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Efficacy of Different Fungicide Spraying Techniques on the Infestation with *Kabatiella zeae* and Formation of *Fusarium* Mycotoxins in Forage Maize

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Abstract: The application of fungicides in maize by the commonly used overhead spraying technique is more challenging than in small-grain cereals. Especially in later development stages, when the plant has reached a considerable height, lower plant organs (e.g., ears) may be insufficiently protected, since a large part of the applied fungicide is deposited on the upper leaves. In contrast, lower plant organs can be reached by the dropleg spraying technique, which allows sub-canopy applications. This study investigated the efficacy of fungicide applications during flowering in forage maize using the overhead and dropleg spraying techniques as well as a combination for the control of *Kabatiella zeae* and mycotoxin-producing *Fusarium* species, which can affect leaves and ears, respectively. The efficacy was evaluated in field trials under natural *K. zeae* and artificial *Fusarium* inoculum conditions in Northern Germany by measuring disease severities of *K. zeae* on maize leaves, dry matter yields, and concentrations of the *Fusarium* mycotoxins deoxynivalenol (DON) and zearalenone (ZEN) at harvest. *Fusarium* inoculations of main ears at full flowering resulted in significantly higher DON and ZEN concentrations compared to natural *Fusarium* infections, whereas the dry matter yield was not affected. The strongest disease progressions of *K. zeae* were determined after flowering on the upper leaves and leaves around the main ear. Disease severities were significantly reduced on the upper leaves by the overhead application and the combination of the overhead and dropleg spraying technique, whereby the three spraying techniques were equally able to decrease the infestation on the yield-essential leaves around the main ear. No differences in dry matter yield were found between the application techniques, but they were significantly higher than in the untreated control. The contamination with DON and ZEN was most effectively reduced by sub-canopy applications using the dropleg technique, whether as a solo application or in combination with the overhead technique. The main ears were reached better with the applied fungicide, reducing *Fusarium* infections, and therefore, contamination with mycotoxins. The dropleg technique offers an opportunity for improved protection of lower plant organs, especially in tall growing crops. In combination with the overhead spraying technique, the protection of various plant organs along the entire plant with the applied fungicide could be advantageous, especially when different parts of the plant are affected by different fungal diseases.

Keywords: eyespot; disease severity; ear rot; deoxynivalenol; zearalenone; fungicide application; overhead spraying technique; dropleg spraying technique; yield

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1. Introduction

The management of fungal leaf and ear diseases in cereal crops is often based on the foliar application of fungicides [1–5]. These pesticides are commonly applied by overhead

application. This spraying technique uses nozzles on a horizontal boom that spray from above the crop in a basically vertical direction down into the crop [6]. In small-grain cereals, important plant organs such as ears and the uppermost, yield-essential leaves that are closely located to the spray boom, can easily be reached by this application technique. However, fungicide applications in maize using the overhead spraying technique are more challenging. Especially in later stages of plant development (e.g., flowering), the overhead application reaches its limits since the plant height increases drastically with an increasing distance between nozzles on the spray boom and the target site of application, and an increasing leaf density [7] due to the large number of leaves along the maize plant [8]. Several plant organs such as main ears, and rudimentary and lower inserted leaves are located under a dense canopy of the upper leaves. Consequently, a large part of the applied pesticide is deposited on the upper leaves, whereby less pesticide reaches the lower plant organs [7,9]. Therefore, this spraying technique may be insufficient for the adequate biological control of fungal pathogens that affects lower parts of the plant.

In contrast, the so-called dropleg technology offers an opportunity to reach lower plant organs of the maize plant with the fungicide, which are difficult to achieve with the overhead spraying technique. This spraying technique generally allows sub-canopy treatments with various pesticides, e.g., in row crops in horticulture [10,11] as well as field crops such as oilseed rape [11–13]. Compared to the overhead spraying technique, the nozzle carriers are not directly attached to the spray boom, but to the lower end of the dropleg, which are prolonged elastic nozzle holders that hang freely floating underneath the boom down into the crop canopy during spraying [12,13]. The spray of the nozzles is directed toward the ground and sideways. In contrast to the overhead technique, the dropleg technique avoids spraying the crop canopy, whereby the upper leaves are only insufficiently protected from fungal infections.

Nevertheless, for a maximum biological control efficacy of fungal diseases, a good coverage of various plant organs along the entire maize plant with the fungicide can be necessary, since important fungal pathogens such as *Kabatiella zeae* and *Fusarium* species are capable to affect different parts of the maize plant at the same time.

K. zeae is the causal agent of eyespot disease, a common foliar disease in many regions where maize is grown [14–20]. Symptoms are small, 1 to 4 mm, circular, tan-colored, necrotic spots, which are surrounded by a red-brown ring with a chlorotic yellow halo (Figure 1A). Eyespots are initially single, but can rapidly spread to cover large areas of the leaf (Figure 1B) resulting in large areas of necrotic leaf tissue (Figure 1C) [14,21].

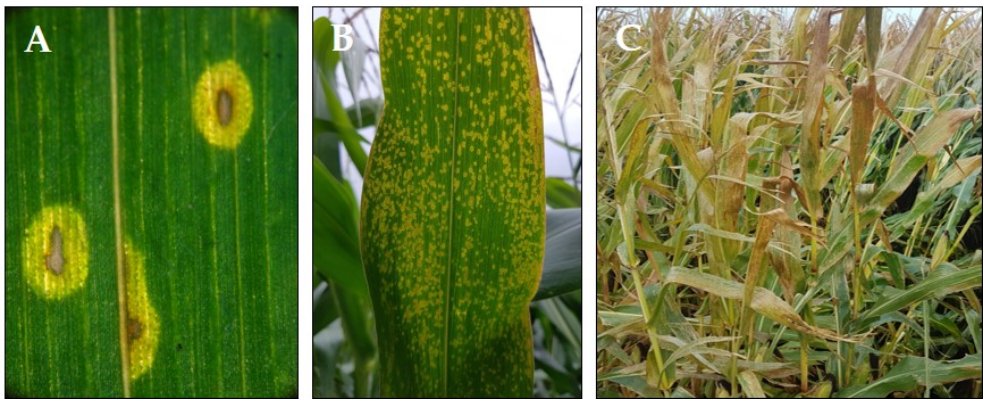

Figure 1. Symptoms of eyespot disease on maize leaves. (**A**) Typical eyespots caused by *Kabatiella zeae*. (**B**) Eyespots spread over the entire leaf during disease progression. (**C**) Eyespots join together into large necrotic areas causing premature drying and maturity of leaves.

Infections can occur throughout the growing season, whereby the disease progressed generally very slowly in the early vegetation period until flowering. From then on, the level of disease can increase rapidly. The strongest disease progressions are mainly observed

on the leaves of the upper and middle parts of the plant [14,18,22,23]. The disease causes premature drying and maturity of leaves, and therefore, when much of the leaf area is blighted, decreases the yield of both grain and forage maize [16,18,19,23].

Fungi of the genus *Fusarium* causes various rot diseases of ears (ear rot; Figure 2A), rudimentary ears (Figure 2B), and stalks (stalk rot; Figure 2C). Symptoms are characterized by a white or reddish discoloration with rotting symptoms on the ears and inside the stalk [24].

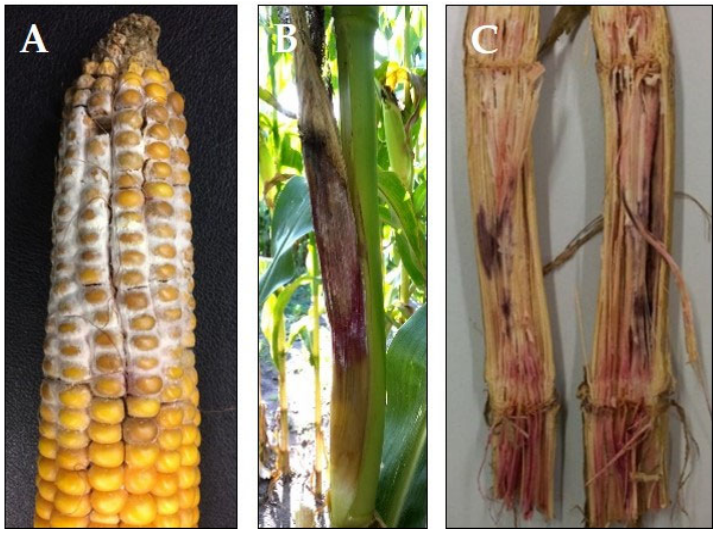

Figure 2. Symptoms of *Fusarium* rot diseases in maize. (**A**) Ear rot—White-colored mold layers on main ear. (**B**) Rotted rudimentary ear. (**C**) Stalk rot—Disintegrated and reddish discolored pith of the stalk.

Along with *Fusarium* infections, mycotoxins accumulated in affected tissues, which could pose a significant health risk for animals and humans [25]. In many maize production areas, the often co-occurring mycotoxins deoxynivalenol (DON) and zearalenone (ZEN), both produced by *F. graminearum* and *F. culmorum*, are among the most important *Fusarium* mycotoxins due to their frequent occurrence in toxicologically relevant concentrations [26]. Compared to contaminated stalks and rudimentary ears, the main ears are responsible for the highest amount of mycotoxins in forage maize (harvested whole plant) due to the high proportion of ears in the total aboveground dry matter at silage maturity, whereas the mycotoxin contamination of grain maize is only caused by ear rot. Infections of maize ears mainly result from fungal entry via silks at the stage of flowering [24,27,28].

Due to the late occurrence and late increase in infestation of important fungal pathogens such as *K. zeae* and *Fusarium* species during plant development, when the maize plant has already reached a considerable height with a large number of leaves, the choice of a suitable spraying technique is of particular interest for the application of fungicides to protect different plant organs along the entire maize plant for the prevention of quantitative (e.g., yield) and qualitative losses (e.g., accumulation of mycotoxins).

The present study investigated the efficacy of different fungicide spraying techniques, namely the overhead and dropleg spraying technique as well as their combination for the control of *K. zeae* and *Fusarium* in forage maize by applying fungicides at flowering. For this purpose, field trials were carried out under natural *K. zeae* and artificial *Fusarium* inoculum conditions in Northern Germany. In order to evaluate the efficacy of the different fungicide spraying techniques, disease severities of *K. zeae* as well as dry matter yields and *Fusarium* mycotoxin concentrations (DON, ZEN) in harvested whole-crop plants at silage maturity were analyzed.

2. Materials and Methods

2.1. Experimental Sites and Fungicide Spraying Treatments

Field trials were conducted in a one-year study at three locations in the northernmost federal state of Germany, Schleswig-Holstein (Table 1). The region between the Baltic and the North Sea is characterized by maritime weather conditions, with an average annual temperature of 8.9 °C and an annual precipitation of 823 L/m^2 [29]. Arable crops were grown on 651,000 ha in 2017 with winter wheat and forage maize as dominant crops in crop rotation, accounting for 28.9% and 24.7% of the arable land, respectively, followed by winter oilseed rape (14.9%) and winter barley (10.3%) [30].

Table 1. Coordinates, agronomic practices (previous crop, soil cultivation), planting and harvest dates of forage maize of the three trial locations in Northern Germany.

Parameter		Location		
		Barkhorn	Hemdingen	Hohenschulen
Coordinates [1]	x	1,073,958	1,091,656	1,110,278
	y	7,211,748	7,122,399	7,229,975
Previous crop		Forage maize	Forage maize	Forage maize
Soil cultivation		Reduced tillage	Plough	Reduced tillage
Planting date		24 April	3 May	2 May
Harvest date		17 October	16 October	9 October

[1] Coordinate System: WGS 1984 Web Mercator Auxiliary Sphere (EPSG 3857).

At all trial locations the commercial forage maize cultivar "SY Werena" was cultivated after pre-crop maize, more precisely, forage maize was the only crop in crop rotation (continuous maize). In Barkhorn and Hohenschulen trials were established in reduced tillage fields, whereas in Hemdingen soil cultivation was conducted by plowing (Table 1). Forage maize was sown at the end of April and the beginning of May, respectively, with a sowing density of nine plants/m^2. Harvest took place at silage maturity in mid-October. Fertilization as well as weed management were based on common agricultural practices.

All locations were set up under natural *K. zeae* inoculum conditions, whereas artificial *Fusarium* inoculations were conducted according to Reid et al. [31], in order to ensure, infection, and therefore a contamination of forage maize with mycotoxins. Approximately ten days prior to inoculation a macroconidia spore suspension of the two DON- and ZEN-producing *F. culmorum* isolates VIII 18 (isolated from wheat in Northern Germany 2012) and Fu 13 (isolated from forage maize in Northern Germany 2012) [32] was prepared as described by Reid et al. [31]. Two milliliters of the prepared spore suspension with a concentration of 5×10^5 conidia/mL were injected into the silk channel of maize main ears seven days after silk emergence using a self-filling vaccinator [31].

At each location, field trials were arranged in a randomized complete block design with five treatments (T1–T5) and four replications (plots) per treatment. Each field plot had a size of 30 m^2 (3 × 10 m) and consisted of four rows with a row spacing of 0.75 m. For *Fusarium* inoculations, each main ear of all maize plants of the two middle rows were considered. Disease assessment of *K. zeae*, harvest and sampling for mycotoxin analysis was performed on the two middle rows of each plot. All trials were integrated into the farmers' fields.

Treatments T1 and T2 were both fungicide-untreated controls, whereby T1 was the only treatment of the five investigated treatments (T1–T5) which was not inoculated with *Fusarium* spores in order to measure the success of the silk channel inoculation by comparing DON and ZEN concentrations between the two fungicide-untreated controls T1 (natural mycotoxin contamination) and T2. In addition to these two untreated controls, three fungicide treatments were established using different spraying techniques, namely overhead spraying technique (T3), dropleg spraying technique (T4), and the combination of the overhead and dropleg spraying technique (T5) (Table 2). In each of these three treatments the fungicide Prosaro®, containing the two systemic triazoles (demethylation

inhibitors) tebuconazole and prothioconazole, was applied once two days after silk channel inoculation corresponding to full flowering, at BBCH stage 65 (for further details of BBCH stages, see Lancashire et al. [33]). Fungicides were applied with 0.125 kg a.i./ha each (corresponding to 1 L of commercial product per ha) and a spray volume of 400 L/ha. The fungicide applications were performed using a self-propelled ground-based boom sprayer with an adjustable working height of the mounted horizontal spray boom from 0.7 to 4.0 m [23]. The application speed was 4 km/h.

Table 2. Fungicide application parameters in forage maize at BBCH 65 (full flowering) using the overhead spraying technique (T3), dropleg spraying technique (T4), and the combination of the overhead and dropleg spraying technique (T5).

Parameter	Treatment		
	T3—Overhead Spraying	T4—Dropleg Spraying (Sub-Canopy)	T5—Combination of T3 and T4
Spray volume (L/ha)	400	400	200 [1,2]; 200 [1,3]
Amount of Prosaro® (L/ha)	1.0	1.0	0.5 [1,2]; 0.5 [1,3]
Nozzle	IDK 120-03	2 × FT 1.5-408 [4]	IDK 120-02 [1,2]; 2 × FT 0.75-348 [1,3,4]
Nozzle spray angle (°)	120	140	120 [1,2]; 140 [1,3]
Nozzle spacing (m)	0.50	0.75	0.50 [1,2]; 0.75 [1,3]
Spray pressure (bar)	2.1	2.0	2.1 [1,2]; 2.1 [1,3]
Nozzle output (L/min)	1.0	1.0 (2 × 0.5) [4]	0.67 [1,2]; 0.5 (2 × 0.25) [1,3,4]

[1] Combination of [2] overhead (T3) and [3] dropleg spraying techniques (T4). [4] Two nozzles at the lower end of the dropleg.

The fungicide application of treatment T3 was carried out by a commonly used standard overhead application technique with a distance of 0.5 m between the spray boom and the top of the crop (Figure 3(A_I,A_{II})). Flat-fan nozzles (IDK 120-03; spray angle 120°; Lechler GmbH, Metzingen, Germany) with a standard nozzle spacing of 0.5 m on the spray boom were used to spray the application volume of 400 L/ha at a pressure of 2.1 bar from above the crop in a vertical direction down into the crop (Table 2).

The dropleg spraying technique (Lechler GmbH) was used for the fungicide application of treatment T4. These prolonged elastic nozzle holders hang freely floating underneath the boom of the sprayer down into the crop (Figure 3(B_I,B_{II})). Droplegs were installed on the spray boom due to a universal mounting plate with a distance of 0.75 m from each other. With this attachment, the droplegs can only move sideways, but not in the direction of forward and backward movement. Droplegs were guided centrally between two maize rows. At the lower end of the dropleg, two flood nozzles (2 × FT 1.5-408; Lechler GmbH) were installed in a twin-spray-cap (Lechler GmbH). The spray of both nozzles was directed toward the ground and sideways with a spray angle of 140°. The distance between the spray boom and the two nozzles at the lower end of the dropleg was 1.0 m (dropleg length: 0.9 m, mounting plate length: 0.1 m). The application volume of 400 L/ha was applied at a pressure of 2.0 bar (Table 2).

The fungicide application of treatment T5 was carried out as a combination of treatments T3 and T4 (Table 2). With each of the techniques, a spray volume of 200 L/ha with a fungicide amount of 0.5 L/ha was applied, resulting in a total application volume of 400 L/ha and a fungicide amount of 1 L/ha, respectively. Both flat-fan nozzles (overhead; IDK 120-02; Lechler GmbH) and flood nozzles (dropleg; 2 × FT 0.75-348; Lechler GmbH) were used at a pressure of 2.1 bar.

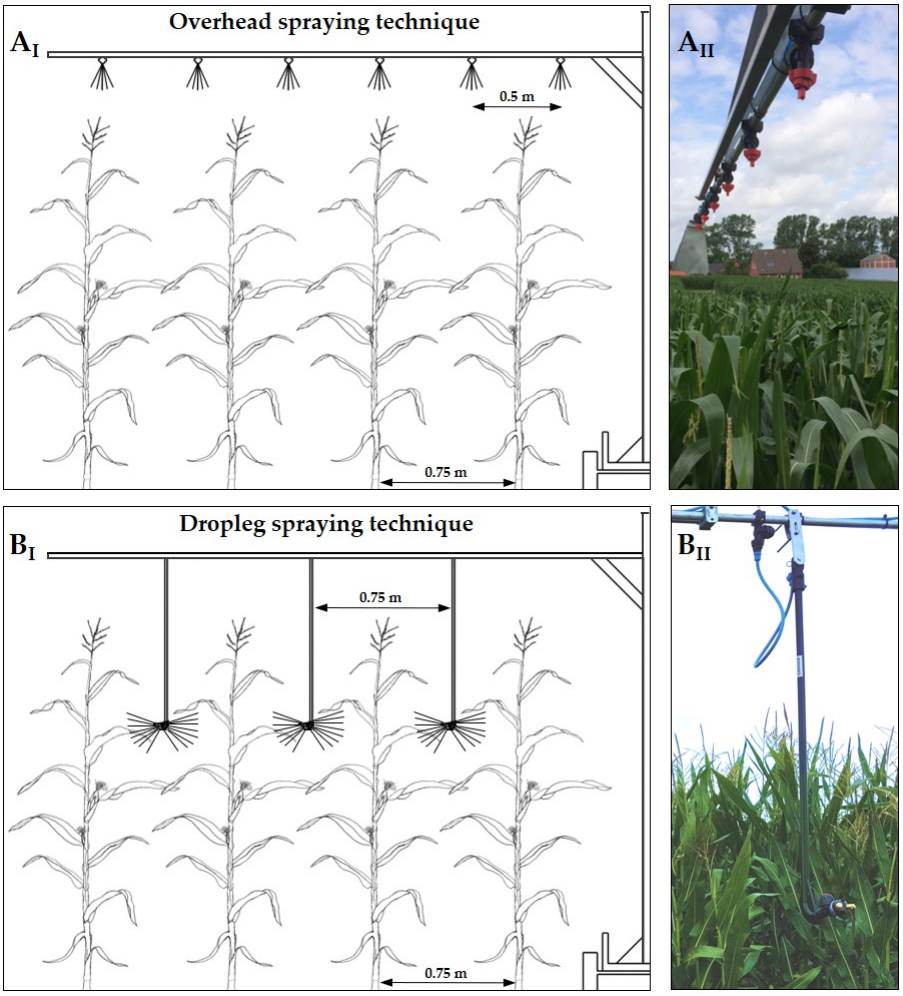

Figure 3. Schematic comparison between the (**A**) overhead and (**B**) dropleg spraying technique used for the fungicide applications in forage maize at BBCH 65. (**A_I**,**A_{II}**) In the conventional spraying treatment (T3) fungicides were applied from above the crop in a basically vertical direction down into the crop with a standard nozzle spacing of 0.5 m on a horizontal spray boom and a distance 0.5 m between spray boom and the top of the crop. (**B_I**,**B_{II}**) In the dropleg spraying treatment (T4) two nozzles in a twin-spray-cap at the lower end of the dropleg were guided in the crop centrally between two maize rows with a distance of 1 m underneath the spray boom spraying towards the ground and sideways.

Treatments T3 to T5 were compared with the fungicide-untreated control T2 in order to determine the efficacy of the different fungicide spraying techniques on disease severities of *K. zeae*, dry matter yield, and concentrations of DON and ZEN.

2.2. Disease Assessment of Kabatiella zeae

Ten plants per plot of treatments T2, T3, T4 and T5 were visually evaluated during flowering at BBCH 65, during fruit development at BBCH 75 and ripening immediately before harvest at BBCH 85 (silage maturity) in order to establish the disease severity of *K. zeae*. In accordance with the EPPO guideline PP 1/272 (2) [34], disease assessment was made on five labeled successive plants in the center of each of the two inner rows in each plot, whereby every plant was divided into three leaf segments. The middle leaf segment was defined by the main ear and five leaves around the main ear, consisting of the main ear leaf (L0), two leaves above the main ear (L+1, L+2), and two below (L-1, L-2). The lower and upper leaf segments were below and above the middle segment of the plant, respectively. The visual rating for the disease severity of *Kabatiella* eyespot, i.e., the percentage of leaf

area affected by symptoms of *K. zeae*, was conducted following the scheme from the EPPO guideline PP 1/272 (2) [34]. All leaves of the upper, middle and lower leaf segments were separately assessed for symptoms of *K. zeae* and a mean disease severity value was estimated for each leaf segment considering all leaves of the corresponding segment.

2.3. Harvest, Yield Assessment and Sample Preparation

The two middle rows of each plot of treatments T1 to T5 were harvested at silage maturity (BBCH 85) using a Haldrup forage harvester. The freshly harvested and chopped material from each plot was weighed automatically by the harvester to determine plot yields, which were converted into dt/ha fresh matter yield. In addition, a sample of approximately 1000 g was taken from the freshly harvested material of each plot, weighed and immediately dried for 48 h in an oven at 60 °C for the determination of dry matter yields [8]. The dry matter percentage calculated for each plot was used for converting dt/ha fresh matter yield into dt/ha dry matter yield. Dried samples were ground in a mill (Fritsch, Idar-Oberstein, Germany) to 1 mm particle size for further mycotoxin analysis.

2.4. Analysis of Mycotoxins

All dried forage maize samples were analyzed for their concentrations of DON and ZEN by SYNLAB Analytics and Services Germany GmbH (now: SGS Analytics Germany GmbH; Jena, Germany) according to the method described by Sulyok et al. [35].

2.5. Statistical Analysis

The statistical software R, version 4.0.1 (R Foundation for Statistical Computing, Vienna, Austria) [36], was used to evaluate the data. For each measurement variable (disease severity of *K. zeae* on the lower, middle and upper leaf segment; dry matter yield; DON and ZEN concentrations), the data evaluation started with the definition of an appropriate statistical mixed model [37,38]. The model included the treatment (T1–T5) as a fixed factor. The location and the block within the location were regarded as random factors. The residuals were assumed to be normally distributed and to be heteroscedastic. These assumptions are based on graphical residual analysis. Based on this model, a Pseudo R^2 was calculated [39] and an analysis of variances (ANOVA) was conducted, followed by multiple contrast tests [40] in order to compare the several treatments. Statistical significance was evaluated at $p \leq 0.05$.

3. Results

3.1. Effect of Artificial Fusarium Inoculations of Maize Main Ears on Dry Matter Yield, DON and ZEN Concentrations

Artificial *Fusarium* inoculations of maize main ears were carried out at BBCH 65 to ensure an infection, and therefore, a contamination of forage maize with mycotoxins. However, the inoculation of ears with *F. culmorum* did not affect dry matter yields compared to natural *Fusarium* infections (Figure 4A). In the non-inoculated (T1) and inoculated (T2) fungicide-untreated control similar dry matter yields of 167.0 and 167.8 dt/ha were determined. At all trial locations, dry matter yields were comparable between the two fungicide-untreated controls (Table S1). Under natural *Fusarium* inoculum conditions mean DON and ZEN concentrations of 3628 and 520 µg/kg were detected in forage maize samples at silage maturity of the fungicide-untreated control T1 (Figure 4B,C), ranging from 2598 to 4453 µg DON/kg and 478 to 585 µg ZEN/kg between locations (Table S1). In contrast, the silk channel inoculation of maize ears with *F. culmorum* seven days after silk emergence resulted in significantly higher DON and ZEN concentrations of 22,947 and 2160 µg/kg in the inoculated fungicide-untreated control T2 (Figure 4B,C). Between the three trial locations mean DON and ZEN concentrations ranged from 14,992 to 28,355 µg DON/kg and from 1966 to 2260 µg ZEN/kg, respectively (Table S1).

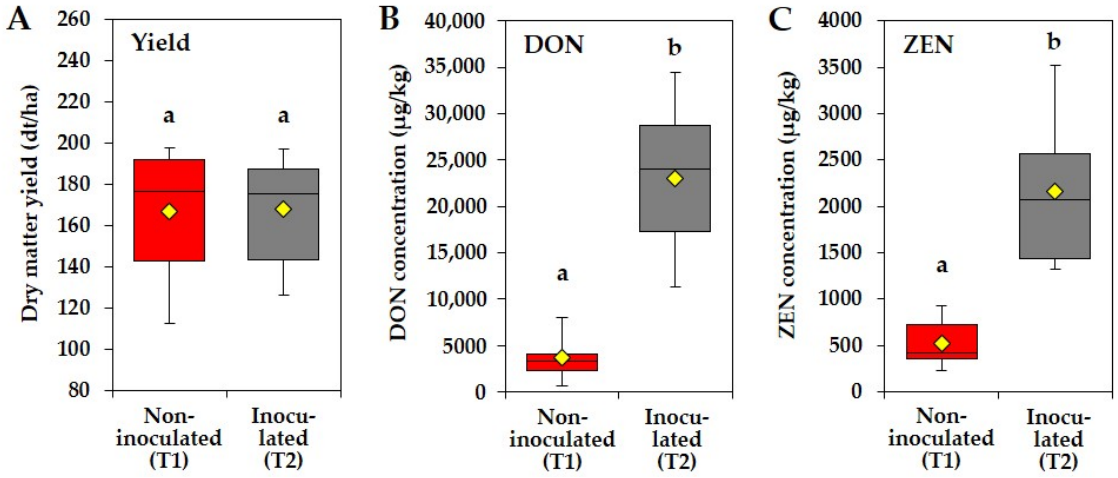

Figure 4. Boxplots and means (yellow rhombus) of (**A**) dry matter yield (dt/ha), (**B**) DON and (**C**) ZEN concentrations (µg/kg) in forage maize samples at silage maturity of the cultivar "SY Werena" in the two fungicide-untreated controls without (T1) and with (T2) silk channel inoculation of main ears with macroconidia of *Fusarium culmorum* seven days after silk emergence at BBCH 65 summarized for the three trial locations. Five statistics are represented in each boxplot from bottom to top: The smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Different letters describe significant differences in dry matter yield, DON and ZEN concentrations between the non-inoculated (T1) and inoculated (T2) fungicide-untreated control. Statistical significance was evaluated at $p \leq 0.05$. DON = deoxynivalenol; ZEN = zearalenone.

3.2. Effect of Different Fungicide Spraying Techniques on Disease Severities of Kabatiella zeae

The first disease assessment of *K. zeae* was performed at BBCH 65. At this development stage, the eyespot disease was detected in the fungicide-untreated control T2 with low mean disease severities of 1.2% on the upper, 1.9% on the middle, and 1.6% on the lower leaf segment (Figure 5).

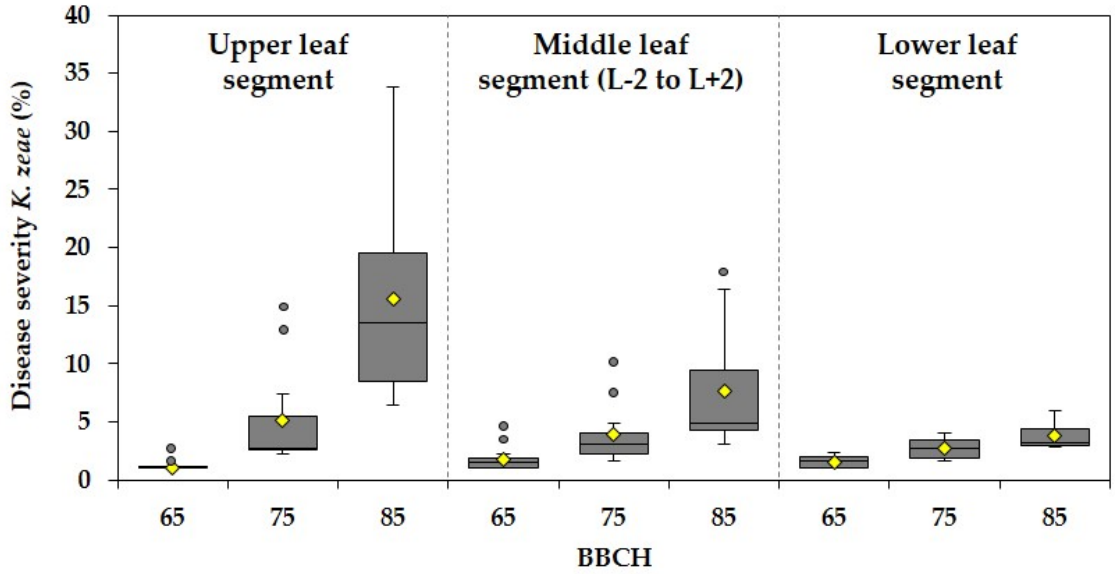

Figure 5. Boxplots and means (yellow rhombus) of disease severities of *Kabatiella zeae* (percentage of leaf area affected by symptoms) on the upper, middle and lower leaf segment of forage maize at BBCH 65, BBCH 75 and BBCH 85 of the cultivar "SY Werena" in the fungicide-untreated control (T2) summarized for the three trial locations. Five statistics are represented in each boxplot from bottom to top: The smallest observation, lower quartile, median, upper quartile, and largest observation, respectively.

On the upper leaf segment, the mean disease severity increased to 5.1% at BBCH 75 and 15.6% at BBCH 85. Mean disease severities ranged from 7.6 to 25.6% between locations at BBCH 85 (Table S2). The percentage of leaf area affected by symptoms of *K. zeae* on the middle leaf segment increased from 1.9% at BBCH 65 to 3.9% at BBCH 75 and to a mean maximum of 8.3% at BBCH 85 (Figure 3), ranging from 4.6 to 15.6% between the three trial locations (Table S2). In contrast to the upper and middle leaf segment, a significantly lower level of disease progression was observed in the lower leaf segment, reaching a mean disease severity of 3.8% at BBCH 85 (Figure 5, Table S2). In general, the strongest disease progression of *K. zeae* was determined on the upper leaf segment followed by the middle and lower leaf segments (Figure 5, Table S2).

The efficacy of the fungicide application at BBCH 65 on *K. zeae* disease severities at BBCH 85 using the three different fungicide spraying techniques is shown in Figure 6.

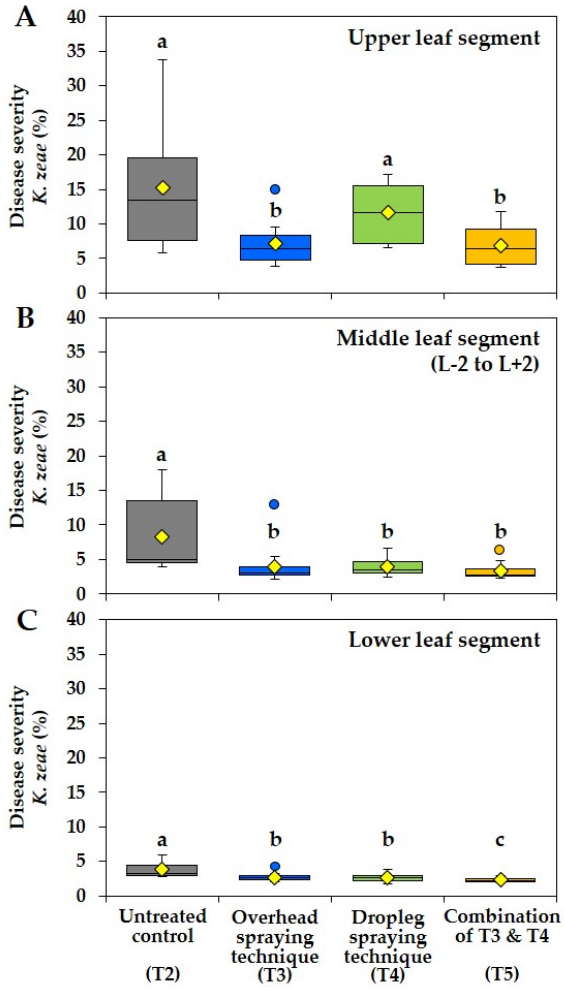

Figure 6. Boxplots and means (yellow rhombus) of disease severities of *Kabatiella zeae* (percentage of leaf area affected by symptoms) on the (**A**) upper, (**B**) middle (L-2 to L+2) and (**C**) lower leaf segment of forage maize at BBCH 85 of the cultivar "SY Werena" depending on different fungicide spraying techniques (treatments T2–T5) summarized for the three trial locations. Treatments: T2—Fungicide-untreated control, T3—Overhead spraying technique, T4—Dropleg spraying technique, T5—Combination of T3 and T4. The fungicide Prosaro® was applied (T3–T5) with 1.0 L/ha and a spray volume of 400 L/ha at BBCH 65. Five statistics are represented in each boxplot from bottom to top: The smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Different letters describe significant differences in disease severities between treatments T2–T5. Statistical significance was evaluated at $p \leq 0.05$.

Compared to the fungicide-untreated control T2 with a mean disease severity of 15.6% on the upper leaf segment, the fungicide application with the overhead spraying technique (T3) and the combination of the overhead and dropleg spraying technique (T5) significantly reduced the disease severity to a similar extent by 53.6% (7.1% disease severity) and 54.9% (6.9%), respectively (Figure 6A). In contrast, the fungicide application using the dropleg spraying technique (T4) did not result in a significant reduction in *K. zeae* disease severities on the upper leaf segment, reaching a mean disease severity of 11.6% (reduction of 25.6%). This was observed at all trial locations (Table S2). As shown in Figure 6B, the fungicide application with the three different spraying techniques significantly lowered the disease severities on the middle leaf segment. The percentage of affected leaf area was reduced in a similar manner by 50.6% (T3; 4.1% disease severity), 51.8% (T4; 4.0%), and 60.2% (T5; 3.3%) compared to the untreated control T2 with a mean disease severity of 8.3%. On the lower leaf segment disease severities were significantly decreased by the three fungicide spraying techniques (Figure 6C). However, a significantly lower disease level was determined compared to the upper and middle leaf segment. Between the four treatments, T2 to T5 disease severities varied in a narrow range from 2.1 (T5) to 3.8% (T2) on the lower leaf segment.

3.3. Effect of Different Fungicide Spraying Techniques on Dry Matter Yield

The fungicide application at BBCH 65 with the three different fungicide spraying techniques (T3–T5) significantly increased the dry matter yield compared to the untreated control T2 with a mean dry matter yield of 167.8 dt/ha (Figure 7). The dry matter yield increased by 10.0% (184.7 dt/ha) using the overhead spraying technique (T3), by 8.8% (180.8 dt/ha) using the dropleg spraying technique (T4), and by 13.9% (191.1 dt/ha) using the combination of the overhead and dropleg spraying technique (T5). No significant differences were observed between the three spraying techniques. At all locations, the fungicide application with the three different spraying techniques resulted in higher dry matter yields compared to the untreated control T2 (Table S3).

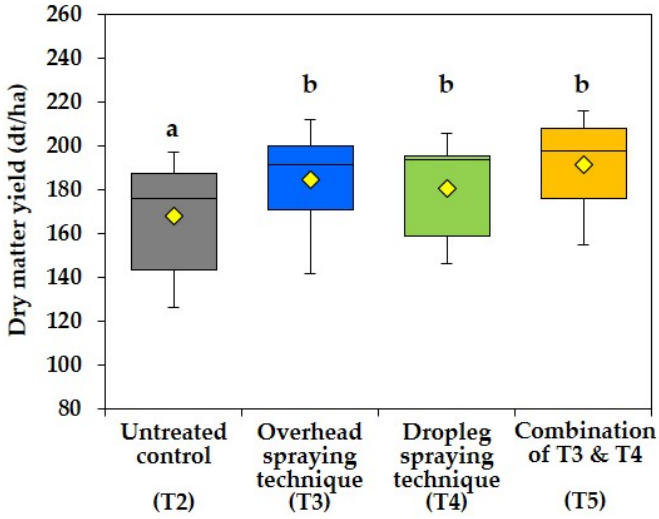

Figure 7. Boxplots and means (yellow rhombus) of dry matter yield (dt/ha) of forage maize of the cultivar "SY Werena" depending on different fungicide spraying techniques (treatments T2–T5) summarized for the three trial locations. Treatments: T2—Fungicide-untreated control, T3—Overhead spraying technique, T4—Dropleg spraying technique, T5—Combination of T3 and T4. The fungicide Prosaro® was applied (T3–T5) with 1.0 L/ha and a spray volume of 400 L/ha at BBCH 65. Five statistics are represented in each boxplot from bottom to top: The smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Different letters describe significant differences in dry matter yield between treatments T2–T5. Statistical significance was evaluated at $p \leq 0.05$.

3.4. Effect of Different Fungicide Spraying Techniques on DON and ZEN Concentrations

The fungicide application with the three different fungicide spraying techniques (T3–T5) two days after silk channel inoculation at BBCH 65 significantly reduced the DON and ZEN concentrations in harvested forage maize compared to the untreated control T2 with 22,947 µg DON/kg and 2160 µg ZEN/kg, respectively (Figure 8). Concentrations of DON and ZEN decreased by 56 and 53% using the overhead spraying technique (T3). The fungicide application with the dropleg spraying technique (T4) and the combination of the overhead and dropleg spraying technique (T5) significantly reduced the DON and ZEN content to a greater extent than the overhead spraying technique (T3), reducing DON concentrations by 81% (T4; 4320 µg/kg) and 78% (T5; 5021 µg/kg) compared to the untreated control T2 (Figure 8A), and ZEN concentrations by 76% (T4; 515 µg/kg) and 78% (T5; 484 µg/kg) (Figure 8B), respectively. At all trial locations, the highest DON and ZEN reductions were observed using the dropleg spraying technique (T4), and the combination of the overhead and dropleg spraying technique (T5) (Table S4).

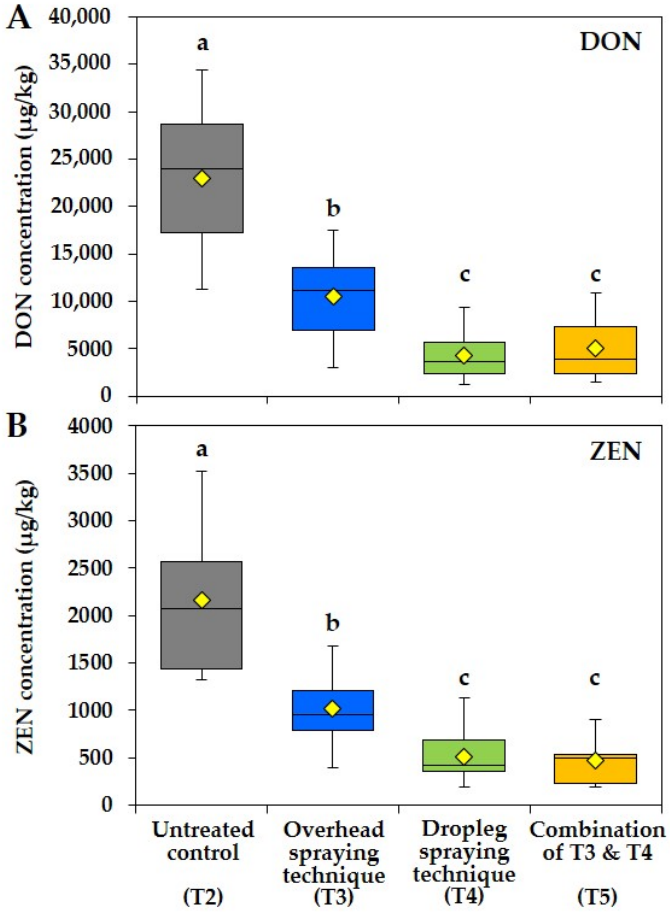

Figure 8. Boxplots and means (yellow rhombus) of (**A**) DON and (**B**) ZEN concentrations (µg/kg) in forage maize samples at silage maturity of the cultivar "SY Werena" depending on different fungicide spraying techniques (treatments T2–T5) summarized for the three trial locations. Treatments: T2—Fungicide-untreated control, T3—Overhead spraying technique, T4—Dropleg spraying technique, T5—Combination of T3 and T4. The fungicide Prosaro® was applied (T3–T5) with 1.0 L/ha and a spray volume of 400 L/ha two days after silk channel inoculation of main ears with macroconidia of *Fusarium culmorum* at BBCH 65. Five statistics are represented in each boxplot from bottom to top: The smallest observation, lower quartile, median, upper quartile, and largest observation, respectively. Different letters describe significant differences in DON and ZEN concentrations between treatments T2–T5. Statistical significance was evaluated at $p \leq 0.05$. DON = deoxynivalenol; ZEN = zearalenone.

4. Discussion

For a maximum biological control efficacy of the applied fungicide, good coverage of plant organs to be protected is required. This is especially important for non-systemic fungicides (e.g., multi-site inhibitors) that only act as protectants on the plant surface where they were deposited before the infection has been observed. On the other hand, a good coverage is also important for systemic fungicides, which get absorbed by the plant at the site of deposition and are translocated within the plant tissue, whereby they can act in a protective and especially curative way, thus affecting the fungal pathogen after infection. However, local-systemic fungicides are only redistributed over short distances within the plant tissue, whereas other systemic fungicides such as triazoles (e.g., tebuconazole, prothioconazole) are transported more extensively through the plant by xylem vessels, i.e., acropetal. Due to the upward translocation with the transpiration stream from the point of entry, xylem-mobile systems cannot protect deeper-located plant organs that have been insufficiently covered with the applied fungicide [3,41–43]. Only a few fungicides can be partially translocated basipetally in the phloem [43]. Nevertheless, foliar fungicides are commonly applied by the overhead spraying technique, spraying top-down [6]. This application technique may result in high deposits of active ingredients on the upper parts of the plant but in rather low deposits on the lower parts, where protection can also be necessary [7,9,10]. Due to the reduced coverage of deeper-located plant organs and the insufficient downward transport of systemic fungicides within the plant from the site of application, lower-located plant parts are generally insufficiently protected from fungal infections. In contrast, the dropleg spraying technique allows sub-canopy treatments, and therefore, an accumulation of active ingredients on plant parts that are difficult to achieve by the overhead spraying technique [10,11].

In our present study, we investigated the efficacy of the overhead and dropleg spraying technique as well as their combination for the control of important fungal pathogens in forage maize. The choice of a suitable spraying technique is of particular interest for the application of fungicides, especially in a tall-growing crop such as maize. This is due to the fact that several parts along the maize plant can be affected by fungal pathogens such as *K. zeae*, mainly affected affecting leaves, and mycotoxin-producing *Fusarium* species, which can cause various rot diseases of deeper located plant organs such as ears. Due to the late increase in infestation by the *Kabatiella* eyespot disease and the late occurrence of *Fusarium* diseases (especially ear rot) during the vegetation period of maize, fungicide applications are mainly useful at later stages of plant development, particularly at flowering, when the plant has already reached a considerable height with a large number of leaves along the maize plant and an increasing leaf density. Therefore, good coverage of various plant organs along the entire maize plant or deeper located plant organs with the applied fungicide can be necessary for a maximum biological control efficacy of fungal diseases in maize [7,44].

As shown in our study, the *Kabatiella* eyespot disease progressed generally very slowly in the early vegetation period of maize, reaching low mean disease severities at flowering on the upper, middle and lower leaf segments. However, disease severities increased drastically with the end of flowering. These observations are in line with other studies analyzing the disease progression of *K. zeae* [14,15,18,22,23]. At this development stage, the translocation of soluble carbohydrates from leaves to the ears begins [45]. For certain perthotrophic pathogens, a low carbohydrate content in the leaf tissue is responsible for an increased susceptibility [46], which can also be expected for *K. zeae*. After flowering, the disease progression is mainly determined by weather conditions. The production of conidia and the development of the disease are favored by long periods of cool, wet weather conditions [14,16], so regions with cool, moist environments are most affected by the disease. In our field trials, the uppermost leaves of the plant were most severely affected by symptoms of *K. zeae* during fruit development and ripening, followed by leaves of the middle (i.e., around the main ear) and lower part of the plant. Thermal air currents within the plant crop, which transport spores formed on the lower leaves upwards by solar radiation after cool nights, are mentioned as an explanation for these differently occurring

infestation levels along the maize plant. In addition, the nightly cooling will increase dew formation in the upper parts of the plant and contribute to the development of the pathogen [23]. Furthermore, the direct exposure of the upper leaf layers to the sunlight may be another factor for higher disease severities on the upper leaves. A number of photoactive toxins (e.g., cercosporin) have been described in the literature, which enables the colonization of the host tissue by phytopathogenic fungi. Cercosporin can be induced by light into an active state and, by reacting with oxygen, produce reactive oxygen species, which are capable of destroying the cell components of the plant [47,48]. A similar process is conceivable for *K. zeae*.

For the control of *K. zeae* a fungicide application with the overhead spraying technique should be suitable for an effective control of this pathogen on the upper leaves, which is confirmed by our results. The overhead spraying technique deposits the most active ingredient on the upper leaves, resulting in a higher biological control efficacy of the eyespot disease compared to the dropleg spraying technique. In all three analyzed leaf segments, namely upper, middle and lower, the investigated spraying techniques significantly reduced the disease severities compared to the untreated control, whereby the differences between the spraying techniques became greater the higher the leaf layer was inserted along the plant. The sole fungicide application with the dropleg spraying technique was less effective on the uppermost leaves compared to the overhead spraying technique, whereas the control efficacy was equal in the middle leaf segment around the main ear (L-2 to L+2). The fungicide application with the different spraying techniques significantly increased the dry matter yield compared to the untreated control, whereby no significant differences were observed between the overhead and dropleg spraying techniques, as well as their combination. This suggested that the yield effect of *K. zeae* on the uppermost leaves may be overestimated and the control of the pathogen on the leaf layers L-2 to L+2 is more important to avoid yield losses. Therefore, it seems to be irrelevant for the control of the eyespot disease which of the three spraying techniques is used. On the other hand, it can also be concluded that if no spraying technique provides significant advantages, no significant disadvantages arise from a specific technique. This provides greater flexibility in the choice of a suitable spraying technique if a certain technique is preferred for the effective control of other fungal diseases.

In contrast to *K. zeae*, fungi of the genus *Fusarium* generally affect the lower parts of the plant, causing various rot diseases of ears (ear rot), rudimentary ears and stalks (stalk rot). The primary infection pathway is via silks of maize ears, which are highly susceptible to *Fusarium* infections during the first week after emergence [24,27]. After spore germination on silks, the fungus enters the silk channel and subsequently infects the rachis from the tip towards the bottom as well as kernels connected to the rachis. Furthermore, rudimentary ears underneath the main ear, as well as adjacent stalk sections, can be colonized [24]. The main problem of *Fusarium* infections is the quality losses resulting from the accumulation of mycotoxins in affected tissues, which causes a potential health risk for animals and humans because their occurrence in feed and food is often associated with chronic or acute mycotoxicosis [25,49–52]. The co-occurring mycotoxins DON and ZEN are two of the most important *Fusarium* mycotoxins in maize production due to their frequent occurrence in toxicologically relevant concentrations [26]. These mycotoxins are produced by *F. graminearum* and *F. culmorum*, both associated with temperate and moist environmental conditions [53]. To avoid human and animal health risks, the European Union has defined maximum levels for DON and ZEN in foodstuffs [54] and guidance values for DON and ZEN in products intended for animal feeding [55]. In our study, DON and ZEN occurred with high concentrations in forage maize under natural *Fusarium* inoculum conditions, reaching concentrations near or above the guidance value of 5000 µg/kg for DON and 500 µg/kg for ZEN in complementary and complete feeding stuffs. These values were significantly exceeded by DON and ZEN concentrations resulting from silk channel inoculations of maize main ears seven days after silk emergence, reaching DON and ZEN concentrations of up to 28,355 µg/kg and 2260 µg/kg, respectively. Although

these very high concentrations were caused by artificial infections, such high levels are also found more frequently after natural *Fusarium* infections in forage maize [56–59] as well as grain maize [59–62]. It is important to note that in forage maize, which is mainly used as feedstuff for dairy cows and beef cattle, all potentially *Fusarium*-infected, and therefore, mycotoxin-contaminated organs (ears, rudimentary ears, stalks) of the maize plant are included. Nevertheless, mycotoxin-contaminated ears are responsible for the greatest amount of mycotoxins in harvested whole plants due to the high proportion of the ears in the total aboveground dry matter yield at silage maturity [24]. Therefore, ear rot is of particular importance in affecting the toxicogenic quality of grain-based products used for animal and human nutrition. In consequence, the protection of maize ears from *Fusarium* infections is of major importance in order to prevent a risk to human and animal health from mycotoxins.

Compared to the fungicide-untreated control, *Fusarium* infections, and therefore, the contamination with mycotoxins were significantly reduced by the three different fungicide spraying techniques. Concentrations of DON and ZEN were most effectively reduced by sub-canopy fungicide applications using the dropleg spraying technique, whether as a solo application or in combination with the overhead technique. Especially the main ears, which were inoculated with a macroconidia spore suspension of *F. culmorum*, were reached more effectively with the applied fungicide using the dropleg technique. It was possible to halve the amount of mycotoxins in the harvested crop with the same active ingredient content simply by changing the spraying technique. The dropleg technique generally deposits higher amounts of the applied fungicide on deeper located plant organs, whereas higher amounts of the fungicide are deposited on the upper leaves using the overhead spraying technique, whereby less active ingredient reaches the lower located plant organs [10]. Due to the reduced coverage of lower plant organs and the insufficient downward transport of the xylem-mobile systemics tebuconazole and prothioconazole within the plant from the site of application, the main ears are less protected from *Fusarium* infections using the overhead spraying technique. In addition to the main ears, other *Fusarium*-infected plant organs such as rudimentary ears, which are located underneath the main ear, can also contribute to the total mycotoxin contamination in forage maize. When the rudimentary ears are infected, mycotoxin concentrations may reach extraordinarily high levels compared to other parts of the maize plant including ears, kernels and stalks [63,64]. Non-germinated *Fusarium* spores on leaf blades as well as nutrient-rich pollen residues of the maize plant can be washed down into the leaf sheaths of the plant by rainwater after pollination. The rainwater cannot drain off as the leaf sheaths tightly enclose the maize stalk, resulting in constant moist conditions around the rudimentary ears, which offers favorable infection conditions for *Fusarium* in a persistent humid environment [24]. Fungal mycelium derived from infected rudimentary ears can infect adjacent stalk sections to subsequently colonize and contaminate the remaining stalk. Therefore, the use of the dropleg technique may lead to better protection by depositing higher amounts of active ingredients on these plant organs.

5. Conclusions

The dropleg spraying technique offers an opportunity to protect deeper-located plant organs more effectively by depositing larger amounts of active ingredients on plant parts that are difficult to achieve by the overhead spraying technique, especially in tall-growing row crops like maize. In combination with the overhead spraying technique the protection of various plant organs along the entire plant with the applied fungicide could be advantageous, especially when different parts along the plant are affected simultaneously by different fungal diseases.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture13061269/s1, Table S1: Dry matter yield (±SD; dt/ha), DON and ZEN concentrations (±SD; µg/kg) in forage maize samples at silage maturity of the cultivar "SY Werena" in the two fungicide-untreated controls without (T1) and with (T2) silk channel inoculation of main

ears with macroconidia of Fusarium culmorum seven days after silk emergence at BBCH 65 at the three trial locations Barkhorn, Hemdingen, and Hohenschulen; Table S2: Disease severities of Kabatiella zeae (±SD; percentage of leaf area affected by symptoms) on the upper, middle (L-2 to L+2) and lower leaf segment of forage at BBCH 65, BBCH 75 and BBCH 85 of the cultivar "SY Werena" depending on different fungicide spraying techniques (treatments T2–T5) at the three trial locations Barkhorn, Hemdingen, and Hohenschulen. Treatments: T2—Fungicide-untreated control, T3—Overhead spraying technique, T4—Dropleg spraying technique, T5—Combination of T3 and T4. The fungicide Prosaro®was applied (T3–T5) with 1.0 L/ha and a spray volume of 400 L/ha at BBCH 65; Table S3: Dry matter yield (±SD; dt/ha) of forage maize of the cultivar "SY Werena" depending on different fungicide spraying techniques (treatments T2–T5) at the three trial locations Barkhorn, Hemdingen, and Hohenschulen. Treatments: T2—Fungicide-untreated control, T3—Overhead spraying technique, T4—Dropleg spraying technique, T5—Combination of T3 and T4. The fungicide Prosaro®was applied (T3–T5) with 1.0 L/ha and a spray volume of 400 L/ha at BBCH 65; Table S4: Concentrations of DON and ZEN (±SD; µg/kg) in forage maize samples at silage maturity of the cultivar "SY Werena" depending on different fungicide spraying techniques (treatments T1–T5) at the three trial locations Barkhorn, Hemdingen, and Hohenschulen. Treatments: T1—Fungicide-untreated control, T2—Fungicide-untreated control, T3—Overhead spraying technique, T4—Dropleg spraying technique, T5—Combination of T3 and T4. Treatments T2 to T5 were inoculated with macroconidia of Fusarium culmorum seven days after silk emergence by silk channel inoculation of forage maize main ears. Treatment T1 was not inoculated with Fusarium spores in order to measure the success of the silk channel inoculation by comparing DON and ZEN concentrations between the two fungicide-untreated controls T1 and T2. The fungicide Prosaro®was applied (T3–T5) with 1.0 L/ha and a spray volume of 400 L/ha two days after inoculation at BBCH 65.

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References

1. Poole, N.F.; Arnaudin, M.E. The role of fungicides for effective disease management in cereal crops. *Can. J. Plant Pathol.* **2014**, *36*, 1–11. [CrossRef]
2. Jalli, M.; Kaseva, J.; Andersson, B.; Ficke, A.; Nistrup-Jørgensen, L.; Ronis, A.; Kaukoranta, T.; Ørum, J.-E.; Djurle, A. Yield increases due to fungicide control of leaf blotch diseases in wheat and barley as a basis for IPM decision-making in the Nordic-Baltic region. *Eur. J. Plant Pathol.* **2020**, *158*, 315–333. [CrossRef]
3. Jørgensen, L.N.; Heick, T.M. Azole use in agriculture, horticulture, and wood preservation—Is it indispensable? *Front. Cell. Infect. Microbiol.* **2021**, *11*, 730297. [CrossRef] [PubMed]
4. Różewicz, M.; Wyzińska, M.; Grabiński, J. The most important fungal diseases of cereals—Problems and possible solutions. *Agronomy* **2021**, *11*, 714. [CrossRef]
5. Willocquet, L.; Meza, W.R.; Dumont, B.; Klocke, B.; Feike, T.; Kersebaum, K.C.; Meriggi, P.; Rossi, V.; Ficke, A.; Djurle, A.; et al. An outlook on wheat health in Europe from a network of field experiments. *Crop Prot.* **2021**, *139*, 105335. [CrossRef]
6. Matthews, G.; Bateman, R.; Miller, P. *Pesticide Application Methods*, 4th ed.; Wiley & Blackwell: Hoboken, NJ, USA, 2014; ISBN 9781118351307.
7. Bayat, A.; Bolat, A.; Soysal, A. Performance of different types of spraying methods in second crop maize production. *J. Agric. Machin. Sci.* **2011**, *7*, 53–59.
8. Taube, F.; Vogeler, I.; Kluß, C.; Herrmann, A.; Hasler, M.; Rath, J.; Loges, R.; Malisch, C.S. Yield progress in forage maize in NW Europe—Breeding progress or climate change effects? *Front. Plant Sci.* **2020**, *11*, 1214. [CrossRef]

9. Menechini, W.; Maggi, M.F.; Jadoski, S.O.; Leite, C.D.; Camicia, R.d.M. Aerial and ground application of fungicide in corn second crop on diseases control. *Eng. Agric.* **2017**, *37*, 116–127. [CrossRef]
10. Rueegg, J.; Eder, R.; Anderau, V. Improved application techniques: Ways to higher efficacy of fungicides and insecticides in field grown vegetables. *Outlooks Pest. Manag.* **2006**, *17*, 80–84. [CrossRef]
11. Brandes, M. Einsatzmöglichkeiten der Dropleg-Technik in Gemüse- und Ackerbaukulturen. *J. Kult.* **2021**, *73*, 309–315. [CrossRef]
12. Hausmann, J.; Brandes, M.; Heimbach, U. Effects of dropleg application technique during flowering of oilseed rape on insect pests. *Crop Prot.* **2019**, *126*, 104917. [CrossRef]
13. Hausmann, J.; Heimbach, U.; Rostás, M.; Brandes, M. The effect of insecticide application by dropleg sprayers on pollen beetle parasitism in oilseed rape. *BioControl* **2021**, *66*, 765–777. [CrossRef]
14. Arny, D.C.; Smalley, E.B.; Ullstrup, A.J.; Worf, G.L.; Ahrens, R.W. Eyespot of maize, a disease new to North America. *Phytopathology* **1971**, *61*, 54–57. [CrossRef]
15. Schneider, R.; Krüger, W. *Kabatiella zeae* Narita et Hiratsuka als Erreger einer Blattfleckenkrankheit an Mais in Deutschland. *J. Phytopathol.* **1972**, *74*, 238–248. [CrossRef]
16. Reifschneider, F.J.B.; Arny, D.C. Yield loss of maize caused by *Kabatiella zeae*. *Phytopathology* **1983**, *73*, 607–609. [CrossRef]
17. Flett, B.; Nowell, D. First report of maize eyespot caused by *Aureobasidium zeae* in South Africa. *Afr. Plant Prot.* **1995**, *1*, 49–50.
18. Pronczuk, M.; Bojanowski, J.; Warzecha, R. Effect of leaf infection by *Kabatiella zeae* on stalk rot prevalence and grain yield of maize hybrids. *J. Phytopathol.* **2004**, *152*, 410–415. [CrossRef]
19. Jørgensen, L.N. Significant yield increases from control of leaf diseases in maize—An overlooked problem?! *Outlooks Pest. Manag.* **2012**, *23*, 162–165. [CrossRef]
20. Chen, N.; Xiao, S.; Sun, J.; He, L.; Liu, M.; Gao, W.; Xu, J.; Wang, H.; Huang, S.; Xue, C. Virulence and molecular diversity in the *Kabatiella zeae* population causing maize eyespot in China. *Plant Dis.* **2020**, *104*, 3197–3206. [CrossRef]
21. Dingley, J.M. 'Eye spot' disease of maize in New Zealand. *N. Z. J. Agric. Res.* **1973**, *16*, 325–328. [CrossRef]
22. Chinchilla, C.M. Eyespot (*Kabatiella zeae* Narita and Hiratsuka) disease progression curves in ten maize hybrids. *Turrialba* **1987**, *37*, 37–43.
23. Algermissen, C. Überregionale Studien zum Einfluss von Witterungsparametern auf die Epidemiologie und Schadensdynamik von Maispathogenen (Kabatiella zeae, Exserohilum turcicum, Fusarium spp.) und deren Bekämpfung durch Integrierte Pflanzenschutzmaßnahmen. Ph.D. Thesis, Christian-Albrechts-University, Kiel, Germany, 27 January 2016.
24. Oldenburg, E.; Höppner, F.; Ellner, F.; Weinert, J. *Fusarium* diseases of maize associated with mycotoxin contamination of agricultural products intended to be used for food and feed. *Mycotoxin Res.* **2017**, *33*, 167–182. [CrossRef] [PubMed]
25. Ferrigo, D.; Raiola, A.; Causin, R. *Fusarium* toxins in cereals: Occurrence, legislation, factors promoting the appearance and their management. *Molecules* **2016**, *21*, 627. [CrossRef]
26. Leite, M.; Freitas, A.; Silva, A.S.; Barbosa, J.; Ramos, F. Maize food chain and mycotoxins: A review on occurrence studies. *Trends Food Sci. Technol.* **2021**, *115*, 307–331. [CrossRef]
27. Munkvold, G.P. Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. *Eur. J. Plant Pathol.* **2003**, *109*, 705–713. [CrossRef]
28. Oldenburg, E.; Ellner, F. Distribution of disease symptoms and mycotoxins in maize ears infected by *Fusarium culmorum* and *Fusarium graminearum*. *Mycotoxin Res.* **2015**, *31*, 117–126. [CrossRef] [PubMed]
29. Deutscher Wetterdienst. Klimareport Schleswig-Holstein: Fakten bis zur Gegenwart—Erwartungen für die Zukunft. Available online: https://www.dwd.de/DE/leistungen/klimareport_sh/download_report_2017.pdf?__blob=publicationFile&v=5 (accessed on 7 February 2023).
30. Statistisches Amt für Hamburg und Schleswig-Holstein. Die Bodennutzung in Schleswig-Holstein. 2017. Available online: http://epub.sub.uni-hamburg.de/epub/volltexte/2018/78253/pdf/C_I_1_j_17_SH_e.pdf (accessed on 7 February 2023).
31. Reid, L.M.; Hamilton, R.I.; Mather, D.E. *Screening Maize for Resistance to Giberella Ear Rot*; Technical Bulletin SE; Research Branch Agriculture and Agri-Food Canada: Ottawa, ON, Canada, 1996.
32. Birr, T. Überregionales Monitoring zur Epidemie- und Schadensdynamik von Fusariumerregern sowie Strategien zur Befalls- und Risikominimierung der Mykotoxinbelastung in der Weizen- und Maiskultur Schleswig-Holsteins (2008–2012). Ph.D. Thesis, Christian-Albrechts-University, Kiel, Germany, 6 November 2013.
33. Lancashire, P.D.; Bleiholder, H.; van den Boom, T.; Langelüddeke, P.; Stauss, R.; Weber, E.; Witzenberger, A. A uniform decimal code for growth stages of crops and weeds. *Ann. Appl. Biol.* **1991**, *119*, 561–601. [CrossRef]
34. European and Mediterranean Plant Protection Organization. PP 1/272 (2) Foliar diseases on maize. *EPPO Bull.* **2016**, *46*, 388–391. [CrossRef]
35. Sulyok, M.; Berthiller, F.; Krska, R.; Schuhmacher, R. Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2649–2659. [CrossRef]
36. R Core Team. R: A language and Environment for Statistical Computing (R Foundation for Statistical Computing, Vienna, Austria). Available online: https://www.R-project.org/ (accessed on 22 June 2020).
37. Carroll, R.J.; Ruppert, D. *Transformation and Weighting in Regression*; CRC Press: London, UK, 1988; ISBN 9781351407267.
38. Pinheiro, J.C.; Bates, D.M. *Mixed-Effects Models in S and S-PLUS*; Springer: New York, NY, USA, 2000; ISBN 9780387227474.

39. Nakagawa, S.; Schielzeth, H. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* **2013**, *4*, 133–142. [CrossRef]
40. Bretz, F.; Hothorn, T.; Westfall, P.H. *Multiple Comparisons Using R*; Chapman & Hall/CRC Press: Boca Raton, FL, USA, 2011; ISBN 9781584885740.
41. Lehoczki-Krsjak, S.; Varga, M.; Szabó-Hevér, Á.; Mesterházy, Á. Translocation and degradation of tebuconazole and prothioconazole in wheat following fungicide treatment at flowering. *Pest Manag. Sci.* **2013**, *69*, 1216–1224. [CrossRef] [PubMed]
42. Oliver, R.P.; Hewitt, H.G. *Fungicides in Crop Protection*, 2nd ed.; CABI: Wallingford, UK, 2014; ISBN 9781780641669.
43. Carmona, M.; Sautua, F.; Pérez-Hérnandez, O.; Reis, E.M. Role of fungicide applications on the integrated management of wheat stripe rust. *Front. Plant Sci.* **2020**, *11*, 733. [CrossRef] [PubMed]
44. Nuyttens, D.; Baetens, K.; de Schampheleire, M.; Sonck, B. Effect of nozzle type, size and pressure on spray droplet characteristics. *Biosyst. Eng.* **2007**, *97*, 333–345. [CrossRef]
45. Ettle, T.; Schwarz, F.J. Effect of maize variety harvested at different maturity stages on feeding value and performance of dairy cows. *Anim. Res.* **2003**, *52*, 337–349. [CrossRef]
46. Horsfall, J.G.; Dimond, A.E. Interactions of tissue sugar, growth substances and disease susceptibility. *Z. Pflanzenk. Pflanzen.* **1957**, *64*, 415–421.
47. Daub, M.E.; Ehrenshaft, M. The photoactivated Cercospora toxin cercosporin: Contributions to plant disease and fundamental biology. *Annu. Rev. Phytopathol.* **2000**, *38*, 461–490. [CrossRef]
48. Daub, M.E.; Herrero, S.; Chung, K.-R. Photoactivated perylenequinone toxins in fungal pathogenesis of plants. *FEMS Microbiol. Lett.* **2005**, *252*, 197–206. [CrossRef]
49. Pestka, J.J. Deoxynivalenol: Toxicity, mechanisms and animal health risks. *Anim. Feed Sci. Technol.* **2007**, *137*, 283–298. [CrossRef]
50. Metzler, M.; Pfeiffer, E.; Hildebrand, A. Zearalenone and its metabolites as endocrine disrupting chemicals. *World Mycotoxin J.* **2010**, *3*, 385–401. [CrossRef]
51. Bryła, M.; Waśkiewicz, A.; Ksieniewicz-Woźniak, E.; Szymczyk, K.; Jędrzejczak, R. Modified *Fusarium* ycotoxmins in cereals and their products—Metabolism, occurrence, and toxicity: An updated review. *Molecules* **2018**, *23*, 963. [CrossRef]
52. Freire, L.; Sant'Ana, A.S. Modified mycotoxins: An updated review on their formation, detection, occurrence, and toxic effects. *Food Chem. Toxicol.* **2018**, *111*, 189–205. [CrossRef] [PubMed]
53. Logrieco, A.; Mulè, G.; Moretti, A.; Bottalico, A. Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. *Eur. J. Plant Pathol.* **2002**, *108*, 597–609. [CrossRef]
54. European Commision. Commission Regulation (EC) No 1126/2007 of 28 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. *Off. J. Eur. Union* **2007**, *L255*, 14–17.
55. European Commission. Commission Recommendation (2006/576/EC) of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT- 2 and fumonisins in products intended for animal feeding. *Off. J. Eur. Union* **2006**, *L229*, 7–9.
56. Oldenburg, E.; Ellner, F. *Fusarium* mycotoxins in forage maize—Detection and evaluation. *Mycotoxin Res.* **2005**, *21*, 105–107. [CrossRef] [PubMed]
57. Birr, T.; Jensen, T.; Preußke, N.; Sönnichsen, F.D.; de Boevre, M.; de Saeger, S.; Hasler, M.; Verreet, J.-A.; Klink, H. Occurrence of *Fusarium* mycotoxins and their modified forms in forage maize cultivars. *Toxins* **2021**, *13*, 110. [CrossRef]
58. Vandicke, J.; de Visschere, K.; Ameye, M.; Croubels, S.; de Saeger, S.; Audenaert, K.; Haesaert, G. Multi-mycotoxin contamination of maize silages in Flanders, Belgium: Monitoring mycotoxin levels from seed to feed. *Toxins* **2021**, *13*, 202. [CrossRef]
59. Weaver, A.C.; Weaver, D.M.; Adams, N.; Yiannikouris, A. Co-occurrence of 35 mycotoxins: A seven-year survey of corn grain and corn silage in the United States. *Toxins* **2021**, *13*, 516. [CrossRef]
60. Goertz, A.; Zuehlke, S.; Spiteller, M.; Steiner, U.; Dehne, H.W.; Waalwijk, C.; De Vries, I.; Oerke, E.C. *Fusarium* species and mycotoxin profiles on commercial maize hybrids in Germany. *Eur. J. Plant Pathol.* **2010**, *128*, 101–111. [CrossRef]
61. Dorn, B.; Forrer, H.R.; Jenny, E.; Wettstein, F.E.; Bucheli, T.D.; Vogelgsang, S. *Fusarium* species complex and mycotoxins in grain maize from maize hybrid trials and from grower's fields. *J. Appl. Microbiol.* **2011**, *111*, 693–706. [CrossRef]
62. De Boevre, M.; Landschoot, S.; Audenaert, K.; Maene, P.; Di Mavungu, D.; Eeckhout, M.; Haesaert, G.; De Saeger, S. Occurrence and within field variability of *Fusarium* mycotoxins and their masked forms in maize crops in Belgium. *World Mycotoxin J.* **2014**, *7*, 91–102. [CrossRef]
63. Oldenburg, E.; Höppner, F.; Weinert, J. Distribution of deoxynivalenol in Fusarium-infected forage maize. *Mycotoxin Res.* **2005**, *21*, 196–199. [CrossRef] [PubMed]
64. Schollenberger, M.; Müller, H.-M.; Ernst, K.; Sondermann, S.; Liebscher, M.; Schlecker, C.; Wischer, G.; Drochner, W.; Hartung, K.; Piepho, H.-P. Occurrence and distribution of 13 trichothecene toxins in naturally contaminated maize plants in Germany. *Toxins* **2012**, *4*, 778–787. [CrossRef] [PubMed]