Increasing the concentration of film antitranspirant increases yields of rapeseed under terminal drought by improving plant water status

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Increasing the concentration of film antitranspirant increases yields of rapeseed under terminal drought by improving plant water status

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ABSTRACT

Film antitranspirant (AT) can effectively reduce yield losses of rapeseed crops under drought by blocking stomata if applied at the critical stage. However, the physiological mechanism by which film AT mitigates drought damage remains unclear. To investigate the effect of different concentrations of AT from 1% to 3% on rapeseed yields and its components of rapeseed under terminal drought, we carried out two field experiments at two locations in the year 2021, using rain shelters to simulate terminal drought at Bird's Nest (BN) and Flat Nook (FN). The study was conducted in a randomised complete block design with different concentrations of film AT (Vapor Gard, a.i., di-1-*p*-menthene) from 0% to 3% applied at the flowering stage of water-stressed rapeseed. Soil and plant water status, leaf gas exchange, seed yield and yield components, etc., were examined. Drought depressed leaf gas exchange and resulted in large yield losses. Aboveground biomass, seed yield, pod number and oil yield showed linear increases with AT concentrations consistently from both sites. With every 1% increase in concentration, seed yield was predicted to increase by 0.61 and 0.23 t ha^{-1} at BN and FN, respectively. The improvement in seed yield was strongly associated with pod number ($R^2 = 0.97$ and 0.76, respectively; $p < 0.001$). Further, pod number and leaf relative water content were positively correlated, albeit with differences between the two sites. It was concluded that increasing concentrations can enhance yield benefits of film AT on rapeseed subjected to drought, and the greater yield from film AT appeared to be mediated through the improvement in leaf water status. As high concentrations of film AT are less cost-effective and conventional spraying methods only cover the adaxial surface, improving leaf coverage considering both sides of the leaf surface would help lower the cost and extend the commercial use of film AT.

1. Introduction

Rapeseed (*Brassica napus* L.), one of the most important oilseed crops, plays a vital role in meeting global demands for edible oil, biofuels and fodder to keep up with the growing global population (Wu et al., 2018). Numerous research studies have revealed that agricultural drought greatly impacts rapeseed production. This detrimental effect will be amplified by the increased intensity and severity of drought from climate change (Pachauri et al., 2014). The flowering stage is commonly recognised as the critical period for rapeseed, when plants have the greatest number of newly opened flowers and near-open buds which are highly dependent on the assimilate availability for the development of ovules (Kirkegaard et al., 2018). Impaired assimilation supply caused by water stress reduces sink size, thus leading to yield losses (Weymann et al., 2015). Therefore, it is imperative to develop effective agronomy tools to mitigate drought damage in addition to breeding methods.

Film ATs (detailed abbreviations and definitions used in the paper are listed in Table 1) are water-emulsifiable polymers that form a physically waterproof layer to block stomata, thereby reducing water loss through transpiration (Kettlewell and Holloway, 2010). The reduction in stomatal conductance is expected to depress the photosynthesis that depends on the diffusion of CO_2 through stomata. So, it is more applicable to ornamental horticultural plants with more emphasis on reducing water loss than assimilation (Das and Raghavendra, 1979). However, film AT can give yield benefits on crops under drought if applied at the right time, i.e., most sensitive to water stress, which was first reported on wheat (Kettlewell et al., 2010). More robust evidence has helped understand the underlying physiological mechanism (reviewed by Mphande et al., 2020, and referenced therein), with increasing research on the timing effect of film AT on wheat and a wider variety of plants.

However, there are few articles about AT application on brassica

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Table 1
List of abbreviations used throughout the manuscript.

Abbreviation	Definition
A	Net photosynthesis rate
ABA	Abscisic acid
AGB	Aboveground biomass
AT(s)	Antitranspirant(s)
AWC	Available water content
BD	Bulk density
BN	Bird's Nest
DAP	Days after planting
DAS	Days after spraying (film antitranspirant)
DW	Dry weight
Endo-ABA	Endogenous abscisic acid
FC	Field capacity
FN	Flat Nook
FW	Fresh weight
GDD	Growing degree days
GS	Growth stage
g_s	Stomatal conductance
GWC	Gravimetric water content
L_T	Difference in leaf temperature between treatments and benchmark
PWP	Permanent wilting point
RH	Relative humidity
RWC	Relative water content
SMP	Soil matric potential
SR	Solar radiation
SWRC	Soil water retention curve
TSW	Thousand seed weight
TW	Turgid weight
VWC	Volumetric water content
WS	Water stressed
WUEi	Intrinsic water use efficiency
WW	Well-watered

crops. Patil and De (1978) sprayed film AT (Mobileaf, 10%) once at the early flowering stage of *Brassica campestris* under dryland conditions in addition to two other types of ATs. All AT applications resulted in yield benefits, among which film AT improved the seed yield by 26%, accompanied by greater leaf relative water content, compared to 0% AT. More recently, Faralli et al. (2016) applied film AT 1% (di-*p*-menthene) on winter rapeseed (*Brassica napus*) at the flowering stage under controlled drought, and they reported that AT improved seed dry weight by 17%, primarily by increasing pod number. Later, the same authors found similar improvement in winter rapeseed from the field conditions, and AT efficacy for yield benefits was highly related to the magnitude of water stress (Faralli et al., 2017a, 2017b). They used three concentrations, 0.5% (1 L ha^{-1}), 1% (2 L ha^{-1}) and 2% (4 L ha^{-1}), of di-*p*-menthene in one experiment and there was an indication that increasing concentration at the beginning of flowering increased yield, but this was not significant. Therefore, there is a need for further work to investigate rapeseed responses to film AT when applied at various concentrations under both controlled and field conditions.

We firstly investigated the response of spring rapeseed to different concentrations of film AT in the glasshouse (Xiang et al., 2022), showing significant physiological responses to AT concentrations in terms of gas exchange. However, the lack of consistent responses in yield and yield components strongly indicates the need for further work on AT with higher concentrations, and field studies under terminal drought are required in future research. Terminal drought is an important pattern of water supply dynamics and is well-known to occur more frequently in Mediterranean climates around the world and stored soil moisture systems of the semi-arid tropics (Berger et al., 2016).

Therefore, we conducted two field experiments with the aims of: i) investigating the responses of rapeseed under terminal drought to different concentrations of AT; and ii) understanding the physiological mechanism of AT-related yield improvement. The null hypothesis tested is that there is no significant difference in physiological responses and yield and its components of rapeseed treated with film antitranspirant with increasing concentrations from 1% to 3%. Essential physiological parameters are determined to estimate plant water status, gas exchange and endogenous ABA content in plant tissues in addition to yield and yield components.

2. Materials and methods

2.1. Experimental sites and agronomic management

Two field experiments were conducted at BN and FN, Harper Adams University (HAU) (52°46′N, 2°25′W) in 2021. The previous crop of both was wheat. Ten soil samples of the top 30 cm were collected randomly from both sites using a rotary corer drill prior to the start of trials, and they were mixed up in a sealable plastic bag to form one representative sample for each. The two soil samples were then stored at 4 °C and sent for physicochemical analysis in a private laboratory (NRM Coopers Bridge, Bracknell, UK). Results and methods used for analysis are shown in Table 2. The soil at BN and FN was ploughed and power-harrowed before sowing. To simulate terminal drought by keeping the rain out, one fixed polytunnel at BN covered all plots and the soil was cultivated inside the polytunnel, while four polythene shelters were installed at FN on fixed metal frames, one for each block, after the soil was cultivated. Two TinyTag loggers (Gemini Data Loggers, UK) monitored air temperature and RH at BN and two selected polytunnels at FN. Daily SR data were obtained from the meteorological station based at HAU, located within a one-kilometre distance from both research sites. The size of one plot was 1.5 m long (L) × 1 m wide (W) at both sites except for the benchmark plot (L:1 m × W: 1 m) at FN (see more details in Fig. A1).

Rapeseed seeds (*Brassica napus* L. var. Mirakel; NPZ-Lembke, Germany) were sown by hand in four rows with ~15 cm between rows and ~8 cm between plants within rows at 1–2 cm depth. Seedlings at the early leaf development stage were thinned to ~50 plants m^{-2}. The sowing dates of the two experiments are listed in Table 3, alongside AT application and harvesting dates. All treatments received light irrigations to ensure uniform seed germination and plant emergence. Following the establishment of seedlings, irrigation in experimental plots was withheld from ~1 month after planting (i.e., GS12/13), whereas benchmark plots were irrigated with three 1 m-dripper tapes (dripper spacing: 20 cm, output: 250 L h^{-1}, TSX 506 T-Tape, Access Irrigation Ltd, Northampton, UK) for 1 h (approximately 17 mm h^{-1}) on Mondays, Wednesdays, and Fridays at 9:00–10:00 am for optimal growth until harvest.

At BN, nitrogen fertiliser (ammonium nitrate, 34.5% N) at 100 kg N ha^{-1} was incorporated into the soil using a tractor-drawn plough on 02

Table 2
Soil physical and chemical characteristics (0–30 cm in depth) at Bird's Nest and Flat Nook. Note that chemical analysis was conducted on a dry matter basis.

Parameters	Bird's Nest	Flat Nook	Methods
Sand (% W/W)	62	73	Particle size analysis
Silt (% W/W)	20	14	
Clay (% W/W)	18	13	
Texture class	Sandy loam	Sandy loam	
Field capacity (0.03 MPa)	26	21	Pressure membrane apparatus on undisturbed soil samples
Permanent wilting point (1.5 Mpa)	13	8	
pH	7.3	7.3	[1:2.5] soil water suspension
Organic matter (%)	3.8	2.3	Loss on ignition
Mineral nitrogen (kg ha^{-1})	300	40	2 M Potassium Chloride extraction method
Available phosphorus (mg L^{-1})	86.6	61.4	Olsen's extractable method
Exchangeable potassium (mg L^{-1})	353	156	Ammonium nitrate extraction method
Exchangeable magnesium (mg L^{-1})	120	68	Ammonium nitrate extraction method

Table 3
Dates of planting, film antitranspirant (AT) application and harvest at Bird's Nest (BN) and Flat Nook (FN).

Sites	Planting	AT application				Harvest			
		GS[a]	Date	DAP[b]	GDD[c]	GS	Date	DAP	GDD
BN	19 March 2021	63	02 June 2021	75	644	89	02 August 2021	136	1669
FN	19 April 2021	62	15 June 2021	57	557	89	17 August 2021	120	1478

[a] GS: growth stage.

[b] DAP: days after planting.

[c] GDD: growing degree days at a base temperature of 5 °C (Wintermantel et al., 2020).

March 2021; fungicide (Propulse, a.i. carboxamide and triazolinthione, Bayer Crop Science Ltd, UK) at 1 L ha^{-1} applied on 20 May 2021. At FN, nitrogen fertiliser was applied at 100 kg ha^{-1} on 21 May 2021 and foliar multi-nutrient fertiliser (3X Solution, OMEX Agriculture Ltd, UK) at 5 L ha^{-1} on 01 July 2021. Due to an unexpected delay in receiving soil analysis results from the laboratory, the same amount of nitrogen fertiliser was applied to both sites to reduce soil variance and to ensure plant optimal growth. A mixture of insecticide (Hallmark Zeon, a.i. lambda-cyhalothrin, Syngenta UK Ltd, UK) at 75 mL ha^{-1} and fungicide – Propulse at 1 L ha^{-1} was applied on 02 July 2021. Weeding was manually done at both sites.

2.2. Experimental design

The study was conducted in a randomised complete block design with a single factor, including eight blocks at BN and four blocks at FN. Details of treatments in each block are summarised in Table 4. At BN, each block consisted of four drought plots; four well-watered benchmark plots were located on one side of the polytunnel to estimate the magnitude of drought. At FN, each rain shelter was treated as one block, and each block comprised six drought plots and two benchmark plots located at one end of each rain shelter. Note that benchmark plots of two experiments were not part of randomisation so they were not included in statistical analyses. The layout of drought and benchmark plots from both sites is presented in Fig. A1.

2.3. Spray application

Film AT (Vapor Gard, a.i. di-1-*p* enthane, Miller Chemical and Fertilizer Corp., Hanover, PA) was applied to the canopy at a 0.5 m distance from the nozzle at the early flowering stage. Di-1-*p*-menthene is a terpene polymer, also known as pinolene, produced by distillation from conifer resins. A range of concentrations of AT from 1% to 3% was applied, and unsprayed plots (i.e., 0AT) were treated with water only, using a hand-held boom sprayer (flat-fan 110/03, 2 bar, 200 L ha^{-1}, Lunch Box Sprayer, Trials Equipment (UK) Ltd, Essex, UK). Details of treatments are listed in Table 4. To prevent cross-contamination between treatments, solutions/water were prepared in individual tanks, and nozzles were brushed thoroughly using corresponding solutions/water prior to spraying on plants.

Table 4
Details of treatments sprayed with film antitranspirant (AT) within one block at Bird's Nest (BN) and Flat Nook (FN).

Sites	Treatments	Concentration of AT (%)	Dose rate of AT (L ha^{-1})	Sprayer tank	
				Volume of AT (mL)	Volume of water (mL)
BN	0AT (control)	-	-	-	5000
	1AT	1	2	50	4950
	3AT	3	6	150	4850
FN	0AT (control)	-	-	-	5000
	1AT	1	2	50	4950
	1.5AT	1.5	3	75	4925
	2AT	2	4	100	4900
	3AT	3	6	150	4850

2.4. Soil moisture dynamics

Access tubes were installed in the central part of representative plots within each block at both sites to monitor the dynamic soil moisture in both drought and benchmark plots as shown in Fig. A1. Soil VWC in the depths of 0–20 cm, 20–40 cm and 40–60 cm was measured by time domain reflectometry (TDR, TRIME-TDR, IMKO Micromodultechnik GmbH, Ettlingen, Germany) approximately twice a week throughout the growing season. The TDR probe was calibrated by the manufacturer before use. To compare the magnitude of drought between the two sites, soil VWC was converted to SMP according to the equations of corresponding SWRC.

The equations of SWRC for the two sites were determined on undisturbed soil using the pressure membrane apparatus (0700CG23F1 Manifold, Soil Moisture Equipment Corp., USA) (Bittelli and Flury, 2009). Low-pressure steps, including 0.01, 0.1, 0.5, and 1 bar, were applied with a 1-bar pressure plate, and 3, 5, 10, and 15 bars were used for high-pressure steps with a 5-bar pressure plate. Soil samples were collected at a depth of 0–30 and 30–60 cm using the steel corer (diameter: 5 cm, height: 5 cm), and then samples were shaped using a knife to fit the size of the ring (diameter: 5 cm, height: 1 cm) for the pressure application. A minimum of two days without any outflow were required for equilibration to be attained. Once the equilibrium was established, samples were removed from the pressure plates to measure GWC. There were three replicates for each depth and each pressure step. The porous plates were cleaned thoroughly with deionised water to prevent cross effects between pressures. BD (g/cm^3) was measured on sub-soil samples ($n = 3$) for SWRC at depths of 0–30 cm (mean = 1.47, SD = 0.05 at BN; mean = 1.45, SD = 0.8 at FN) and 30–60 cm (BN: mean = 1.66, SD = 0.05 at BN; mean = 1.52, SD = 0.03 at FN) respectively. Soil VWC was then calculated by multiplying GWC by the mean of the two depths of BN, followed by the linear model fitted with VWC against log transformed SMP to obtain the equation of SWRC (Fig. A2).

2.5. Thermal image collection and analysis

Thermal images were taken at 2 and 7 DAS using a FLIR T420bx camera (Model: FLIR-T62101, Teledyne FLIR, Kent, UK) with a ~0.5 m distance above the canopy at 40–45° during 11:00–13:00 (Table 5). A software called FLIR Tools was used for temperature analysis. Before collecting data, thermal images were standardised according to the air temperature and relative humidity obtained from the logger, and the distance (0.5 m). After calibration, the temperatures of five randomly selected leaves per plot were acquired, and the means of five recordings were recorded for each plot. L_T was used for data analysis, calculated as the mean of benchmark plots subtracted from the individual plot for WS treatments.

2.6. Gas exchange analysis

g_s (mol m^{-2} s^{-1}) and A (μmol m^{-2} s^{-1}) were determined by using an

Table 5
Summary dates of thermal images, gas exchange, leaf relative water content and leaf/pod sampling for endogenous ABA concentration (Endo-ABA) at Bird's Nest (BN) and Flat Nook (FN).

Sites	Thermal images			Gas exchange			Leaf relative water content			Sampling for Endo-ABA		
	Date	DAP[a]	DAS[b]	Date	DAP	DAS	Date	DAP	DAS	Date	DAP	DAS
BN	04 June 2021	77	2	05 June 2021	78	3	04 June 2021	77	2	09 June 2021_leaf	82	7
	09 June 2021	82	7	10 June 2021	83	8	16 June 2021	89	14	16 June 2021_leaf	89	14
										30 June 2021_pod	10	28
FN	17 June 2021	59	2	18 June 2021	60	3	17 June 2021	59	2	22 June 2021_leaf	64	7
	22 June 2021	64	7	23 June 2021	65	8	29 June 2021	71	14	29 June 2021_leaf	71	14
										13 July 2021_pod	85	28

[a] DAP: days after planting.
[b] DAS: days after spraying film antitranspirant.

infrared gas analyser- LC pro-SD (ADC BioScientific Ltd, UK) on the youngest fully expanded leaves (the 5th/6th leaf counting down from the top of canopy) during 10:00–13:00 at 3 DAS and 8 DAS (Table 5). The temperature of the leaf chamber (6.25 cm^2) was maintained at ~25 °C, and the photosynthetically active photon flux density was 1044 $\mu mol\ m^{-2}\ s^{-1}$, provided by an attached mixed red/blue LED array, with a flow rate of 300 $\mu mol\ s^{-1}$. The CO_2 concentration of inlet air through the leaf chamber was ~380 ppm. All the data were recorded when steady-state photosynthesis was achieved after 3–5 mins. The flow check was calibrated before the first sampling time for each of the two experiments to check if the cycle times were long enough for the gas through the analysis cell to become stable before taking readings. WUEi ($\mu mol\ (CO_2)\ mol\ (H_2O)^{-1}$) was calculated from A divided by g_s.

2.7. Endogenous ABA concentration and leaf relative water content

The first fully expanded leaf and one pod from the bottom of the terminal raceme of five plants per plot were collected for Endo-ABA assay. Leaves were sampled at 7 and 14 DAS, and pods at 28 DAS during 12:00–14:00 (Table 5). Samples from each plot were placed into individual 50 mL vials, and flash frozen in liquid nitrogen, then stored in a − 80 °C freezer for Endo-ABA assay. Frozen leaves and pods were freeze-dried for five days and further processed in accordance with the Cusabio ABA ELISA protocol, code CSB-E09159Pl (Cusabio Biotechnology Co., Ltd, Wuhan, Hubei Province 430206, China http://www.cusabio.com).

Leaves were collected for the analysis of RWC at 2 and 14 DAS at BN ($n = 8$) and FN ($n = 4$) (Table 5). Two leaf discs per leaf were collected from fully expanded leaves at 12.00–14:00, using a punch (diameter: 32 mm) and placed individually in a 50 mL tube. FW was determined using a four-decimal balance (Kern ABS120–4, Germany). Leaves were then soaked in the tube with distilled water and placed in a refrigerator at 4 °C for 24 h. Next, TW was recorded by carefully blotting the leaf discs on a paper towel before weighing them. DW was then determined by placing them in a labelled paper envelope and oven drying at 105 °C for 24 h. Leaf RWC was calculated according to Barrs and Weatherley (1962): $RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100$.

2.8. Yield and yield components analysis

At maturity, plants within 1 m × 1 m were harvested from each plot and plant population ha^{-1} was determined. Subsamples of 10 plants were randomly selected to determine pod number by hand, followed by oven drying at 105 °C for 48 h to determine aboveground biomass based on 0% moisture. Pod number per hectare and AGB ($t\ ha^{-1}$) were then calculated by dividing the number of subsampled plants (i.e., 10) by corresponding plant populations ha^{-1}. Seeds were obtained by threshing all pods manually, followed by oven drying at 60 °C for 72 h to determine seed yield ($t\ ha^{-1}$) at 0% moisture. Six seed subsamples (7–8 g) per plot were randomly sampled and weighed. Subsequently, seed number per subsample was determined by analysing pictures of seeds spread out on white paper, using "Analyse particles" programme in Image J software (https://ij.imjoy.io/). Individual seed weight was calculated as the mean of the six samples by dividing seed weight by seed number per sample. TSW was then calculated by multiplying individual seed weight by 1000. Seed number per pod (seed pod^{-1}) was calculated from seed number ha^{-1} (derived from seed yield and individual seed weight) divided by pod number per hectare, and the harvest index was calculated as the ratio of seed yield to AGB. Oil content (%) was determined using the Soxtec system (Soxtec 1043 fat extraction unit, Foss in Britain & Ireland, UK). Dried samples were prepared by milling ~10 g seeds per replicate using a coffee grinder. Duplicates (~0.5 g samples with 10 g sand and 40 mL petroleum) were performed for extraction procedures, including boiling for 10 mins, and rinsing for 20 mins. Oil yield ($t\ ha^{-1}$) was then calculated as the product of oil content and seed yield.

2.9. Statistical analysis

Data were analysed using Genstat 18th Edition (VSN International, Hemel Hempstead, UK). Shapiro-Wilk and Levene tests were used for estimating normality and homogeneity of variance prior to conducting an analysis of variance (*ANOVA)*. One-way *ANOVA* with polynomial contrasts was conducted to explore plant responses to AT in terms of leaf temperature, gas exchange, Endo-ABA in plant tissues, leaf RWC and yield-related parameters. *Post hoc* analyses were performed using Tukey's test at $p = 0.05$ to compare the difference between treatments. Simple linear regression analysis with treatments as groups using replicates was applied separately for two sites to test the relationship of gas exchange, AGB, seed yield, pod number and oil yield with AT concentrations. Combined data of yield and yield components were fitted with a linear regression model against leaf RWC using means.

3. Results

3.1. Environmental conditions

The mean daily air temperature at both sites was ~17 °C through the growing seasons, and the minimum and maximum RH were 36.1%, 95.5% and 43.4%, 97.0% at BN and FN, respectively (Fig. 1a–b). After spray application until harvest, the daily air temperature on average was 21.8 °C at BN, slightly higher than FN (19.6 °C). However, min- and max-RH were lower at BN than at FN, i.e., 36.8%, 95.6% and 43.0%, 97.1%, respectively. During the whole season, the average SR received was 16.3 and 16.7 $MJ\ m^{-2}\ day^{-1}$ at BN and FN, respectively; it was 17.7 and 16.1 $MJ\ m^{-2}\ day^{-1}$ after spraying (Fig. 1c). Compared to BN with early planting, rapeseed plants at FN started flowering earlier and had a shorter growing season by 11% in terms of thermal time (Table 3).

3.2. Soil water status

Average soil VWC (SMP) in benchmark plots was maintained at 27.5% (< 0.01 MPa) and 19.8% (−0.01 MPa) throughout the growing

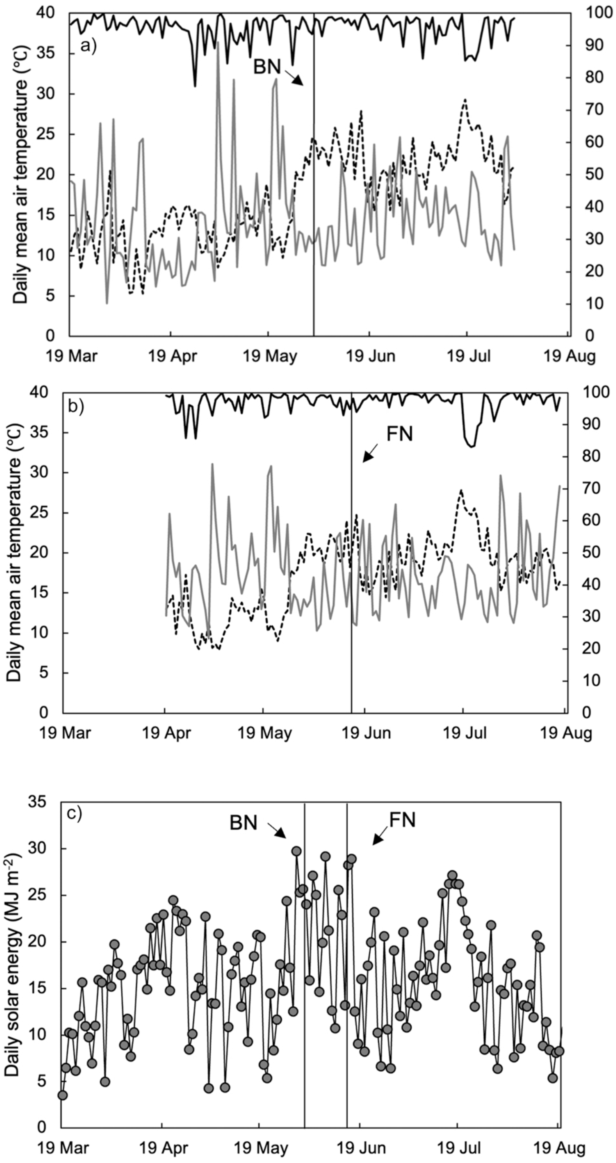

Fig. 1. Daily mean air temperature (°C, ▬ ▬), minimum (▬) and maximum (▬) relative humidity (%) during the growing season of rapeseed plants at Bird's Nest (BN; a) and Flat Nook (FN; b); and daily solar radiation (MJ m^{-2}, —●—) from both sites (c). Vertical lines represent the day of spraying film antitranspirant as indicated by arrows.

season at BN and FN, respectively. In contrast, soil moisture (VWC/SMP) in the top 60 cm significantly decreased with increasing DAP at BN and FN, showing no differences between AT-treated plots relative to 0AT from regression analysis in groups (data not shown). Overall, soil water deficits were greater at BN than at FN. At harvest, soil moisture (VWC/SMP) decreased to below PWP at BN, but it was not the case at FN, particularly in SMP (Fig. 2). At BN, soil VWC decreased from an average of 21.6% at 41 DAP to 14.8%, just above PWP, at 74 DAP (one day before spraying), and it further gradually declined to 12.6% at harvest (i.e., 136 DAP) (Fig. 2a). The corresponding readings of SMP were from − 0.04 MPa to − 0.77 MPa, and ultimately to − 2.08 MPa (Fig. 2b). At FN, soil VWC decreased from the average of 13.2% at 36 DAP to 12.2% at 56 DAP (one day before spraying), and then it continually reduced to 9.69% at 112 DAP near harvest, with corresponding SMP values of − 0.10, − 0.22 and − 0.67 MPa, respectively (Fig. 2c–d).

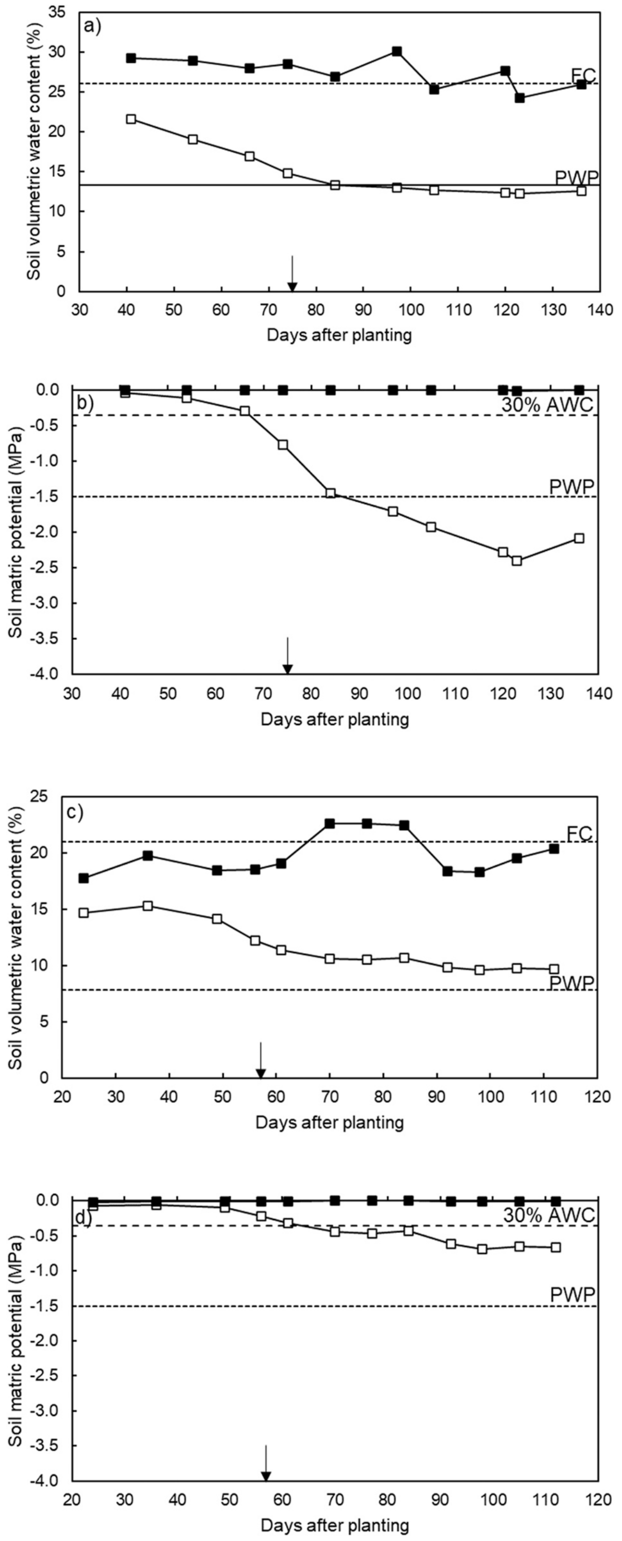

Fig. 2. Soil volumetric water content and matric potential in the top 60 cm from well-watered (WW) benchmark (—■—) and water-stressed plots (—□—) at Bird's Nest (BN; a–b) and Flat Nook (FN; c–d). Dashed lines represent field capacity (FC) and permanent wilting point (PWP) as indicated on the graph, and densely dashed lines represent the level of water stress, i.e., 30% available water content (AWC) imposed in the glasshouse study (Xiang et al., 2022). Arrows represent the day of spraying film antitranspirant. Data are means of replicates (WS: n = 8 at BN and n = 4 at FN; WW: n = 2 at BN and n = 4 at FN).

3.3. Leaf temperature

Leaf temperature fluctuated considerably depending on the weather conditions at sampling (Fig. 3). Relative to the well-watered benchmark, spray treatments increased leaf temperature significantly to varying extents at both sites, except for BN at 2 DAS (Table 6). L_T at 2 DAS in 0AT was 4.3 °C and 1.3 °C at BN and FN, respectively, while at 7 DAS, L_T was 1.5 °C and 1.4 °C (Fig. 3). AT had significant effects on L_T at three sampling dates at both sites, and a linear relationship was observed at FN, although deviations from FN at 7DAS were significant. When compared to 0AT, AT increased L_T by 67% and 35% at BN and FN, respectively, by averaging concentrations from the two sampling times.

3.4. Gas exchange

Drought inhibited g_s and A, and improved WUEi substantially relative to the benchmark at both sites (without statistical comparisons due to the non-randomisation of benchmark plots (Fig. 4). With the development of terminal drought, responses in gas exchange to AT application were more prominent at 8 DAS over 3 DAS across two trials (Table 6). At 8DAS in BN, there was a significant and linear relationship between g_s, WUEi and AT concentrations. Readings of g_s, A and WUE$_i$ at 8DAS were 0.3 mol m^{-2} s^{-1}, 16.5 μmol m^{-2} s^{-1} and 57.6 μmol (CO_2) mol (H_2O)$^{-1}$ in 0AT, respectively. When AT increased from 0% to 3% at 8 DAS, g_s decreased by an average of 16%, while WUE$_i$ increased by 12% (Fig. 4b, j). At FN, g_s, A and WUE$_i$ in 0AT were 0.7 mol m^{-2} s^{-1}, 19.8 μmol m^{-2} s^{-1} and 32.2 μmol (CO_2) mol (H_2O)$^{-1}$. Only A had a significant and linear relationship with AT concentrations (Table 6). AT reduced A by an average of 18% across all concentrations at FN (Fig. 4h).

3.5. Yield and yield components

At both sites, the application of AT significantly influenced AGB, seed yield and pod number per hectare, although with borderline significances in AGB and seed yield at BN (Table 7). Compared to the benchmark, drought decreased AGB and seed yield by an average of 43% and 37% at BN, respectively (Fig. 5a–b), and more markedly by 49% and 56% at FN (Fig. 6a–b), although non-randomised benchmark plots were not included in statistical analysis. AGB and seed yield in 0AT were 13.5, 5.6 t ha^{-1} and 5.6, 1.5 ha^{-1} at BN and FN, respectively. AT at 1%– 3% increased AGB and seed yield by an average of 12% and 14% at BN, respectively, and to a greater extent, by 31% and 34% at FN relative to 0AT (Table 7). Among all concentrations applied, 3% AT significantly increased AGB and seed yield at FN only, by 48% and 52% respectively.

Compared to the benchmark, terminal drought decreased pod number by an average of 38% and 34% at BN (Fig. 5d) and FN (Fig. 6d), respectively. Average AT concentrations increased pod number per hectare by 7% and 25% at BN and FN, respectively, compared to 0AT (i.e., 57 and 15.1 million ha^{-1} at BN and FN, respectively). 3% AT was significantly higher than 0AT, increased by ~42% from both sites (Table 7). The oil content from the benchmark was ~42% and ~47% at BN and FN, respectively. Compared to the benchmark, oil content from drought plots was slightly lower, decreased by 6% and 2% at BN and FN, respectively (Fig. 5 g, Fig. 6 g). Compared to 0AT, 3% AT increased oil yield by 31% and 45% at BN and FN, respectively, with the latter showing significance. Across all concentrations, AT application increased oil yield by 15% and 28% at BN and FN, respectively. However, there were no significant effects observed on HI or TSW (Table 7).

Seed yield at BN and FN was linearly associated with AT concentrations, and so were AGB, pod number and oil yield (Table 7). With a

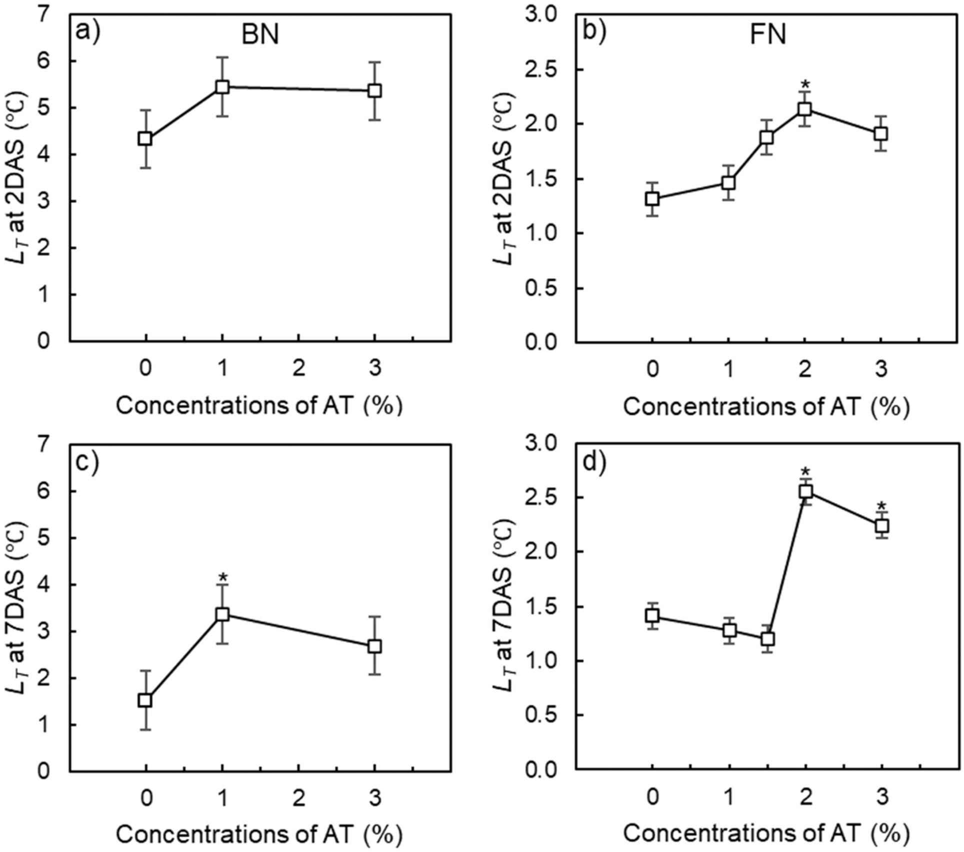

Fig. 3. Difference in leaf temperature between water-stressed and benchmark plots (L_T) at 2 and 7 days after spraying (DAS) film antitranspirant (AT) at Bird's Nest (BN; a–b) and Flat Nook (FN; c–d). Data are means (n = 8 at BN and n = 4 at FN) ± standard error of the mean. Asterisks (*) represent the significance compared to drought control (0AT) according to Tukey's test at $p = 0.05$.

Table 6

Probability values from *ANOVA* for L_T (leaf temperature of [treatments − benchmark]), stomatal conductance (g_s), photosynthesis rate (A) and intrinsic water use efficiency (WUEi) as affected by the application of film antitranspirant (AT) at Bird's Nest (BN) and Flat Nook (FN). Polynomial contrasts were conducted between concentrations of AT, including water-stressed plots treated with water (0AT). Bold numbers indicate significant differences at $p < 0.05$.

Sites	Factors	d.f.	L_T		Gas exchange					
			2 DAS	7 DAS	3 DAS			8 DAS		
					g_s	A	WUEi	g_s	A	WUEi
BN	Treatments	3	0.388	**0.036**	0.211	0.352	0.936	**0.040**	0.123	0.056
	Linear	1	0.332	0.190	0.086	0.192	0.757	**0.018**	0.099	**0.022**
	Deviations	1	0.330	**0.022**	0.825	0.550	0.861	0.331	0.205	0.473
FN	Treatments	1	**0.014**	**< .001**	0.934	0.856	0.842	0.995	**0.027**	0.159
	Linear	5	**0.004**	**< .001**	0.415	0.354	0.357	0.778	**0.005**	0.287
	Quadratic	1	0.149	0.101	0.941	0.866	0.805	0.755	0.081	0.275
	Deviations	1	0.158	**< .001**	0.961	0.851	0.825	0.995	0.679	0.104

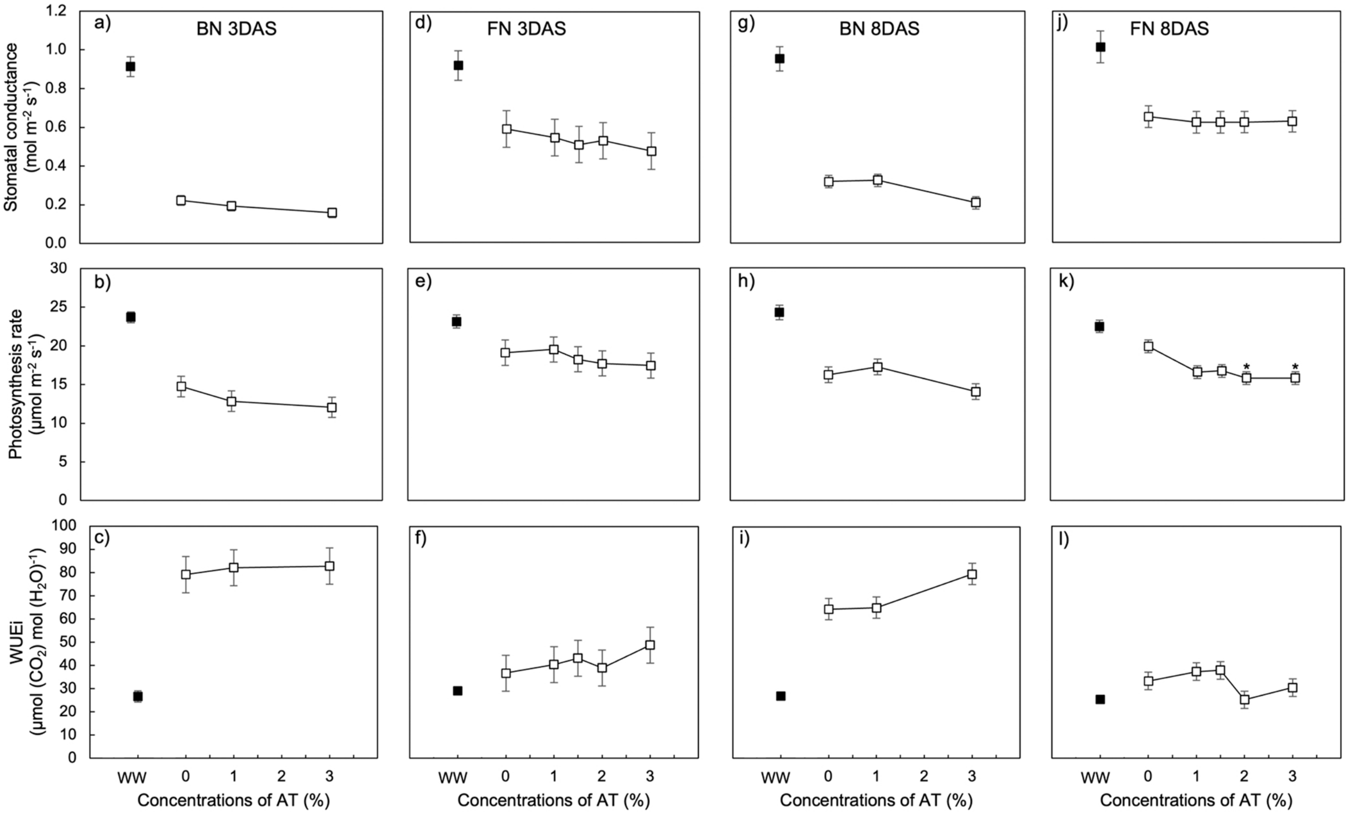

Fig. 4. Stomatal conductance, photosynthesis rate and intrinsic water use efficiency (WUEi) of rapeseed plants at 3 and 8 days after spraying (DAS) film antitranspirant (AT) from well-watered (WW) benchmark (—■—) and water-stressed plots (—□—) at Bird's Nest (BN; a–c, g–i) and Flat Nook (FN; d–f, j–l). Data are means of replicates (n = 8 at BN; n = 4 at FN) ± standard error of the mean. Asterisks (*) represent the significance compared to drought control (0AT) according to Tukey's test at $p = 0.05$.

Table 7

Probability values from *ANOVA* for aboveground biomass (AGB), seed yield (SY), harvest index (HI), pod number per hectare (Pod), seed number per pod (SP), thousand-seed weight (TSW), oil content and oil yield (OY) as affected by application of film antitranspirant (AT) at Bird's Nest (BN) and Flat Nook (FN). Polynomial contrasts were conducted between concentrations of AT, including water-stressed plots treated with water (0AT). Bold numbers indicate significant differences at $p < 0.05$.

Sites	Factors	d. f.	*p* values							
			AGB ($t\ ha^{-1}$)	SY ($t\ ha^{-1}$)	HI (%)	Pod ($10^6\ ha^{-1}$)	SP	TSW (g)	Oil (%)	OY ($t\ ha^{-1}$)
BN	Treatments	2	0.055	0.067	0.783	**0.005**	0.151	0.463	0.909	0.092
	Linear	1	**0.029**	**0.032**	0.514	**0.011**	0.116	0.309	0.693	**0.042**
	Deviations	1	0.274	0.348	0.831	**0.016**	0.234	0.487	0.866	0.423
FN	Treatments	4	**0.023**	**0.035**	0.959	**0.037**	0.297	0.160	0.509	**0.025**
	Linear	1	**0.002**	**0.003**	0.646	**0.008**	0.460	0.171	0.267	**0.002**
	Quadratic	1	0.908	0.803	0.743	0.972	0.622	0.939	0.266	0.957
	Deviations	2	0.740	0.642	0.881	0.159	0.138	0.092	0.691	0.521

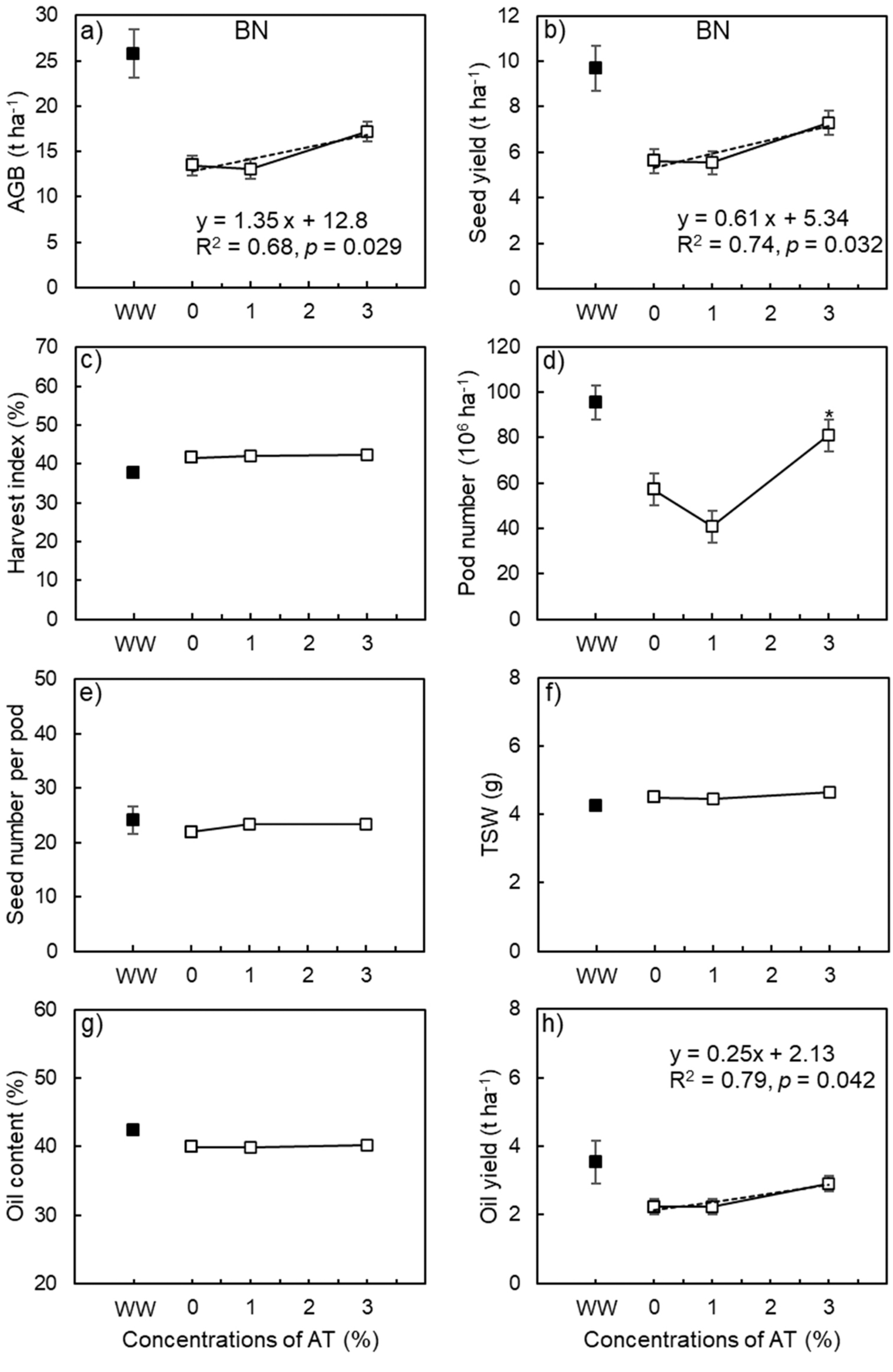

Fig. 5. Aboveground biomass (AGB, a), seed yield (b), harvest index (c), yield components (d–f), oil content (g) and oil yield (h) of rapeseed plants from well-watered (WW) benchmark (—■—) and water-stressed plots (—□—) following the application of film antitranspirant (AT) at Bird's Nest (BN). The linear regression model was fitted with AGB, seed yield and oil yield against concentrations of AT, where parameters had significant and linear relationships with AT concentrations (Table 7). Note that pod number at BN had significant deviations in addition to the linear contrast between concentrations, so a linear fitted line with low R-squared is not present. Data are means of replicates (n = 8) ± standard error of the mean. Asterisks (*) represent the significance compared to drought control (0AT) according to Tukey's test at $p = 0.05$.

1% increase in the concentration of AT, the projected increase in AGB, seed yield and oil yield were, respectively: 1.35, 0.61, 0.25 t ha^{-1} at BN (Fig. 5a–b, h) and 0.85, 0.23, 0.10 t ha^{-1} at FN (Fig. 6a–b, h). Pod number at BN showed very high variabilities due to significant deviations, so it was not included in the linear regression model (Table 7). At FN, every 1% increase in AT concentrations was predicted to increase pod number per hectare by 1.92 × 10^6 (Fig. 6d).

From the regression of yield with its components, we found that seed yield was highly associated with pod number, followed by seeds per pod at BN (Fig. 7a, c). This is also consistent with FN in seed yield, showing the highest positive correlation with pod number (Fig. 7b) but a negative correlation with seeds per pod with large variabilities (Fig. 7d). Although leaf RWC was not significantly affected by AT application at either sampling of the two sites (Fig. A3), pod number was strongly associated with leaf RWC from the regression analysis on combined data, with BN and FN showing different linear trends (Fig. 8b). Pod number was predicted to increase at BN nearly six times more than it would be at FN, with every 1% improvement in leaf RWC.

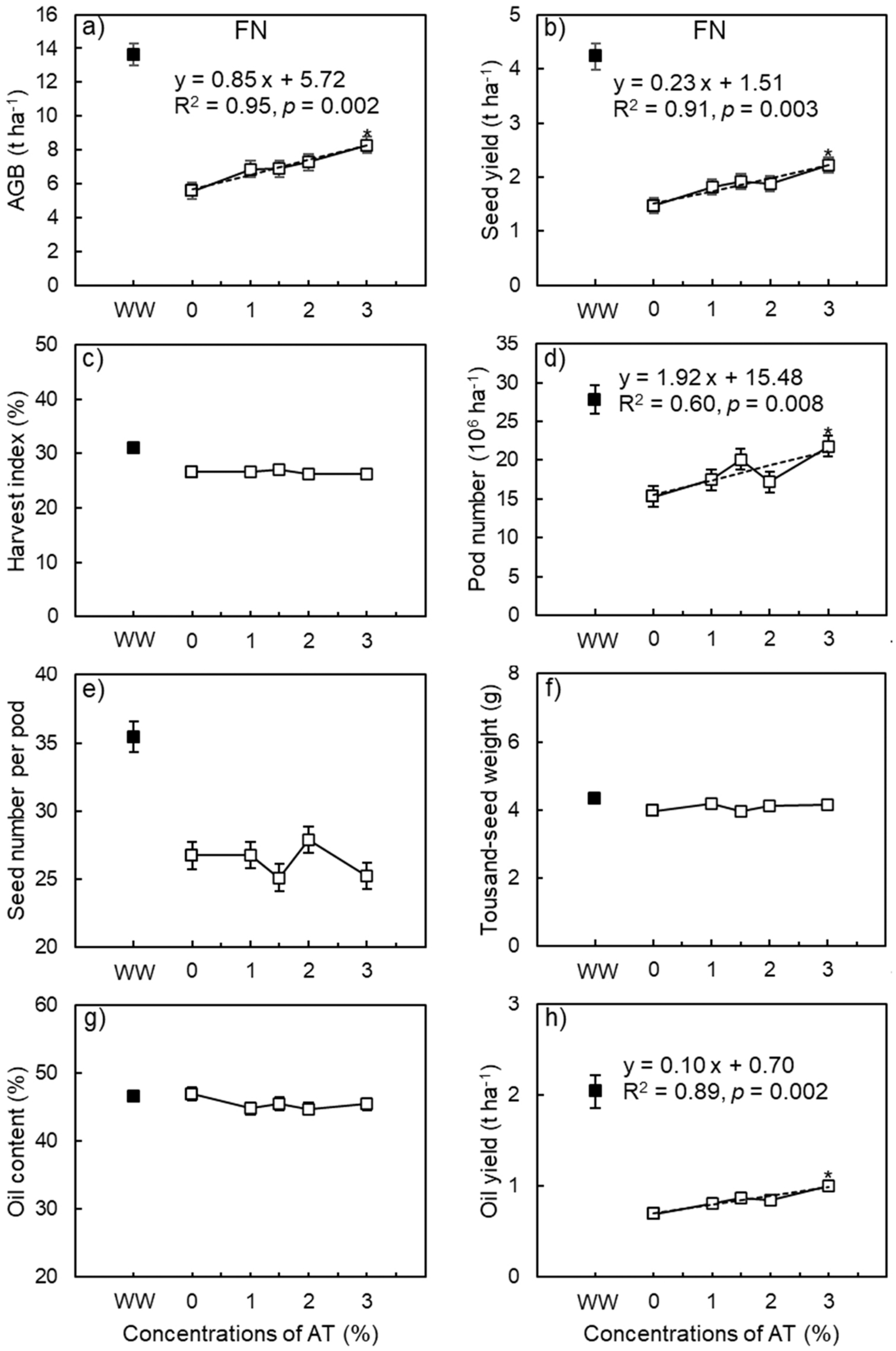

Fig. 6. Aboveground biomass (AGB, a), seed yield (b), harvest index (c), yield components (d– f), oil content (g) and oil yield (h) of rapeseed plants from well-watered (WW) benchmark (—■—) and water-stressed plots (—□—) following the application of film antitranspirants (AT) at different concentrations at Flat Nook (FN). The linear regression model was fitted with AGB, seed yield, pod number and oil yield against concentrations of AT, where parameters had significant and linear relationships with AT concentrations (Table 7). Data are means of replicates (n = 4) ± standard error of the mean. Asterisks (*) represent the significance compared to drought control (0AT) according to Tukey's test at $p = 0.05$.

4. Discussion

Previous studies demonstrated that rapeseed plants exhibited significant physiological responses to AT 0.25–1% and improvements in some yield components under glasshouse conditions (Xiang et al., 2022). In the present field study with higher concentrations of AT 1–3%, we reject our null hypothesis and show that when applied at the flowering stage, AT improved seed yield of water-stressed rapeseed by an average of 24% across concentrations and two sites. This yield improvement was also reported in winter rapeseed (Michele et al., 2017) and other crops like wheat (Mphande et al., 2021b). Furthermore, seed yield and AT concentrations were linearly correlated, indicating that the optimum concentration is above 3% and increasing concentrations of AT above 3% may result in greater yield for rapeseed under terminal drought.

Stomatal closure is the earliest response to drought for the maintenance of leaf water potential, which restricts the diffusion of water and CO_2 into the leaf (Flexas and Medrano, 2002). We showed that drought reduced g_s and A by an average of 73%, 37% and 39%, 22% across two

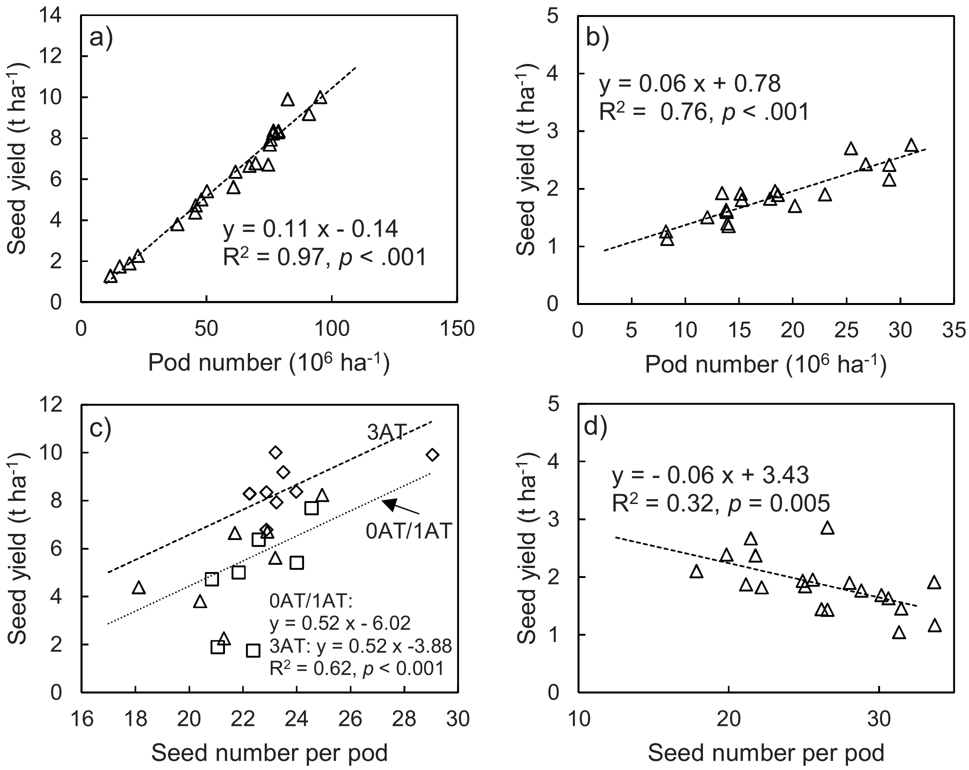

Fig. 7. Relationships between seed yield and yield components at Bird's Nest (BN; a, c) and Flat Nook (FN; b, d). In panel C, symbols of triangle (△), square (□) and tilted square (◇) represent the concentrations of film antitranspirant (AT) at 0% (0AT), 2% (2AT) and 3% (3AT), respectively parallel/common dotted lines are fitted with the linear regression model with/without treatments as groups. Data are replicates from BN (n = 9) and FN (n = 4).

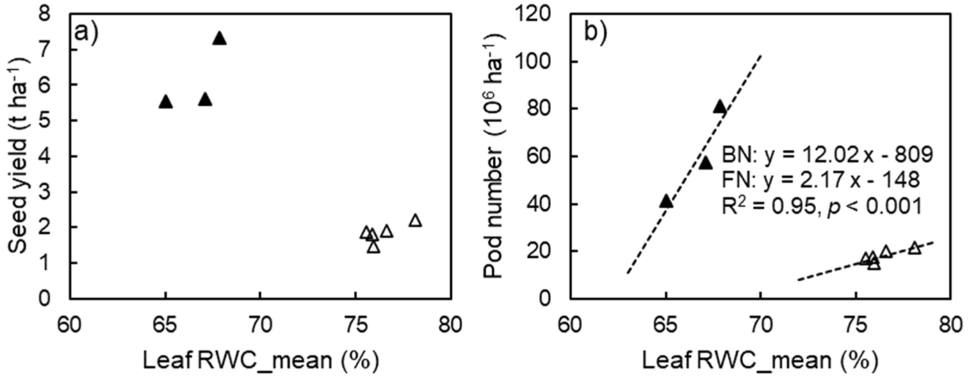

Fig. 8. Relationships in seed yield (a) and pod number (b) against the mean of leaf relative water content (RWC) at 2 and 14 days after spraying film antitranspirant from Bird's Nest (BN - ▲) and Flat Nook (FN - △) combined data. Simple linear regression analysis with experiments as groups are conducted. Based on results from regression analysis at the level of 5% Separate lines are fitted with pod number against leaf relative water content (RWC) in Panel b, along with equations, adjusted R^2 and probability values of fitted linear regression model. Data points are means of replicates (n = 8 at BN; n = 4 at FN).

sampling times at BN and FN, respectively; and improved WUEi by 176% and 31% as compared to the well-watered benchmark (Fig. 4). Negative effects of drought on rapeseed have been widely reported by previous research (Hess et al., 2015; Yan et al., 2016), the degree of which is positively associated with the magnitude of water stress (Faralli et al., 2016). An increase in WUEi of water-stressed rapeseed has also been reported by Elferjani and Soolanayakanahally (2018) and Biswas et al. (2019). Compared to FN, the further suppression of g_s at BN could be explained by the greater and longer-lasting soil water deficit, and drought at BN developed about three times faster than at FN in terms of soil SMP (Fig. 7).

From the previous glasshouse study under controlled drought conditions, we found *that* g_s and *A* of AT-treated rapeseed plants had a significant and negative relationship with specific AT concentrations (0.25–1%). The greater detrimental effects of increasing concentrations from 1% to 3% were also observed from some sampling times at the two sites in the present study under terminal drought, but with large variabilities between them due to variable environmental conditions in the field (Table 6). As concentrations increased, more stomata were blocked with higher leaf coverage, which augmented the suppression of g_s (Xiang et al., 2021). To confirm this, a preliminary pot experiment was conducted to estimate leaf coverage of different AT concentrations by arranging four replicates at an interspace the same as plants in the field experiment. Results showed that leaf coverage and AT concentrations were positively correlated, and leaf coverage increased approximately from 20% to 30% when AT was applied from 1% to 3% (Fig. A4). Apart from concentrations, drought intensity would also impact gas exchange responses to AT application (del Amor et al., 2010).

Under drought conditions, rapeseed plants adopting a "conventional" strategy via stomatal closing would result in less leaf cooling (Elferjani and Soolanayakanahally, 2018), which could also be confirmed by increased leaf temperature from water-stressed plots (Fig. 3). After AT application, reduced g_s is expected to reduce leaf transpiration, thus increasing leaf temperature (Gale and Hagan, 1966).

This is also confirmed in the present study, when compared to 0AT, the application of AT increased leaf temperature by 1.1–1.8 °C averaging all concentrations at both sites (Fig. 3). This is consistent with previous studies by Faralli et al. (2016) on winter rapeseed and Gatti et al. (2016) on grapevines. Faralli et al. (2016) found that VG at 1% increased leaf temperature significantly compared to well-watered control, and leaf temperature increased by < 1 °C compared to unsprayed control. Similarly, Gatti et al. (2016) applied a higher concentration of VG at 2% and found that sprayed leaves were moderately warmer by 1–2 °C than unsprayed ones. The heating effect can be explained by greater resistance to the passage of water vapour from AT application, thereby increasing the turgidity of guard cells and slowing evaporative cooling effects (Davenport et al., 1972). In addition, L_T increased linearly as AT concentrations increased from 1% to 3%, but with highly significant deviations at both sites (Table 6). This suggests that the relationship between L_T and AT concentrations may not always be linear, and a more complex relationship may involve leaf metabolic processes following blocked stomata, or canopy structure could also impact the relationship.

The ABA accumulation in guard cells triggers stomatal closure, thus preventing plants from losing excessive water under drought conditions (Kollist et al., 2014). Previous studies conducted by Faralli et al. (2017a) showed that AT application at the flowering stage of winter rapeseed reduced Endo-ABA concentration in the leaf and pod at 7 and 16 DAS. Similar findings have also been reported on wheat (Mphande et al., 2021b). In our study, however, differences between treatments in Endo-ABA of leaves at 7, 14 DAS or of pods at 28 DAS were not significant (Fig. A5). This might be explained by the dynamic responses of Endo-ABA to different environmental conditions. Leaf-synthesised ABA might be loaded to the phloem and transported to the roots (Wilkinson and Davies, 2002). Alternatively, our colleagues compared VG and fluridone (ABA inhibitor) on wheat and found no significant difference in Endo-ABA from fluridone application, but VG-sprayed reduced Endo-ABA due to the conservation of plant water by suppressing transpiration (Mphande, 2021, unpublished data). Nevertheless, the role of ABA in the activity of film AT remains elusive and requires further research.

During flowering and pod set, the relationship between source and sink regulates the availability of assimilates necessary for seed filling (Diepenbrock, 2000). Numerous studies have reported that terminal drought can cause massive yield losses in rapeseed (Faralli et al., 2017b; Elferjani and Soolanayakanahally, 2018), which is also supported by our study, showing that terminal drought resulted in ~46.6% seed yield losses across two sites, compared to the benchmark (Fig. 5b, Fig. 6b). The large decrease was strongly associated with reductions in AGB and pod number by 46.6% and 36.4%, respectively (Fig. 7), which can be explained by a substantial decline of available assimilates during the flowering stage, which in turn negatively affected the formation of pods and seed size (Johnston et al., 2002; Wang et al., 2011), leading to abortion and/or abscission of pods particularly (Tesfamariam et al., 2010).

However, yield losses caused by drought were lower at BN than at FN, which were 36.5% and 56.7% relative to the benchmark, respectively (without statistical comparisons because of non-randomisation in benchmark plots). One possible explanation would be the huge difference in initial nutrients available for plants in the soil, as both sites showed similar levels of air temperature and RH. Initial available N from BN was almost eight times higher than from FN (300 vs 70 kg ha^{-1}, respectively). Greater N supply can improve individual plant growth through a higher leaf area index and a prolonged period of photosynthetic activity, thereby producing high yields (Wang et al., 2014). Additionally, plants at BN had a 13% longer growth period than at FN in terms of thermal time due to early planting, which resulted in greater dry matter accumulation (Sieling et al., 2017). Taken together, these two important factors boosted plant growth at BN and produced a much higher yield at BN than at FN and other related parameters (Figs. 5–6). Although fungicide and insecticide were applied at both sites, plants at FN were still affected by pollen beetles and fungus disease, particularly during the reproductive stage, which may have partially accounted for yield losses.

In the current study, we first demonstrated that seed yield had a linear and positive relationship with concentrations of AT from 1% to 3%. Similar relationships were also observed in AGB, pod number/ha and oil yield with AT concentrations (Table 7). An increase in AGB by ~22% across AT concentrations and two sites relative to 0AT is consistent with Faralli et al. (2016), who studied winter rapeseed and found two types of film AT at 1% increased aboveground dry matter by an average of about 17% under terminal drought. This improvement in shoot biomass has also been demonstrated in other crops such as sweet corn (Shekour et al., 1987), fava bean (Davenport et al., 1972), wheat (Mphande et al., 2021a) and olives (Cirillo et al., 2021). From the glasshouse study, however, we only observed improvements in some yield components (Xiang et al., 2022). The individual plants in a controlled environment can experience different light and water conditions compared to those in the field, resulting in different responses to AT application. There may be two reasons for the difference in yield response in our glasshouse and field studies. First, soil hydraulics that affect the expansive growth of reproductive organs (pod and seed) in varying degrees, leading to pod and seed abortion varied between these two studies (Turc and Tardieu, 2018). Soil moisture decreased gradually throughout the whole season in the field experiments while potted plants in the glasshouse experienced rapid onset of water deficit and soil moisture was maintained at a similar level (i.e., 30% AWC equal to ~20% VWC) only at the flowering stage, albeit with fluctuations (Fig. 2). A non-limiting shoot (leaf and pod) water status could have been maintained for longer with AT application under slower soil drying, thereby preventing some of the pod abortion seen in drought-stricken plants without AT. Second, different ways of imposing drought can impact the capability of yield compensation during seed filling stage. As terminal drought developed with increasing DAP in the field, drought may have imposed more restrictions on the capacity of surviving pods and seeds for compensatory growth at the later seed filling stage (Kirkegaard et al., 2018).

Improving leaf water status, particularly during the flowering stage, is vital in determining final crop production as it is closely associated with gas exchange (Raza et al., 2017). The application of AT physically blocks leaf stomata and reduces water loss through transpiration, improving leaf water status (Davenport et al., 1972). This is also reported by Faralli et al. (2017a) on winter rapeseed, showing that plants treated with AT 1% exhibited significantly higher RWC relative to unsprayed control. However, in the present study, we failed to see a significant increase in leaf RWC at 2 or 14 DAS from two sites compared to 0AT (Fig. A3). The lack of statistical significance in leaf RWC might be attributed to variable environmental conditions at the time of sampling; different types of drought and plant varieties may also result in the discrepancy compared with Faralli et al. (2017a).

Nevertheless, pod number and leaf RWC were significantly and positively correlated, with separate lines from the two experiments (Fig. 8b), indicating the greater pod number from increasing AT concentration appeared to be mediated by leaf RWC. Since seed yield was strongly related to pod number (Fig. 7a–b), it can be deduced that greater seed yield from increasing AT concentration may also have been mediated by RWC (Fig. 8a).

Our study showed a greater yield from increasing AT concentrations, which confirms findings from a previous study conducted at FN on winter rapeseed (Faralli et al., 2017b). Researchers sprayed three concentrations of film AT (VG) on winter rapeseed subject to terminal drought, showing that AT at 1 (0.5%), 2 (1%) and 4 L ha^{-1} (2%) increased seed yield by 14%, 14% and 21%, respectively when AT applied at the flowering stage (although with no significant differences between concentrations). This strong relationship with AT concentrations was also reported by Fahey and Rogiers (2019) on grape bunches dipped in 0%, 1%, 2% and 3% VG solutions, showing a linear and positive relationship between the bunch mass loss and AT concentrations. For water-stressed rapeseed, therefore, we can improve seed yield by increasing concentrations of film AT. Based on the current price of rapeseed, and the average cost of spraying an AT product (Vapor Gard), the improvement from 1% (2 L ha^{-1}) and 3% (2 L ha^{-1}) at flowering of rapeseed under drought would approximately result in an economic benefit of £ 29.36/ha and £ 555.88/ha respectively (more details available in Table A1). Higher concentrations, however, would increase the cost of products and reduce economic profits, which is less cost-effective and practical. Since rapeseed stomata are unequally distributed between the surfaces (with ~44% of total stomata on the adaxial and 56% on the abaxial surface (Table A2)), an alternative to increasing the concentration is to optimise the spraying method to achieve higher leaf coverage, i.e., stomatal blockage of both adaxial and abaxial surface. Results from a preliminary study showed that leaf coverage was approximately 20% and 30% at the concentration of 1% and 3% AT, respectively (Fig. A4). On the other hand, only one genotype was used in our study, further work is recommended on different genotypes at more than one location/year when water supply and demand vary to verify the response of water-stressed rapeseed to film AT at different concentrations.

5. Conclusion

In the present study, drought caused yield losses as compared to well-watered benchmark (although benchmark was not included in the statistical test). After film AT was applied at the flowering stage, leaf temperature increased as expected due to blocked stomata, accompanied by the inhibition of gas exchange but improved intrinsic water use efficiency. A linear and positive relationship was found in seed yield, aboveground biomass, pod number and oil yield with AT concentrations. This AT-involved yield improvement was predominantly determined by increased pod number, and it was shown that pod number was closely correlated to leaf water status (i.e., relative water content). Therefore, we conclude that rapeseed yield can be improved by increasing AT concentrations when plants are under terminal drought. The yield benefit from AT application appeared to be mediated by improving leaf water status. Future research on the root-shoot water relations and osmotic responses to film AT would be worthwhile for understanding the relationship of AT with plant water status, and the role of ABA involved in the mode-of-action of film AT merits further investigation. Improving leaf coverage, considering both sides of leaf surfaces, would also help lower the cost and extend the commercial use of film AT.

CRediT authorship contribution statement

Jie Xiang was involved in methodology, formal analysis, investigation, writing – original draft, writing – review and editing, visualization, funding acquisition; **Laura H. Vickers** was involved in methodology, writing – review and editing, supervision; Martin C. Hare was involved in methodology, writing – review and editing, supervision; **Peter S. Kettlewell** was involved in conceptualization, methodology, formal analysis, writing – review and editing, supervision. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jie Xiang reports financial support was provided by China Scholarship Council.

Data availability

Data will be made available on request.

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Appendices

Table A1
Economic benefits from film antitranspirant (AT) at 1% and 3% on rapeseed yield in the UK.

AT concentrations	AT cost (VG)[a]	Spraying cost[b]	Yield benefit[c]	Rapeseed price[d]	Economic benefit
1% (2 L ha^{-1})	£ 15.25 L^{-1}	£ 16.30 ha^{-1}	0.14 t ha^{-1}	£ 544 ha^{-1}	£ 29.36 ha^{-1}
3% (6 L ha^{-1})	£ 15.25 L^{-1}	£ 16.30 ha^{-1}	1.22 t ha^{-1}	£ 544 ha^{-1}	£ 555.88 ha^{-1}

[a] Price of film antitranspirant product - Vapor Gard (VG, a.i., di-1-*p*-menthene) from Seed Ranch, 2022 URL (https://www.seedranch.com/Millers-Vapor-Gard-Concentrate-1-Gallon-p/vapor-gard-gallon.htm) (accessed 18 October 2022).

[b] NAAC CONTRACTING PRICES SURVEY 2022 (https://stmaaprodfwsite.blob.core.windows.net/assets/sites/1/2022/05/NAAC-contracting-prices2.pdf?_ga=2.120630152.1661377273.1655111315-735006727.1653313056) (accessed 18 October 2022).

[c] means of two field experiments at two sites, Bird's Nest and Flat Nook.

[d] AHDB UK delivered oilseed prices (https://ahdb.org.uk/cereals-oilseeds/uk-delivered-prices) (accessed 18 October 2022).

Table A2
Adaxial and abaxial stomatal density (stomata/mm) of rapeseed leaves from the first fully expanded leaf and two leaves below. Data are means (n = 20) ± standard deviations.

Leaf surface	Three positions of leaves		
	1st	2nd	3rd
Adaxial	178.37 ± 27.75	154.32 ± 17.15	138.26 ± 23.88
Abaxial	237.51 ± 21.54	221.56 ± 23.20	224.77 ± 30.75

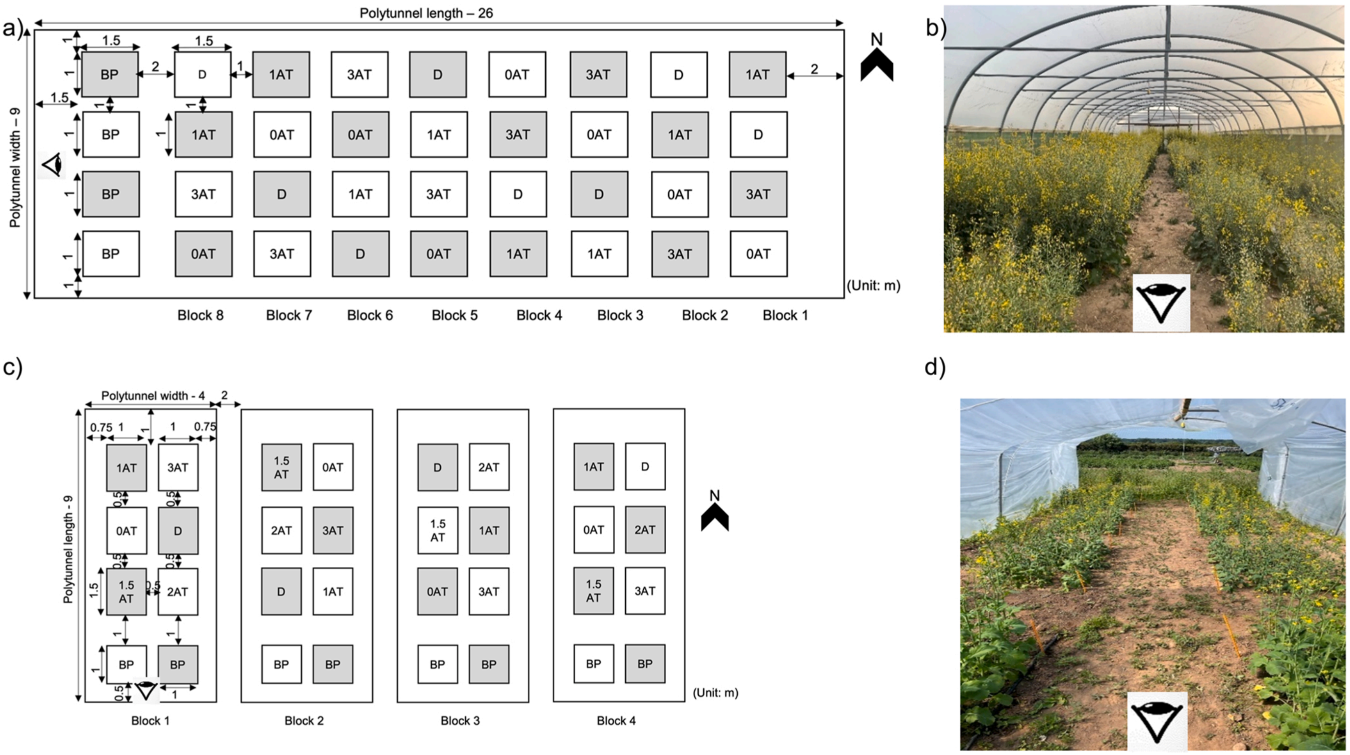

Fig. A1. The experimental layout of well-watered benchmark plots (BP) and experimental plots treated with film antitranspirant (AT) at different concentrations at Bird's Nest (BN; a) and Flat Nook (FN; c); photos of the whole experiment at BN (b) and block 4 at FN (d) when rapeseed plants were at flowering stage. Plots in grey represent the locations where access tubes were installed. Note that plots labelled with the capital letter D were not used for any experiment.

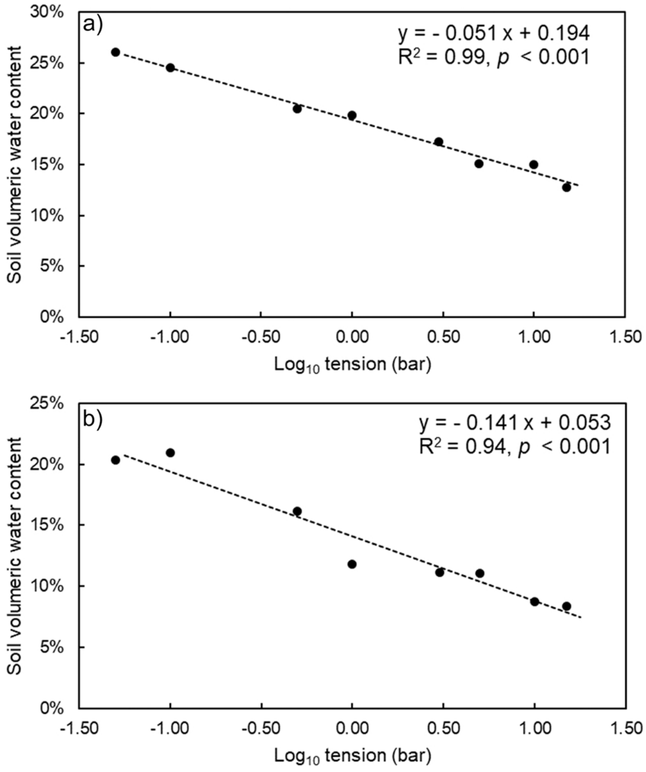

Fig. A2. Soil water retention curve at Bird's Nest (a) and Flat Nook (b). A linear regression model was fitted with soil volumetric water content against the common logarithm of tension applied using the pressure membrane. Data points are means of 0–30 cm and 30–60 cm depths (n = 3).

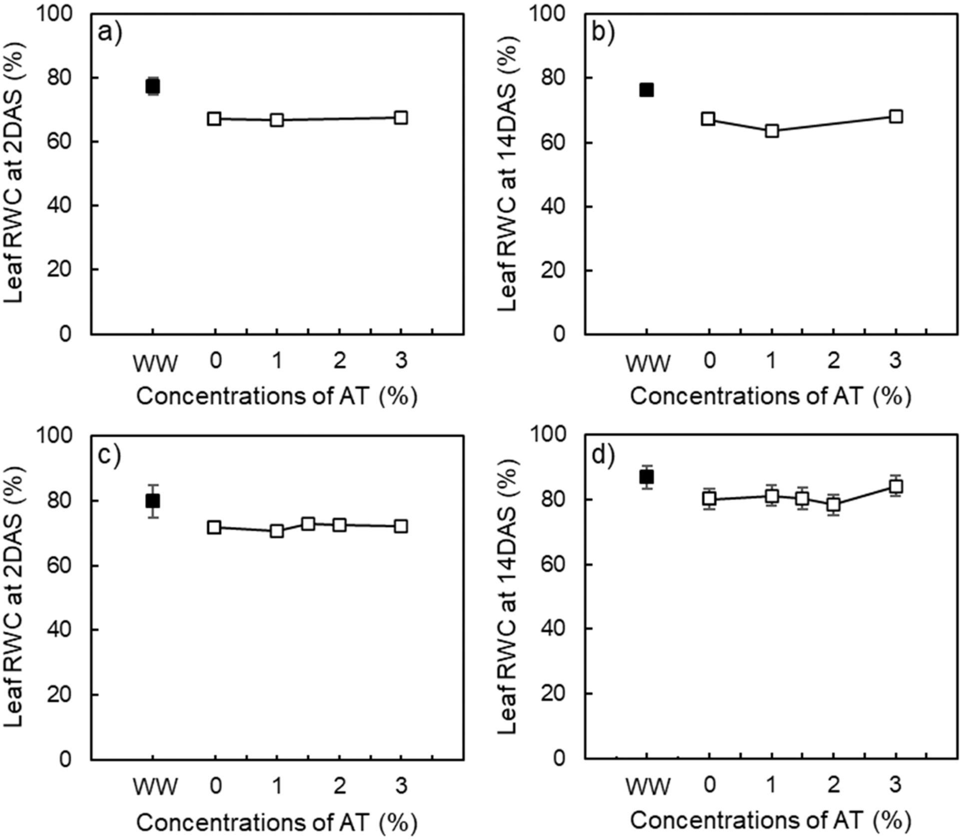

Fig. A3. Leaf relative water content (RWC) from well-watered (WW) benchmark (—■—) and water-stressed plots (—□—) at Bird's Nest (BN; a, b) and Flat Nook (FN; c, d). Samples were collected at 2 and 14 days after spraying (DAS) film antiitranspirant (AT). Data are means of replicates (n = 8 at BN; n = 4 at FN) ± standard error of the mean.

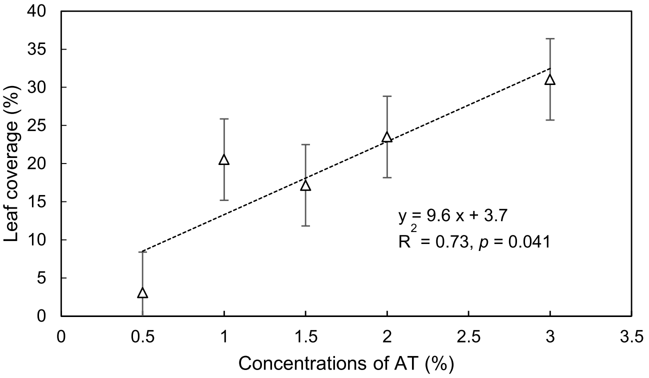

Fig. A4. Relationship between leaf coverage of film antitranspirant (AT) estimated using spray marker TiO_2 and concentrations of AT (0.5%, 1%, 1.5%, 2% and 3% with corresponding dose rates of 1, 2, 3, 4 and 6 L ha^{-1}, respectively). A linear model was fitted and presented with a broken straight line. Data points are means (n = 12), and error bars represent standard deviations.

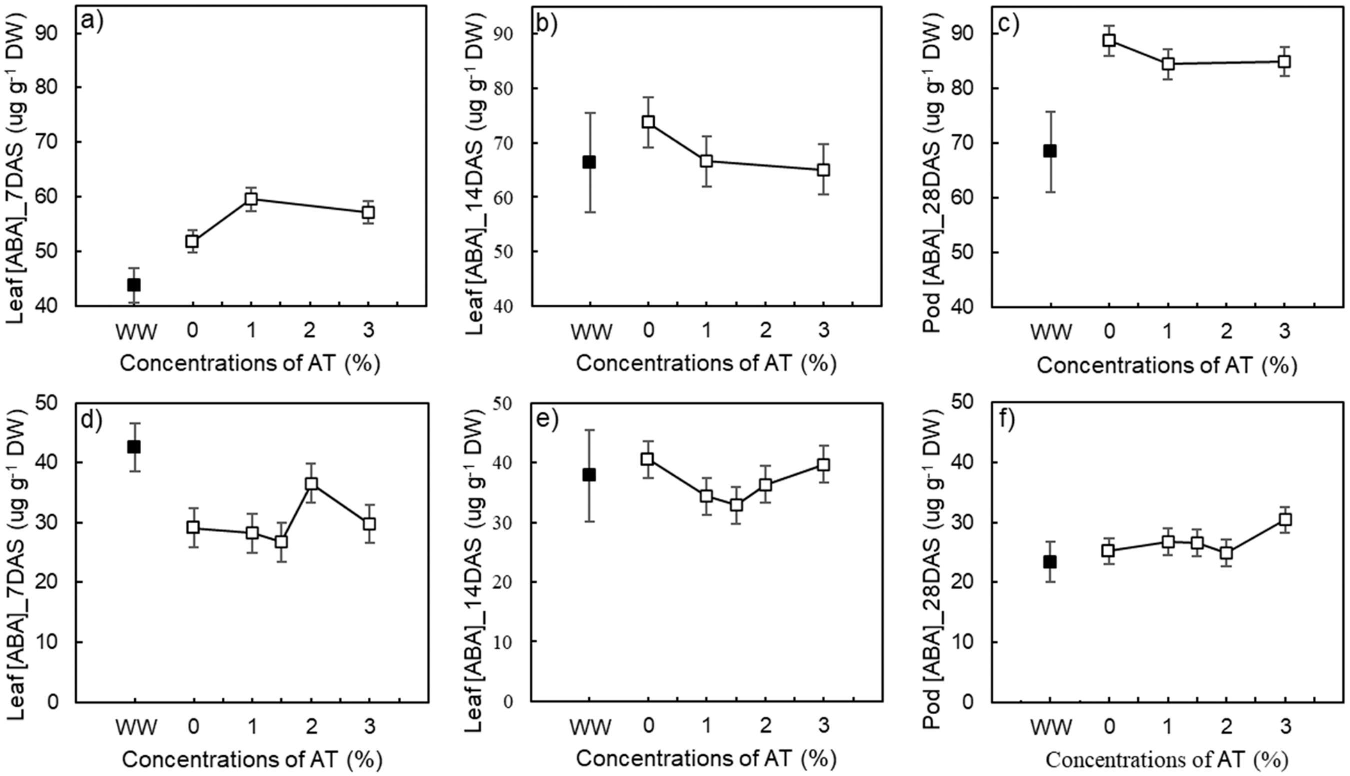

Fig. A5. Endogenous ABA concentration ([ABA]) in leaf and pod from well-watered (WW) benchmark (—■—) and water-stressed plots (—□—) at Bird's Nest (BN; a-c) and Flat Nook (FN; d-f). Leaf samples were collected at 7 and 14 days after spraying (DAS) film antitranspirant (AT); pod samples were collected at 28DAS. Data are means of replicates (n = 8 at BN; n = 4 at FN) ± standard error of the mean.

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