. Duration of leaf persistence on plants was more dependent on the meteorological conditions (in the case of moisture shortage rape plants defoliate earlier compared with wet years) rather than DS on leaves. By early applications of fungicides, after the spread of disease on lower or middle leaves and thus reducing DS on medium and upper leaves, we cannot expect to prevent the spread of *Alternaria* blight on siliques. Therefore to protect spring rape siliques and seeds against *Alternaria* blight it is advisable to apply fungicides later – at the end of rape flowering, during the formation of siliques or after appearance of the first *Alternaria* blight spots on siliques.

The findings of previously conducted research also demonstrated that *Alternaria brassicae* in oilseed rape was more effectively controlled by the later fungicide treatments (Bolton and Adam 1992; Evans et al. 1988). However, later (post flowering) fungicidal applications were less efficient in controlling *Pyrenopeziza brassicae* and *Phoma lingam* stem infection (Bolton and Adam 1992). Prochloraz and tebuconazole applied at the latest terms – end of spring rape flowering or after appearance of the first spots of *Alternaria* blight on siliques inhibited DS not only on siliques but also significantly declined the number of infected seeds per silique. Fewer disease infected seeds means a higher 1000 seed weight and yield. Effective protection against *Alternaria* blight is vital in seed production farms involved in spring rape seed multiplication.

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POLISH SUMMARY

DYNAMIKA ROZPRZESTRZENIANIA I ZMIENNOŚĆ PARAMETRÓW CZERNI KRZYŻOWYCH NA LIŚCIACH I ŁUSZCZYNACH RZEPAKU JAREGO POD WPŁYWEM PROCHLORAZU I TEBUKONAZOLU W ZALEŻNOŚCI OD TERMINU ICH UŻYCIA

Podczas badań polowych z rzepakiem jarym odmiany "Star" przeprowadzonych w latach 1997–1999 w Litewskim Instytucie Rolnictwa zbadano dynamikę rozprzestrzeniania czerni krzyżowych na liściach dolnych, środkowych i górnych oraz łuszczyach rzepaku. Zbadano również rozprzestrzenianie i intensywność choroby na liściach, łuszczynach, łodygach i nasionach pod wpływem prochlorazu i tebukonazolu. Porównano efektywność fungicydów w zależności od czasu ich używania (terminy zabiegów ustalano na podstawie rozprzestrzeniania czerni krzyżowych). Najlepsze rezultaty uzyskano w wyniku pryskania tebukonazolem na początku pojawu czerni krzyżowych (pierwsze plamy) na łuszczynach, albo w końcu kwitnienia rzepaku jarego.