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Performance of Symmetric Double Flat Fan Nozzles against Fusarium Head Blight in Durum Wheat

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Abstract: Four types of nozzles were tested on large-scale trials with a 40 m^2 plot unit size. The Avi Twin 110-01 (80 L ha^{-1}), 110-02 (160 L ha^{-1}), 110-03 (240 L ha^{-1}), and 110-04 (320 L ha^{-1}) symmetric double fan injector nozzles were tested during the 2020/2021 and 2021/2022 growing seasons. This study aimed to evaluate the performance of spray nozzles with regard to deoxynivalenol (DON) accumulation in durum wheat grains. Artificial inoculation with *Fusarium* spp. was performed after durum heads were protected with fungicide. The percentage of heads covered with fungicide droplets, grain yield, yield-related traits, technological quality parameters, and concentrations of DON were determined. Compared to the control (without fungicide treatment), the Avi Twin 04 nozzle caused a reduction of 45.0% in the DON concentration on average across both growing seasons. This positively corresponded to the percentage of heads covered with fungicide droplets, which was highest when this nozzle was utilized. In both trial years, the DON reduction caused by the 110-04 twin nozzle was higher than that caused by the 110-01 nozzle. Treatment with the 110-04 nozzle more effectively improved the grain yield, 1000-kernel weight, and test weight compared to treatment with the 110-01 nozzle and the untreated control. The differences in technological quality were less pronounced when different spray nozzles were used.

Keywords: deoxynivalenol; durum wheat; technological application; grain yield; spray nozzle

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1. Introduction

Durum wheat (*Triticum turgidum* sp. *durum*) is an economically important crop in many areas worldwide, especially in the Mediterranean [1]. However, the grain yield and end-use quality of durum wheat are threatened by many diseases, with one very common example being fusarium head blight (FHB). FHB contaminates the grain with the fungal mycotoxin deoxynivalenol, also known as DON [2]. The most common *Fusarium* species is the *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* Schw. (Petch)) species complex, but *F. culmorum* and *F. avenaceum* are also highly representative species [3]. FHB is particularly severe in humid and moderately warm areas, in which it can cause severe epidemics.

The best way to combat FHB and the associated mycotoxins is to integrate FHB-resistant varieties, agronomic practice, and chemical control. It was previously concluded that DON levels for wheat are mainly affected by tillage, crop rotation, varieties, and fungicide application [4]. However, fungicides are not completely effective against FHB; they are highly dependent on year and weather conditions during the flowering stage [5]. Many factors may influence the effectiveness of fungicide application, including the fungicide's efficacy, the timing of application, the orientation of the spray nozzle, and the water volume. Thus, to increase the efficiency of fungicide control, various important factors should be considered, such as the timing of application, the application rate, the selection of fungicides, and the coverage of the heads with fungicides [6].

Fungicides used for FHB control are locally systemic, which means that they only protect the site at which the fungicide makes contact on the wheat head. The coverage of heads using fungicide droplets should be increased to maximize the fungicide's effects [7]. Different parameters influence the head coverage, such as the nozzle type, droplet size, spraying speed, use of adjuvants, etc. [8,9]. There is an indirect relationship between head coverage and reductions in FHB, and consequently, DON content. Higher and more uniform head coverage, paired with the right timing of fungicide application, can decrease DON content. With the best fungicide, optimal timing, and best application, DON can be reduced by about 50% in winter wheat. If the coverage is 100%, then the efficacy could be close to 100% [10]. In previous investigations, the DON content was reduced by 22.5% when using XR single flat fan nozzles, 23% when using TwinJet double flat fan nozzles, and 41.5% when using alternating Turbo FloodJet nozzles [11]. In laboratory experiments, double flat fan nozzles achieved higher levels of coverage of wheat heads than single fan nozzles, and better results were observed when using double fan injector nozzles compared to standard double fan nozzles [12]. Furthermore, an increase in water volume per hectare was shown to increase head coverage [11]. It has been shown that angled nozzles with coarser droplets improve head coverage as they travel faster, resist evaporation, and are less prone to drift, compared to those that spray fine droplets. Symmetrical double fan nozzles with 30° angles were shown to improve the coverage of wheat heads versus single fan nozzles. Nozzles with steeper backward angles (70°) also improved the coverage of the rear parts of wheat heads [11]. The coverage of the rear part of wheat heads improved from 8 to 22%, the coverage of the sides improved from 18 to 37%, and the coverage of the front part of wheat heads improved from 35 to 52% when using alternating Turbo FloodJet nozzles, compared to standard XR single flat nozzles [13]. New nozzle combinations from TeeJet, QJ 90, TT F, and XR B reduced the visual symptoms of FHB by 50% compared to standard XR nozzles [14].

The objective of this study was to analyse the effects of spray quality and spray quantity applied to durum wheat heads on the DON accumulation, grain yield, yield-related traits, and technological quality traits under high FHB inoculum pressure using fungicide protection with four symmetric double flat fan injector nozzles (110-01, 110-02, 110-03, and 110-04). The hypotheses were that (1) better fungicide coverage on durum wheat heads can decrease DON content in the grain and (2) decreased DON content in the grains can increase the grain yield and improve yield parameters using less than 100 L ha^{-1} of water with these nozzles.

2. Materials and Methods

2.1. The Experimental Layout

Field research was conducted during 2020–2021 and 2021–2022 with the winter durum wheat variety 'Auradur', produced by Probstdorfer Saatzucht (Probstdorf, Austria), with a density of 420 plants/m^2. This variety varies from being susceptible to very susceptible to FHB. The experiment was conducted using five treatment combinations in a randomized complete block design with four replications. Each individual plot was 16 m long and 2.5 m wide. The total area per replication per plot was 200 m^2, and the whole trial area was 800 m^2. The research was conducted at the Biotechnical Faculty of the University of Ljubljana, Slovenia (46.0569° N, 14.5058° E), on heavy soil (>30% of clay).

The trial plot was ploughed with a reversible plough and cultivated with a rotary harrow. Basic mineral fertilization with nitrogen, phosphorus, and potassium (NPK 7-20-30) was applied in 500 kg/ha. After that, sowing was completed with an Amazone D9 (Amazonen-Werke H. Dreyer Se & Co. KG, Hasbergen, Germany) mechanical seeding machine at a rate of 210 kg/ha. The sowing took place on 4 November 2020 and on 28 September 2021. In October 2020, there was 202 mm of precipitation, resulting in a later sowing date. The pre-crop in 2020 was winter wheat (*Triticum aestivum* L.), while in 2021, the pre-crop was grassland. In the spring, calcium ammonium nitrate (CAN) mineral fertilizer was added in BBCH 25, BBCH 31, and BBCH 51 in 200 kg/ha amounts. The BBCH (Biologische Bundesanstalt, Bundessortenamt, and Chemische Industrie) scale is a system used for the uniform coding of growth stages, which has been widely used to describe the phenological stages of plants. All other agrotechnical procedures in the trial were carried according to good agricultural practice. The temperatures in May 2021 were 2.3 °C colder than the long-term average from 1981 to 2010, and there was 139 mm more rainfall compared to the long-term average for May [15]. In June 2021, the temperature was 4 °C higher than the long-term average. In May 2022, the average air temperature was 2.3 °C higher than the long-term average from 1981 to 2010, and there was 58 mm less rainfall compared to the long-term average (Table 1).

Table 1. Average air temperature and rainfall (October 2020–July 2021; October 2021–July 2022) and long-term average 1981–2010 for Ljubljana, Slovenia [15].

Season	**2020/2021**		**2021/2022**		**1981–2010**	
Month	**Temp. (°C)**	**Rainfall (mm)**	**Temp. (°C)**	**Rainfall (mm)**	**Temp. (°C)**	**Rainfall (mm)**
October	11.9	202	9.8	47	11.2	147
November	5.3	48	5.9	165	5.6	129
December	2.9	183	1.3	122	1.2	107
January	1.2	141	0.8	32	0.3	69
February	5.9	85	5.1	53	1.9	70
March	6.7	57	6.6	7	6.5	88
April	9.1	129	10.4	113	10.8	99
May	13.5	248	18.1	51	15.8	109
June	23.1	25	23.4	36	19.1	144
July	23.3	150	24.5	87	21.3	115
Sum		1268		713		1077

2.2. Fungicide Application against FHB and Nozzles

Fungicide application against FHB was performed using a mechanized set formed of a mounted commercial sprayer (Agromehanika 600 EN, Kranj, Slovenia) with a 600 L nominal tank and a standard tractor Fendt 208 S (AGCO-Fendt, Marktoberdorf, Germany) with 60 kW nominal power. Prosaro fungicide (a.i. prothioconazole 125 g/L + tebuconazole 125 g/L) (Bayer AG, Leverkusen, Germany) was applied against FHB in a dose of 1.0 litre per hectare at BBCH 61–63. The heading date was 31 May 2021 and 17 May 2022, while the fungicide was applied a few days later (8 June 2021 and 24 May 2022). A spraying speed of 6.0 km/h and a spraying pressure of 3.0 bar were used with all four nozzles.

For fungicide application, Avi Twin symmetric double flat fan injector nozzles were used. The four nozzles used in the four treatments were the 110-01 nozzle (80 L ha^{-1}), the 110-02 nozzle (160 L ha^{-1}), the 03 nozzle (240 L ha^{-1}), and the 04 nozzle (320 L ha^{-1}). The Avi Twin 110-01 nozzle was specified as the control nozzle. The angle between the spray jets was 65° and the individual spray angle was 110°. The boom height at spraying was 50 cm above the wheat durum heads. The droplet size of Avi Twin nozzles 01, 02, 03, and 04 at a spraying pressure of 3.0 bar was extremely coarse (VMD 484–553 µm) according to the manufacturer [16].

2.3. Inoculum Production and Artificial Inoculations

A conidial inoculum of *Fusarium graminearum* (PIO 31), isolated from winter wheat collected in the eastern part of Croatia, and *F. culmorum* (IFA 104), obtained from IFA-Tulln (Tulln an der Donau, Austria), was produced using a mixture of wheat and oat grains (3:1 *v*/*v*) in the Phytopathological Laboratory of the Agricultural Institute Osijek (Osijek, Croatia). They were identified morphologically and with molecular markers; after this, we assigned them with an abbreviated label which included the institution where they originated from. Macroconidia were washed off the colonized grains, the suspension was diluted, and the final conidial concentrations were set to 10×10^4 mL^{-1} using a hemocytometer (Bürker-Türk, Hecht Assistent, Sondheim vor der Rhön, Germany) for both species.

Plants were sprayed with inoculum on the same day as fungicide application against FHB. Directly after the inoculation, irrigation was applied to provoke FHB infection. The time interval between fungicide application and artificial inoculation was about 8 to 9 h. The Solo Accu Power 416Li electrical backpack sprayer (SOLO Kleinmotoren GmbH, Sindelfingen, Germany) with the 110-03 Avi Twin nozzle was utilized. Artificial inoculation and irrigation was repeated after 48 h.

2.4. Measurement of Head Coverage

In the individual trial plot, three plants were selected, and the measurement of head coverage was executed. Water-sensitive paper (WSP) was attached to special holders over the front of the winter durum heads (facing the sprayer's advance) and over the back side (facing the sprayer's retreat) in the direction of spraying. After the FHB fungicide application, the WSP was collected from the plants, and the coverage was analysed using Wise Node (Wise Technologies, Ljubljana, Slovenia). This is a purpose-built measuring device which uses machine vision technology to detect particles on paper samples; thus, it can be utilized in applications such as statistics of sprayed surface coverage. On each WSP, three measurements were performed, which provided the percentage of the area covered with droplets.

2.5. Measurement of Deposit Quantity

The day after FHB fungicide application, filter paper (76 mm × 26 mm) was fixed on the front and rear sides of the holders around the durum heads in the spraying direction. The front side faced the sprayer's approach, and the back side faced the sprayer's retreat. Next, the trial plots were sprayed with a water solution of UV dye Helios SC 500. First, the UV dye was dissolved in water in a spray tank, and it underwent hydraulic agitation for 15 min. Each trial plot was sprayed in different ways according to the types of nozzles and plot design, using the same Agromehanika 600 EN sprayer used for the FHB fungicide application. From each trial unit, three samples were taken for deposit quantity analysis on the durum heads and filter paper. Immediately after the application, the samples were put in black plastic foil and put in a refrigerator at 4 °C. Next, the samples were taken to a chemical laboratory in the Slovenian Institute of Hop Research and Brewing for tracer quantity analysis. The method was based on determining the amount of tracer Helios SC 500, which has fluorescent properties on collectors.

In short, 0.2 g of durum heads was cut using scissors previously cleaned with ethanol of p.a.-grade purity (Sigma-Aldrich, Darmstadt, Germany) for each new individual sample to prevent cross-contamination. Then, 7 mL of solvent diethylene glycol monoethyl ether (Sigma-Aldrich, Darmstadt Germany) was added and extracted for 15 min in an ultrasonic bath (Bandelin Sonorex, Berlin, Germany) at room temperature. After extraction, the liquid extract was transferred into a high-performance liquid chromatography (HPLC) vial after extraction and subjected to further analysis via HPLC immediately after extraction. For the determination of the tracer concentration in the extracts, a liquid chromatograph, equipped with a fluorescence detector (Agilent Technologies 1200 series, Santa Clara, CA, USA) and without any column, was used. The isocratic mobile phase (2-ethoxyethanol for paper collectors and diethylene glycol monoethyl ether for plant tissues) with a flow of 0.7 mL/min was used, and 10 μL of the extract sample was injected. The excitation energy with a wavelength of 375 nm was used, and emitted light was detected at 435 nm. The amount of tracer on the collector was calculated externally using the calibration curve prepared with standard solutions of tracer in solvents used for extraction. The results were presented in μg g^{-1} of deposit on the durum head and ng cm^{-2} of deposit on the filter paper. The same procedure as that described for the durum heads was used to determine the tracer quantity on the filter paper (the whole filter paper area).

2.6. Field Control Efficacy

For the assessment of FHB, 50 durum heads per trial plot were chosen and an assessment key was used according to [17]. Field control efficacy was calculated based on the percentage of head area infected with FHB (visual symptoms) (Equation (1)). The results were compared to the untreated control, where no fungicide against FHB was used.

$$\begin{gathered}\text{Field control efficacy (\%)} \\ = 100 - (\text{percentage of head area infected}_{\text{nozzle treatment}}/\text{percentage of head area} \\ \text{infected}_{\text{ untreated control}}) \times 100\%\end{gathered} \quad (1)$$

2.7. Measurement of Deoxynivalenol Content

Prior to harvesting, 200 winter durum heads were randomly picked by hand from each individual plot. Then, the grains from the sampled durum heads were threshed and cleaned using a Wintersteiger threshing–cleaning device. Approximately 100 g of grains was sent to a laboratory for DON content analysis. The quantitative test used to determine DON content (lateral flow) was based on immunochromatographic principles. Test strips used for the determination of DON were coated with specific antibodies that were conjugated to colloidal gold. The diluted filtrate of the sample was added to the incubation container for the DON determination, where the test strip with two display lines, tight and control, was soaked. The mixture of filtrate and reagents in the incubation vessel began to travel vertically up the test strip, where it crossed 2 lines. During this upward flow, the mycotoxin DON to be determined adhered to the conjugated antibodies. A valid test always had a red upper control line. If the sample did not contain DON, a colour developed in the area of the control line, indicating the absence of the mycotoxin. Otherwise, the presence of DON in the sample resulted in the test line being stained. The intensity of the colour in the test line was indirectly proportional to the DON concentration in the sample. By using the Lateral logic S-Flow reader (ProGnosis Biotech, Larissa, Greece) and symmetrical quantification technology, the DON content in the sample was appropriately measured. The DON content of mycotoxins was given in ppb per kg of air-dried substance. The DON analysis was carried out in a chemical laboratory at KGZS—Zavod Ptuj (Ptuj, Slovenia).

2.8. Harvest and Grain Quality

The trial plots were harvested with small plot combines Wintersteiger Quantum on 29 July 2021 and Wintersteiger Elite (WINTERSTEIGER Seedmech GmbH, Ried im Innkreis, Austria) on 19 July 2022. The harvested area was 6 m × 1.5 m. The grain mass of each individual plot was weighed with a Kern weighing device (Balingen, Germany), and the grain moisture was measured using an HE 50 moisture meter (Pfeufer, Kitzingen Germany). Then, the grain yield at 14% grain moisture was calculated. The 1000-kernel weight was determined by counting 100 grains per sample, weighting them, and multiplying the weight by 10. Eight samples were taken from each plot unit for the measurement of 1000-kernel weight. Afterwards, the grain quality was analysed using Bureau Veritas. An AgriCheck device (Bruins Instrument, Weiler bei Bingen, Germany) was used for the analysis of protein content, sedimentation value, and test weight, while the FN 1700 device (Perten, Waltham, MA, USA) was used for the analysis of the Hagberg falling number.

The AgriCheck instrument is a high-performance transmission spectrometer (it measures the amount of transmitted light) and its use is intended for the analysis of the composition of a sample based on the excitation of a sample with wavelengths close to the near-infrared (NIR) spectrum. During the measurement, the instrument exposes the sample to a specific wavelength from the near-infrared spectrum determined with the monochromator. As the light passes through the sample, it collides with the molecules, where part of the light is absorbed, and the other part diffusely penetrates through. This transmitted light is detected by a detector behind the sample. The method is relative, so the apparatus must be calibrated on identically prepared and measured samples with classical analysis in order to find the protein content (ISO 5983-2:2009) [18], sedimentation value (internal method MET/K/054), and test weight (Schopper weight device). In this study, calibration analysis was carried out by the Agricultural Institute of Slovenia.

The method used to determine the Hagberg falling number—the Perten method, according to Hagberg—is simple (it is defined by ISO standard 3093, ICC standard no. 107/1, and AACC 56-81B) [19]. It is an international standard method used for the determination of alpha amylase activity in starch-containing cereal grains and flours. The grains were ground to a certain granulation, and the time taken by a special mixer to fall through a suspension of flour and water heated to 100 °C was measured. The time expressed in seconds was a descending number. With the increased activity of enzymes, this value was lower.

2.9. Statistical Analysis

The data used to determine the percentage of coverage of fungicide droplets, deposit quantity, grain yield, test weight, 1000-kernel weight, protein content, sedimentation value, Hagberg falling number, and DON content were presented as the means of four replications. The determination of differences among treatments and years was conducted using factorial analysis of variance (ANOVA), followed by Duncan's post hoc test ($p < 0.05$). Data analysis was performed within STATGRAPHICS ® Centurion (v. 17) software (Statpoint Technologies, Inc., Warrenton, VA, USA).

3. Results

3.1. Head Coverage

In 2021, the front and back head coverage using the 110-04 nozzle was the highest of all the nozzles (Table 2). In the second year of investigation, the 110-03 and 110-04 nozzles resulted in better front and back head coverage than the other two nozzles. In both trial years, the 110-01 nozzle resulted in front and back head coverage below 10%.

Table 2. Head coverage (%) of durum wheat.

Nozzle	Coverage (%)	
Position	**2021**	**2022**
Front		
110-01 (control nozzle)	9.8 a [1]	9.1 a
110-02	14.5 ab	17.1 a
110-03	16.6 b	25.8 b
110-04	33.3 c	33.5 b
Back		
110-01 (control nozzle)	6.1 a	2.7 a
110-02	20.2 b	7.0 a
110-03	18.1 b	14.9 b
110-04	36.6 c	14.0 b

[1] Different letters within the same year and position denote a significant difference (Duncan's test, $\alpha = 0.05$).

3.2. Deposit Quantity

The tracer mass on durum heads significantly increased with the nozzle flow rate. In 2021, no significant differences appeared between the 110-02 and 110-03 nozzles, with the smallest tracer mass being recorded when the 110-01 nozzle was used and the largest being recorded when the 110-04 nozzle was used. In 2022, no significant differences emerged between the 110-03 and 110-04 nozzles, but these two nozzles resulted in larger tracer masses on the durum heads compared to the 110-01 and 110-02 nozzles (Table 3).

Table 3. Tracer mass (µg g^{-1}) on durum heads caused by different nozzles.

Nozzle	Tracer Mass on Durum Head (µg g^{-1})	
	2021	**2022**
110-01 (control nozzle)	0.106 a [1]	0.304 a
110-02	0.202 b	0.384 b
110-03	0.241 b	0.537 c
110-04	0.362 c	0.576 c

[1] Different letters within the same year denote a significant difference (Duncan's test, α = 0.05).

The filter paper was positioned on the front and back side of the special holders around the durum heads. The tracer mass on the front side significantly increased with the nozzle flow rate from 110-01 to 110-04. In 2022, there were only no significant differences in tracer mass on the front side between the 110-03 and 110-04 nozzles. In 2021, on the back side, the tracer mass was significantly larger with the 110-03 and 110-04 nozzles compared to the 110-01 and 110-02 nozzles. A year later, the tracer mass significantly increased with nozzle flow rate from 110-01 to 110-04 (Table 4).

Table 4. Tracer mass (ng cm^{-2}) on filter paper.

Nozzle	Tracer Mass on Filter Paper (ng cm^{-2})	
Position	**2021**	**2022**
Front		
110-01 (control nozzle)	1.540 a [1]	2.958 a
110-02	4.692 b	9.057 b
110-03	8.315 c	14.898 c
110-04	13.178 d	16.060 c
Back		
110-01(control nozzle)	2.405 a	2.086 a
110-02	3.707 a	7.384 b
110-03	8.298 b	17.430 c
110-04	8.346 b	23.977 d

[1] Different letters within the same year and position denote a significant difference (Duncan's test, α = 0.05).

The relative distribution of the tracer mass on the front and back side of the head was calculated (Table 5). In 2021, a slightly higher percentage of tracer was obtained on the front of the head, except when the 110-01 nozzle was used. A year later, there was a slightly higher percentage of tracer on the front side when the 110-01 and 110-02 nozzles were used, while there was a slightly higher percentage of tracer on the rear side of the head when the 110-03 and 110-04 nozzles were applied. In general, the distribution of the tracer was fairly uniform, both on the front (40–60%) and back (40–60%) sides of the head, in both trial years.

Table 5. Tracer mass ratio (%) of front and back sides on filter papers.

Nozzle	Front/Back Side of Durum Head (%)	
	2021	**2022**
110-01 (control nozzle)	39/61	59/41
110-02	56/44	55/45
110-03	50/50	46/54
110-04	61/39	40/60

3.3. Field Control Efficacy

In the first trial year, field control efficacy was the lowest when the control nozzle was used (110-01). Between other nozzle treatments, no differences in control efficaccy appeared that year. In 2022, the highest control efficacy was recorded when the 110-04 nozzle was used, while the lowest was recorded when the 110-01 control nozzle was used (Table 6).

Table 6. Field control efficacy (%) with nozzles compared to untreated control.

Treatment	Field Control Efficacy (%)	
	2021	**2022**
110-01 (control nozzle)	27 a [1]	26 a
110-02	61 b	54 b
110-03	73 b	69 c
110-04	85 b	86 d

[1] Different letters between treatments in the same year denote significant differences at $p < 0.05$.

3.4. Grain Yield and Yield-Related Traits

The grain yield at 14% moisture was significantly increased by all nozzles compared to the control in both trial years. In 2021, there were no significant differences in grain yield between nozzles, while in 2022, the 110-04 nozzle facilitated a significantly higher yield compared to the 110-01 and 110-02 nozzles. In both trial years, the 1000-kernel weight was significantly decreased in the control treatment compared to the nozzle treatments. The use of the 110-04 nozzle resulted in significantly increased 1000-kernel weights compared to the 110-01 and 110-03 nozzles in 2021 and the 110-01 nozzle in 2022. The test weight was significantly increased by the 110-02, 110-03, and 110-04 nozzles compared to the 110-01 nozzle and the control treatment in 2021 and 2022 (Table 7).

Table 7. Grain yield (kg ha^{-1}), 1000-kernel weight (g), and test weight (kg hL^{-1}) with four different nozzles and control treatments in a two-year study.

Treatment	Grain Yield (kg ha^{-1})		1000-Kernel Weight (g)		Test Weight (kg hL^{-1})	
	2021	**2022**	**2021**	**2022**	**2021**	**2022**
110-01 (control nozzle)	3013 b [1]	3438 b	36 b	26 b	68.8 a	62.4 a
110-02	3392 b	4607 c	40 cd	28 bc	72.3 b	70.1 b
110-03	3327 b	4885 cd	37 bc	28 bc	70.6 b	70.5 b
110-04	3382 b	5544 d	41 d	29 c	75.0 b	70.9 b
Control	2430 a	2506 a	30 a	22 a	62.5 a	58.4 a

[1] Different letters denote significant differences at $p < 0.05$ between treatments.

3.5. Technological Quality of Tested Wheat Grains

No significant differences appeared in the protein content between the nozzles and the control treatment in both trial years (Table 8). The protein content ranged between 13.0 and 14.3%. Also, no significant differences were found in the sedimentation value in both trial years. In 2021, the sedimentation value varied between 65 and 71 mL, while in the second year of investigation, it varied between 49 and 57 mL. The Hagberg falling number was significantly increased by the 110-01 nozzle compared to the 110-04 nozzle in 2021. In 2022, the 110-04 nozzle resulted in a significantly increased Hagberg falling number compared to the 110-01 nozzle.

Table 8. Protein content (%), sedimentation value (ml), and Hagberg falling number (s) with four different nozzles and control treatments in a two-year study.

Treatment	Protein Content (%)		Sedimentation Value (mL)		Falling Number (s)	
	2021	**2022**	**2021**	**2022**	**2021**	**2022**
110-01 (control nozzle)	13.6 a	13.6 a	66 a	51 a	356 b [1]	319 a
110-02	13.4 a	13.4 a	65 a	49 a	337 ab	336 ab
110-03	14.1 a	13.9 a	71 a	53 a	336 ab	322 ab
110-04	13.0 a	13.1 a	66 a	48 a	309 a	352 b
Control	14.3 a	14.2 a	67 a	57 a	338 ab	322 ab

[1] Different letters denote significant differences at $p < 0.05$ between treatments.

3.6. DON Accumulation of Tested Wheat Grains

The DON content was significantly increased in the control treatment compared to the DON content in the treatments with fungicides using different nozzles in 2021. No significant differences in DON content appeared among nozzles that year. However, a year later, the 110-04 nozzle significantly decreased the DON content compared to the 110-01 nozzle and the control treatment (Table 9).

Table 9. DON content (μg kg^{-1}) with four nozzles and control treatments in a two-year study.

Treatment	DON Content (μg kg^{-1})	
	2021	**2022**
110-01 (control nozzle)	4315 a [1]	19,790 b
110-02	3966 a	18,027 ab
110-03	3871 a	18,965 ab
110-04	3313 a	14,311 a
Control	8606 b	22,350 b

[1] Different letters between treatments in the same year denote significant differences at $p < 0.05$.

The DON reduction was most significantly increased by the 110-04 nozzle and the least by the 110-01 nozzle compared to the untreated control in both trial years. In 2021, the DON reduction ranged from 50 to 63%, and a year later, it ranged from 11 to 36% (Table 10).

Table 10. DON reduction (%) with nozzles compared to untreated control.

Treatment	DON Reduction (%)	
	2021	**2022**
110-01 (control nozzle)	50 a [1]	11 a
110-02	54 ab	19 ab
110-03	55 ab	15 ab
110-04	63 b	36 b

[1] Different letters between treatments in the same year denote significant differences at $p < 0.05$.

4. Discussion

Durum wheat has extreme susceptibility to FHB from anthesis to the soft dough stage of grain development [20]. There is limited information about the influence of spray nozzles on DON accumulation in durum wheat. Therefore, this study aimed to evaluate the performance of different spray nozzles to control DON content in durum wheat grains. The head coverage of durum wheat with fungicide is one of the factors influencing DON content in the grain and application efficiency of FHB control [6]. In the current investigation, symmetrical double flat fan injector nozzles with coarse droplets and a 65-degree angle between the front and back jets were used. The especially low coverage of durum heads both at the front and back side in the direction of spraying was found when 110-01 nozzles were used. The coverage did not exceed 10%, which is under the 10–15% limit necessary for the good efficiency of foliar fungicides [21]. This is directly related to the flow rate of the nozzle and the water rate per hectare. When the 110-01 nozzle was used, the water rate was 80 L ha^{-1}, while the usage of the 110-04 nozzle resulted in a water rate of 320 L ha^{-1}. Many authors reported in their investigations that increased water volumes improved coverage [8,11,12]. In 2021, there was largely uniform coverage on the front and back sides of durum heads using all nozzles. However, a year later, the level of coverage on the back side was lower when using all nozzles compared to that of the front side. This could have been due to different boom heights or positions in each trial year. It was reported that symmetrical double fan nozzles with shallow angles (30 degrees from vertical) improved the coverage uniformity, while nozzles with steeper backward facing angles (70 degrees from vertical) increased the coverage on the back side or on the sprayer's retreat side [11,22]. As the active ingredients of the fungicide, tebuconazole and prothioconazole, are classified as semi-systemic, application with double-angled nozzles inevitably increases the fungicide's efficacy [14]. At full coverage, the efficacy of fungicides is 2–3 times higher and reaches 90% or more for the best fungicides. Under field conditions, the efficacy of the same fungicide can be maximally 50%, and therefore, we need better head coverage [23].

The results showed that the tracer mass increased on durum heads from the 110-01 nozzle, with the lowest flow rate, to the 110-04 nozzle, with the highest flow rate, which is directly connected to the coverage on water-sensitive paper. From these results, we can predict that fungicides used for FHB control will be more effectively deposited when using Avi Twin nozzles with higher nozzle flow rates and water rates per hectare, such as 110-03 and 110-04. In this way, the durum head will be more effectively covered, and fungicide application will be more effective. A similar trend was also noticed on the filter paper. On filter paper facing the front and the back, it was noticeable that with the increase in the nozzle flow rate, the tracer quantity increased. Therefore, using a lower water volume per hectare will result in the poor deposition of durum heads. From the results, it was noticeable that the distribution of the amount of tracer was fairly uniform both on the front and back sides of the durum heads, and these findings do not match the results of some investigations which reported that symmetric double flat fan nozzles resulted in the poorer deposition of the back sides of heads compared to the front sides [10]. In the current research, this was not the case. Both of the active ingredients of fungicide used in this study (prothioconazole and tebuconazole) inhibit demethylation in fungi processes in the process of sterol biosynthesis. A fungicide with this mode of action is called a demethylation inhibitor (DMI). Several authors have reported contradictory results regarding the efficiency of these fungicides [24–26]. This is related to the degree and duration of the pre- and post-infection activity of the fungicide used and the time of fungal infection relative to the time of fungicide application [27]. One investigation found that through the use of side-spraying nozzles, there was a much larger ratio of the active agent of fungicides in the heads than in the flag leaves compared to vertical-spraying nozzles where the a.i. concentration was much higher in the leaves than in the heads [28]. For this reason, the active ingredients in the fungicide tested (tebuconazole and prothioconazole) should be classified as semi-systemic, as the nodes in the stem and the heads inhibit the transfer of the fungicide. Therefore, side spraying inevitably increases fungicide efficiency in the control of FHB, and we should find the best solution possible for its application [14].

Grain contamination with DON in durum wheat is highly problematic because the grain becomes unsuitable for human consumption [29]. In the current research, we expected the high occurrence of type B-trichothecene, specifically DON, as this mycotoxin is primarily produced by *F. graminearum* and *F. culmorum*, which were used for artificial inoculation. The significant decrease in DON content in comparison to the DON content in 110-01 nozzle treatment and the untreated control in 2022 indicated that the poor fungicide coverage of durum heads can increase the DON content in grain, which was contrary to our second hypothesis. All nozzles significantly improved the level of DON reduction compared to the control. In both trial years, it was noticeable that the level of DON reduction caused by the 110-04 nozzle was higher compared to that caused by the 110-01 nozzle. Our results indicated that very good and uniform fungicide coverage on durum heads can significantly decrease the DON content in grains compared to the very poor coverage of durum heads, which was in accordance with our first hypothesis. In particular, water rates below 100 L ha^{-1} should be avoided in conditions favourable for FHB and grain contamination with DON. Such low water volumes are only reasonable to use when there are resistant durum varieties planted in the field or in drier conditions with low temperatures during the heading stage.

In the current study, lower temperatures (average 13.5 °C) in May 2021 could have slowed down the FHB infection and consequently decreased the level of DON accumulation, although high humidity was present as a result of increased rainfall. The increase in temperatures during May 2022 favoured the increase in the frequency and intensity of FHB disease. For example, the perithecia of FHB only matures at 20 and 25 °C [30]. Also, it was reported that temperatures above 25 °C and moist periods longer than 24 h favour infection when both *F. culmorum* and *F. graminearum* are used [31]. It should be stated that in both years in our study, the DON concentration was above the DON limit of 1750 ppb for durum wheat [32]. This was due to artificial inoculation with *F. culmorum* and *F. graminearum* and the highly susceptible wheat durum variety used in the current research. In France, only 3.6% of analysed durum wheat samples exceeded DON content over 6000 ppb, while in 60.2% of samples with DON content, the content was below 500 ppb [29]. Furthermore, 38% variance in DON content was explained by year, 16% by the previous crop, and 43% was unexplained. The same author reported that in addition to the previous crop, tillage, and the susceptibility of the variety, the quality of fungicide treatment is an important factor in reducing DON content in durum wheat. DON contamination was reduced by 45% with two fungicide treatments during the stem elongation and heading stage and with the addition of a foliar N fertilizer in naturally infected durum wheat in north Italy [33]. In a three-year study in northern and central Italy, the average DON content in durum wheat was 240 ppm, which ranged from 119 to 9129 ppm [34]. The DON levels in 464 durum wheat samples from different Italian regions varied from <50 ppm to 16,000 ppm [35].

These were all DON contents from natural infections, while in our trial, artificial inoculation was performed, and much higher DON values were reached. The heads in the current research were sampled manually before harvesting so that all shrivelled grains were gathered, which is not the case in combine harvesting, where small grains could be blown out by air in the combine cleaning system and DON values could potentially be decreased, as shown in a previous investigation [14]. In natural conditions of FHB disease, such high DON values as those in 2022 could occur. Increased levels of DON contamination in organic durum wheat were found in samples from Tunisia, with the DON content ranging between 12,800 and 30,500 ppb [36]. In Argentina, the values of DON contamination ranged from <50 to 15,141 ppb [37], and in Canada, they ranged from <50 to 4700 ppb in conventional durum wheat in 2010 [38]. However, some investigations reported that a high sowing rate (400 seeds/m^2) decreased the DON content from 13,200 to 9800 ppb in high disease conditions and from 1800 to 1200 ppb in low disease conditions compared to a low sowing rate (75 seeds/m^2) in artificially inoculated spring durum wheat plants [39]. Also, the fact that the DON content in durum wheat is four times higher than that in winter wheat is very important [40].

With the best nozzle, the Avi Twin 110-04 nozzle, DON reduction reached 63% in 2021 and 36% in 2022. Field control efficacy, which is based on visual symptoms, reached over 80% with the 110-04 nozzle in our trial, while it was reported that it can reach 70–90% for the best fungicides [14]. Another study reported about 40.3% field efficacy for visual symptoms and 21.6% for DON reduction compared to control treatments [41]. Our results confirmed that with the best fungicides, DON reduction could reach 40–60%, as was reported previously [10]. The same author also showed that with head coverage of around 100%, DON reduction could reach 100%. Some investigations reported an 81% DON reduction rate with the best fungicide and a 31% reduction rate with the least effective fungicide in winter wheat [14]. Such a large reduction was not achieved in the current research. The higher coverage of wheat heads using fungicides decreased the DON content and FHB symptoms with the right timing of application [8]. The same authors also reported that the best application could reduce the DON content by about 50%, which is in accordance with the current research. Also, the breeding of FHB-resistant varieties of durum wheat was not successful due to the lack of effective sources of resistance in the cultivated gene pool [42]. For this reason, other factors such as head coverage using fungicides with appropriate nozzles should be optimized in the future.

In our opinion, a higher grain yield is related to the better coverage of heads and leaves with fungicides and lower DON content when using the 110-03 nozzle and especially the 110-04 nozzle. Many authors reported a reduction in DON accumulation in grain and increasing grain yield when triazole fungicides were applied, which was also confirmed in the current study [43–45]. The reason for the increased grain yield obtained using nozzles in 2022 compared to the grain yield in the previous investigation year could have been the late sowing date in November 2020, which worsened germination, and heavy rain during the winter months, which prevented better starting conditions for plant growth and development. Thus, lower plant density could have resulted in a decreased grain yield in 2021. Furthermore, over 500 mm less rainfall was recorded in 2022 (October 21–July 22) compared to the year before. Some reports have shown that a high sowing rate of spring durum wheat (400 seeds/m^2) increased the grain yield and decreased the DON content, compared to the low sowing rate (75 seeds/m^2) accompanied by artificial inoculation with *F. graminearum*, which was due to higher plant density and an increased number of durum heads/m^2 [39].

The infection of grains with FHB primarily decreased the 1000-kernel weight [40]. The decreased 1000-kernel weight observed in both trial years was expected because FHB development is often associated with kernel damage (shrivelling), directly affecting the 1000-kernel weight and test weight, which is in accordance with previous findings [44,46]. It was previously reported that a higher 1000-kernel weight was achieved with a low sowing rate in low disease conditions compared to a high sowing rate [39].

In both trial years, the untreated control treatment and the use of the 110-01 nozzle caused a significantly decreased test weight compared to the other three nozzles. As stated previously, due to shrivelled kernels, the test weight was lower. Some investigations showed that the test weight of durum wheat was higher in treatments with high sowing rates compared to low sowing rates, while low sowing rates increased tillering, resulting in nonuniform crop and variation in crop maturity [39]. This was valid in both low- and high-pressure conditions. These findings were comparable to other investigations [47–49].

Similar results were observed regarding the nonsignificant effect of fungicide application and foliar N application on the grain protein content [33]. In that study, the protein content was higher (14.4–18.6%) than in the current study due to higher amounts of nitrogen being used for fertilization. Some authors [50] reported increased protein content in infected grains with FHB in winter wheat, while in the current study, this was not the case. The protein levels were higher in the low sowing rate treatment, as fewer plants were competing for the nitrogen [39].

Similar to the protein content, no significant differences appeared between treatments in terms of sedimentation values in both trial years due to the relation between sedimentation values and the quality of proteins. Sedimentation value, an indicator of gluten strength, depends on wheat's protein composition and wet gluten content [51]. In previous research, the sedimentation value of durum wheat genotypes ranged from 26 to 44 mL, with an overall mean value of 35 mL, while in our study, it ranged from 48 to 71 mL. To make good-quality pasta products, durum wheat needs to have protein content greater than 13% and a sedimentation value above 40 mL [52], which were both achieved in the current research.

As the activity of amylolytic enzymes increases, the Hagberg falling number decreases. It was reported that fungal enzymes of α-amylase decompose starch in grain, which can decrease the Hagberg falling number [40]. In the current research, this was not the case, as the Hagberg falling number ranged from 309 to 356 s, which is a recommended range. Critical Hagberg falling number values range below 300–350 s, or even below 200–250, and usually occur because of sprout damage [53]. Wheat with a low Hagberg falling number due to high α-amylase activity causes substantial economic losses to growers and significant processing and storage problems [54].

5. Conclusions

Our research showed that the 110-04 symmetrical injector double flat fan nozzle with a water rate of 320 L ha^{-1} significantly increased the durum wheat head coverage, grain yield, 1000-kernel weight, and test weight and significantly reduced DON content compared to the 110-01 nozzle with a water rate of 80 L ha^{-1}. Also, the Avi Twin 110-02 nozzle at 3.0 bar and a forward speed of 6.0 km/h is prospective while a reduced distributed volume of 160 L ha^{-1} makes the treatment less expensive and more sustainable. We proved the relation between durum head coverage using fungicides and the analysed parameters. For more efficient FHB control and reduced DON contamination, in addition to varietal resistance, effective fungicides, and timing, another critical measure is the good coverage of durum heads using symmetrical double flat fan nozzles with sufficient water rates. Further research should continue in the direction of improving head coverage using new nozzle types and new application technology.

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