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Development of a field biotest using artificial inoculation to evaluate resistance and yield effects in sugar beet cultivars against *Cercospora beticola*

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Abstract *Cercospora beticola* resistance and disease yield loss relationships in sugar beet cultivars are best characterised under field conditions with heavy natural infection; this does not occur regularly under German climatic conditions. Since Cercospora resistance reduces the rate of pathogen development, high yield loss was observed in studies using artificial inoculation. Our study, therefore aimed to optimise inoculum density to obtain cultivar differentiation, which correlates to natural infection. In 2005 and 2006, field trials were carried out to determine the effect of different inoculum densities on Cercospora resistance of three sugar beet cultivars possessing variable resistance. The epidemic progress and white sugar yield loss (WSY_{loss}) were determined and their relationship evaluated. An optimal inoculum concentration range (between 10,000–20,000 infectious Cercospora units ml^{-1} inoculum suspension) was determined which allowed maximum resistance parameter differentiation in terms of *C. beticola* disease severity (DS), area under the disease progress curve (AUDPC) and WSY_{loss}. The correlation between AUDPC and WSY_{loss} was identical for all cultivars independent of the resistance level, demonstrating that tolerant reactions of the cultivars under study were not detectable. This study provides evidence that even under optimal inoculum levels necessary to obtain maximum differentiation between cultivars, climatic conditions are important for disease management, but remain unpredictable, indicating that artificial inoculation needs to be optimised, but that single field locations are not sufficient and reliable to evaluate Cercospora resistance.

Keywords Beta vulgaris · Area under disease progress curve · Quantitative resistance · Inoculum density

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Introduction

The most destructive foliar disease in sugar beet (*Beta vulgaris* spp. *vulgaris*) is Cercospora leaf spot (CLS) caused by the fungus *Cercospora beticola* (Rossi et al. 2000b) and occurs in all sugar beet-growing areas worldwide (Bleiholder and Weltzien 1972b). Yield reductions due to CLS in susceptible cultivars range from 10 to 50% in Austria, and from 15 to 40% in France (Byford 1996). There are conflicting reports of yield losses caused by *C. beticola* in Germany. Byford (1996) reported losses <10%, whereas Wolf et al. (1995) observed yield losses up to 50% in diseased areas. In general, the first leaf spots appear after canopy closure, which is usually at the begin-

ning of July in Germany. Before canopy closure the climate in the plant stand is less conducive for fungal infection (Wolf and Verreet 1997; Wolf et al. 2001). The pathogenesis of *C. beticola* depends strongly on weather conditions. The optimal conditions for infection and subsequent spreading in the plant are high temperatures (27–30°C) and high relative humidity (RH) (98–100%) (Bleiholder and Weltzien 1971, 1972a).

Resistant sugar beet cultivars adapted to the different sugar beet-growing areas worldwide where *C. beticola* occurs regularly are available (Byford 1996; Mechelke 2000). Breeding efforts to generate Cercospora resistance in sugar beet started in the 1920s by Munerati (1920). Resistance against *C. beticola* was introgressed from the wild sea beet (*Beta vulgaris* spp. *maritima*); additional resistant accessions were also found in other subspecies of *B. vulgaris* and in other sections of the genus *Beta*, namely *Corollinae*, *Nanae* and *Procumbentes* (Asher et al. 2001). Resistance against *C. beticola* is a quantitative trait based on the additive effects of at least four to five major resistance genes (Smith and Gaskill 1970). However, the exact number of host genes involved is unknown (Weiland and Koch 2004). Host resistance is not efficient to prevent infection by *C. beticola* entirely but reduces the pathogen's development (infection efficiency, spore production, lesion size) (Rossi et al. 1999, 2000a). Therefore, especially at the late stages of epidemics, leaf damage in combination with leaf regrowth and subsequent yield reduction cannot be prevented (Rossi et al. 2000b).

Disease control in susceptible cultivars is achieved with fungicide application. Because of the quantitative character of the resistance, under a certain but yet unknown infection pressure, fungicide treatment may also become necessary to prevent yield losses in resistant cultivars (Harveson et al. 2002). The development of fungicide-resistant Cercospora strains (Karaoglanidis et al. 2002) and the need to use fungicides only when necessary in integrated crop protection strategies (BMVEL 2004) necessitates the cultivation of Cercospora-resistant cultivars. To evaluate Cercospora resistance and resulting yield losses under field conditions, an adequate infection level is necessary (Ruppel and Gaskill 1971; Ossenkop et al. 2002). The estimation and comparative evaluation of cultivar performance in the field is possible, if evaluation is done in parallel thereby minimising the risk of weak or late-occurring epidemics (Ossenkop et al. 2004). However, due to highly variable climatic conditions on a single location, Cercospora field resistance tests in Germany are still unreliable (Pfleiderer and Schäufele 2000). Therefore, in commercial breeding, sugar beet lines are selected for resistance against *C. beticola* in the greenhouse using artificial inoculation or in regions where natural infection occurs annually, namely Italy and Greece in southern Europe (Byford 1996). As the climatic conditions in these countries are different from Germany, selected sugar beet lines do not correspond to the demands of the German sugar beet growers who need high yielding sugar beet cultivars with moderate resistance to *C. beticola* (Märländer et al. 2003). In addition, yield evaluation of cultivars adapted to north-European demands under Mediterranean conditions with reliable Cercospora incidence is not appropriate. Hence, it was necessary to develop a method for testing for resistance against *C. beticola* in the field under the climatic conditions of Germany. Adams et al. (1995) field-tested artificial Cercospora inoculation by spraying a spore suspension of *C. beticola* at the time of canopy closure. A high inoculum density (one fungal overgrown agar-plate homogenised with 400 l ha^{-1} water per 4.5 m^2) was used, whereas the inoculum density in this study was not determined by counting infectious units of *C. beticola* (conidial and mycelium fragments) and resulted in high infection levels and severe epidemics. Resistant genotypes were infected similarly to susceptible ones. Consequently, differences in white sugar yield (WSY) between susceptible and resistant cultivars were not pronounced (Ossenkop et al. 2004). Pfleiderer and Schäufele (2000) proposed that the inoculum density obtained using the method of Adams et al. (1995) should be reduced for a widespread testing system. Pundhir and Mukhopadhyay (1987) showed that (i) in greenhouse infection experiments, a minimum inoculum concentration was required to cause visible lesions and (ii) that inoculum concentration, final disease index and sugar yield reduction were positively related.

We therefore aimed to improve the field inoculation method of Adams et al. (1995) by varying the Cercospora inoculum density and by quantifying the number of infectious units in order to determine the inoculum parameters which allow the differentiation of susceptible and resistant cultivars based

not only on epidemic progress but also on WSY losses. In 2005 and 2006 Cercospora resistance field trials using artificial inoculation were conducted to test different inoculum densities, starting with the lowest inoculum density tested by Pfleiderer and Schäufele (2000), in sugar beet cultivars with varying levels of resistance to *C. beticola*. The epidemic progress was monitored and analysed; additionally WSY and its correlation with infection level were evaluated.

Materials and methods

Field trials

In 2005 and 2006, field trials using artificial inoculation were carried out, testing one susceptible (ZR registration identifier of the Bundessortenamt (German cultivar office) 1464) and two resistant (1374 and 1341) registered sugar beet cultivars. Additionally, these cultivars were evaluated under natural infection at seven locations in a German and Austria-wide cultivar trial series in 2004 and 2005 (Kaiser 2007). In this study, one location of this trial series with severe disease occurrence in 2004 was selected to analyse the epidemic progress of *Cercospora* under strong natural infection. For artificial inoculation, three (2005) and four (2006) different inoculum densities, respectively, were tested in comparison to a fungicide-treated healthy control. Field trials with artificial inoculation were conducted at one site per year in the south of Lower Saxony. The experimental design was a complete randomised block with three (natural infection) and four (artificial inoculation) replicates, respectively. Field trials were sown between mid-and end of April with 45 cm (artificial inoculation) and 50 cm (natural infection) distance between rows, determined by the harvesting technology available. The distances between plants within rows in the artificial inoculation trials were 18 cm in 2005 and 12 cm in 2006, and in the natural infection trial 6 cm, and the trials were manually thinned to a density of 75,000–100,000 plants ha^{-1}. Fertilisation and weed control were carried out according to local standards. Inoculation and harvest were conducted in a three-row core of the six-row plots to avoid neighbourhood effects. WSY was calculated as described by Märländer et al. (2003), whereas in this study loss of WSY (WSY_{loss}) at any inoculum density in relation to the healthy control was determined using equation (1):

$$\text{relative } WSY_{loss} = \frac{(WSY_{hc} - WSY_{id}) \times 100}{WSY_{hc}} \quad (1)$$

where WSY_{hc} = WSY of the healthy control and WSY_{id} = WSY at different inoculum densities. However, the results for WSY_{loss} were evaluable only for 2005. In 2006, we observed a late and extremely non-uniform homogeneous field emergence due to strong environmental fluctuations during germination and early plant development, leading to an incomplete plant stand with considerable heterogeneities in plant development throughout the growing season. Severely hail-damaged leaves and bacterial infections (*Pseudomonas* spp.) were frequently observed. In addition, insufficient weed control and nematode attack (*Heterodera schachtii*) occurred.

Fungal isolates, inoculum production and inoculation

A mixture of four German field isolates from three different geographical regions (Mecklenburg, Lower Saxony and two isolates from Bavaria) and one Greek field isolate (Thessaloniki) of *C. beticola* was used for inoculation in both years. The isolates were kept as long-term cultures before mass spore production; all isolates were host-passaged to ensure pathogenicity. Host-passage was carried out as follows: three sugar beet plants per isolate were spray-inoculated with conidial suspensions and incubated under plastic bags in a climate chamber at temperatures of 24°C for 14 h under supplementary assimilation light, and 18°C for 10 h in the dark with a RH of 100%. After the appearance of the first Cercospora leaf spots, the pathogen was re-isolated and grown on V8 agar (50% Grannini® Vegetable Juice, 50% demineralised water, 1.5% Agar, pH 5.8). For mass production of the fungus, an agar plug (2 cm^2 surface) overgrown with fungal mycelium was homogenised with 3 ml of sterile tap water, and 300 µl of the homogenate was plated onto solid fresh V8 agar in Petri dishes (92 mm Ø) incubated at 24°C under diffuse daylight. After 8–14 days the overgrown plates were homogenised for 90 s in a mixer (Stephan, Type UM44A, Stephan und Söhne, Hameln, Germany) and diluted with tap water to adjust the inoculum concentration, determined by counting *C. beticola* conidia and

mycelial fragments with a minimum size of one septum. In 2005, inoculum density 1 was adjusted to about 30,000 infections units (iu) ml^{-1}, density 2 to about 20,000 iu $ml^{-1}$and density 3 to about 10,000 iu ml^{-1}. Due to the heavy infection in plots treated with the highest inoculum density 1 in 2005, this inoculum density was not used in 2006. Inoculum densities 2 and 3 were used in 2006; additionally inoculum densities 4 (5,000 iu ml^{-1}) and 5 (2,500 iu ml^{-1}) were included. Inoculation was carried out when canopy closure was fully established under warm temperatures and humid conditions. Inoculum was sprayed at a rate of 400 l ha^{-1} with a six-row plot hand-driven air-pressure sprayer in three core rows of the plots, starting with the lowest inoculum density. Inoculation was done during the early morning with natural leaf wetness at about 20°C. In all treatments, no rainfall occurred during the next 12 h.

Assessment of disease parameters

Infection with *C. beticola* was assessed weekly starting at 7 days post-inoculation (dpi) and at the beginning of July under natural infection conditions. One hundred plants per cultivar and per inoculum density were used for the assessment of disease severity (DS) as percentage of infected leaf area on one leaf of the middle leaf apparatus (Wolf and Verreet 1997). The assessment of DS on every single leaf was determined according to Battilani et al. (1990). As a descriptor for epidemics, the area under the disease progress curve (AUDPC) was calculated in accordance to Shaner and Finney (1977) using equation (2):

$$\mathrm{AUDPC} = \sum_{i}^{n-1} \frac{(y_i + y_{i+1})}{2} \times (t_{i+1} - t) \qquad (2)$$

where n = number of assessment times, i = assessment time, y = DS (%) and t = dpi. Due to the weekly assessment of DS, AUDPC has units of ('% DS × weeks') but this not used in the text.

CLS progress over time follows a typical sigmoid curve and is adequately described by a logistic model (Rossi 2000). Therefore, the following equation (3) was fitted to the observed DS values:

$$y = \frac{100}{\left(1 + \left(\frac{(100-a)}{a}\right)\right)} \times \exp(-b \times t) \qquad (3)$$

where y = DS (%), a = DS at the beginning of the epidemic, b = the expansion rate (% DS per unit time) and t = dpi. Parameters a and b were estimated.

Assessment of weather data

Weather data were monitored with a weather station on the field where the trials were situated. Temperature was measured as °C in the crop (0.05 m above ground) and at 2 m above ground. RH was measured as % at 2 m above ground. Precipitation was determined in mm at 1 m above ground. All weather data were recorded hourly. The monthly means were calculated and are given in Table 1.

Statistics

Analysis of variance (ANOVA) was carried out with the programme SAS version 8.1 (SAS Institute Inc., Cary, NC, USA) using the GLM procedure followed by a multiple comparison of means according to Tukey (Dufner et al. 2002). Significant differences are indicated with different letters for probabilities of $P \leq 0.05$, whereas n.s. indicates non-significant. The logistic model was fitted to observed DS values and was carried out with the NLIN procedure. The disease-loss relationship was described with a linear regression using the REG procedure. For each cultivar all four replicate values of the three inoculum densities were used to calculate the disease-loss relationship. Significance of the coefficient of determination (r^2) of the fitted regression model are indicated with *, ** or *** for probabilities of $P \leq 0.05$, 0.01 or 0.001, respectively.

Results

Disease progress and yield effects under natural infection

In a German and Austria-wide cultivar trial series in 2004 and 2005, the three cultivars used in this study were evaluated under natural *C. beticola* infection at seven locations. One location in 2004 with the best differentiation of cultivar resistance was selected. First symptoms of *C. beticola* appeared at the beginning of July (Fig. 1). DS increase was slow in August and gained momentum in September. Subse-

Table 1 Climatic conditions of the field trial location in 2005 and 2006

Month	Ta in 2 m (°C)	T in 0.05 m (°C)	Hb in 2 m (%)	Pc in 1 m (mm)
April 2005	9.3	9.7	78.7	43.4
May 2005	12.6	11.5	82.3	80.1
June 2005	15.7	17.2	81.1	40.3
July 2005	17.8	18.6	83.4	56.3
August 2005	15.3	15.2	84.6	67.2
September 2005	14.6	14.2	83.7	32.6
October 2005	10.7	10.1	88.8	48.3
November 2005	5.1	n.m.[d]	92.7	29.1
December 2005	1.8	2.1	94.2	39.3
January 2006	−2.3	−2.4	92.3	14.7
February 2006	0.1	−0.2	92.4	28.8
March 2006	1.8	2.2	87.6	58.7
April 2006	8.2	8.5	83.2	44.1
May 2006	13.0	13.8	77.7	74.2
June 2006	16.5	18.0	78.7	85.1
July 2006	21.5	23.3	73.9	30.6
August 2006	15.7	16.0	85.2	51.4
September 2006	16.8	17.6	78.4	4.8
October 2006	12.5	12.2	87.9	38.7

[a] T = temperature, [b] H = relative humidity(RH), [c] P = precipitation, [d] n.m. = no measurement

quently, DS increased rapidly, particularly the susceptible cultivar which reached a higher DS than the resistant cultivars. However, the stationary phase of the expected sigmoid growth curve was not observed until harvest. Because only three values per cultivar were available, the mean AUDPC values of the cultivars (susceptible cultivar: 243, resistant cultivar 1: 194 and resistant cultivar 2: 127) were not significantly different. However, statistical analysis revealed a significantly higher WSY_{loss} for the susceptible cultivar (approximately 12%) than for resistant cultivars (1.4% resistant cultivar 1 and 3.6%, resistant cultivar 2).

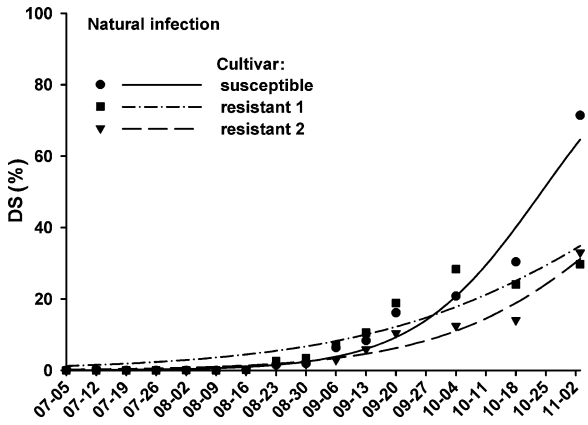

Fig. 1 Progress of disease severity (DS) of *C. beticola* under natural infection in sugar beet cultivars with different susceptibilities after fitting a logistic growth curve to the measured values; three cultivars, Germany, 2004

Disease progress in field trials following artificial inoculation

In trials with artificial inoculation, first *C. beticola* symptoms appeared 1 week after inoculation (disease index data not shown). As expected, the DS increase followed a typical sigmoid curve consisting of lag (slow growth), log (exponential growth) and stationary phases, and the maximum of 100% DS was only reached under very conducive climatic conditions in 2005 (Figs. 2, 3). The exponential increase of DS was not observed until six (in 2005) or eight weeks (in 2006) post-inoculation depending on the cultivar, indicating an extended incubation period between infection and first appearance of leaf spots. In July and the first half of August 2005, daily mean temperatures in the crop (at a height of 0.05 m) varied between 12°C and 27°C and the RH ranged between 70% and 99% (Table 1). Initial pathogen

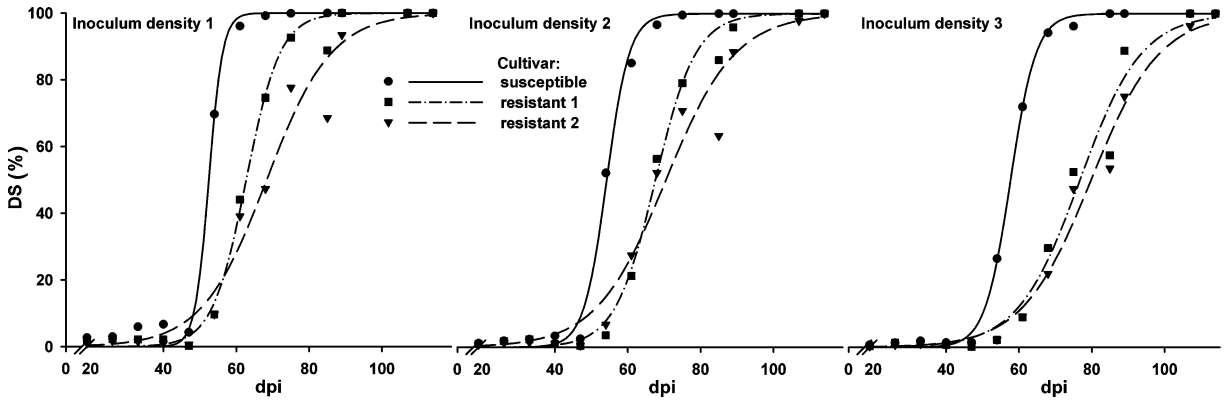

Fig. 2 Progress of disease severity (DS) of *C. beticola* following artificial inoculation in sugar beet cultivars with different susceptibilities depending on inoculum density after fitting a logistic growth curve to measured values; three cultivars, Germany, 2005, dpi days post-inoculation

development could have been inhibited or delayed, which might explain the extended incubation period. Subsequently, climatic conditions became more favourable for the fungus and DS increased exponentially. In July and August 2006, climatic conditions were less favourable than in 2005. At the beginning of July 2006, temperatures and RH were high; however, inoculation was carried out after mid-July, when canopy closure was fully established. Afterwards, daily mean temperatures varied between 20°C

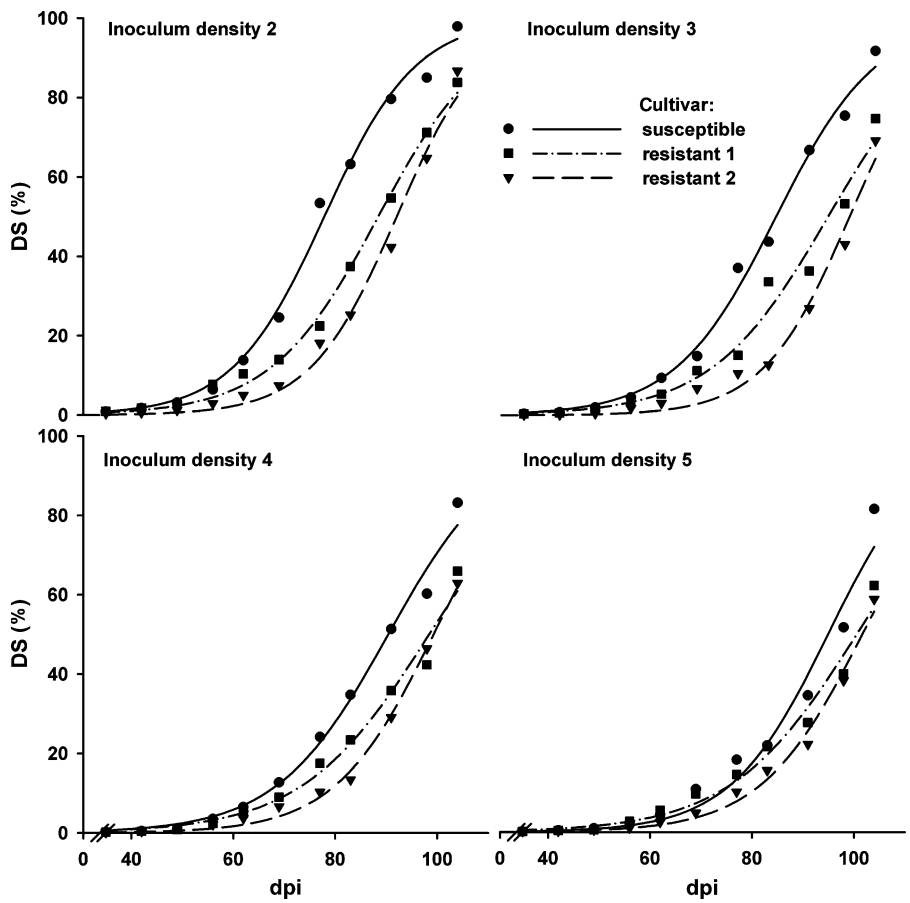

Fig. 3 Progress of disease severity (DS) of *C. beticola* following artificial inoculation in sugar beet cultivars with different susceptibilities depending on the inoculum density after fitting a logistic growth curve to the measured values; three cultivars, Germany, 2006, dpi days post-inoculation

and 27°C, but the RH mostly ranged between 60% and 70% with a maximum of 87% for 1 day. Temperatures in August 2006 ranged from 12 to 20° C but it was very dry (RH between 72% and 97%). At the end of August, rainfall and increasing RH occurred. Thus, in 2006, pathogen development was delayed by suboptimal climatic conditions for a considerable length of time during the crop growth period.

In 2005, DS differences between resistant and susceptible cultivars across the different inoculum densities were clear (Fig. 2). The DS progress of the susceptible cultivar, however, was nearly identical at the different inoculum levels. In resistant cultivars, the DS increase in the log phase was less with decreasing inoculum density and hence the stationary phase of 100% DS was reached later compared to the susceptible cultivar. In 2006, as a result of unfavourable climatic conditions, the entire sigmoid curve of DS progress, including the stationary phase, was observed only in the high inoculum density treatment and in the susceptible cultivar (Fig. 3). In contrast to 2005, the beginning of the exponential phase of DS progress started earlier in the susceptible cultivar than in both resistant cultivars, when the highest inoculum density was applied. This difference was reduced with decreasing inoculum densities.

In both years, the differentiation of DS development between resistant and susceptible cultivars was most obvious in the treatments with inoculum densities 2 and 3. However, in 2005 when the log phase of the growth curve displayed maximum slope, the differences in slope and delay of DS progress between the cultivars were obviously higher. In all environments and at all different inoculum densities applied, the log phase of the DS progress in resistant cultivar 1 started earlier than in resistant cultivar 2.

In order to determine the inoculum density that produced the maximum DS differentiation between resistant and susceptible cultivars at several times post-inoculation, the following calculations were carried out. After fitting a logistic growth equation to DS values, an ANOVA was conducted to determine DS values at different dpi (Table 2). Interestingly, a significant interaction of cultivar and inoculum density was only detected at 75 dpi in both years and additionally at 50 dpi in 2005. Therefore, a multiple comparison test was carried out with the DS values of these dates to determine differences between the susceptible and resistant cultivars at different inoculum densities (Table 3). In 2005, this resulted in a clear differentiation of DS between susceptible and resistant cultivars, at 50 dpi for all inoculum densities. However, at 75 dpi, only at the lower inoculum density 3 were the differences between susceptible and resistant cultivars significant, whereas at the higher densities, only one resistant cultivar differed significantly from the susceptible one. In contrast, in 2006, a significant differentiation between cultivars was observed only at the high inoculum densities 2 and 3. At the lower inoculum densities 4 and 5, no significant differences between cultivars were detectable at any time.

Area under the disease progress curve (AUDPC)

AUDPC was calculated for all treatments of the trials in both years (Fig. 4). AUDPC of *C. beticola* varied between 837 and 6,264 depending on cultivar, inoculum density and year. In general, the susceptible cultivar had considerably higher AUDPC values than the resistant cultivars. However, the differences among cultivars were significant only in treatments with inoculum densities 1, 2 and 3 but not with inoculum densities 4 and 5. In both years, the differentiation between susceptible and resistant cultivars was most apparent at inoculum densities 2 and 3. The AUDPC differences between resistant and susceptible cultivars increased with decreasing inoculum density in 2005. In inoculum density 1, a significant difference in AUDPC between the two resistant cultivars was also observed, mirroring the earlier DS progress of the resistant 1 cultivar as shown in Fig. 2. In 2006 due to unfavourable climatic conditions, AUDPC values were low and significant differences among cultivars were not detected.

Yield effects

In 2005 WSY_{loss} differed between cultivars and inoculum densities (Fig. 5). The susceptible cultivar reached a significantly higher WSY_{loss} than the resistant cultivars in all inoculum densities except when compared to resistant cultivar 1 with inoculum density 1. Thus with increasing inoculum densities, the quantitative resistance in resistant cultivar 1 seemed to be more strongly affected than in resistant cultivar 2. Again, as observed for AUDPC in 2005,

Table 2 ANOVA for disease severity (DS) of *C. beticola* in sugar beet cultivars (cv.) with different levels of susceptibility following artificial inoculation with different inoculum densities (id) at different days post-inoculation (dpi), Germany, 2005 and 2006

dpi	Source	2005			2006		
		DF	F-value	*P*	DF	F-value	*P*
14	cv.	2	11.02	0.0004	2	6.22	0.0051
	id	2	0.74	0.4855	3	0.10	0.9612
	rep	3	2.73	0.0659	3	1.73	0.1803
	cv. × id	4	1.48	0.2390	6	0.35	0.9051
20	cv.	2	12.16	0.0002	2	6.62	0.0038
	id	2	0.78	0.4692	3	0.18	0.9064
	rep	3	2.78	0.0626	3	1.67	0.1921
	cv. × id	4	1.63	0.1985	6	0.42	0.8574
50	cv.	2	57.63	<0.0001	2	15.42	<0.0001
	id	2	15.83	<0.0001	3	3.63	0.0227
	rep	3	0.06	0.9817	3	1.15	0.3446
	cv. × id	4	3.95	**0.0133**[a]	6	1.83	0.1240
75	cv.	2	53.25	<0.0001	2	39.44	<0.0001
	id	2	22.05	<0.0001	3	16.59	<0.0001
	rep	3	2.84	0.0593	3	0.52	0.6735
	cv. × id	4	5.64	**0.0024**	6	4.37	**0.0024**
100	cv.	2	13.98	<0.0001	2	24.98	<0.0001
	id	2	7.29	0.0034	3	19.85	<0.0001
	rep	3	2.94	0.0534	3	2.74	0.0589
	cv. × id	4	1.84	0.1543	6	0.40	0.8714
125	cv.	2	10.21	0.0006	2	10.07	0.0004
	id	2	2.61	0.0946	3	4.27	0.0118
	Rep	3	3.04	0.0485	3	2.31	0.0942
	cv. × id	4	0.70	0.5979	6	0.43	0.8525

DS was calculated using a logistic growth equation; rep=replication

[a] Values in bold indicate significant interaction of cultivar × inoculum density

the differences in WSY_{loss} increased with decreasing inoculum density. In inoculum densities 2 and 3, the differentiation between the susceptible and resistant cultivars was significant.

The relationship between AUDPC and WSY_{loss} was positively correlated with an identical slope independent of the genotype (Fig. 6). However, the susceptible cultivar showed a higher AUDPC than

Table 3 Differences in disease severity (DS) of *C. beticola* in sugar beet among cultivars at different days post-inoculation (dpi) depending on inoculum density (1–5); Germany, 2005 and 2006

	2005												2006							
	50 dpi						75 dpi						75 dpi							
	Inoculum density																			
Cultivar	1	T[a]	2	T	3	T	1	T	2	T	3	T	2	T	3	T	4	T	5	T
Susceptible	20.99	a[b]	18.16	a	9.69	a	100.00	a	99.53	a	99.10	a	41.96	a	27.68	a	19.36	n.s.	11.02	n.s.
Resistant 1	6.28	b	2.87	c	3.77	b	91.23	ab	78.81	ab	45.26	b	21.81	b	14.58	b	12.69		10.80	
Resistant 2	8.81	b	9.35	b	3.49	b	70.57	b	63.86	b	38.30	b	10.89	c	5.06	c	7.26		5.73	

DS was calculated using a logistic growth equation

[a] multiple comparison test carried out according to Tukey (T), $P \leq 0.05$

[b] different letters indicate significant differences between cultivars, n.s. non-significant effects

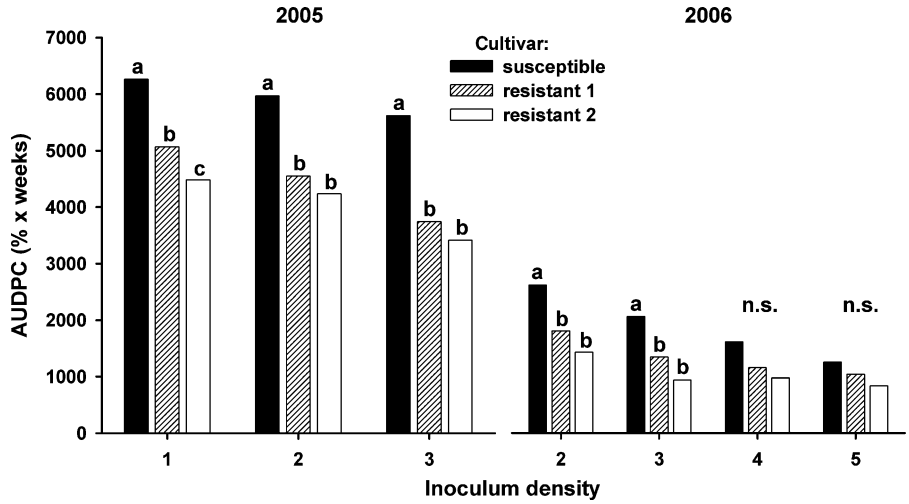

Fig. 4 Area under the disease progress curve (AUDPC) of *C. beticola* in sugar beet cultivars with different susceptibilities depending on the inoculum density; Germany, 2005 and 2006. Multiple comparison test carried out according to Tukey ($P \leq 0.05$), different letters indicate significant differences among cultivars for each inoculum density, n.s. non-significant effects

both resistant cultivars. This resulted in a higher WSY_{loss} of the susceptible cultivar than the resistant cultivars. The resistant cultivars had a high determination coefficient (r^2) of 0.74 and 0.68 while r^2 of the susceptible cultivar reached only 0.45.

Discussion

In this study, we aimed to estimate optimal *C. beticola* inoculum densities in field tests using artificial inoculation, suitable for the differentiation of commercially-cultivated sugar beet hybrids possessing variable degrees of Cercospora resistance. We quantified for the first time that in our artificial inoculation experiments the disease progress, in addition to climatic

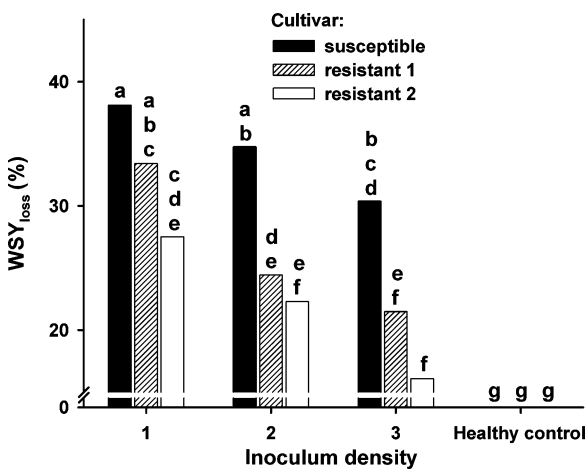

Fig. 5 Loss of white sugar yield (WSY_{loss}) in sugar beet cultivars with different susceptibilities to *C. beticola* depending on inoculum density, Germany, 2005, healthy control per cultivar = 100. Multiple comparison test carried out according to Tukey ($P \leq 0.05$), different letters indicate significant differences among cultivars for each inoculum density, n.s. non-significant effects

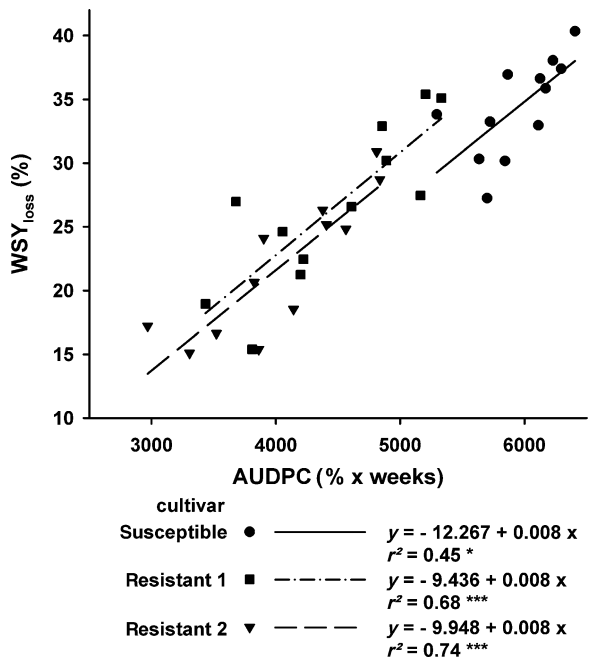

Fig. 6 Correlation between area under the disease progress curve for *C. beticola* (AUDPC) and loss of white sugar yield (WSY_{loss}) in sugar beet cultivars with different susceptibilities, Germany, 2005. For each cultivar all four replicate values of the three inoculum densities, were used for calculation. WSY of healthy control per cultivar = 100. r^2 coefficient of determination, *, **, *** indicate significant results for probabilities of error at $P \leq 0.05$, 0.01, 0.001

conditions, strongly depended on the inoculum density. Additionally, for the cultivars tested, AUDPC and WSY_{loss} were strongly correlated independently of the cultivar's resistance traits. Artificial inoculation can result in rapid disease progress of *C. beticola* and can have an effect on yield similar to heavy natural infection, and can therefore be used to evaluate the possible impact of a severe epidemic. Because climatic conditions cannot be foreseen in field experiments we conclude that resistance tests applying varying inoculum densities would be the correct procedure to use to evaluate Cercospora resistance, and might help to reduce the high number of field locations required to evaluate and compare the resistance of cultivars under natural infection as described by Ossenkop et al. (2004).

In both years inoculation with the fungus was successful and at 7 dpi first symptoms were found in all cultivars independently of their resistance. This incubation period is in contrast to former field studies using artificial inoculation (Pfleiderer and Schäufele 2000) where first symptoms of *C. beticola* appeared not earlier than 15 dpi and 30 dpi depending on the cultivar's susceptibility, although the inoculum density in their trials was higher than in the experiments described here. The authors applied an inoculum density of one *Cercospora* overgrown Petri dish per plot, which can only be roughly estimated to represent a density of 100,000 iu ml^{-1}. The variable length of incubation period observed might be explained by the different climatic conditions that were not documented. The observation that the first *Cercospora* leaf spots, independently of the genotype, appeared simultaneously, is in agreement with previous descriptions of Rossi and Battilani (1990; cited in Rossi 2000) who demonstrated that the time of disease onset on susceptible and resistant cultivars was nearly identical. In addition, no influence of the inoculum density on the time of first spot appearance was detected.

An exponential increase of DS was observed not earlier than 6 or 8 weeks post-inoculation; this might be explained by the suboptimal climatic conditions for fungal development and sporulation in the field in both years. Infection with *C. beticola* as well as incubation period, lag phase and conidial production strongly depend on climatic conditions. High temperatures with an optimum of 30°C and a high RH ranging between 98% and 100% as well as adequate leaf wetness duration accelerate fungal development and spread (Bleiholder and Weltzien 1971, 1972a). RH is the most important climatic factor influencing infection; at a RH of 96% the conidial production is already inhibited and at <88.5% RH conidial production is completely inhibited (Bleiholder and Weltzien 1971, 1972a). These conditions were assumed to be the reasons for the long lag phase and the late spread of the fungus in the field observed in our investigations.

The DS progress of *C. beticola* varied between cultivars and inoculum densities in our experiments. In contrast, Pfleiderer and Schäufele (2000) did not detect differences in final DS at harvest, when applying two different inoculum densities (one and one quarter *Cercospora* overgrown Petri dishes per plot). Probably both inoculum concentrations were at a very high level, inducing no differential genotypic reactions. In both years the resistant cultivars obviously showed a delayed DS development in comparison to the susceptible cultivar. This was probably the result of the rate-reducing resistance character as described by Rossi et al. (1999, 2000a). The DS development of both resistant cultivars displayed the same effect of the climatic conditions but the relationship to DS development of the susceptible cultivar varied with the inoculum density. DS differentiation between the susceptible and both resistant cultivars at different dpi was most pronounced at inoculum densities 2 and 3 in 2005 as well as in 2006. The DS development of the tested cultivars at these two inoculum densities was similar to DS development under natural infection in 2004. Evaluating DS development at all other inoculum densities applied in both years clearly showed that densities higher and lower than 20,000 iu ml^{-1} and 10,000 iu ml^{-1} resulted in less or no detectable differentiation between resistant and susceptible genotypes. Because inoculum density 1 was not applied in 2006, we can only speculate whether a better differentiation between the genotypes would have been observed. Therefore, we conclude that the components of partial resistance that reduce the development of the pathogen's disease-cycle (infection rate, lag phase and infectious period), perform best at a certain primary inoculum density.

Njiti et al. (2001) evaluated quantitative cultivar resistance against soybean sudden death syndrom in greenhouse experiments. Similar to our results, low inoculum densities did not allow a sufficiently clear

differentiation among cultivars. Since AUDPC is a result of DS and time, the maximum AUDPC differentiation was observed at the same inoculum densities (2 and 3) in both years as for DS. In addition, maximum AUDPC differentiation between susceptible and resistant cultivars was only achieved if an epidemic occurred at optimal inoculum densities. This supports the previous observations of Pfleiderer and Schäufele (2000) and Ossenkop et al. (2004) and provides further evidence for the necessity of optimised inoculum densities in resistance screening.

The fact that DS differentiation between resistant and susceptible cultivars varied over time has consequences for field disease assessment. In order not to underestimate the resistance of the cultivar, disease assessment should be made at the time when maximum differentiation between resistant and susceptible cultivars is observed. Assessment during the whole of disease progress and comparison of AUDPC values between cultivars, however, are the most appropriate measurements to evaluate resistance. Therefore, assessing DS just one or two times during the whole of disease progress may give misleading results. Assessing DS regularly during the whole of disease progress and subsequent calculation of the AUDPC gives an advantage over single DS values as the whole of disease progress is reflected in just one value. We conclude that AUDPC is more appropriate to detect a clear differentiation between cultivars. However, time and labour costs for regular DS assessment may be too high to be feasible if many cultivars have to be tested in parallel. Additionally, single DS measurements do not cover changing climatic conditions that affect epidemic development (Danielsen et al. 2001). This is in agreement with Broers et al. (1996), who analysed resistance of spring wheat cultivars against yellow rust and demonstrated that AUDPC is a suitable parameter to characterise quantitative resistance in host-pathogen interactions.

For sugar beet, the determination of WSY_{loss} induced by the destruction of leaf green area and subsequent leaf regrowth (Rossi et al. 2000b) is a very important parameter to evaluate cultivar performance under disease conditions. Only in 2005, under optimal conditions for Cercospora disease progress, were yield effects observed. In 2006, where the stationary phase of DS of the susceptible cultivar was reached close to harvest, no yield differences were observed. Only if densities lower than inoculum density 1 of *C. beticola* infectious units under favourable climatic conditions caused an epidemic, was maximum differentiation of WSY_{loss} between resistant and susceptible cultivars observed. The optimal inoculum density for cultivar differentiation in disease progress as well as in WSY_{loss} ranged between 10,000 and 20,000 iu ml^{-1} and a similar differentiation between resistant and susceptible cultivars was found under natural infection. In contrast to the artificially inoculated trial, resistant cultivar 2 displayed a higher WSY_{loss} than resistant cultivar 1, although the DS progress in the former was delayed in comparison to the latter.

There was a positive linear correlation of AUDPC with WSY_{loss}, supporting previous observations (Wolf et al. 1995). This leads to the conclusion that at an optimal inoculum density, maximum differentiation of WSY_{loss} can be expected at high AUDPC values. However, as all cultivars tested here showed an identical slope in this relationship, we conclude that identical AUDPC will produce similar WSY_{loss} independent of genotypes. Therefore, yield tolerance to infection with *C. beticola* of the cultivars in this study, was not detectable. Lower WSY_{loss} of the tested resistant cultivars was the result of lower AUDPC values, based on resistance traits. This is in contrast to the findings of Ossenkop et al. (2004) who detected differences in symptom expression and yield reaction between resistant cultivars; however AUDPC values were not determined. Further studies with more cultivars are needed to prove if this is a general correlation for sugar beet. It is important to mention the fact that WSY_{loss} in this study is a relative value, and does not consider the absolute yield of a specific cultivar. Most Cercospora-resistant cultivars still suffer from a yield penalty under non-diseased conditions (Mechelke 2000), although some comparable high-yielding cultivars are now available (BSA, Bundessortenamt 2007). As long as the above relationship is true for all resistant cultivars, it has to be considered when comparing the performance of resistant and susceptible cultivars.

This study has clearly demonstrated that the inoculum density for testing cultivar resistance has to be carefully optimised. The optimal concentration of 10,000 to 20,000 iu ml^{-1} for maximum AUDPC differentiation under the environmental conditions of our field tests represents only one important factor for

resistance screening. The influence of post-inoculation climatic conditions on epidemic progress is equally important and remains unpredictable. Initial infection is no guarantee for successful disease spread and rapid disease progress, but the use of optimal inoculum densities will render Cercospora field resistance tests more reliable and reproducible under variable climatic conditions.

References

Adams, H., Schäufele, W. R., & Märländer, B. (1995). A method for the artificial inoculation of sugarbeet with *Cercospora beticola* under field conditions. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*, *102*, 320–322.

Asher, M. J. C., Luterbacher, M. C., & Frese, L. (2001). Wild *Beta* species as a source of resistance to sugar-beet pests and diseases. *International Sugar Journal*, *103*, 447–456.

Battilani, P., Beltrami, G., Meriggini, P., Ponti, I., Rossi, A., Rossi, V., Rosso, F., Tugnoli, V., & Zocca, A. (1990). Nuovo indirizzi, di difesa anticercosporica. *L'Informatore Agrario*, *46*, 53–70.

Bleiholder, H., & Weltzien, H. C. (1971). Beiträge zur Epidemiologie von *Cercospora beticola* Sacc. an Zuckerrübe. I. Die Inkubations- und Fruktifikationszeit. *Phytopathologische Zeitschrift*, *72*, 344–353. doi:10.1111/j.1439-0434.1971.tb03207.x.

Bleiholder, H., & Weltzien, H. C. (1972a). Beiträge zur Epidemiologie von *Cercospora beticola* Sacc. an Zuckerrübe. II. Die Konidienbildung in Abhängigkeit von den Umweltbedingungen Temperatur, relative Luftfeuchtigkeit und Licht. *Phytopathologische Zeitschrift*, *73*, 46–68. doi:10.1111/j.1439-0434.1972.tb02524.x.

Bleiholder, H., & Weltzien, H. C. (1972b). Beiträge zur Epidemiologie von *Cercospora beticola* Sacc. an Zuckerrübe. III. Geopathologische Untersuchungen. *Phytopathologische Zeitschrift*, *73*, 93–114. doi:10.1111/j.1439-0434.1972.tb02532.x.

Broers, L. H. M., Cuesta Subias, X., & López Atilano, R. M. (1996). Field assessment of quantitative resistance to yellow rust in ten spring bread wheat cultivars. *Euphytica*, *90*, 9–16.

BMVEL, Bundesministerium für Verbraucherschutz, Ernährung und Landwirtschaft (2004). Reduktionsprogramm chemischer Pflanzenschutz. Nachhaltige Landwirtschaft—Vorsorgender Verbraucherschutz—Schutz des Naturhaushalts.

BSA, Bundessortenamt (2007). *Beschreibende Sortenliste Getreide, Mais, Ölfrüchte, Leguminosen, Hackfrüchte*. Deutscher Landwirtschaftsverlag: Hannover.

Byford, W.-J. (1996). *A survey of foliar diseases of sugar beet and their control in Europe*. Paper presented at the 59th IIRB Congress, Brussels.

Danielsen, S., Jacobsen, S. E., Echegaray, J., & Ames, T. (2001). *Impact of downy mildew on the yield of quinoa. In Program Report 1999–2000* pp. 397–401. Lima: International Potato Center.

Dufner, J., Jensen, U., & Schumacher, E. (2002). *Statistik mit SAS*. Stuttgart: B.G. Teubner.

Harveson, R. M., Hein, G. L., Smith, J. A., Wilson, R. G., & Yonts, C. D. (2002). An integrated approach to cultivar evaluation and selection for improving sugar beet profitability. *Plant Disease*, *86*, 192–204. doi:10.1094/PDIS.2002.86.3.192.

Kaiser, U. (2007). Epidemiology of *Cercospora beticola* Sacc. and disease-loss relationship in sugar beet (*Beta vulgaris* L.) depending on the susceptibility of cultivars and consequences for cultivar specific threshold systems. Ph.D. thesis, University of Goettingen.

Karaoglanidis, G. S., Ioannidis, P. M., & Thanassoulopoulos, C. C. (2002). Changes in sensitivity of *Cercospora beticola* populations to sterol-demethylation-inhibiting fungicides during a 4-year period in northern Greece. *Plant Pathology*, *51*, 55–62.

Märländer, B., Hoffmann, C., Koch, H.-J., Ladewig, E., Merkes, R., Petersen, J., & Stockfisch, N. (2003). Environmental Situation and Yield Performance of the Sugar Beet Crop in Germany: Heading for Sustainable Development. *Journal Agronomy & Crop Science*, *189*, 201–226. doi:10.1046/j.1439-037X.2003.00035.x.

Mechelke, W. (2000). Züchtungs-und Sortenstrategien zur Resistenz bei Zuckerrüben gegenüber *Cercospora beticola*. *Zuckerindustrie*, *125*, 688–692.

Munerati, O. (1920). Osservazione e Richerche sulla Barbabietola da Zucchero. *Observations and Research on Sugar Beet—Part 1. reprint 1999*. Tipografica della R. Accademia dei Lincei.

Njiti, V. N., Johnson, J. E., Torto, T. A., Gray, L. E., & Lightfoot, D. A. (2001). Inoculum rate influences selection for field resistance to soybean sudden death syndrome in the greenhouse. *Crop Science*, *41*, 1726–1731.

Ossenkop, A., Ladewig, E., & Manthey, R. (2002). Performance of Cercospora resistant cultivars—consequences for cultivar testing and advisory service. *Zuckerindustrie*, *127*, 867–871.

Ossenkop, A., Ladewig, E., & Manthey, R. (2004). Variety performance of sugarbeet under the attack of foliar diseases—opportunities and limitations of the variety trial system. *Zuckerindustrie*, *130*, 25–31.

Pfleiderer, U. E., & Schäufele, W. R. (2000). Development of a testing method for resistance against *Cercospora beticola* in sugar beet. In M. J. C. Asher, B. Holtschulte, M. R. Molard, F. Rosso, G. Steinrücken, & R. Beckers (Eds.), *Cercospora beticola Sacc. Biology, agronomic influence and control measures in sugar beet. Advances in Sugar Beet Research vol. 2* (pp. 147–153). Brussels: International Institute for Beet Research.

Pundhir, V. S., & Mukhopadhyay, A. N. (1987). Epidemiological studies on cercospora leaf spot of sugar beet. *Plant Pathology*, *36*, 185–191. doi:10.1111/j.1365-3059.1987.tb02220.x.

Rossi, V. (2000). Cercospora leaf spot infection and resistance in sugar beet. In M. J. C. Asher, B. Holtschulte, M. R. Molard, F. Rosso, G. Steinrücken, & R. Beckers (Eds.), *Cercospora beticola Sacc. Biology, agronomic influence and control measures in sugar beet. Advances in Sugar Beet Research vol. 2* (pp. 17–48). Brussels: International Institute for Beet Research.

Rossi, V., & Battilani, P. (1990). Dinamica delle epidemie di *Cercospora beticola* Sacc. su barbabietola da zucchero. III. Ruolo della suscettibilità varietale. *Phytopathologia Mediterranea*, *29*, 114–119.

Rossi, V., Battilani, P., Chiusa, G., Giosuè, S., Languasco, L., & Racca, P. (1999). Components of rate-reducing resistance to Cercospora leaf spot in sugar beet: incubation length, infection efficiency, lesion size. *Journal of Plant Pathology*, *81*, 25–35.

Rossi, V., Battilani, P., Chiusa, G., Giosuè, S., Languasco, L., & Racca, P. (2000a). Components of rate-reducing resistance to Cercospora leaf spot in sugar beet: conidiation length, spore yield. *Journal of Plant Pathology*, *82*, 125–131.

Rossi, V., Meriggi, P., Biancardi, E., & Rosso, F. (2000b). Effect of Cercospora leaf spot on sugar beet growth, yield and quality. In M. J. C. Asher, B. Holtschulte, M. R. Molard, F. Rosso, G. Steinrücken, & R. Beckers (Eds.), *Cercospora beticola Sacc. Biology, agronomic influence and control measures in sugar beet*. *Advances in Sugar Beet Research vol. 2* (pp. 49–76). Brussels: International Institute for Beet Research.

Ruppel, E. G., & Gaskill, J. O. (1971). Techniques for evaluating sugarbeet for resistance to *Cercospora beticola* in the field. *Journal of the American Society of Sugar Beet Technology*, *16*, 384–389.

Shaner, G., & Finney, R. E. (1977). The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox-wheat. *Phytopathology*, *67*, 1051–1056.

Smith, G. A., & Gaskill, J. O. (1970). Inheritance of resistance to Cercospora leaf spot in sugarbeet. *Journal of the American Society of Sugar Beet Technology*, *16*, 172–180.

Weiland, J., & Koch, G. (2004). Sugarbeet leaf spot disease (*Cercospora beticola* Sacc.). Pathogen profile. *Molecular Plant Pathology*, *5*, 157–166. doi:10.1111/j.1364-3703.2004.00218.x.

Wolf, P. F. J., & Verreet, J.-A. (1997). Epidemiologische Entwicklung von *Cercospora beticola* (Sacc.) in Zuckerrüben. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*, *104*, 545–556.

Wolf, P. F. J., Weis, F.-J., & Verreet, J.-A. (1995). Grundlagen einer integrierten Bekämpfung von *Cercospora beticola* in Zuckerrüben. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*, *102*, 574–585.

Wolf, P. F. J., Heindl, M., & Verreet, J.-A. (2001). Zum Einfluss des Bestandesklimas auf die Prädisposition der Zuckerrübe gegenüber Infektionen von *Cercospora beticola* (Sacc.). *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*, *108*, 578–592.