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In-field film antitranspirants application shows potential yield protection from flowering stage drought periods in winter canola (*Brassica napus* L.)

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Summary text

Previous work has shown antitranspirant efficacy at protecting *Brassica napus* and other major food crops from drought damage in glasshouse conditions. Two experiments were carried out in the same field over consecutive years to evaluate the effectiveness of chemicals with antitranspirant activity applied over different growth stages and at different dose rates at sustaining canola yield under drought. The results showed yield protection when antitranspirant was applied at 1 L ha^{-1} just before flowering therefore encouraging further work in different environments and spraying conditions.

Abstract

Crop management solutions that simulate plant water-saving strategies might help to mitigate drought damage in crops. Winter canola is significantly drought-sensitive from flowering to mid-pod development and drought periods lead to significant yield losses. In this work the drought-protection efficacy of different chemicals with antitranspirant activity applied just prior to key drought-sensitive phenological stages was tested on field-grown canola in two years. Drought was artificially imposed with rain-shelters. The results suggest that in-field application of 1 L ha^{-1} antitranspirant (Vapor Gard, a.i. di-1-*p* menthene, VG) at GS 6.0 (initiation of flowering) mitigated drought-induced yield loss leading to a 22%seed yield benefit on average over two years of experiments when compared to the un-sprayed un-irrigated plots. No significant yield responses were found from application at GS 7.0, from increasing VG concentrations (i.e. 2 and 4 L ha^{-1}), or from an antitranspirant with short-lasting effectiveness. The data suggest that in field conditions where drought occurs during the flowering stage, application of 1 L ha^{-1} VG just prior to the drought event can reduce yield loss. This result should encourage further work on water-saving management strategies during key drought-sensitive phenological stages as drought mitigation tools in canola and under different environments.

Introduction

There is significant evidence that a major factor determining the yield of winter canola (*Brassica napus* L., BN) is the amount of soil water available over the reproductive stages (Jensen *et al.* 1996; Berry and Spink, 2006; Istanbulluoglu *et al.* 2010). The yield components of the crop (pod number, seed number, and seed weight) are determined over a crucial period between flowering and mid-pod development (Mendham *et al.* 1981). This period often occurs in a seasonal time-frame (i.e. spring) of high crop water use (Vadez *et al.* 2014), elevated soil evaporation (Vadez *et al*. 2014) and low precipitation (Berry and Spink, 2006) in turn lowering the yield potential of the main commercially-available varieties.

It has been extensively hypothesized that by maintaining high soil water availability and/or plant water status over these key-periods, arable crops may exhibit a yield benefit (e.g. Salter and Goode, 1967). In Wang *et al.* (2009) and Wang *et al.* (2005), down-regulation of the farnesyltransferase subunit, a protein involved in stomatal sensitivity to ABA, gave a yield benefit in field-grown BN under drought due to a significant reduction in transpiration. Similarly, intracuticular and epicuticular wax accumulation under water-limited conditions reduces leaf transpiration leading to a sustained photosynthetic rate (Cossani and Reynolds, 2012). Thus, further exploitation of water-saving strategies or wax-simulating tools may significantly reduce the drought damage to BN yield at sensitive growth stages.

The ability of a film antitranspirant (AT) to reduce transpiration through stomatal occlusion for a temporary period is well documented (Solarova *et al.* 1981). Recently, the mechanisms of the yield benefit from AT under drought conditions on wheat and BN, in particular in relation to the reproductive development, have been explored (Weerasinghe *et al.* 2016; Faralli *et al.* 2016; Faralli *et al.* 2017a). The main physiological factors involved in reduced yield loss from drought following AT application are i) a higher leaf water potential (Weerasinghe *et al*. 2016;

Faralli *et al.* 2016), ii) a higher pollen fertility at pollen development stage and/or a lowered ABA signalling (Weerasinghe *et al.* 2016; Faralli *et al.* 2016; Faralli *et al.* 2017a) and iii) a sustained photosynthetic rate (Abdullah *et al.* 2015; Faralli *et al.* 2016) leading to more grains/seeds production when compared to the un-treated and stressed control (Abdullah *et al.* 2015; Weerasinghe *et al.* 2016; Faralli *et al.* 2016; Faralli *et al.* 2017a).

BN has been shown to be more drought sensitive than wheat (Hess *et al.* 2015) and AT application around flowering was beneficial for the yield of pot-grown BN subjected to water stress, although a substantial difference in efficacy between two AT was recorded (Faralli *et al.* 2016). Application of AT on field-grown *Brassica campestris* gave a grain yield increase following improved plant water status and water-use efficiency under dryland conditions (Patil and De, 1976 and 1978). However, no additional work has been published on field experiments so far and there is no work in the literature investigating the effectiveness of film antitranspirants at avoiding winter BN yield losses under drought conditions in the field. Thus, two field experiments under rain-shelters investigated the effectiveness of AT at sustaining the yield of droughted BN over different phenological stages: in 2015 (Experiment I) two chemicals with antitranspirant activity were applied at three different phenological stages, whereas in 2016 (Experiment II) the chemical (di-1-*p* menthene) which showed the best yield response in four glasshouse experiments and in the field in 2015, was used in a dose-response experiment and sprayed at two phenological stages.

Materials and methods

Site, soil analysis and crop sowing

The two field experiments were carried out in Flat Nook field, a field site at Harper Adams University, Shropshire (52°46′ N, 2°25′ W). Soil profile, bulk density and soil texture were analysed on 20 January 2015. A 1 m^3 soil profile pit was excavated inside the experimental area. Four bulk density samples, at 20, 30, 60, 80 cm depths, were collected inside the pit with a 300 cm^3 tin, adapted from Rowell (1994). Texture samples were collected at the same depths as bulk density samples. The soil profile was used to determine soil depth (~90 cm). Texture samples were analysed according to Toogood (1958).

Previous crops at the site were fallow (no crops) for the 2014/2015 experiment area and potatoes for the 2015/2016 experiment area. Winter canola seeds (cv. Excalibur, Dekalb, UK) were sown on 29 August 2014, 15 cm row spacing and 80 seeds m^{-2} (Experiment I) and on the 04 September 2015 with row spacing at 15 cm and a seed rate of 50 seeds m^{-2} (Experiment II). Soil preparation for sowing and crop management followed the standard UK agronomic practices including insecticide, fungicide, herbicide and fertilizer application.

Design and treatments in 2014/2015 (Experiment I)

The experiment was a factorial randomized block design composed of three blocks with each block in a separate rain-shelter. There were eight plots per block and the plots were ~5 m length and ~3 m width. The treatments consisted of two antitranspirant products each sprayed at three growth stages according to the BBCH growth scale: bud emerging (23 March 2015; flower buds visible from e above, GS 5.1), flowering (17 April 2015; 50% of plants have the first flower open, GS 6.0), pod development (15 May 2015; 10% of pods on the main stem reached the final size, GS 7.0). There were two additional control treatments in each block: irrigated with no AT (WW) and unirrigated with no AT (WS). Rain-shelters were moved into position on the 26 February 2015 when plants were still at rosette stage and from this stage until harvest water was applied only on the WW plots. The two antitranspirants chosen for the experiments (Nu-Film P, a.i. poly-1-*p* menthene 96%, NFP; Vapor Gard, a.i. di-1-*p* menthene 96%, VG. Miller Chemicals and Fertilizer, Hanover, USA) were sprayed in a volume of 200 L ha^{-1} of water using a hand-held knapsack sprayer (Flat Fan 110/03, 0.3 MPa, 1 m s^{-1}). For each spray treatment the boom was maintained ~0.5 m above the leaf (GS 5.0 and 6.0) and pod (GS 7.0) canopy.

Design and treatments in 2015/2016 (Experiment II)

The experiment was a factorial randomized block design composed of six blocks with eight treatments per block and the plots were ~6 m length and ~1 m width. Each rain-shelter contained two blocks and in each block the treatments were three VG dose rates (1, 2 and 4 L ha^{-1}) sprayed at two growth stages (08 April 2016, GS 6.0; 19 May 2016, GS 7.0) using the spray conditions of the 2015 experiment and two control treatments in each block: irrigated with no AT (WW) and the unirrigated with no AT (WS). Rain-shelters were moved into position the 1st of February 2016 until harvest and water was applied only to the WW plots.

Soil moisture measurements, irrigation and environmental conditions

In Experiment I, 80-90cm aluminium alloy neutron probe access tubes for soil moisture data collection were placed in each plot. Soil moisture measurements were taken with a neutron probe (Institute of Hydrology Neutron Probe System, Wallingford, UK) of 80 cm length. Soil moisture readings were taken from all plots (one reading per tube per plot) at 20, 30, 50 and 80 cm depth in both the experiments. Volumetric water content (VWC) was calculated for all the experiment according to the Neutron Probe handbook (Bell 1987) for sandy soil as:

$$\text{VWC (\%)} = \left[0.79 \text{ x } \frac{\text{counts per second}}{\text{neutron probe reading}} - 0.024\right] \text{x } 100$$

Field capacity for the different soil depths was determined by taking readings on the 15 December 2014 and the 14 January 2015, whilst the soil was at field capacity. Soil moisture data were taken on the 16 December 2014, 14 January 2015, 02 March 2015, 16 March 2015, 26 March 2015, 07 April 2015, 17 April 2015, 24 April 2015, 01 May 2015, 13 May 2015, and

04 June 2015. Irrigation was applied only to WW plots over the whole experimental period through a pipe installed in the WW plots. Water was applied from the installation of the rain-shelter until complete maturity (i.e. before harvest) every two days to avoid soil moisture deficit to the WW plots (Fig. 1A).

In Experiment II, one aluminium alloy tube was placed in a WW and one in a plot subjected to drought stress (regardless of antitranspirant application) randomly selected for each rain-shelter (n=3 for WS and WW). Soil moisture readings and calculations for VWC were done as for Experiment I and for each tube on the 19 January 2016, 21 January 2016, 26 February 2016, 24 March 2016, 26 April 2016, 23 May 2016, and 21 June 2016. Irrigation was applied to the WW-rain-shelter plots by installing irrigation tapes to each WW plot (Fig. 1C). Tapes had 1 mm diameter emitters (two for each set) positioned 10 cm apart from each other and ensuring ~200 mm H_2O m^{-2} h^{-1}.

Stomatal conductance and gas-exchange

In both the experiments, leaf stomatal conductance to water vapour (*gs*) was collected AT GS 6.0 and GS 7.0 using a transient state diffusion porometer (AP4, Delta-T Devices, Cambridge, UK). The device was calibrated before every use with the calibration plate provided. Measurements of the abaxial *gs* and adaxial *gs* were collected from three randomly selected fully expanded leaves at the top of the canopy per plant and then averaged (n=4 of averaged measures for Experiment I and n=6 of averaged measures for Experiment II). Total *gs* was then calculated as adaxial *gs* + abaxial *gs*. Data were collected between 09:30 and noon. Pod *gs* was analysed with the same porometer on main stem pods positioned at mid-distance between the first internode and the plant tip (n=4 for Experiment I and n=6 for Experiment II).

In Experiment I, the light-saturated CO_2 assimilation (A_{max}, µmol CO_2 m^{-2} s^{-1}) and the leaf transpiration rate (E, mmol H_2O m^{-2} s^{-1}) were measured on the first fully expanded leaf of the top canopy of randomly selected plants for each treatment/plot (n=4) using a CIRAS portable photosynthesis system (PP system, MA, USA) with a 2.5 cm^2 cuvette ensuring a saturating 1200 µmol m^{-2} s^{-1} PAR; all the data were recorded after 3–4 min at 400 ppm CO_2 level, when steady-state photosynthesis was achieved. The data were recorded after GS 6.0. The leaf water-use efficiency (WUE) was then calculated as A_{max}/E (n=6).

Chlorophyll fluorescence

A FluorPen 100 MAX (PSI, Czech Republic) was used to evaluate dark-adapted chlorophyll fluorescence parameters. From 09:00 to 16:00, the tagged first fully expanded leaf of the top canopy was used for a 30 min dark-adaptation provided by leaf clips in Experiment I and Experiment II (n=6). The maximum quantum efficiency of photosystem II photochemistry ($F_v/F_m = [F_m - F_o / F_m]$) was recorded according to Murchie and Lawson (2013).

Leaf and pod water potential

Plants were used for leaf water potential (LWP, over GS 6.0) and flower/pod water potential (PWP, over GS 7.0) analysis in Experiment I. Between 11:00 and 14:00, leaves or pods were excised with a scalpel from five plants for each treatment (n=5) and water potential was immediately analysed by a Scholander pressure chamber (SKPM 1405/50, Skye Instruments Ltd, UK). The tissues were analysed on the cut end of the petiole 1 cm from the base (leaf or flower/pod). The water potential value (MPa) was collected when water was exuding from the cut surface, seen by using a magnifying lens.

Yield assessments

At maturity (the 1 July 2015 for Experiment I and the 19 July 2016 for Experiment II), plots were harvested with a plot-combine harvester (Wintersteiger Nursery Master, Germany) (in total 7.5 m^2 area harvested for each plot in Experiment I and 6 m^2 in Experiment II) and the seeds for each plot were collected and stored in a drying room (~35 °C temperature). Seed moisture was collected daily with a moisture meter and seed were weighed by balance. The values were considered correct when all the seed samples reached the 9% moisture (~3-4 days after drying). Yield (t ha^{-1}) was then calculated by adjusting the area of the harvested plot to a hectare. Then, 1000-seed weight (TSW) was determined by taking the mean weight of three 100 seed lots per replicate and extrapolated TSW. Seed per m^2 was then calculated as the total plot seed number (calculated from TSW and yield) divided by the area of the plots.

Statistical analyses and data presentation

Temperature and rainfall for Experiment I and II are presented as daily data collected at a weather station approximately 650 metres from the field site. The volumetric water content (VWC) of each experiment is shown as plot means. Since in Experiment I no statistically significant differences were recorded between droughted antitranspirant sprayed and un-sprayed plots, all the data from droughted plots (+ or - antitranspirant) were pooled and presented as “un-irrigated” means. Stomatal conductance, gas-exchange and water potential data were subjected to one-way ANOVA for each day of data collection and means were separated by using a Tukey’s test (P=0.05). Yield data were subjected to one-way ANOVA and means were separated by using a Tukey’s test (P=0.05). Yield data were then subject to contrast analysis to evaluate additional statistical differences between treatment combinations. In Experiment I, plots were subjected to significant lodging in two of the rain-shelters, and this was scored as % of the total plot area. For Experiment I, data from GS 5.0 are not presented since the soil moisture deficit applied at the time of the antitranspirants application was very similar to the irrigated one (no soil moisture deficit) and therefore, a valid test of the effect of AT on droughted BN was not conducted. Yield data from Experiment I and II of un-irrigated un-sprayed, 1 L ha^{-1} VG GS 6.0 and 1 L ha^{-1} VG GS 7.0 were pooled and a Tukey’s test was used to test the differences over two years in seed yield. Since in Experiment II block 1 was significantly damaged by pigeons and block 6 was subjected to edge effects, only block 2, 3, 4 and 5 were used for the Tukey's test (therefore, *n*=7). All the statistical analyses were performed by using GenStat (17th edition, VSN International Ltd, UK)

Results

Weather, soil and VWC

The monthly weather data for Experiment I (2014-2015) and Experiment II (2015-2016) are shown in Figure 1. In Experiment II, the winter and the spring were warmer (~8 °C on average) than that of Experiment I (~7 °C on average) following by higher total precipitations (~2.32 mm day^{-2} in Experiment II and 1.77 mm day^{-2} in Experiment I on average). Analysis of the soil texture showed that Flat Nook soil is typically a sandy loam soil according to Toogood (1958). At a soil depth of 20 cm the percentage of sand was 75.8% with 20.8% silt and 3.4% clay and a bulk density of 1.74 g/cm^3. At 40 cm depth the percentage of sand increased compared to the 20 cm depth to 78.9% and decreasing to 71.2% and 72.1% for 60 and 80 cm depth respectively. Silt percentage remained relatively stable at ~20% whereas clay concentration increased to 6.4 and 5.4% at 60 and 80 cm depth respectively. Bulk density steadily increased to 1.76, 1.78 and 1.84 g/cm^3 at 40, 60 and 80 cm depth respectively.

In both Experiment I and II, well-watered plots grown under rain-shelters exhibited similar VWC values that fluctuated between 40-45% for 20 and 40 cm depth and 30-35% for 60 and 80 cm depth (Figure 2). Rain-shelter and un-irrigated plots exhibited a steep decrease in VWC during both Experiments I and II. When compared to the irrigated plots, un-irrigated plots showed an average (20, 40, 60 and 80 cm depth) decrease in VWC from an initial 40% to 38%, 28% and 21% at GS 5.0, 6.0 and 7.0 respectively in Experiment I. In Experiment II it was from an initial 43% to 30% and 24% on average at GS 6.0 and 7.0.

Stomatal conductance, gas-exchange and chlorophyll fluorescence over GS 6.0

In both the experiments, total *gs* of WW plots over GS 6.0 fluctuated from ~1200 to 500 mmol m^{-2} s^{-1}. Over GS 6.0 WS plots exhibited a decrease in total *gs* at all the DAS when compare to the WW plots (Figure 3). Compared to the WW un-sprayed plots, the WS un-sprayed exhibited a lower total *gs* by ~50% in Experiment I and by ~25% in Experiment II. Indeed at all the DAS, WS significantly decreased abaxial and adaxial *gs* with the latter showing a smaller reduction. At the same time, gas-exchange analysis in Experiment I showed that WS plots exhibited a lower capacity at assimilating CO_2 compared to the WW plots leading to higher leaf WUE values when compared to the WW plots.

In Experiment I, application of NFP significantly reduced adaxial *gs* on DAS 3 and DAS 6 without affecting abaxial *gs* compared to the WS un-sprayed. However, no significant differences were found in total *gs* and CO_2 assimilation rate when compared to the WS plots. Application of NFP decreased the transpiration rate compared to the droughted un-sprayed plots by 13% leading to slightly higher leaf WUE values.

In both the Experiments, VG (1 L ha^{-1} dose rate) significantly reduced adaxial *gs* throughout GS 6.0 compared to the WS un-sprayed plots. However, a small increase, although not significant, was found in the abaxial surface values compared to the WS un-sprayed on DAS 6 and DAS 16. Total *gs* was significantly reduced by VG treatment on most of the DAS. When the experiments showed low conductance values (i.e. DAS 10 and 12 of Experiment I and DAS 6 of Experiment II) the effect was not significant. Steady lower total *gs* values compared to the WS un-sprayed were recorded even at DAS 18 and DAS 20. In Experiment II, higher VG dose rate (2 and 4 L ha^{-1}) did not show any additional *gs* reduction when compared to the 1 L ha^{-1}. VG application in Experiment I did not affect CO_2 assimilation showing similar trends to the WS un-sprayed plots but it was accompanied by an overall 15% reduction in transpiration rate leading to significantly higher WUE values (Figure 3H) when compared to the WS plots. For both the Experiments and all the treatments, no differences were found between chlorophyll fluorescence traits (data not presented).

Stomatal conductance over GS 7.0

In WW plots and over the two Experiments, the pod *gs* was between ~120 and ~150 mmol m^{-2} s^{-1} on average whereas adaxial *gs* fluctuated between ~150 mmol m^{-2} s^{-1} and ~200 mmol m^{-2} s^{-1} in Experiment I and II respectively (Figure 4). In WS plots, the average pod *gs* was ~70 and 100 mmol m^{-2} s^{-1} in Experiment I and II respectively, that was ~40% less of the WW plots. Similarly, the adaxial *gs* of the WS plots was ~45% lower than that of the WW plots.

In Experiment I, NFP application did not have a significant effect on pod *gs*. In contrast a slight reduction of adaxial *gs* was recorded on DAS 1 that however was not statistically significant.

Application of VG at 1 L ha^{-1} had a strong and significant effect at reducing pod *gs* in Experiment I, whereas no significant differences were recorded in Experiment II. Similarly, 1 L ha^{-1} VG decreased adaxial *gs* on DAS 1, 4 and 6 in Experiment I whereas in Experiment II no statistical significant differences were recorded. Increasing dose rate (i.e. 2 and 4 L ha^{-1}) had a negligible effect at reducing both adaxial and pod *gs* in Experiment II, despite pod *gs* being significantly lower than that of the WS un-sprayed plots on DAS 1, 4 and 6.

Leaf and pod water potential

LWP of WW plots was between -1 and -1.2 MPa whereas the PWP in WW plants was slightly less negative (~ -0.9 on average) (Figure 5). Drought had an effect on both LWP and PWP leading to lower values by ca. 2-fold on average respectively. While no differences in LWP and PWP were found between NFP sprayed and un-sprayed plots, statistically significant less negative values were found in VG-sprayed plots by 33% and 25% respectively averaged over all the dates when compared to WS plots.

Yield and yield components analysis

In Experiment I watered un-sprayed plots showed an average seed yield of 3.56 t ha^{-1} (Figure 6). Water deprivation decreased the seed yield and seed m^{2} yield component by 43% compared to the watered plots leading to an average seed yield of 2.01 t ha^{-1}. NFP sprayed at GS 6.0 and GS 7.0 onto droughted canola increased the seed yield compared to the droughted un-sprayed plots leading to 2.87 and 2.42 t ha^{-1} seed yield respectively. In particular, NFP application at GS 6.0 increased seed m^{2} yield component by 27% when compared to the droughted un-sprayed plots. With respect to the droughted un-sprayed plots, VG-treated plots at GS 6.0 and GS 7.0, showed an increase in seed yield leading to 2.49 and 2.26 t ha^{-1} respectively, accompanied at GS 6.0 by a 25% seed m^{2} yield component increase.

In Experiment II watered un-sprayed plots showed an average seed yield of 4.22 t ha^{-1} and a seed m^{2} of 85,000 (Figure 6). Water deprivation decreased the seed yield and seed m^{2} yield component by 33% compared to the watered plots leading to an average seed yield of 2.85 t ha^{-1}. TSW was not affected by water deprivation leading to similar values (~4.92 g). VG applied over GS 6.0, despite not being significant, appeared to increase seed yield by 14%, 14% and 23% at 1, 2 and 4 L ha^{-1} respectively when compared to the un-irrigated un-sprayed plots. In contrast and when compared to the un-irrigated un-sprayed plots, the VG application over GS 7.0, although not significant, increased seed yield by 12% and 14% when sprayed at 1 and 2 L ha^{-1} whereas a 7% decrease was recorded at 4 L ha^{-1} application. Since TSW was never affected by both watering regimes and VG, the seed yield variation was governed only by a similar reduction/increase in seed m^{2}.

On average the two field experiments showed that un-irrigated plots have an average decrease in seed yield by 40% (Figure 7). Application of 1 L ha^{-1} VG just prior to GS 6.0 did have a significant effect at sustaining the yield of un-irrigated BN plots by 0.71 t ha^{-1} on average when compared to un-sprayed plots. In contrast, the effect of 1 L ha^{-1} VG application just prior to GS 7.0 was not significant.

Discussion

The effect of water deficit on field-grown canola at GS 6.0 and GS 7.0

The VWC recorded in this work is high for a sandy loam soil. Indeed the VWC for a sandy loam top-soil would be expected to be in the range of 31% ± 8.6 (SD) (Hall *et al.* 1977). However, bulk density and organic matter variations could explain some of this variation as they are both known to influence VWC (Hall *et al.* 1977) and relative readings should be reliable, allowing legitimate comparisons between treatments. In our experiments the crop was grown under rain-shelters (built at the end of the winter) to decrease the soil moisture and therefore artificially induce water stress to the crop. As in Weerasinghe *et al.* (2016), an average of 2-3 °C differences in temperature between the inside and the outside of the rain-shelter were recorded on days with high temperatures and elevated light irradiance. However, since in this work only plots grown under rain-shelters are compared, the temperature differences are unlikely to affect this comparison.

Data of *gs* from Experiment I and II and water potential analysis from Experiment I showed that, at the dates of AT application, the un-irrigated plots were significantly stressed. In addition, soil moisture data showed significant decreases in VWC in both top and sub-soil that match with the *gs* reduction of un-irrigated plots. Since the rain-shelters were built at the end of winter for both the years, the VWC reduction was much larger at GS 7.0 antitranspirant application than GS 6.0. In Experiment II, the VWC of the un-irrigated plots was higher than that of Experiment II at GS 6.0 and GS 7.0. This was due to the significantly lower temperatures of March and April 2016 (Figure 1) that led to lower evaporative demand and thus a possible lower total evapotranspiration. At the same time and in both the experiments, the irrigated plots showed constant VWC at all the soil depths that were very similar to the winter values. This suggests that on irrigated plots, plants had access to high water availability throughout the experimental period.

In both the experiments, total *gs* of un-irrigated plots was significantly lower than that of the irrigated plots. Despite that, in Experiment II the reduction was less evident throughout the GS 6.0 stage. Our data showed that stomatal closure occurred at field scale when water availability decreased, but the reduction was much lower than for an artificial drought stress imposed in pots (Faralli *et al.* 2016). Similarly, lower CO_2 assimilation capacity was found in un-irrigated plots when compared to the irrigated one and this may be accompanied by lower assimilate production over flowering stage. However, the non-significant differences in chlorophyll fluorescence traits between un-irrigated and irrigated plots suggests that photosynthetic down-regulation is only stomatal-driven (at least at the soil moisture deficit applied in this work) and drought does not directly affect photochemistry efficiency (as already reported by Muller *et al.* 2010). To confirm this, leaf WUE was increased in un-irrigated plots with respect to the irrigated one (Figure 3H) therefore showing a water-stress induced water-saving strategy triggered by stomatal closure. Similarly, in Jensen *et al.* (1996), canola plots grown in a sandy soil and stressed over reproductive stages showed gas-exchange and water potential reductions that match with our data. Indeed, in our experiments drought affected water potential data, and led to more negative values in un-irrigated plots. Altogether, the data showed overall significant detrimental effects on field-grown BN at a physiological level, that were clearly less prominent when compared to glasshouse work (e.g. Faralli *et al*. 2016; Champoliver and Merrien, 1996), but consistent with other field reports (e.g Jensen *et al.* 1996; Morgensen *et al.* 1997; Istanbulloglu *et al.* 2010).

In both the Experiments, un-irrigated plots showed a significant decrease in seed yield when compared to the irrigated ones. The reduction was due mainly to a significant decrease in seed m^{-2}, in accordance with many other reports (Berry and Spink, 2006; Berry and Spink, 2009) where seed m^{-2} is a main target to increase BN yield. In contrast, no significant differences were found in TSW in contradiction with other reports that show significant TSW compensation under drought (e.g. Champolivier and Merrien, 1996). However, since in our experiments un-irrigated plots did not received supplementary watering until harvest, it is possible that the TSW compensation was significantly reduced due to the prolonged stress conditions. In this work, we confirm that soil moisture deficit during the BN reproductive period is a key factor for seed number determination and therefore further efforts should focus at improving BN resilience to drought focusing on reproductive physiology, a field that has not been particularly studied in BN.

The effect of film antitranspirant on canola at GS 6.0 and GS 7.0

Our data on BN physiology show that AT application at 1 L ha^{-1} decreased *gs* and did not affect CO_2 assimilation. One major problem related to the use of AT is that often the reduction in water loss was accompanied by a reduction in CO_2 assimilation (Solarova *et al.* 1981). However, it has been shown that the increase in atmospheric CO_2 may counteract the reduction in CO_2 uptake (del Amor *et al.* 2010). Moreover, the recent literature shows an increasing amount of successful work using biotechnological approaches that focus on triggering water-saving strategies in crops leading to ameliorative physiological responses under drought (especially BN and Arabidopsis; e.g. Wang *et al.* 2005 and 2009 and Yang *et al.* 2016) thus confirming the importance of water-saving strategies and their success to improve crops resilience to water deficit especially in conditions (e.g. the present atmospheric CO_2 concentration ~404 ppm) where Rubisco is less limited when compared to the past (e.g. 1960 with an atmospheric CO_2 concentration of ~300 ppm) (Faralli *et al*. 2017b).

Collectively, the data over GS 6.0 suggests that VG had a major effect on seed m^2 and therefore it is possible to hypothesize that the higher plant water status during GS 6.0 following AT application significantly sustained seed set (as already reported on wheat by Weerasinghe *et al.* 2016). In Experiment I, lodging was present in the last part of the season with higher intensity on irrigated plants and to the GS6.0 sprayed plants potentially because of the higher water available that allowed plant growth and therefore plant with higher possibility of lodging effects. At the same time, contrast analysis showed no significant effect of the dose rate (P=0.12), suggesting that no yield benefit can be achieved by increasing VG rate at the magnitude of stress applied in this work. In addition, since the yield gain at GS 6.0 of this work exceeds the cost of most of the chemicals with antitranspirant activity available (e.g. ~20-30£ per L for VG), the 1 L ha^{-1} may be relatively inexpensive if the application is done prior to the onset of terminal drought conditions (therefore enhancing the water-saving effect of VG during flowering). The potential integration with the standard crop protection treatments (e.g. Sclerotinia, pollen beetle and plant growth regulator treatment applications) can be an additional value that might significantly eliminate the cost of the spray application. In contrast, no statistically significant effects were recorded when antitranspirants were applied at GS 7.0. One reason of this could be the fact that at GS 7.0 the artificial soil moisture deficit applied with the rain-shelter was much stronger than that applied at GS 6.0 and therefore it is possible to speculate that VG is not efficient when a strong drought-induced stomatal closure is triggered (as shown in Faralli *et al.* 2017a). In addition the dose response experiment, showed slight (not significant) decreases in seed yield at 4 L ha^{-1} when compared to the un-treated un-irrigated plots. Since previous work showed that application of VG on both stressed and un-stressed plants significantly reduced ABA concentrations in both leaf and reproductive organs (Iriti *et al.* 2009; Faralli *et al.* 2016; Faralli *et al.* 2017a), it is possible that the different yield response to VG over GS 6.0 and GS 7.0 could be due to the sensitivity to ABA of the two phenological stages. Indeed, while ABA has been reported to be involved in early reproductive failure on wheat (Westgate *et al.* 1996) and soybean (Liu *et al.* 2004) the accumulation of ABA in wheat spikelets during the grain filling stage is considered a desirable trait (Foulkes *et al.* 2001). This is because ABA counteracted the detrimental effect of ACC (thus ethylene) on grain filling thus leading to higher seed weight and lower seed abortion under stress. Despite the fact that no work has been done on the effect of ABA/ACC ratio during pod development/seed filling stage in BN, we can speculate that VG application over GS 7.0 mitigated the ABA accumulation on pods and seeds and therefore reduced the beneficial effects of ABA during the seed filling stage. Indeed, in de Bouille *et al.* (1986), ABA accumulated in BN seeds during the late stage of pod development/ initiation of seed filling, suggesting that, as for other crops, ABA may possibly modulate assimilate flux to seeds and thus induce seed maturation.

Application of film antitranspirant has been previously used in a broad range of crops to mitigate drought induced yield losses (e.g on sorghum in Fuehring, 1975) and recently in field-grown wheat (Weerasinghe *et al.* 2016) and pot-grown oilseed rape (Faralli *et al.* 2016; Faralli *et al.* 2017a). There is only one publication available testing the efficacy of different AT to avoid yield losses on a crop belonging to the same BN family (*Brassica campestris*) (Patil and De, 1978). Mobileaf (the film forming chemical), increased seed yield irrespective of the N supply in both years with an average of 0.41 t ha^{-1} following an ameliorative effect on plant water status. In these experiments the control un-irrigated and un-treated showed a lower seed yield than in our work on average (1.60 t ha^{-1}). The lower seed yield found by Patil and De

(1978) when compared to our work may be for two reasons. First, the crop was a spring variety, and it is well known that spring varieties generally exhibit lower yield than the winter crop. Second, the crop was grown under dry-land conditions with high temperature (~25 °C whilst in the present work the average spring temperature was ~12 °C) in both years and low precipitation.

Conclusions

Consistent with previous work, the efficacy of an antitranspirant treatment is confined to the most drought-sensitive stages where maintaining high plant water status can sustain the reproductive capacity under reduced water availability. In addition, our work has been carried out under relatively cool springs where the loss of evaporative cooling following the reduction in stomatal conductance did not have a detrimental effect on the physiological traits analysed. Therefore, further investigations on the efficacy of AT should be done under different environmental conditions and on a broader range of crops to better define their use and potential. To conclude, our work suggests a potential use of the antitranspirant VG to reduce yield losses when applied at 1 L ha^{-1} just prior to GS 6.0 on BN subjected to water stress.

References

Abdullah AS, Aziz MM, Siddique KHM, Flower KC (2015) Film antitranspirants increase yield in drought stressed wheat plants by maintaining high grain number. *Agricultural Water Management* **159**, 11-18.

Bell JP (1987) Neutron probe practice. 3rd edition. Wallingford, Institute of Hydrology, (IH Report No.19)

Berry PM, Spink JH (2009) Understanding the effect of a triazole with anti-gibberellin activity on the growth and yield of oilseed rape (*Brassica napus*). *The Journal of Agricultural Science* **147**, 273-285.

Berry PM, Spink JH (2006) A physiological analysis of oilseed rape yields: Past and future. *Journal of Agricultural Science* **144**, 381-392.

Champolivier L, Merrien A (1996) Effects of water stress applied at different growth stages to *Brassica napus* L. var. oleifera on yield, yield components and seed quality. *European Journal of Agronomy* **5**, 153-160.

Cossani CM, Reynolds MP (2012) Physiological traits for improving heat tolerance in wheat. *Plant physiology* **160**, 1710-1718.

de Bouille P, Sotta B, Miginiac E, Merrien A (1989) Hormones and pod development in oilseed rape (*Brassica napus*). *Plant physiology* **90**, 876-880.

Del Amor FM, Cuadra-Crespo P, Walker DJ, Cámara JM, Madrid (2010) Effect of foliar application of antitranspirant on photosynthesis and water relations of pepper plants under different levels of CO_2 and water stress. *Journal of plant physiology* **167**, 1232-1238.

Faralli M, Grove I, Hare M, Boyle R, Williams K, Corke FMK, Kettlewell P (2016) Canopy application of film antitranspirants over the reproductive phase enhances yield and yield-related physiological traits of water stressed oilseed rape (*Brassica napus* L.). *Crop and Pasture Science* **67**, 751-765

Faralli M, Grove IG, Hare MC, Kettlewell PS, Fiorani F (2017b) Rising CO_2 from historical concentrations enhances the physiological performance of *Brassica napus* seedlings under optimal water supply but not under reduced water availability. *Plant, Cell & Environment* **40**, 317-325.

Faralli M, Grove IG, Hare MC, Alcalde-Barrios A, Williams KS, Corke FMK, Kettlewell PS (2017). Modulation of *Brassica napus* source–sink physiology through film antitranspirant induced drought tolerance amelioration that is dependent on the stress magnitude. *Journal of Agronomy and Crop Science.*

Foulkes MJ, Slafer GA, Davies WJ, Berry PM, Sylvester-Bradley R, Martre P, ... & Reynolds MP (2011) Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. *Journal of Experimental Botany* **62**, 469-486.

Fuehring HD (1975) Yield of dryland grain sorghum as affected by antitranspirant, nitrogen, and contributing micro-watershed. *Agronomy Journal* **67**, 255-257.

Hall DGM, Reeve MJ, Thomasson AJ, Wright VF (1977) Water retention, porosity and density of field soils. Soil Survey Technical Monograph No. 9. Soil Survey of England and Wales, Harpenden.

Hess L, Meir P, Bingham IJ (2015) Comparative assessment of the sensitivity of oilseed rape and wheat to limited water supply. *Annals of Applied Biology* **167**, 102-115

Iriti M, Picchi V, Rossoni M, Gomarasca S, Ludwig N, Gargano M, Faoro F (2009) Chitosan antitranspirant activity is due to abscisic acid-dependent stomatal closure. *Environmental and Experimental Botany* **66**, 493-500.

Istanbulluoglu A, Arslan B, Gocmen E, Gezer E, Pasa C (2010) Effects of deficit irrigation regimes on the yield and growth of oilseed rape (*Brassica napus* L.) *Biosystems Engineering* **105**, 388-394

Jensen CR, Mogensen VO, Mortensen G, Andersen MN, Schjoerring JK, Thage JH, Koribidis J (1996) Leaf Photosynthesis and drought adaptation in field-grown oilseed rape (*Brassica napus* L.). *Australian Journal of Plant Physiology* **23**, 631-644

Liu F, Jensen CR, Andersen MN (2004) Pod set related to photosynthetic rate and endogenous ABA in soybeans subjected to different water regimes and exogenous ABA and BA at early reproductive stages. *Annals of Botany* **94**, 405-411.

Mendham NJ, Shipway PA, Scott RK (1981) The effect of delayed sowing and weather on growth, development and yield of winter oilseed rape (*Brassica napus*). *Journal of Agricultural Science* **96**, 389-416

Mogensen VO, Jensen CR, Mortensen G, Andersen MN, Schjoerring JK, Thage J H, Koribidis J (1997) Pod photosynthesis and drought adaptation of field grown rape (*Brassica napus* L.). *European journal of agronomy* **6**, 295-307.

Müller T, Lüttschwager D, Lentzsch P (2010) Recovery from drought stress at the shooting stage in oilseed rape (*Brassica napus*). *Journal of Agronomy and Crop Science* **196**, 81-89.

Murchie EH, Lawson T (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of experimental botany* **64**, 3983-3998.

Patil BB, De R (1976) Influence of antitranspirants on rapeseed (*Brassica campestris*) plants under water-stressed and non-stressed conditions. *Plant Physiology* **57**, 941-943.

Patil BB, De R (1978) Studies on the effect of nitrogen fertilizer, row spacing and use of antitranspirants on rapeseed (*Brassica campestris*) grown under dryland conditions. *The Journal of Agricultural Science* **91**, 257-264.

Rowell DL (1994) Soil science: methods and applications. Essex: Longman Group.

Salter PJ, Goode JE (1967) Crop responses to water at different stages of growth. Commonwealth Agricultural Bureaux, Farnham Royal, UK.

Solarova J, Pospisilova J, Slavik B (1981) Gas exchange regulation by changing of epidermal conductance with antitranspirants. *Photosynthetica* **15**, 365-400.

Toogood JA (1958) A simplified textural classification diagram. *Canadian Journal of Soil Science* **38**, 54-55.

Vadez V, Kholova J, Medina S, Kakkera A, Anderberg H (2014) Transpiration efficiency: new insights into an old story. *Journal of Experimental Botany* **65**, 6141-6153.

Wang Y, Ying J, Kuzma M, Chalifoux M, Sample A, McArthur C, Huang Y (2005) Molecular tailoring of farnesylation for plant drought tolerance and yield protection. *The Plant Journal* **43**, 413-424

Wang Y, Beaith M, Chalifoux M, Ying J, Uchacz T, Sarvas C, ... Huang Y (2009) Shoot-specific down-regulation of protein farnesyltransferase (α-subunit) for yield protection against drought in canola. *Molecular Plant* **2**, 191-200.

Weerasinghe MM, Kettlewell PS, Grove IG, Hare MC (2016). Evidence for improved pollen viability as the mechanism for film antitranspirant mitigation of drought damage to wheat yield. *Crop and Pasture Science* **67**, 137-146.

Westgate ME, Passioura JB, Munns R (1996) Water status and ABA content of floral organs in drought-stressed wheat. *Functional Plant Biology* **23**, 763-772.

Yang Z, Liu J, Tischer SV, Christmann A, Windisch W, Schnyder H, Grill E (2016) Leveraging abscisic acid receptors for efficient water use in Arabidopsis. *Proceedings of the National Academy of Sciences*, 201601954.

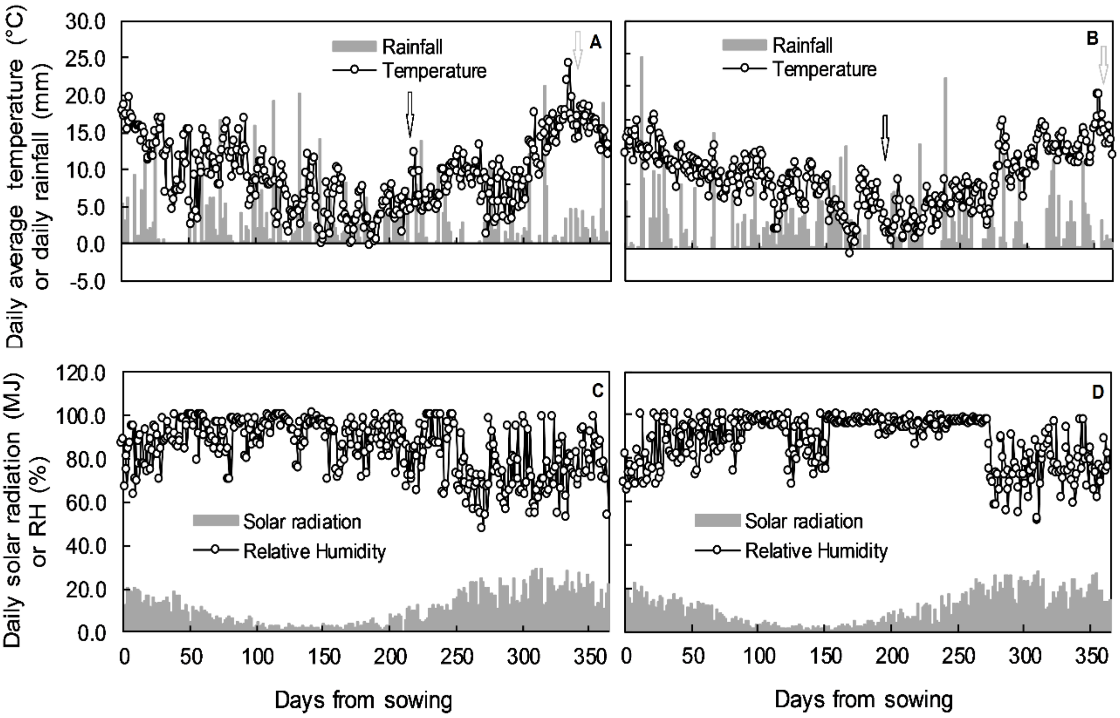

Figure 1. Daily average temperature (°C), daily rainfall (mm), daily solar radiation (MJ) and relative humidity (RH, %) for Experiment I (2014-2015, A and C) and for Experiment II (2015-2016, B and D). The data are shown as sowing date as 0. Black arrows represent the date for rain shelter application for Experiment I (A) and Experiment II (B). Grey arrows represent the harvest for Experiment I (A) and Experiment II (B).

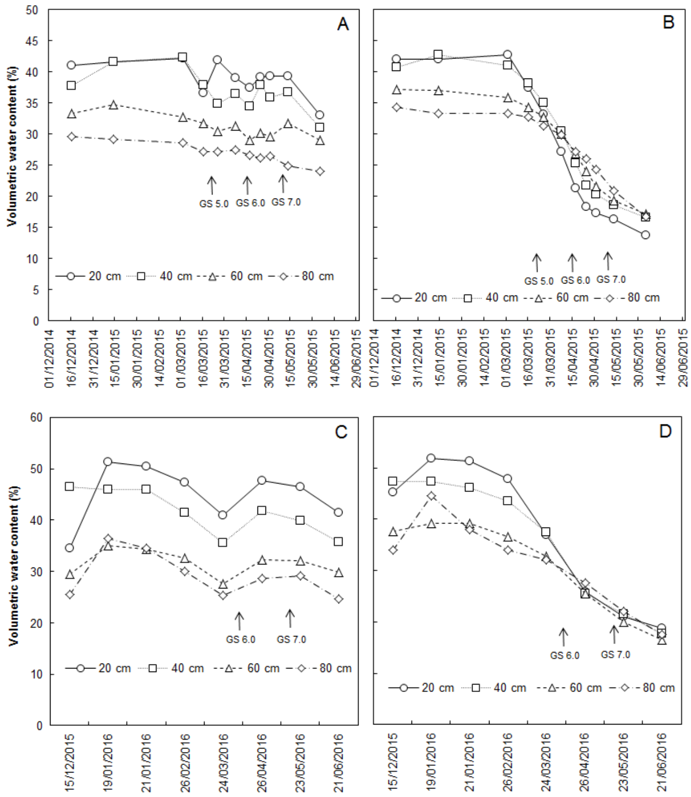

Figure 2. Volumetric water content (VWC, %) for Experiment I (A, irrigated plots, B un-irrigated plots) and Experiment II (C, irrigated plots, D un-irrigated plots) collected with the neutron probe at 20, 40, 60 and 80 cm depth. Arrows represent the growth stages at which chemicals were applied. Data are means (n=3 for A and D and n=21 for B; in C, all the means are n=3 except for 80 cm depth where n=2)

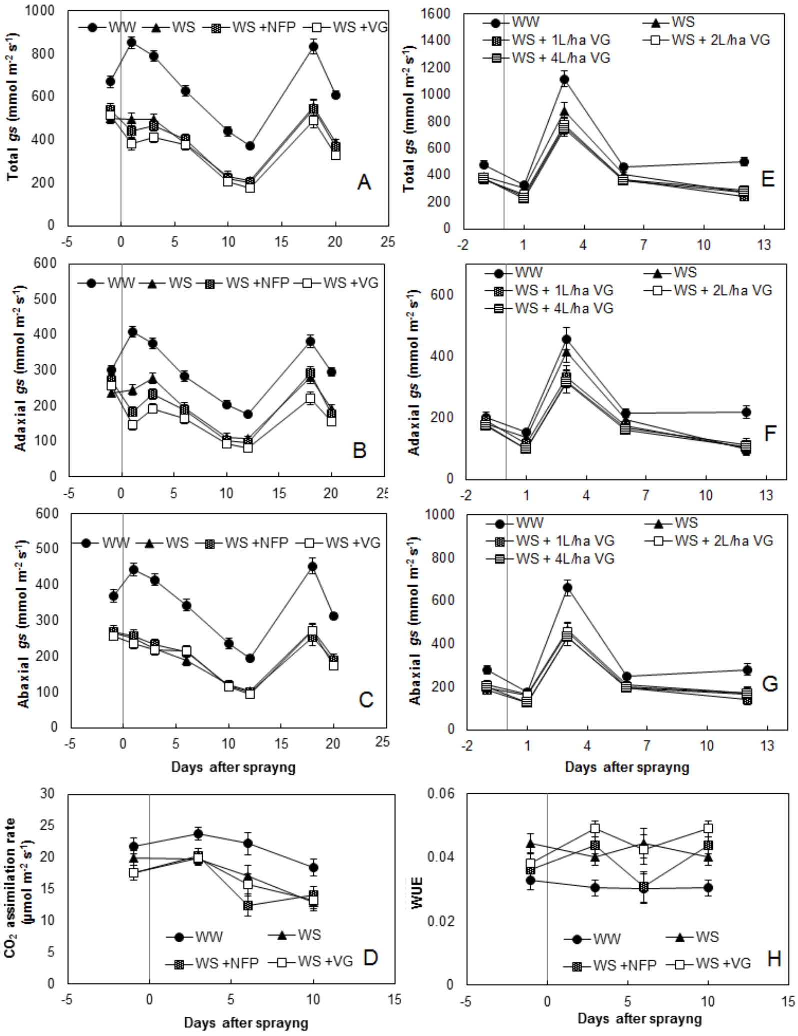

Figure 3. Total, adaxial and abaxial stomatal conductance (*gs*) for canola plots over GS 6.0 of Experiment I (A, B and C) and Experiment II (E, F and G). For Experiment I data are means (n=4) ± SE collected in irrigated (WW), un-irrigated (WS), un-irrigated treated with 1 L ha^{-1} Nu Film P (WS+NFP) and un-irrigated treated with 1 L ha^{-1} Vapor Gard (WS+VG). In Experiment II data are means (n=6) ± SE collected in irrigated (WW), un-irrigated (WS), un-irrigated treated with 1 L ha^{-1} Vapor Gard (WS+ 1L/ha VG), un-irrigated treated with 2 L ha^{-1} Vapor Gard (WS+ 2L/ha VG) and un-irrigated treated with 4 L ha^{-1} Vapor Gard (WS+ 4L/ha VG). CO_2 assimilation rate (D) and leaf water-use efficiency (H, WUE) calculated as the ratio between CO_2 assimilation rate and transpiration for canola plots over GS 6.0. Data are means (n=4) ± SE and collected in Experiment I.

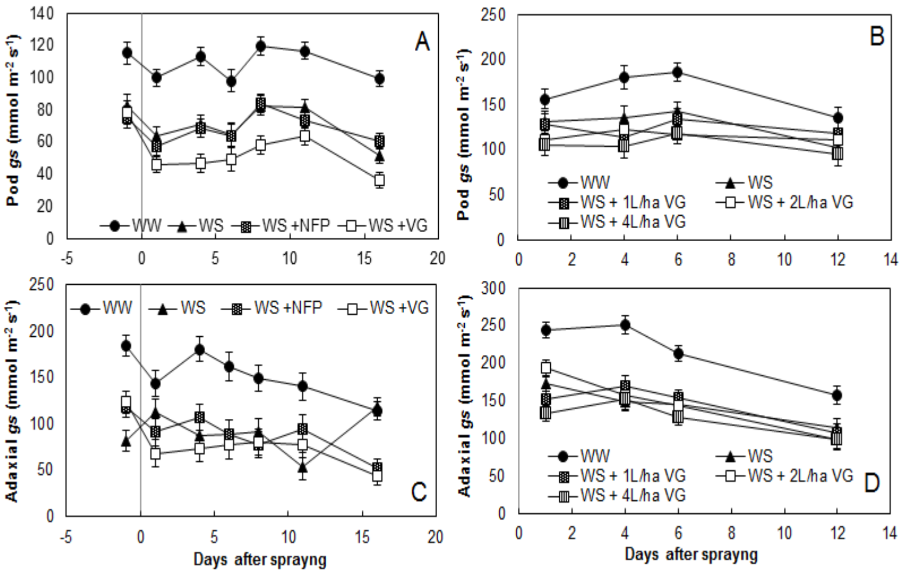

Figure 4. Pod and adaxial stomatal conductance (*gs*) for canola plots over GS 7.0 of Experiment I (A and C) and Experiment II (B and D). For Experiment I data are means (n=4) ± SE collected in irrigated (WW), un-irrigated (WS), un-irrigated treated with 1 L ha^{-1} Nu Film P (WS+NFP) and un-irrigated treated with 1 L ha^{-1} Vapor Gard (WS+VG). In Experiment II data are means (n=6) ± SE collected in irrigated (WW), un-irrigated (WS), un-irrigated treated with 1 L ha^{-1} Vapor Gard (WS+ 1L/ha VG), un-irrigated treated with 2 L ha^{-1} Vapor Gard (WS+ 2L/ha VG) and un-irrigated treated with 4 L ha^{-1} Vapor Gard (WS+ 4L/ha VG).

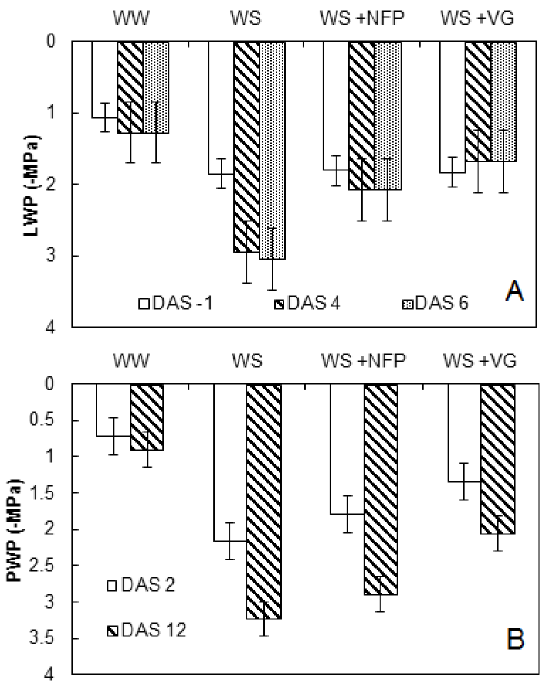

Figure 5. Leaf water potential (LWP, A) and pod water potential (PWP, B) for canola plots over GS 6.0 and GS 7.0 respectively. Data are means (n=5) ± SE collected in irrigated (WW), un-irrigated (WS), un-irrigated treated with 1 L ha^{-1} Nu Film P (WS+NFP) and un-irrigated treated with 1 L ha^{-1} Vapor Gard (WS+VG). DAS represents days after spray application. Data from Experiment I.

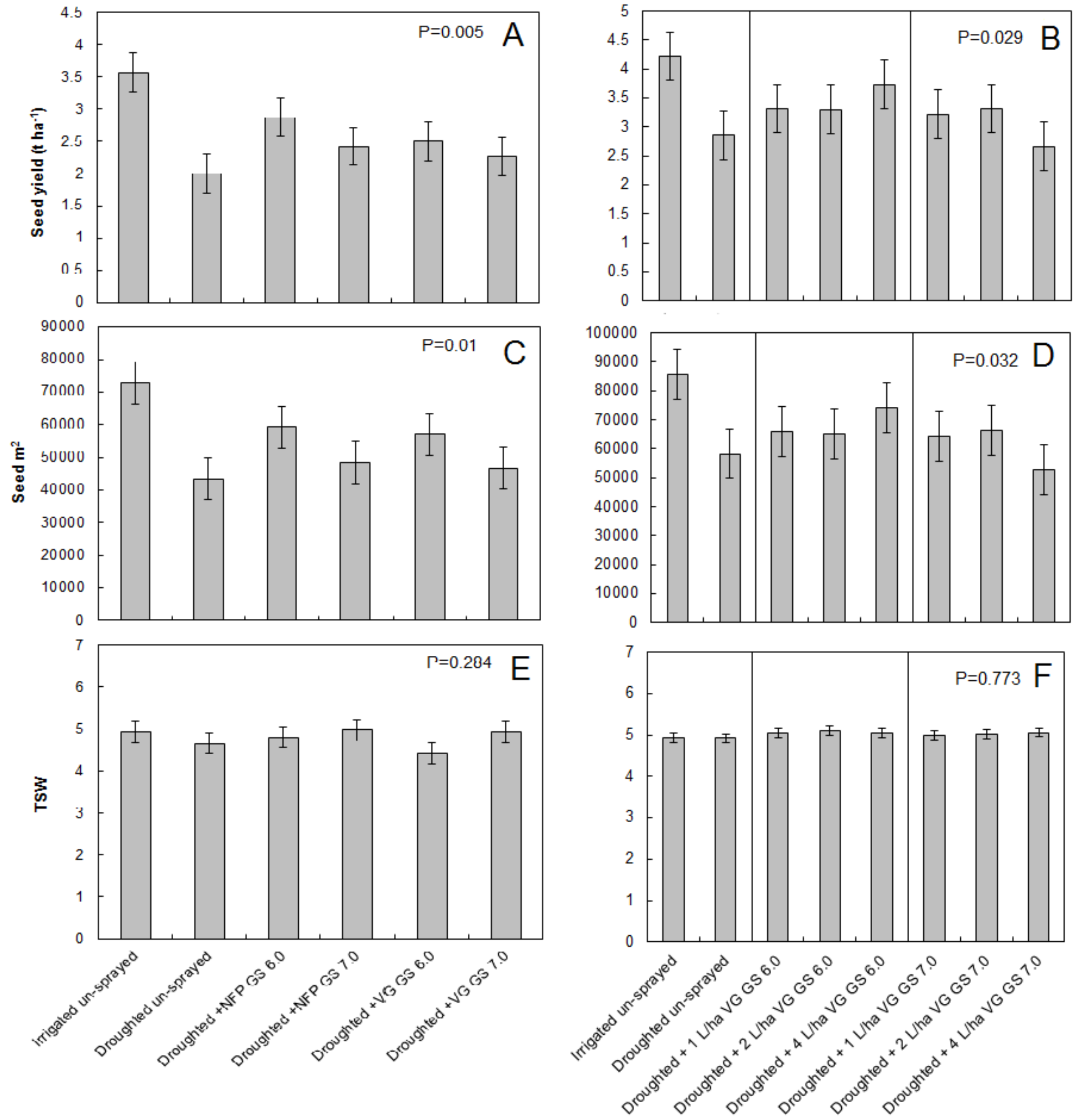

Figure 6. Seed yield (t ha^{-1}, A and B), seed per m^2 (C and D) and thousand-seed weight (TSW, E and F) of canola plots grown under irrigated and un-irrigated (droughted) conditions and sprayed at flowering (GS 6.0) or pod development (GS 7.0) stages with 1 L ha^{-1} of Nu-Film P (NFP) or Vapor Gard (VG) for Experiment I (A, C and E). On B, D and F (Experiment II), canola plots were grown under irrigated and un-irrigated (droughted) conditions and sprayed at flowering (GS 6.0) or pod development (GS 7.0) stages with 1 L ha^{-1}, 2 L ha^{-1} and 4 L ha^{-1} of Vapor Gard (VG). Data were analysed with ANOVA. Data are means (n=3) ± standard error of the differences of the means (SED) for Experiment I and means (n=5) ± SED for Experiment II.

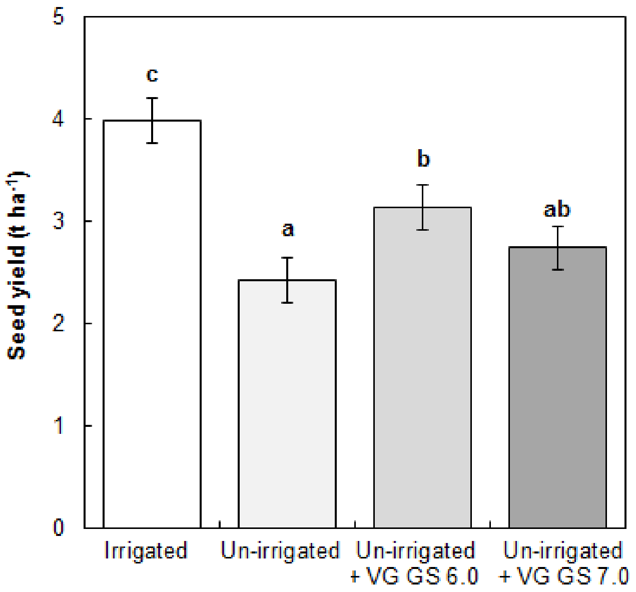

Figure 7. Pooled seed yield (t ha^{-1}) data for Experiment I and Experiment II of canola plots subjected to irrigation, reduced water availability through rain-shelters and treated with 1 L ha^{-1} Vapor Gard (+VG) or not (-VG) just prior to flowering (GS 6.0) or pod development (GS 7.0). Data are means (*n*=7) and error bars represent standard error of the differences of the means according to the ANOVA ($P<0.001$). Different letters represent significant differences according to the Tukey's test ($P<0.05$).