# Epidemic progress of *Cercospora beticola* Sacc. in *Beta vulgaris* L. under different conditions and cultivar resistance

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<sup>2</sup>Department of Soil and Crop Management The present study was designed to evaluate the characteristics of the incidence of Cercospora leaf spot, caused by Cercospora beticola Sacc. in sugar beet (Beta vulgaris L.) cultivars differing in resistance as affected by the environmental conditions. The trials were conducted during the period 2001-2005 at the Lithuanian Institute of Agriculture in Dotnuva. In the field experiments we grew 5-10 cultivars of sugar beet annually. The cultivars were susceptible or resistant. The disease incidence in resistant cultivars was significantly lower than in susceptible ones, except for the year 2002. The disease severity was significantly higher in susceptible sugar beet cultivars (by 29.1 to 89.8%) than in resistant ones. In 2004 and 2005, the epidemic progress of C. beticola in the B. vulgaris susceptible cultivar Millennium and resistant cultivar Dorena depended on the weather conditions. The first symptoms of the incidence of C. beticola in the susceptible cultivar Millennium appeared two weeks earlier than in the resistant cultivar Dorena. In 2004, in the resistant cultivar Dorena, 50% of plants were affected by C. beticola 20 days later and in 2005 5 days later compared with the susceptible cultivar Millennium. AUDPC (Area Under the Disease Progress Curve) calculated by trapezoid integration in 2004 and 2005 was substantially higher in the susceptible cultivar Millennium than in the resistant cultivar Dorena. The white sugar yields were significantly higher for the resistant sugar beet cultivar compared with the susceptible cultivar in 2001 (12.3%) and 2002 (5.5%). Our results suggest that white sugar content in both sugar beet cultivars was significantly dependent on the disease severity.

Key words: Beta vulgaris L., Cercospora beticola Sacc., resistance, yield

## INTRODUCTION

Cercospora leaf spot in sugar beet (*Beta vulgaris* L.) caused by *Cercospora beticola* Sacc. occurs worldwide and may cause a 25–50% reduction of gross sugar yield. In severe epidemics the foliage will be totally destroyed and the beet starts to produce new leaves. Root weight and sugar content are strongly negatively influenced by the extent of new growth [1, 2]. The organism *Cercospora beticola* is an imperfect filamentous fungus with no known sexual stage and infects species of the genus *Beta* and a number of species in the *Chenopodiaceae* [3]. Control of host weeds such as winged pigweed, lambsquarter, red root pigweed, mallow and wild buckwheat is also important in reducing primary inoculum [4]. Cercospora leaf spot is known to be a disease of warm and wet weather. Increase in time of exposure to

high humidity from 24 to 72 h resulted in a great increase in the number of lesions for the same amount of inoculum [5]. Due to the current reduction in soil tillage and shorter cycles of crop rotation, residues of sugar beets are becoming an efficient source of inoculums for *C. beticola* risk [6].

Sugar beet varieties with resistance to leaf spot disease are now available in all countries of the world where *C. beticola* occurs regularly [1, 7]. Resistant cultivars play an important role in the control of Cercospora leaf spot epidemics on sugar beet, because they reduce the rate of disease progress in the field. In sugar beet resistant to Cercospora leaf spot, the efficiency of infection is reduced, the incubation period is lengthened and the number of conidia produced on necrotic lesions is reduced [8, 9]. Breeding for resistance can help to maintain crop yield even under severe disease pressure while reducing the levels of fungicide used. The development of tolerant or resistant varieties can increase sugar yield up to 45% in the presence of Cercospora

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leaf spot infection [10]. The use of resistant cultivars of sugar beet reduced both production costs and the impact of pesticides on the environment. The breeding of sugar beet varieties that combine resistance to C. beticola and high yield under non-diseased conditions is a major challenge to the breeder. However, the breeding of resistant cultivars is difficult owing to the polygenic inheritance of this trait. The genetics of Cercospora resistance is not well understood; it is inherited quantitatively, with the main effects controlled by at least four or five major genes [11]. Resistance currently implemented in the field is quantitatively inherited and exhibits a low to medium heritability [3]. The resistance to this pathogen is only partial and not widely available in commercial varieties, so it does not replace the need for a fungicide treatment. Because of the difficulty in producing high-yielding sugar beet with a high resistance to Cercospora leaf spot, fungicides of the triazole class, the dithiocarbamate class and others have been used to provide a cost-effective means of controlling the disease [12]. The control of C. beticola by fungicide application incurs added costs and has selected for fungicide-tolerant strains. However, resistance in C. beticola to benzimidazole fungicides developed in many regions where members of the fungicide class had been used [13]. A reduced sensitivity of C. beticola isolates to sterol-demethylation-inhibiting (DMI) fungicides has been reported. Repeated application of triazole flutriafol (DMI fungicide) at full or reduced doses favoured the selection of highly resistant strains [14, 15]. In consideration of the detriment of Cercospora leaf spot on sugar beet yield and quality, a new approach to disease prevention was needed, including a variety's resistance and reducing chemical load on the environment. An integrated pest management (IPM) system based on disease epidemic and economic damage thresholds and weather data was established in Germany. The scientific innovation of IPM was the linking of the fungicide treatment and damage thresholds to develop forecasts of damage risk [6].

The current study was designed to evaluate the characteristics of the incidence of Cercospora leaf spot on sugar beet cultivars differing in resistance, as affected by the environmental conditions.

## MATERIALS AND METHODS

The trials were conducted during 2001–2005 at the Lithuanian Institute of Agriculture in Dotnuva. For the field experiments were grown 2–3 resistant and 3–8 susceptible cultivars of sugar beet (*Beta vulgaris* L.) annually. The experimental design was a randomised block with four replicates in all experimental years. Each plot had a surface area of 32.4 m<sup>2</sup> (6 rows 12 m long). Sugar beet was sown at the end of April with 45 cm row spacing, at 15 cm distances.

In trials, symptoms of Cercospora leaf spot were recorded weekly from the beginning of August until October. The percentage of leaf area showing symptoms of Cercospora leaf spot was used to quantify the disease severity. Disease severity was assessed on each plot in four randomly selected places on five plants along the row (20 plants per plot) using a percentage scale 0, 1, 5, 10, 25, 50, 75. The degree of infection on the middle-aged leaves of each plant was assessed. The percentage of affected plants was used to quantify the disease incidence. Plant growth stages were identified according to the BBCH scale [16]. The weather data from the Dotnuva weather station were used. The daily temperature °C, sum total of precipitation and air humidity were recorded.

AUDPC was calculated by trapezoidal integration in accordance with 10–15 day interval disease severity data over the season: AUDPC =  $\sum_{i=1}^{n-1} \left(\frac{y_i + y_i + 1}{2}\right)(t_i + 1 - t_i)$ , where:  $y_i$  – disease severity %,  $t_i$  – interval of data records (days), n – number of assessments [17].

Root yield and quality were determined at harvesting in October. Beets were harvested manually. White sugar percent (WSP) was calculated according to Reinefeld et al. [18]: WSP = Pol – [0.343 (K + Na) +  $0.094 \times N + 0.29$ ], where Pol – sugar %, K and Na – the concentration of potassium and sodium in mmol 100 g<sup>-1</sup> fresh beet, N – the concentration of  $\alpha$ -amino nitrogen in mmol 100 g<sup>-1</sup> fresh beet. White sugar yield for every plot was calculated from the product of root yield and white sugar percent.

The data were processed by the analysis of variance (ANOVA) according to Fisher's protected least significant difference (PLSD) test at p = 0.05 and 0.01 to indicate statistically significant differences between treatments [19].

#### **RESULTS AND DISCUSSION**

The onset and course of the epidemic of Cercospora beticola on Beta vulgaris as affected by the environmental conditions and cultivar resistance are described. In the field C. beticola is usually observed after row closure in shady areas, probably due to a high humidity and temperature in the crop. The fungus produces conidia that are principally splash- and also wind-dispersed, but only over short distances, e.g., to adjacent fields [20, 21]. After the crop is harvested, all organic debris, including the infected leaves, were ploughed under the field and served as inoculums when a new B. vulgaris crop was planted. The incidence of C. beticola in sugar beet fields with different crop rotation was investigated in Poland [22]. It was found that in each case shortening of rotation stimulated the infection of plant by this pathogen and the differences were significant. When sugar beet was followed by sugar beet, the increase of plant infection was double in comparison with a twoyear crop rotation. The incidence and severity of C. beticola varied from year to year depending on weather conditions. Leaf spot caused by C. beticola was not

economically important to the sugar beet crop produced in Lithuania before 2001. In 1994–1996, the incidence and severity of *C. beticola* were investigated on 26 sugar beet varieties, and 19 of them were affected by the fungus [23]. According to literature [24], during the period 1999– 2003 Cercospora leaf spot on average affected 85.2% of sugar beet leaves annually (severity 34.8%).

Our results suggest that the epidemic development of *C. beticola* affected from 90.7 to 100% of plants in susceptible sugar beet cultivars (Table 1). The disease incidence on resistant cultivars was significantly lower than in susceptible ones, except for the year 2002.

Due to the rainy and warm July the appearance of *C. beticola* in 2001 and 2002 was early and abundant, therefore Cercospora leaf spot severity was the highest in these years (Table 2). The lower disease severity on both resistant and susceptible cultivars in 2003–2005 was the result of unfavourable weather conditions. In all ex-

perimental years the severity of Cercospora leaf spot was significantly higher on susceptible (by 29.1 to 89.8%) than on resistant sugar beet cultivars. According to Wolf et al. [25] and Wolf, Verret [26], meteorological factors had a predominant impact on the development of the disease. Disease development is favoured

Table 1. Incidence of *C. beticola* on different cultivar of *B. vulgaris* in different years

Cultivars	Affected plants %					
	2001	2002	2003	2004	2005	Average
Resistant	20.0a	85.0a	10.0a	90.0a	66.7a	54.3a
Susceptible	99.2b	100.0a	90.7b	100.0b	93.3b	96.6b

Values followed by the same letter in the same column for a parameter are not significantly different at p < 0.05. n = 8-12 (resistant); n = 12-24 (susceptible).

Table 2. Severity of C. beticola on different cultivars ofB. vulgaris in different years

Cultivars	Severity %					
	2001	2002	2003	2004	2005	Average
Resistant	2.95a	8.50a	1.00a	9.00a	6.83a	5.66a
Susceptible	23.42b	21.57b	9.79b	12.69b	10.62b	15.62b

Values followed by the same letter in the same column for a parameter are not significantly different at p < 0.05. n = 8-12 (resistant); n = 12-24 (susceptible).



both resistant and susceptible cultivars **Fig. 1.** Epidemic progress of *C. beticola* in *B. vulgaris* susceptible cultivar Millenin 2003–2005 was the result of unfanium and resistant cultivar Dorena as affected by the weather conditions in 2004 yourable weather conditions. In all ex-

by temperatures between  $20^{\circ}$  and  $30 {\circ}$ C, but strongly inhibited by temperatures <10 {\circ}C.

In 2004 and 2005, the epidemic progress of *C. beti*cola in the *B. vulgaris* susceptible cultivar Millennium and resistant cultivar Dorena as affected by the weather conditions was described. In 2004 the primary symptoms of the incidence of *C. beticola* in the susceptible cultivar Millennium appeared two weeks earlier than in the resistant cultivar Dorena (Fig. 1). The appearance of *C. beticola* in crop was associated with rain at the end of July and beginning of August and warm weather conditions. In the middle of September, due to the rainy August and high relative air humidity (RH), the incidence of *C. beticola* in the susceptible cultivar Millennium became severe. Conversely, in the resistant cultivar Dorena only 25% of plants were slightly infected at that time.

In 2005, in the trial field *C. beticola* appeared after the rainy first half of August when it rained day after day and a high RH prevailed (Fig. 2). Like in 2004, *C. beticola* in the susceptible cultivar Millennium appeared two weeks earlier than in the resistant cultivar Dorena. Due to the high RH, especially at night, the disease progress in both susceptible and resistant cultivars was intense, but the disease severity in the resistant cultivar Dorena was noticeably lower than in the susceptible Millennium. According to Volf and Verreet (2002), the beginning of the epidemic is defined as the time when 50% of the beet plants are infected. In 2004, in the resistant cultivar Dorena, 50% of plants were affected by *C. beticola* 20 days and in 2005 – 5 days later compa-



Fig. 2. Epidemic progress of *C. beticola* in *B. vulgaris* susceptible cultivar Millennium and resistant cultivar Dorena as affected by the weather conditions in 2005



Fig. 3. Epidemic progress (AUDPC) of *C. beticola* in susceptible Millennium and resistant Dorena cultivars of sugar beet

red with the susceptible cultivar Millennium. Arita et al. [27] showed that when *C. beticola* was prevalent, resistant sugar beet cultivars were 50% infected approximately one week later than susceptible cultivars. When *C. beticola* broke out moderately, the time it took to infect resistant cultivars increased. Since the disease development in resistant cultivars was slower than in susceptible cultivars, the difference in disease severity became prominent between them as time elapsed.

AUDPC (Area under Disease Progress Curve) allows expression of entire season's *C. beticola* epidemic on different sugar beet cultivars with one value (Fig. 3). In both 2004 and 2005, the Cercospora leaf spot AUDPC value was substantially higher in the susceptible variety Millennium than in the resistant Dorena.

Under no fungicide conditions, the decrease in yield of the resistant cultivar was less than in the susceptible cultivar. When *C. beticola* occurred moderately, the yield of the resistant cultivar did not decrease. However, in an experiment when C. beticola occurred severely, the effect of fungicides lasted approx. 10-20 days longer than on the susceptible cultivars [27]. The results of evaluation of leaf dynamics of sugar beet plants infected by C. beticola in Italy revealed that an attack of the disease affects all the considered parameters of leaf growth. The chemical protection and genetic resistance compliment each other as they are both necessary to maintain almost normal levels of photosynthetic activity. Fungicide treatments limit the loss of active surface area, reduce the leaf appearance rate, increase the life span of the leaves and noticeably delay the senescence processes. Genetic resistance and normal chemical defence have similar effects. However, the former is generally less effective [28]. In severe epidemics, the entire foliage may become necrotic and the beet starts to produce new leaves. These flushes of growth occur at the expense of carbon investments of the plant into the root. If leaf spots cover

at least 3% of the foliage by harvest, economic losses occur through reduced root tonnage and sucrose content and increased impurities. Also, roots of infected plants do not store as well as roots of healthy plants [12].

Sugar beet yield varied in the experimental years, and no significant differences were observed in the yields of the susceptible resistant cultivars, except for an apparent decrease in 2004 (Table 3). The average sugar beet root yield of the susceptible cultivars was by 3.6% higher than that of the resistant cultivar. The lower yield of the resistant cultivar was recorded in three experimental years and of the susceptible cultivar in two years. The resistant cultivars are known to be lower yielding under diseasefree conditions as compared with susceptible cultivars [3, 7]. But it has been noted that the yield of the resistant cultivar did not decrease when Cercospora leaf spot occurred moderately [27]. The white sugar yields were significantly higher in the resistant sugar beet cultivar compared with the susceptible cultivar in 2001 (12.3%) and 2002 (5.5%) (Table 4). This result might have occurred due to the different C. beticola infection levels on sugar beet cultivars. A similar causality of losses of white sugar yield has been reported by many authors [11, 29].

The impact of the incidence and severity of *C. beticola* was used to estimate the relationship between disease pressure and sugar beet yield and quality (Table 5). A significant effect of the disease incidence and severity on quality parameters in the resistant cultivar was determined. The effect of *C. beticola* severity was highly significant in many cases. Our results suggest that

Table 3. Comparison of root yield of sugar beet cultivars in different years

Vear	Root yield t ha-1				
i cai	Resistant	Susceptible	Difference		
2001	60.86	61.31	-0.45		
2002	42.18	41.09	1.09		
2003	56.55	55.71	0.84		
2004	58.15	69.43	-11.28**		
2005	64.16	64.65	-0.49		
Average	56.38	58.44	-2.06*		

\* Significant at p < 0.05. \*\* Significant at p < 0.01. n = 8-12 (resistant); n = 12-24 (susceptible).

Table 4. Comparison of white sugar yield of sugar beet cultivars in different years

Vear	White sugar yield t ha-1			
1 cai	Resistant Susceptible		Difference	
2001	9.40	8.25	1.15**	
2002	7.94	7.51	0.43*	
2003	9.18	9.37	-0.19	
2004	9.86	11.34	-1.88**	
2005	12.12	12.14	-0.02	
Average	9.70	9.80	-0.10	

\* Significant at p < 0.05; \*\* Significant at p < 0.01.

n = 8-12 (resistant); n = 12-24 (susceptible).

Table 5. Effects of *C. beticola* incidence and severity on the quality and yield of sugar beet cultivars

Cultivars	Root yield t ha <sup>-1</sup>	Sugar content %	White sugar content %	White sugar yield t ha <sup>-1</sup>	
Incidence (%)					
Resistant	NS	**	**	NS	
Susceptible	NS	NS	NS	NS	
Severity (%)					
Resistant	NS	**	**	NS	
Susceptible	**	NS	*	**	

\* Significant at p < 0.05. \*\* Significant at p < 0.01. NS – not significant. n = 8-12 (resistant); n = 12-24 (susceptible).

white sugar content in both sugar beet cultivars was significantly dependent on the disease severity.

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## EPIDEMIOLOGINIAI CERCOSPORA BETICOLA SACC. TYRIMAI ĮVAIRAUS JAUTRUMO BETA VULGARIS L. GENOTIPUOSE SKIRTINGOMIS APLINKOS SĄLYGOMIS

#### Santrauka

2001–2005 m. Lietuvos žemdirbystės institute, Dotnuvoje, buvo įvertinti *Cercospora beticola* Sacc. (rudmargė) plitimo ypatumai skirtingo jautrumo cukrinių runkelių (*Beta vulgaris* L.) veislėse ir ligos išplitimo priklausomybė nuo aplinkos sąlygų. Kasmet tirtos 2–3 jautrios ir 3–8 atsparios rudmargei cukrinių runkelių veislės. Tyrimų metais ligos išplitimas tarp atsparių veislių augalų buvo statistiškai mažesnis nei tarp jautrių veislių, išskyrus 2002 m., kai rudmargė pažeidė visus augalus. Ligos intensyvumas buvo patikimai didesnis jautrių (nuo 29,1 iki 89,8%) nei atsparių veislių augaluose. Pirmieji ligos simptomai "Millennium" veislės augaluose pasirodė dviem savaitėmis anksčiau nei atsparios "Dorena" veislės augaluose. 2004 m. rudmargė pažeidė 50% "Dorena" veislės augalų 20 dienų vėliau, o 2005 m. – 5 dienomis vėliau nei "Millennium" veislės augalų 20 dienų vėliau. Tiek 2004, tiek 2005 tyrimų metais "Millennium" veislės augalų AUDPC (ligos vystymosi indeksas per tam tikrą laiką) buvo ženkliai didesnis nei "Dorena" veislės augalų. Baltojo cukraus derlius 2001 m. (12,3) ir 2002 m. (5,5%) statistiškai buvo didesnis atsparių veislių grupės nei jautrių. Mūsų tyrimų duomenimis, baltojo cukraus kiekis priklausė nuo rudmargės intensyvumo.