European Journal of Plant Pathology (2005) 112: 201–210
DOI 10.1007/s10658-004-4172-y

Root infection of sugar beet by *Cercospora beticola* in a climate chamber and in the field

Jessica Vereijssen[1], Johannes H.M. Schneider[1,*] and Aad J. Termorshuizen[2]
[1]*IRS (Institute of Sugar Beet Research), P.O. Box 32, 4600 AA, Bergen op Zoom, The Netherlands;*
[2]*Biological Farming Systems, Wageningen University, Marijkeweg 22, 6709 PG, Wageningen, The Netherlands;*
**Author for correspondence (Phone: +31164274400; Fax: +31164250962; E-mail: schneider@irs.nl)*

Accepted 21 September 2004

Key words: Cercospora leaf spot, disease, root incubation

Abstract

Sugar beet root infection by *Cercospora beticola*, the causal agent of Cercospora leaf spot (CLS), was studied in a climate chamber and in the field. In the climate chamber, root incubation of susceptible seedlings with a conidial suspension resulted in disease incidences that were significantly different for two sugar beet cultivars (Auris: 0.8 ± 0.14 and A00170: 0.5 ± 0.18; $P < 0.05$) with regard to the control treatment 35 days after root incubation in a standard potting soil-fine river sand mixture. In a field trial with susceptible cv. Savannah with soil-incorporated CLS-infested leaf material, disease developed four weeks earlier in the infested plots than in the control plots. The probability that disease develops in the field was significantly higher for the infested than for the control plots ($P < 0.05$). Symptomless plants from infested field plots transferred to the glasshouse to induce leaf spot symptoms showed a significantly higher probability to induce symptom development (0.4 ± 0.08), than plants from control plots (0.02 ± 0.02) ($P < 0.05$) 14 days after transfer. This probability was significantly higher than for plants that remained in three of the infested field plots (0.2 ± 0.04; 0.2 ± 0.05 and 0.2 ± 0.04 respectively), except for one infested field plot (0.4 ± 0.05) on July 5. We conclude that *C. beticola* is able to infect sugar beet seedlings through their roots and that latent CLS infections in sugar beet lead to symptom development at high temperatures (>20 °C) and high relative humidity (>95%) in our climate chamber or after canopy closure in the field. Quantification of root infection and long term survival in soil is necessary to assess its contribution to the epidemiology and life cycle of *Cercospora beticola*. Cultural methods such as a wider crop rotation, management of crop debris and ploughing systems may provide control strategies alternative to or reducing fungicide input.

Introduction

Cercospora beticola causes Cercospora leaf spot (CLS) in sugar beet (*Beta vulgaris*) and is the major fungal foliar pathogen of sugar beet worldwide (Holtschulte, 2000). The fungus causes well-defined, circular lesions on the leaves, enlarging to a maximum of 2–5 mm. Lesions are tan to light brown with dark brown or reddish purple margins and elongated lesions occur on petioles (Ruppel, 1986; Vereijssen, 2004). Minute black dots, pseudostromata, are often visible at the centre of mature lesions, by which *C. beticola* lesions can easily be distinguished from those caused by *Pseudomonas syringae* or *Ramularia beticola*. The disease is usually first observed after row closure, which is probably due to a more favourable microclimate (higher temperature and relative humidity; Mischke, 1960), and in shady locations, which is probably due to longer periods with high relative humidity. It has been demonstrated that *C. beticola* spreads mainly through splash-dispersal, e.g. rain and irrigation water (Pool and McKay, 1916; Carlson, 1967; Meredith, 1967).

Wind-dispersal is considered of minor importance (McKay and Pool, 1918; Meredith, 1967) and occurs only over short distances, e.g. to adjacent fields (McKay and Pool, 1918).

The source of initial infection prior to the first lesion development after canopy closure, however, is unclear. There is a possibility that seedlings and mature plants harbour latent *C. beticola* infections before canopy closure, but that symptom development only occurs under (sub-)optimal temperature and relative humidity. Even so, this still raises the question as to what the primary source of infection is, but leaves possibilities open for primary infection of new sites. Three aboveground sources of primary infections have been described. First, the fungus has been reported to survive on weeds and other crops (Frandsen, 1955; El-Kazzaz, 1977; Soylu et al., 2003). However, in the Netherlands, in a joint survey conducted in 2003 with the Centraal Bureau Schimmelcultures (Utrecht, The Netherlands), *C. beticola* was not encountered on weeds (J. Vereijssen, IRS, The Netherlands, unpublished). Second, the role of wild beet plants (*B. vulgaris* spp. *maritima*) in the survival of *C. beticola* is unknown. In the Netherlands, the role of wild beet in the life cycle of *C. beticola* is negligible as it is fairly uncommon and grows only along the seashore (van der Meijden et al., 1990). In addition, severe outbreaks of CLS were first recorded in the southeast of the Netherlands, far from the coastline. Third, seed contamination by *C. beticola* has been suggested (Schürnbrand, 1952) and observed microscopically (Plotho, 1951), which offers the pathogen a niche for between-crop survival (Schürnbrand, 1952). Nowadays, seed contamination cannot be excluded as a source; even after polishing, processing and pelleting, conidia have been found on sugar beet seeds. Inexplicable CLS epidemics can develop (M. Nihlgård, Syngenta, Sweden, pers. comm.), but the role of seed infection in the CLS epidemiology is unknown.

Several studies have indicated that soil might be a source of primary infection. First, crop debris has repeatedly been demonstrated to serve as a source of inoculum when a new sugar beet crop is planted (McKay and Pool, 1918; Nagel, 1938; Knapp, 1954; Koch, 1958; Giannopolitis, 1978). At the start of a CLS epidemic, patches of diseased plants can be observed (Vereijssen, 2004), which may indicate the presence of soil-borne inoculum. Second, Stolze (cited in Plotho, 1951) reported infection of beet plants in the field when infected leaf material was buried up to 25 cm deep. Despite these results, splash dispersal was regarded as the primary dispersal mechanism. Third, the pathogen has been demonstrated to spread to the cotyledons by splash dispersal of conidia from the soil, and subsequently to the leaves (Nagel, 1938). Fourth, Kimmel and Potyondi (1999), observed less CLS symptoms in plots where soil had been treated with triazole fungicides (Impact and Milstar) in Hungary. Another indication that soil is a site of primary infection has recently been reported (Vereijssen et al., 2004). In the latter study, the sugar beet root was shown to be the primary site of infection in preliminary glasshouse experiments, but without full control of environmental conditions of temperature, relative humidity and light intensity, and conclusive field data were still lacking.

In this paper, root infection of sugar beet by CLS was studied in a controlled climate chamber and a field experiment. The objectives were to (i) repeat the glasshouse experiments described by Vereijssen et al. (2004) under better controlled conditions, (ii) induce CLS symptoms in the field with soil-incorporated CLS-infested leaf material and (iii) show whether *C. beticola* causes latent infections of sugar beet plants in the field, with symptom expression depending on favourable environmental conditions.

Materials and methods

Climate chamber experiment

Sugar beet seedlings were produced by sowing the CLS susceptible cv. Auris (30 seeds) and A00170 T99 (30 seeds) in seedling trays filled with a standard soil (fine river sand, 0–1 mm; pH = 6–6.5; 0% organic matter) in the glasshouse in March 2001. This cultivar is genetically identical, but the seed lot younger, than Auris. Plants were incubated for 14 days at 22 °C in 16:8 h light:dark regime until used in the root incubation experiment. A monoconidial culture of *C. beticola* isolate IRS 00-2, originally isolated from a field at Veendam, province of Groningen, The Netherlands, was used to produce conidia according to a method modified from L.W. Panella (USDA-ARS, USA, pers.

comm.). In short, *C. beticola* was grown on Potato Dextrose Agar (PDA) for 7 days and flooded with sterile water. Mycelium was scraped off, and the mycelial suspension was poured onto sugar beet leaf agar (SBLA; 50 g of fresh cut leaves in 450 ml of demi water and 10 g PDA was autoclaved at 121 °C for 35 min) still containing pieces of leaves, and left for 4 h for spores to settle. Excessive water was then decanted and the SBLA-plates were incubated at 23 °C for 7 days. The cultures were each flooded with 5 ml of tap water and the conidia were gently brushed off the culture plate using a hairbrush, into a jar. A conidial suspension was prepared from all cultures and divided over six black photo cans (diam. × height = 33 mm × 49 mm), with approximately 10^4 conidia/ml. Seedlings were gently removed with water from the standard soil. Ten seedlings (30 per cv.) were tied together with Parafilm (American National Can, USA) and the roots, not the hypocotyl, were immersed in the conidial suspension for 3 days at ambient temperature (18–22 °C) and under laboratory lighting. During the incubation period, air was gently blown through with a pipette to agitate the suspension for 30 s twice daily. Care was taken not to splash water to the hypocotyl and cotyledons. After root incubation, seedling roots were gently washed with tap water to wash off the largest part of attached conidia and mycelium. Control treatment seedlings (10 plants) were immersed with their roots in tap water for 3 days under similar conditions. After 3 days, each seedling was transferred to a pot (180 ml) containing a potting soil (30% dry matter; 27% organic matter; pH range 5–6.5) – fine river sand mixture (1:9 v/v). Control seedlings were transplanted first. Incubated seedlings were handled only at the cotyledons and stems and care was taken not to touch the upper soil with the incubated roots by making large transplanting-holes in the potting soil–sand mixture, to prevent future splash dispersal. Control and treated seedlings were separated to prevent cross-contamination. Plants, mostly at the cotyledon stage, were placed in a walk-in climate chamber at Advanta/VanderHave Sugar Beet Seed (Rilland, The Netherlands) with 14:10 h light:dark, 23 °C and 100% relative humidity created by fine mist from March 18 till April 3, and 16:8 h light:dark, 23 °C and 100% relative humidity till April 22. Where symptoms developed that were atypical of CLS, leaf spots were examined with a loupe or microscope. Symptoms on seedlings often differ from those on older plants by not displaying the reddish-purple margins, and leaf spots being on the leaf tip or elongated on leaves.

Field trial experiment

Field trial establishment. On March 14, 2001, a farmer's field that had never been planted with sugar beet and remote (>5 km) from any sugar beet crop before 2001, was selected as a trial site at Halsteren, province of Noord-Brabant, The Netherlands. The field trial bordered potatoes on the west-side, a strip of bare soil and a *Rhizoctonia* sugar beet trial on the east-side, and hedges surrounded these fields. A fungicide application (Score 250EC, Syngenta, The Netherlands) was applied on the *Rhizoctonia* field trial to prevent CLS infection or cross contamination. The experimental design (Figure 1) was laid out in plots 3 m wide (6 rows) and 10 m nett length, with 18 cm between plants in the rows. Each plot was

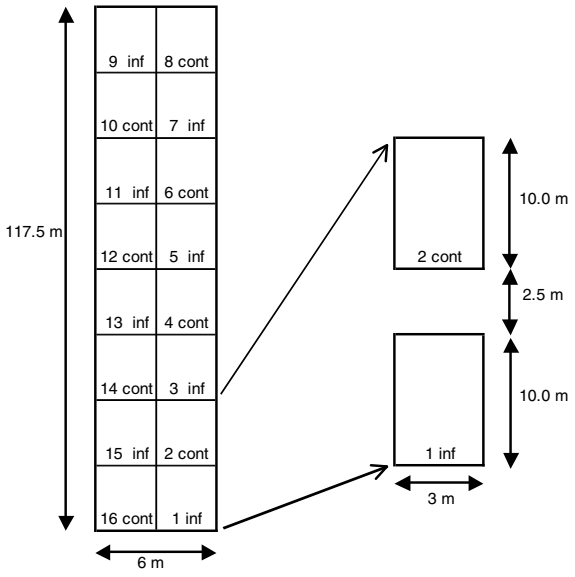

Figure 1. Lay out of the Cercospora leaf spot root infection field trial at Halsteren in 2001. The infested (inf) and control plots (cont) were 10 m long and 3 m (6 rows of sugar beet) wide and in the length separated by 2.5 m of sugar beet. In the infested plots dried CLS-infected leaf material was incorporated at 5 cm in soil on March 14; the control treatment contained no incorporated leaf material. Cultivar Savannah was sown above the infested leaf debris on April 24.

separated by 2.5 m sugar beet from another plot. The control plot was always adjacent to an infested plot in eight replications. Rows and row distances were marked out with a planter containing no sugar beet seed by driving with the coulter to the above dimensions. The marked out rows were later used as a seed furrow. The marked out rows were deepened to 5 cm using a spade. Dried, crushed, heavily CLS infested sugar beet leaves (60 g), collected at Wijnandsrade, province of Limburg, The Netherlands, in 2000, were spread evenly in the 10 m furrows and carefully dug in to a depth of approximately 5 cm. The furrow was closed with soil immediately after incorporation of the infested leaf material. These plots are referred to as infested plots, the non-infested plots as control plots. In the laboratory 10 g of dried infested leaves, kept separately for each plot, was soaked in 250 ml water and viability of conidia was determined for six plots. A large percentage of conidia were still viable as indicated by the development of germination tubes (data not shown). On April 24, sugar beet seed of the CLS susceptible cv. Savannah was drilled precisely on the marked out rows, with and without infestation, at a 3 cm depth, which was 2–3 cm above the soil-incorporated infested leaf material. Thus it was ensured that infested leaf material was below the planted seeds.

Disease assessment. CLS assessments started two weeks after sowing. Whole-plant disease severity of all individual sugar beet plants per treatment was assessed according to the pictorial Agronomica disease severity index (Vereijssen et al., 2003, modified after Battilani et al., 1990), which covers a scale from 0 (healthy) to 5 (totally destroyed foliage). When the first cercospora lesions were observed, early July 2001, we started the disease assessment of all plants in all plots on July 5. Due to extremely hot weather that week, only half the trial could be assessed that day due to the fact that the canopy wilted under those conditions and the first disease assessment was completed at July 11. The second assessment was conducted when diseased plants were found in the control plots (beginning of August), and disease in four plots was assessed, only to get an impression of the development of disease in the control treatments. Disease incidence, defined as the proportion of plants per plot with CLS symptoms, was used to assess the influence of incorporated infested leaves on the occurrence and development of CLS in a field.

Induction of leaf spots by high humidity in the glasshouse. The possibility that CLS can be latently present in a sugar beet plant, and that symptoms can be induced when temperature and relative humidity are optimal for symptom development was investigated by removing 18 arbitrarily chosen plants per plot (16×18 plants) from the field before CLS symptoms were visible in the field trial (May 22). Care was taken not to spill soil onto the leaves and the plants were taken into the glasshouse. The roots were gently washed to remove soil residues. Individual plants were transplanted immediately into plastic pots of 180 ml, containing the potting soil – fine river sand mixture described above. The plants were allowed to grow for one week at ambient glasshouse conditions. On May 29, plastic covers were put over the plants to create an ambient 100% relative humidity, and were removed on June 5 to prevent other fungi from developing and to give plants better growth conditions. All plants were checked daily for CLS symptoms till June 8.

Statistical analysis

Climate chamber experiment. Incidence data were analysed to calculate the confidence interval for the probability of a plant becoming diseased based on the binomial distribution, with parameters n indicating the number of plants per cultivar, and p the probability of a plant becoming diseased. The binomial distribution was approximated by a normal distribution, because both $n{\cdot}p$ and $n{\cdot}(1-p)$ were greater than 5 (Oude Voshaar, 1995). A confidence interval for p was calculated as $\hat{p} \pm 1.96 \times \sqrt{\hat{p}(1-\hat{p})/n}$ at the 5% significance level.

Field trial experiment. Incidence data were analysed to calculate the confidence interval for the probability of a plant becoming diseased between dates, between plots, between infested and control plots and in specific rows again assuming a binomial distribution with parameters n indicating the number of assessed plants in a plot, and p the probability of a plant becoming diseased in a plot or specific rows.

The same procedure was followed for estimating the probability of plants in the infested and control plots becoming diseased in the glasshouse. The binomial distribution was approximated by a normal distribution in both analyses.

Significance of disease severity according to the Agronomica disease severity index was calculated using the mean disease severity per plot. A confidence interval for the mean was calculated at the 5% significance level.

Results

Climate chamber experiment

Twenty-nine days after root incubation, disease incidences of 0.57 and 0.37 were recorded for cv. Auris and A00170 T99, respectively, in the root incubation treatment (Table 1). On one plant of the control treatment on cv. A00170 T99, one leaf spot caused by *C. beticola* was observed, possibly due to cross-infection or seed contamination. The proportion of plants that became diseased was not significantly different between cv. Auris (0.57 ± 0.18) and A00170 T99 (0.37 ± 0.17) ($P > 0.05$), but treated plants were significantly different from the control treatment (0.1 ± 0.09). Thirty-five days after root incubation, disease incidences of 0.80 and 0.53 were recorded for cv. Auris and A00170 T99, respectively, in the root incubation treatment (Table 1). The number of leaf spots recorded per plant ranged from 1 to 3 (mean = 1.5) for cv. A00170 T99 and from 1 to 5 (mean = 2.4) for cv. Auris, except for one plant having over more than 15 leaf spots (overall mean = 2.9). The number of spots on this plant could not be counted exactly as large necrotic areas were formed as a result of the many leaf spots. The one plant in the control treatment that showed symptoms at the first assessment still had only one leaf spot at the second assessment. The maximum likelihood estimator $\hat{p}$ of the binomial distribution was 0.80 ± 0.14 for cv. Auris and 0.53 ± 0.18 for A00170 T99, respectively, for the root incubation treatment. There was no significant difference between the two cultivars in this estimator ($P = 0.05$). The confidence interval for the control treatment of cv. A00170 T99 was 0.1 ± 0.09. The probability of becoming diseased was statistically significantly different from the two root incubation treatments ($P < 0.05$). Leaf spots in the root incubation treatment were visible on cotyledons as well as on newly formed leaves, however the number of cotyledons with symptoms was higher (A00170 T99 = 10; Auris = 14) than the number of cotyledons as well as newly formed leaves with symptoms (A00170 T99 = 4; Auris = 7). In a minority of cases, leaf spots only developed on the newly formed leaves (A00170 T99 = 0; Auris = 3) and not on the cotyledons. In contrast to the root infection experiment previously described by Vereijssen et al. (2004), lesions were not observed on the stem.

Table 1. Disease incidence in the root incubation climate chamber experiment with the sugar beet cultivar Auris and A00170 T99

Cultivar	Incidence	
	16 April (29 d.a.i.)[1]	22 April (35 d.a.i.)
Auris ($n = 30$)	0.57[2] a	0.80 a
A00170 T99 ($n = 30$)	0.37 a	0.53 a
Control Auris ($n = 10$)	0 b	0 b
Control A00170 T99 ($n = 10$)	0.10[3] b	0.10 b

Roots were immersed in a conidial suspension of *Cercospora beticola* for 3 days and consequently potted in a standard soil and incubated at 100% relative humidity and 23 °C.

[1] d.a.i. = days after incubation.

[2] Different letters in a column indicate significant difference at $P = 0.05$ using the binomial distribution approximated by a normal distribution.

[3] Possibly due to cross-infection.

Field trial experiment

On June 20 the first Cercospora leaf spot was observed in an infested plot. At the first disease assessment on July 5, ten weeks after sowing, disease incidences ranging between 0.22 and 0.37 were observed in the infested plots with no disease in the control plots (data not shown). Maximum likelihood estimators for the probability of a plant becoming diseased, $\hat{p}$, in infested plots 1, 3 and 7 (one half of the field trial) were not significantly different ($P > 0.05$) between plots, $\hat{p} = 0.2 \pm 0.04$, 0.2 ± 0.04 and 0.2 ± 0.05, respectively (Figure 2). The probability of becoming diseased in infested plot 5, was significantly higher (0.4 ± 0.05) than in infested plots 1, 3 and 7 (Figure 2), which could not be explained.

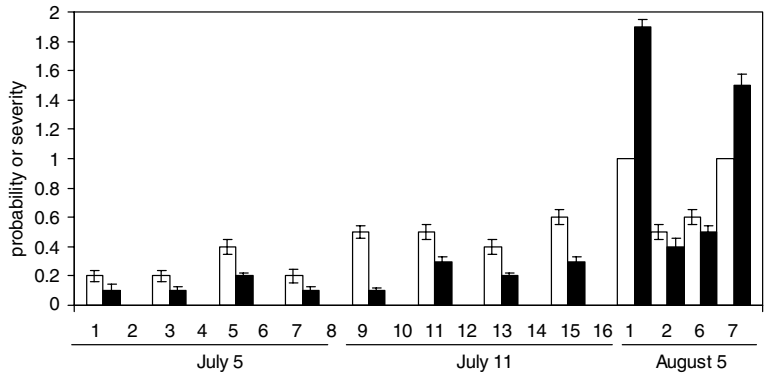

Figure 2. The estimated probability of a plant becoming diseased by *Cercospora beticola* (white bars) and mean disease severity per plot according to the Agronomica disease severity index (black bars) in infested and control plots at Halsteren 2001 at three assessment dates (July 5, July 11 and August 5). In the infested plots dried CLS-infected leaf material was incorporated at 5 cm in soil on March 14; the control treatment contained no incorporated leaf material. Cultivar Savannah was sown above the infested leaf debris on April 24.

On July 11 disease incidences in the infested plots varied between 0.25 and 0.63 (data not shown). The probabilities of plants becoming diseased in plots 9, 11, 13 and 15 differed significantly between the plots, $\hat{p} = 0.2 \pm 0.04$, 0.5 ± 0.05, 0.4 ± 0.05 and 0.6 ± 0.05, respectively. In the control plots no CLS symptoms were observed (Figure 2). Disease severities of plots 1 (0.1 ± 0.02), 3 (0.1 ± 0.03), 7 (0.1 ± 0.03), and 9 (0.1 ± 0.02) did not differ significantly ($P > 0.05$), as did disease severities of plots 5 (0.2 ± 0.02) and 13 (0.2 ± 0.03). Disease severities of plots 11 (0.3 ± 0.03) and 15 (0.3 ± 0.03) were significantly different from each other and significantly higher than of the other plots ($P < 0.05$) (Figure 2).

At the second assessment date on August 2, disease incidence in the infested plots reached 1 and severity had increased with regard to the first assessment (Figure 2). In the control plots, CLS symptoms were observed and disease incidence was 0.5 and 0.6 for plot 2 and 6 respectively (Figure 2). The probabilities of plants becoming diseased in control plots 2 (0.5 ± 0.05) and 6 (0.6 ± 0.05), were significantly lower than in infested plots 1 ($p = 1$) and 7 ($p = 1$) (Figure 2). The difference between plot 2 and 6 was not statistically significant ($P = 0.05$). Disease severities of control plots 2 (0.4 ± 0.06) and 6 (0.5 ± 0.04) were not significantly different ($P > 0.05$). Disease severities of infested plots 1 (1.9 ± 0.06) and 7 (1.5 ± 0.08) were significantly different from each other and from the control plots (Figure 2). The probabilities of plants becoming diseased in the first two rows of beet, adjacent to infested plots, of the control plots 2 and 6, were significantly higher than in rows three to six (Table 2). For the infested plots 1 and 7, no significant difference between the first two rows and three to six was observed.

Induction of leaf spots by high relative humidity in the glasshouse

On June 1, two weeks after removing plants from the field, CLS symptoms were visible on plants of

Table 2. The estimated probability of a plant becoming diseased ($\hat{p}$) by *Cercospora beticola* in the first two rows and in rows three till six of the infested and control plots at Halsteren 2001 at 2 August

Plot	Rows 1 and 2	Rows 3 till 6
	$p \pm$ CI[1]	$p \pm$ CI
1 Infested	1	1
2 Control	0.81 ± 0.07	0.36 ± 0.06
6 Control	0.86 ± 0.06	0.49 ± 0.07
7 Infested	1	1

The first two rows of the control plots were adjacent to infested plots. In the infested plots dried CLS-infected leaf material was incorporated at 5 cm in soil on March 14; the control treatment contained no incorporated leaf material. Cultivar Savannah was sown above the infested leaf debris on April 24.

[1] $\hat{p}$ is the maximum likelihood estimator, in this study the probability that a plant becomes diseased, $\pm$ the confidence interval (CI) for the binomial distribution approximated by a normal distribution at $P = 0.05$.

the infested treatment, resulting in 0.11 disease incidence (Table 3). The control treatment was free of CLS symptoms. On 5 June one leaf spot was visible in the control treatment, possibly due to cross infection caused by glasshouse air circulation in the period without plastic cover. The probability of becoming diseased in the glasshouse when a plant originated from the infested field plots at June 5 was estimated as 0.4 ± 0.08, significantly higher than when a plant originated from the control field plots (0.02 ± 0.023). The probability of a plant becoming diseased in the glasshouse at June 5 was significantly higher than for plants in the infested individual field plots 1, 3 and 7 in the field trial at July 5 ($\hat{p} = 0.2 \pm 0.04$; 0.2 ± 0.05; 0.2 ± 0.04, respectively). The estimated probability for the infested field plot 5 (0.4 ± 0.05) was not significantly different from the estimated probability for the glasshouse.

Discussion

In this paper, we discuss the occurrence of root infection by *C. beticola* of sugar beet under both climate chamber and field conditions. We were able to repeat the experiments reported by Vereijssen et al. (2004) under better controlled conditions. We obtained significantly higher disease incidences in the root infected treatment compared to the previous study, which is probably due to the more favourable conditions of temperature and relative humidity maintained during the experiments. In contrast to the previous study, we did not observe stem lesions on the seedlings. If the infection occurs at the roots and mycelium grows within the plant or epiphytically along with the plant, a slower growth of the pathogen is expected under sub-optimal conditions, which might result in lesions on the stem as a kind of survival/propagative mechanism of the pathogen. Epiphytic growth of *C. beticola* cannot be excluded in either the climate chamber or field experiment and deserves further research. However, it seems almost impossible that the fungus could grow and survive epiphytically on the plant for up to four months. Stem infections of seedlings were not observed in the field trial, nor in farmers' fields. CLS symptoms on above-ground hypocotyls and upper parts of the main root of older sugar beet were observed by Giannopolitis (1978) in Greece in late August. Whether these CLS lesions are a result of early season seedling stem infection or rain/dew run-off containing conidia from foliage to the root is uncertain. Root infection may offer new options in breeding. Currently, breeding for CLS reaction focuses on tolerance; disease still develops but with disease severities a little lower than on susceptible varieties and a higher financial return (Anonymous, 2003). Another option for plant breeders might be to focus on root resistance. Another important implication is that, although beet growers control CLS by spraying fungicides, root infection may require other control methods than spraying foliar fungicides.

Table 3. Disease incidence of sugar beet plants transferred from the field trial on 22 May 2001 to the glasshouse and incubated at 20 °C and more than 95% relative humidity for *Cercospora beticola* development

Assessment date	Incidence		Avg # leaves/ plant
	Infested plot (n = 144)	Control plot (n = 144)	
June 1	0.11[1] a	0 b	5
June 5	0.35 a	0.07[2] b	6
June 8	0.40 a	0.07[2] b	6

[1] Different letters for the same assessment date indicate significant difference at P = 0.05 using the binomial distribution approximated by a normal distribution.
[2] Possibly due to cross-infection.

The observed disease in the control plots in the field on August 5 was probably not a result of root infection by soil-borne inoculum, since in the glasshouse experiment, latent infections were only demonstrated in plants from the infested plots. Conclusively, the infection came from above-ground. However, air-borne dispersal is considered to be very unlikely (McKay and Pool, 1918; Meredith, 1967) and we can hypothesise that splash dispersal from adjacent infested plots caused the disease in the control plots. We demonstrated for the control plots only that plants in the first two rows, neighbouring the infested plots, had a higher probability becoming diseased than plants in rows further away from the infested plots, demonstrating a short range nature of dispersal of CLS.

The ability of *C. beticola* to infect sugar beet plants via the root and to survive in soil merits consideration of this pathogen as having a significant soil-borne phase. Survival of *C. beticola* on

infected leaf debris buried 2.5–12.5 cm in soil through winter has been demonstrated (McKay and Pool, 1918) and may continue for at least two years (Jones and Windels, 1991). Longevity of *C. beticola* in field soil declined in successively a greenhouse, cold room (5 °C), and freeze room (−7 °C) experiment of 20 months, but the pathogen was still able to infect sugar beet seedlings that were grown in the field soil (Nagel, 1938). The pathogen can be recovered from above-ground leaf debris left behind in the field, in January in the Netherlands (J. Vereijssen, IRS, The Netherlands, unpublished). Other airborne *Cercospora* spp., affecting other crops, such as soybean, maize, peanut, lettuce, and other legume crops, survive the winter on crop residues and can infect the crop in subsequent years (Kilpatrick, 1956; Payne and Waldron, 1983; Alabi, 1986; de Nazareno et al., 1993). *C. asparagi* on asparagus is also known to survive winter, but the pathogen survived better in above ground debris than in buried debris (Cooperman et al., 1986). For a range of leaf pathogens, tillage involving the burying of infested leaf debris reduces disease severity in the next season in comparison with non-tillage (Payne et al., 1987; Elen, 2003). By contrast, in Germany, different tillage systems did not influence CLS infection, and conservation tillage (non-inverting over a depth of 10 cm) did not enhance survival of *C. beticola* over a three year period (Koch et al., 2003). For other pathogens considered to be typically airborne, infection from inoculum (oospores) residing in soil has been reported, e.g. from downy mildew (*Peronospora viciae* f. sp. *fabae* infecting faba bean seedlings; van de Gaag and Frinking, 1997a). This pathogen can survive in significant numbers one growing season (van de Gaag and Frinking, 1997b), but to what extent survival over multiple years is important is unclear. It also occurs in *Magnaporthe grisea* causing both rice blast and gray leaf spot in cereals (Sesma and Osbourn, 2004). In most of the above-mentioned examples, pathogens germinate at the root surface and subsequently the fungal hyphae enter the root and thus colonise the plant. The process of root infection in *C. beticola*, through conidia, hyphae or epiphytic growth, merits further research.

Long-term survival in soil might be less relevant for airborne pathogens that have a short disease cycle and efficient wind dispersal, such as is the case for downy and powdery mildews and rusts. However, for *C. beticola* the relevance of survival in soil is more relevant as wind dispersal appears inefficient and splash dispersal predominates (McKay and Pool, 1918; Carlson, 1967; Meredith, 1967). A study on the long-term survival of *C. beticola* in infects plant material in soil is essential for new control measures. If a significant proportion of *C. beticola* inoculum survives the duration of a typical crop rotation of 4 years, disease onset may occur earlier following row closure and thus damage may be more significant. This phenomenon has been observed in the second year in fields with two years of sugar beet (Nagel, 1938; Jones and Windels, 1991; J. Vereijssen, IRS, The Netherlands, unpublished). De Nazareno et al. (1993) concluded that for *C. zeae-maydis* 'that there was a positive association between disease severity and amount of infested residue on the soil surface, although the environment had a major effect on the level of disease variability'. If it appears necessary to reduce inoculum of *C. beticola* residing in soil, only few options are available. Extensification of crop rotation is difficult because of economic reasons and limits on the available land for growers. Perhaps survival of *C. beticola*, in the form of conidia, mycelium and pseudostromata, can be influenced by the timing of incorporation of beet material in soil. When infested debris is incorporated before winter, the chances of the pathogen to survive are limited because of natural breakdown of the debris. Control of the disease can also focus on CLS-specific fungicides incorporated within the pelleted seed. Seed-bed treatment with a fungicide (Kimmel and Potyondi, 1999) is not an option in the Netherlands, where they are not allowed on a large scale. Future research should quantify how long *C. beticola* can survive on beet residue in soil, if *C. beticola* is able to grow vegetatively in soil as demonstrated with sterile soil cultures in Petri dishes and in field soil by Nagel (1938), and the length of time beet residues can survive in soil, providing a niche for vegetative growing *C. beticola*.

Symptomless plants from infested plots in the field trial that were placed in the glasshouse, showed CLS symptoms after 10 days. Subsequently on June 5, we calculated a significantly higher probability for plants had become diseased in the glasshouse than in the field for 3 of 4 plots at July 5. This supports the hypothesis that

C. beticola can be present latently within an infected plant without showing symptoms due to sub-optimal conditions. The question arises as to whether a fungicide spray on infected, but symptomless, beet would contribute to the control of CLS. Fungicides approved for CLS control have both a curative and preventive mode of action. If the curative mode of action acts against mycelium or conidia inside the plant, this might offer a new approach for the development of supervised control systems. A fungicide spray can then be applied when no symptoms are visible as in the Dutch Cercospora Advisory Model (CAM) being developed at the IRS (Vereijssen, 2004). CLS development appears to depend on favourable weather conditions, which are included in the CAM. A calculation of the risk on CLS through the CAM then would allow a minimised and optimal input of fungicides.

We have shown that, under controlled conditions, root infection of sugar beet seedlings by *C. beticola* is possible. In a field experiment, disease symptoms in infested plots were present earlier in the season than in the non-infested control plots. Inoculum causing the disease in the control plots most likely came from the adjacent infested plots. Symptomless plants, grown in infested plots in the field, developed symptoms under optimal environmental conditions, suggesting the possibility of latent infections. Molecular detection techniques and histological studies will be needed to confirm the presence of *C. beticola* in symptomless but infected plants, only when epiphytic growth can be excluded.

Acknowledgments

Many thanks to R. van den Maegdenbergh, E. van Lakwijk and O. de Nijs for their help preparing, maintenance and harvesting the field trial. The help of J. Schoone and R. Munning during the glasshouse experiment is greatly appreciated. We would like to thank A.C. van Spijk and M.C. Rijstenbil of VanderHave Sugar beet Seeds (Rilland, The Netherlands) for their useful suggestions, help during the climate chamber experiment, use of their climate chambers, and all the efforts to make the experiments work. The help of N. van Swaaij with the statistical analysis is greatly appreciated. Prof. Dr. M. Jeger is thanked for reading the manuscript and constructive discussion.

References

Alabi RO (1986) Dispersal and survival of conidia of *Cercospora arachidicola* Hori. Archives of Phytopathology and Plant Protection 22: 33–39

Anonymous (2003) Jaarverslag 2002. Publicatie van Stichting IRS, Bergen op Zoom, The Netherlands

Battilani P, Beltrami G, Meriggi P, Ponti I, Rossi A, Rossi V, Rosso F, Tugnoli V and Zocca A (1990) Nuovi indrizzi di difesa anticercosporica. L'Informatore Agrario 46: 53–70

Carlson LW (1967) Relation of weather factors to dispersal of conidia of *Cercospora beticola* (Sacc.). Journal of the American Society of Sugar Beet Technologists 14: 319–323

Cooperman CJ, Jenkins SF and Averre CW (1986) Overwintering and aerobiology of *Cercospora asparagi* in North Carolina. Plant Disease 70: 392–394

de Nazareno NRX, Lipps PE and Madden LV (1993) Effect of levels of corn residue on the epidemiology of gray leaf spot of corn in Ohio. Plant Disease 77: 67–70

Elen O (2003) Long-term experiments with reduced tillage in spring cereals. III. Development of leaf diseases. Crop Protection 22: 65–71

El-Kazzaz MK (1977) Cercospora leaf spot disease of chard in Egypt. Egyptian Journal of Phytopathology 9: 81–82

Frandsen NO (1955) Über den Wirtskreis und die systematische Verwandschaft von *Cercospora beticola*. Archiv für Mikrobiologie 22: 145–174

Giannopolitis CN (1978) Lesions on sugarbeet roots caused by *Cercospora beticola*. Plant Disease Reporter 62: 424–427

Holtschulte B (2000) *Cercospora beticola* – worldwide distribution and incidence. In: Asher MJC, Holtschulte B, Richard Molard M, Rosso F, Steinrücken G and Beckers R (eds) Advances in Sugar Beet Research Vol. 2: *Cercospora beticola* Sacc. biology, agronomic influence and control measures in sugar beet (pp 5–16) IIRB, Brussels, Belgium

Jones RK and Windels CE (1991) A management model for Cercospora leaf spot of sugarbeets. *University of Minnesota Extension Service AG-FO-5643-E*

Kilpatrick RA (1956) Longevity of *Cercospora kikuchii* on soybean stems. Phytopathology 46: 58

Kimmel J and Potyondi L (1999) Seed-bed treatment against *Cercospora beticola*. Proceedings of the 62nd IIRB congress, Seville, Spain: 301–309

Knapp E (1954) Können Cercospora-Infektionsquellen im Feld über mehrere Jahre erhalten bleiben? Zucker 8: 169–170

Koch F (1958) Ein Feldversuch zur Frage der Lebensdauer von *Cercospora beticola* im Boden. Zucker 10: 237–238

Koch HJ, Pringas C and Scherer J (2003) Conservation tillage for sustainable sugarbeet production in Germany – environmental and phytopathological aspects. Zuckerindustrie 128: 810–813

McKay MB and Pool VW (1918) Field studies of *Cercospora beticola*. Phytopathology 8: 119–136

Meredith DS (1967) Conidium release and dispersal in *Cercospora beticola*. Phytopathology 57: 889–893

Mischke W (1960) Untersuchungen über den Einfluß des Bestandsklimas auf die Entwicklung der Rüben-Blattfleckenkrankheit (*Cercospora beticola* Sacc.) im Hinblick auf die Einrichting eines Warndienstes. Bayerischer Landwirtschaft Jahrbuch 37: 197–227

Nagel CM (1938) The longevity of *Cercospora beticola* in soil. Phytopathology 28: 342–350

Oude Voshaar JH (1995) Statistiek voor onderzoekers. Wageningen Pers, The Netherlands

Payne GA and Waldron JK (1983) Overwintering and spore release of *Cercospora zeae-maydis* in corn debris in North Carolina. Plant Disease 67: 87–89

Payne GA, Duncan HE and Adkins CR (1987) Influence of tillage on development of gray leaf spot and number of airborne conidia of *Cercospora zeae-maydis*. Plant Disease 71: 329–332

Plotho, v. O (1951) Untersuchungen zur Morphologie und Physiologie der *Cercospora beticola*. Zucker 22: 461–467

Ruppel EG (1986) Foliar diseases caused by fungi. In: Whitney ED and Duffus JE (eds) Compendium of Beet Diseases and Insects (pp 8–9) APS Press, St Paul, Minnesota

Schürnbrand E (1952) Ein Beitrag zur Frage der Bedeutung der Sameninfektion durch *Cercospora beticola*. Zucker 13: 295–299

Sesma A and Osbourn AE (2004) The rice leaf blast pathogen undergoes developmental processes typical of root-infecting fungi. Nature 431: 582–586

Soylu S, Soylu EM and Kurt S (2003) First report of Cercospora leaf spot on Swiss chard caused by *Cercospora beticola* Sacc. in Turkey. Plant Pathology 52: 804

van der Gaag DJ and Frinking HD (1997a) The infection court of faba bean seedlings for oospores of *Peronospora viciae* f.sp. *fabae* in soil. Journal of Phytopathology 145: 257–260

van der Gaag DJ and Frinking HD (1997b) Survival characteristics of oospore populations of *Peronospora viciae* f.sp. *pisi* in soil. Plant Pathology 46: 978–988

van der Meijden R, Weeda EJ, Holverda WJ and Hovenkamp PH (1990) Heukels' Flora van Nederland. Wolters-Noordhoff, Groningen, The Netherlands

Vereijssen J (2004) Cercospora leaf spot in sugar beet. Epidemiology, life cycle components and disease management. PhD thesis Wageningen University, Wageningen, The Netherlands

Vereijssen J, Schneider JHM, Termorshuizen AJ and Jeger MJ (2003) Comparison of two disease assessment keys to assess *Cercospora beticola* in sugar beet. Crop Protection 1: 201–209

Vereijssen J, Schneider JHM and Termorshuizen AJ (2004) Possible root infection of *Cercospora beticola* in sugar beet. European Journal of Plant Pathology 110: 103–106