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Effect of Aviation Spray Adjuvant on Improving Control of *Fusarium* Head Blight and Reducing Mycotoxin Contamination in Wheat

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Abstract: *Fusarium* head blight (FHB) and its mycotoxin contamination are among the main factors affecting wheat yield and quality. There is an urgent need to develop an efficient strategy to prevent and control the FHB disease and reduce the mycotoxin level in the wheat product. As a triazolinthione fungicide, prothioconazole is an effective broad-spectrum fungicide to control various diseases of wheat by foliar spraying. However, prothioconazole has potential harm to the female reproductive system, and its metabolism prothioconazole-desthio has teratogenicity. Considering this point, the plant protection unmanned aerial vehicles (UAVs) are undoubtedly a suitable choice for the field application of prothioconazole. In this work, by spraying 30% prothioconazole dispersible oil suspensions, we report that aviation spray adjuvant of methylated vegetable oil influences the control effect of wheat head blight, wheat yield, prothioconazole residues, and mycotoxin deoxynivalenol (DON) content. Adding 1.0% aviation spray adjuvant to the spray solution can significantly increase the droplet density and deposition amount in different layers of wheat canopy. The wheat yield increased by 6.94% compared with the treatment areas without spray adjuvant. Meanwhile, the prothioconazole and DON mycotoxin were not detected in the wheat grains. Based on these results, we conclude that the addition of aviation spray adjuvant can also not only ensure the high control effect of prothioconazole on FHB in wheat and increase wheat yield, but also greatly reduce the content of DON mycotoxin and ensure the safety of wheat production. This study is expected to provide theoretical guidance and data support for applying spray adjuvants in the field of plant protection UAVs in modern intensive sustainable agriculture.

Keywords: aviation spray adjuvant; UAV; prothioconazole; *Fusarium* head blight; mycotoxin; pesticide residue

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple food crops globally [1], which greatly contributes to the food security. *Fusarium* head blight (FHB) is an important cereal disease that seriously threatens the safety of cereal production worldwide, resulting in reduced yields and a deterioration of grain quality because of the contamination by harmful mycotoxins [2,3]. FHB is the most important and devastative fungal disease affecting wheat cultivation [4]. In general epidemic years, it may cause a reduction in yield by 15–20%, and in extreme cases, even by 60% [5]. In addition to the yield loss, *Fusarium* can also produce potent mycotoxins, such as deoxynivalenol (DON), which has a significant impact on the immune function of humans and animals [6]. The threshold for DON in wheat grain destined for human consumption is set at 1.00 mg kg^{-1} by the

Chinese government [7]. Therefore, it is highly desirable to develop an efficient strategy to prevent and control the FHB disease and reduce the DON level in wheat products.

The application strategy to control wheat head blight is spraying control in the early stage of wheat blooming. Among various sprayers, unmanned aerial vehicles (UAVs), also called drones, have aroused considerable attention in precision pest management owing to their fascinating features, mainly including flexibility, high efficiency, and lower labor intensity [8]. No specific take-off and landing sites are required; multi-rotor UAVs can operate efficiently and flexibly in complex and variable terrains and respond quickly to crop disease and insect outbreaks [9]. In recent years, the rapid innovation and development of flight control, real-time high-precision positioning, obstacle avoidance, and terrain-following technologies have promoted the large-scale application of UAVs in the field of crop protection [10]. Considering the characteristics of large-scale continuous planting of wheat, UAVs are especially suitable for preventing and controlling FHB in wheat.

Chemical control is still the most promising and effective method to control FHB in wheat [11]. However, to obtain increased chemical control of FHB, the fungicide selection and the timing of fungicide application would be important factors, as well as application method and rate, and good coverage of the spike [12]. Due to the increased pathogen resistance to the applied fungicides (e.g., tebuconazole, metconazole, and pyraclostrobin) and unsatisfactory application method, the control of FHB in the field has not always been consistently effective [7]. Thus, it is important to explore the effects of new fungicides and their application methods for efficient FHB management. Recently, chitosan and chitosan nanoparticles have been developed to prevent the development of FHB disease, and good control effects were achieved [13–15]. However, commercial fungicides are still the priority for the widespread field application to control FHB in wheat. As a triazolinthione fungicide with a unique chemical structure, prothioconazole, developed by Bayer Crop Science, is an effective broad-spectrum fungicide to control various wheat diseases (e.g., FHB) and other crops by foliar spraying [16]. However, prothioconazole has potential harm to the female reproductive system, and its metabolism prothioconazole-desthio has teratogenicity [17], limiting its wide registration in China due to the potential health risk for operators. Considering this point, the plant protection UAVs are undoubtedly a very suitable choice for the field application of prothioconazole.

Every coin has two sides. Although UAVs have many intriguing advantages, they still have some problems, such as pesticide loss originating from droplet drift and bouncing from the target leaves, bringing about environmental risk. The elaborate selection and application of spray adjuvants are one of the most effective methods to reduce the droplet drift and improve the deposition efficiency as well as the control effect. Wang et al. found that the type of adjuvants can significantly decrease the droplet drift by widening the droplet spectra and reducing the percentage of fine droplets [18]. Meng et al. reported that the addition of spray adjuvant could improve the efficiency of UAVs spraying by reducing the required dosage of imidacloprid by 20% [19]. Xiao et al. found that using a vegetable oil adjuvant could significantly increase the droplet coverage rate and the defoliation rate of cotton leaves [20]. However, the effects of the application of aviation spray adjuvants on pesticide residues and the content of pathogenic bacteria toxic metabolites in crops have less been exploited.

In the present study, biocompatible vegetable oil was used as an aviation spray adjuvant, and 30% prothioconazole oil-based suspension concentrate (OD) was applied as a fungicide. The effect on the droplet deposition and distribution, the control effect of FHB in wheat, and the prothioconazole residues and DON mycotoxin content in wheat samples were investigated. The present experiments were performed to verify a research hypothesis that the mycotoxin contamination in wheat could be reduced while improving the control of FHB when aviation spray adjuvant was used. This study is expected to provide theoretical guidance and data support for applying spray adjuvants in the field of plant protection UAVs in modern intensive sustainable agriculture.

2. Materials and Methods

2.1. Reagents and Materials

Prothioconazole 30% OD was purchased from Anhui JiuYi Agriculture Co., Ltd. (Hefei, China). Aviation spray adjuvant (methylated vegetable oil, trade name of Beidatong) was kindly provided by Mingshun Agricultural Technology Co., Ltd. (Shijiazhuang, China). With the purity of 85%, Allura Red was obtained from Zhejiang Jigaode Pigment Technology Co., Ltd. (Zhejiang, China). Prothioconazole standards (98.5%) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Mycotoxin standard with a purity of 98.0% was obtained from Sigma Aldrich (St. Louis, MO, USA). Chromatographic-purity acetonitrile and methanol were acquired from Fisher Scientific (Pittsburgh, PA, USA). Water was purified using a Sartorius H_2O-AOV-50 (Sartorius Lab Instruments GmbH & Co. KG, Goettingen, Germany). All other chemicals and reagents were commercially available and used as received.

2.2. Instruments

The type of aviation platform used was the XAG P20 aircraft (XAG Technology Co., Ltd., Guangzhou, China), which is a quadcopter with centrifugal atomization nozzles and 10 kg load capacity. and GNSS RTK (global navigation satellite system, real-time kinetic) high-precision navigation. The spraying system uses four spinning disc rotary atomizers, one located under each rotor. Droplet size is altered by changing the disc rpm and/or flow rate. The type of ground mechanical equipment is a tractor-mounted boom sprayer (3WPZ-1000, Zhongnong Fengmao Plant Protection Machinery Co., Ltd., Beijing, China), which has 32 Teejet nozzles that operate at a typical pressure of 300 kPa. The spraying equipment is shown in Figure 1.

Figure 1. The UAV sprayer and tractor-mounted boom sprayer.

2.3. Plants and Diseases

The wheat cultivar used in this study is Huaimai 22, one of the China's most popular winter wheat cultivars, and the planting density is 678 plants m^{-2}. Plants were grown until harvest according to local standard agronomic practices. FHB caused by *F. asiaticum* is the main pathogen in the local wheat plant area throughout the growing season. The pesticides were applied in the flowering period of wheat, and the critical period for FHB control when the wheat plants were approximately 80 cm.

2.4. Field Trials

This field experiment was conducted on April 19, 2019, in Yingzhou District, Fuyang City, Anhui Province, China (115°35′46.99″ E, 32°54′50.21″ N), with the meteorological conditions of field temperature of 21–30 °C, wind speed of 1.29–1.93 m s^{-1}, and relative humidity of 48–62%. There are four treatments in this experiment. Each treatment consisted of three replicate plots. The area of each plot for UAV application was 14 m (width) × 100 m (length) = 1400 m^2, and the area of the tractor-mounted boom sprayer application was set as 32 m (width) × 50 m (length) = 1600 m^2. A 10 m buffer zone separated each plot. The area of the blank control treatment is arranged randomly, with each plot area of 10 m (width) × 10 m (length). Fungicide (prothioconazole 30% OD) and aviation spray adjuvant were applied at the dosage recommended by the regulation guidelines. A blank control plot was sprayed with water. The detailed working parameters of the UAV and the tractor-mounted boom sprayer and the dosage of pesticide and adjuvant for field control against FHB were set in Table 1. The spray droplet deposition on wheat canopy and the control effects of different pesticide treatments on FBH were examined.

The droplets collection sampling points were arranged to evaluate the deposition of spray droplets on the wheat canopy, as shown in Figure 2. Before spraying, three parallel droplet sampling belts are arranged at 10 m, 20 m, and 30 m from the starting spraying position. The sampling points are perpendicular to the direction of the walking direction of the spraying equipment and are in the middle of the spraying area. There are six sampling points in each sampling belt, and the interval between the two sampling points was 0.7 m. The sampling points were marked from left to right, the first sampling point was marked as 1, and the last one was marked as 6 (Figure 2a). A PVC tube, a similar height with the wheat plant, was inserted at the sampling point to simulate a wheat plant. The droplets collectors, including a Kromekote® card and a filter paper, were fixed on the PVC tube with a double-ended clip to collect droplets. The upper droplets collector is fixed on the same level position with the top of the wheat canopy, the middle-level droplets collector is fixed on the middle of the PVC tube, and the lower-level droplets collector is fixed on the PVC tube 10 cm higher than the ground (Figure 2b).

Table 1. The detailed working parameters and dosage of different treatments in the field experiment of control against *Fusarium* head blight (FHB) in wheat.

Treatment [a]	Spraying Equipment	Dosage [b] (mL ha^{-1})	Adjuvant [c] (mL ha^{-1})	Water (L ha^{-1})	Flight Speed (m s^{-1})	Height [d] (m)
T1	UAV	675	0	15	5	2
T2	UAV	675	150	15	5	2
T3	Boom sprayer	675	0	300	/	0.5
T4	UAV	0	0	15	5	2

[a] T1 is the abbreviation of treatment 1, and so on. [b] prothioconazole 30% OD; [c] aviation spray adjuvant (methylated vegetable oil); [d] the distance between the nozzle and the top of the wheat canopy; UAV: unmanned aerial vehicle.

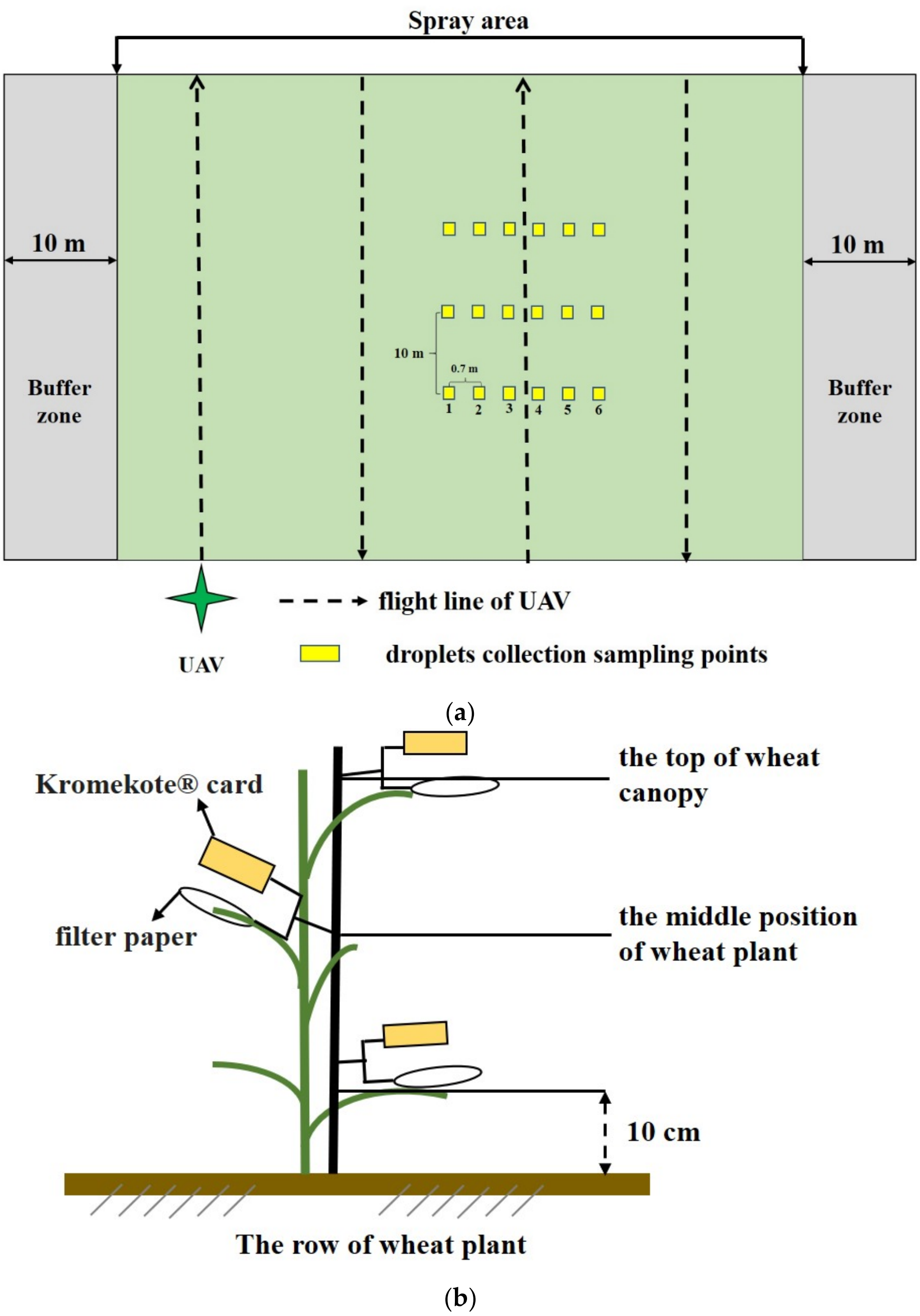

Figure 2. The droplets collection sampling points (**a**) and the droplets collectors (**b**) arrangement.

2.5. Determination of Droplets Deposition

Before application, Allura Red with the concentration of 20.0 g L^{-1} was added into the spray solution as the tracer. Allure red, a water-soluble food dye, was frequently used as a tracer for spray deposition assessment [21]. After spraying, waiting for the droplets on the Kromekote® cards and filter papers to dye, the Kromekote® cards and filter papers on different sampling points were collected in a zip-lock bag separately and taken to the laboratory for detection. Kromekote® cards were scanned at a resolution of 600 dpi with a scanner (Shanghai Zhongjing Technology Co., Ltd., Shanghai, China). Imagery software DropletScan (USDA, UAS) was used to analyze the droplet density and deposition [22,23].

Each filter paper placed in a separated zip-lock bag was washed with 5 mL of distilled water and shaken for 10 min. Afterward, the washing solution was filtered with a syringe with a 0.45 μm water-based filter membrane. The concentration of Allura Red in each extract solution was determined at 541 nm with a microplate reader (FlexStation 3, Molecular Devices Shanghai Ltd., Shanghai, China). The linear regression equation of the calibration curve of Allura Red was $y = 0.0191x + 0.0345$ ($R^2 = 0.9985$).

2.6. Control Efficacy

The disease incidence and severity of FHB disease and the control effect (%) against FHB were determined according to the method of Guidelines on efficacy evaluation of pesticides Part 15: Fungicides against *fusarium* head blight of wheat (NY/T 1464.15-2007), issued by the Institute for the Control of Agrochemicals, Ministry of Agriculture and Rural Affairs, China.

Disease incidence and severity in each plot were rated 8 days before wheat harvest using an FHB disease scale. One hundred spikes from each of five locations (four corners and the middle of the plot) were assessed for severity and incidence. FHB severity (the proportion of affected spikelets on infected spikes) was recorded visually on a scale of 0–7 representing the percentage of surfaces exhibiting visible symptoms, where 0 = 0% (no infection); 1 = 0–25%; 3 = 25–50%; 5 = 50–75%; 7 =75–100% (all spikes infected). The disease index and control efficacy for each treatment were calculated according to the Equations (1) and (2).

$$DI = \frac{\sum n_i \times DS_i}{n \times DS_{max}} \times 100 \tag{1}$$

$$CE = \frac{DI_0 - DI_n}{DI_0} \times 100 \tag{2}$$

where DI is the disease index, DS_i is the different disease scale, n_i is the total number of plants categorized in that scale, DS_{max} is the largest disease scale; CE is the control efficacy, DI_0 is the disease index of no fungicide treatment control, DI_n is the disease index of fungicide treatment.

2.7. Wheat Yield Assessment

Grain yields were determined 2 days before wheat harvest. There are five 1.0 m × 1.0 m areas selected in each plot. Then, 20 wheat ears were grabbed randomly from the roots in each area, and the grain numbers were counted. Harvest mature wheat plants from five different locations in each plot, and dry them in a ventilated room, and then thresh and collect the plants and the grains. The thousand-grain weight of each plot is determined with 20 times repeatedly, and then the theoretical wheat yield is calculated.

A total of 1 kg of grain samples were taken from well-mixed wheat grains of each plot for pesticide residues and mycotoxin determinations. The wheat plants were cut into small pieces and was triturated as a powder using a household blender, and homogenized for pesticide residue analysis.

2.8. Pesticide Residues Analysis

To determine the residual content of prothioconazole in the wheat plant, 2.0 g of the homogenized plant sample (5.0 g for crushed wheat kernels) was weighed into a 50-mL stoppered centrifuge tube, 5 mL of water and 10 mL of acetonitrile (containing 1% acetic acid) were added. After shaking for 30 min, 3.0 g of anhydrous sodium acetate was added immediately. The mixture was cooled in an ice-water bath, vortexed for 1 min, and centrifuged at 5000 r min^{-1} for 5 min. Then 1.5 mL of the supernatant was transferred to a 2-mL tube containing 50 mg of C_{18} packing and 150 mg of anhydrous magnesium sulfate, vortexed for 1 min, and centrifuged at 5000 r min^{-1} for 5 min. The supernatant extract was filtered through a 0.22-μm polyethersulfone membrane for ultra-performance liquid chromatography-tandem quadrupole mass spectrometry (UPLC-MS/MS) analysis.

The HPLC system comprised an Acquity UPLC-TQS LC/MS/MS system (Waters Corporation, Milford, MA USA) with an Acquity UPLC BEH C_{18} column (2.1 × 100 mm, 1.7 μm). The linear gradient elution using a binary gradient composed of water containing 0.2% (*v*/*v*) formic acid (A) and acetonitrile (B) was as follows: 10–90% B (0–1.5 min); 90% B (1.5–3.0 min); 90–10% B (3.0–3.1 min); and 10% B (3.1–5.0 min). The flow rate and injected volume were set to 0.3 mL min^{-1} and 5 μL, and the column temperature was kept at 30 °C. The mass spectrometer was equipped with an electrospray ionization ion source. Prothioconazole was detected in multiple reaction monitoring (MRM) mode using negative ionization, and the m/z values of parent and quantification ions were 342.2 and 125.0, respectively, and the collision potential was 40 eV. Prothioconazole-desthio was also detected in MRM mode but with positive ionization, and the m/z values of parent and quantification ions were 312.2 and 70.0, respectively, and the collision potential was 36 eV. Other source parameters were as follows: capillary voltage, 3.1 kV; ion source temperature, 150 °C; dry gas temperature, 200 °C; drying gas flow, 80 L min^{-1}.

2.9. Mycotoxin Analysis

To investigate the mycotoxin contamination in the wheat grain, each homogenized grain (5 g) was weighed into a 50-mL polypropylene centrifuge tube to which 10 mL of acetonitrile and 1 mL of water were added as extraction solvent. The tubes were then homogenized for 2 min at 20,627 × g before adding 1.0 g of NaCl and 2.0 g of $MgSO_4$ to separate the acetonitrile from the water. After centrifugation for 5 min at 4000 × g, 2.0 mL of the supernatant extract was filtered through a 0.22-μm polyethersulfone membrane for UPLC-MS/MS analysis.

The UPLC system comprised an Agilent 1290 Infinity/6495 LC/MS/MS system (Agilent, Quantum Analytics, Inc., Foster City, CA, USA) with an XDB-C_{18} column (2.1 × 100 mm, 1.7 μm). The mobile phase was water containing 2 mM ammonium formate with 0.1% formic acid (phase A) and acetonitrile with 0.1% formic acid (phase B). The mycotoxins were eluted following a gradient elution program with a flow rate held at 0.2 mL min^{-1} as follows: 0 min 90% B, 1.5 min 90% B, 3.5 min 65% B, 5.5 min 25% B, 7.0 min 90% B, and kept constant for 2 min for column re-equilibration; yielding a total chromatographic run of nine minutes. The sample injection volume was set at 10 μL. The column and autosampler were maintained at 25 °C. DON was detected in MRM mode using positive electrospray ionization. Other MS parameters were as follows: the de-solvation temperature at 300 °C, source temperature at 120 °C, the capillary voltage at 3.0 kV, de-solvation gas (nitrogen) at 600 L h^{-1}. The m/z values of parent and quantification ions were 297.2 and 248.9, respectively.

2.10. Statistical Analysis

Statistical analysis was performed using the Origin software (Origin Lab 2018, Northampton, MA, USA). One-way ANOVA was performed followed by Duncan's multiple range test to study the effect of aviation spray adjuvant on control effect against FHB in wheat and wheat yield (For Disease Index, $F = 21.835$, $DF = 3$, $p < 0.005$; for Efficacy, $F = 4.439$, $DF = 2$, $p > 0.05$; for Yield, $F = 1168.782$, $DF = 3$, $p < 0.005$).

The Shapiro-Wilk test was applied to evaluate the normality of the distribution of spray droplets. $p < 0.05$ was accepted as significant.

3. Results and Discussion

3.1. Effect of Aviation Spray Adjuvants on Droplet Deposition

Spray adjuvants greatly influence on the deposition characteristics of spray droplets [24]. Xiao et al. found that adding aviation spray adjuvant vegetable oil could significantly improve the defoliant droplet deposition on cotton leaves [20]. Wang et al. [25] and He et al. [26] found that reasonable adjuvant addition helps to improve the droplet density and deposition rate during the aerial spraying. When 0.1% pinolene spray adjuvant was added into the tebuconazole solution, the droplet deposition on leaves of *Photinia* × *fraseri* Dress. and *Carya cathayensis* Sarg. with UAVs spraying was obviously improved [27]. Table 2, Figures 3 and 4 display the effect of aviation spray adjuvant on droplet deposition characters, including droplets density and deposition amount of pesticide. The in-swath distribution patterns of spray droplets are similar with or without adjuvant added in the spray solution through drone spraying. The relationship between the distribution of spray droplets and the sampling location in one spray swath followed a normal distribution (DF = 6, $p > 0.05$) (Figures 3 and 4).

The droplet density of prothioconazole 30% OD with the spray adjuvant in the wheat canopy (65.23 ~ 242.30 droplets cm^{-2} for the upper layer, 22.10 ~ 74.90 droplets cm^{-2} for the middle layer, and 7.50 ~ 24.70 droplets cm^{-2} for the lower layer) was significantly higher than that of no adjuvant (51.97 ~ 172.40 droplets cm^{-2} for the upper layer, 18.30 ~ 70.70 droplets cm^{-2} for the middle layer and 3.90 ~ 15.90 droplets cm^{-2} for the lower layer) (Table 2 and Figure 3). The deposition amount of pesticide in the wheat canopy (1.23 ~ 2.93 μg cm^{-2} for the upper layer, 0.22 ~ 0.72 μg cm^{-2} for the middle layer, and 0.07 ~ 0.16 μg cm^{-2} for the lower layer) was mostly higher than that of no adjuvant added (1.05 ~ 3.02 μg cm^{-2} for the upper layer, 0.06 ~ 0.65 μg cm^{-2} for the middle layer, and 0.02 ~ 0.11 μg cm^{-2} for the lower layer) (Table 2 and Figure 4).

The spray droplets of spray liquid are both mainly deposited in the upper layer of the wheat canopy, and the droplet density in the upper layer of the wheat canopy was also significantly higher than that of in the middle layer and lower layer of the wheat canopy, whether the aviation spray adjuvant added or not (Table 2, Figures 3 and 4). However, with the addition of spray adjuvant to the spray liquid, compared with no spray adjuvant, the proportion of droplets deposition on the upper layer of the wheat canopy in the total deposition amount is reduced from 70.9% ~ 93.8% to 75.0% ~ 82.0%, the proportion of droplets deposition on the middle layer of the wheat canopy increased significantly, and, the proportion of droplets deposition on the lower layer of the wheat canopy increased slightly in-swath. Meanwhile, the RSD value of droplets density and deposition amount between different sample points were decreased with the adjuvant added (Table 2). These results suggest that the addition of aviation spray adjuvant during UAVs spraying can increase the droplet density and deposition amount in different layers of wheat canopy, increase the penetration performance of spray droplets, and improve spray uniformity in-swath.

Table 2. The effect of aviation spray adjuvant on droplet density and deposition in different layers of wheat canopy.

Wheat Canopy	Treatment [a]	Sample Points	Droplets Density (Droplets Number cm^{-2})		Deposition (μg cm^{-2})	
			Mean	RSD	Mean	RSD
Upper layer	Pro	1	51.97 ± 1.39	9.91	1.18 ± 0.18	43.09
		2	157.70 ± 1.55		1.47 ± 0.20	
		3	172.40 ± 3.90		3.02 ± 0.13	
		4	142.30 ± 3.48		1.60 ± 0.07	
		5	81.87 ± 0.67		1.55 ± 0.27	
		6	62.07 ± 1.59		1.05 ± 0.07	

	Pro + AD	1	77.93 ± 3.88	3.28	1.49 ± 0.14	30.98
		2	242.30 ± 3.96		2.33 ± 0.11	
		3	184.20 ± 4.84		2.93 ± 0.17	
		4	104.30 ± 3.41		2.69 ± 0.21	
		5	95.50 ± 3.73		2.48 ± 0.45	
		6	65.23 ± 3.55		1.23 ± 0.09	
Middle layer	Pro	1	21.27 ± 1.42	60.35	0.05 ± 0.03	79.34
		2	47.30 ± 0.46		0.19 ± 0.03	
		3	70.70 ± 5.84		0.65 ± 0.02	
		4	34.30 ± 0.26		0.55 ± 0.05	
		5	24.30 ± 2.71		0.45 ± 0.08	
		6	18.30 ± 0.85		0.06 ± 0.02	
	Pro + AD	1	27.00 ± 0.47	14.41	0.26 ± 0.04	41.12
		2	45.80 ± 2.87		0.51 ± 0.05	
		3	74.90 ± 1.42		0.56 ± 0.04	
		4	36.30 ± 5.33		0.72 ± 0.05	
		5	31.00 ± 8.11		0.63 ± 0.07	
		6	22.10 ± 1.15		0.22 ± 0.07	
Lower layer	Pro	1	7.80 ± 0.58	46.54	0.03 ± 0.01	63.59
		2	15.90 ± 2.86		0.05 ± 0.01	
		3	8.50 ± 1.08		0.07 ± 0.02	
		4	8.10 ± 1.00		0.11 ± 0.02	
		5	5.30 ± 0.15		0.03 ± 0.01	
		6	3.90 ± 0.31		0.02 ± 0.01	
	Pro + AD	1	14.80 ± 2.66	23.36	0.07 ± 0.02	43.86
		2	24.70 ± 3.27		0.08 ± 0.02	
		3	14.40 ± 1.04		0.16 ± 0.03	
		4	13.10 ± 2.63		0.18 ± 0.04	
		5	8.80 ± 0.45		0.12 ± 0.01	
		6	7.50 ± 0.49		0.08 ± 0.02	

[a] Pro: prothioconazole 30% OD only, working parameters and dosage of fungicide applied are shown in T1 (Table 1); Pro + AD: prothioconazole 30% OD and aviation spray adjuvant (methylated vegetable oil), working parameters and dosage of fungicide and adjuvant applied are shown in T2 (Table 1).

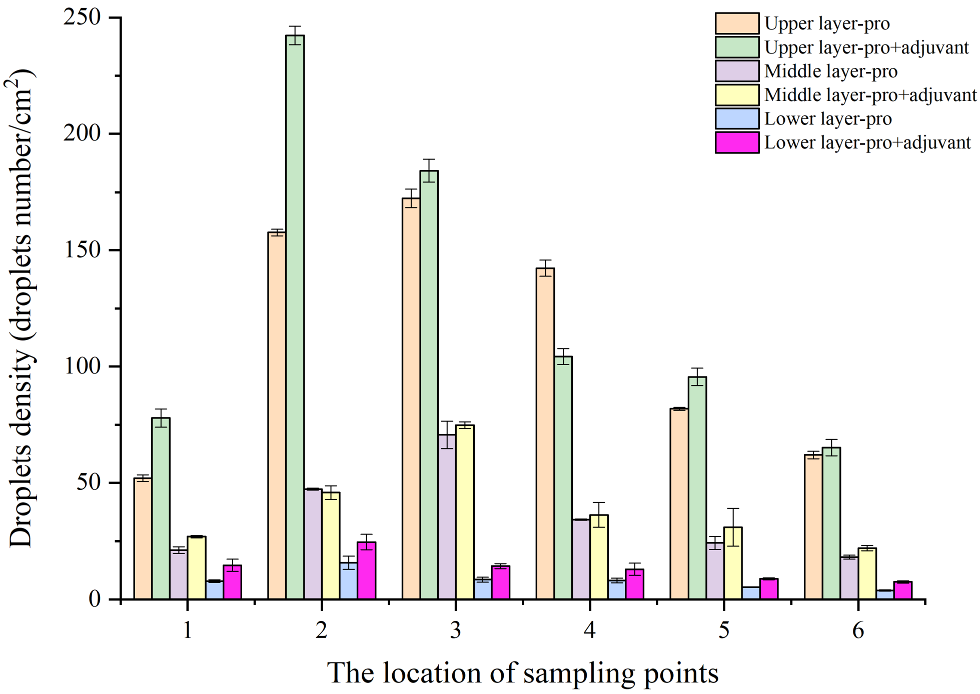

Figure 3. Effect of the aviation spray adjuvant on droplets density in the wheat canopy.

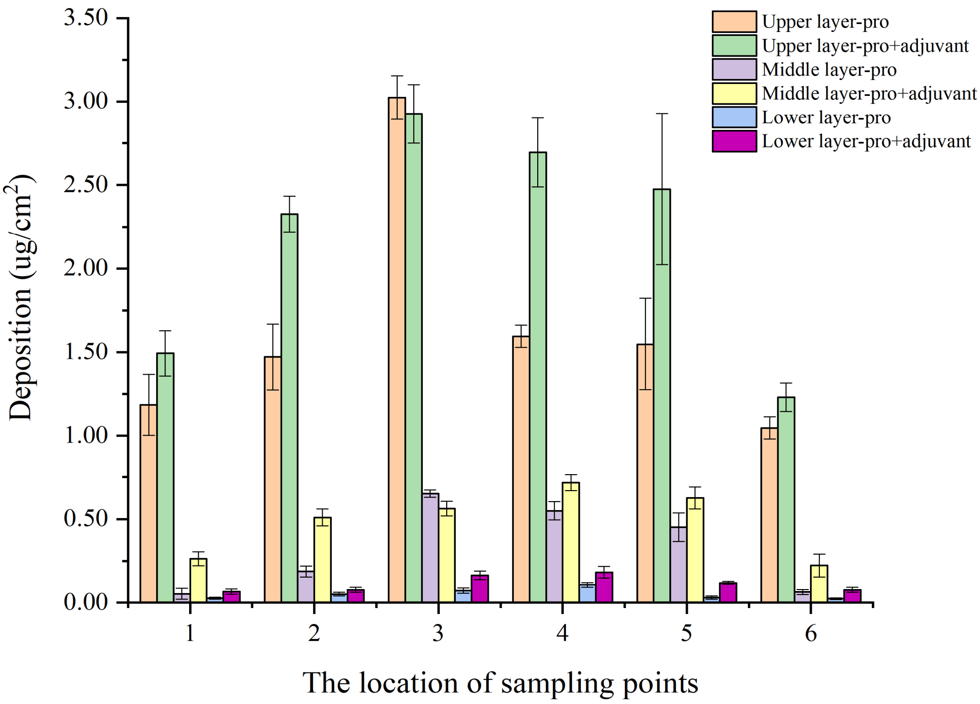

Figure 4. Effect of the aviation spray adjuvant on deposition in the wheat canopy.

3.2. Effect of Aviation Spray Adjuvants on FHB Control and Wheat Yield

As shown in Table 3, when the dosage was 15 L hm^{-2}, the application of prothioconazole 30% OD by UAV had a good control effect of 96.64% on FHB in wheat (T1). The disease index of 1.52 was lower than that of only water applied (T4). When aviation spray adjuvant of methylated vegetable oil was added, the disease index was reduced to 0.38 and the control effect increased to 99.16% (T2), demonstrating that spray adjuvant could improve the control efficiency against disease and pests. The results were consistent with the reported findings that the addition of aviation spray adjuvants could enhance the control effect against the target to some extent. The control efficiency of wheat aphid at wheat flowering and grain filling stage was improved by adding adjuvants methylated vegetable oil and organosilicone even with reduced insecticide imidacloprid dosage [19]. It has been reported that the defoliation rate of cotton leaves increased by 3.12–34.62%, and the boll opening rate increased by 6.67–29.56% after the addition of aviation spray adjuvant vegetable oil [20]. Zang et al. reported that when a hyperbranched polymer adjuvant was added in water-dispersible granules at a volume rate of 12 L hm^{-2} sprayed with UAV, the droplet deposition on maize leaves and maize borer control was increased by 104% and 46%, respectively [28]. The spraying application by boom sprayer had a comparable control effect with UAV spraying (T3).

In addition to improve the control efficiency with the addition of spray adjuvant, the effect on the wheat yield was also explored. As indicated in Table 3, the theoretical wheat yields under the chemical treatment with prothioconazole (T1, T2, and T3) were significantly higher than that of the blank control (T4). Indeed, control of FHB of wheat with appropriate fungicides can improve the wheat yield. Cromey et al. reported that FHB incidence was reduced by up to 90% and wheat yield increased by 14% after two applications of tebuconazole at a range of crop growth stages around flowering [29]. The addition of spray adjuvant had a significant effect on the thousand-grain weight and wheat yield but had no significant effect on the number of grains per spike. When 1.0% vegetable oil additives were added, the thousand-grain weight and theoretical yield of wheat reached the highest, which were 47.2 g and 9981.6 kg hm^{-2} (T2), which were higher than the treatment without spraying additives (T1). Similarly, the spraying application by boom sprayer had a comparable wheat yield with that of UAV spraying (T3).

Table 3. The effect of aviation spray adjuvant on control effect against FHB in wheat and wheat yield.

Treatment [a]	Disease Index	Control Effect (%)	Yield (kg hm^{-2})
T1	1.52 ± 0.72 b	96.64 ± 1.58 b	9334.1 ± 148.8 c
T2	0.38 ± 0.44 b	99.16 ± 0.96 a	9981.6 ± 114.9 a
T3	0.48 ± 0.33 b	98.94 ± 0.72 a	9644.4 ± 117.7 b
T4	45.41 ± 16.52 a	/	7832.6 ± 111.1 d

[a] The working parameters and dosage of fungicide and adjuvant applied of each treatment are shown in T1–T4 (Table 1). Different letters in the same column are significantly different at $p < 0.05$.

3.3. Effect of Aviation Spray Adjuvants on Prothioconazole Residue and DON Content

Prothioconazole is easily metabolized in plants and converted to prothioconazole-desthio [30]. According to the National Food Safety Standard-Maximum Residue Limits for Pesticides in Food (GB 2763-2021), the maximum residue limit (MRL) value of prothioconazole in wheat is identified with the concentration of prothioconazole-desthio, which is 0.1 mg kg^{-1}. It's reported that the application method and formulation could affect the prothioconazole degradation and residue in wheat [7]. This study determined the effect of aviation spray adjuvant on the prothioconazole residue in wheat (Table 4). The residual amount of prothioconazole-desthio in wheat samples treated without spray adjuvant was 0.12 mg kg^{-1} (T1). After adding spray additives, the residual amount of prothioconazole-desthio in wheat samples was 0.13 mg kg^{-1} (T2), which was a little higher than that without adjuvant. This phenomenon is easy to understand. As described above, the addition of spray adjuvant vegetable oil could enhance the deposition of spray droplets on the target plant. Meng et al. also found that when organosilicone adjuvant was added for UAV spraying, the initial imidacloprid residues on wheat leaves and heads after 2 h were higher than those of spraying without adjuvant [19]. The prothioconazole-desthio in wheat plant samples sprayed by UAV was less than that with boom sprayer (0.22 mg kg^{-1}) (T4). Importantly, there were no prothioconazole-desthio detected in wheat grain with or without the addition of spray adjuvant vegetable oil, indicating the food safety of wheat production with the application of prothioconazole.

Deoxynivalenol, also called vomitoxin, is one of the most common mycotoxins produced by *Fusarium* species, prevalent worldwide in crops used for food and feed production [31]. All over the world, the economic losses caused by DON through contaminating crops are in the range of billions of dollars every year [32]. DON affects animal and human health, causing acute temporary nausea, vomiting, abdominal pain, dizziness, diarrhea, headache, and fever [33]. Because of DON's toxicity, MRLs for DON in food and feed have been established to protect consumers. Due to the origin of DON from FHB, the effective control of FHB can eliminate the content of DON. As shown in Table 4, the DON in wheat grain without any treatment was 0.30 mg kg^{-1} (T4). When UAV used prothioconazole, the value was decreased to 0.18 mg kg^{-1} (T1), far below the established threshold for DON in a wheat grain of 1.00 mg kg^{-1}. However, when spray adjuvant vegetable oil was applied, the DON was not detected in wheat grain (T2). The decreased tendency was consistent with the increased FHB control effect with the addition of spray adjuvant. Váňová et al. reported that the addition of Silwet L-77 adjuvant with azole fungicides could reduce the mycotoxins content in grain and malt of spring barley [34]. The present result demonstrated that the reasonable utilization of aviation spray adjuvant could provide a useful strategy to enhance the control effect against FHB and decrease the mycotoxins in wheat grain.

Table 4. The effect of aviation spray adjuvant on residues of prothioconazole-desthio and deoxynivalenol (DON) in wheat plant and grain.

Treatment [a]	Prothioconazole-Desthio in the Wheat Plant (mg kg^{-1})	Prothioconazole-Desthio in Wheat Grain (mg kg^{-1})	DON in Wheat Grain (mg kg^{-1})
T1	0.12	ND	0.18
T2	0.13	ND	ND
T3	0.22	ND	0.13
T4	—	—	0.30

[a] The working parameters and dosage of fungicide and adjuvant applied of each treatment are shown in T1–T4 (Table 1). The detection limits for prothioconazole-desthio and DON were 0.01 and 0.02 mg kg^{-1}, respectively. ND: not detected.

4. Conclusions

In the present study, biocompatible methylated vegetable oil was used as an aviation spray adjuvant and 30% prothioconazole OD was applied as a fungicide to control FHB in wheat. The effect of aviation spray adjuvant on the droplet deposition and distribution, the control effect of FHB in wheat as well as the prothioconazole residues and DON mycotoxin content in wheat samples were explored, which was to verify a research hypothesis that the mycotoxin contamination in wheat could be reduced while improving the control of FHB when aviation spray adjuvant was used. Based on the investigation, the addition of aviation spray adjuvant methylated vegetable oil during UAV spraying has the following advantages: (1) increase the droplet density and deposition amount in different layers of the wheat canopy; (2) enhance the penetration performance of spray droplets; (3) ensure the high control effect of prothioconazole on FHB in wheat and increase wheat yield; (4) reduce the content of DON mycotoxin and ensure the safety of wheat production. However, this is just one case-by-case study. Further research should be conducted to confirm whether different types of aviation spray adjuvants can generally decrease the mycotoxin contamination in wheat. Even so, this study gives valuable information on disease control, and is expected to provide theoretical guidance and data support for the application of spray adjuvants in the field of plant protection UAVs in modern intensive sustainable agriculture.

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