Evidence for improved pollen viability as the mechanism for film antitranspirant mitigation of drought damage to wheat yield

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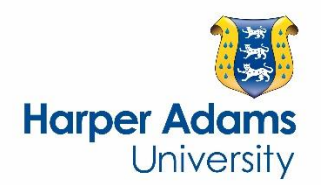

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Evidence for improved pollen viability as the mechanism for film antitranspirant mitigation of drought damage to wheat yield

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Abstract. Application of film antitranspirant to wheat during late stem extension reduces drought damage to yield, but the mechanism is unknown. Field experiments under rain shelters were conducted over three years to test the hypothesis that film antitranspirant applied before meiosis alleviates drought-induced losses of pollen viability, grain number and yield. The film antitranspirant di-1-*p*-menthene was applied at third node stage, and meiosis occurred at the early boot stage, with a range from 11 to 16 days after spray application in different years. Irrigated, unsprayed plots were included under the rain shelters, and pollen viability, measured in two years in these plots, averaged 95.3%. Drought reduced pollen viability to 80.1% in unirrigated, unsprayed plots, but only to 88.60% in unirrigated plots treated with film antitranspirant. Grains m^{-2} and yield of irrigated plots, measured in all three years, were 16,529 and 9.55 t ha^{-1} respectively on average. These were reduced by drought to 11,410 and 6.31 t ha^{-1} in unirrigated, unsprayed plots, but only to 12,878 and 6.97 t ha^{-1} in unirrigated plots treated with film antitranspirant. Thus compared with unirrigated, unsprayed plots, antitranspirant gave a grain yield benefit of 0.66 t ha^{-1}. Further work is needed to validate the pollen viability mechanism in different climatic zones and with a wide range of cultivars.

Summary Text for the Table of Contents

Yield loss from drought in wheat, one of the world's major food crops, is an increasing problem with climate change where irrigation is not possible. However, a spray application of an antitranspirant polymer before the sensitive reproductive stage of the wheat crop has been shown to enhance yield by reducing drought damage to pollen viability. This knowledge will help farmers to time application of these polymers to droughted wheat so that protection of yield from drought damage is more reliable.

Additional keywords: BBCH GS 33, pollen mother cells, polymer, water deficit, water potential, water stress

Running head: Film antitranspirant and droughted wheat pollen

Introduction

The importance of reproductive development in the physiology of drought damage to wheat is increasingly recognised (e.g. Ji *et al*. 2010). For yield formation, the stage of meiosis in pollen mother cells is the most sensitive stage of the crop to drought stress. Drought during this stage reduces pollen viability and hence number of grains and yield (Saini and Westgate 2000). Potential for exploitation of this knowledge in breeding drought tolerant wheat has been described for Australian germplasm (Ji *et al*. 2010).

It may also be possible to exploit this knowledge in improved crop management by using film antitranspirants (Kettlewell *et al.* 2010). Research into film antitranspirants carried out mainly in the period from the 1950s to the 1970s on a range of plant species showed clearly that although transpiration from the leaf could be reduced, the antitranspirant films are also less permeable to carbon dioxide entering the leaf and as a result photosynthesis and growth are reduced (Solarova *et al*. 1981). Subsequent text books on plant water relations (e.g. Jones 1992; Kramer and Boyer 1995) have concluded that the use of film

antitranspirants is only practical for situations, such as ornamentals, for which photosynthesis is less important but reduction in transpiration is advantageous. Use of film antitranspirants on cereal and other food crops, such as wheat, has not been recommended because photosynthesis is important in yield formation (Whitmore 2000).

Applying a film antitranspirant around the sensitive stage of booting has, however, been shown to be beneficial for wheat yield under high soil moisture deficits (Kettlewell *et al*. 2010), outweighing the detriment of reducing photosynthesis. Although it is speculated that a reduction in drought-induced pollen sterility is the cause of this yield increase, there is no previous published work investigating this mechanism. The research described in this paper aimed to test the hypothesis that applying a film antitranspirant before the stage of meiosis in pollen mother cells will lead to less negative leaf water potential, and alleviate the effect of drought on pollen viability, grain set and yield.

Field experiments to test this hypothesis were carried out at one site in the UK in the three years of 2009, 2010 and 2011 under rain shelters. A preliminary study was also conducted to determine the growth stage at which meiosis occurs in pollen mother cells in the cultivar of wheat used.

Materials and Methods

Meiosis and growth stage

The relationship between meiosis in pollen mother cells in winter wheat cultivar Claire and both the crop growth stage (GS) defined by the BBCH code (Meier 2001) and the external morphology of the shoot was explored using plants collected from field experimental plots of irrigated controls, which were free from drought stress and not sprayed with antitranspirants in 2009. The developing spikes were monitored for the initiation of anthers

by observing dissected shoots. Anthers were sampled every day after the stage where anthers could be distinguished from other floral parts. Photographs of the shoots were taken before dissecting so that the development stage and external appearance of the shoots could be related to the development stage of the anthers as identified after microscopic studies. Shoot morphology was characterised, at the beginning, in terms of the emerged length of the flag leaf blade and later on, in terms of the distance between the auricles of the flag leaf and the penultimate leaf, which is a measure of spikelet development (Ji *et al*. 2010). The length of the spikes at sampling was also noted. Only the spikelets from the middle of the spike, which are the most advanced in development (Bennett *et al*. 1973), were used in the study and anthers were collected only from the two most developed florets of the spikelets, which are the first and the second florets (Bennett *et al*. 1973). Care was taken to note down which anthers were from which floret; i.e. from the first floret or from the second floret. Whenever it was possible, anthers were used in microscopic studies on the same day of sampling after fixing and staining in one step using 1% acetocarmine solution (Li *et al*. 2005). If this was not possible due to time constraints, sampled spikelets were fixed in 1:3 Carnoy's solution (ethanol:acetic acid = 3:1) prior to staining (Bennett *et al*. 1973). The fixed or fresh anthers were separated from the florets; each anther was placed in a drop of 1% acetocarmine solution on a clean glass slide, covered with a coverslip and squashed gently by tapping the cover slip (Bennett *et al*. 1973) so that the columns of archesporial cells were extruded on to the slide. Then the slides were observed under the light microscope (Leitz DMRB, Leica, Nussloch, Germany) and photographs were taken from freshly prepared slides with a digital camera (Infinity-2, Lumenera Corporation, Ottawa ON, Canada) fixed to the microscope.

Site and sowing

All the field experiments were located within 100 m of each other in Flat Nook field, an experimental site at Harper Adams University, Shropshire (52°46′N, 2°25′W) on a loamy sand soil. The soil had 92% sand in the sub soil and 80% sand in the top soil, quantified by particle size distribution analysis (MAFF/ADAS 1987). The field capacity for a depth of 80 cm was determined to be 160 mm from neutron probe measurements (Institute of Hydrology Neutron Probe System, Wallingford). For a depth of 80 cm, the permanent wilting point was quantified to be 62 mm (Hall *et al*. 1977) and the available soil water capacity (AWC), which is the difference between the field capacity and the permanent wilting point (Hall *et al*. 1977), was thus calculated to be 98 mm. Although all the experiments were on the same experimental site, the exact location of the experiments was different from year to year. The previous crops on the location of the experiments were: 2009 maize, 2010 fallow (no crop), 2011 oilseed rape (canola).

Soil preparation for sowing consisted of conventional ploughing and power-harrowing, and seeds were sown at 2 cm depth on 26 November 2008, 19 October 2009 and 18 January 2011. The row spacing was 15 cm and the seed rate 350 seeds m^{-2} in all years.

Design and treatments in 2009

The experiment was a 2 x 6 factorial split-plot design with three randomised blocks, with each block in a separate rain shelter. Each block had two main plots, each main plot consisting of six sub plots. There were two winter wheat cultivars, Claire and Einstein, randomised to the two main plots in each block, and four antitranspirant treatments and two controls randomised to the six sub plots. Sub plots were approximately 10 × 1.5 m. Rain shelters were moved into position at the beginning of GS 25 (25 April 2009) and from that stage until harvest water was only applied to the irrigated, unsprayed control (IUC). The other control was unsprayed with no irrigation (UC). The four antitranspirant (di-1-p-menthene 96%; Emerald; Intracrop Ltd, Lechlade) treatments were sprayed at GS

33 (15 May 2009), GS 39 (22 May 2009), GS 41 (28 May 2009) and GS 59 (11 June 2009). Potential evapotranspiration and SMD were calculated using the IMS irrigation scheduling programme (Hess 1996) and SMD of the IUC plots was allowed to rise to 50% of field capacity (80 mm). The amount of water equivalent to two days water use was estimated and then applied on alternate days to the IUC plots to ensure that the SMD did not exceed 80 mm, i.e. at least 18 mm of water available at the driest point in time, since the AWC was calculated to be 98 mm.

Design and treatments in 2010

The experiment was conducted using one winter wheat cultivar, Claire, and was a 2 x 5 factorial split-plot design with three randomised blocks, with each block in a separate rain shelter. Each block had two main plots, each main plot consisting of five sub plots. The two main plots were randomly allocated to either a 'low SMD regime' i.e. less-stress, or a 'high SMD regime' i.e. more-stress. The five subplots were randomly allocated to four antitranspirant treatments and the UC. An additional plot, the same size as the sub plots, was randomised in each block for an IUC. Sub plots were approximately 10 × 1.5 m. Rain shelters were moved into position at the beginning of GS 25 (28 April 2010). The plots of the high SMD regime received no water until GS 69 when the SMD had risen to 115 mm, i.e. no plant-available water, since the AWC was calculated as 98 mm. All high SMD plots were then irrigated to field capacity at GS 69 (20 June 2010) and irrigation was continued to maintain the SMD near field capacity until irrigation for the whole experiment was ceased at GS 85 (15 July 2010) to enhance grain ripening. The SMD of the low SMD regime plots was allowed to rise to 90 mm at GS 37 (20 May 2010). From GS 37 to GS69, the amount of water equivalent to two days water use was estimated and then applied on alternate days to all the plots in the low SMD regime to ensure that the SMD did not exceed 90 mm, i.e. at least 8 mm available water at the driest point in time. All low SMD

144 plots were then irrigated to field capacity at GS 69 and irrigation was continued to
145 maintain the SMD near field capacity until irrigation for the whole experiment was ceased
146 at GS 85 to enhance grain ripening. The four antitranspirant treatments were di-1-p-
147 menthene sprayed at GS 31 (8 May 2010), GS 33 (16 May 2010) and GS 41 (25 May
148 2010) and latex (98%; Neo-Tex; Intracrop Ltd, Lechlade) sprayed at GS 41. For the IUC
149 plots, soon after the rain shelters were installed, the amount of water equivalent to two
150 days water use was estimated and then applied on alternate days until GS 69 to ensure that
151 the SMD did not exceed 40 mm (25% of field capacity), i.e. at least 58 mm available water
152 at the driest point in time. IUC plots were irrigated to field capacity at GS 69 and irrigation
153 was continued to maintain the SMD near field capacity until irrigation for the whole
154 experiment was ceased at GS 85 to enhance grain ripening.

155 *Design and treatments in 2011*

156 Two single factor experiments with antitranspirant treatments using cultivar Claire were
157 conducted in 2011. Experiment 1 had no irrigation from installation of the rain shelters at
158 GS 23 (1 April 2011) until GS 69 and Experiment 2 had no irrigation from installation of
159 the rain shelters to harvest. . Both experiments had three randomized blocks and each of
160 three rain shelters contained two blocks, one block of Experiment 1 and one block of
161 Experiment 2. Plot size for both experiments was 4 x 1.2 m and additional replication
162 within blocks was used to attempt to reduce the standard errors. There were six plots of
163 each treatment in each block of Experiment 1 and two plots of each treatment in
164 Experiment 2. . There were two antitranspirant treatments and an unsprayed, unirrigated
165 control in both Experiment 1 and Experiment 2. The antitranspirant treatments were di-1-
166 p-menthene sprayed at GS 33 (12 May 2011) and latex sprayed at GS 33. This growth
167 stage was chosen since it appeared to give the greatest effect of antitranspirant in the
168 previous two years. In addition to these treatments, two plots were located at one end of

each rain shelter (not randomized with the treatments), and were irrigated as a reference for comparison with the treatment plots (irrigated unsprayed control: IUC). For these plots, the amount of water equivalent to two days water use was estimated and then applied on alternate days until GS 69 to ensure that the SMD did not exceed 40 mm (25% of field capacity), i.e. at least 58 mm available water at the driest point in time. None of the treatments in Experiment 1 were irrigated until GS 69 (15 June 2011) when the whole experiment was irrigated to field capacity. Water was then withheld at GS 85 (10 July 2010) to enhance grain ripening. Experiment 2 was not irrigated at any time.

Antitranspirant spray application, irrigation and crop management

Antitranspirants were applied at 2.5 l ha^{-1} in a volume of 200 l (1.25% v/v antitranspirant product in water), using a hand held sprayer at a sprayer pressure of 0.2 MPa and a sprayer speed of 1 m s^{-1} with Flat Fan nozzles (Agratech; Lancashire; f110 03). The height of the boom was maintained at 0.5 m above the crop canopy while spraying. Irrigation was applied through irrigation tapes with 5 mm diameter emitters, each 10 cm apart, with one tape on each side of every crop row. Every 100 mm of length of the tapes delivered 750 l h^{-1}. Prior to moving the rain shelters into position the application of fertilizers and nutrients followed typical practice for intensively-grown wheat in the UK, but no applications of either fertilizers or nutrients were made after positioning the rain shelters to avoid a possible difference in nutrient uptake between irrigated plots and non-irrigated/droughted plots. Insecticides, herbicides and fungicides were applied whenever necessary.

Soil moisture measurements

The IMS irrigation scheduling programme was updated continuously with data from October 2008 to August 2011. The main inputs were: the maximum root length (80 cm

193 from the neutron probe readings); the "texture class" for both top-soil and sub-soil (loamy
194 sand; from particle size distribution analysis; MAFF/ADAS 1987); top soil depth (40 cm).
195 Weather data was obtained from the weather station at Harper Adams University
196 approximately 0.5 km from the experimental site.

197 In 2011, soil moisture measurements were taken with the neutron probe (Institute of
198 Hydrology Neutron Probe System, Wallingford). Access tubes were inserted in five
199 randomly-selected unsprayed control plots and in five randomly-selected irrigated
200 unsprayed control plots of the experiment under rain shelters. Soil moisture readings were
201 taken at 10 cm intervals from 2.5 cm to a maximum depth of 100 cm.*Leaf water potential*
202 *measurements*

203 Leaf water potential of the penultimate leaf and unrolled flag leaf of cultivar Claire was
204 measured in 2009 and 2010 with a Scholander pressure bomb (SKMP 1405/50, Skye
205 Instruments Ltd) using five randomly-selected shoots from the unsprayed control and five
206 from the GS 33 treatment one day before spraying, one day after spraying and three days
207 after spraying. Five shoots were also used for leaf water potential measurement of the
208 irrigated unsprayed control plots in 2009. In 2010, lack of time only permitted five shoots
209 to be measured for the first block, three shoots for the second block and two shoots for the
210 third block.

211 *Pollen viability assessment*

212 Pollen viability was assessed by the presence or absence of accumulated starch in 2010
213 and in the experiment irrigated after GS 69 in 2011. From each plot 10 anthers, which had
214 just dehisced, were excised and transferred into an Eppendorf tube with 1.5 ml of Lugol's
215 solution (Sigma-Aldrich, Dorset, UK). The tube was closed and shaken gently so that the
216 pollen grains were released into the solution. Only one anther excised from a first floret in

the middle of the spike was used, and the 10 spikes were selected from random locations. Dark colour eppendorf tubes were used, since Lugol's solution decomposes in the presence of direct sunlight. Immediately after the completion of pollen collection from each plot, the tube was placed in the dark in a box. The tubes were transferred to the laboratory and stored at 4ºC in the dark.

Within a week of collection, each eppendorf tube was shaken gently so that the pollen grains distributed evenly within the solution, and 1 ml of the sample was transferred into 2.5 ml of distilled water in a watch-glass. A 1 ml sub-sample was then transferred into a Sedgwick Rafter counting chamber using a disposable plastic pipette. The pollen grains in the Sedgwick Rafter counting chamber were observed under the light microscope (40x10). Pollen grains stained fully with a dark blue/black colour were considered fertile and pollen grains which were unstained or partially stained were considered sterile (Nelson 1968). In 2010, the total number of pollen grains and the number of fertile pollen grains within a randomly selected grid cell were counted and the percentage of fertile pollen in the grid cell was calculated. From each diluted sample, three replicates of pollen samples were observed. In 2011, ten randomly selected grid cells were counted.

Yield and yield component assessment

At maturity, and a few days before harvest, all head-bearing shoots from one 1 m^2 quadrat in a randomly selected position per plot were removed and counted. The samples were threshed using an electric thresher (Wintersteiger, Austria), cleaned to remove chaff, the moisture content measured with a moisture analyser (AP 6060, Sinar, Surrey, UK) and weighed. Grain yield in t ha^{-1} was calculated at 15% moisture. After weighing, subsamples of about 40 g were separated from each sample, hand-cleaned, weighed again, and the number of grains in each sub sample was counted with a grain counter

(CountAmatic Console, Farm-Tec, Whitby, UK). The grains head^{-1} and thousand grain weight (TGW) were calculated according to Sylvester-Bradley *et al*. (1985). The grains m^{-2} were calculated by multiplying the heads m^{-2} by grains head^{-1}.

Statistical analysis

Data was analysed by ANOVA with using GenStat 13th edition (VSN International, Hemel Hempstead UK). For some analyses, additional data were used as covariates in the ANOVA and if significant the output from these analyses was presented. Where covariates were not significant, the output from ANOVA without the covariate was presented. After the completion of all the field experiments, the soil electrical conductivity was measured by Soyl Precision Farming (Newbury, United Kingdom) using electromagnetic induction at the locations of the field experiments to assess spatial differences in water-holding capacity. The plot layouts were drawn to scale on the soil electrical conductivity maps to quantify the electrical conductivities of each plot. The soil electrical conductivity of each plot was then used as a covariate in the analyses of yield and yield component data from the three years, but was only significant for yield for 2011. In 2009, there was a row of plots (3 out of 36 plots) with a lower sowing rate compared to the other plots. When used as a covariate, this difference was significant only in the ANOVA of grains head^{-1}. In 2011, 18 out of 54 plots within Experiment 1 and 2 out of 18 plots within Experiment 2 were of low crop density (due to lower sowing rate and invasion of weeds). This difference was included as a covariate, but was significant only for yield and heads m^{-2}.

Although the design of the four experiments in the three years differed, the unsprayed control and antitranspirant application at GS 33 were common for all experiments. In order to provide an overall test of the effect of an antitranspirant applied at GS 33, mean values for these treatments, pooled standard errors of difference and t tests were calculated over

all four experiments for yield components and yield. The overall effect of drought was not tested, since the irrigated unsprayed control means were not part of the randomisation in 2011.

Presentation of data

The means and probabilities are presented in Tables 2 and 3 for two orthogonal contrasts: (a) comparing the irrigated treatment with the mean of the unirrigated, unsprayed and unirrigated, antitranspirant application at GS 33 treatments, and (b) within the unirrigated treatments, comparing antitranspirant application at GS 33 with unsprayed. These contrasts were chosen because the GS 33 timing appeared to give the largest response to antitranspirant across the two years 2009 and 2010 (although timing differences were not statistically significant),. and GS 33 was the only timing common to all three years, The results for antitranspirant and control treatments are presented using means for the two cultivars in 2009 and means for the two SMD regimes in 2010, because the interactions between cultivar and antitranspirant treatment in 2009 and between SMD regime and antitranspirant treatment in 2010 were not significant. The results presented for 2011 yield and yield components were from analyses of the two experiments combined, since there was little difference in response to antitranspirant between the two experiments. In addition the means are presented of latex and di-1-p-menthene at GS 33 since there was no significant difference between the two treatments.

Results

Meiosis and growth stage

Anthers which were at meiotic stages were from shoots at early GS 41 and the mean length between the auricles of the flag leaf and the auricles of the penultimate leaf, of the 18

shoots which were bearing anthers at meiotic stages, was 4.5 cm (Fig. 1a), and the mean length of the spikes was 6 cm (Fig. 1b). All the stages of meiosis were visible at early GS 41, and some of the observed stages of meiosis from anaphase 1 to tetrad stage (Bennett *et al*. 1973) are shown in Fig. 2. The value 4.5 cm for the length between the auricles of the flag leaf and the penultimate leaf was used to estimate the date that meiosis in pollen mother cells occurred in each experiment on the assumption that the meiosis timing and auricle length relationship was similar in subsequent years. The estimated date of meiosis was 30 May, 28 May and 26 May respectively in the three years, equivalent to 16, 11, and 14 days after the GS 33 spray.

[Figs 1 and 2 near here]

Weather and SMD

The monthly mean temperature and rainfall for the external environment for each growing season are shown in Table 1. The three years varied in mean temperature during the main period of water use from April (when rain shelters were moved into position) to maturity in August, with 2009 being the warmest (14.1°C) and 2010 being the coolest (13.6°C), with 2011 intermediate (13.9°C).

[Table 1 near here]

The changes in SMD with time inside the rain shelters in all the three years, as calculated by the IMS irrigation scheduling programme, are shown in Fig. 3. The SMDs at the spray application time of GS 33 had not risen to the level of the available water capacity of 98 mm, but in all years exceeded the easily available water capacity (60% of the total available water capacity, equivalent to 59 mm SMD). This indicates that plants would have been beginning to experience stress in all three years, although this was greatest in 2011

311 and least in 2009. At the time of meiosis, the SMDs had risen above the total available
312 water capacity of 98 mm in 2011 and in 2010 for the high SMD regime, and the SMD in
313 2009 was not far below 98 mm, indicating severe stress in all three years. The low SMD
314 regime clearly reduced the deficit as intended.

315 In 2011, SMD was also calculated from the soil moisture measurements obtained from the
316 neutron probe as well, and regression analysis showed that the results from the two
317 methods are very closely related (Fig. 4; $p<0.001$; $R^2 = 0.993$), giving confidence in the
318 IMS calculations. The equation of the fitted line indicates, however, that the IMS
319 algorithm slightly overestimated the SMD compared with the neutron probe, because the
320 intercept is above zero and the slope is slightly greater than one.

321 [Figs 3 and 4 near here]

322 *Leaf water potential*

323 The water potential values indicated that plants in the unirrigated plots were much more
324 stressed in 2010 than in 2009 (Table 2). Irrigation clearly reduced the stress in both years,
325 and the leaf water potential of the plants given antitranspirant treatment was less negative
326 than that of the unsprayed control plants three days after the spray application (borderline
327 significant in 2009). Averaged over both years, three days after spraying, drought reduced
328 the water potential from -0.40 to -0.88 in unsprayed plots, but only to -0.69 in sprayed
329 plots.

330 [Table 2 near here]

331 *Pollen viability, yield components and yield*

332 Drought gave a much greater reduction in pollen viability in 2010 than in 2011, but in both
333 years the treatment means indicated that pollen viability of the antitranspirant-treated

plants was greater than that of the unsprayed control, although this was only significant in 2010 (Table 3). Statistical significance for the yield components varied between years, but generally the negative effect of drought on yield was from reduced grains m^{-2}, whereas the TGW was not affected or was increased. Both heads m^{-2} and grains $head^{-1}$ appeared to contribute to the grains m^{-2} reduction. Yield of antitranspirant-treated plots was only significantly improved in 2011, but there was an indication that yield benefited from antitranspirant in all three years.

Combining data from all three years (Table 3) showed that drought reduced yield by 3.24 t ha^{-1}, but in plots sprayed with antitranspirant drought only reduced yield by 2.58 t ha^{-1}, a benefit from antitranspirant of 0.66 t ha^{-1}. This effect of the antitranspirant derived from reduced loss of grains m^{-2}, with thousand grain weight unaffected. In turn, the response of grains m^{-2} derived from a combination of effects on grains $head^{-1}$ and heads m^{-2}.

[Table 3 near here]

Discussion

Since the rain shelters were necessary to ensure reliable imposition of drought, it is possible that the protected environment within the rain shelters, in particular an increased temperature, may have influenced the results. Two measures were taken to reduce temperature rise inside the rain shelters: in all three years the lowest 1 m either side of the rain shelters was not covered by polythene, and in 2010 and 2011 one open end of each rain shelter faced the prevailing wind direction. Under dull conditions the difference in temperature between the two environments was almost undetectable. When the solar radiation was above 5 W m^{-2} and when there was no wind passing through the rain shelters the maximum difference between air temperature inside and outside the shelters was 3 ºC. However, since results were always compared between plots within the same shelter and

358 exposed to the same environment, then environmental differences between the external
359 and internal environment should not affect the validity of the treatment comparisons.

360 The water potential values indicate that in both 2009 and 2010 the plants were stressed,
361 when compared with other studies on imposed drought in wheat plants e.g. Quarrie and
362 Jones (1979). Wheat leaf water potential displays anisohydric behaviour, i.e. the leaf water
363 potential changes according to the changes in the rate of transpiration during the day
364 (decreases with increasing transpiration rate and increases with decreasing transpiration
365 rate), and is lower in plants growing under high SMDs compared to plants under low
366 SMDs (Henson *et al*. 1989). Thus the less negative leaf water potential in antitranspirant-
367 treated plants found in the experiments in this paper is assumed to reflect more water
368 conserved within the plant as a result of reduced transpiration. These results are consistent
369 with the work of other researchers who have demonstrated that leaf water potential in other
370 anisohydric species is increased as a result of reduced transpiration by film antitranspirants
371 e.g. potato (Win *et al*. 1991), and with increases in leaf turgor from antitranspirant found in
372 a recent study on wheat (Abdullah *et al*. 2015) . Abdullah *et al*. (2015) also reported that in
373 their pot experiment application of an antitranspirant reduced water use and it is possible
374 that in the experiments described in this paper that moisture was conserved in the soil by
375 reduced transpiration and may have slowed the development of the SMD.

376 Wheat floral organs maintain high internal water status even in the period of substantial
377 leaf drying during drought stress; therefore, it appears that inhibition of pollen
378 development under drought stress results from a yet undefined signal from the roots or
379 other vegetative organs affected by drought stress (Saini and Westgate 2000). There are
380 reports that abscisic acid, cytokinins, reactive oxygen species and various other molecules
381 act as sporocidal signals in triggering drought stress response reactions in plants (Huang *et*
382 *al*. 2012), and it can be speculated that antitranspirant may reduce the production of

383 sporicidal signals and thereby mitigate drought effects on pollen viability. Indeed in
384 droughted canola, it has been shown that abscisic acid concentration in leaf and
385 reproductive organs is depressed after antitranspirant application (M. Faralli, personal
386 communication).

387 The antitranspirant was applied during late stem extension between 11 and 16 days before
388 the stage at which meiosis occurs, and sprays closer in time to this stage are generally less-
389 effective at reducing yield loss from drought (Kettlewell *et al*. 2010; Weerasinghe 2013).
390 The reason why a period of several days needs to elapse between spraying and meiosis to
391 achieve the best response is not clear. One possible hypothesis is that sufficient time is
392 needed for drought-induced sporocidal signals to degrade in response to the less negative
393 water potential. An alternative hypothesis could be that, during late stem extension
394 photosynthate supply is in excess and surplus photosynthate is being stored in stem
395 internodes (Schnyder 1993) and photosynthate supply is thus not critical for growth of
396 yield components. Therefore a reduction in photosynthesis from antitranspirant application
397 at late stem extension may be of relatively little consequence for yield, and by the time of
398 meiosis photosynthesis may not have reduced as much as in unsprayed plants. In support
399 of this, Abdullah *et al*. (2015) found that in wheat after a few days the decline in
400 photosynthesis in antitranspirant=treated plants stabilised compared with unsprayed plants
401 which continued to reduce in photosynthesis. Faralli *et al*. (2015) found similar results in
402 canola. In contrast, a reduction in photosynthesis from antitranspirant application at
403 meiosis may have detrimental effects on pollen viability which counteract the benefit from
404 reduced water loss.

405 The mitigation of drought damage to yield by antitranspirant resulting from alleviation of
406 drought damage to grains m^{-2} is consistent with the results of Abdullah *et al*. (2015) in
407 glasshouse-grown wheat. They found that reduced damage to yield from antitranspirant

408 resulted mainly from effects on grains head^{-1} with little effect on head number or grain
409 weight. The antitranspirant effect on increasing heads m^{-2} in the experiments in this paper
410 may have occurred indirectly through development of grains in tillers which would
411 otherwise have been infertile from drought effects on pollen, and therefore not counted as
412 fertile, grain-bearing heads. The lack of effect of drought (and antitranspirant) on TGW, is
413 presumed to be a result of yield component compensation (Egli 1998), since grain weight
414 is determined after the number of grains. This is consistent with the suggestion of Liu *et al*.
415 (2005) that drought damage to pollen has evolved because it confers a selective advantage
416 by reducing demand for the limited assimilate supply. The smaller number of grains set
417 can then each achieve sufficient carbohydrate reserves to enable successful seed
418 germination.

419 Some studies report yield improvements from film antitranspirants in other seed crops
420 including corn (Fuehring and Finckner 1983), sorghum (Fuehring 1973) and rapeseed
421 (Patil and De 1978). These studies, however, have not attempted to explore the effects of
422 film antitranspirants on yield components or on the underlying mechanisms of the yield
423 increase by antitranspirants. Our finding that the antitranspirant treatment alleviated the
424 effect of drought on pollen viability at the stage of meiosis in pollen mother cells appears
425 to be the first report of a study of the effect of film antitranspirants on pollen in relation to
426 yield. This finding is, however, an association and it is possible that there is not a causal
427 relationship between pollen viability and yield. Further studies are needed to explore the
428 association between antitranspirant application, reduced damage to pollen viability and
429 yield to clarify whether the relationship is causal.

430 A reduction in photosynthesis by an antitranspirant treatment at any growth stage may
431 reduce the total amount of photosynthate available for grain filling (i.e. the source). Yield
432 of modern wheat is, however, more sink limited than source limited during grain filling,

433 and it has been suggested that sink strength during grain filling is the main factor limiting
434 yield potential in wheat (Reynolds *et al*. 2009). This might be one of the reasons why an
435 increase in sink, even with a concomitant decrease in source, from an antitranspirant
436 application may ultimately bring about yield increases in wheat. Further research to
437 understand the dynamics of source-sink relations in antitranspirant-treated wheat may be
438 helpful in optimising the use of film antitranspirant for reducing drought damage to wheat
439 crops in semi-arid and arid areas.

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528

529 **Table 1. Monthly mean temperature and total rainfall for 2008/9 to 2010/11 growing**

530 **seasons**

	Mean Temperature (ºC)			Mean rainfall (mm/day)		
Month	2008/9	2009/10	2010/11	2008/9	2009/10	2010/11
October	9.95	11.95	10.1	3.3	1.7	1.9
November	7.05	8.50	4.80	2.0	4.1	1.3
December	3.30	2.70	-1.95	1.5	1.6	0.8
January	2.80	1.20	3.55	2.2	1.7	1.4
February	4.60	2.90	6.85	0.8	1.0	1.9
March	7.15	6.00	6.65	0.8	1.4	0.4
April	10.20	8.95	11.65	1.4	0.9	2.4
May	12.35	11.10	12.50	1.6	0.9	1.7
June	15.05	15.70	13.80	3.1	2.0	1.7
July	16.25	16.85	15.60	4.6	1.3	1.8
August	16.75	15.30	16.05	1.2	1.5	1.0

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539 **Table 2**. **Leaf water potential (MPa) before and after antitranspirant application**

Year	Measurement time relative to spray date	IUC[A]	UC	GS33	SEM	*P* irrigated vs mean unirrigated	*P* antitranspirant vs unsprayed
2009	-1	- 0.11	- 0.23	- 0. 24	0.025	0.014	0.676
	+1	- 0.12	- 0.22	- 0.14	0.034	0.192	0.142
	+3	- 0.26	- 0.73	- 0.56	0.042	0.002	0.051
2010	-1	- 0.49	- 1.09	- 1.07	0.077	0.003	0.823
	+1	- 0.40	- 1.09	- 0.85	0.063	0.002	0.055
	+3	- 0.54	- 1.03	- 0.81	0.044	0.002	0.026

540 [A]IUC = irrigated unsprayed control; UC = unsprayed control; GS33 = antitranspirant
541 treatment at GS33; SEM = standard error of mean (26 degrees of freedom).

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545 **Table 3. Pollen viability, yield components and yield in response to antitranspirant**

Year	Measurement	Treatment			SEM[B] (DF)	*P*[C] irrigated vs mean unirrigated	*P* antitranspirant vs unsprayed
		IUC[A]	UC	GS 33			
2009	Heads m^{-2}	361	248	281	12.6 (20)	<0.001	0.086
	Grains $head^{-1}$	47.9	41.4	45.9	0.71 (19)	0.001	<0.001
	Grains m^{-2}	17050	10692	12638	563 (20)	<0.001	0.024
	TGW (g)	51.8	51.3	48.4	0.88 (20)	0.011	0.030
	Yield (t ha^{-1})	8.84	5.44	6.09	0.314 (20)	<0.001	0.158
2010	Pollen viability (%)	94.19	67.55	83.40	1.052 (10)	<0.001	<0.001
	Heads m^{-2}	462	356	392	17.8, 12.6 (16)	<0.001	0.056
	Grains $head^{-1}$	41.2	40.6	41.4	1.95, 1.38 (16)	0.941	0.670
	Grains m^{-2}	18995	14437	16278	925, 654 (16)	<0.001	0.061
	TGW (g)	53.1	58.2	58.0	0.65, 0.55 (16)	<0.001	0.758
	Yield (t ha^{-1})	10.07	8.41	9.40	0.563, 0.398 (16)	0.029	0.095
2011	Pollen viability (%)	96.45	92.61	93.78	0.508, 0.360 (49)	NA	0.186
	Heads m^{-2}	431	286	291	5.8, 4.1 (63)	NA	0.418
	Grains $head^{-1}$	43.1	32.0	33.2	0.57, 0.40 (64)	NA	0.099
	Grains m^{-2}	13542	9101	9718	215, 150 (62)	NA	0.023
	TGW (g)	52.4	56.4	56.1	0.39, 0.28 (64)	NA	0.513

	Yield (t ha^{-1})	9.74	5.08	5.42	0.116, 0.080 (62)	NA	0.022
Mean	Heads m^{-2}	418	297	321	5.8, 4.1 (103)	NA	0.004
	Grains head^{-1}	44.1	38.0	40.2	0.57, 0.40 (103)	NA	0.004
	Grains m^{-2}	16529	11410	12878	212, 150 (102)	NA	<0.001
	TGW (g)	52.4	55.3	54.2	0.39, 0.28 (104)	NA	0.074
	Yield (t ha^{-1})	9.55	6.31	6.97	0.115, 0.081 (102)	NA	<0.001

546

547 [A]IUC = irrigated unsprayed control; UC = unsprayed control; GS 33 = antitranspirant
548 treatment at GS 33

549 [B] SEM = standard error of mean; for 2010, first SEM applicable only to IUC and second
550 SEM applicable only to UC and GS 33 mean; for 2011 and year mean, first SEM
551 applicable only to UC, second SEM applicable only to GS 33

552 [C]NA = not applicable.

553

554 **Figure captions**

555 **Fig. 1. a)** A Claire shoot at early GS 41 (meiosis) **b)** A Claire spike bearing cells at
556 meiotic stages in the 1st and 2nd florets of the spikelets at the middle of the spike

557 **Fig. 2.** Nuclei of pollen mother cells of the cultivar Claire at **a)** anaphase I (late) **b)** dyad
558 stage (first telophase) **c)** just after metaphase II **d)** anaphase II (early) **e)** anaphase II (late)
559 **f)** tetrad stage; c.w. = callose wall; s.f. = spindle fibres; Estimated magnification is x6000
560 for a) and x4000 for b), c), d), e) and f).

561 **Fig. 3.** SMD calculated using the IMS irrigation scheduling program with nearby
562 meteorological station data, but with zero rainfall, from the dates rain shelters were
563 installed to the dates crops reached GS 69 in 2009, 2010 and 2011. H-SMD regime = high
564 SMD regime; L-SMD regime = low SMD regime; diamond symbol = antitranspirant
565 application date (GS 33); triangle symbol = date of meiosis.

566 **Fig. 4.** The relationship between the results from the IMS irrigation scheduling programme
567 and the neutron probe (NP) in 2011.

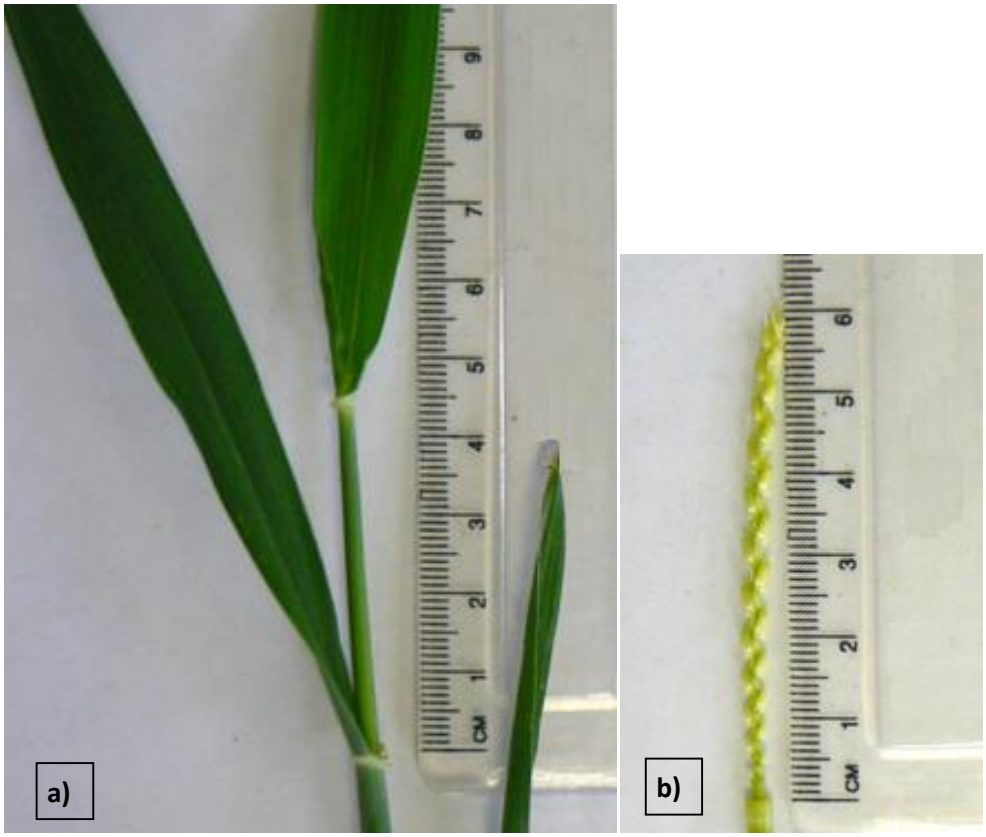

Fig. 1.

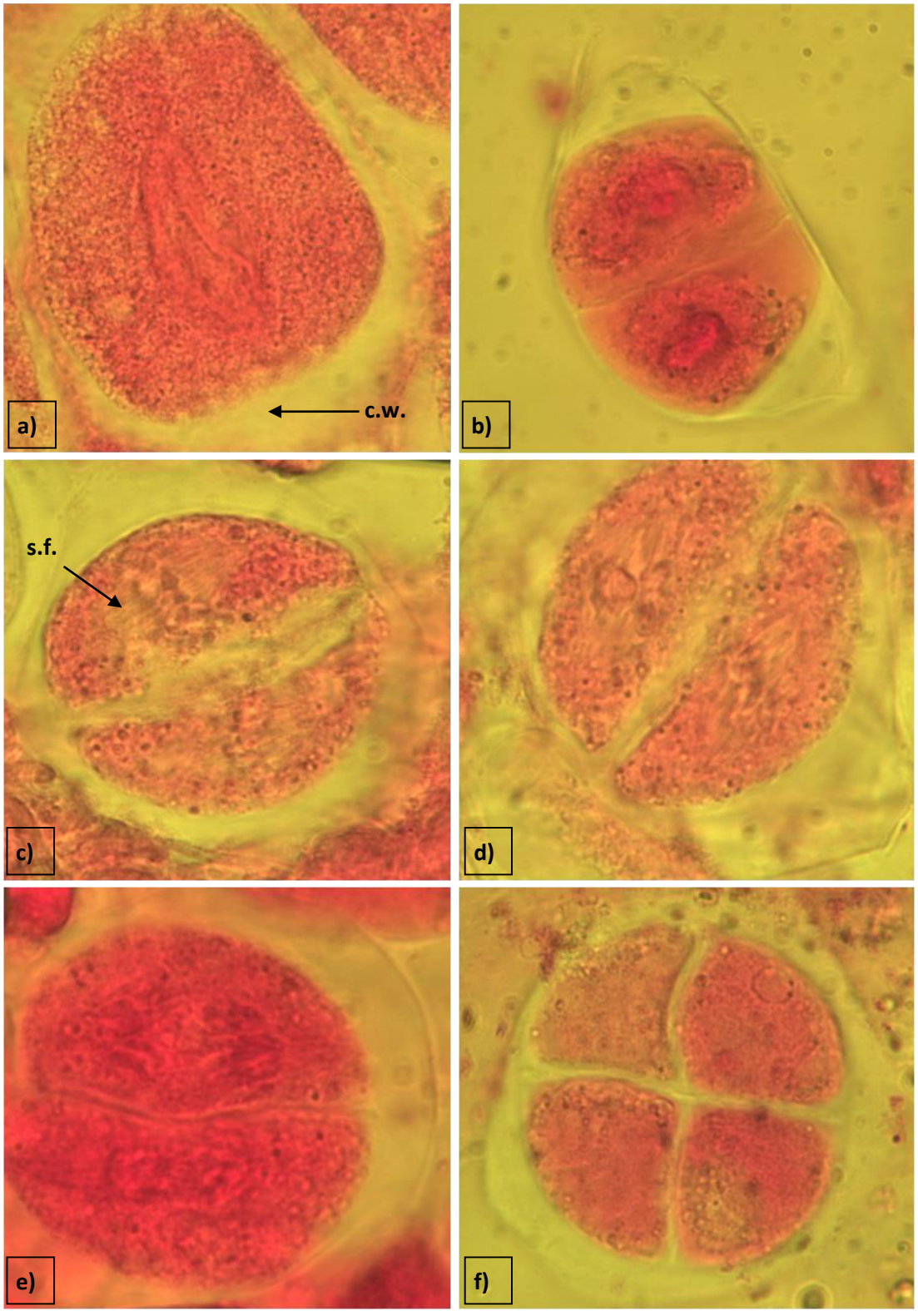

Fig. 2.

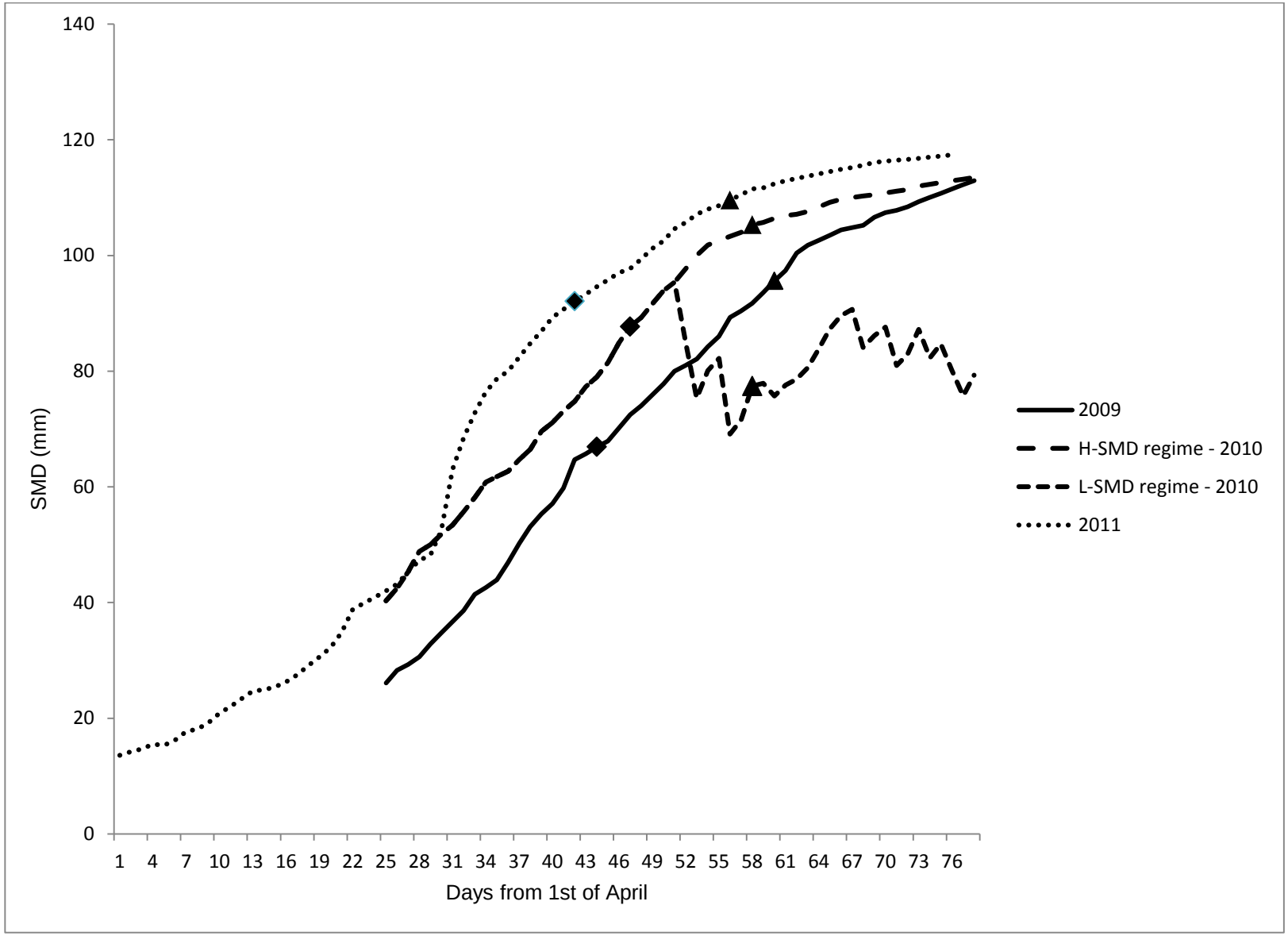
140
120
100
80
60
40
20
0
SMD (mm)
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76
Days from 1st of April
2009
H-SMD regime - 2010
L-SMD regime - 2010
2011

Fig. 3.

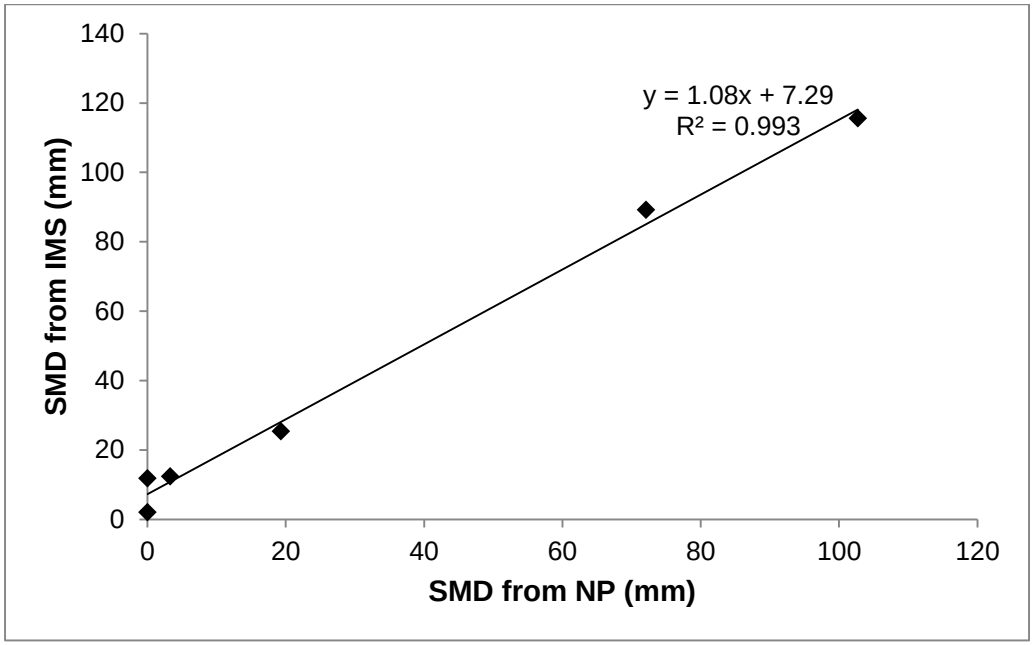
y = 1.08x + 7.29
R² = 0.993
SMD from IMS (mm)
SMD from NP (mm)
0
20
40
60
80
100
120
140

Fig. 4.