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Estimation of film antitranspirant spray coverage on rapeseed (*Brassica napus* L.) leaves using titanium dioxide

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Abstract

Film antitranspirant (AT) forms a physical layer to block stomata on the leaf surface and thus improve plant water status under drought. There is little understanding of how leaf coverage relates to the physiological mechanism, so a reliable method of evaluating AT spray deposition is needed. Titanium dioxide (TiO_2) is widely applied to heighten the whiteness, brightness and opacity of materials, which can be potentially used as an inert marker to visualize AT deposited on leaves. This study aimed to evaluate the effect of TiO_2 on the spray characteristics and coverage of film AT (a.i. di-1-*p*-menthene) on water-sensitive papers (WSP) and its subsequent use to explore the dose-response relationship between this AT and leaf coverage. Spray characteristics when applied through standard 110° flat fan nozzles were assessed using a droplet analyzer and coverage was measured using image analysis of deposition on water sensitive paper (WSP) and oilseed rape leaves. There was no significant difference observed with TiO_2 added to film AT and water in droplet size spectra. Spray coverage averaged 46.8% and 57.3% respectively when WSP were positioned at 70 cm and 50 cm below nozzles. Adding TiO_2 to AT solutions with different dose rates had no significant effects on WSP spray coverage at either nozzle height. Leaf coverage was positively correlated with the dose rates of AT at the distance of 50 cm from nozzles to the canopy. Overall, results suggest that TiO_2 did not affect droplet size spectra or deposition on WSP of the AT when applied through a flat fan nozzle.

Therefore, TiO_2 can be effective as a tool to estimate the leaf coverage of film AT on rapeseed for use in future physiological studies.

Keywords: oilseed rape; canola; volume median diameter; dose response

1. Introduction

To achieve the expected efficacy of any crop protection chemical, sufficient chemical deposited on the target area is necessary (Hill and Inaba, 1989). Spray coverage is widely accepted as the percentage of the target area covered by the spray, which can show the proportion of targeted area in contact with chemicals directly (Holownicki et al., 2002). As reviewed by Hilz and Vermeer (2013), the biological efficacy of chemicals as a function of impaction and retention, is affected by many factors such as droplet size and physical properties of liquids. The magnitude and uniformity of canopy deposition, as well as spray drift are dependent on a series of operation parameters (nozzle type and configuration, spray pressure, application volume rate, etc.), tank mix properties and so forth, which in turn influence the number, size and velocity of droplets, and thus determine final spreading behaviors of sprays (Ozkan et al., 2012).

Film antitranspirants (AT) are polymers, which are generally wax and plastic-based emulsions sprayed on the surface of leaves to create a waterproof layer to block stomata and thereby reduce water loss (Patil and De, 1976; Kettlewell, 2014). Studies have shown that the yield of droughted crops can be improved when sprayed with film AT at the most sensitive growth stage (Kettlewell, 2014; Abdullah et al., 2015), such as wheat (Weerasinghe et al., 2016) and rapeseed (Faralli et al., 2017). The physiological mechanism by which AT increases yields is not yet clear, and there is almost no published information to help understand the physiology of the optimum dose. Since the mode of action of film AT is by blocking stomata physically on the leaf surface, estimating the spray coverage is essential for understanding the dose-response relationship of film AT.

In practice, methods of estimating deposition of sprays are mainly categorized into two groups with their own limitation: dye tracers mixed with spray liquid that visualize the liquid; and sensitive papers or cards, which detect spray droplets with a color change (Jaeken et al., 2000). Dye tracers are commonly used to determine spray retention (i.e. total mass retained per leaf area or plant area) as they can provide clear contrast between spray deposits and the background (Nairn and Forster, 2019), such as fluorescence dyes like Rhodamine (Bueno et al., 2017). They are less useful, however, in determining the distribution of spray droplets on the target areas. Water-sensitive paper (WSP), which has been used for more than 30 years to assess spray qualities in agriculture, is another conventional method of visualizing and quantifying the distribution of deposited spray droplets because an aqueous droplet can leave a dark blue stain on WSP with a yellow surface (Salyani et al., 2013). Droplet spot analysis such as spray coverage and number of spots per unit area can subsequently be determined by image analysis techniques (Zhu et al., 2008). Fox et al. (2003) compared three methods of evaluating spot distributions on WSP. They found that the imaging system could provide consistent measurements of droplet size and spray coverage. Further relationship between stain diameter and coverage on WSP was addressed by Cerruto et al. (2019). With a high degree of coverage, the spread factor needs to be adjusted without considering overlapped stains.

Leaf coverage can be estimated with WSP and image analysis software (Owen-Smith et al., 2019), though deposition of spray on a leaf surface is likely to be different from that on WSP. Thus, adding an appropriate marker to the spray could be a useful tool to help estimate spray coverage directly from leaves, which helps to understand the relationship between the coverage and efficacy of chemicals. Wise et al. (2010) initially used kaolin as a suspended solid spray marker to study spray deposition from two types of sprayer on grapefruit clusters using image analysis. However, authors did not evaluate effects of additional solid marker on the spray characteristics of chemicals.

Titanium dioxide (TiO_2) is a white and inorganic pigment which has been applied to a wide range of products to heighten the whiteness, brightness, and opacity of materials including plastics, coatings, papers and so forth (Khataee and Kasiri, 2010). It is also stable and non-toxic, extracted from various naturally occurring ores (Chen and Mao, 2007). TiO_2 can be a potentially useful material to evaluate the deposit distribution of AT on the leaf surface, which without a marker would not be visible to the naked eyes/standard scanner. Generally, ideal markers need to be chemically bonded to the substance of interest as is done with many common biological markers such as Green Fluorescent Protein (Zimmer, 2002). The location and concentration of the substance of interest can then be exactly determined using the marker because the marker and substance of interest are exactly co-located. Chemically bonded markers are rarely available for spraying studies, and the alternative is to use a marker which is sufficiently similar in physical properties that it approximately co-locates with the active substance. Thus it is necessary to keep the concentration of marker proportional to the concentration of active substance in studies which vary the quantity of active substance applied (e.g.van Zyl et al., 2013; da Cunha et al., 2018). We adopted the same approach in our study, keeping the concentration of marker (i.e. TiO_2 in our study) proportional to the concentration of AT at different dose rates. However, little is yet known about applying TiO_2 as a marker to assess spray deposition on artificial targets or natural leaves.

Therefore, to validate TiO_2 as an inert marker for estimation of the spray coverage, we conducted an experiment (Expt 1) on the spray characteristics of one commercial film AT product (a.i di-1-*p*-menthene) and two experiments (Expt 1 and Expt 2) on spray coverage on WSP. Additionally, two experiments (Expt 3 and 4) investigated the dose-response relationship between this AT and leaf coverage on rapeseed natural leaves. The null hypotheses were:

1) TiO_2 had no effect on droplet size spectra and class size distribution in Expt 1;

2) TiO_2 had no effects on WSP coverage of film AT with different concentrations at 70 cm from nozzles to WSP in Expt 1 and 50 cm in Expt 2;

3) There is no difference in leaf coverage of film AT with additional TiO_2 at increasing dose rates sprayed on leaves of rapeseed in Expt 3 and Expt 4.

2. Material and methods

2.1 Design and application parameters for Expt 1 and Expt 2

Expt 1 and Expt 2 were conducted as randomized single factor designs with seven treatments in Expt 1 and ten treatments in Expt 2 on 4 December 2018 and 17 January 2019, respectively. There were three replicates for each. Water-sensitive papers (WSP, 26x76 mm, Teejet, USA) were used as artificial spray targets to assess spray coverage. WSP was positioned horizontally at a specific height below the nozzles (70 cm in Expt 1, 50 cm in Expt 2). Film antitranspirant Vapor Gard (a.i. di-1-*p* menthene 96%, Miller Chemicals and Fertilizer, Hanover, USA) and water as control were sprayed with the amounts of water-insoluble titanium dioxide (TiO_2, CI 77891, ReAgent, Cheshire, UK) as a spray marker shown in Table 1. The proportion of AT and TiO2 was 1:1 across all the AT-related treatments. A custom-built automatic pot sprayer with a pair of nozzles (Hypro Flat Fan 110–03, Retrofitparts, UK) was used at 0.2 MPa pressure and nominal 1 m s^{-1} forward speed (Fig. 1.). The volume of application in both experiments was nominal 200 L ha^{-1}. The actual application volume was estimated at 70 cm and 50 cm height below nozzles with ten replicates using filter paper in a Petri dish. There was no significant difference between the two heights (p = 0.342, data not shown), so the actual application volume was averaged over the two heights and was approximately 250 L ha^{-1}. WSP was allowed to dry in several minutes after spraying, followed by storage in sealable plastic bags separately for the image analysis. Both experiments were conducted in an enclosed chamber to reduce variation in air and droplet movement.

Fig. 1. The customized built-in pot sprayer inside an enclosed chamber with a pair of nozzles.

Table 1. Overview of treatment composition including the nominal and actual dose rates of film antitranspirant, and the corresponding amount of TiO_2 in four experiments. The volume of the sprayer tank used was 100 mL.

Expts	Treatments	Dose rates of AT (L ha^{-1})		Mixture in the tank		
		Nominal	Actual	TiO_2 (g)	AT (mL)	water (mL)
Expt 1	Water	0.0	0.0	0.0	0.0	100.0
	Water +1 g TiO_2	0.0	0.0	1.0	0.0	100.0
	Water + 2 g TiO_2	0.0	0.0	2.0	0.0	100.0
	1AT	1.0	1.3	0.0	0.5	99.5
	2AT	2.0	2.5	0.0	1.0	99.0
	1AT + 1 g TiO_2	1.0	1.3	1.0	0.5	99.5
	2AT + 2 g TiO_2	2.0	2.5	2.0	1.0	99.0
Expt 2	Water	0.0	0.0	0.0	0.0	100.0
	Water +1 g TiO_2	0.0	0.0	1.0	0.0	100.0
	Water + 2 g TiO_2	0.0	0.0	2.0	0.0	100.0

	Water + 3 g TiO_2	0.0	0.0	3.0	0.0	100.0
	1 AT	1.0	1.3	0.0	0.5	99.5
	2 AT	2.0	2.5	0.0	1.0	99.0
	3 AT	3.0	3.8	0.0	1.5	98.5
	1 AT + 1 g TiO_2	1.0	1.3	1.0	0.5	99.5
	2 AT + 2 g TiO_2	2.0	2.5	2.0	1.0	99.0
	3 AT + 3 g TiO_2	3.0	3.8	3.0	1.5	98.5
Expt 3	0.5 AT + 0.5 g TiO_2	0.5	0.6	0.5	0.3	99.7
	1 AT + 1 g TiO_2	1.0	1.3	1.0	0.5	99.5
	1.5 AT + 1.5 g TiO_2	1.5	1.9	1.5	0.8	99.2
	2 AT + 2 g TiO_2	2.0	2.5	2.0	1.0	99.0
Expt 4	0.5 AT + 0.5 g TiO_2	0.5	0.6	0.5	0.3	99.7
	1 AT + 1 g TiO_2	1.0	1.3	1.0	0.5	99.5
	2 AT + 2 g TiO_2	2.0	2.5	2.0	1.0	99.0

2.2 Spray coverage analysis in Expt 1 and Expt 2

In Expt 1 and Expt 2, water-sensitive papers stored in the sealable plastic bags were scanned by a TASKalfa 3252 ci scanner (Kyocera, UK) with high resolution (600 × 600 dpi) and files were saved as the color JPEG. The image analysis was processing in MATLAB (R2018a). Firstly, the whole area of each paper was extracted by cropping the scanned images, followed by the image segmentation in RGB color space. Next, segmented images were thresholded by defining the range of RGB values based on specific color image. Before that, at least ten points from blue dyes and yellow background papers, respectively, were selected to determine the range of RGB of the area of interest in each paper to eliminate the human errors. Accordingly, spray coverage was determined as the percentage of white pixels (blue dye area) relative to total pixels of corresponding specific WSP.

2.3 Droplet size analysis in Expt 1

In Expt 1, the Dropcounter (Fig. 1. in supplementary material) (Billericay Farm Services Ltd, Essex, UK) was placed 50 cm below the nozzles. The device is designed to use infrared light to measure the number and droplet size within an area of 0.7 cm^2 (Kateley et al., 2016). The volumetric droplet size spectra parameters for analysis were $D_{V0.1}$, $D_{V0.5}$, $D_{V0.9}$, relative span (RS) and uniformity of the spray distribution was determined by the coefficient of variation (CV) of $Dv_{0.5}$ (see more details in Ferguson et al., 2015). Additionally, the analysis of droplet class size distributions was carried out in 12 class sizes with a range from 30 μm to >200 μm, with the relative increment of 10 μm between class sizes (Cunha et al., 2012).

2.4 Leaf coverage analysis in Expt 3 and Expt 4

In Expt 3 and Expt 4, seeds of rapeseed (cv. Mirakel; NPZ-Lembke, Germany) were sown into seedling-planter trays filled with John Innes No. 2 compost (loam, peat, coarse sand and base fertilizer, John Innes Manufacturers Association, Reading, UK) on 26th April and 25th July 2019, respectively. Seedlings at the fourth true leaf stage were transplanted into 1 L pots (one plant per pot). Each pot contained ~500 g John Innes No. 2 compost at 22±1% volumetric water content measured with a soil moisture probe (ML2X theta probe; Delta-T-device, Cambridge, UK). Pots were arranged in the glasshouse in Harper Adams University with sodium vapour lamps supplemented (16 h-8 h light-dark photoperiod) and daily temperature on average was approximately 17 °C in Expt 3 and 21 °C in Expt 4 before treatments started.

Both experiments were conducted using a complete randomized block design and treatments are shown in Table 1. Each treatment was replicated three times in each experiment. AT was applied at the flowering stage (GS 6.0) (Lancashire et al., 1991) with/without TiO_2 on 12th July in Expt 3 and 14th November 2019 in Expt 4. The adaxial surface of leaves was sprayed with AT solutions uniformly using the same custom-built automatic pot sprayer (Flat Fan 110-03, 0.2 MPa, 1 m s^{-1} forward speed) at nominal 200 L ha^{-1} while the actual volume rate was about 250 L ha^{-1} as described above. The distance between nozzles and plant canopy was kept at ~50 cm. After spraying, the first fully expanded leaf and two leaves below were collected for leaf coverage analysis. In both experiments, we estimate that the distance from nozzles to the first fully expanded leaf was approximately 70-90 cm and the interval between two leaves was ~5cm.

In Exp 3 and Exp 4, leaves collected were scanned by the TASKalfa 3252ci Printer (Kyocera, UK) with high resolution (600 × 600 dpi). Files were saved as the color JPEG. Three representative parts from each leaf were selected ranging from 0.4 to 1.0 cm^2 to avoid the main and lateral veins as possible. Then, selected areas were saved as new color images for leaf coverage analysis in MATLAB (R2018a). The following procedures about image segmentation and thresholding were the same as spray coverage analysis in 2.2. Therefore, leaf coverage was calculated as the percentage of pixels of the white area of interest to the total number of pixels of the whole image (examples from Expt 3 also shown in Fig. 5). Data from three leaves and means were used for the statistical analysis.

2.5 Statistical analysis

All the data were checked for normality by examining residual plots and presented as means ± standard error of means (SEM). A one-way analysis of variance (ANOVA) was carried out to analyze differences among treatments in spray coverage and droplet size spectra in Expt 1 and Expt 2, based on Tukey's test at the level of $p = 0.05$. Residual plots after ANOVA were inspected and any data not showing approximate normality and equality of variance was reanalyzed with Friedman's non-parametric ANOVA. Droplet number in Expt 1 was analyzed in a contingency table using the Chi-square test at the level of $p = 0.05$. As position of leaves was not randomly allocated, repeated measures ANOVA was conducted on the leaf coverage from three leaves with the consideration of one leaf position as equivalent to the data from one measurement time in Expt 3 and 4. Means of three leaves of leaf coverage was then analyzed with polynomial regression in groups to test the dose-response relationship between AT and leaf coverage. All the data analysis was performed by GenStat 18th edition (VSN International, Hemel Hempstead, UK).

3 Results and discussion

3.1 Effects of TiO_2 on droplet size spectra and class size distribution

According to the ISO draft standard (ISO 25358, 2018), six spray quality boundaries are defined based on the combination of different nozzles and specific pressures for classification of droplet size spectra. Despite minor changes in $Dv_{0.1}$, $Dv_{0.5}$ and $Dv_{0.9}$ across treatments as shown in Table 2, spray quality of droplets was all classified as fine. We found that TiO_2 had no significant effects on the droplet size spectra of AT solutions or water control in Expt 1. In terms of spray distribution uniformity, CV values ranged from 3.43% to 16.22% while all treatments had similar values in relative span.

Table 2. Droplet size spectra with three replicates (n = 3) measured by the Dropcounter, relative span and ISO 25358 spray quality classification based on $Dv_{0.1}$, $Dv_{0.5}$ and $Dv_{0.9}$, and the output of one-way ANOVA (degrees of freedom (df) = 20) in Expt 1.

Treatments	$Dv_{0.1}$	$Dv_{0.9}$	$Dv_{0.5}$		Relative span	ISO classification
	µm	µm	µm	CV (%)		
Water	93.00	345.67	193.46	9.71	1.30	Fine
Water+ 1 g TiO_2	93.00	406.67	206.46	16.22	1.49	Fine
Water+ 2 g TiO_2	87.00	315.67	180.97	3.68	1.26	Fine
AT 1 L ha^{-1}	85.00	357.33	187.74	11.14	1.44	Fine

AT1 L ha^{-1} + 1 g TiO_2	105.00	441.67	212.65	8.98	1.57	Fine
AT 2 L ha^{-1}	93.00	355.00	193.62	3.43	1.35	Fine
AT 2 L ha^{-1} + 2 g TiO_2	111.67	357.33	203.07	11.65	1.20	Fine
ANOVA						
SEM	†	39.4	11.8	-	0.11	-
P-values	0.15	0.39	0.53	-	0.25	-

† $Dv_{0.1}$ was not normally distributed and analyzed using Friedman's test.

To evaluate the droplet class size distribution, droplets were grouped into 12 class sizes according to the diameter, ranging from 30 µm to >200 µm. Fig. 2 shows the profile of the droplet class size distribution of AT and AT+TiO_2. The diameter of most droplets was within the range of 30 µm-40 µm, accounting for 59%-64% of the total number of droplets measured, followed by the group of 40-50 µm droplets with the percentage of 9%-11%. According to the Chi-square test, it showed that there was no significant difference between these two groups of treatments ($p = 0.332$). Different chemical compounds with similar physical properties can produce similar spray characteristics including droplet size and droplet number (Butler Ellis et al., 1997). The advantage of TiO_2 would be that it can be visible in ordinary light, compared to fluorescein dyes that require special light to be visualized.

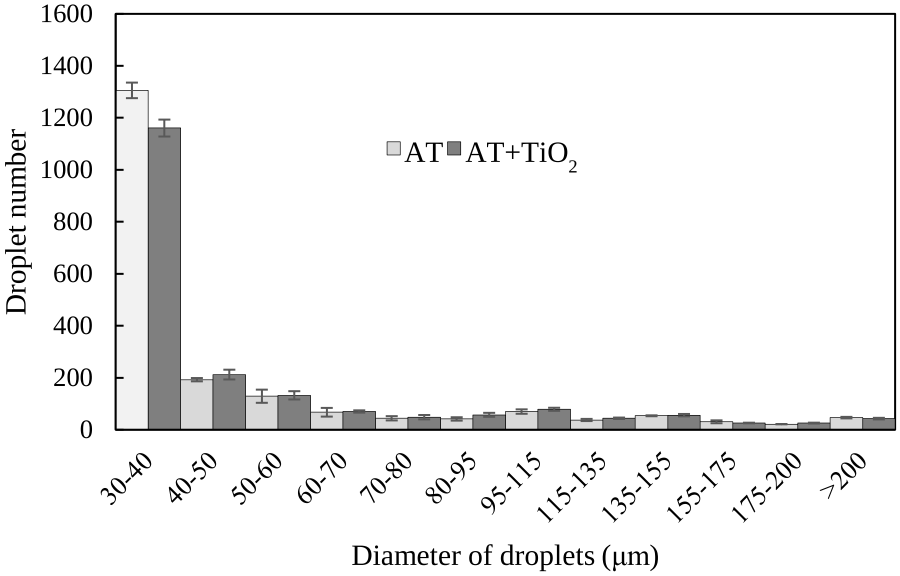

Fig. 2. Number of droplets for each class size diameter measured by the Dropcounter in Expt 1 (n = 3). Data are means of film antitranspirant with/without TiO_2 and error bars represent standard error of means.

Regarding the evidence above, the null hypothesis was accepted that there were no effects of TiO_2 on droplet size and spray distribution. It cannot be denied that differences in nozzle types and operation settings of measuring system result might result in considerable variation in spray droplet characteristics (Nuyttens et al., 2009). As Kateley et al. (2016) indicated that the Dropcounter was able to discriminate droplet size from different nozzles in a similar way to laser techniques.

3.2 Effects of TiO_2 on WSP spray coverage

We found that spray coverage on WSP was about 46.8% on average in Expt 1 as shown in Fig. 3. The overall ANOVA showed that all treatments were borderline significant (*p* = 0.048). Except for the treatment of water with 2 g TiO_2, observing that 31% difference between 1 L ha^{-1} and treatment of water with 2 g TiO_2; there were no significant differences among the remaining treatments on spray coverage. In Expt 2, spray coverage averaged about 57.3% (Fig. 4). Except for the treatment of water with 1 g TiO_2 and 2 g TiO_2, no significant differences in the coverage were observed between treatments (*p* = 0.018). Additionally, compared to water control, TiO_2 with 1 g or 2 g mixed with AT did not affect coverage significantly. These results indicate the null hypothesis should be accepted that TiO_2 had no significant effects on WSP spray coverage at different dose rates of AT.

Results from the present study showed a decrease in the height from nozzles to WSP (Expt 1: 70 cm; Expt 2: 50 cm) led to an increase of spray coverage by ~22% with the same application volume rate, suggesting that volume of spray deposited per unit area changed with the boom height. This is consistent with Ferguson et al. (2016), that the higher coverage on WSP was observed from the top card than the ground card when volume rate was consistent. Hanna et al. (2009) also found that fungicide deposition coverage on WSP reduced from the top to the middle and bottom by degrees.

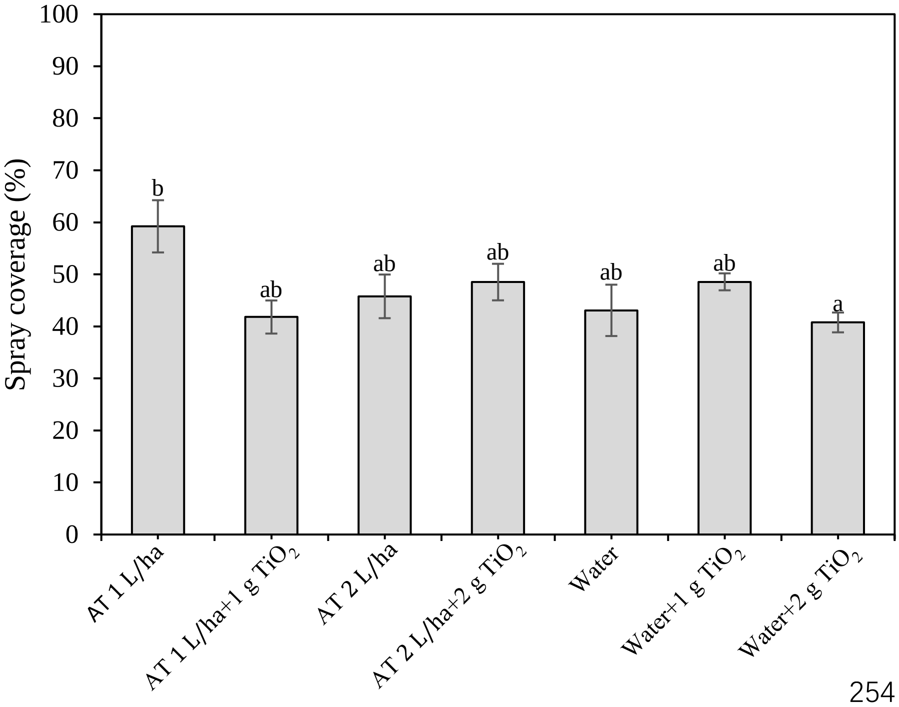

Fig. 3. Spray coverage using water sensitive papers with film antitranspirant (AT) application with/without TiO_2 in Expt 1. Data are means of replicates (n = 3) and error bars represent standard error of means. Treatments with the same letters are not significantly different according to Tukey's test at *p* = 0.05.

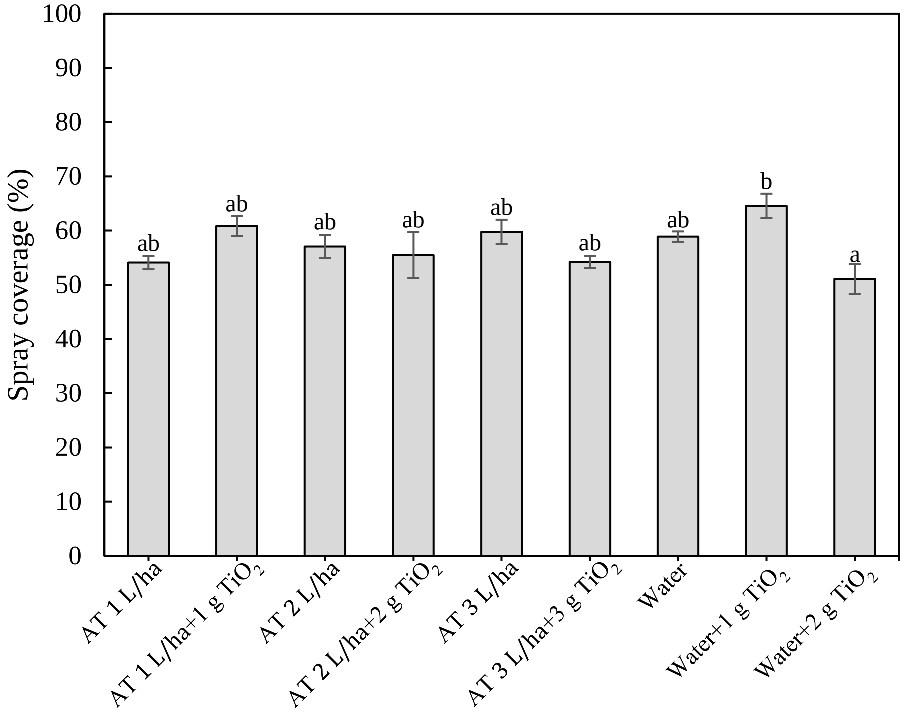

Fig. 4. Spray coverage using water sensitive papers with film antitranspirant (AT) application with/without TiO_2 in Expt 2. Data are means of replicates (n = 3) and error bars represent standard error of means. Treatments with the same letters are not significantly different according to Tukey's test at *p* = 0.05.

Considering the droplet size between AT at 1 L ha^{-1} and water with 2 g TiO_2 (Table 2), a great difference between them makes it difficult to explain from the present data, but it may be attributed to the variation from measurements between replicates (Berger-Neto et al., 2017). The result from water with 1 g TiO_2 and with 2 g TiO_2 observed significantly different in Expt 2 was not found in Expt 1, suggesting a chance occurrence. One possible reason for that can be that TiO_2 was not mixed up in the tank with water adequately before spraying for the 2 g treatment. On the other hand, fluctuations observed from different treatments can result from slight changes in spread factor, influencing the spot size on WSP (Fox et al., 2001). However, some variation from image processing software (i.e. Matlab in this study) using pixel recognition may affect results directly. Accuracy decreases along with the decreased spot size on WSP, and mistakes would be made when deposits on WSP are too dense, leading to plenty of overlapped deposits which cannot be discriminated by the program (Zhu et al., 2011). In Expt 1 and 2, spray coverage was nearly ~50%, at which plenty of overlapping deposits were observed by eyes or the imaging software system. The contrast between stains and background will be lost when the coverage is heavy (Panneton, 2002).

The two experiments aimed to explore the effects of TiO_2 on the droplets spray characteristics (spray quality in Expt 1 and spray coverage in Expt 1-2) of AT through two fixed nozzles. Those findings suggest that TiO_2 can be considered a viable and direct method to evaluate the coverage by the application of AT based on artificial targets (i.e. WSP). It is possible that when AT and TiO_2 are sprayed on leaves, that the TiO2 coverage is not a good estimate of AT coverage because the TiO2 physically separates from AT and is no longer exactly co-located on the leaf surface. We believe that there is sufficient evidence from the above droplet and WSP studies that this effect will be small and that TiO2 will also be a valid marker to estimate coverage of natural leaves by AT.

3.3 Relationship between film antitranspirants and leaf coverage

To explore the dose-response between leaf coverage and dose rates of AT, regression analysis in groups showed that both experiments could be displayed in parallel lines (Fig. 6). Leaf coverage was 14% and 9% on average in Expt 3 and 4 respectively. Despite the two experiments being conducted at different times, the data shows that there was significantly positive relationship between leaf coverage and dose rates of AT ($p < 0.001$, $R^2 = 0.99$). In Expt 3, the highest and lowest leaf coverages were observed with 2.0 L ha^{-1} AT (18.62%) and 0.5 L ha^{-1} AT (6.61%) respectively. In Expt 4, the highest and lowest value were 14.64% from 2.0 L ha^{-1} and only 2.12% from 0.5 L ha^{-1}.

Compared to WSP coverage averaging approximately 50% in Expt 1 and 2, leaf coverage showed a substantial decline averaging 14% and 9% in Expt 3 and 4 respectively. There can be three reasons for that, one of which is the roughness of the catching surface that can affect the efficiency of deposition on targets. Where the surface is rougher, the less easily droplets would bounce (Spillman, 1984). Secondly, there is a cuticle and waxes on the surface of leaves which are hydrophobic, while on the contrary WSP can absorb any aqueous droplet with enough water in it. As discussed above, droplets are expected to spread on WSP, but the spread factor on leaves is usually negligible because of these hydrophobic characteristics of the leaf surface. A third possible explanation could be the difference in contact angle. Without the effect from wind in an enclosed chamber within an automatic sprayer, the catch efficiency on horizontal surfaces is 100% if no bounce occurs because the only motion of one droplet is downwards due to sedimentation (Spillman, 1984). A difference in contact angle exists because WSP was positioned horizontally under the nozzles, but the angle between spray droplets and leaves depends on the leaf orientation which was not completely horizontal.

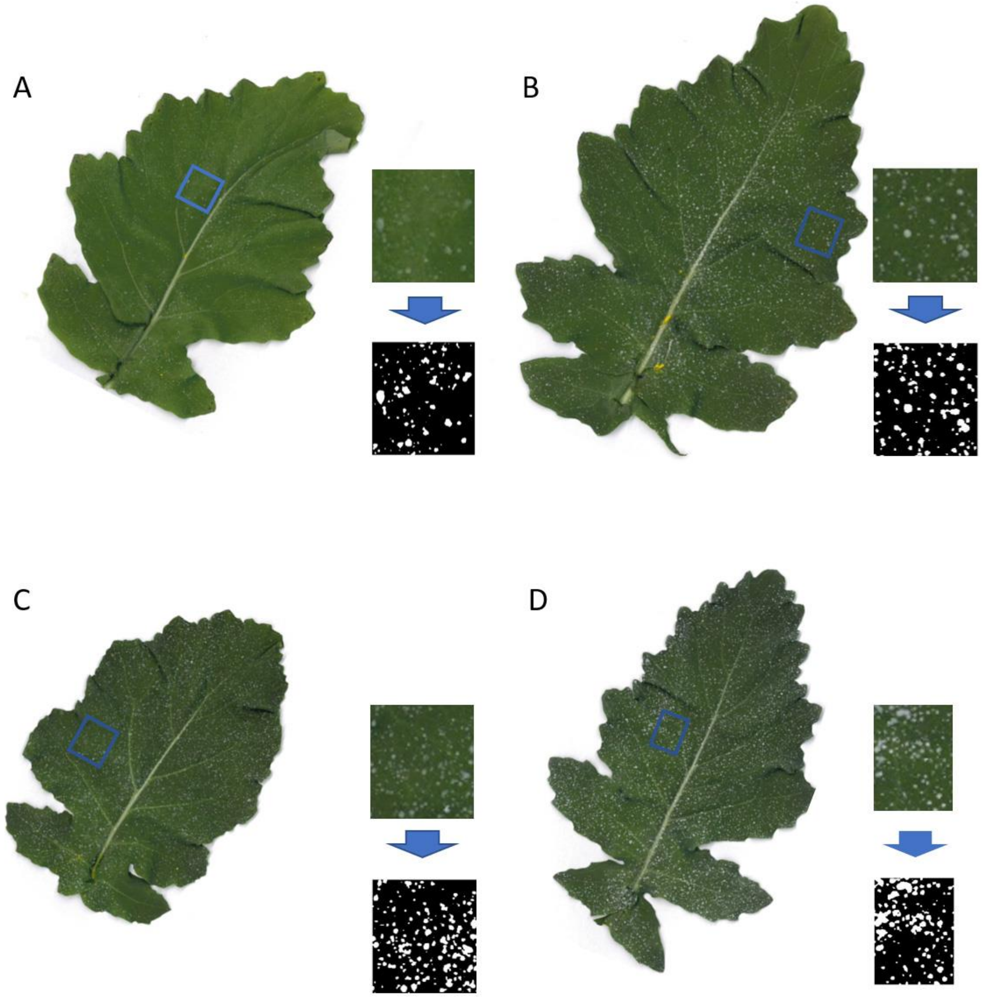

Fig. 5. Images of the scanned 1st leaf and corresponding representative selections after thresholding at 3x magnification when rapeseed was sprayed at nominal dose rates of 0.5 L ha^{-1} (A), 1.0 L ha^{-1} (B), 1.5 L ha^{-1} (C) and 2.0 L ha^{-1} (D) in Expt 3.

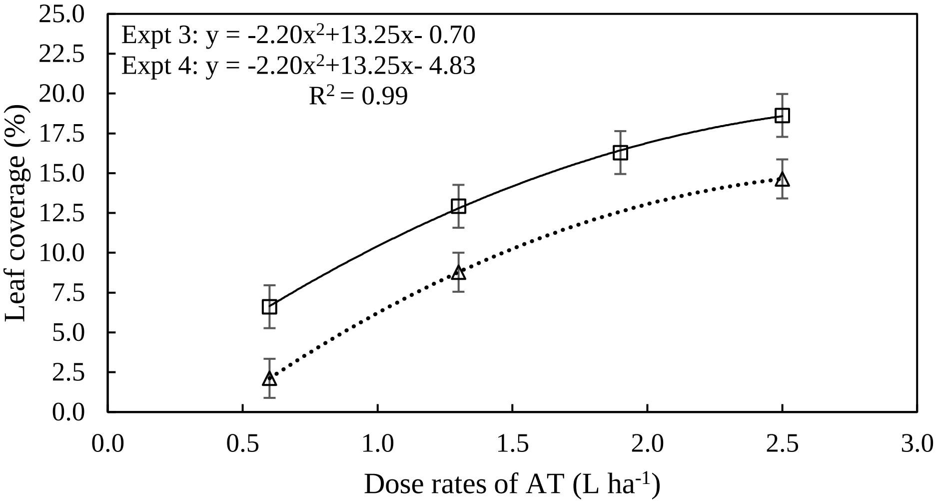

Fig. 6. Relationship between leaf coverage of film antitranspirant (AT) estimated from TiO_2 and actual dose rates of AT in Expt 3 (open squares) and Expt 4 (open triangles). Lines were fitted by using polynomial regression analysis in groups (solid and dashed for Expt 3 and 4 respectively). Data points are means and error bars represent standard error of means.

Our results showed that there was a positive relationship between AT and leaf coverage, albeit large differences between two experiments mainly resulted from the difference in the canopy characteristics which plays a role in the deposition of sprays on the plant (Duga et al., 2015). The thicker canopy with more leaf surface area might probably intercept more sprays and exhibit decreased spray penetration at high plant densities (Owen-Smith et al., 2019). Zhu et al., (2004) also found canopy penetration in dense peanut that spray deposits at the bottom of canopies tended to be linearly related to the leaf area index for all four nozzle types used in that research. In the present study with well-spaced plants, one possible contributor to the reduction that occurred in Expt 4 may have been the difference of leaf orientation due to different growing seasons. Leaf orientation resulted in the droplets flux per unit leaf area under constant operating conditions, and subsequently, affected the spray retention on the surface (Spillman, 1984).

As shown in Table 3, only AT had consistently significant effects on the leaf coverage in both Expts ($p < 0.001$ in Expt 3 and $p = 0.009$ in Expt 4), while no significant effects were observed from leaf position alone or interaction between AT and leaf position. It implies that differences in the volume of application for the different layers cannot account for the variability in coverage between leaves. This can also be confirmed by the observation during the research. Despite that, assessed leaves of interest were partially obstructed by the inflorescence and leaves above them, which can change the general route of flow liquid and thus affect the retention on the surface.

Table 3. Leaf coverage from three leaves of each treatment in Expt 3 and 4, and probability values from two-way *ANOVA* as affected by actual dose rates of AT and leaf position (LP).

Experiments	Dose rates of AT (L ha^{-1})		Leaf coverage at three leaf positions (%)		
	Nominal	Actual	1st	2nd	3rd
Expt 3	0.5	0.6	6.64	6.77	6.43
	1.0	1.3	13.61	15.05	11.37
	1.5	1.9	17.09	14.79	16.99
	2.0	2.5	18.28	20.09	17.50
Expt 4	0.5	0.6	1.96	2.04	1.87
	1.0	1.3	7.44	10.41	6.76
	2.0	2.5	10.68	12.03	13.07
		ANOVA			
P values					
Expt 3	AT			0.001	
	Leaf position (LP)			0.619	
	AT*LP			0.621	
Expt 4	AT			0.009	
	Leaf position (LP)			0.088	
	AT*LP			0.442	

Leaf coverage increased with an increased dose rate of AT at constant volume rate, indicating that deposition efficiency (i.e. leaf coverage) was highly related to the concentration of AT involved under the same spray operating conditions. This is in line with Herrington et al. (1981) that there was a positive relationship between the volume retained on the various component zones of apple tree like trunk, branches and shoots, and the volume of copper fungicide sprayed corresponding to the same level of application rates. van Zyl et al. (2013) also obsered an increase in the percentage covered by fluorescent pigments, i.e., deposition quantity, with increased concentrations of copper oxychloride on detached mandarin leaves. Changes in the coverage can mainly result from the mode-of-action of chemicals, which undoubtedly affect the performance on the target plants, as well as nozzle types and operation parameters (Wise et al., 2010).

Theoretically, the larger leaf coverage, the more stomata must be blocked by AT to reduce water loss. In terms of di-1-*p*-menthene, it is usually recommended to be applied in a spray at a concentration of 1%-2% depending on the specific plant species. In our study, four dose rates of AT at 0.5, 1.0, 1.5 and 2.0 L ha^{-1} were corresponding to four concentrations which were 0.25%, 0.5%, 0.75% and 1% respectively. We found that there was limited improvement in leaf coverage with increased AT when exceeding 1 L ha^{-1}. This is consistent with Fahey and Rogiers (2019). They showed that three levels of film AT (di-1-*p*-menthene at 1%, 2% and 3%) were applied to explore the effect on transpiration of grape. It showed that the optimum concentration of AT reducing the cuticular transpiration was dependent on the growth developmental stage, but there were only slight improvements by increasing the concentration above 1%. van Zyl et al. (2013) developed a model between coverage of fungicide and desease control based on detached mandarin leavs. It showed that disease control increased with an increase of fungicide concentration, but acompanied by the decline of the proportional contribution to disease control. It was predicted that 50% and 75% of disease control would be achieved 0.34 and 0.68 times of the registered concentration with corresponding leaf coverage of 2.07% and 4.14% respectively. This highlighted the importance of correct use of fungicide to varying degrees of disease to avoid over spray and reduce detrimental effects on the environment. The aforementioned findings indicate that the best performance can be achieved by selecting an appropriate concentration and corresponding type of sprayer, depending on the specific liquid with its unique mode of action. Therefore, further studies are ongoing to explore an optimal dose rate of AT with minimum level of biologically effective coverage while mitigating drought damage to an acceptable level on rapeseed in the glasshouse and field.

4. Conclusions

In this study, we demonstrated that TiO_2 did not have significant effects on the droplet size spectra with flat fan nozzles (110/03, 0.3 Mpa) at an application volume of 250 L ha^{-1}. With similar operating parameters, AT and AT with TiO_2 produced similar spray distribution. It suggests that TiO_2 can be considered as a valid marker to visualize AT on artificial targets (WSP) and natural leaves, for an estimation of coverage. Leaf coverage was positively correlated with an increase in the dose rate of AT when conducted in the glasshouse. It should be noted that leaf coverage assessed by the image analysis can be variable attributed from many factors such as the structure of plant canopy (e.g. curling of the leaf). Further investigation will be carried out in the field to evaluate the effect of AT on leaf coverage to relate to the physiological response of rapeseed to drought damage.

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CRediT author statement

Jie Xiang: Methodology, Formal analysis, Investigation, Writing – Original Draft, Writing - Review and Editing, Visualization, Funding acquisition **Martin Hare:** Conceptualization, Methodology, Writing - Review and Editing, Supervision **Laura Vickers:** Writing - Review and Editing, Supervision **Peter Kettlewell:** Conceptualization, Methodology, Formal analysis, Writing - Review and Editing, Supervision

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Supplementary data

Fig. 1. Measuring unit of the Dropcounter and the imaging system.