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ORIGINAL ARTICLE

Timing of fungicide application against *Cercospora* leaf spot disease based on aerial spore dispersal of *Cercospora beticola* in sugar beet

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Abstract

Cercospora leaf spot is an important foliar disease in sugar beet caused by *Cercospora beticola*. Tolerant cultivars are available, but application of fungicides is still mandatory for disease control. The timing of the fungicide application is crucial as it determines the outcome of disease epidemiology. A disease incidence (DI) of 5% is widely used as a threshold for fungicide application. Recently a method was developed that allows the quantification of aerial spore dispersal of *C. beticola* for measuring spore flight intensity. It was aimed in this study to prove if fungicide application based on spore flight might improve disease control compared to DI. In a field trial with artificial inoculation, a single fungicide application at the onset of spore flight slowed down disease development as indicated by reduced disease severity and aerial spore dispersal. However, it did not provide sufficient control in terms of sugar yield. Only a second fungicide application based on spore flight detection achieved an efficacy similar to two fungicide applications based on DI. In contrast, a single fungicide application based either on spore flight or DI was sufficient in two on-farm trials under natural infection with moderate disease pressure. This highlights the necessity of an early timed first fungicide application followed by a second application under high disease pressure induced by artificial inoculation. Although fungicide application based on spore flight achieved sufficient control success in on-farm trials, it seems not to improve disease control compared to the usage of DI as threshold.

Keywords Spore trap · Real-time PCR · Integrated pest management · CLS

Introduction

Cercospora leaf spot (CLS) is caused by the fungal pathogen *Cercospora beticola* and represents the most destructive foliar disease in sugar beet production (Rangel et al. 2020). CLS causes losses in root and sugar yield up to 50% under strong disease pressure (Wolf et al. 1998). The fungus is a member of the genus *Mycosphaerella* within the phylum *Ascomycota*. No sexual stage has been identified yet, although there is indirect evidence for sexual reproduction (Bolton et al. 2012). Disease symptoms are restricted to leaves and are characterized by grey to brown necrotic spots with reddish to brown margins. The spots increase in size and number as disease progresses resulting in completely necrotic leaves. The fungus survives as pseudostromata on infected plant debris, and there is experimental evidence that the fungus can persist in soil for at least 2 years depending on soil depth (Khan et al. 2008). Spores (conidia) produced on plant debris serve as primary inoculum in the next seasons. The disease is characterized by a polycyclic spread with several asexual cycles within a single growing season. After primary infection in spring during canopy closure, spores are produced on the surface of necrotic spots and are dispersed mainly by wind (Khan et al. 2009) and rain (Pool and McKay 1916) leading to secondary infections. A high relative humidity (> 60%) and warm temperature (27–32 °C) favour infection (Jacobsen et al. 2009; Skaracis et al. 2010). Similarly, suitable conditions for spore production are 27–32 °C with a relative humidity above 60% lasting for 15–18 h (Pool and McKay 1916).

In the past decades, major improvements in sugar beet breeding were achieved leading to the availability of CLS-resistant cultivars that display less fitness penalty in the

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absence of disease (Vogel et al. 2018). Currently available resistant varieties delay disease development but cannot prevent infection and subsequent spread of CLS in the field (Kaiser et al. 2010; Kaiser and Varrelmann 2009). Therefore, fungicide application is still mandatory and part of an integrated CLS management. Fungicides belonging to the classes of methyl-2-benzimidazole carbamate (MBC), sterol demethylation inhibitor (DMI), and quinone outside inhibitor (QoI) have been predominantly used in most sugar beet growing areas (Rangel et al. 2020). The frequent and extensive application of these fungicides along with the polycyclic lifestyle and high sporulation rate of *C. beticola* favoured the development of resistant strains in the past (Rangel et al. 2020). A glutamic acid to alanine amino acid change at codon 198 in the target gene β-tubulin mediates high resistance against MBCs (Davidson et al. 2006; Trkulja et al. 2013, 2015). Similarly, a substitution of glycine by alanine at codon 143 in the target cytochrome b occurred rapidly in geographically distinct *C. beticola* populations and mediates resistance towards QoIs (Birla et al. 2012; Bolton et al. 2013; Trueman et al. 2013). In contrast, the resistance mechanisms against DMIs cause a sensitivity shifting rather than a complete loss of efficacy as reported for MBCs and QoIs. The target of DMIs is the lanosterol 14α-demethylase (CYP51) which catalyses a crucial step during fungal ergosterol biosynthesis. Target site modifications (Leroux et al. 2007; Muellender et al. 2020) as well as overexpression of *CYP51* (Bolton et al. 2016; Leroux et al. 2007) have been found in *C. beticola* isolates displaying sensitivity shifting. Considering recent reports on *C. beticola* isolates with multi-resistance against MBC, DMI and QoI fungicides, there is a high risk of losing major fungicidal compounds as part of an integrated management (Shrestha et al. 2020; Trkulja et al. 2017).

In Germany, decision for fungicide application against CLS is based on a threshold system using disease incidence (DI) (Wolf and Verreet 2002). The DI is determined by randomly sampling 100 leaves in the field and calculating the number of leaves showing at least one CLS typical lesion. A DI of 5% (before 15 August) is defined as the threshold at which expected yield losses are no longer tolerable, and therefore a first fungicide application is required. This strategy provides sufficient control of CLS, but it mostly requires at least a second application a few weeks later when fungicide efficacy ceases and infection pressure under conducive environmental conditions continues. A reduction of the application frequency is desirable to reduce the risk of fungicide resistance development; however, the treatment must still guarantee full disease control, otherwise it will favour the development of resistant isolates. Spore release and their dispersal by wind is one major force driving secondary infections of CLS within the field. The onset and intensity of *C. beticola* spore flight is positively correlated with the average temperature of daily hours and relative humidity (Khan et al. 2009). Disease development is accompanied by several spore flight events during the growing season (Imbusch et al. 2021; Khan et al. 2009). Moreover, the increase in disease severity (DS), measured as the percentage of infected leaf area (average of 100 leaves), is responsible for severe yield losses. Furthermore, the DS is strongly correlated with the aerial spore concentration (Imbusch et al. 2021; Khan et al. 2009). Therefore, it was hypothesized in this study that a fungicide application shortly after the onset of spore flight might improve the control success of CLS and allow a reduction of fungicide applications. Recently we developed a method that enables quantification of *C. beticola* aerial spore dispersal by means of spore traps coupled with real-time PCR (Imbusch et al. 2021). This method allows a timely analysis of many samples in parallel which is required for fungicide application based on aerial spore dispersal. To prove our hypothesis, aerial spore dispersal of *C. beticola* was determined in a field trial with artificial inoculation and in two on-farm trials with natural infection. Fungicides were applied depending either on DI or aerial spore dispersal, and the control success was evaluated by measuring different disease parameters as well as sugar yield.

Materials and methods

Experimental field trial (trial 1)

In 2018 a field trial (trial 1) was conducted to analyse the effect of different fungicide treatments on CLS disease development and yield parameters of sugar beet (Table 1). Fungicides were applied either once or twice using DI or the occurrence of spore flight as threshold for application. The trial was located near Göttingen (Holtensen), Germany, and comprised four plots (replicate) per treatment organised in a randomized block design. One plot comprised 24 rows with a length of 12 m. Distance between rows was 45 cm and within row spacing was 18 cm. Side effects between plots were minimized by 6-m stripes between all plots. All spore traps were placed in one plot from each treatment within the same block. No spore trap was placed in the plot from treatment 'threshold + threshold' as no spore flight detection was necessary for fungicide application. Inoculation of plots was done by hand before sowing with effectively 4 g per m^2 CLS-infected sugar beet air-dried leaf material. Therefore, heavily infected sugar beet leaves were collected in the previous growing season, air-dried, and stored in potato bags over winter in a barn. A few days before inoculation, leaves were ground and mixed with semolina at a ratio of 1:6. The variety with registration number ZR 2313 (Cercospora susceptibility score 4; Federal Plant Variety Office Germany) was sown, and field management was conducted according

Table 1 Overview of fungicide treatments and thresholds used to determine the time point of first and second application in trials 1–3

Trial	Treatment	First application	Second application
1	Untreated	No application	No application
	Threshold + threshold	At 5% disease incidence	Three weeks after first application
	Threshold + spore flight	At 5% disease incidence	Presence of spore flight
	Spore flight + spore flight	Presence of spore flight	Presence of spore flight
	Spore flight	Presence of spore flight	No application
2–3	Untreated	No application	No application
	Threshold	At 5% disease incidence	No application
	Spore flight	Presence of spore flight	No application

Second fungicide application was done earliest 3 weeks after first application if respective application criteria were fulfilled

to common agricultural practice. Fungicide (Duett Ultra at 0.6 L per ha) was applied depending on the treatment. The second application was done at the earliest three weeks after the first application. At the end of the growing season, two rows from each plot were harvested and root as well as sugar yield was determined as described before (Hoffmann 2019).

On-farm trials (trial 2–3)

Two on-farm trials (trial 2–3) were conducted in 2018 to study the effects of the different fungicide treatments under natural infection conditions. A single fungicide application was done using either DI or the occurrence of spore flight as threshold (Table 1). Trial 2 was located in South Hesse (Bensheim) and trial 3 in Lower Bavaria (Buchhofen). These sites were chosen due to the strong natural occurrence of CLS in the past growing season. The trials were placed within growers' fields. Each treatment comprised a single stripe with 50 m length and 21–27 m width. Stripes from different treatments were placed in 40–50 m distance from each other. Three plots (10 × 10 m) for disease rating were located in each stripe and the middle plot contained the spore trap. The variety with registration number ZR 2313 (Cercospora susceptibility score 4; Federal Plant Variety Office Germany) was sown and field management was conducted according to common agricultural practice. Fungicides were applied depending on the treatment using Duett Ultra (0.6 L per ha).

Disease assessment

In all trials, disease assessment was done transversally on a weekly basis for each plot. Disease rating was conducted in trial 1 from 4 June to 27 August 2018, in trial 2 from 26 May to 8 September 2018 and in trial 3 from 30 May to 19 September 2018. Older and not fully developed leaves were not taken into account for the assessment. For determination of DI and DS, 25 leaves from each plot (100 leaves per treatment) were randomly selected and inspected for CLS symptoms. DS was measured on a metric scale by estimating the infected leaf area in per cent and was started after the first observation of CLS symptoms in the field.

Airborne spore trapping

Rotorod samplers (Burkard manufacturing Co Ltd, Hertfordshire, UK) supplied with exchangeable sampling sticks were used for spore trapping as described before (Imbusch et al. 2021). In all trials, one spore trap was placed in 50 cm height in the centre of one plot from each treatment. In trial 1, the treatment 'threshold + threshold' was not included for spore sampling. Sampling was conducted in trial 1 from 4 June to 31 August 2018, in trial 2 from 23 May to 10 September 2018 and in trial 3 from 28 May to 5 September 2018. Sampling sticks were covered with a thin layer of petroleum jelly. Spore traps rotated with 3500 rpm. Samples were collected on Mondays, Wednesdays, and Fridays and stored at − 20 °C until DNA extraction. For data analysis, only sampling dates were considered when data from all treatments was available.

Detection and quantification of *C. beticola* spore DNA

DNA was extracted from the sampling sticks as described before using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany) (Imbusch et al. 2021). The quantity and quality of the DNA was checked with a DS-11 Series Spectrophotometer (Denovix). Real-time PCR was performed in the CFX Real-Time PCR Detection System (Bio-Rad, Hercules, USA) using 96-well plates. *C. beticola*-specific primers targeting the *CALMODULINE* gene were used (forward primer: 5′-AGGCAGAGCTAACGACAG CAAC-3′; reverse primer: 5′-TTGTCGGCGTCGACTTCG -3′) in combination with a TaqMan probe (FAM-5′-TGC GAATGTACTGAACTAACCTCGACCG-3′-TAMRA). The reaction mix contained 12.5 µl 2 × Takyon No Rox dTTP

Mastermix (Eurogentec, Liege, Belgium), 500 nM of forward and reverse primer, respectively, and 100 nM probe in a final volume of 25 µl with 5 µl DNA sample per well. PCR programme comprised an initial denaturation at 95 °C for 4 min followed by 40 cycles consisting of 95 °C for 15 s and 60 °C for 30 s. Samples were analysed as duplicates, whereas two real-time PCR plates were prepared for each spore trap sample from the field, resulting in two sets of two technical replicates each. A sixfold dilution series of pure mycelial *C. beticola* DNA ranging from 10 ng µl-1 to 1 pg µl-1 was included in all runs as real-time PCR standard. *C. beticola* DNA contents in samples were automatically calculated by the Bio-Rad CFX Manager software (Bio-Rad, Hercules, USA) based on the regression function of the Ct values and log10 initial *C. beticola* DNA contents of the real-time PCR standard. DNA contents were converted to nanograms of *C. beticola* DNA per sampling period.

Statistical analysis

Statistical analysis was done with R version 4.0.3 (R Core Team 2014). The R packages 'agricolae' (de Mendiburu and de Mendiburu 2019) and 'dplyr' (Wickham et al. 2015) were used to calculate area under disease progress curve (AUDPC) based on DS measurements in trials 1–3. Statistical analysis of AUDPC and sugar yield was only done for trial 1 as the assumptions for statistical testing were violated in the on-farm trials 2–3. Variance homogeneity was checked with the Levene's test ($p > 0.1$) and normality distribution of residuals was confirmed by the Shapiro–Wilk test ($p > 0.1$). Analysis of variance (ANOVA) and multiple comparison ($p < 0.05$) of means were done using the R packages 'emmeans' (Lenth et al. 2018), 'lsmeans' (Lenth and Lenth 2018) and 'multcomp' (Hothorn et al. 2016). All data in this article were visualized with the R package ggplot2 (Wickham 2009).

Results

Aerial spore dispersal and effect of fungicide treatments after artificial inoculation of *C. beticola* (trial 1)

A randomized plot trial with four replicates per treatment was conducted in 2018 on a field near Göttingen (Germany). All plots were artificially inoculated with *C. beticola* during sowing to ensure homogeneous disease occurrence and to enhance effects of the different fungicide treatments. The earliest fungicide application was conducted on the 7 June in treatments using DI as threshold for the first application (Table 2). A second application followed either on 27 June 2018 based on DI (threshold + threshold) or three weeks later, on 17 July 2018 when spore flight occurred (threshold + spore flight). In contrast, the first application based on spore flight was done on 20 June 2018 (spore flight; spore flight + spore flight) followed by a second application on 25 July 2018 (spore flight + spore flight). The date for the first detection of spores in traps was similar between treatments (13 June–20 June 2018) and always after the first detection of CLS symptoms. In the untreated control, the aerial spore dispersal ranged between 0 and 337 ng/sample and peaked several times during the growing season illustrating the polycyclic nature of *C. beticola* (Fig. 1). In contrast, the concentration of *C. beticola* spores in traps from fungicide treated plots was considerably lower (ranging between 0 and 63 ng/sample) missing the characteristic peaks over

Table 2 Time points of first CLS symptom and spore detection in treatments from trial 1–3 as well as application dates for the first and second fungicide application

Trial	Treatment	First detection of symptoms	First detection of spores[a]	First fungicide application[b]	Second fungicide application[b]
1	Untreated	06.04.18	13.06.18	–	–
	Threshold + threshold	06.04.18	n. d	07.06.18	27.06.18
	Threshold + spore flight	06.04.18	20.06.18	07.06.18	17.07.18
	Spore flight + spore flight	06.04.18	13.06.18	20.06.18	25.07.18
	Spore flight	06.04.18	18.06.18	20.06.18	–
2	Untreated	09.06.18	01.08.18	–	–
	Threshold	16.06.18	08.08.18	22.06.18	–
	Spore flight	16.06.18	03.08.18	13.08.18	–
3	Untreated	04.07.18	18.07.18	–	–
	Threshold	04.07.18	10.08.18	06.07.18	–
	Spore flight	04.07.18	30.07.18	09.08.18	–

[a]"n.d.": Aerial spore dispersal was not determined in the fungicide treatment "threshold + threshold"

[b]"–": No fungicide application

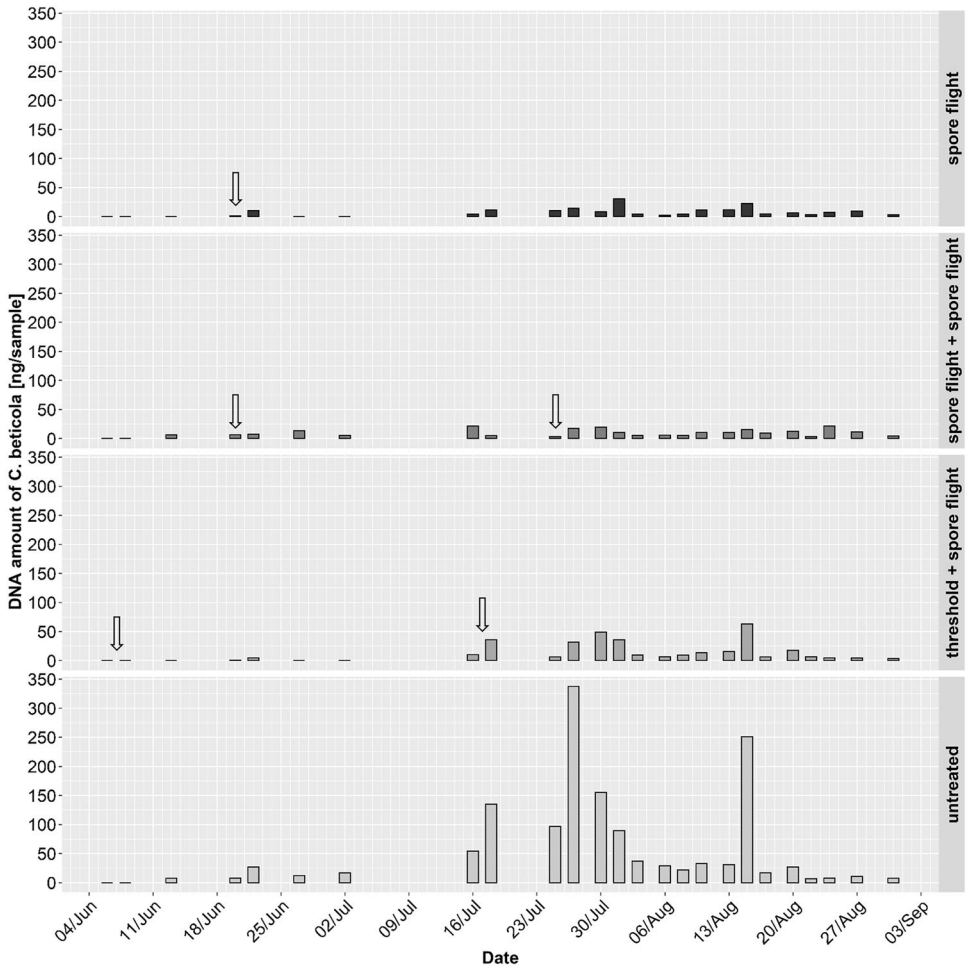

Fig. 1 Amount of *C. beticola* DNA [ng/sample] detected in spore traps located in field plots of trial 1. Fungicides were sprayed either once (spore flight) or two times (spore flight + spore flight, threshold + spore flight) using spore flight or disease incidence as threshold. An untreated variant served as control. Arrows indicate timing of fungicide applications. Only sampling days are shown when spore trap data from all treatments was available

Table 3 Cumulative DNA amount of *C. beticola* measured in spore traps during the vegetation period

Trial	Treatment	Number of samples[a]	Cumulative DNA amount [ng]
1	Untreated	25	1418.04
	Threshold + threshold	–	–
	Threshold + spore flight	25	337
	Spore flight + spore flight	25	234
	Spore flight	25	183
2	Untreated	31	359
	Threshold	31	53
	Spore flight	31	367
3	Untreated	36	1965
	Threshold	36	532
	Spore flight	36	829

[a]"–": Aerial spore dispersal was not determined in the fungicide treatment "threshold + threshold"

time observed in the untreated control. There were no apparent differences in the spore flight onset and intensity between the fungicide treatments. Consequently, the cumulative DNA amount considering all sampling points was highest for the untreated control with 1418 ng and ranged between 183 and 337 ng for the fungicide treatments (Table 3).

Disease rating showed no differences between treatments in case of DI, but there was a clear effect of the fungicide treatment when considering DS (Fig. 2). The earliest and sharpest increase in DS could be observed in the untreated control with an average value of 88% at the last rating date. A single fungicide application based on spore flight slowed down DS development, but still 73% was rated at the last date. A similar DS (67%) was also observed for the treatment with two applications using DI as threshold ('threshold + threshold'). In contrast, DS was further reduced by the treatments 'spore flight + spore flight' (51%) and 'threshold + spore flight' (53%) in a similar manner. The AUDPC was calculated for statistical comparison between treatments as it considers DS from all rating dates (Table 4). All fungicide treatments led to AUDPC values statistically

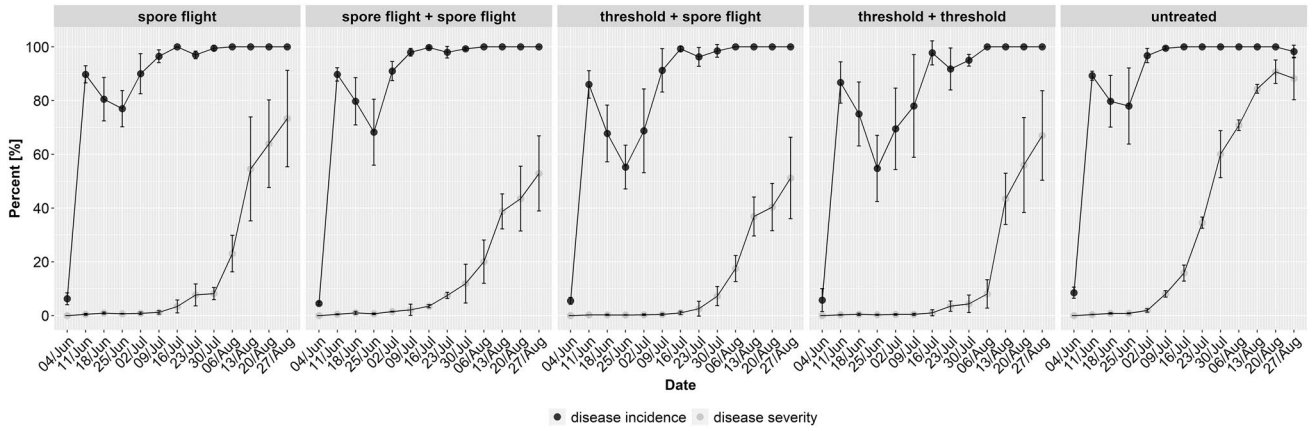

Fig. 2 Development of *Cercospora* leaf spot disease incidence and severity within field plots of trial 1. Fungicides were sprayed either once (spore flight) or two times (spore flight + spore flight, threshold + spore flight, threshold + threshold) using spore flight or disease incidence as threshold. An untreated variant served as control. Disease rating was done once a week from the onset of disease development. Error bars indicate standard deviation from three independent field replicates

Table 4 Mean values of area under disease progress curve (AUDPC) and white sugar yield for the different treatments in trial 1

Treatment	AUDPC	White sugar yield [t ha^{-1}]
Untreated	412.3 (±12.4)^{a}	7.4 (±1.1)^{a}
Threshold + threshold	151.7 (±46.9)^{b}	10.1 (±0.5)^{b}
Threshold + spore flight	132.5 (±24.9)^{b}	9.3 (±1.1)^{b}
Spore flight + spore flight	157.2 (±36.2)^{b}	9.8 (±0.3)^{b}
Spore flight	201.3 (±55.7)^{b}	8.7 (±1.1)^{ab}

Number in brackets indicates standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$)

lower than the untreated control that displayed the highest AUDPC value (412.3 week %). The sugar yield dropped to 7.4 t ha^{-1} in the untreated control due to the strong DS. A single fungicide application based on spore flight increased the sugar yield to 8.7 t ha^{-1}, but this effect was statistically not different from the untreated control. Only the treatments with two applications significantly increased the sugar yield with the highest value (10.1 t ha^{-1}) in treatment 'threshold + threshold'.

Aerial spore dispersal and effect of fungicide treatments after natural infection of *C. beticola*

Two on-farm trials were conducted in 2018 (trial 2 and 3) to evaluate the different fungicide treatments under natural infection conditions. At both sites, each treatment ('untreated control', 'spore flight', 'threshold') comprised a single stripe containing a spore trap to measure the spore flight during the growing season. In trial 2, the overall spore flight detected by the traps in the different treatments was very low (Fig. 3). In the untreated control, the amount of *C. beticola* DNA ranged between 0 and 99 ng/sample with a few peaks during the season. Furthermore, first spores were detected late in season on 1 August 2018 (Table 2). The spore flight intensity in the fungicide treatment 'spore flight' was similar to the untreated control with the highest peak (179 ng *C. beticola* DNA/sample) late in the season. In contrast, the spore flight in the fungicide treatment 'threshold' was lower and ranged only between 0 and 21 ng *C. beticola* DNA/sample. Consequently, the cumulative DNA amount considering all sampling points was lowest for the treatment 'threshold' with 53 ng *C. beticola* DNA followed by the untreated control (359 ng *C. beticola* DNA) and the treatment 'spore flight' (367 ng *C. beticola* DNA) (Table 3). The low disease pressure in trial 2 was also reflected by only 16% DI and 3% DS at the last rating date in the untreated control (Fig. 4). In the treatment using DI as threshold, the first fungicide application was done on the 22 June that was nearly two months before the first detection of spores in the trap (8 August 2018). The DI (15%) and DS (3%) measured at the last rating date were similar to the untreated control. Fungicide application in the treatment 'spore flight' was done very late in the season on 13 August 2018, a few days after first detection of spores in the trap (3 August 2018). Thus, the fungicide treatment was delayed by approximately two months compared to the usage of 5% DI as threshold. However, the DI (16%) and DS (3%) at the last rating date was on the same level as in the other treatments. Additionally, the AUDPC value was calculated for each treatment, but there were no obvious differences (Fig. 4).

The disease pressure in trial 3 was considerably stronger as indicated by two peaks with a high concentration of *C. beticola* DNA (871 and 422 ng/sample) found in the trap from the untreated control in mid-August (15 August 2018) and at the beginning of September (5 September 2018)

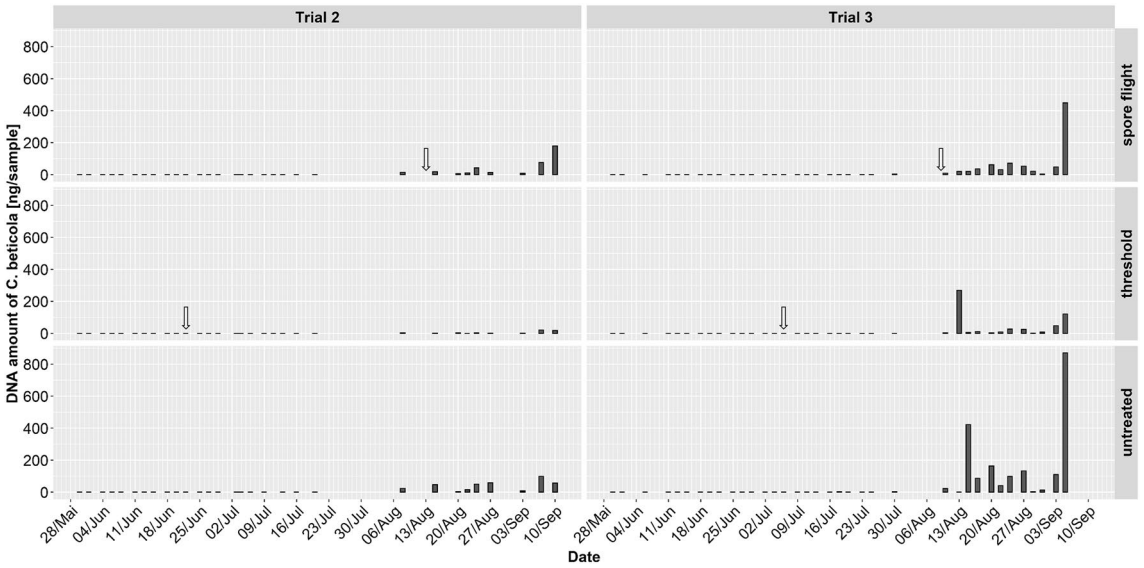

Fig. 3 Amount of *C. beticola* DNA [ng/sample] detected in spore traps located in on-farm trials 2 and 3. Fungicides were sprayed using either spore flight or disease incidence as threshold. Arrows indicate timing of fungicide applications. An untreated variant served as control. Only sampling days are shown when spore trap data from all treatments was available

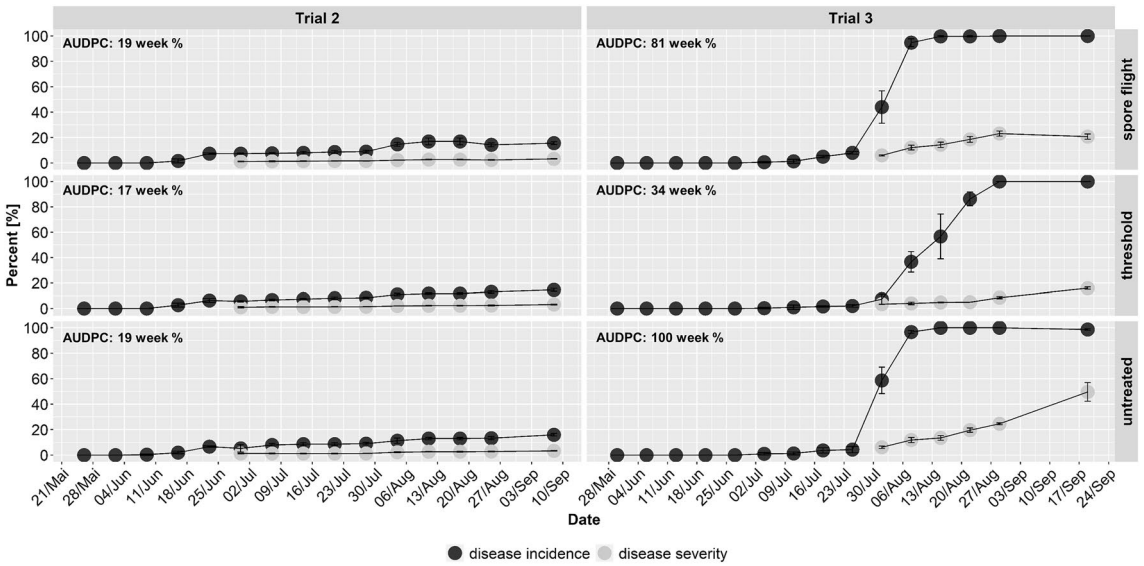

Fig. 4 Development of *Cercospora* leaf spot disease incidence and severity of on-farm trials 2 and 3. Fungicides were sprayed using either spore flight or disease incidence as threshold. An untreated variant served as control. Disease rating was done once a week at three different positions within each treatment. Error bars indicate standard deviation. Additionally, mean values of area under disease progress curve (AUDPC) are provided for each treatment

(Fig. 3). Furthermore, the first spore flight occurred earliest in the untreated control on 18 July 2018 (Table 2). Similarly, to the untreated control, highest peaks in the treatment 'threshold' were observed on the 13 August 2018 (269 ng *C. beticola* DNA/sample) and 5 September 2018 (121 ng *C. beticola* DNA/sample). In the treatment 'spore flight', the two highest peaks were measured on 24 August 2018 (70 ng *C. beticola* DNA/sample) and 5 September 2018 (449 ng *C. beticola* DNA/sample). Consequently, the cumulative DNA amount considering all sampling points was highest for the untreated control with 1965 ng *C. beticola* DNA followed by the treatments 'spore flight' (829 ng *C. beticola* DNA) and 'threshold' (532 ng *C. beticola* DNA) (Table 3). The disease rating followed a similar trend with 100% DI and 50% DS at the last rating date in the untreated control (Fig. 4). The first fungicide application was done on 6 July 2018 using

5% DI as threshold and spore flight was detected for the first time on 10 August 2018 in this treatment. The DI also reached 100%, but the DS (16%) was lower compared to the untreated control at the last rating date (Fig. 4). In contrast, fungicide application in the treatment 'spore flight' was done later, on 9 August 2018, a few days after first spore detection (30 July 2018). The DI (100%) and DS (21%) at the last rating date were similar to the treatment 'threshold' (Fig. 4) although the fungicide application was delayed by one month. The AUDPC value for the treatment 'threshold' (34 week %) was lower compared to the treatment 'spore flight' (81 week %) and untreated control (100 week %) (Fig. 4).

Discussion

The release and dissemination of spores is an important factor for severe epidemic outbreaks caused by fungal plant pathogens. Thus quantification of the airborne inoculum using spore traps coupled with molecular tools has been developed for several fungal pathogens like *Alternaria solani*, *Magnaporthe oryzae*, *Phytophthora infestans, Ramularia beticola,* and *Sclerotinia sclerotiorum* (Lees et al. 2019; Rogers et al. 2009; Villari et al. 2017; Wieczorek et al. 2014). Moreover, it has been shown that fungicide applications can be saved when the aerial spore concentration is used as threshold (Dhar et al. 2020; Thiessen et al. 2017). Therefore, it was the objective of this study to evaluate the potential of aerial spore dispersal of *C. beticola* as threshold for fungicide application against CLS. In all trials, the aerial spore dispersal peaked several times during the growing season in the absence of any fungicide application. This illustrates the polycyclic nature of CLS as described before (Imbusch et al. 2021; Khan et al. 2009; Tedford et al. 2018). Already a single fungicide application dramatically reduced the subsequent spore flight intensity and disease severity highlighting the importance of the airborne inoculum as one main driver of CLS epidemic in the field.

In all trials, the first fungicide application was done earliest for DI as threshold, whereas the first fungicide application based on aerial spore dispersal was delayed by 13 (trial 1), 53 (trial 2), and 34 days (trial 3). Consequently, the second application based on spore flight was also done later in trial 1 (20–28 days) compared to the treatment based on DI threshold. Despite these differences in the number and timing fungicide applications, the control success of CLS in terms of DS was similar between treatments as shown in the statistical analysis of AUDPC values in trial 1. In contrast, the analysis of sugar yield clearly showed that two fungicide applications were required to obtain a yield that is statistically higher than the untreated control. Although there was no statistical difference between fungicide treatments, the single fungicide application based on spore flight resulted in the highest AUDPC and lowest sugar yield. Nevertheless, it remains remarkable that a single application in the growing season and under high disease pressure as in trial 1 can nearly achieve a similar control success as two applications. Moreover, it did not lead to a severe epidemic outbreak of CLS. The disease pressure in trial 2 was very low, and therefore, the effects of the two different fungicide treatments (DI vs. spore flight threshold) were indistinguishable from the untreated control. In contrast, the disease pressure in trial 3 was higher and the lowest AUDPC was measured in the treatment using DI as threshold. However, a similar control success was achieved when the fungicide application was delayed by 34 days using spore flight as threshold. Nevertheless, it has to be pointed out that a statistical analysis was not possible due to the on-farm trial design.

Using aerial spore dispersal as threshold for fungicide application requires a detection method that is sensitive enough to detect the airborne inoculum before symptom development has started. The spore trap in combination with the real-time qPCR used in this study only detects the secondary spore flight and not the primary spores originating from the inoculum in soil (Imbusch et al. 2021). This was also observed in this study as symptom development has started before the detection of the first spore flight. In a previous study, *C. beticola* spores could be collected in traps before symptom development, but the quantification was based on time-consuming microscopic analysis (Khan et al. 2009). The primary spores originating from soil initiate the epidemic onset by the infection of a few plants within the field (Khan et al. 2008). A DI of 5% as application threshold targets this initial infection and the secondary spore flight at an early stage of the epidemic onset. In contrast, the method applied in this study requires a certain disease development in order to detect the spores in the trap, and therefore the first fungicide application was done delayed. Furthermore, there was always a time gap of a few days between first spore detection and fungicide application that further contributes to this problem. Although there is the possibility to improve the sensitivity of the spore trap, it remains questionable whether it will have a benefit over the DI threshold system as it will probably result in a similar application date.

Finally, it can be concluded that fungicide application based on aerial spore dispersal of *C. beticola,* as done in this study will neither improve CLS control nor allow a reduction of the application frequency. The first fungicide application is crucial for the control success as indicated by results from trial 1–3 whereas a second application is only required under strong disease pressure. Although the first application was dramatically delayed in all trials when spore flight was used as threshold, the subsequent disease development did not lead to a severe CLS epidemic outbreak as discussed above. This indicates a certain tolerance for the timing of the first

application that must not exactly match the 5% DI. However, the weather conditions during the onset of the epidemic are crucial and probably determine how long the first application can be delayed. A connection of aerial spore concentration with weather variables suitable for CLS infection may could help to optimize the first fungicide application (Tedford et al. 2018). Apart from that, the past progress in breeding CLS-resistant sugar beet cultivars (Vogel et al. 2018) implies that there is still potential for further improvements. The availability of highly resistant cultivars may allow a reduction of fungicide treatments when the DI threshold for application is shifted to the end of the growing season. Thus further research should focus on the integration of new resistant cultivars into fungicide strategies against CLS.

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Declarations

Conflict of interest One of the co-authors (TE) is working for the company (BASF SE) who funded this project.

Ethical approval This study does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication All authors consent to this submission.

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