Harper Adams University

A Thesis Submitted for the Degree of Doctor of Philosophy at

Harper Adams University

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FILM ANTITRANSPIRANTS TO INCREASE YIELD OF DROUGHTED WHEAT

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

Harper Adams University

January 2013

Abstract

Drought stress results in large yield losses in wheat. One way of alleviating the effect of drought stress on crops may be to suppress transpiration with antitranspirants. Film antitranspirants sprayed onto plants reduce transpiration by increasing the resistance to diffusion of water vapour from stomata, and are also of low permeability to carbon dioxide entering the leaf and thus photosynthesis and growth are restricted. Previous work has indicated that the most sensitive stage to wheat yield formation to drought stress, the stage of meiosis in pollen mother cells, may respond positively to film antitranspirant applications irrespective of reduced photosynthesis. The main objectives of this study were to determine the most effective growth stage to receive a film antitranspirant application targeted to increase yield under drought conditions and to explore the underlying mechanisms by which film antitranspirants increase yield.

Field experiments were carried out in three consecutive years 2008/2009, 2009/2010 and 2010/2011 under polytunnels. The experiments indicated that, among the growth stages included within the experiments (GS31, GS33, GS39, GS41 and GS59), GS33 is the most effective growth stage to apply film antitranspirants in order to increase yield of droughted wheat. There was a significant mean yield increase across all three years of 0.57 t/ha from film antitranspirants, di-1-p-menthene and latex when sprayed at GS33 at SMDs above 66 mm, under the conditions of this study. The yield increase by antitranspirant treatments was due to an increase in grains m^{-2} either by increasing grains ear^{-1} or tiller survival.

The antitranspirant treatments significantly decreased transpiration, significantly increased leaf water potential and indicated a reduction in photosynthesis which was not significant. The antitranspirant treatments did not increase leaf temperature significantly. The antitranspirant treatments at GS33 and GS31 increased pollen viability. The observation of anthers using a light microscope collected from different growth stages showed that, in the variety Claire, meiosis in pollen mother cells occurs at early GS41. Therefore, the increased pollen viability by the antitranspirant treatments at GS33 may be attributed to an alleviation of the effect of drought stress on the crop during meiosis in pollen mother cells. The study performed to understand the effect of film antitranspirants on drought stress sensitive invertase genes, down regulated under drought stress did not show promising results may be due to a lack of representativeness of pollen/anther samples collected from different treatments.

It was concluded that an antitranspirant treatment at GS33 can increase yield of droughted wheat by increasing grains m^{-2} possibly via increased pollen viability at the stage of meiosis in pollen mother cells.

Acknowledgement

I am grateful to my director of studies, Professor Peter S. Kettlewell for his continuous guidance, support and encouragement throughout my PhD studies. I am obliged to him for his expert advice on statistics and his time devoted to improve my writing skills.

I am also grateful to my second supervisors Dr. Ivan Grove and Dr. Martin Hare for their valuable suggestions and expert guidance provided during my PhD studies. My sincere gratitude is due to Dr. Ivan Grove, especially, for the help provided in quantifying the soil moisture content in the field experiments.

I would like to express my gratitude to Dr. Nigel Hall for taking measurements from the Neutron probe for me in my field experiments, and his advices on the equipment. I would also like to thank Professor Simon Edwards for his advices on molecular studies.

I am thankful to all the staff members in Crop and Environmental Research Centre and Princess Margaret Science Laboratories who helped me in my PhD studies.

Finally I would like to thank my parents, my husband and colleagues for their encouragement and support throughout this work.

Declaration

This report has been written by myself and describes the work carried out by myself unless otherwise stated. Information from other sources has been fully acknowledged and referenced in the text. No part of this thesis has been previously submitted for examination leading to the award of degree.

Minuka Madhubhashini Weerasinghe

January, 2013

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List of Abbreviations

ANOVA	Analysis of variance
AT	Antitranspirant
ATP	Adenosine-5'-triphosphate
DNA	Deoxyribonucleic acid
CV	Coefficient of variation
DF	Degree of freedom
GS	Growth stage
HSD	Honestly significant difference
IC	Irrigated control
IMS	Irrigation management services
ISC	Irrigated sprayed control
ISP	Irrigated sprayed plot
IUC	Irrigated unsprayed control
IUP	Irrigated unsprayed plots
LS	Loamy sand
PCR	Polymerase chain reaction
RT-PCR	Reverse transcriptase polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
RNA	ribonucleic acid
RUBP	Ribulose-1,5-bisphosphate
SEM	Standard error of mean
SMD	Soil moisture deficit
UC	Unsprayed control
UV	Ultra violet
TGW	Thousand grain weight

1 General introduction and literature review

1.1 General introduction to the project

Research into film antitranspirants carried out mainly in the period from the 1950s to the 1970s on a range of plant species pointed out 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 restrained (Kettlewell *et al*., 2010). Subsequent reviews on film antitranspirants (Gale and Hagan, 1966; Das and Raghavendra, 1979 and Solarova *et al*., 1981) and the text books on plant water relations (Kramer and Boyer, 1995 and Jones, 1992) have concluded that the use of film antitranspirants is important for species, 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 was not recommended as photosynthesis is important in yield formation.

Kettlewell *et al*. (2010) identified that the above conclusion of the limited usefulness of film antitranspirants on cereal and food crops is based on a reductionist approach where only the physiology of the processes of transpiration and photosynthesis is considered. It has been pointed out that, a more-holistic approach towards exploring the potential of film antitranspirants should consider the fact that growth and yield depend on the integration of physiological and metabolic processes with the stage of development of the plant, and thus the factors affecting the physiological processes in turn might change growth and/or yield to a greater or lesser extent, adversely or favourably, depending on the stage of development of the plant at which the factors occurred.

The literature review of chapter 1.2 explains that the response and the sensitivity of the wheat crop to drought stress are not similar at all the growth stages and there are growth stages which are highly sensitive to drought stress towards yield formation. The most sensitive stage of the crop to drought stress towards yield formation is the stage of meiosis in pollen mother cells. Drought during this stage reduces pollen viability and hence number of grains and yield (Saini and Aspinall, 1981; Koonjul *et al*., 2005; Dorion *et al*., 1996; Lalonde *et*

al., 1997). It has been proposed by Kettlewel *et al*. (2010) that ameliorating the effect of drought via reduced transpiration by means of an antitranspirant during the stage of meiosis in pollen mother cells might be beneficial towards yield formation irrespective of reduced photosynthesis. The experiments previously carried out at Harper Adams University College by Kettlewell *et al*. (2010) have evaluated this concept on droughted field-grown wheat plants using the antitranspirant di-1-p-menthene. Two adjacent experiments were conducted each year in 2002/2003, 2003/2004 and 2004/2005 one in natural environment and one under polytunnels which was used to restrict rain water reaching the crop. The antitranspirant was applied at GS 69 in 2002/2003, GS 37 and GS 55 in 2003/2004 and GS 39 and GS 45 in 2004/2005. The application rate of the antitranspirant was 5 l/ha in 2002/2003 and 2.5 l/ha in 2003/2004 and 2004/2005. In all three years the spray volume was 200 l/ha. The control treatment was unsprayed. The SMD at the time of spraying was different in the experiment under the natural environment to the experiment under the polytunnels in each year, and from year to year, ranging from 41 mm to 118 mm, giving 10 different combinations of SMD and GS over the three years. The difference of mean yield between antitranspirant-treated and control plots was considered as the yield response of each experiment, and combined data of the three years were analysed using multiple regression analysis, in which the yield response was used as the response variate and the SMD and the numerical value of the Zadoks code for the growth stage at the time of spraying were used as two explanatory variates.

When the yield response was plotted against the SMD at the time of spraying, adjusted for GS, yield was substantially increased by the antitranspirant treatments made at high soil moisture deficits. In contrast, yield decreased substantially when the antitranspirant treatments were applied at low soil moisture deficits. Furthermore, multiple regression analysis showed a significant relationship when the yield response, adjusted for SMD, was plotted against the GS at which the antitranspirant was applied. Yield was reduced by the antitranspirant treatments at GS 55 and GS 69. In contrast, yield was increased by the

antitranspirant treatments at GS 37 and GS 39. Antitranspirant treatment at GS45 had little effect on yield.

According to the study described in chapter 3, in the life cycle of the winter wheat variety Claire, GS 37 and GS 39 occur before meiosis in pollen mother cells which occurs at GS41, GS 45 after meiosis in pollen mother cells and GS 55 and GS 69 are well after meiosis in pollen mother cells. The reason for the yield decrease from the antitranspirant treatments at low soil moisture deficits and from the antitranspirant treatments at GS 55 and GS 69, two of the growth stages which are comparably less sensitive to drought stress, is similar to what is described in the reviews and text books on the topic of film antitranspirants. The film restricts CO_2 intake by the leaf, reducing photosynthesis and thus the supply of assimilates to the developing grain and ultimately the yield. A paradigm, which was unseen by the reviews and text books was exposed by the results, which indicated that the film antitranspirant applications to the crop before the most sensitive stage to drought stress may increase yield. It is suggested that this yield increase may have taken place as a consequence of a reduction in drought-induced pollen sterility.

Chapter 1 (1.4.2.2) reviews some of the other studies which reported yield improvements from film antitranspirants in other seed crops including corn (Fuehring and Finckner, 1983), sorghum (Fuehring, 1973) and rapeseed (Patil and De, 1978).

The results of the study by Kettlewell *et al*. (2010) provide preliminary evidence supporting the concept that reducing water loss before meiosis in pollen mother cells by applying a film antitranspirant is beneficial for wheat yield under high soil moisture deficits, outweighing the detriment of reducing photosynthesis. Further research is required, however, to confirm the benefit of film antitranspirants for increasing wheat yield and to define the optimal application strategy for film antitranspirants.

The antitranspirant treatments in Kettlewell *et al*. (2010) were applied in a number of experiments over three years. Changes in environmental conditions between years, other

than the SMD, might have interfered with the results when the treatments of different experiments carried out in different years were combined for analysis. It is important to explore the concept using all treatments in one experiment in order to increase the reliability of the results. The public acceptance and recognition of the concept could be established by confirming the reproducibility of the results in several experiments repeated with or without slight changes in several years.

The effect of film antitranspirants on yield components, which are number of grains per ear (grains ear^{-1}), number of ears per square meter (ears m^{-2}) and thousand grain weight (TGW), was not explored by Kettlewell *et al*. (2010) in relation to this concept. If the effects of film antitranspirants on yield components are known, the mechanisms contributing to the yield increase could be identified.

Identifying the best growth stage before meiosis for the spray application is necessary to define optimal application strategy. How long before meiosis in pollen mother cells should the spray application be done may depend on the prevailing weather conditions and soil moisture deficit, but more research into this may give an idea which is the best growth stage to receive the spray application under at least one defined set of conditions.

Although it is speculated that a reduction in drought-induced pollen sterility is the cause of the yield increase observed by Kettlewell *et al*., (2010), the mechanism by which film antitranspirants increase yield is yet to be studied and discovered. Further research aimed to explore the physiological effects of film antitranspirants on the crop, especially effects on gas exchange, plant water status and pollen development is likely to provide evidence of the mechanisms involved.

Considering the above developments which could be done in order to optimise and establish the use of film antitranspirants on droughted wheat, this research project was conducted under the supervision of Professor P.S. Kettlewell the main author of Kettlewell *et al*. (2010) with the following main objectives, by which the above proposed developments are covered:

1. To explore the effect of film antitranspirants at different growth stages in relation to meiosis in pollen mother cells on yield and yield components of droughted wheat, with the purpose of determining the most effective growth stage to apply a film antitranspirant to increase yield under drought conditions.

2. To determine the underlying mechanism by which antitranspirants increase yield by exploring the physiological effects of antitranspirants on gas exchange, plant water status and pollen development

1.2 Drought stress and world wheat production

Wheat is the first most produced cereal crop in the world in terms of area harvested and the third most produced cereal crop in the world in terms of yield (FAOSTAT, 2010). Wheat provides about one fifth of the calories consumed by humans (Dubcovsky and Dvorak, 2007). Winter wheat (*Triticum aestivum L.*) is the most widespread arable crop in the UK, grown on an area of about 2 million hactares (DEFRA, 2012). Common wheat/bread wheat is a hexaploid species (genome AABBDD) that originated from hybridization events involving three different diploid progenitors, *Triticum urartu* (genome AA), *Aegilops speltoides* (genome BB) and *Aegilops tauschii* (genome DD) classified in the genera Triticum and Aegilops (Feldman *et al*., 1995). Two types of common wheat varieties, differ in growing season, are in cultivation. Winter wheat requires vernalisation treatment, i.e. exposure to a period of low temperature, in order to enter the flowering stage. Winter wheat is sown in the autumn, induced to flower during the winter (vernalised), flowers in the increasing day length of the following spring and matures in the summer. Spring wheat does not need vernalisation to induce flowering. Spring wheat is sown in the spring and is induced to flower by the increasing daylength only. Spring wheat can be harvested in late summer or autumn (Loukoianov et al., 2005).

The availability of soil water is a major factor limiting wheat production in most regions of the world and water deficits result in large yield losses in wheat, especially under semiarid and arid environments (Foulkes *et al*., 2002; Hongbo *et al*., 2005). About 70% of wheat cultivation areas are located in arid and semiarid zones in the world (Hongbo *et al*., 2005). Approximately 30% of the wheat area in the UK is on drought-prone soils (Foulkes *et al*., 2001). Drought-related yield losses in the UK can range between 2 and 4.5 t/ha, and the annual yield loss to drought is in the region of 15% (Foulkes *et al*., 2007).

With predicted climate change and more frequent summer droughts maximum soil moisture deficit is likely to increase in the future and hence drought-related reduction of wheat yield

(Richter and Semenov, 2004). On the other hand, total wheat demand is continuously increasing with increasing population growth (Hongbo *et al*., 2005). Drought related studies on wheat are of great importance in increasing wheat production under limited soil moisture conditions.

1.3 The sensitivity of different growth stages of wheat towards drought

The effect of drought at different growth stages of wheat on growth and yield are reviewed in order to clarify the sensitivity of different growth stages of wheat towards drought. Here the growth stages were identified according to the Zadoks scale (Zadoks *et al*., 1974). The Waddington scale quantifies the stages of spike initial and pistil development (Waddington *et al*., 1983), and it would be beneficial to know the effects of drought on the crop at different stages of spike and pistil development. However, no much studies focused on the effect of drought on the crop at the stages described according to the Waddington scale could be found from the literature. The Figure 1.3.1 summarises wheat plant development in Zadoks scale, floret development in Waddington scale and meristem development.

Plant growth and development can be affected by drought at any time during the crop life cycle (Saini and Westgate, 2000). However, The extent and nature of damage, the underlying mechanisms involved with the injury, the capacity for recovery, and the impact on yield (Hassan *et al*., 1997) depend on the developmental stage at which drought stress occurs and the intensity, rate and duration of exposure to drought (Beltrano *et al*., 2006; Hassan *et al*., 1997).

Because it is difficult to compare plants of different developmental stages at an equivalent water status, the studies which attempt to compare the effects of drought during various stages of crop growth do not provide a uniform scale for assigning differences in sensitivity in responses to comparable tissue water deficits. However, taken together they allow us to identify the stage specific nature of drought effects from which we can draw conclusions about the relative sensitivity of different stages with respect to the potential for an impact on yield.

A number of attempts have been made to define the soil moisture deficit at which wheat yields become limited. But none of the attempts were completely successful, partly because

the stage of crop development, at which a soil moisture deficit occurs, influences its effect on yield (Foulkes *et al*., 2001).

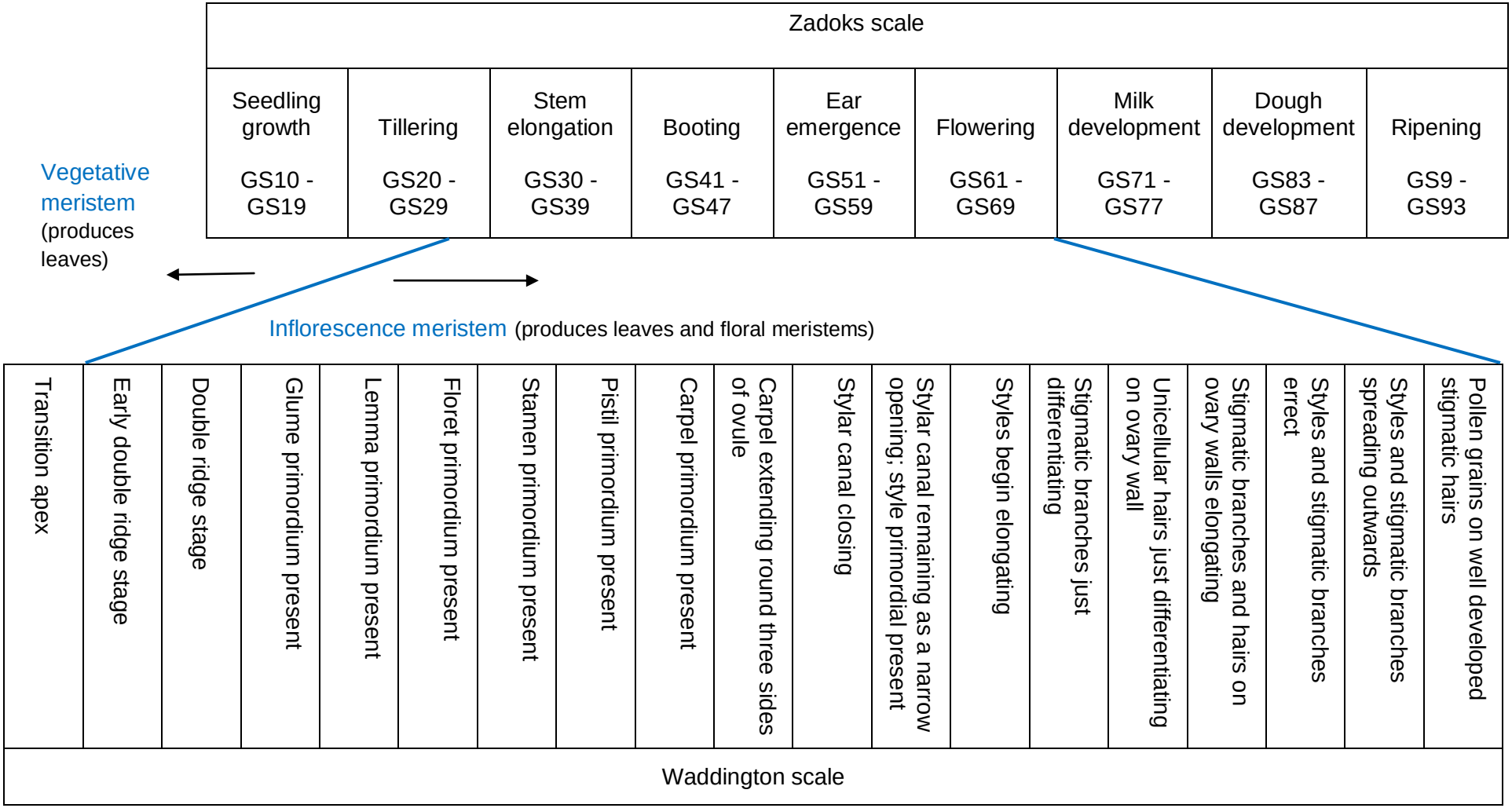


Figure 1.3.1: A diagram that summarises wheat plant development in Zadoks scale, floret development in Waddington scale and meristem development (According to Zadoks *et al*. (1974) and Waddington *et al*. (1983))

1.3.1 The effect of drought during pre anthesis growth stages

The crop's sensitivity to water deficit is greater before than after flowering, since, the size of the root system is smaller then, and since leaves are still expanding (Foulkes *et al*., 2001). However, water stress is less detrimental to grain yield when it occurs early in the crop cycle (Beltrano *et al*., 2006).

Drought stress at any growth stage prior to anthesis has detrimental effects on plant growth**.** It has been reported that drought stress during pre anthesis growth stages reduces plant height, total leaf area and leaf area index (Hassan *et al*., 1987; Choudhury and Kumar, 1979; Gupta *et al*., 2001). Decreased total leaf area and leaf area index lead to the production of comparatively less crop biomass through reductions in both radiation interception and the radiation use efficiency and finally result in low grain yields (Foulkes *et al*., 2001; Day and Intalap, 1970). Drought stress during all the pre anthesis growth stages decreases number of ears per m^2 at maturity and hence yield (Hassan *et al*., 1987; Beltrano *et al*., 2006). The reduced number of ears per m^2 caused by drought stress during germination, seedling growth and tillering is attributed to the reduced number of tillers produced per shoot (Hassan *et al*., 1987; Choudhury and Kumar, 1979; Gupta *et al*., 2001). However, on rewatering, before the beginning of the stage of stem elongation, plants showed recovery of tiller number and in consequence, yield (Choudhury and Kumar, 1979). Under natural conditions some tillers die between GS33 and GS61 (HGCA, 2007), and drought stress at stem elongation (Hassan *et al*., 1987; Day and Intalap, 1970) and booting (Gupta *et al*., 2001) increases the rate of tiller death. When drought stress was applied to a wheat community at the beginning of stem elongation, tiller death occurred at a mean rate of 11 tillers m^{-2} day^{-1} compared to 3 tillers m^{-2} day^{-1} in well-irrigated wheat (Turner and Begg, 1981). The reduced number of ears per m^2 caused by drought stress during stem elongation and booting is attributed to the increased rate of tiller death (Turner and Begg, 1981; Hassan *et al*., 1987; Day and Intalap).

Apical morphogenesis in cereals is quite sensitive to water deficit during vegetative development. Water stress during vegetative development in cereals slows the rate of inflorescence development, resulting in a delay or even inhibition of anthesis (Saini and Westgate, 2000).

Apart from the above described effects of drought on the crop generally during the pre anthesis stages, some of the experimental results showing the effect of drought stress occurred/imposed specifically during some of the pre anthesis growth stages are explained in the following page.

Germinating spring wheat (*Triticum aestivum L*.) seeds (Miazek *et al*., 2001) and hard red winter wheat seeds (Guedira *et al*., 1997) remained drought tolerant up to the 3^{rd} -4^{th} day following imbibition, which coincide with the rapid growth of the coleoptile and appearance of the first leaf, and from the 4^{th} day the seedling survival decreased. Dehydration reduced coleoptile lengths by 17 to 58% compared with the control. Seminal roots also were highly sensitive to dehydration, except at early stages, but were replaced quickly when seedlings were rehydrated (Guedira *et al*., 1997). The seedling stage when dehydration occurs is more important than the duration of the stress (Guedira *et al*., 1997).

The heterotrophic seedling growth (mg per seedling) could be quantitatively described as the product of the following two components: (1) the weight of mobilized seed reserve, and (2) the conversion efficiency of mobilized seed reserve to seedling tissue, i.e. the production of seedling dry matter per unit of usage of seed reserve (Soltani *et al*., 2006). Soltani *et al*., (2006) subjected growing wheat seedlings to different levels of soil water deficit, and observed that the seedling growth, fraction of seed reserve utilization and weight of mobilized seed reserve decreased with increasing drought intensity. However, drought had no effect on the conversion efficiency. It was concluded that the sensitive component of seedling growth is the weight of mobilized seed reserve (Soltani *et al*., 2006; Miazek *et al*., 2001). Glucose supplied either to dry seeds or to 4 d old seedlings increased survival of dehydrated

seedlings. Changes in seed weight suggest that reserves were important for recovery of seedlings from dehydration (Miazek *et al*., 2001).

Drought stress during tillering delays tiller development and this stress induced delay is likely to have a significant impact on the yield potential of affected tillers (Stark *et al*., 1986). Furthermore, drought stress during tillering reduces lipid content per grain (Singh *et al*., 1971). Tillers of spring wheat which developed under optimal soil moisture conditions exhibited uniform appearance patterns and reach maximum populations over relatively short degreeday intervals, whereas, soil water deficits in the period of tillering decreased the rate of appearance of all main stem tillers and caused appearance to occur over longer intervals. Water deficits severely reduced the development of tillers at the coleoptile node. When stressed plants were finally irrigated, the appearance rate of affected tillers frequently increased (Stark *et al*., 1986).

Hassan *et al*. (1987) reported that irrigation withheld at two consecutive irrigations during stem elongation reduced grain yield by 65% compared to the control irrigated at 10 day intervals, as a result of a decreased number of ears per m^2 and number of grains per ear. However, the level of the stress related to the yield loss in this experiment is not very clear. Furthermore, drought stress at stem elongation resulted in fewer days from planting to flowering and more lodging (Day and Intalap, 1970).

Drought stress during both stem elongation (Hassan *et al*., 1987) and booting (Gupta *et al*., 2001) results in fewer grains per head.

It has been reported that the stage of stem elongation is the most sensitive pre anthesis growth stage towards water deficit (Day and Intalap, 1970; Hassan *et al*., 1987; Choudhury and Kumar, 1979). Both seedling growth and stem elongation are equally sensitive to drought stress as far as total leaf area and number of tillers per plant are concerned (Hassan *et al*., 1987).

1.3.2 The effect of drought during the reproductive phase

The reproductive phase starts with the transformation of a vegetative meristem into inflorescence and flower primordia; it ends when the seed reaches physiological maturity. The transformation of a vegetative meristem into inflorescence and flower primordia occur at early stages of crop development, for e.g., at GS30 the ear is 1 cm long (Tottman, 1987). The growth stage coincides with meiosis in pollen mother cells is still a matter of controversy; according to Tottman (1987) it is the stage of stem elongation (Tottman, 1987), but, according to some others (Lalonde *et al*., 1997; Dorion *et al*., 1996; Koonjul *et al*., 2005), it is the stage of booting. The effects of drought at these reproductive stages are described separately in the following sections, since the growth stages that coincide with these early reproductive stages are not clearly defined. The above described responses to drought stress during the stem elongation and booting stages might also have been influenced by the effects of drought stress on one of these early reproductive stages that coincide with the growth stage under study, however, none of the authors have described the stage of reproductive development in relation to the growth stage under their study.

The most acute effects on yield of cereal crops have been recorded when stress coincides with the period in between the onset of meiosis and early grain initiation (Saini and Westgate, 2000; Salter and Goode, 1967; Saini, 1997). Within this phase, pollen mother cell meiosis and tetrad break up is identified as the most sensitive stage in the life cycle of wheat (Saini, 1997; Koonjul *et al*., 2005; Dorion *et al*., 1996; Saini and Westgate, 2000). Although anthesis and initial stages of grain development are also identified as stages that are highly sensitive to water stress in rice and maize, a much lower level of sensitivity at these stages, generally evident only under severe stress, has been observed in wheat (Saini and Westgate, 2000).

1.3.2.1 The effect of drought during flower initiation and development

The effect of drought on flower initiation and early development in wheat is not clearly understood (Saini and Westgate, 2000). At the initial stages of floral induction and differentiation, the apex can survive at a water potential as low as -6 MPa, which is lethal to leaves. Although the water potential of the cereal apex declines in parallel with that of leaves, apex turgor is maintained by the accumulation of solutes such as sucrose and amino acids imported from the vegetative tissues. Water stress during flower/inflorescence initiation and development in cereals slows the rate of inflorescence development, resulting in a delay or even inhibition of anthesis (Saini and Westgate, 2000).

1.3.2.2 The effect of drought during the development of male reproductive organs

Male reproductive organs are more sensitive to water deficit than female reproductive organs (Salter and Goode, 1967; Dorion *et al*., 1996; Saini, 1997). Pollen development in wheat often fails causing male sterility when a short period of even moderately severe drought stress coincides with the period of pollen mother cell meiosis and tetrad breakup, incidents that last approximately 24 hours in a wheat anther. Therefore, fertilization and hence grain set is inhibited (Saini, 1997; Koonjul *et al*., 2005; Dorion *et al*., 1996). The grain set of wheat plants subjected to meiotic stage drought stress for 3-4 days was not affected until the xylem water potential fell to -1.2 MPa, and grain set decreased linearly with declining water potential to reach zero at xylem water potential of -2.4 MPa. A more rapid drought stress of equal magnitude can cause a slightly greater injury to grain set (Dorion *et al*., 1996; Saini, 1997).

Although meiotic division in wheat pollen mother cells proceeds normally under drought stress, subsequent pollen development is arrested a few days later. The dislocation of microspores from their normal peripheral position is the most prominent sign of developmental failure. This dislocation can takes place at any time between the young microspore stage and the first mitosis of the pollen grain, depending on the cultivar. An

abnormal vacuolization of the tapetum can be seen in some anthers almost immediately after meiosis. Therefore, it is possible that tapetal dysfunction leads to the dislocation of microspores. The disoriented pollen grains have little or no intine but normal exine, dilute cytoplasm, and these pollen grains fail to accumulate starch which is necessary in subsequent pollen development (Koonjul *et al*., 2005; Dorion *et al*., 1996; Saini, 1997; Lalonde *et al*., 1997).

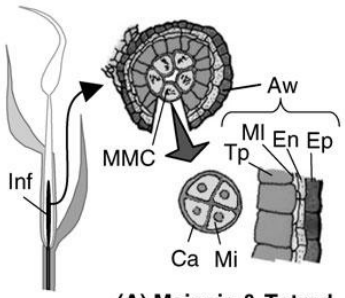
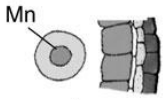
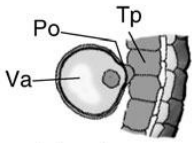
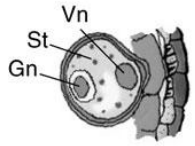
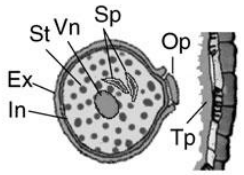
Stages during normal development	Normal Development	Development following water stress
 **(A) Meiosis & Tetrad**	Inflorescence protected by two uppermost leaf sheaths. A callose envelope isolates each MMC, which undergoes meiosis. Starch in anther wall.	**In plants stressed during meiosis and then re-watered:** Callose envelope present, meiosis is completed.
	Meiosis produces a tetrad of microspores.	Anther development normal. Tetrads formed.
 **(B) Young microspore**	Callose envelope dissolves, releasing thin-walled microspores, which align along tapetual cells. Degeneration of tapetum begins.	Occasional microspore disorientation or abnormal vacuolation of tapetum, otherwise development proceeding normally.
 **(C) Vacuolate microspore**	Microspores with a prominent vacuole, always in contact with tapetum via a pore. Tapetum degenerating, microspore-wall forming. Starch disappears from anther-wall. Anthers start enlarging.	Tapetal vacuolation progresses. Microspores losing contact with-tapetum, which degenerates too fast or persists. *No more sensitivity to concurrent water deficit.*
 **(D) 1st Pollen Grain Mitosis**	Microspore nucleus divides into vegetative and generative nuclei. A separate cell forms around the generative nucleus. Pollen grains begin accumulating starch. Rapid pollen-wall formation. Pore remains positioned towards tapetum.	Pollen grains fail to accumulate starch, have poorly-formed or no intine. All abnormal pollen grains are detached from tapetum. Anther smaller and paler than normal.
 **(E) 2nd Pollen Grain Mitosis**	Generative nucleus divides to form two ovoid sperms. Exine and intine formation advanced, pollens full of starch, and tapetal degeneration nearly complete. Pollen ready for shedding within a day.	Shriveled pollen grains, devoid of starch and with thin or no intine. Pollen grains non-viable and fail to germinate.

Figure 1.3.2: The major events during normal and drought stress-induced abortive development of the male reproductive organs of wheat (adapted from Koonjul *et al*., 2005 and edited); inf = inflorescence; MMC = microspore mother cells; Mi = microspore; Ca = callose (a cell wall component); Aw = anther wall; Tp = tapetum, Ml = middle layer, En = endothecium and Ep = epidermis (layers of anther wall); Mn = microspore nucleus; Po = pore; Va = vacuole; Gn = generative nucleus; St = starch; Vn = vegetative nucleus; Sp = sperm; Ex = exine, In = intine (layers of pollen wall)

Koonjul *et al*. (2005) reviewed and summarised the major events during normal and drought stress-induced abortive development of the male reproductive organs of wheat as shown in Figure 1.3.2.

Metabolic events associated with the failure of pollen development under meiotic stage drought are poorly understood (Koonjul *et al*., 2005). The most prominent sign of metabolic failure in drought-stress-affected wheat pollen grains is their failure to accumulate starch (Dorion *et al*., 1996). During normal development, pollen grains accumulate starch, which serves as the energy source for subsequent pollen germination and pollen-tube growth (Dorion *et al*., 1996; Saini, 1997; Lalonde *et al*., 1997). The pattern of distribution of starch in anthers is also changed by drought stress (Saini, 1997). It has been shown that instead of an impairment of enzymes directly involved in starch biosynthesis or a restriction of sugar import into anthers, an inability to metabolize incoming sucrose to hexoses, due to an impairment of invertase under drought stress, may be involved in this reproductive failure (Dorion *et al*., 1996; Koonjul *et al*., 2005).

Sucrose is the principal sugar imported into sinks in wheat. Prior to the utilization in physiological or metabolic processes, sucrose is generally converted to hexoses by invertase and/or sucrose synthase. The resulting hexoses are channelled into several important metabolic routes, including starch synthesis (Dorian *et al*., 1996). Therefore, an inhibition of any of the steps in sucrose metabolism could limit starch accumulation in pollen (Dorian *et al*., 1996). Invertase is the main enzyme of sucrose cleavage in pollens and anthers of wheat and several other species (Koonjul *et al*., 2005). In wheat and rice anthers affected by meiotic stage drought, a significant and immediate decline in the activity of vacuolar (Dorion *et al*., 1996; Koonjul *et al*., 2005) and cell-wall bound invertase (Koonjul *et al*., 2005) precedes any other sign of developmental failure. The enzyme is affected only when drought occurs during meiosis (Dorion *et al*., 1996; Koonjul *et al*., 2005).

Dorion *et al*. (1996) observed that the activity of vacuolar invertase declined 4-fold following meiotic stage drought and never recovered even after the stress is relieved.

Koonjul *et al*. (2005) isolated three invertase cDNAs, two encoding the cell wall (*Ivr1, Ivr3*) and one the vacuolar (*Ivr5*) invertase isoform, from an anther cDNA library. A transitory water deficit, which dropped the mean leaf water potential to -2.3 MPa (-0.5 MPa in the controls), during meiosis affected invertase activity by down regulating the transcription of *Ivr5* and *Ivr1* genes, without affecting the transcription of *Ivr3* gene. The expression of the two stress sensitive genes did not recover upon irrigation, and these two genes were found to be expressed within pollen whereas the insensitive one was not. The stress effects on the gene transcripts were consistent with the stress effects on the developmental profiles of the corresponding enzyme isoforms. The effect of drought stress is highly selective: the stress does not affect other closely related enzymes and even discriminates among different genes encoding the same class of invertases; only the invertase isoforms expressed within the pollens are suppressed (Koonjul *et al*., 2005).

When wheat plants are drought-stressed during meiosis, water potential in the floral organs either does not change or declines much less than in the leaf, because at this stage, the inflorescence is completely covered by the sheaths of the two uppermost leaves (Saini, 1997). Therefore, the effects of drought on invertase within anthers must be distantly regulated by some signal from plant parts with low water status. Although, much research has focused on abscisic acid, its role as a sporocidal signal remains inconclusive (Koonjul *et al*., 2005). The inhibition of photosynthesis under drought can affect sugar supply to anthers, and the expression of various genes, including invertase, which is modulated by sugars; hence sugars could be involved in this signalling (Saini, 1997; Koonjul *et al*., 2005). As glucose, drought stress, and abscisic acid all enhance the expression of genes encoding invertase in vegetative tissues, an interaction between sugars and abscisic acid in regulating invertase activity in anthers is possible (Koonjul *et al*., 2005).

1.3.2.3 The effect of drought during the development of female reproductive organs, ear emergence and anthesis

The development and fertility of the female gametophyte are much less drought sensitive than those of the male gametophyte. Comparatively few attempts have been made to determine if female infertility also contributes to the decline in yield in response to meiotic-stage drought (Saini and Westgate, 2000). Reciprocal crosses between stressed and unstressed wheat plants showed that female fertility was not affected by a drought stress treatment that caused complete male sterility in approximately 40% of the florets (Saini and Westgate, 2000).The leaf water potential of these stressed plants declined to approximately -2.3 MPa (control water potential = -0.8 MPa), which verges on being a severe stress for wheat (Saini and Westgate, 2000).

There are no published experiments focused specifically on the effect of drought during ear emergence. Drought stress during anthesis causes a variety of abnormalities in floral organs, which interfere with pollination or fertilization, and induces abscission of flowers or abortion of newly formed grains (Saini, 1997). Furthermore, the stress during anthesis decreased plant height, leaf area, days from planting to maturity and TGW (Day and Intalap, 1970; Choudhury and Kumar, 1980; Hassan *et al*., 1987). None of these authors has clearly specified the level of drought stress which caused these impairments. When drought stress was imposed during anthesis, the reduced yield was produced, primarily by lighter seeds/decreased TGW (Day and Intalap, 1970; Hassan *et al*., 1987). The number of grains per ear was also significantly decreased only under more severe drought stress than the stress which caused significant reductions in number of grains per ear when coincided with the stage of stem elongation (Hassan *et al*., 1987; Saini and Westgate, 2000).

1.3.2.4 The effect of drought during kernel growth and maturation

Kernel development has been divided into three phases. Phase I, often known as “lag phase”, is an active phase of cell division and differentiation. During this phase a rapid increase in kernel fresh weight occurs, primarily as a result of a water influx driven by a rapid accumulation of solutes. Overlapping and following is Phase II, during which reserves, predominantly starch, are deposited in kernel cells, resulting in a rapid increase in kernel dry weight. During Phase III, dry matter accumulation discontinues and the kernel undergoes maturation drying and approaches a “quiescent state” (Saini and Westgate, 2000). The grain watery ripe stage (GS71-GS73) is within Phase I; the stages, milk development (GS73-GS77) and dough development (GS83-GS87) are within Phase II, the period of grain filling; and the ripening stage which starts from GS91 is within Phase III (Tottman, 1987; HGCA, 2008).

1.3.2.4.1 The effect of drought during Phase I of the grain development

In wheat, Phase I of the grain development typically extends from 14 – 20 days after anthesis (Nicolas *et al*., 1985; Saini and Westgate, 2000). The sink potential of cereal grains is determined during Phase I, and drought stress during this period significantly reduces the sink potential of the grain (Nicolas *et al*., 1985; Saini and Westgate, 2000; Plaut *et al*., 2004; Beltrano *et al*., 2006). During Phase I, cell division occurs in the wheat grain endosperm and large A-type starch granules, which account for up to 60% of the total mass of starch at maturity, are initiated. The sink potential in kernels is a function of the number of endosperm cells and the number of starch granules initiated in endosperm cells (Nicolas *et al*., 1985). Drought stress during Phase I reduces kernel sink potential by forming fewer endosperm cells and decreasing the number and the size of starch granules (Nicolas *et al*., 1985; Yang and Zhang, 2006; Saini and Westgate, 2000).

The decrease in kernel sink potential affects both the rate and duration of dry matter accumulation/grain filling during Phase II (Nicolas *et al*., 1985; Beltrano *et al*., 2006) decreasing the kernel growth rate (Plaut *et al*., 2004), final kernel size (Saini and Westgate,

2000) and dry weight at maturity/TGW thereby limiting final grain yield (Plaut *et al*., 2004; Nicolas *et al*., 1985; Beltrano *et al*., 2006; Yang and Zhang, 2006; Saini and Westgate, 2000).

The reduction of yield was found to be more severe when the stress occurred suddenly rather than gradually. There was a 33% yield difference between plants subjected to 0.10 and 0.18 MPa d^{-1} of increasing plant water deficits during Phase I of the grain development (Kobata *et al*., 1992).

1.3.2.4.2 The effect of drought during Phase II of the grain development

Water deficit during the stage of grain filling causes physiological maturity to occur earlier shortening the duration of kernel filling, and hence reducing the kernel size and dry weight at maturity/TGW (Plaut *et al*., 2004; Saini and Westgate, 2000). Prevailing drought during grain filling results in a decrease in kernel water volume causing a premature decline in kernel water potential and kernel solute potential late during grain filling (Saini and Westgate, 2000). This leads to endosperm and embryo desiccation ultimately limiting the duration of grain filling by affecting metabolism of incoming assimilates (Kobata *et al*., 1992; Saini and Westgate, 2000).

Under drought stress, the production of new photosynthetic products become limited as a result of stomatal closure (affecting the intake of CO_2) and the depression of leaf area, chloroplasts and enzyme activity (Begg and Turner, 1976; Siddique *et al*., 1999). The stored carbohydrates may, thus, become the predominant source of grain filling (Gavuzzi *et al*., 1997; Plaut *et al*., 2004; Yang and Zhang, 2006). Under water deficit conditions the contribution of stored assimilates could be 75-100% of grain yield, as compared with 37–39% under high rainfall conditions (Gavuzzi *et al*., 1997). Nonetheless, in water-stressed plants, dry matter stored in vegetative organs is a much more limited source of grain filling, as it was retained probably to sustain osmotic adjustment/stress adjusting processes requiring assimilates (Plaut *et al*., 2004). Therefore, assimilates supply for the kernel is reduced by drought stress (Siddique *et al*., 1999; Plaut *et al*., 2004).

Water deficit during grain filling alters the grain composition and influences quality characteristics (Beltrano *et al*., 2006; Ozturk and Aydin, 2004). The two major components of wheat grain are protein and starch (Beltrano *et al*., 2006). In general, the proteins are synthesized essentially from photosynthate produced before grain filling, whereas starch is produced from the net assimilation of CO_2 after anthesis. Therefore, protein and starch deposition in the process of grain filling do not proceed simultaneously, that is, the rate of protein accumulation may reach a peak before that of starch. Hence, drought stress during the grain filling period mainly affects starch accumulation, and has little effect on nitrogen/protein accumulation (Beltrano *et al*., 2006). Therefore, drought during grain filling alters grain protein/starch ratio affecting the grain quality (Beltrano *et al*., 2006; Ozturk and Aydin, 2004).

Drought stress during the grain-filling period increases the remobilization of non-structural carbohydrates from the vegetative tissues to the grain up to a certain level of the stress (Yang and Zhang, 2006). In determinate crops such as wheat where the leaf area is fixed at flowering, yield under drying conditions has been inversely related to the rate of leaf senescence after flowering, which in turn was related to plant drought stress (Begg and Turner, 1976) up to a certain level of the stress (Yang and Zhang, 2006). Extensive studies have demonstrated that mild post-anthesis water deficits result in early senescence and more remobilization of pre-anthesis stored assimilates to grains in cereals (Yang and Zhang, 2006).

1.4 The effect of drought at different growth stages on wheat yield – a summary

The two yield components of crop yield are grains per m^2 and TGW (Egli, 1998). As described in section 1.3, drought can affect crop yield either by effecting grains per m^2 or TGW. According to the literature review of section 1.3, the effect of drought at different growth stages on grains per m^2 and TGW (and intern on yield) can be summarised as follows.

Figure 1.4.1 summarises the physiological processes that affect grain number per m^2 as a consequence of drought. Drought during germination, seedling growth and tillering decreases the number of tillers per shoot and hence grains per m^2 (Choudhury and Kumar, 1979; Gupta *et al*., 2001). The rate of tiller death is increased by drought during stem elongation and booting decreasing grains per m^2 (Hassan *et al*., 1987; Day and Intalap, 1970; Gupta *et al*., 2001). Drought during pre anthesis growth stages decreases total leaf area and leaf area index affecting radiation interception. Photosynthesis is decreased as a result and as a consequence of direct effects of drought on photosynthesis. This may limit pre anthesis biomass partitioning to the developing ear resulting in low numbers of floral primodia/florets followed by low grain numbers (Foulkes *et al*., 2001; Day and Intalap, 1970). When drought coincides with pollen mother cell meiosis pollen development is disrupted and as a consequence the number of fertile pollen grains is decreased resulting in low grain numbers (Saini and Westgate, 2000; Koonjul *et al*., 2005; Dorion *et al*., 1996).

The physiological processes that affect TGW as a consequence of drought are not shown in a figure, as there are no much to describe. When drought coincides with Phase I of grain development kernel sink potential is reduced by forming fewer endosperm cells and decreasing the number and the size of starch granules, and as a result TGW is decreased (Nicolas *et al*., 1985; Yang and Zhang, 2006; Saini and Westgate, 2000). Apart from that the decreased assimilate production due to drought during pre anthesis stages and anthesis stages can decrease TGW if the affect is not compensated by decreased grains per m^2 (Saini and Westgate, 2000).

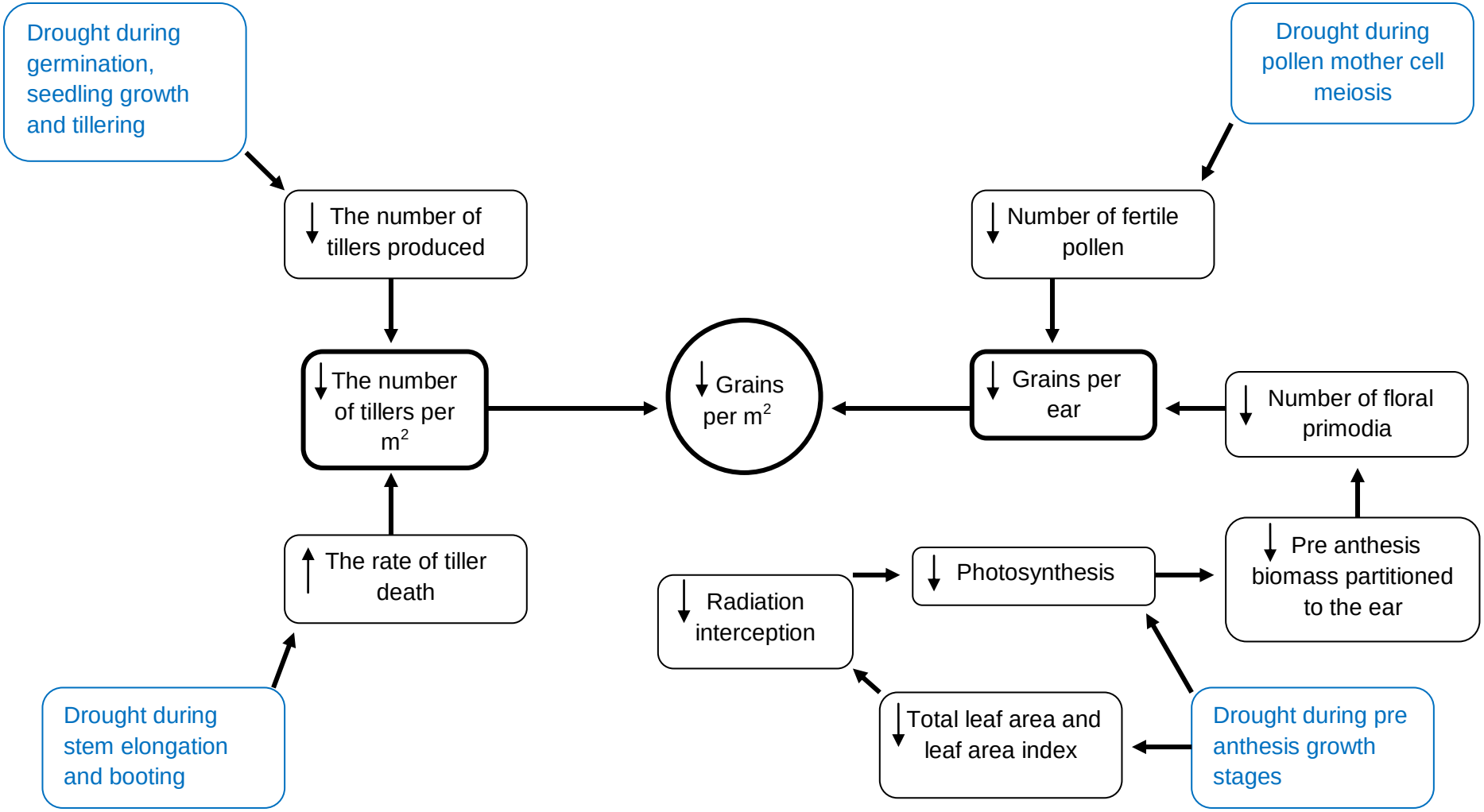


Figure 1.4.1: The physiological processes that affect number of grains per m^2 as a consequence of drought

1.5 The effects of drought stress on physiological processes.

The knowledge on the effects of drought stress on plant physiological processes is important in this study. The effects of drought stress, mainly, on photosynthesis and some other physiological processes are discussed.

1.5.1 The effects of drought stress on photosynthesis

Water deficit is one of the most important environmental factors inhibiting photosynthesis (Begg and Turner, 1976; Chaves *et al*., 2002).

Since stomata act as regulators for CO_2 exchange, as well as regulators of water loss, water deficit sufficient to close stomata must also depress CO_2 entering to the leaf (Begg and Turner, 1976). It has been shown that Internal CO_2 concentration is decreased by drought induced stomatal closure, and rate of photosynthesis is decreased with the lack of internal CO_2 (Flexas and Medrano, 2002; Lawlor and Cornic, 2002).

It has been shown that non-stomatal limitations to photosynthesis also exist under drought stress (Siddique *et al*., 1999; Tezara *et al*., 1999; Vu *et al*., 1999; Loggini *et al*., 1999; Tambussi *et al*., 2000; Parry *et al*., 2002; Chaves *et al*., 2002; Flexas and Medrano, 2002).

The exposure of plants to drought stress leads to decrease in mesophyll conductance to CO_2 (Siddique *et al*., 1999; Begg and Turner, 1976). However, in wheat, the internal conductance of CO_2 was unaffected at water deficits much higher than the deficits which caused stomatal closure (Begg and Turner, 1976).

Decreased photosynthesis under drought conditions is also caused by inhibition of the photosynthetic carbon reduction (Calvin) cycle (Tezara *et al*., 1999). The photosynthesis rate in higher plants, mainly in C3 plants, depends on the activity of the enzyme, ribulose-1, 5-bisphosphate carboxylase/oxygenase (rubisco) and synthesis of ribulose-1, 5-bisphosphate (RuBP), which is the substrate of the photosynthetic reaction (Tezara *et al*., 1999; Parry *et al*., 2002). Drought stress decreases rubisco quantity (Vu *et al*., 1999),

rubisco activity (Parry *et al*., 2002) as well as RuBP regeneration (Tezara *et al*., 1999; Vu *et al*., 1999). The rate of synthesis and degradation determines the quantity of rubisco in leaves, and drought stress affects the quantity of rubisco by decreasing the rate of synthesis of the enzyme (Vu *et al*., 1999). It has been evident that drought stress mediated decrease in rubisco activity is due to binding of inhibitors with rubisco, blocking the catalytic sites of the enzyme for RUBP (Parry *et al*., 2002). Rubisco activase is an enzyme which is responsible in the maintenance of the catalytic sites of rubisco by removing tight-bound inhibitors, and it has been reported that activity of rubisco activase is also decreased with increasing drought stress (Chaves *et al*., 2002). Drought stress affects the enzyme, ATP synthase, involved with photophosphorylation, the process by which ATP is produced, thus reduces the rate of production of ATP (Tezara *et al*., 1999). The removal of inhibitors from rubisco binding sites by rubisco activase is known to be impaired by reduced ATP concentrations under drought stress conditions (Tezara *et al*., 1999). The Calvin cycle uses ATP and NADPH to synthesize RuBP, and the decrease in RuBP regeneration under drought conditions is due to an inadequate supply of ATP (Tezara *et al*., 1999). According to Tezara *et al*. (1999), among rubisco quantity, rubisco activity, RuBP regeneration and ATP production, the one which was most sensitive to drought stress is ATP production, and the inhibition of Calvin cycle under mild drought stress was mainly by the reduction in RuBP regeneration which was caused by inadequate supply of ATP; Rubisco activity was decreased only by relatively severe stress with no change in rubisco quantity.

Under drought stress leaves could be exposed to excess energy. If not safely dissipated, this excess energy may cause over-reduction of reaction centers (Demmig-Adams and Adams, 1992) and increased production of reactive oxygen species (Takeda *et al*., 1995; Loggini *et al*., 1999; Tambussi *et al*., 2000; Sharma *et al*., 2010). Although, reactive oxygen species act as important molecules in cell metabolism (Sharma *et al*., 2010), the dramatic increase in the reaction oxygen species under drought stress inhibits Calvin-cycle enzymes (Takeda *et al*., 1995), causes damages to the reaction centers (Loggini *et al*., 1999; Tambussi *et al*., 2000), protein degradation, membrane lipid peroxidation, DNA

and RNA damage, and ultimately cell death (Sharma *et al*., 2010) all of which together contribute to reduced photosynthesis. In particular, photosystem II has been shown to be damaged by drought stress (Tambussi *et al*., 2000; Loggini *et al*., 1999). Tambussi *et al*., (2000) imposed water stress on wheat plants in the vegetative stage (4-week old plants) to reach soil water potential about ¯2.0 MPa and observed an increase in oxidative damage to thylakoid proteins in water-stressed leaves, associated with a considerable decrease in photosynthetic electron transport activity and photosystem II efficiency.

Flexas and Medrano (2002) reanalysed data from a large number of publications in literature using stomatal conductance as a parameter indicative of water deficit in plants, and suggested that although, stomatal closure is the earliest response to drought and the main limitation to photosynthesis at mild to moderate drought, in parallel inhibition of metabolic processes leads to decreased RuBP content, which becomes the main limitation at severe drought. Furthermore, sever dough stress (around wilting point) can lead to inhibition of the rubisco activity (Tezara *et al*., 1999) and content (Vu *et al*., 1999) and, finally, chloroplast damage and cell death due to reaction oxygen species (Sharma *et al*., 2010).

1.5.2 The effects of drought stress on translocation and distribution of assimilates

The transport of assimilates from source to sink or site of utilisation is influenced by the assimilation rate, utilisation rate, the rate of assimilate loading to and unloading from the sieve elements and speed of assimilate movement in the sieve tubes. Therefore, an effect of drought stress on any of these processes will be apparent as an effect on overall translocation (Begg and Turner, 1976). Although, the velocity of assimilate translocation through the sieve tubes was highly resistant to drought stress, the stress decreases the translocation of assimilate in droughted plants due to inhibition of leaf growth by the stress (Wardlaw, 1969). The phloem translocation is less susceptible to drought than leaf photosynthesis (Wardlaw, 1969; Wardlaw, 1971; Yang and Zhang, 2006). In wheat, the decreased rate of photosynthesis and decreased rate of loading of assimilates to the

sieve tubes were considered to be the most likely factors limiting the translocation (Wardlaw, 1971).

The stage of development of the crop is important in the distribution of assimilates under drought stress conditions. In Darnel grass (*Lolium temulentum*), labelled assimilates moved preferentially to young roots, leaves and sheaths under vegetative stage drought stress. Whereas, in wheat, reduced leaf photosynthesis, by drought stress at the grain filling stage, resulted in assimilates moving from the roots, leaves, stems, and crown to the ear (Begg and Turner, 1976).

1.5.3 The effects of drought stress on cell division (mitotic) and enlargement

Both cell division (mitotic) and enlargement are sensitive processes to drought stress (Begg and Turner, 1976; Schuppler *et al*., 1998; Tardieu *et al*., 2000). A mild drought stress (¯0.3 MPa water potential in growth media) imposed on wheat seedlings decreased the leaf-elongation rate by one-half and the mitotic activity of mesophyll cells to 42% of well irrigated controls within one day. The zone of cell division was also restricted to a length of 4 mm in stressed leaves, compared to 8 mm in control leaves. The phase of division continued longer in the stressed leaves than in the control leaves. Furthermore, the final cell number in the stressed leaves was 85% of that of control leaves (Schuppler *et al*., 1998). Showing a similarity to above results, Tardieu *et al*. (2000) reported that both the cell division rate and the length of the zone of cell division in maize were decreased by drought stress. Although, Begg and Turner (1976) reports that cell division appears less sensitive to water deficit than cell enlargement, Tardieu *et al*. (2000) suggests that the cell enlargement rate and cell division rate are affected to the same extent by drought.

One of the most important consequences of the sensitivity of meiotic division and cell enlargement to drought stress is the distinct reduction in leaf area and subsequent reduction in crop growth. Leaf area is generally more sensitive to water stress than stomatal resistance and CO_2 assimilation. Therefore crop growth can be affected even by a SMD, which does not directly decrease stomatal aperture and photosynthesis. One of

the most detrimental features of a reduction in leaf area is the fact that the effect is permanent and in the case of a determinate crop there is no scope for compensation through an increase in the number of leaves (Begg and Turner, 1976).

1.5.4 The effects of drought stress on some other processes related to plant growth and development

Whenever the water deficit is sufficiently great to close stomata and decrease photosynthesis, dark respiration is also decreased (Begg and Turner, 1976), but respiration is relatively less affected by drought stress than photosynthesis (Begg and Turner, 1976; Tezara *et al*., 1999).

Although overall plant growth is reduced during drought stress, root growth is generally supported relative to shoot growth as signified by several reported increases in the root-to-shoot ratio (Hsiao and Xu, 2000). Some studies have indicated that drought stress enhances root growth not only relative to shoot growth but absolutely (Begg and Turner, 1976). The mechanisms underlying the continued root growth under drought stress include osmotic adjustment (Saab, 1992: Hsiao and Xu, 2000).

1.6 The use of film antitranspirants to alleviate the effect of drought stress on crops

Antitranspirants are materials applied to plants for the purpose of retarding transpiration (Gale and Hagan, 1966). The literature reports three major types of antitranspirants, namely film-forming types, those that act by forming a film on the leaf surface; stomatal closing types, those that act by preventing complete stomatal opening; reflecting types, those that reflect a part of the incident solar radiation. The effect of stomatal closing and reflecting types of antitranspirants on plants have been extensively studied. These will not be discussed in this literature review, since these types of antitranspirants are not relevant to this study.

Commercially available film antitranspirants are generally polymers sprayed as emulsions in water and include hydrocarbons and terpenoids (Kettlewell *et al*., 2010), which form a tightly and symmetrically arranged monomolecular layer (Gale and Hagan, 1966) on the surface on to which it is sprayed. Various applications of a number of commercially available film antitranspirants and the effect of these film antitranspirants on transpiration, plant water status, photosynthesis, plant growth, yield, yield quality characteristics, leaf temperature and nutrient uptake are described in the following text. The chemical names or main chemical ingredients or the type of chemical of the antitranspirants specified in the following text are; **Nu-film, Pinolene, Vapor Gard and Wilt Pruf** – di-1-p-menthene; **Plantco** and **Dyroton**– acrylic emulsions; **Mobileaf** – a wax emulsion; **Clearspray** and **Folicote** –hydrocarbon emulsions; **Dow X2-1337** – a silicon emulsion; **Tag** – a polyethylene based emulsion; **S-789** – a copolymer dispersion of acetate acrylate esters; **S-4000** – a copolyacrylic emulsion. Some of the film antitranspirants discussed in this text include di-1-p-menthene, which is the film antitranspirant used in the field experiment with the commercial name of **Emerald**.

1.6.1 The effect of film antitranspirants on plant water relations

A film-forming antitranspirant, sprayed on to plant surfaces, curtails transpiration by providing a physical barrier over some, if not all, the stomata, offering resistance to the passage of water vapour so that the escape of water vapour from the plant surface to the atmosphere is reduced (Davenport *et al*., 1974; Davenport *et al*., 1972). The effect of different film forming antitranspirants on transpiration, plant water status and water uptake rate of different plant species have been recorded.

Plantco (an acrylic emulsion), Vapor Gard (di-1-p-menthene) and Dow X2-1337 (a silicon emulsion) effectively reduced transpiration from black spruce seedlings, whereas, Clearspray (a hydrocarbon emulsion) and Folicote (a hydrocarbon emulsion) were unable to reduce transpiration (Colombo and Odlum, 1987). The application of linseed oil at a concentration of 7.5% significantly reduced foliar transpiration in durum wheat under water deficit conditions (Mokhtari *et al*., 2006).

The effect of reduced transpiration by a film antitranspirant on plant water status is described in some studies. The significant decrease of daily transpiration rate by the application of Mobileaf (a wax emulsion) on oilseed rape (*Brassica campestris*) at vegetative stage resulted in a significant increase in relative water content by 6 - 8% under 75% of available moisture depletion (Patil and De, 1976). Furthermore, a significant decrease in transpiration rate and significant increases in leaf water potential, osmotic potential and turgor potential were recorded in Tuberose (*Polianthes tuberose* L.), a flowering ornamental sprayed with Vapor gard (di-1-p-menthene) at vegetative and flowering stages, under water deficit conditions (Moftah and Al-Humaid, 2005). An application of 2% of Folicote (a hydrocarbon emulsion) at 300 l/ha significantly increased the leaf water potential of potato (*Solanum tuberosum L*.) plants under water deficit conditions (Win *et al*., 1991). Daytime shrinkage of tree trunks occurs when water uptake lags behind transpiration, and this indicates a water deficit in the plant, and showing the importance of film antitranspirants on conserving water in tree crops, Mobileaf (a wax emulsion) on red pine (*Pinus resinosa*), almonds (*Prunus amygdalus*) and some other

trees, including peaches (*Prunus persica*) reduced daytime trunk shrinkage, often by over 50%, indicating an improvement in the water status of the trees (Davenport *et al*., 1972).

Showing evidence of the effect of film antitranspirants on water uptake, Steinberg *et al*. (1990) reported that an application of a 10% solution of Wilt Pruf (di-1-p-menthene) on peach (*Prunus persica*) trees following fruit harvest resulted in an average reduction in water uptake by 30% for a period of 85 days after the treatment. Furthermore, Vapor Gard (di-1-p-menthene) sprayed at 1% on to sweet pepper (*Capsicum annuum* L.) plants grown hydroponically, reduced water uptake by 12.4% (Amor and Rubio, 2009), and Pinolene (di-1-p-menthene), sprayed every fortnight to the aerial part of the plants at the same rate also reduced water uptake significantly without affecting dry weight accumulation, net CO_2 assimilation, cation uptake and fruit yield (Amor and Rubio, 2009).

1.6.2 The effect of film antitranspirants on photosynthesis, plant growth yield and yield components

While the use of a film antitranspirant will reduce transpiration, some reduction of carbon dioxide intake, and hence of photosynthesis, growth and yield, may also be expected, since stomata are common sites not only for loss of water vapour but also for intake of carbon dioxide. This is particularly so if the standard of comparison is an unstressed control plant with open stomata (Davenport *et al*., 1974; Gale and Hagan, 1966; Kettlewell *et al*., 2010; Solarova *et al*., 1981). The three main reviews of antitranspirants (Davenport *et al*., 1974; Gale and Hagan, 1966; Solarova *et al*., 1981) and text books on plant water relations (Kettlewell *et al*., 2010) have, therefore, concluded that the use of film antitranspirants is limited to plants such as ornamentals for which photosynthesis is less important. However, In contrast to this general conclusion there are a number of studies (as described below) which show the importance of antitranspirants even for the crops for which photosynthesis is in great importance in terms of producing economic yield. In contrast to the previous reviews, especially to the review by Solarova *et al*. (1981), among the recent studies, the number of studies reporting on neutral or positive effects of antitranspirants on photosynthesis, growth and yield is very high compared to the studies

reporting on negative effects. It is possible that most of the studies with negative results might have not been published.

1.6.2.1 The effects of film antitranspirants on photosynthesis and plant growth

Solarova *et al*. (1981) reviewed the published effects of 23 film antitranspirants in reducing photosynthesis across 13 plant species. Davenport *et al*. (1974) and Gale and Hagan (1966) also have reported several experiments where different antitranspirants reduced photosynthesis and growth of different plant species. In these reviews, there are records on published effects of film antitranspirants in increasing plant growth as well.

According to Davenport *et al*. (1974) film antitranspirants affect growth positively by increasing water potential and negatively by reducing photosynthesis. The overall effect is therefore dependent on the fact whether current photosynthesis or plant water potential is more important for the growth of a considered plant part e.g. fruit at the time of antitranspirant application. However, there are some records where film antitranspirants were claimed to increase both photosynthetic CO_2 assimilation and plant growth. The number of stomata per unit leaf area and photosynthetic CO_2 assimilation rate (μmol CO_2 $m^{-2}s^{-1}$) and the number of leaves significantly increased by the application of Vapor gard (di-1-p-menthene) on 8 weeks old, Tuberose (*Polianthes tuberose* L.), a flowering ornamental under water deficit conditions. The water use efficiency estimated as the ratio between photosynthetic CO_2 assimilation rate and transpiration rate was significantly higher in Vapour gard treated plants compared to the control (Moftah and Al-Humaid, 2005; Moftah and Al-Humaid, 2006). An increase in photosynthetic CO_2 assimilation in eggplants (*Solanum melongena* L.) plants from Vapor gard (di-1-p-menthene) under water deficit conditions was reported by Prakash and Ramachandran (2000). Furthermore, the application of Mobileaf (a wax emulsion) on rapeseed (*Brassica campestris*) at vegetative stage significantly increased dry matter production and WUE under 75% of available moisture depilation. The increase in WUE is attributed to the increased dry matter production and decreased transpiration by the antitranspirant (Patil and De, 1976). Irmak and Jones (2000) observed a significant increase in plant developmental rate and the leaf

area expansion rate upon the application of Vapor Gard (di-1-p-menthene) on tomato (*Solanum lycopersicum*) plants under water deficit conditions. However, the author stated that Vapor Gard film is impermeable to water vapor, but permeable to CO_2 and O_2. El-Aal *et al*. (2008) reported that foliar application of dyroton (an acrylic emulsion), on 60 days old (i.e., during vegetative stage) of eggplants (*Solanum melongena*) significantly increased the plant height, the number of leaves and shoots and the fresh and dry weight of leaves and shoots under water deficit conditions, and these increases were attributed to increased plant water potential upon the application of antitranspirants when plant growth is more dependent on water status than on photosynthesis. According to these results a film antitranspirant may possibly increase growth not only by increasing plant water potential, but also by increasing both plant water potential and photosynthesis if the antitranspirant is of higher permeability for CO_2 than for water vapour and/or if leaf water potential is increased by the antitranspirant applied under severe drought stress conditions, under which otherwise chloroplasts are damaged and photosynthetic enzyme activity is depressed. Furthermore, film antitranspirants increase growth when growth of a particular plant part or plant is more dependent on water status than on photosynthesis.

The effect of a film antitranspirant on CO_2 uptake seems to depend on the time duration from the application. 3-year-old *Pinus resinosa* seedlings treated with Wilt Pruf (di-1-p-menthene) at 20% exhibited an increased rate of C^{14} uptake upon exposure to $C^{14}O_2$, 1 day after the antitranspirant treatments under water deficit conditions. However, when seedlings were exposed to $C^{14}O_2$, 11 days after the application Wilt Pruf (di-1-p-menthene), Folicote (a hydrocarbon emulsion) and Vapor gard (di-1-p-menthene) a decreased rate of C^{14} uptake exhibited compared to the control.

1.6.2.2 The effect of film antitranspirants on yield, yield components and quality characteristics

Davenport *et al*. (1974) and Gale and Hagan (1966) have reported several records of film antitranspirants reducing yield and yield components of different plant species. There are

also several reports showing positive effects of film antitranspirants on yield, yield components and quality characteristics.

Antitranspirants are important to increase plant water potential at a time when plant yield is more dependent on water status than on photosynthesis. The relative importance of optimum plant water potential and the photosynthesis depends on the plant growth stage (Kettlewell *et al*., 2010). For example, the stage of development in wheat, which appears to be most sensitive to drought stress is the period of meiosis in pollen mother cells (Koonjul *et al*., 2005; Dorion *et al*., 1996; Lalonde *et al*., 1997). The application of Emerald (di-1-p-menthene) around the stage of meiosis in pollen mother cells enhances the yield of droughted wheat (Kettlewell *et al*., 2010). This increase in yield by film antitranspirants was attributed to increased plant/soil water potential upon the application of antitranspirants when plant growth is more dependent on water status than on photosynthesis (The study in Kettlewell *et al*. (2010) is further discussed in the general introduction of section 1.5). Application of the antitranspirant, Folicote (a hydrocarbon emulsion) at a rate of 2 l/ha on sorghum just prior to the stage of booting resulted in yield increases between 5 to 17% under water deficit conditions (Fuehring, 1973). Application of the same antitranspirant at a rate of 1.93 l/ha on droughted corn just prior to tasseling resulted in yield increases between 11 to 17% (Fuehring and Finkner, 1983). Furthermore, it has been reported that the antitranspirant, Mobileaf (a wax emulsion), increased seed yield of droughted rapeseed by 26% (Patil and De, 1978). The effects of antitranspirants, used at different stages of crop growth, on progressive vegetative and reproductive growth and final yield of crops, however, have received little study.

The effect of film antitranspirants on yield depends on the level of soil moisture deficit/drought stress at the time of application. According to Kettlewell *et al*. (2010), the soil moisture deficit at the time of application of Emerald (di-1-p-menthene) was linearly related to the yield response of wheat. Lipe and Thomas, (1980) reported that, although, total yield of Red Lasoda potatoes (*Solanum tuberosum*) was increased by the application of Folicote (a hydrocarbon emulsion) under water deficit conditions, there was no significant increase in irrigated plots.

There are a number of other studies which show the importance of antitranspirants even for the crops for which photosynthesis is in great importance in terms of producing economic yield. Folicote (a hydrocarbon emulsion) and Vapor gard (di-1-p-menthene), reduced water uptake by Norgold Russet potato (*Solanum tuberosum*) plants by 20-40% and increased yield by 2352-5040 kg/ha (Lipe and Thomas, 1980). An antitranspirant, Pinolene (di-1-p-menthene), sprayed every fortnight to the aerial part of hydroponically cultivated sweet pepper (*Capsicum annuum* L.) plants at 1% (v/v) reduced the fructose and glucose concentration in the leaves but no effect was found in the fruits. The antitranspirant significantly reduced fruit firmness but no effect was found on fruit colour, shape index, total soluble solids, or pericarp thickness (Amor and Rubio, 2009). Cranberry vines (*Vaccinium macrocarpon*) receiving an application of Vapor gard (di-1-p-menthene) produced more berries and greater total fruit mass compared to the non treated plants (Sandler, 1998).

Reduction of the degree and length of periods of moisture stress through the use of antitranspirants may increase the amount of time when photosynthesis takes place thus increasing crop yield with the water available (Fuehring, 1973).

1.6.3 The effect of film antitranspirants on leaf temperature

Transpiration is important in leaf cooling. It has been calculated that the percentage of the net energy input removed by transpiration is quite high (Gale and Hagan, 1966). Although, transpiration may remove a considerable portion of the absorbed net energy, a reduction in transpiration will not cause a proportionate rise in the difference between the plant leaf and air temperatures (Gale and Hagan, 1966). Experimental and theoretical analysis of the thermal balance of plant leaves under field conditions indicated that transpiration could not lower leaf temperatures by more than about 5°C (Gale and Poljakoff-Mayber, 1966).

As transpiration is reduced by antitranspirants leaf temperature is increased. Even at the environmental conditions which cause the transpiration rate to be very low, an antitranspirant may not increase leaf temperature to a level which causes adverse effects,

since antitranspirants do not cause a complete cessation of transpiration (Gale and Hagan, 1966; Gale and Poljakoff-Mayber, 1966).

Gale & Poljakoff-Mayber (1966) measured leaf temperature of a number of species treated with the antitranspirants, Tag (a polyethylene based emulsion), S-789 (a copolymer dispersion of acetate acrylate esters) and S-4000 (a copolyacrylic emulsion), which reduced transpiration by about 30 per cent, and found no significant difference in temperature between treated leaves and untreated leaves. Irmak and Jones (2000) observed a significant increase in temperature (by 3.5%) upon the application of Vapor Gard (di-1-p-menthene) on tomato (*Solanum lycopersicum*) plants.

The increase in leaf temperature by an antitranspirant application has been seen to have advantageous effects on plants. Han (1990) suggested reduction of heat loss by antitranspirants may help promote plant health under stressful environmental situations. Irmak and Jones (2000) explored how different plant growth processes are affected by Vapor Gard (di-1-p-menthene) using tomato (*Solanum lycopersicum*) plants and postulated that the observed increases in plant growth characteristics (plant developmental rate and the leaf area expansion rate) upon the antitranspirant application were for an increase in plant temperature (by 3.5°C) made by the antitranspirant by reducing transpiration.

Although it was believed that antitranspirants may also affect leaf temperatures by altering their albedo, by trapping long wave radiation or by modifying the convective sensible cooling of the air by changing the surface texture of the leaf, their total effect is very small (Gale and Poljakoff-Mayber, 1966).

2 The effect of film antitranspirants at different growth stages in relation to meiosis in pollen mother cells on yield and yield components of droughted wheat

2.1 Introduction to the chapter 2

The objective 1 of the project: the effect of film antitranspirants applied at different growth stages around the time of meiosis in pollen mother cells on yield and yield components of winter wheat was explored in field experiments carried out in the three years of 2008/2009, 2009/2010 and 2010/2011.

In 2008/2009 and 2010/2011 two adjacent experiments were carried out, one in natural environmental conditions and one under polytunnels (Figure 2.1.1) which were used to restrict rain water reaching the crop. In 2010/2011 there were three adjacent experiments two under polytunnels and one in natural environment. In total, over the three years there were seven field experiments, four experiments under polytunnels and three in a natural environment.

It was not the same experiment repeated in all the three years. Depending on the results of the previous experiments some developments and changes were made to the experiments in each following year. The objectives of each year, which are relevant to this section, are given below. The hypotheses tested by each of the experiments are listed at the end of this section.

The objectives of the field experiments in 2008/2009 were:

1. To explore the effect of the film antitranspirant di-1-p-menthene (Emerald; Intracrop Ltd, Lechlade) applied at GS33, GS 39, GS41 and GS59 on yield and yield components of winter wheat.
2. To compare two winter wheat varieties to investigate possible differences in their response in yield and yield components to the above antitranspirant treatments.

It was shown by the study explained in chapter 3, in the winter wheat variety Claire, meiosis occurs at early GS41. In order to conserve enough water in the plant at meiosis, antitranspirant should be applied prior to meiosis. The length of time prior to meiosis that the antitranspirant should be applied may depend on SMD and weather conditions which determine the rate of evapotranspiration. According to Kettlewell *et al*. (2010) yield was

increased when the film antitranspirant was applied at GS39 and the authors stated application at an earlier stage might be more effective. In the experiments in 2008/2009, the four growth stages, GS33 (well before meiosis), GS39 (just before meiosis), GS41 (at the time of meiosis) and GS59 (well after meiosis) were chosen for antitranspirant-treatment application so that a wide range of growth stages in relation to the time of meiosis in pollen mother cells is covered.

One hard milling winter wheat variety (Einstein) and one soft milling winter wheat variety (Claire) was chosen for the experiments in 2008/2009. Both the varieties are in the HGCA Recommended List, 2013/214, and Claire is higher yielding than Einstein. According to the information in HGCA Recommended List, 2013/214 Claire is a nabim group 3, soft, milling, high-yielding variety with a high disease resistance and use in UK biscuit making (HGCA, 2013). Whereas Einstein is a nabim group 2, hard, milling wheat with a high disease resistance and used in UK bread making (HGCA, 2013). No information on the relative drought resistance of the two varieties was presented in the list. One of the reasons Claire was chosen for the first field experiment is the fact that it was one of the varieties that was used in previous experiments conducted in Harper Adams University. These two varieties were chosen because the varieties are from two different groups.

The antitranspirant which has been used in the previous experiments conducted in Harper Adams University is di-1-p-menthene (Emerald; Intracrop Ltd, Lechlade). The same antitranspirant was used so that the results of the experiment can be compared with the results of previous experiments.

The objectives of the field experiments in 2009/2010 were:

1. To explore the effect of the film antitranspirant di-1-p-menthene applied at GS31, GS 33 and GS41, and latex (Neo-Tex; Intracrop Ltd, Lechlade) applied at GS41 on yield and yield components of winter wheat (variety Claire).
2. To determine the effect of above antitranspirant treatments on yield and yield components of winter wheat (variety Claire) under two different SMD regimes; high SMD regime and low SMD regime.

The objectives of the experiments in 2009/2010 were decided depending on the results of the experiments in 2008/2009. The reasons behind the modifications in the objectives of the experiments in 2009/2010 compared to those in 2008/2009 are explained in chapter 2.4.

The objective of the field experiments in 2010/2011 was:

- To explore the effect of the film antitranspirant di-1-p-menthene applied at GS33 and latex applied at GS33 on yield and yield components of winter wheat (variety Claire).

Unlike in previous two years, it was decided to have two experiments inside polytunnels in 2010/2011 with the same objective described above. The difference between the two experiments was, one experiment was irrigated after GS69 to field capacity but the other was without irrigation until harvest. The experiments in 2010/2011 were decided depending on the results of both the experiments in 2008/2009 and 2009/2010, and rationale behind the decisions is described in chapter 2.4.

The first objective of both the experiments in 2008/2009, 2009/2010 and the only objective of the experiments in 2010/2011 are based on the first, second and third hypotheses given below. The second objective of the experiment in 2008/2009 is based on the fourth hypothesis given below, and the second objective of the experiment in 2009/2010 is based on the fifth hypothesis given below.

The hypotheses are:

1. Film antitranspirants increase yield of droughted wheat when applied before GS41, the growth stage that meiosis in pollen mother cells occurs in the winter wheat cultivars, Claire and Einstein
2. The most effective growth stage to apply a film antitranspirant to increase yield under drought conditions may be GS31, GS33, GS39 or GS41
3. The increase of yield is by an increase in the number of grains

4. The two varieties, Claire and Einstein are different in their response in yield and yield components to the antitranspirant treatments made around meiosis in pollen mother cells
5. The responses in yield and yield components to antitranspirant treatments made under different SMDs at application are different in winter wheat (variety Claire)

Figure 1.6.1: Polytunnels used to restrict rain water

2.2 Materials and Methods

2.2.1 Experimental site

All the field experiments were located in Flat Nook field, an experimental site at Harper Adams University College, Shropshire (52°46′N, 2°25′W) on a loamy sand soil with good drainage. The particle size distribution was 80% sand (2 mm – 200 µm) to 0.3 m (top soil) and 92% sand below this. The remainder was predominately silt (quantified by particle size distribution analysis; MAFF/ADAS, 1987). Medium sized, rounded quartzite stones were common throughout the soil profile. The field capacity for a depth of 80 mm was determined to be 160 mm from neutron probe measurements (Institute of Hydrology Neutron Probe System, Wallingford). For a depth of 80 mm, the permanent wilting point was quantified to be 62 mm (Hall *et al*., 1977) and the available soil water content, which is the difference between the field capacity and the permanent wilting point (Hall *et al*., 1977), was calculated to be 98 mm. Although 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: 2008/2009 maize, 2009/2010 fallow (no crop), 2010/2011 oil seed rape.

2.2.2 Sowing and the layout of the experiments

Seeds were sown on 26.11.08 and 19.10.09 respectively for the experiments in 2008/2009 and 2009/2010. For the experiments in 2010/2011 at first seeds were sown in October 2010. Since most of those seedlings were destroyed by pigeons, the whole trial was redrilled on 18.01.2011. The depth of sowing, row spacing and the seed rate were 2 cm, 15 cm and 350 seeds/m^2 respectively in all years.

All of the experiments were composed of three blocks. Each year the three blocks of the experiment under the polytunnels were in three different polytunnels. In 2008/2009 and 2009/2010 the whole of one polytunnel was occupied by one block and in 2010/2011,

where there were two experiments under polytunnels, two blocks one from each experiment shared one polytunnel.

2.2.3 Experimental design and treatments

On all the occasions mentioned below antitranspirants were applied at 2.5 l ha^{-1} (1.25% v/v antitranspirant in water), using a hand held sprayer at a sprayer pressure of 0.2 MPa and a sprayer speed of 1 ms^{-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. On every occasion mentioned below water was applied by a trickle irrigation system with irrigation tapes with 10 cm spaced, 5 mm diameter emitters, aligned either sides of the crop rows. GenStat 12th edition (VSN International, Hemel Hempstead UK) was used to randomise treatments. Prior to moving the polytunnels into the position the application of fertilizers and nutrients followed typical practice for intensively-grown wheat in the UK, but no application of either fertilizers or nutrients were done after positioning the polytunnels to avoid a possible difference in nutrient uptake between irrigated plots/plots exposed to rain and non-irrigated/droughted plots. The crop management inputs of the three years are listed in Appendix I.

2.2.3.1 Field experiments in 2008/2009

In 2008/2009 both the experiment in the natural environment and the experiment under the polytunnels were split plot designs with two factors and three replicates (Figure 2.2.1). Each replicate was a block, and each experiment was composed of 36 plots of approximately 10 × 1.5 m. Two winter wheat varieties Claire and Einstein were randomised on main plots and four antitranspirant treatments and two controls were randomised on sub plots (Table 2.2.1). The four antitranspirant treatments of the experiment under the polytunnels were the antitranspirant di-1-p-menthene (96%; Emerald; Intracrop Ltd, Lechlade) sprayed at GS33, GS39, GS41 and GS59. In 2008/2009 the temperature and hence the development rate of the crop under the polytunnels were higher than that of the natural environment. Therefore on the dates on

which the antitranspirant treatments were made the growth stages of the crop in natural environment were GS32, GS37, GS39 and GS55 respectively, instead of GS33, GS39, GS41 and GS59. The two controls were; the unsprayed control (UC) which was not sprayed with the antitranspirant and also without irrigation the same as the experimental sprayed plots; the unsprayed irrigated control (IUC) which was also unsprayed with the antitranspirant but with irrigation to maintain the SMD above 50% of field capacity (field capacity was 160 mm for 80 cm depth). Polytunnels were moved into position at the beginning of growth stage GS25, on 25.04.09. From this date until harvest there was no water supply to the antitranspirant treated and the unsprayed control plots of the experiment under the polytunnels.

Table 2.2.1: The factors and levels of the field experiments in 2008/2009

Factor 1: Variety	Factor 2: Antitranspirant/control treatment
Claire Einstein	di-1-p-menthene treatment at GS33 di-1-p-menthene treatment at GS39 di-1-p-menthene treatment at GS41 di-1-p-menthene treatment at GS59 Unsprayed control Irrigated unsprayed control

Note that: on the dates on which the antitranspirant treatments were made the growth stages of the crop in natural environment were GS32, GS37, GS39 and GS55 respectively, instead of GS33, GS39, GS41 and GS59.

Ex. 2 Block 1	Ex. 1 Block 1	Ex. 1 Block 2	Ex. 2 Block 2	Ex. 1 Block 3	Ex. 2 Block 3
C-IUC	**C**-IUC	**E**-IUC	**C**-IUC	**C**-GS41	**E**-GS55
C-GS32	**C**-UC	**E**-GS39	**C**-GS37	**C**-UC	**E**-IUC
C-GS55	**C**-GS59	**E**-GS33	**C**-GS39	**C**-IUC	**E**-UC
C-UC	**C**-GS41	**E**-GS41	**C**-GS55	**C**-GS33	**E**-GS39
C-GS39	**C**-GS33	**E**-UC	**C**-GS32	**C**-GS39	**E**-GS32
C-GS37	**C**-GS39	**E**-GS59	**C**-UC	**C**-GS59	**E**-GS37
E-GS32	**E**-GS41	**C**-IUC	**E**-GS32	**E**-IUC	**C**-UC
E-UC	**E**-IUC	**C**-GS39	**E**-UC	**E**-UC	**C**-GS37
E-GS55	**E**-GS39	**C**-GS33	**E**-IUC	**E**-GS39	**C**-GS39
E-IUC	**E**-GS59	**C**-GS41	**E**-GS37	**E**-GS59	**C**-IUC
E-GS37	**E**-GS33	**C**-GS59	**E**-GS55	**E**-GS33	**C**-GS32
E-GS39	**E**-UC	**C**-UC	**E**-GS39	**E**-GS41	**C**-GS55

Figure 2.2.1: Layout of the field experiments in 2008/2009; Highlighted in yellow: blocks under polytunnels; **Ex 1:** experiment under polytunnels; **Ex 2:** experiment in the natural environment; **C:** variety Claire; **E:** variety Einstein; **UC:** unsprayed control; **IUC:** irrigated unsprayed control; **GS32/GS33/GS37/GS39/GS41/GS55/GS59:** di-1-p-menthene treatment at the respective growth stages

2.2.3.2 Field experiments in 2009/2010

The experiments in 2009/2010 were modified based on the results of the experiments in 2008/2009 as explained in chapter 2.4. The major changes done to the experiments are: Instead of varieties, SMD regimes were used as the first factor and only the variety Claire was used; unlike in 2008/2009, the whole experiment under the polytunnels was irrigated to field capacity when the crop was at GS69. Some changes in antitranspirant treatments and in control treatments were also done as described in the following text.

Both, the experiment in the natural environment and the experiment under the polytunnels in 2009/2010 were randomised as split plot designs with two factors and three replicates. Each replicate was a block, and each experiment was composed of 36 plots of

approximately 10 × 1.5 m. Polytunnels were moved into the position at GS25, on 28.04.2010.

Two soil moisture deficit regimes were randomised in main plots and four antitranspirant treatments and two control treatments were randomised in the sub plots. The two SMD regimes were the “low SMD regime” and the “high SMD regime”. In the experiment under the polytunnels the highest SMD of the low SMD regime was 90 mm and that value was reached when the crop is at GS37. From GS37 to GS69, all the plots in the low SMD regime were irrigated to maintain the SMD around 90 mm. SMD was predicted using the IMS irrigation scheduling programme and the amount of water that should be applied to the low SMD regime every other day to maintain SMD at 90 mm was then calculated. The SMD of the high SMD regime by the time of irrigation at GS69 was 115 mm therefore that was the highest SMD of the high SMD regime. The low and high soil moisture deficit regimes were included in the experimental design of the experiment under the natural environment to be used in case of a possible occurrence of an extended non-rainy episode during the experimental period. There were, however, rains from time to time and it was not possible to have a difference in the SMD of the two regimes. Treatments were applied presuming there were two SMD regimes so that the experimental design was not compromised. The four antitranspirant treatments were di-1-p-menthene sprayed at GS31, GS33 and GS41 and latex (98%; Neo-Tex; Intracrop Ltd, Lechlade) sprayed at GS41. One of the controls, unsprayed control (UC) was unsprayed and received irrigation to the same extent as the antitranspirant treated plots of the same SMD regime. The other control treatment, irrigated unsprayed control (IUC) was also unsprayed but received irrigation to maintain the SMD above 75% of field capacity (field capacity is 160 mm for 80 cm depth), until irrigation for the whole experiment was ceased at GS85 to enhance grain ripening.

After randomising the experiments in a split plot design with the treatments described above, a change was made to the controls. Since there was no difference in the irrigated unsprayed controls of the two SMD regimes it was decided to change one of the irrigated unsprayed controls to an irrigated sprayed plot (ISP). Therefore, in each polytunnel, either

the IUC in the low SMD regime or the high SMD regime, as selected randomly, was changed into an ISP. Irrigation of the ISP was the same as for the IUC and the only difference was that it was sprayed with di-1-p-menthene at GS33. The IUC was used as a common control for all of the treatments in both the SMD regimes. The ISPs were compared with IUC just to see whether there is any effect of antitranspirant spray at GS33 on plants under no SMD. Table 2.2.2 shows the treatments and Figure 2.2.2 shows the final experimental layout.

Table 2.2.2: The factors and levels of the field experiments in 2009/2010

Factor 1: SMD regime	Factor 2: Treatment
Low SMD regime High SMD regime	di-1-p-menthene treatment at GS31 di-1-p-menthene treatment at GS33 di-1-p-menthene treatment at GS41 latex treatment at GS41 Unsprayed control
Irrigated unsprayed control (IUC) which is common to both SMD regimes	

Ex. 2 Block 1	Ex. 1 Block 1	Ex. 2 Block 2	Ex. 1 Block 2	Ex. 2 Block 3	Ex. 1 Block 3
ISC	**L**-di-GS33	**H**-di-GS41	ISC	**L**-la-GS41	IUC
L-di-GS31	IUC	**H**-di-GS31	**L**-la-GS41	**L**-di-GS33	**H**-la-GS41
L-la-GS41	**L**-UC	**H**-di-GS33	**L**-UC	**L-**di-GS41	**H**-UC
L-di-GS33	**L**-la-GS41	**H**-UC	**L**-di-GS31	IUC	**H**-di-GS41
L-UC	**L**-di-GS31	**H**-la-GS41	**L**-di-GS41	**L**-di-GS31	**H**-di-GS33
L-di-GS41	**L**-di-GS41	IUC	**L**-di-GS33	**L**-UC	**H**-di-GS31
IUC	**H**-di-GS33	**L**-di-GS31	**H**-di-GS31	**H**-la-GS41	**L**-di-GS41
H-di-GS31	**H**-di-GS41	**L**-la-GS41	IUC	ISC	**L**-di-GS31
H-UC	**H**-di-GS31	**L**-di-GS41	**H**-UC	**H**-di-GS41	**L**-la-GS41
H-la-GS41	ISC	**L**-UC	**H**-di-GS41	**H**-di-GS31	**L-**di-GS33
H-di-GS33	**H**-UC	ISC	**H**-la-GS41	**H**-UC	**L**-UC
H-di-GS41	**H**-la-GS41	**L**-di-GS33	**H**-di-GS33	**H**-di-GS33	ISC

Figure 2.2.2: Layout of the field experiments in 2009/2010; Highlighted in yellow: blocks under polytunnels; **Ex 1:** experiment under polytunnels; **Ex 2:** experiment in natural environment; **L:** low SMD regime; **H:** high SMD regime; **UC:** unsprayed control; **IUC:** irrigated unsprayed control; **ISC:** Irrigated sprayed control; **di-GS31/GS33/GS41:** di-1-p-menthene treatment at the respective growth stages; **la-GS41:** latex treatment at GS41

2.2.3.3 Field experiments in 2010/2011

Compared to the previous experiments in 2010/2011 the number of replicates was increased. Unlike the previous experiments in 2010/2011 only the GS 33 was included in terms of spray application, only the unsprayed control was included as a control treatment, and all the experiments were one-factor experiments. Polytunnels were moved into the position earlier compared to the last two years at GS 23, on 01.04.2011. The rationale behind these decisions is discussed in chapter 2.4. The length of the polytunnels was increased to 26 m (previous years it was 24 m) the plots were half the length of the

plots of previous years and the number of treatments reduced to increase the number of replicates still using only three polytunnels.

The variety Claire was used for the field experiments in 2010/2011 and all the three experiments; the experiment in the natural environment, the experiment under the polytunnels irrigated after GS 69 (Experiment 1) and the experiment under the polytunnels with no irrigation (Experiment 2), used a complete randomised block design (Figure 2.2.3). The experiment in the natural environment and Experiment 1 under the polytunnels had 6 replicates within a block and a total of 18 replicates. Experiment 2 under the polytunnels had 2 replicates within a block and a total of 6 replicates. The size of the plots inside the polytunnels (in Experiment 1 and 2) was 4 x 1.2 m and that of the plots in the natural environment was 5 x 1.2 m.

All the experiments were composed of two antitranspirant treatments and one control (Table 2.2.3). The two antitranspirant treatments were di-1-p-menthene sprayed at GS33 and latex sprayed at GS33. The control, unsprayed control (UC) was not sprayed with any of the antitranspirants.

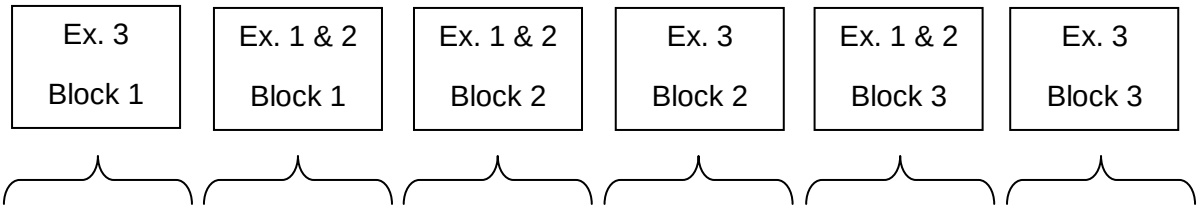
In addition to these treatments, two plots per each block of the experiment in the natural environment and per each polytunnel were irrigated (Irrigated unsprayed plots: IUP) whenever necessary to maintain SMD above 75% of field capacity (field capacity is 160 mm for 80 cm depth) as a reference point to give an indication of the effect of natural drought and the drought imposed by the polytunnels. Irrigated plots were not included when the treatments within the each experiment were randomised to the plots, therefore, irrigated plots were not considered as a part of any of the experiments statistically.

None of the treatments in Experiment 1 under the polytunnels were irrigated until the whole Experiment 1 was irrigated at GS 69 to field capacity. Irrigation to Experiment 1 was withheld at GS85 to enhance grain ripening. The difference between Experiment 1 and Experiment 2 was that Experiment 2 was not irrigated at any time until harvest. The experiment in the natural environment did not receive any irrigation except rain from sowing to harvest.

Table 2.2.3: The experiments and the treatments in 2010/2011

Experiments	Treatment
Experiment 1: inside polytunnels; irrigated after GS69 Experiment 2: inside polytunnels; without irrigation until harvest The experiment in the natural environment	di-1-p-menthene treatment at GS33 latex treatment at GS33 Unsprayed control

Note that: Apart from the treatments there were irrigated unsprayed plots inside polytunnels common to Experiment 1 and Experiment 2, and irrigated unsprayed plots outside polytunnels for the experiment in the natural environment. Irrigated unsprayed plots were not randomised with in experiments, therefore, not considered as a treatment.



IUP	**IUP**	**IUP**	**IUP**	**IUP**	**IUP**	discard		**UC**	**di**	**IUP**	**IUP**
discard		discard		discard		discard		**UC**	**UC**	discard	
UC	**di**	**la**	**UC**	**UC**	**di**	discard		**di**	**di**	**UC**	**di**
di	**UC**	**UC**	**la**	**di**	**la**	**UC**	**la**	**UC**	**di**	**la**	**di**
UC	**di**	**UC**	**di**	**UC**	**la**	**UC**	**di**	**la**	**UC**	**di**	**UC**
la	**UC**	**di**	**UC**	**di**	**UC**	**la**	**la**	**la**	**la**	**UC**	**la**
di	**la**	**di**	**la**	**UC**	**la**	**di**	**di**	**la**	**di**	**di**	**di**
la	**UC**	**la**	**UC**	**la**	**di**	**di**	**la**	**la**	**UC**	**di**	**la**
la	**di**	**la**	**di**	**la**	**UC**	**di**	**di**	**la**	**di**	**UC**	**la**
di	**UC**	**di**	**di**	**UC**	**di**	**UC**	**la**	**UC**	**UC**	**di**	**di**
la	**la**	**UC**	**la**	**di**	**la**	**UC**	**UC**	**di**	**la**	**di**	**la**
discard		**la**	**di**	**di**	**la**	**la**	**UC**	**di**	**la**	discard	
discard		**UC**	**la**	**UC**	**di**	discard		discard		discard	
discard		**UC**	**di**	**la**	**UC**	**IUP**	**IUP**	**IUP**	**IUP**	discard	

Figure 2.2.3: Layout of the field experiments in 2010/2011; Highlighted in yellow: blocks under polytunnels; **Ex.1/green font:** experiment under polytunnels irrigated after GS69; **Ex. 2/blue font:** experiment under polytunnels without irrigation until harvest; **Ex. 3:** experiment in the natural environment; **UC:** unsprayed control; **IUP:** irrigated unsprayed plots; **di:** di-1-p-menthene treatment at **GS33**; **la:** latex treatment at **GS33**

2.2.4 Soil moisture measurements

In all the three years SMD was calculated using the IMS irrigation scheduling programme (Hess, 1996) (Silsoe College, UK). The IMS irrigation scheduling programme was continuously fed with data since October 2008 to August 2011. Apart from weather data the other information which is crucial for the output from the irrigation programme are as follows; the maximum root length = 80 cm (as deduced by the neutron probe readings); the “texture class” for both top-soil and sub-soil = LS (loamy sand; from particle size

distribution analysis; MAFF/ADAS, 1987); top soil depth = 40 cm. Weather data was obtained from the weather station at Harper Adams University College approximately 1/2 km from the field.

In 2008/2009 and 2009/2010, apart from the results obtained from the IMS irrigation scheduling programme, soil moisture measurements were taken with the soil moisture monitoring device, Diviner 2000 (Sentek Technologies; Stepney, Australia). In 2008/2009 and 2009/2010 readings were taken respectively from four and six locations per block in both the experiment in the natural environment and the experiment under polytunnels.

In 2010/2011, apart from the results obtained from the IMS irrigation scheduling programme, soil moisture measurements were taken with the neutron probe (Institute of Hydrology Neutron Probe System, Wallingford) with a probe 100 cm in length. Soil moisture readings were taken from five randomly selected locations belongs to UC treatment and five randomly selected locations belongs IUP plots of the experiment under polytunnels. From the experiment in the natural environment, readings were taken from two randomly selected locations belong to UC treatments and two randomly selected locations IUP plots. Soil moisture readings were taken at different depths along the soil profile starting from 2.5 cm down the soil surface up to a maximum depth of 100 cm at intervals of 10 cm.

2.2.5 Pre-harvest assessments

Pre-harvest assessments assessed the number of heads per m^2, grain yield per m^2 (at 15% moisture), the number of grains per ear and thousand grain weight of each sub sub-plot.

Two or three days prior to harvest (by combine harvester) shoots which occurred inside a $1m^2$ quadrat on a randomly selected position per sub sub-plot were sampled by harvesting the heads with a pair of scissors. The number of ears in each sample was counted to obtain the number of heads per m^2 of each sub sub-plot. The samples were threshed separately using an electric thresher (Wintersteiger, Austria). Care was taken to collect all

the grains by collecting grains dropped on the floor and blown into the chamber in the thresher into where chaff goes. The samples were cleaned to remove chaff. The moisture content of each sample was measured by a moisture analyser (AP 6060, Sinar, Surrey, UK) and soon after, the samples were weighed. Sample weights were adjusted to 15% moisture to obtain the grain yield per m^2 of each sub sub-plot. After weighing the samples, without delay, subsamples of about 40 g were separated from each sample and hand-cleaned. After cleaning the sub samples were weighed again. The number of grains in each sub sample was counted by a grain counter (CountAmatic Console, Farm-Tec, Whitby, UK). The grain number per ear was calculated as follows (Sylvester-Bradley *et al*., 1985):

$$= \frac{\text{number of grains in subsample} \times \text{weight of whole sample}}{\text{number of ears in whole sample} \times \text{weight of subsample.}}$$

The thousand grain weight was calculated as follows (Sylvester-Bradley *et al*., 1985):

$$= \frac{\text{weight of subsample}}{\text{number of grains in the sub sample}} \times 1000$$

2.2.6 Data analysis

Data obtained were statistically analysed using GenStat 13th edition (VSN International, Hemel Hempstead UK). The skeleton ANOVA of yield and yield components data of the three years are shown below. The skeleton ANOVAs described are for experiments inside polytunnels in the three years. The skeleton ANOVA for an experiment in natural environment is similar to that of the experiment inside polytunnels in the same year, apart from the differences in residual degrees of freedom, which is caused by the presence/absence of covariates in the analysis depending on their significance. However, the skeleton ANOVAs for the experiments in natural environment are not discussed in detail, since, in all the three years the treatments were not significant for yield or any of the yield components.

After the data from each year had been analysed for the first time, it was understood that the CV% for each parameter was high, and if the CV% could be decreased, the effect of the treatments on yield and yield components would be clearer. The spatial differences in available water-holding capacity in a field, which are created by the differences in soil type and characteristics, are a significant contributor for spatial differences in crop yield from the field (Wright *et al*., 1990; Timlin *et al*., 2001). The influence of the spatial differences in available water-holding capacity on yield could be much greater when the crop is under drought conditions (Grisso *et al*., 2009), and the effects of spatially varying soil water-holding capacity on yield might conceal the effects of the treatments on yield. The electrical conductivity of soil correlates with soil water holding capacity, therefore electrical conductivity can be used to detect spatial variations in soil water holding capacity in a field (Grisso *et al*., 2009). Apart from with the soil water holding capacity, electrical conductivity of soil correlates with some other soil properties that affect crop productivity, including soil texture, organic matter level, salinity and cation exchange capacity (Grisso *et al*., 2009). However, most of these properties are directly or indirectly related to soil water holding capacity. Therefore, a soil electrical conductivity map can serve as a substitute for a soil water holding capacity map (Lund *et al*., 2000). Field yield maps frequently correlates with soil electrical conductivity maps, and soil electrical conductivity maps are now widely used in precision farming (Grisso *et al*., 2009; Lund *et al*., 2000). By using the electrical conductivity of soil at each plot as a covariate, the influence of spatially varying soil water holding capacity, on yield and yield components may be eliminated. After the completion of all the field experiments, the soil electrical conductivity maps of the locations of the field experiments were produced and provided by the company, Soyl Precision Farming (Newbury, United Kingdom). The experimental designs were drawn to the scale on the soil electrical conductivity maps to find out the exact electrical conductivities of each plot area from where the crop from each plot was sampled for yield and yield component analyses. The soil electrical conductivity of each plot was used as a covariate in the analyses of yield and yield component data from the three years. The significance of the covariate in the analyses of each parameter (yield and yield components) in each of the

experiments and other covariates particular to any of the experiments, are stated in the following text, when the skeleton ANOVA related to each experiment is discussed. The soil electrical conductivity maps of the experiments are given in Appendix II.

Apart from ANOVA, regression analyses between yield components were performed to detect any correlation/compensatory effect between yield components. The antitranspirant/control treatments were used as the grouping factor.

2.2.6.1 The analysis of yield and yield component data from the experiment in 2008/2009

The skeleton ANOVA of the yield and yield components data from the experiment inside the polytunnels in 2008/2009 is shown in Table 2.2.4. In 2008/2009, the covariate, soil electrical conductivity, was not significant in the ANOVA of yield or any of the yield components. In 2008/2009, there was a row of plots with a lower sowing rate compared to the other plots. There were only three of those plots (total number of plots = 36) within the experiment inside the polytunnels. This difference was included into the ANOVA as a covariate: the covariate, the difference in sowing rate. The covariate, the difference in sowing rate was significant in the ANOVA of grains ear^{-1}. This covariate was not significant in the ANOVA of yield, TGW or ears m^{-2}. The skeleton ANOVA provided is for grains ear^{-1}, where the covariate, the difference in sowing rate was significant. For the parameters, for which the covariate was not significant, ANOVA was repeated without the covariate. In this case the degrees of freedom corresponding to residual value of each stratum is higher by one than the degree of freedom shown in the table of skeleton ANOVA. Tukey's test was performed along with ANOVA of each of the parameters.

Table 2.2.4: The skeleton ANOVA of the yield and yield components data from the experiment inside the polytunnels in 2008/2009

Source of variation	Degree of freedom
Block stratum	2
Block / Variety stratum	
Variety	1
Covariate : Sowing rate	1
Residual	1
Block / Variety / AT-control treatment stratum	
AT-control treatment	5
Variety x AT-control treatment	5
Covariate	1
Residual	19
Total	35

2.2.6.2 The analysis of yield and yield component data from the experiment in 2009/2010

The skeleton ANOVA for yield and yield components data from the experiment inside the polytunnels in 2009/2010 is shown in Table 2.2.5. In 2009/2010, the covariate, soil electrical conductivity, was not significant in the ANOVA of yield or any of the yield components, and there was no any other covariate which was used. GenStat 13th edition did not allow Tukey's test to be performed with this experiment, which is a split plot with a common control.

Table 2.2.5: The skeleton ANOVA for yield and yield components data from the experiment inside the polytunnels in 2009/2010

Source of variation	Degree of freedom
Block stratum	2
Block / Common control stratum	
Common control vs. treatments	1
Residual	2
Block / Common control / SMD regime stratum	
SMD regime	1
Residual	2
Block / Common control / (SMD regime / AT-control treatment) stratum	
AT-control treatment	4
Common control / SMD regime x AT-control treatment	4
Residual	16
Total	32

2.2.6.3 The analysis of yield and yield component data from the experiment in 2010/2011

In 2010/2011, there was a row of plots with a lower sowing rate compared to the other plots, and there were a few plots with low crop density resulted from the invasion of weeds. Altogether there were 18 plots (total number of plots = 54) within Experiment 1 and, two plots (total number of plots = 18) within Experiment 2 with the issue of low crop density. This difference was included into the ANOVA as a covariate. Tukey's test was performed along with ANOVA of each of the parameters to make the analyses consistent with the analyses of the results from the experiment in 2008/2009. ANOVA contrast analyses were also performed in 2010/2011 especially to detect the significance of the difference between the unsprayed control and the two antitranspirant treatments taken as a whole, since both the antitranspirant treatments were made at GS33 (latex treatment at GS33 and di-1-p-menthene treatment at GS33). Although Tukey's test shows the

significance of the differences in parameters between the treatments it does not contrast the results from the unsprayed control from that of the two antitranspirant treatments taken as a whole.

The skeleton ANOVA for yield and yield components data from Experiment 1 is shown in Table 2.2.6. In Experiment 1, the covariate, soil electrical conductivity, was significant only in the ANOVA of yield. The covariate, crop density difference, was significant in the ANOVA of yield and ears m^{-2}, but was not significant in the ANOVA of grains ear^{-1} and TGW. The skeleton ANOVA provided is for yield, where both of the covariates were significant. For the parameters, for which both of the covariates were not significant, the degree of freedom corresponding to residual value of each stratum is higher by 2 than the degree of freedom shown in the table of skeleton ANOVA. For ears m^{-2}, for which only the covariate, crop density difference, was significant, the degree of freedom corresponding to residual value of each stratum is higher by one than the degree of freedom shown in the table of skeleton ANOVA.

The skeleton ANOVA for yield and yield components data from Experiment 2 is shown in Table 2.2.7. In Experiment 2, the covariate, soil electrical conductivity or the covariate, crop density difference, were not significant in the ANOVA of yield or any of the yield components.

Table 2.2.6: The skeleton ANOVA of the yield and yield components data from Experiment 1 (inside the polytunnels in 2010/2011)

Source of variation	Degree of freedom
Block stratum	
Covariates	2
Soil electrical conductivity	1
Crop density difference	1
Block / AT-control treatment "units" stratum	
AT-control treatment	2
Unsprayed control vs. AT treatments	1
Latex AT treatment vs. di-1-p-menthene AT treatment	1
Covariates	2
Soil electrical conductivity	1
Crop density differences	1
Residual	47
Total	53

Table 2.2.7: The skeleton ANOVA of the yield and yield components data from Experiment 2 (inside the polytunnels in 2010/2011)

Source of variation	Degree of freedom
Block stratum	2
Block / AT-control treatment "units" stratum	
AT-control treatment	2
Unsprayed control vs AT treatments	1
Latex AT treatment vs di-1-p-menthene AT treatment	1
Residual	13
Total	17

The skeleton ANOVA of the analyses for which replicate data from Experiment 1 and 2 are combined is shown in Table 2.2.8. The covariate, soil electrical conductivity, was significant only in the ANOVA of yield. The covariate, crop density difference, was significant in the ANOVA of yield and ears m^{-2}, but was not significant in the ANOVA of grains ear^{-1} and TGW. The skeleton ANOVA provided is for yield, where both of the covariates were significant. For the parameters, for which both of the covariates were not significant, the degree of freedom corresponding to residual value of each stratum is higher by two than the degree of freedom shown in the table of skeleton ANOVA. For ears m^{-2}, for which only the covariate, crop density difference, was significant, the degree of freedom corresponding to residual value of each stratum is higher by one than the degree of freedom shown in the table of skeleton ANOVA.

Table 2.2.8: The skeleton ANOVA of the analyses for which Experiment 1 and 2 are combined

Source of variation	Degree of freedom
Experiment stratum	
Covariate	1
Soil electrical conductivity	1
Experiment / Block stratum	
Covariates	2
Soil electrical conductivity	1
Crop density differences	1
Residual	2
Experiment / Block / AT-control treatment "Units" stratum	
AT-control treatment	2
Unsprayed control vs AT treatments	1
Latex AT treatment vs di-1-p-menthene AT treatment	1
Covariates	2
Soil electrical conductivity	1
Crop density differences	1
Residual	62
Total	71

2.2.6.4 The combination and the analysis of yield and yield component data from all the experiments inside the polytunnels

In total there are four experiments carried out inside polytunnels in the three years of 2008/2009, 2009/2010 and 2010/2011. Means of the replicates for each of the parameters from the antitranspirant treatments and controls which were consisted in all the experiments were combined for analysis. The only antitranspirant treatment and the control which were consisted in all the experiments were respectively the antitranspirant treatment at GS33 and the unsprayed control. Table 2.2.9 explains how data from each experiment was combined for the analysis.

Table 2.2.9: The data from the four experiments in the three years, which were combined for the analysis

Experiment	Data	Justification
2008/2009	means of the two varieties for yield and yield components of the antitranspirant treatment at GS33 and unsprayed control from each polytunnel	The interaction between the factor, variety and the factor, antitranspirant/control treatment was not significant for any parameter. Therefore, it is permissible to use the means for the two varieties.
2009/2010	means of the two SMD regimes for yield and yield components of the antitranspirant treatment at GS33 and unsprayed control from each polytunnel	The factor, SMD regime or the interaction between the factor, SMD regime and the factor, treatment were not significant for any parameter. Therefore, it is possible to use the means for the two SMD regimes
2010/2011 – Experiment 1	mean of the two antitranspirant treatments for yield and yield components from each polytunnel and mean yield and yield components of the unsprayed control for each polytunnel	Both the antitranspirant treatments were made at GS33, therefore, it is possible to use the means.
2010/2011 – Experiment 2	mean of the two antitranspirant treatments for yield and yield components from each polytunnel and mean yield and yield components of the unsprayed control for each polytunnel	

No covariate was used in the combined analysis, because all of the covariates used above were not consistent for all experiments. The skeleton ANOVA of the analyses in which the four experiments were combined are shown in Table 2.2.10.

Table 2.2.10: The skeleton ANOVA of the analyses in which the 4 experiments were combined

Source of variation	Degree of freedom
Experiment stratum	3
Experiment / polytunnel stratum	8
Experiment / polytunnel / AT-control treatment "units" stratum	
AT-control treatment	1
Residual	11
Total	23

2.3 Results

Daily weather data: rain fall, minimum and maximum temperature, wind speed and relative humidity for each growing season of field experimentation are shown in Appendix III. Weather conditions on a monthly basis are presented in Appendix IV. Since the conditions inside polytunnels were not measured continuously, the estimated changes in conditions (radiation, air temperature and relative humidity) inside polytunnels compared to the external conditions are explained. Cook *et al*, (2007) measured light irradiance outside and inside a polytunnel at noon using a spectroradiometer (Stellarnet EPP2000C) standardised using a white reflectance standard (Labsphere). In general the difference in photosynthetically active radiation, which is the radiation from 400 nm to 700 nm (Cook et al., 2007) between inside and outside polytunnel was 0.03 – 0.06 $Wm^{-2}nm^{-1}$. The polythene used in their polytunnel is 180 micron-thick horticultural polythene, which is similar to polythene used for ours. Furthermore, their experiment was conducted in the UK. Because of these similarities the difference in irradiance between outside and inside polytunnels in their experiments might be similar to that of our experiment. Air temperature was measured with TPS-2 Portable Photosynthesis system (PP systems, MA, USA) inside and outside polytunnels at the same instances that the other parameters (stomatal conductance, the rate of transpiration and photosynthesis) were measured by the equipment. The difference in air temperature inside and outside polytunnels varied greatly with the wind speed and solar radiation. Under dull conditions the difference in temperature between the two environments was almost undetectable. When the solar radiation was above 5 Wm^{-2} and when there was no wind passing through the polytunnels the air temperature inside polytunnels was 3˚C (maximum) higher than that of the outside. The temperature difference was decreased when wind was passing through the polytunnels. The relative humidity (measured with wet and dry bulb hygrometer (Zeal England, Norfolk, UK) to be used as a covariate for water potential data) was inversely related to the air temperature. Once, when there was a temperature difference of 3˚C between the two environments the difference in relative humidity was 11% (lower inside polytunnels).

The SMDs at antitranspirant spray times, changes in SMD with time during the growing seasons of the three years of field experimentation and results obtained for yield and yield components from the analyses of data collected from experiments inside polytunnels are shown in the following text.

Results obtained from the experiments in natural environment did not show any significant difference in yield or any of the yield components between the treatments in any of the experiments. The increase in yield by a film antitranspirant is linearly related to SMD (Kettlewell, 2011), and the SMDs at most of the antitranspirant spray times of experiments in natural environment might not be high enough (Table 2.3.1) to film antitranspirant treatments to show a significant difference in yield and yield components compared to unsprayed controls. Although at some of the antitranspirant application times the SMDs were relatively high, (antitranspirant treatments at GS41 in 2009/2010 and antitranspirant treatments at GS33 in 2010/2011) there were rains around the antitranspirant application times, which might have cancelled the difference in response of the plants to antitranspirant/control treatments. Therefore, the experiments in natural environment were not a good test of the objectives. The results from the experiments in natural environment are not discussed in detail, and are presented in Appendix V.

2.3.1 The SMDs at antitranspirant spray times and changes in SMD with time

The SMD on each date throughout the period of each experiment was calculated by the IMS irrigation scheduling programme as explained in section 2.2.4. The SMDs at antitranspirant spray times in the experiments in natural environment are shown in Table 2.3.1 and that of the experiments inside polytunnels are shown in Table 2.3.2.

Table 2.3.1: The SMDs at antitranspirant spray times in the experiments in natural environment

Year	Date	Antitranspirant treatment	SMD (mm)
2008/2009	14.05.2009	di - GS33	29.2
	20.05.2009	di - GS39	15.6
	29.05.2009	di - GS41	33.2
	11.06.2009	di - GS59	10.1
2009/2010	08.05.2010	di - GS31	32.6
	17.05.2010	di - GS33	49.4
	25.05.2010	di - GS41	76.7
		la - GS41	
2010/2011	12.05.2011	di - GS33	81.1
		la - GS33	

di = di-1-p-menthene; la = latex

Table 2.3.2: The SMDs at antitranspirant spray times in the experiments inside polytunnels

Year	Date	Antitranspirant treatment	SMD (mm)
2008/2009	14.05.2009	di - GS33	66.9
	20.05.2009	di - GS39	77.8
	29.05.2009	di - GS41	91.7
	11.06.2009	di - GS59	108.4
2009/2010	08.05.2010	LSMD di – GS31	66.5
		HSMD di – GS31	
	17.05.2010	LSMD di – GS33	87.7
		HSMD di – GS33	
	25.05.2010	LSMD di – GS41	82.2
		LSMD la – GS41	
		HSMD di – GS41	102.6
		HSMD la – GS41	
2010/2011	12.05.2011	Ex 1 - di – GS33	90.7
		Ex 1 - la – GS33	
		Ex 2 - di – GS33	
		Ex 2 - la – GS33	

LSMD = low SMD regime, HSMD = high SMD regime, di = di-1-p-menthene; la = latex

The changes in SMD with time inside the polytunnels in all the three years are shown in Figure 2.3.1. In Figure 2.3.1, the line/lines related to each year starts at the date that the polytunnels moved into the position. For 2008/2009, changes in SMD are shown until the crop reached GS69. For 2009/2010, changes in SMD in both the low SMD regime and the high SMD regime are shown until the date that the whole experiment was irrigated to field capacity at GS69. For 2010/2011, changes in SMD are shown until the date that the whole Experiment 1 was irrigated to field capacity at GS69. Note that, since, both Experiment 1 and Experiment 2 were conducted in the same polytunnels; the changes in SMD were similar for both the experiments until the crop reached GS69, when Experiment 1 was irrigated to field capacity. Although Experiment 2 in 2010/2011 and the experiment in 2008/2009 were not irrigated until harvest, the changes in SMD after GS69 for the two experiments are not presented considering the unmanageable long length that the figure would possess if the data were included. The changes in SMD from GS69 to harvest in all the four experiments inside polytunnels in the three years are presented in Appendix VI.

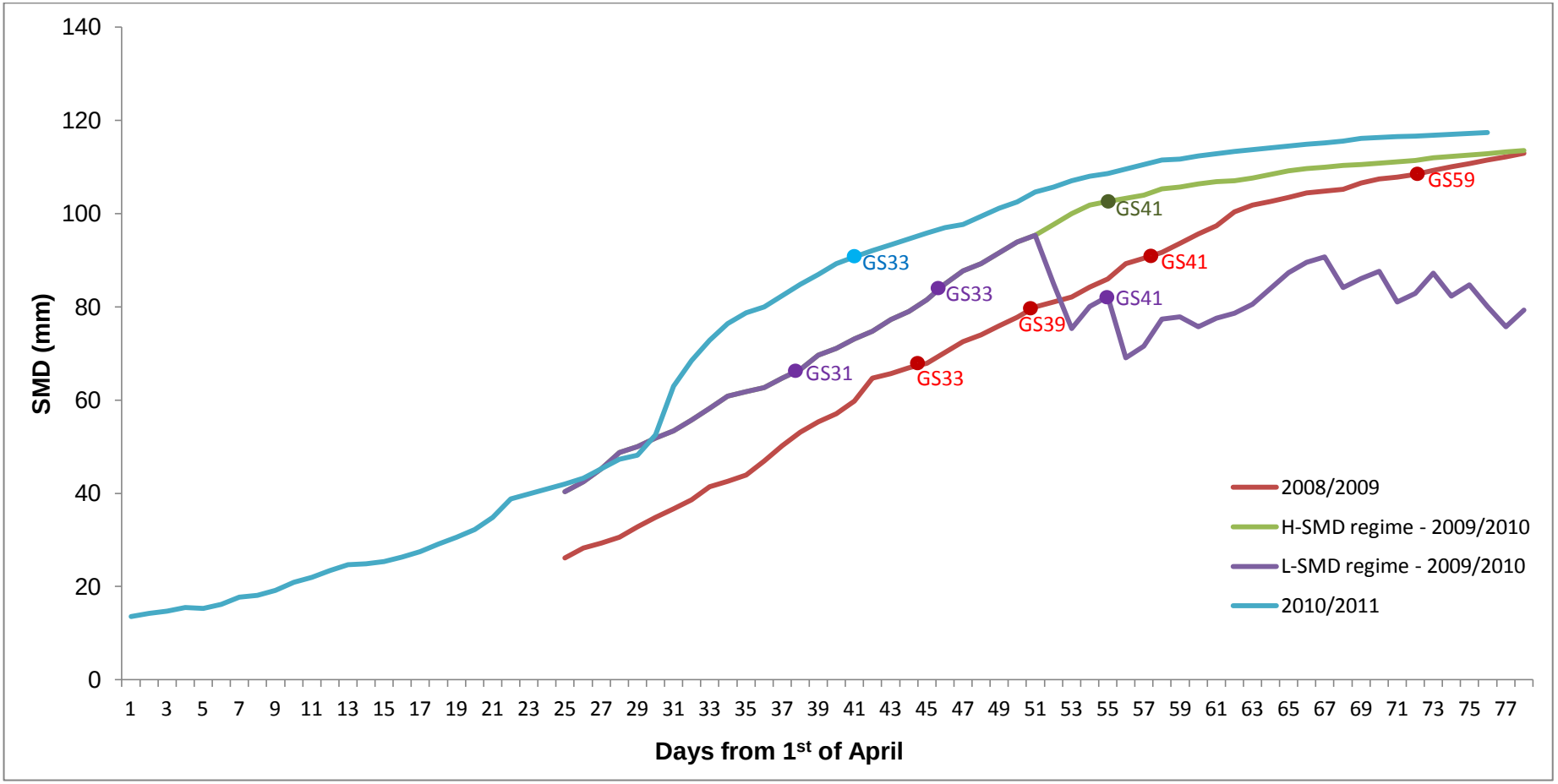


Figure 2.3.1: The changes in SMD with time inside the polytunnels in the three years, 2008/2009, 2009/2010 and 2010/2011 from the dates polytunnels were installed to the dates crop reached GS69. H-SMD regime = high SMD regime; L-SMD regime = low SMD regime; Note that: for the experiments in 2008/2009 and 2009/2010, polytunnels were installed on 25th of April, and for the experiments in 2010/2011, polytunnels were installed on 1st of April.

The graphs showing the changes in SMD in the experiments in natural environment in the three years are presented in Appendix VII. It was not possible to maintain two SMD regimes in 2009/2010 in the experiment in the natural environment.

In 2010/2011, SMD was calculated from the soil moisture measurements obtained from the neutron probe as well (the methods explained in section 2.2.4), and those values were not very different to the values obtained from IMS irrigation scheduling programme. A similar pattern of change in SMD was shown by the results obtained from the irrigation scheduling programme and the neutron probe for the soil inside polytunnels (Figure 2.3.2) as well as for the soil outside the polytunnels (Figure 2.3.3).

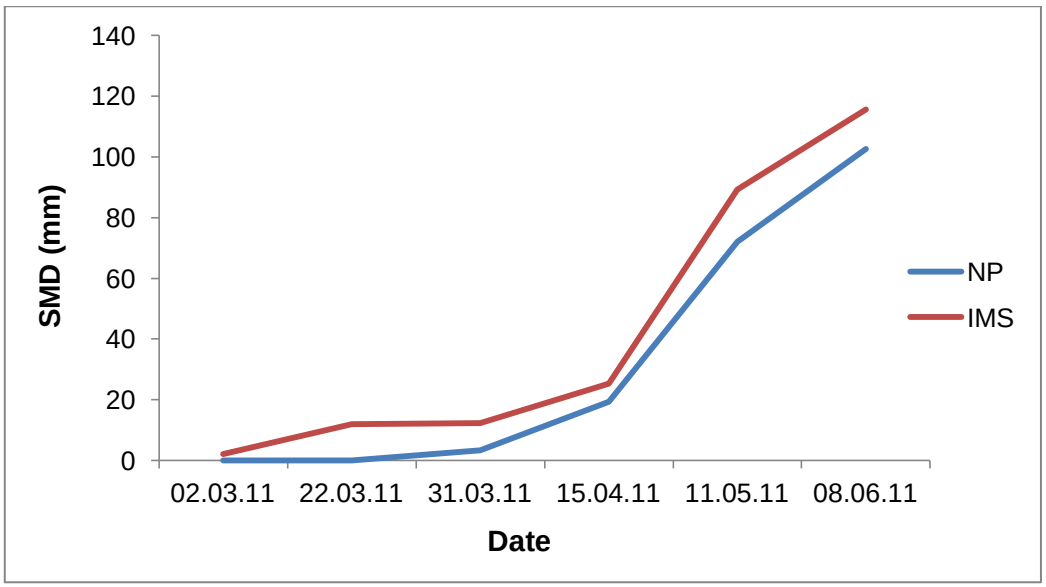


Figure 2.3.2: The comparison of the pattern of change in SMD as shown by the IMS irrigation scheduling programme and the neutron probe for the experiments inside polytunnels in 2010/2011; NP = neutron probe; IMS = IMS irrigation scheduling programme

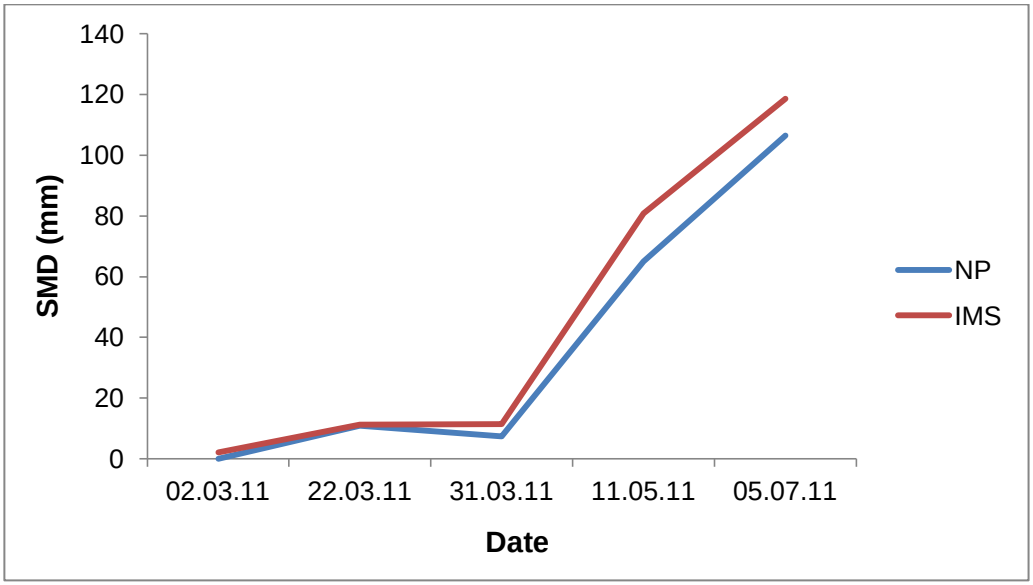


Figure 2.3.3: The comparison of the pattern of change in SMD as shown by the IMS irrigation scheduling programme and the neutron probe for the experiments in the natural environment in 2010/2011; NP = neutron probe; IMS = IMS irrigation scheduling programme

As described in chapter 2.2, In 2008/2009 and 2009/2010, soil moisture measurements were taken with the soil moisture monitoring device, Diviner 2000, as well. However stones present in the soil in the experimental area in large quantities affected the readings from the Diviner 2000 giving a huge variation in the volumetric water content from location to location for the same treatment, and it was concluded that the readings taken from the Diviner 2000 were of little value, therefore results from the Diviner 2000 are not presented.

2.3.2 The experiment inside polytunnels in 2008/2009 – the effect of the antitranspirant treatments on yield and yield components

The results from the hand harvested crop and the combine harvested crop are shown respectively in Table 2.3.3 and Table 2.3.4. Significant and/or important results, which need to be emphasised, are shown by graphs as well.

There was no significant effect of the factor, Variety on yield, grains ear^{-1} or ears m^{-2}. The effect of the factor, Variety was significant (hand harvested $p = 0.021$; combine harvested $p = 0.037$) only for TGW. The interaction between the factor, Variety and the factor, antitranspirant/control treatment was not significant for yield or any of the yield

components. Results for antitranspirant/control treatments are interpreted using means for the two varieties, since the interactive effect of the two factors is not significant. The factor, antitranspirant/control treatment was significant for yield and all the yield components. But according to Tukey's test, except for grains ear^{-1}, significance of the factor, antitranspirant/control treatment for yield and yield components is due to significantly ($p < 0.05$) different results shown by the irrigated unsprayed control compared to other treatments, not because of significant differences in results between unsprayed control and the antitranspirant treatments. The means of the two varieties for grains ear^{-1} of all the antitranspirant treatments and the irrigated control were significantly higher ($p<0.05$) than that of the unsprayed control (Figure 2.3.2).

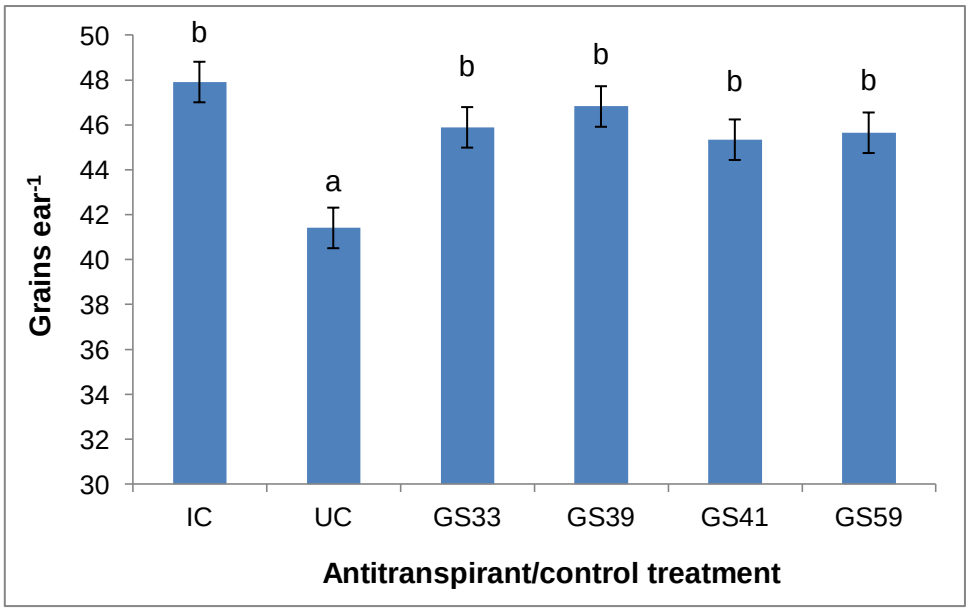


Figure 2.3.4: The effect of antitranspirant/control treatment on the mean grains ear^{-1} of the two varieties (Experiment inside polytunnels 2008/2009); IUC = irrigated unsprayed control; UC = unsprayed control; GS33, GS39, GS41 and GS59 = antitranspirant (di-1-p-menthene) treatments at the stages. Bars accompanied by the same letter are not significantly different at p = 0.05. Error bars show SEM. Refer Table 2.3.3 for p-values, SEM, CV% and DF; bars accompanied by the same letter are not significantly different at p = 0.05.

The mean increase of grains ear^{-1} by the application of the antitranspirant was 11% compared to the unsprayed control. The differences of grains ear^{1} between the four antitranspirant treatments and the irrigated control were not significant. However, the

increases in grains ear^{-1} in antitranspirant treatments compared to the unsprayed control were not directly proportional to the increases in yield. According to the results from the both hand (Table 2.3.3) and combine (Table 2.3.4) harvested crop, the means of the two varieties for yield of all the antitranspirant treatments were higher than that of the unsprayed control but the differences were not significant. The differences of yield between the four antitranspirant treatments were also not significant. The mean of the two varieties for the yield of the irrigated unsprayed control was significantly higher ($p<0.05$) compared to that of all the antitranspirant treatments and the unsprayed control.

The means of the two varieties for TGW of the four antitranspirant treatments were lower than that of the two controls. From the four antitranspirant treatments, the antitranspirant treatments at GS41 and GS59 had the lowest values for mean TGW, which were significantly ($p<0.05$) lower than that of the IC, but not significantly lower than that of the other treatments including the unsprayed control. The means for TGW of the antitranspirant treatments at GS33 and GS39 were not significantly different from that of the other treatments.

The means of the two varieties for ears m^{-2} of all the antitranspirant treatments were higher than that of the unsprayed control but significantly lower ($p<0.05$) than that of the irrigated control. The differences of ears m^{-2} in between the four antitranspirant treatments and the unsprayed control were not significant.

Table 2.3.3: The results for yield and yield components from the experiment inside polytunnels 2008/2009 (from hand harvested crop)

			Antitranspirant/control treatment						Mean	*P* - Var	*P* - Treat	*P* - Var-Treat	s.e.m - Var-Treat	CV% - Var-Treat (res. DF)
			IUC	**UC**	**GS33**	**GS39**	**GS41**	**GS59**						
Yield (t/ha)	**Variety**	**E**	9.30	5.31	6.25	5.34	6.26	6.29	6.46	0.807	<.001	0.214	0.452	12.0 (20)
		C	8.37	5.58	5.93	6.62	6.15	5.63	6.38					
	Mean		8.84 (b)	5.44 (a)	6.09 (a)	5.98 (a)	6.20 (a)	5.96 (a)						
TGW (g)	**Variety**	**E**	54.07	53.45	52.04	51.69	51.26	50.98	52.25	0.021	0.004	0.172	1.263	4.3 (20)
		C	49.63	49.17	44.76	50.20	43.79	44.27	46.97					
	Mean		51.85 (b)	51.31 (ab)	48.40 (ab)	50.94 (ab)	47.53 (a)	47.63 (a)						
Grains ear^{-1}	**Variety**	**E**	49.66	43.71	46.59	49.11	47.77	46.38	47.20	0.187	<.001	0.268	1.169	3.6 (20)
		C	46.19	39.13	45.21	44.55	42.92	44.95	43.83					
	Mean		47.92 (b)	41.42 (a)	45.90 (b)	46.83 (b)	45.35 (b)	45.66 (b)						
Ears m^{-2}	**Variety**	**E**	351.7	231.7	262.0	204.0	257.3	269.0	262.6	0.106	<.001	0.193	20.05	10.8 (20)
		C	370.7	265.3	299.3	300.7	333.3	286.7	309.3					
	Mean		361.2 (b)	248.5 (a)	280.7 (a)	252.3 (a)	295.3 (a)	277.8 (a)						

E = Einstein; C = Claire; IUC = irrigated unsprayed control; UC = unsprayed control; GS33,GS39, GS41 and GS59 = antitranspirant (di-1-p-menthene) treatment at respective growth stages; Var = Variety; Treat = Treatment; res. DF = residual DF; data within rows accompanied by the same letter are not significantly different at p = 0.05.

Table 2.3.4: The results for yield (t/ha) from the experiment inside polytunnels 2008/2009 (from combine harvested crop)

		Antitranspirant/control treatment						Mean	*P* - Var	*P* - Treat	*P* - Var-Treat	s.e.m - Var-Treat	CV% - Var-Treat (res. DF)
		IUC	**UC**	**GS33**	**GS39**	**GS41**	**GS59**						
Variety	**E**	6.70	4.36	4.81	3.62	5.02	4.94	4.91	0.213	<.001	0.220	0.452	15.2 (20)
	C	8.18	3.96	5.36	4.99	5.01	4.82	5.39					
Mean		7.44 (b)	4.16 (a)	5.09 (a)	4.31 (a)	5.01 (a)	4.88 (a)						

E = Einstein; C = Claire; IUC = irrigated unsprayed control; UC = unsprayed control; GS33, GS39, GS41 and GS59 = antitranspirant (di-1-p-menthene) treatment at respective growth stages; Var = Variety; Treat = Treatment; res. DF = residual DF; data within rows accompanied by the same letter are not significantly different at p = 0.05.

The four antitranspirant treatments and the unsprayed control were used as groups and linear regression analyses in groups were performed between ears m^{-2} and grains ear^{-1}; ears m^{-2} and TGW; grains ear^{-1} and TGW. Irrigated unsprayed control was not included in any of the analyses, since the purpose of the regression analyses was to explore possible compensatory effects between the yield components under drought conditions.

Regression analysis between grains ear^{-1} and ears m^{-2} showed that there is a significant negative ($p<0.05$) linear relationship between grains ear^{-1} and ears m^{-2} (Figure 2.3.5) within all the antitranspirant treatments and the unsprayed control. The regression lines of different treatments had the same slope but significantly different ($p<0.05$) intercepts.

Regression analysis between ears m^{-2} and TGW showed that there is a significant negative ($p<0.05$) linear relationship between ears m^{-2} and TGW within all the antitranspirant treatments and the unsprayed control. There was no significant difference in slope or intercept of regression lines for different treatments, therefore, the relationship between ears m^{-2} and TGW within the four antitranspirant treatments and the unsprayed control is denoted by a single line (Figure 2.3.6).

Regression analysis between grains ear^{-1} and TGW showed no significant relationship between grains ear^{-1} and TGW within any of the antitranspirant treatments and the unsprayed control (data not shown).

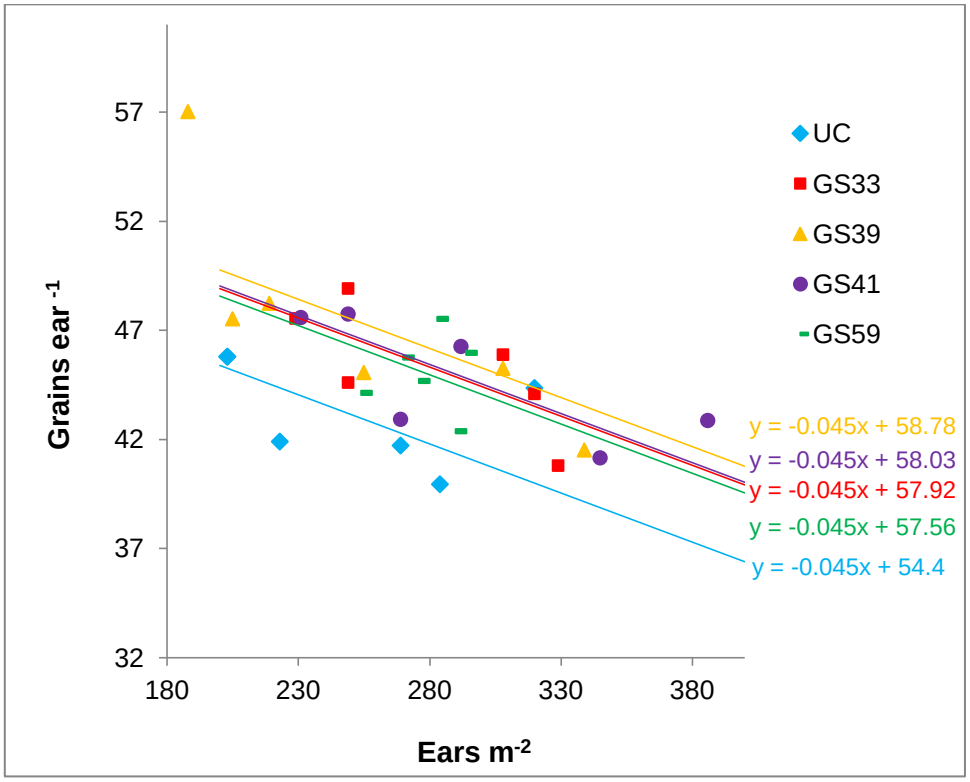


Figure 2.3.5: The relationship between ears m^{-2} and grains ear^{-1} within the four antitranspirant treatments and the unsprayed control (The experiment inside polytunnels 2008/2009); UC = unsprayed control; GS33, GS39, GS41 and GS59 = antitranspirant (di-1-p-menthene) treatments at the stages.

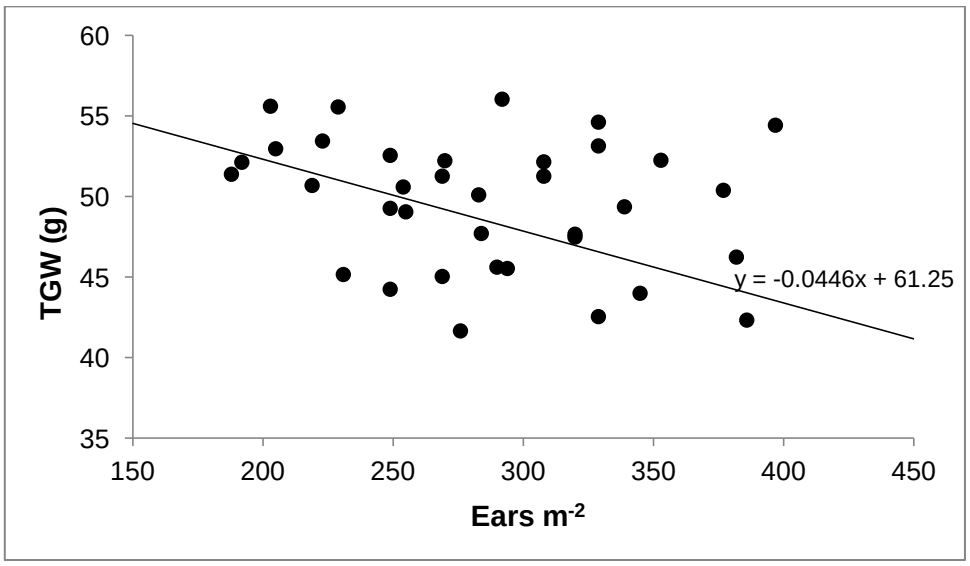


Figure 2.3.6: The relationship between ears m^{-2} and TGW within the four antitranspirant treatments and the unsprayed control (The experiment inside polytunnels 2008/2009).

2.3.3 The experiment inside polytunnels in 2009/2010 – the effect of the antitranspirant treatments on yield and yield components

The results from the hand harvested crop and the combine harvested crop are shown respectively in Table 2.3.5 and Table 2.3.6.

There was no significant effect of the factor, SMD regime on yield or any of the yield components. The interaction between the factor, SMD regime and the factor, antitranspirant/control treatment was also not significant for any of the parameters. The interaction between the two factors was marginally significant only for TGW ($p = 0.061$). Since, the interactive effect of the two factors is not significant, the effects of treatments on yield and yield components are interpreted using the means of the two SMD regimes at each antitranspirant/control treatment.

The factor, antitranspirant/control treatment was not significant for yield or any of the yield components. The means of the two SMD regimes for yield and grains ear^{-1} of all the antitranspirant treatments, except the di-1-p-menthene treatment at GS41, were higher than those of the unsprayed control. The means of the two SMD regimes for ears m^{-2} of all the antitranspirant treatments were higher than that of the unsprayed control. The means of the two SMD regimes for TGW of all the antitranspirant treatments were lower than that of the unsprayed control. The yield and yield components of the irrigated unsprayed control/common control were compared with means of the two SMD regimes for yield and yield components of the antitranspirant treatments and the unsprayed control. The yield of the irrigated unsprayed control /common control was higher than that of the other treatments but the difference was not significant. The ears m^{-2} of the irrigated unsprayed control was higher than that of the other treatments and the difference was marginally significant ($p = 0.055$). The TGW of the irrigated unsprayed control was significantly lower ($p = 0.035$) than that of the other treatments. There was no difference in grains ear^{-1} between the irrigated unsprayed control and the rest of the treatments.

Table 2.3.5: The results for yield and yield components from the experiment inside polytunnels 2009/2010 (from hand harvested crop)

			Treatment						Mean (without IUC)	P - SMD	P - Treat	P – SMD-Treat	S.E.M. - SMD-Treat	CV% - SMD-Treat (res. DF)
			IUC (Common control)	UC	di-GS31	di-GS33	di-GS41	la-GS41						
Yield (t/ha)	SMD	L	10.07	8.65	8.86	10.05	8.88	9.15	9.12	0.084	0.331	0.837	0.563	10.8 (16)
		H		8.18	8.57	8.75	7.70	7.98	8.24					
	Mean		10.07	8.41	8.71	9.40	8.29	8.57						
TGW (g)	SMD	L	53.05	57.24	57.36	56.20	56.63	56.53	56.79	<.128	0.284	0.061	0.647	1.6 (16)
		H		59.18	57.79	59.82	57.56	58.68	58.61					
	Mean		53.05	58.21	57.57	58.01	57.10	57.61						
Grains ear^{-1}	SMD	L	41.21	40.61	43.54	43.34	41.90	44.87	42.85	0.294	0.851	0.358	1.951	6.9 (16)
		H		40.57	40.10	39.53	38.40	37.69	39.26					
	Mean		41.21	40.59	41.82	41.43	40.15	41.28						
Ears m^{-2}	SMD	L	461.7	372.7	356.7	414.0	373.7	361.0	375.6	0.269	0.270	0.453	17.80	7.9 (16)
		H		339.3	370.0	370.3	349.0	361.3	358.0					
	Mean		461.7	356.0	363.3	392.2	361.3	361.2						

L = low SMD regime; H = high SMD regime; IUC = irrigated unsprayed control; UC = unsprayed control; di-GS31, di-GS33 and di-GS41 = the antitranspirant, di-1-p-menthene, treatment at respective growth stages; la-GS41 the antitranspirant, latex, treatment at GS41; Treat = Treatment; res. DF = residual DF.

Table 2.3.6: The results for yield (t/ha) from the experiment inside polytunnels 2009/2010 (from combine harvested crop)

		Treatment						Mean (without IUC)	P - SMD	P - Treat	P – SMD-Treat	S.E.M. -SMD-Treat	CV % - SMD-Treat (res. DF)
		IUC (Common control)	**UC**	**di-GS31**	**di-GS33**	**di-GS41**	**la-GS41**						
SMD	**L**	10.57	8.52	8.78	8.73	8.36	8.70	8.62	0.662	0.972	0.997	0.601	11.4 (16)
	H		8.25	8.43	8.49	8.21	8.16	8.31					
Mean		10.57	8.38	8.60	8.61	8.28	8.43						

L = low SMD regime; H = high SMD regime; IUC = irrigated unsprayed control; UC = unsprayed control; di-GS31, di-GS33 and di-GS41 = the antitranspirant, di-1-p-menthene treatment at respective growth stages; la-GS41 the antitranspirant, latex treatment at GS41; Treat = Treatment; res. DF = residual DF.

The four antitranspirant treatments and the unsprayed control were used as groups and linear regression analyses in groups were performed between ears m^{-2} and grains ear^{-1}; ears m^{-2} and TGW; grains ear^{-1} and TGW. There was no significant relationship between ears m^{-2} and grains ear^{-1}, ears m^{-2} and TGW or grains ear^{-1} and TGW.

The Irrigated sprayed plots were compared against the irrigated unsprayed control by one way ANOVA, and the results are shown in Table 2.3.7. This was performed just to see whether there is any effect of antitranspirant spray at GS33 on plants under none drought conditions. There was no significant difference shown by the analysis between the irrigated sprayed plots and the irrigated unsprayed control in yield or any of the yield components.

Table 2.3.7: The comparison in mean values for yield and yield components between the irrigated unsprayed control and irrigated sprayed plots (with di-1-p-menthene).

	IUC	ISP	*p*	CV% (res. DF)
Yield (t/ha)	10.07	10.18	0.528	6.7 (2)
TGW	53.05	53.15	0.885	1.5 (2)
Grains ear^{-1}	41.21	41.90	0.199	3.1 (2)
Ears m^{-2}	461.7	456.67	0.803	4.7 (2)

IUC = irrigated unsprayed control; ISP = irrigated sprayed plots; res. DF = residual DF

2.3.4 The experiments inside polytunnels in 2010/2011 – the effect of the antitranspirant treatments on yield and yield components

The results for the two experiments inside polytunnels in 2010/2011 (Experiment 1: the experiment irrigated from GS69 onwards and Experiment 2: the experiment without irrigation until harvest) are described. Results are presented for each experiment and for the analyses in which the two experiments are combined. The results from the hand harvested crop are sown in Table 2.3.8, and from the combine harvested crop are shown in Table 2.3.9. The results from ANOVA contrast analyses are presented for the

experiments in 2010-2011 and the reason for using ANOVA contrast analysis is described in Chapter 2.2.6.4.

When the dates that the crop reached each development stage compared to the previous experiments was considered, up to GS30 the development of the crop was faster as a result of the spring drilling. However, after GS30 there was no difference in the rate of development of the crop compared to the previous years. The crop yield was low, since the faster development during the tillering stage did not allow the crop to produce much tillers as previous years.

In Experiment 1, according to both the hand and combine harvested crop results, there is no significant difference in yield between the two antitranspirant treatments. As shown by both the hand and combine harvested crop results, the yields of the two antitranspirant treatments were higher than that of the unsprayed control, but the differences were not significant in a simple ANOVA of three treatments. ANOVA contrast analysis shows that, however, the difference between the yield of the unsprayed control and the mean yield of the two antitranspirant treatments for hand harvested crop is marginally significant ($p = 0.055$). Figure 2.3.7 shows the mean hand harvested yield of the two antitranspirant treatments compared to hand harvested yield of the unsprayed control.

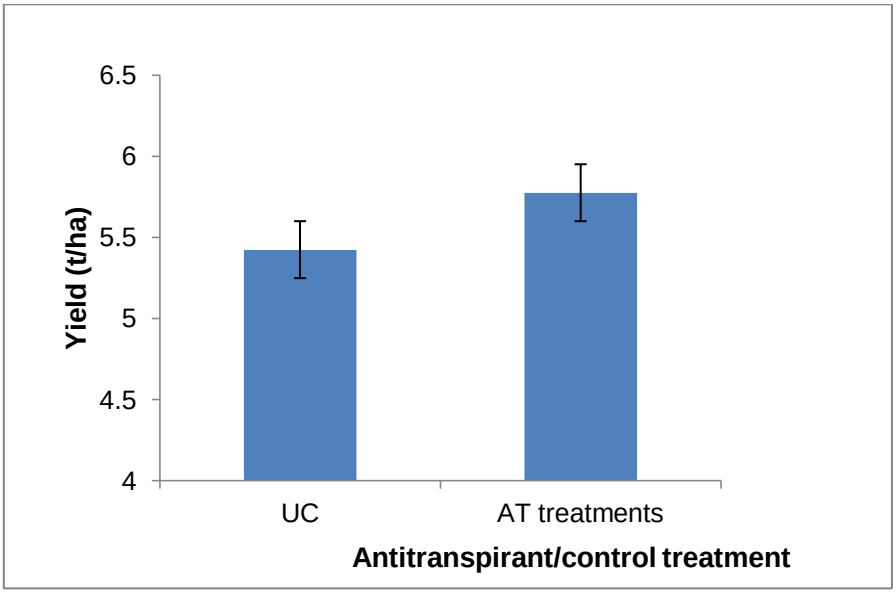


Figure 2.3.7: The mean hand harvested yield of the two antitranspirant treatments compared to that of the unsprayed control (Experiment 1). UC = unsprayed control; AT treatments = combination of the two antitranspirant treatments, which are the latex treatment at GS33 and the di-1-p-menthene treatment at GS33. Error bars show SEM. See Table 2.3.8 for p-values, SEM, CV and DF.

When the results from Experiment 1 for yield components are considered, there was no significant difference in any of the yield components between the treatments according to ANOVA or ANOVA contrast analyses. From the three yield components, ears m^{-2} holds the lowest p-value ($p = 0.122$) for the ANOVA contrast analysis between the unsprayed control and the two antitranspirant treatments. The reason for pointing this out is, it is important to trace which yield component was responsible in the borderline significant difference between the yield of the unsprayed control and the mean yield of the two antitranspirant treatments as shown by hand harvested crop results. As described in the following text there had been compensatory effects between yield components within individual plots. Therefore, the correlation between yield and each yield component, as shown by a linear regression analysis, might not be a true indicator of which yield component is responsible for the increase in yield in antitranspirant treatments compared to the unsprayed control.

The two antitranspirant treatments and the unsprayed control were used as groups and linear regression analyses in groups were performed between ears m^{-2} and grains ear^{-1}; ears m^{-2} and TGW; grains ear^{-1} and TGW.

Regression analysis between ears m^{-2} and grains ear^{-1} showed that there is a significant, positive ($p<0.05$) linear relationship between ears m^{-2} and grains ear^{-1} within each of the treatments. There was no significant difference in slope or intercept of regression lines for different treatments, therefore, the relationship between ears m^{-2} and grains ear^{-1} within the two antitranspirant treatments and the unsprayed control is denoted by a single line (Figure 2.3.8).

Regression analysis between ears m^{-2} and TGW showed that there is a significant negative ($p<0.05$) linear relationship between ears m^{-2} and TGW within the antitranspirant treatments and the unsprayed control. There was no significant difference in slope or intercept of regression lines for different treatments, therefore, the relationship between ears m^{-2} and TGW within the antitranspirant treatments and the unsprayed control is denoted by a single line (Figure 2.3.9).

Regression analysis between grains ear^{-1} and TGW showed that there is a significant, negative ($p<0.05$) linear relationship between grains ear^{-1} and TGW within the antitranspirant treatments and the unsprayed control. There was no significant difference in slope or intercept of regression lines for different treatments, therefore, the relationship between grains ear^{-1} and TGW within the antitranspirant treatments and the unsprayed control is denoted by a single line (Figure 2.3.10).

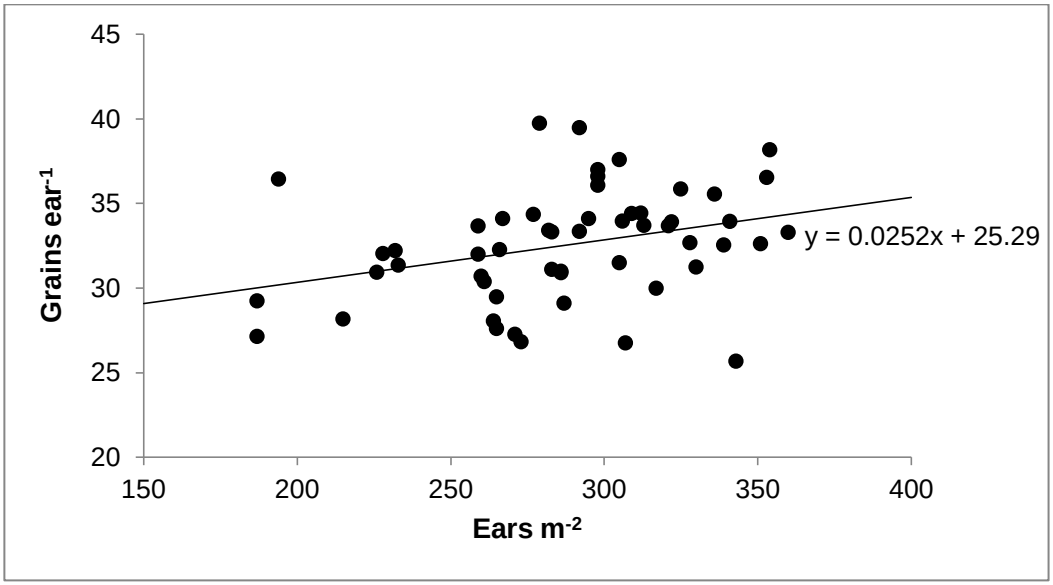


Figure 2.3.8: The relationship between ears m^{-2} and grains ear^{-1} within the two antitranspirant treatments and the unsprayed control (Experiment 1 inside polytunnels 2010/2011)

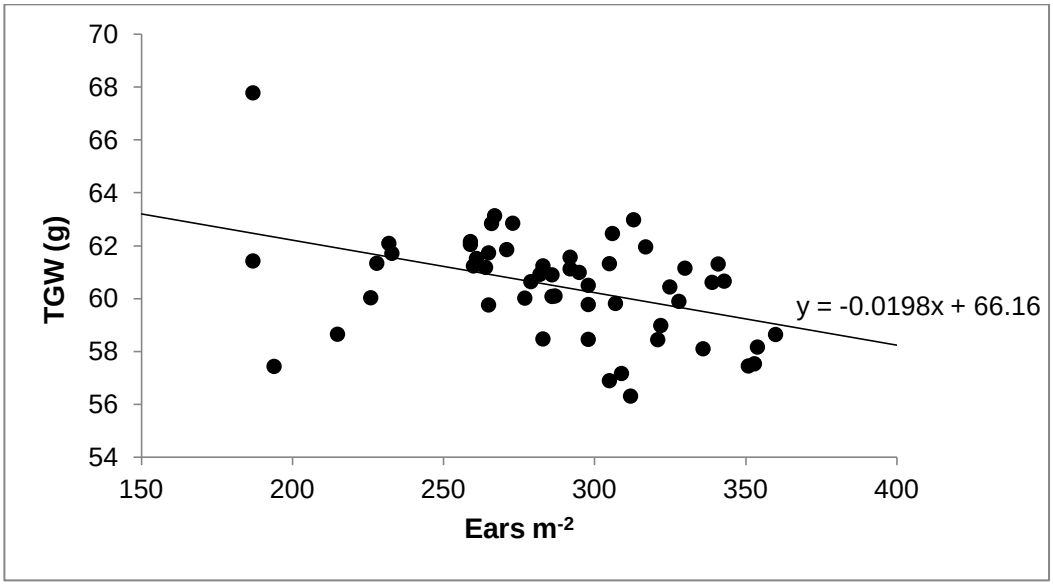


Figure 2.3.9: The relationship between ears m^{-2} and TGW within the two antitranspirant treatments and the unsprayed control (Experiment 1 inside polytunnels 2010/2011)

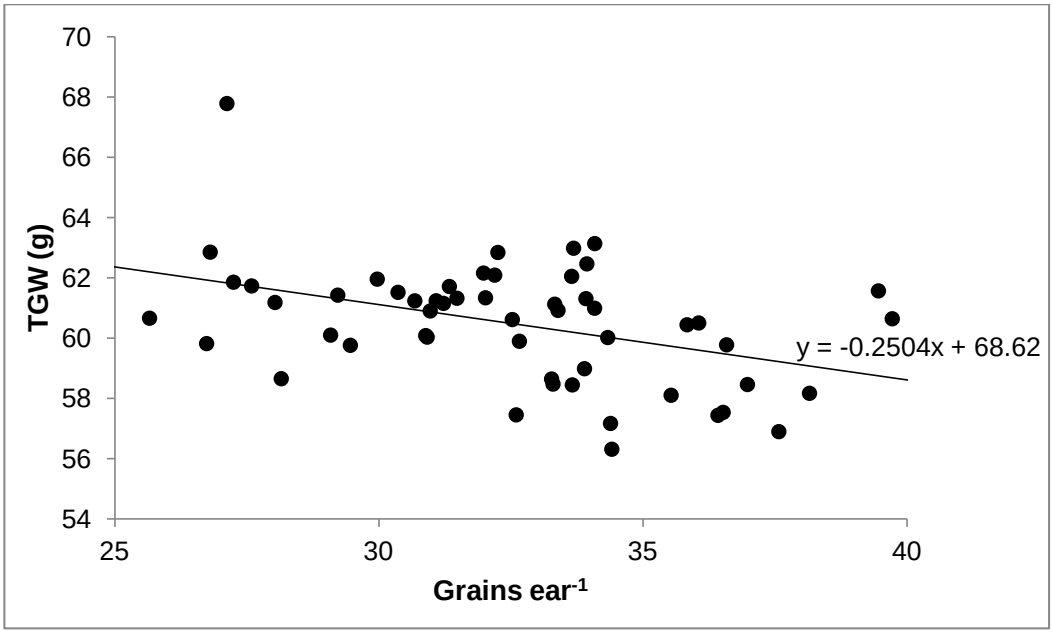


Figure 2.3.10: The relationship between grains ear^{-1} and TGW within the two antitranspirant treatments and the unsprayed control (Experiment 1 inside polytunnels 2010/2011)

In Experiment 2, as shown by the results from the both hand and combine harvested crop, the yields of the two antitranspirant treatments were higher than that of the unsprayed control. According to the combine harvested crop results, there was a significant difference in yield ($p = 0.045$) between the treatments. Furthermore, ANOVA contrast

analysis shows a significant difference ($p = 0.024$) between the mean yield of the two antitranspirant treatments and the yield of the unsprayed control. The hand harvested crop results do not show any significant difference in yield between the treatments. However, it is important to notice that the CV% of the combine harvested yield is lower than that of the hand harvested yield. There is no significant difference in yield between the two antitranspirant treatments according to both combine and hand harvested crop results.

When yield components are considered, ANOVA contrast analysis shows a significant difference ($p = 0.046$) between the mean grains ear^{-1} of the two antitranspirant treatments and the grains ear^{-1} of the unsprayed control. The difference in grains ear^{-1} between the two antitranspirant treatments is not significant. Figure 2.3.11 – a) shows the mean combine harvested yield of the two antitranspirant treatments compared to the combine harvested yield of the unsprayed control. Figure 2.3.11 – b) shows the mean grains ear^{-1} of the two antitranspirant treatments compared to the grains ear^{-1} of the unsprayed control. There was no significant difference in TGW or ears m^{-2} between the treatments.

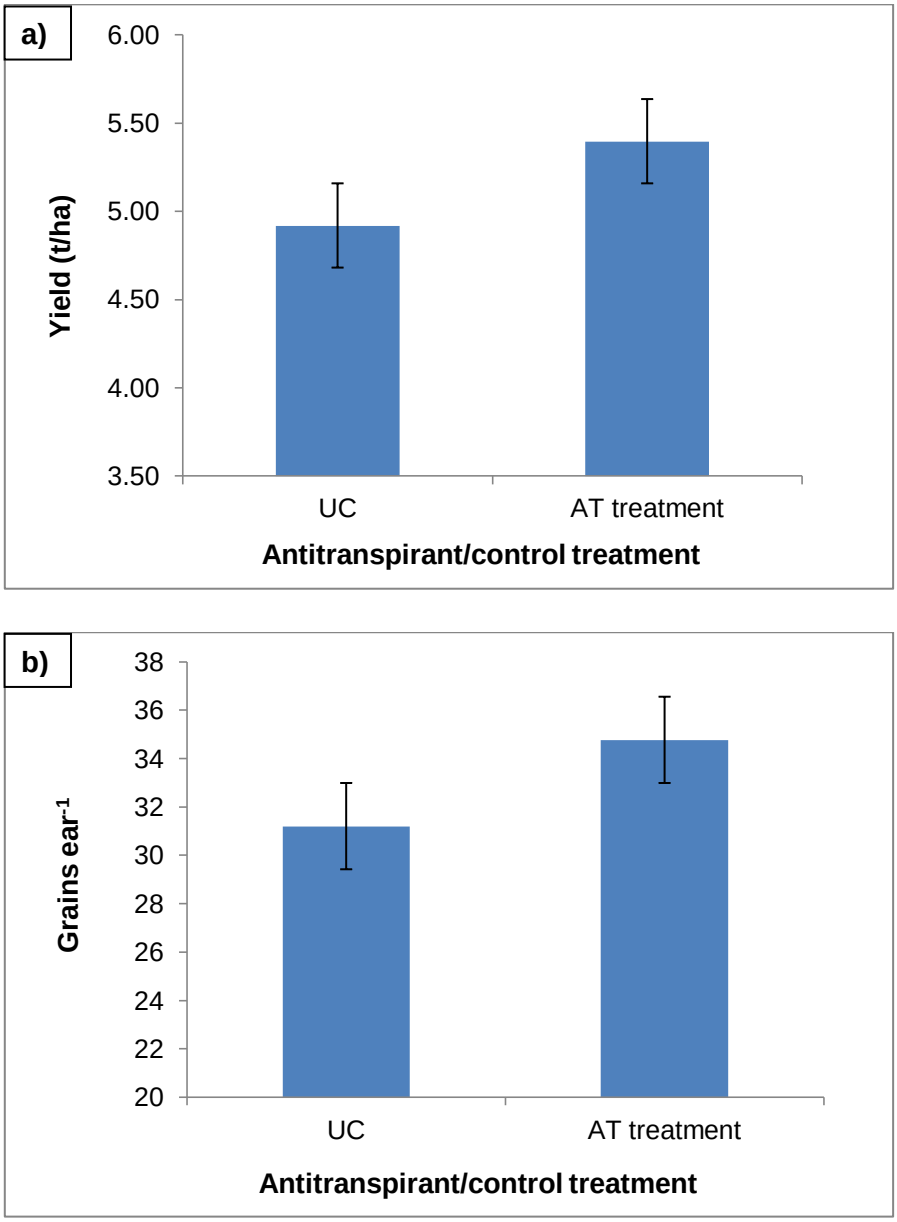


Figure 2.3.11: a) The mean combine harvested yield of the two antitranspirant treatments compared to the combine harvested yield of the unsprayed control (Experiment 2). b) The mean grains ear^{-1} of the two antitranspirant treatments compared to the grains ear^{-1} of the unsprayed control (Experiment 2). UC = unsprayed control; AT treatments = combination of the two antitranspirant treatments, the latex treatment at GS33 and the di-1-p-menthene treatment at GS33. See Table 2.3.8 and 2.3.9 for p-values, SEM, CV and DF.

The two antitranspirant treatments and the unsprayed control were used as grouping factors and linear regression analyses in groups were performed between ears m^{-2} and grains ear^{-1}; ears m^{-2} and TGW; grains ear^{-1} and TGW.

Regression analysis between ears m^{-2} and grains ear^{-1} showed that there is a significant, negative ($p<0.05$) linear relationship between ears m^{-2} and grains ear^{-1} within the

antitranspirant treatments and the unsprayed control. There was no significant difference in slope or intercept of regression lines for different treatments, therefore, the relationship between ears m^{-2} and grains ear^{-1} within the antitranspirant treatments and the unsprayed control was denoted by a single line (Figure 2.3.12).

There was no significant relationship between ears m^{-2} and TGW or grains ear^{-1} and TGW.

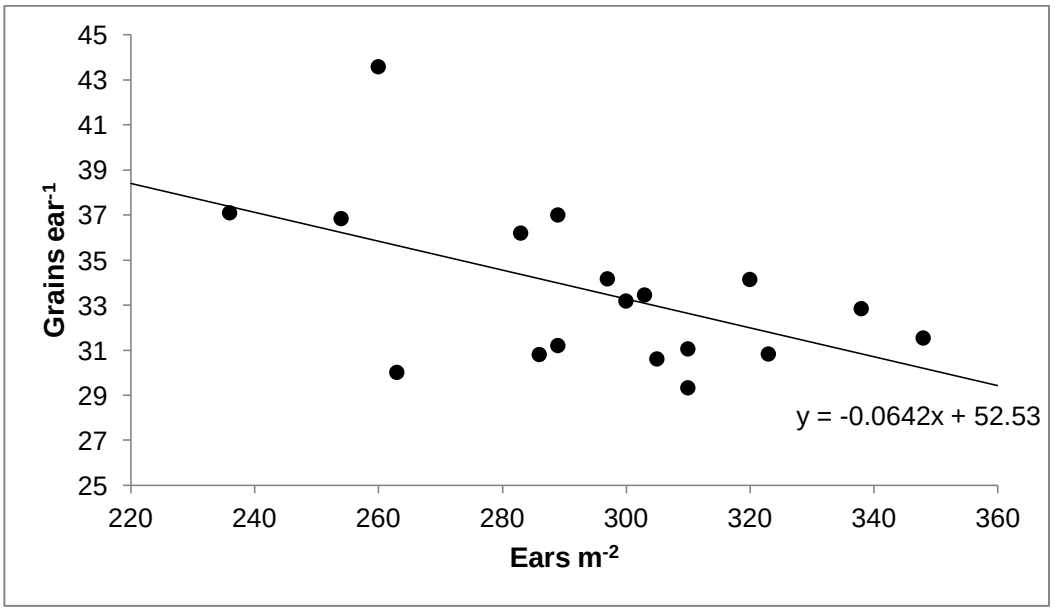


Figure 2.3.12: The relationship between grains ear^{-1} and ears m^{-2} within the two antitranspirant treatments and the unsprayed control (Experiment 2 inside polytunnels 2010/2011)

Since, the CV% in yields and yield components in Experiment 1 are not much different to that of Experiment 2, it is valid to combine the two experiments for analysis. When the two experiments were combined for analysis, the antitranspirant/control treatment was significant for yield giving a p-value of 0.036 for the hand harvested crop and 0.039 for the combine harvested crop. ANOVA contrast analysis shows a significant difference between the yield of the unsprayed control and the mean yield of the two antitranspirant treatments giving a p-value of 0.022 for the hand harvested crop and 0.017 for the combine harvested crop. There is no significant difference in yield between the two antitranspirant treatments according to both combine and hand harvested crop results. Figure 2.3.13 – a)

shows the mean yield of the two antitranspirant treatments compared to the yield of the unsprayed control. When the two experiments are combined for analysis, from the three yield components, the lowest p-value obtained from ANOVA for Treatment was from grains ear^{-1}. There was no significant difference in TGW or ears m^{-2} between the treatments.

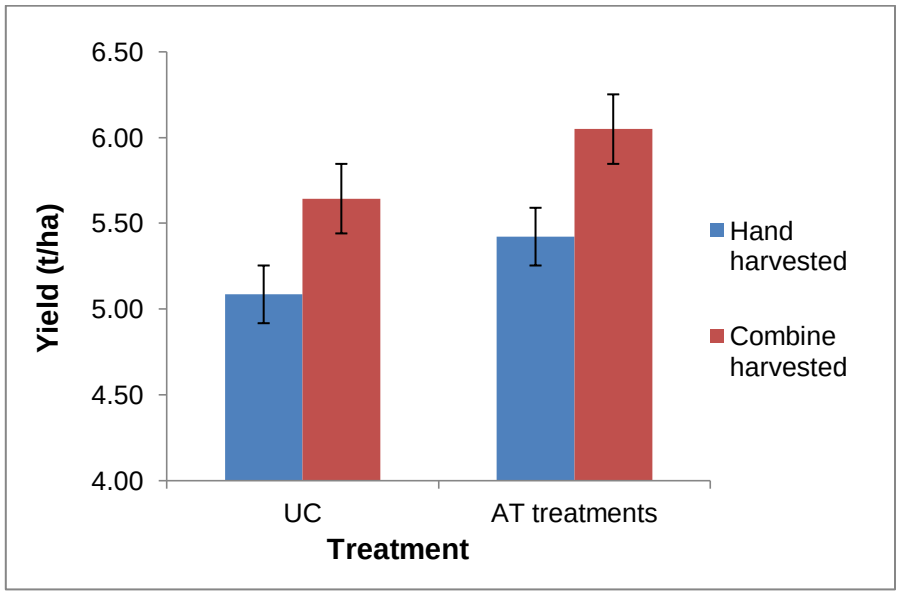


Figure 2.3.13: The mean yield of the two antitranspirant treatments compared to the yield of the unsprayed control (Experiment 2). UC = unsprayed control; AT treatments = combination of the two antitranspirant treatments, the latex treatment at GS33 and the di-1-p-menthene treatment at GS33. See Table 2.3.8 and 2.3.9 for p-values, SEM, CV and DF.

Table 2.3.8: The results for yield and yield components from the experiments inside polytunnels 2010/2011 (from hand harvested crop)

		IUP	Treatment			*P*	*P* – Contrast: At treat vs UC	*P* – Contrast: di-GS33 vs la-GS33	S.E.M.	CV% (res. DF)
			UC	**di-GS33**	**la-GS33**					
Experiment 1	Yield (t/ha)	9.74	5.42	5.66	5.89	0.090	0.055	0.257	0.1448	10.7 (47)
	TGW (g)	52.44	60.89	59.72	60.80	0.127	0.248	0.093	0.443	3.1 (49)
	Grains ear^{-1}	43.12	32.30	32.44	32.86	0.792	0.638	0.623	0.607	7.9 (49)
	Ears m^{-2}	430.5	279.3	290.7	292.5	0.292	0.122	0.839	6.35	9.4 (48)
Experiment 2	Yield (t/ha)	9.74	4.03	4.39	4.38	0.282	0.119	0.961	0.1696	9.7 (13)
	TGW (g)	52.44	42.88	43.92	43.12	0.634	0.522	0.487	0.795	4.5 (13)
	Grains ear^{-1}	43.12	31.16	34.56	34.97	0.124	0.046	0.828	1.336	9.8 (13)
	Ears m^{-2}	430.5	302.0	292.2	291.5	0.803	0.517	0.970	12.46	10.3 (13)
Experiment 1 & Experiment 2 combined	Yield (t/ha)	9.74	5.08	5.32	5.52	0.036	0.022	0.222	0.1159	10.6 (62)
	TGW (g)	52.44	56.39	55.77	56.38	0.448	0.513	0.279	0.391	3.4 (64)
	Grains ear^{-1}	43.12	32.01	32.97	33.39	0.222	0.099	0.600	0.569	8.5 (64)
	Ears m^{-2}	430.5	285.6	289.9	292.8	0.673	0.418	0.717	5.78	9.8 (63)

IUP = Irrigated unsprayed plots; UC = unsprayed control; di-GS33 = di-1-p-menthene treatment at GS33; la-GS33 = latex treatment at GS33; AT treat = combination of the two antitranspirant treatments; Treat = Treatment; res. DF = residual DF.

Table 2.3.9: The results for yield (t/ha) from the experiment inside polytunnels 2010/2011 (from combine harvested crop)

	IUP	**Treatment**			*P*	*P* – Contrast: At treat vs UC	*P* – Contrast: di-GS33 vs la-GS33	S.E.M.	CV% (res. DF)
		UC	**di-GS33**	**la-GS33**					
Experiment 1	9.45	5.88	6.21	6.33	0.172	0.070	0.687	0.1713	11.7 (47)
Experiment 2	9.45	4.92	5.27	5.52	0.045	0.024	0.451	0.1491	6.9 (12)
Experiment 1 & Experiment 2 combined	9.45	5.64	5.96	6.14	0.039	0.017	0.211	0.1349	11.1 (62)

IUP = Irrigated unsprayed plots; UC = unsprayed control; di-GS33 = di-1-p-menthene treatment at GS33; la-GS33 = latex treatment at GS33; AT treat = combination of the two antitranspirant treatments; Treat = Treatment; res. DF = residual DF.

2.3.5 The effect of treatments on grains m^{-2} in all the four experiments under polytunnels

The effects of the treatments on yield and the three yield components, ears m^{-2}, grains ear^{-1} and TGW were shown in the previous text. As explained in the discussion of the section of 2.4.3.4, when there was a yield increase by an antitranspirant treatment it seemed to be from an increase in grains ear^{-1} and/or ears m^{-2}. However, it was difficult to understand from the two yield components, which one was exactly responsible for the yield increase. The reason is, when there was an increase in ears m^{-2} in an antitranspirant treatment compared to an unsprayed control, it was not clear whether the increase in ears m^{-2} was because of an increase in tiller survival or because of an establishment/survival of at least one grain in tillers which otherwise would not bear grains. If the reason was the latter, it is not actually an increase in ears m^{-2}, but an increase in grain number. However, this increase in grain number might decrease grains ear^{-1}, since, when few grains are established/survived by an antitranspirant in tillers which otherwise would not bear grains the mean number of grains per ear is decreased. The two yield components, ears m^{-2} and grains ear^{-1}, are interrelated in the above described manner. Therefore, as explained further in the discussion of the section of 2.4.3.4, the yield increases were attributed to increases in grains m^{-2} (the combination of ears m^{-2} and grains ear^{-1}), rather than increases in ears m^{-2} or grains ear^{-1}. Therefore, it was decided to present the results from the ANOVA of grains m^{-2}, in order to clarify whether the increases in yield by antitranspirant treatments were because of increases in grains m^{-2}. The grains m^{-2} in each plot was calculated by multiplying the ears m^{-2} from grains ear^{-1}.

The results from the experiments inside polytunnels in 2008/2009, 2009/2010 and 2010/2011 are shown respectively in Table 2.3.10, 2.3.11 and 2.3.12.

There was no significant effect of the factor, Variety in 2008/2009 or the factor, SMD regime in 2009/2010 on grains m^{-2}. The interaction between the factor, Variety and the factor, antitranspirant/control treatment in 2008/2009 or the interaction between the factor,

SMD regime and the factor, antitranspirant/control treatment in 2009/2010 was not significant for grains m^{-2}.

In 2008/2009, the antitranspirant/control treatment was significant for grains m^{-2}. But according to Tukey's test the significance of the factor, antitranspirant/control treatment for grains m^{-2} was due to the significantly ($p < 0.05$) different result shown by the irrigated unsprayed control compared to other treatments, not because of significant differences in results between the unsprayed control and the antitranspirant treatments. The grains m^{-2} of all the antitranspirant treatments were, however, higher than that of the unsprayed control.

In 2009/2010 as well, the antitranspirant/control treatment was not significant for grains m^{-2}, although the grains m^{-2} of all the antitranspirant treatments were higher than that of the unsprayed control.

In Experiment 1 in 2010/2011, the grains m^{-2} of the two antitranspirant treatments were higher than that of the unsprayed control, but the differences were not significant in a simple ANOVA of three treatments. ANOVA contrast analysis shows that, however, the difference between the grains m^{-2} of the unsprayed control and the mean grains m^{-2} of the two antitranspirant treatments was nearly significant ($p = 0.060$). In Experiment 2, although the grains m^{-2} of the two antitranspirant treatments were higher than that of the unsprayed control, the differences were not significant in a simple ANOVA of three treatments or ANOVA contrast analysis between the grains m^{-2} of the unsprayed control and the mean grains m^{-2} of the two antitranspirant treatments. When the two experiments were combined for analysis, the antitranspirant/control treatment was borderline significant ($p = 0.056$) for grains m^{-2} in a simple ANOVA of three treatments. ANOVA contrast analysis showed a significant difference ($p = 0.023$) between the grains m^{-2} of the unsprayed control and the mean grains m^{-2} of the two antitranspirant treatments. According to ANOVA contrast analysis there was no significant difference in grains m^{-2} between the two antitranspirant treatments in Experiment 1, Experiment 2 or when the two experiments were combined for analysis.

Table 2.3.10: The results for grains m^{-2} from the experiment inside polytunnels in 2008/2009

		Antitranspirant/control treatment						Mean	*P* - Var	*P* - Treat	*P* - Var-Treat	s.e.m - Var-Treat	CV% - Var-Treat (res. DF)
		IUC	**UC**	**GS33**	**GS39**	**GS41**	**GS59**						
Variety	**E**	17210	9952	12040	10341	12128	12335	12334	0.116	<.001	0.449	1380	10.6 (20)
	C	16891	11432	13235	13166	14094	12710	13588					
Mean		17059 (b)	10692 (a)	12638 (a)	11754 (a)	13111 (a)	12522 (a)						

E = Einstein; C = Claire; IUC = irrigated unsprayed control; UC = unsprayed control; GS33, GS39, GS41 and GS59 = antitranspirant (di-1-p-menthene) treatment at respective growth stages; Var = Variety; Treat = Treatment; res. DF = residual DF; data within rows accompanied by the same letter are not significantly different at p = 0.05.

Table 2.3.11: The results for grains m^{-2} from the experiment inside polytunnels in 2009/2010

		Treatment						Mean (without IUC)	P - SMD	P - Treat	P – SMD-Treat	S.E.M. -SMD-Treat	CV % - SMD-Treat (res. DF)
		IUC (Common control)	**UC**	**di-GS31**	**di-GS33**	**di-GS41**	**la-GS41**						
SMD	**L**	18995	15117	15439	17932	15677	16171	16067	0.089	0.309	0.625	1582.6	10.3 (16)
	H		13757	14826	14623	13388	13601	14039					
Mean		18995	14437	15132	16278	14533	14886						

L = low SMD regime; H = high SMD regime; IUC = irrigated unsprayed control; UC = unsprayed control; di-GS31, di-GS33 and di-GS41 = the antitranspirant, di-1-p-menthene treatment at respective growth stages; la-GS41 the antitranspirant, latex treatment at GS41; Treat = Treatment; res. DF = residual DF.

Table 2.3.12: The results for grains m^{-2} from the experiments inside polytunnels in 2010/2011

	IUP	Treatment			*P*	*P* – Contrast: At treat vs UC	*P* – Contrast: di-GS33 vs la-GS33	S.E.M.	CV% (res. DF)
		UC	**di-GS33**	**la-GS33**					
Experiment 1	13542	8987	9504	9702	0.151	0.060	0.591	206.1	11.6 (47)
Experiment 2	13542	9414	10005	10151	0.432	0.211	0.807	412.6	10.3 (13)
Experiment 1 & Experiment 2 combined	13542	9101	9592	9843	0.056	0.023	0.409	214.8	10.9 (62)

IUP = Irrigated unsprayed plots; UC = unsprayed control; di-GS33 = di-1-p-menthene treatment at GS33; la-GS33 = latex treatment at GS33; AT treat = combination of the two antitranspirant treatments; Treat = Treatment; res. DF = residual DF.

2.3.6 The combination of all the 4 experiments inside polytunnels for analysis – the effect of an antitranspirant treatment at GS33 on yield and yield components

The unsprayed control and an antitranspirant treatment at GS33 were common for all the four experiments. As explained in chapter 2.2.6.4, data from the four experiments for the above two treatments were combined for analysis, and the results obtained from ANOVA for the hand harvested crop and the combine harvested crop are shown in Table 2.3.13. According to the results antitranspirant treatment at GS33 increased yield of droughted wheat significantly (hand harvested $p = 0.004$; combine harvested $p = 0.020$) compared to the unsprayed control. The increase in yield from the antitranspirant treatment compared to the unsprayed control was 0.567 (t/ha) according to the hand harvested yield and 0.48 (t/ha) according to the combine harvested yield. The increase in grains ear^{-1} made by the antitranspirant treatment compared to the unsprayed control was 1.72 per ear. There was a significant difference ($p = 0.038$) in ears m^{-2} between the antitranspirant treatment and the unsprayed control. The increase in ears m^{-2} from the antitranspirant treatment compared to the unsprayed control was 17.6 per m^{-2}. The increase in grains m^{-2} from the antitranspirant treatment compared to the unsprayed control was 1255 per m^2, and the difference in grains m^{-2} between the two treatments was significant ($p=0.003$). The TGW of the unsprayed control was higher than that of the antitranspirant treatment but the difference was not significant.

Table 2.3.13: The results for yield and yield components from the analyses in which all the four experiments inside polytunnels are combined

	UC	**At treatment at GS33**	*P*	S.E.M.	CV% (res. DF)
Yield (t/ha) (hand harvested)	5.840	6.407	0.004	0.1126	6.4 (11)
Yield (t/ha) (combine harvested)	5.85	6.33	0.020	0.1239	7 (11)
TGW (g)	53.32	52.55	0.151	0.355	2.3 (11)
Grains ear^{-1}	36.82	38.54	0.028	0.480	4.4 (11)
Ears m^{-2}	296.5	314.1	0.038	5.28	6 (11)
Grains m^{-2}	10896	12151	0.003	230.5	6.9 (11)

UC = unsprayed control; At treatment at GS33 = antitranspirant treatment at GS33; res. DF = residual DF

2.4 Discussion

2.4.1 The level of drought experienced by the crop and the changes in SMD with time during the experiments in the three years

The experiments under polytunnels were meant to be under drought conditions. Therefore, it is important to know whether the SMDs that occurred at antitranspirant spray application times were sufficient enough to provide an enough stress to drought the crop. There are a number of attempts which have been made to define the SMD at which wheat yields become limited. However, the idea of a specific SMD which is defined to be a threshold for wheat yield has been denied recently by scientists. One of the reasons for this rejection is that the impact of a SMD on yield depends on the developmental stage at which the SMD occurs (Kettlewell *et al*., 2010; Saini, 1997). Furthermore, whether a particular SMD limits yield or not depends on the available water capacity of the soil (Foulkes, *et al*., 2001) and its soil moisture release curve (Hall *et al*., 1977). Therefore there is no single recognised SMD value at which wheat yields become limited.

In this study the SMD values were compared with the easily available water capacity in the soil, which is given as 60% of total available water capacity (Hall *et al*., 1977). If the SMD at an antitranspirant spray application time was higher than the 60% of total available water capacity, the crop was considered to be under “sufficient” drought at the time of the spray application. The decision to use 60% of total available water capacity for this purpose is justified by the following facts. Bailey (1990) suggested a threshold of 50% available water capacity to apply irrigation to wheat in the UK. According to results gathered from six wheat cultivars, Foulkes *et al*. (2001) reported that restricted water availability first affected canopy expansion and canopy senescence respectively at 50% and 64% available water capacity. From the studies related to drought stress responses of winter wheat very few studies have attempted to quantify the level of drought imposed/occurred in terms of available water capacity in the soil. For most of the studies, in which available water capacity was used in quantifying drought, the threshold level had been considered to be below 50% of the available water capacity of the soil.

Since, the field capacity and the permanent wilting point of the soil in the experimental field for a depth of 80 cm is respectively 160 mm and 62 mm, the total available water capacity, which is the difference between field capacity and permanent wilting point (Hall *et al*., 1977; Huisman *et al*., 2001) is 98 mm. The easily available water capacity, which is 60% of the total available water capacity (Hall *et al*., 1977) is 58.8 mm. The SMDs inside the polytunnels at the spray application times in all the years were above 58.8 mm. Therefore, at all the spray application times in all the years water was not easily available, in other words the crop in the experiments inside the polytunnels was under "sufficient" drought during the periods of experimentation.

As described in chapter 2.2, soil moisture deficits were determined by the IMS irrigation programme fed with data obtained from a weather station 1/2 km away from the field. Both the neutron probe and the irrigation scheduling program showed a similar pattern of change in SMD not only in the soil inside polytunnels, but as well as outside polytunnels, which could have been different if the weather received from the weather station, especially the rainfall, was different from that at the experimental site. Furthermore, SMD values obtained from IMS irrigation scheduling programme were not very different to the values calculated from the neutron probe in 2010/2011; all of which shows that the data from IMS irrigation programme were applicable for the experimental site for the period of experimentation in 2010/2011. Since, the IMS irrigation programme was continuously fed with data from the first experiment in 2008/2009 to the final experiment in 2010/2011, data for 2010/2011 are linked with data for 2008/2009 and 2009/2010. Therefore, data from the IMS irrigation programme should be suitable for the experimental site for the period of experimentation in 2008/2009 and 2009/2010.

2.4.2 The response of yield and yield components to the factor variety (2008/2009), the factor SMD regime (2009/2010) and the interactive effect of these two factors with the second factor, antitranspirant/control treatments

Unlike the experiments in 2010/2011, which has only one factor, both the experiments in 2008/2009 and 2009/2010 were factorial designs with two factors. The effect of the factor

Variety in 2008/2009 and the factor SMD regime in 2009/2010 and the interactive effect of these factors with the factor antitranspirant/control treatments on yield and yield components, (according to the results from the experiments inside polytunnels) are discussed first, here in this section.

As described in section 2.1, one of the objectives of the experiments in 2008/2009 was to compare two winter wheat varieties, Claire and Einstein, to investigate possible differences in their response in yield and yield components to the film antitranspirant, di-1-p-menthene, treatments at GS33, GS39, GS41 and GS59, and two control treatments, one unsprayed, and one unsprayed but irrigated. According to the results, there was no significant difference between the two varieties, Claire and Einstein, in their response to antitranspirant/control treatments when yield and most of the yield components were considered. The effect of the factor variety was significant only for TGW ($p = 0.021$). The interaction between the two factors was not significant for yield or any of the yield components. There might be not much difference in the two varieties in the sensitivity to drought encountered at the growth stages at which the antitranspirant treatments were sprayed. In both the varieties, apical development may similarly correlate with the growth stage defined by the decimal code, and therefore, meiosis in pollen mother cells, which is the most sensitive stage of wheat towards drought (as reviewed in section 1.2.1), may occur at the same growth stage. These might be the reasons why the two varieties did not show significant differences in the response to antitranspirants when yield and yield components are considered. However, no evidence to support these facts could be found from literature. According to the results from the study explained in chapter 3, in the variety Claire, meiosis occurs in early GS41. The variety Einstein was not used in that study and no information on the growth stage at which meiosis occurs in Einstein could be found from literature. However, the apparent behaviour of the two varieties was consistent as both the varieties reached main developmental stages about the same time.

After the experiments in 2008/2009, it was decided to use only one variety in subsequent years so that the whole space within polytunnels could be used to include another factor worth exploring or to increase replication to combat the high CV% which was apparent

from the results in 2008/2009. The variety Claire was chosen over the variety Einstein due to the fact that Claire was the variety which was used in the experiments previously carried out at Harper Adams University.

As described in section 2.1, one of the objectives of the experiments in 2009/2010 was to investigate possible differences in the effect of antitranspirant treatments around meiosis on yield and yield components under two SMD regimes. According to the results, there was no significant difference in the mean yield or the yield components between the two SMD regimes. There was also no significant difference between the two SMD regimes, in their response to antitranspirant/control treatments when yield and yield components were considered. It is important to take into consideration that there was no difference in SMD between the two SMD regimes until the crop reached GS37 and after GS69 when the whole experiment was irrigated to field capacity. From GS37 to GS39 the SMD in the low SMD regime was in between about 70 mm to 90 mm, which is 71% to 91 % of the total available water capacity. The SMD in the high SMD regime during this period gradually increased from 90 mm to 115 mm, which is from 91 % of the total available water capacity to a level above permanent wilting point. The drought stress for both the SMD regimes was well beyond the threshold level of 60% of total available water capacity. The difference in SMD in the two SMD regimes from GS37 to GS69 might not have been high enough to create a significant difference in yield or yield components between the two SMD regimes. However, the mean yield, grains ear^{-1} and ears m^{-2} of low SMD regime were higher than those of the high SMD regime, and the difference in yield between the two SMD regimes was nearly 1 t/ha. The high coefficient of variation in yield and yield components within each SMD regime might however be one of the reasons why the differences between the two SMD regimes were not significant.

2.4.3 The effect of antitranspirant/control treatments on yield and yield components

The effect of antitranspirant/control treatments in the three years on yield and yield components, according to the results from the experiments inside polytunnels, are discussed.

2.4.3.1 In 2008/2009

The means of the two varieties for grains ear^{-1} of all the antitranspirant treatments were significantly higher than that of the unsprayed control. The four antitranspirant treatments might have ameliorated the effect of drought on grain initiation related biological processes, which are sensitive to water deficit. The determination of the grain number is a result of a dynamic process that occurs throughout spike development, starting from flower meristem differentiation (Rawson and Evans, 1970), which initiates at the stage of stem elongation (Tottman, 1987). The most sensitive stage of the crop to drought stress towards yield formation is the stage of meiosis in pollen mother cells. Drought during this stage reduces pollen viability and hence number of grains (Saini and Aspinall, 1981; Koonjul *et al*., 2005; Dorion *et al*., 1996; Lalonde *et al*., 1997). As described in section 3 in the winter wheat variety Claire meiosis in pollen mother cells occurs in early GS41. Drought stress at young microspore stage of pollen development leads to abortion of pollen development and reduction in grains ear^{-1} (Ji *et al*., 2010). Furthermore, drought stress during anthesis causes a variety of abnormalities in floral organs, which interfere with pollination or fertilization, and induces abscission of flowers or abortion of newly formed grains (Saini, 1997). The significantly high grains ear^{-1} of the antitranspirant treatments at GS33, GS39 and GS41 compared to the unsprayed control may be from moisture conservation in the plant during any of the above stages, and the antitranspirant treatment at GS59 might have ameliorated the effect of drought at anthesis. It was expected to have significant differences between antitranspirant treatments which were at different times in relation to the time of meiosis in pollen mother cells, the most sensitive

stage towards drought stress. But there was no significant difference between the four antitranspirant treatments. This lack of significance may have been caused by an inability of the treatment replication to overcome the variation between individual plots.

Although, the means of the two varieties for yield of all the antitranspirant treatments were higher than that of the unsprayed control, the differences were not significant. The significant increases in grains ear^{-1} of the antitranspirant treatments, compared to the unsprayed control, were not expressed as significant increases in yield. The high coefficient of variation in yield might be one of the reasons why the differences in yield were not significant despite significant differences in grains ear^{-1}. Although the means of the two varieties for ears m^{-2} of all the antitranspirant treatments were also higher than that of the unsprayed control, there was a significant negative relationship between ears m^{-2} and grains ear^{-1} with in plots. The slope of the regression line of the unsprayed control was not significantly different from that of any of the antitranspirant treatments, which means the antitranspirant treatments had not interacted in the compensation between the two yield components. The negative correlation between ears m^{-2} and grains ear^{-1} provides evidence for the fact that although the antitranspirant treatments increased mean values for both ears m^{-2} and grains ear^{-1} compared to the unsprayed control, in individual plots there had been a compensation between ears m^{-2} and grains ear^{-1} brought about by drought; i.e., the increase in individual plots was either in ears m^{-2} or in grains ear^{-1}. According to regression analyses, although a compensatory effect had not taken place between grains ear^{-1} and TGW, the significant negative relationship between ears m^{-2} and TGW shows a compensatory effect between ears m^{-2} and TGW. Since, when the timing of determination of these three yield components is considered, ears m^{-2} precedes grains ear $^{-1}$ and grains ear $^{-1}$ precedes TGW (Egli, 1998). Where ears m^{-2} was low, grains ear^{-1} might have increased and where ears m^{-2} was high, grains ear^{-1} and TGW might have decreased as compensation. The interrelated compensatory effects between the three yield components might be another reason why the significant differences in mean grains ear^{-1} were not expressed as significant differences in mean yield.

The compensatory effect shown between ears m^{-2} and TGW explains the reason for the unaffected TGW and the significantly lower ears m^{-2} in the unsprayed control, antitranspirant treatments at GS33 and GS39 compared to the unsprayed irrigated control. Even though the TGW of the antitranspirant treatment at GS41 was significantly lower than that of the irrigated unsprayed control, the ear m^{-2} of the treatment was the highest among the four antitranspirant treatments and the unsprayed control. The TGW of the antitranspirant treatment at GS59 was also significantly lower than that of the irrigated unsprayed control, but the ear m^{-2} of the treatment was high compared to the unsprayed control and most of the antitranspirant treatments which showed a non-significant difference in TGW with the irrigated unsprayed control. Therefore, a compensatory mechanism may explain the significantly low TGW obtained for the antitranspirant treatments at GS41 and GS59 compared to the irrigated unsprayed control. Alternatively one can argue that the significantly lower TGW in the antitranspirant treatment at GS41 and GS59 compared to the irrigated unsprayed control might be from a reduction in photosynthesis from the flag leaf and/or the ear caused by the antitranspirant film. Photosynthesis seemed to be reduced by the antitranspirant (chapter 3) to some extent. But this reduction in photosynthesis might have not affected significantly the net amount of assimilates partitioned to yield ultimately, since the yield of the antitranspirant treatment at GS41 or GS59 does not show a reduction in yield compared to the unsprayed control or the other antitranspirant treatments. Nevertheless, the highest yield among the four antitranspirant treatments and the unsprayed control was from the antitranspirant treatment at GS41. As described in section 1.3.1, drought, however, inhibits photosynthesis by decreasing leaf stomatal conductance to CO_2 entering the leaf, inhibiting the Calvin cycle and by producing reactive oxygen species, which causes damages to chloroplasts and cells (Flexas and Medrano, 2002; Tezara *et al*., 1999; Loggini *et al*., 1999; Tambussi *et al*., 2000). Grain filling in wheat depends on carbon from current assimilation (Tambussi *et al*., 2007) and carbon remobilised from stem reserves (Blum, 1998; Foulkes *et al*., 2007). When drought stress coincides with the period of grain filling, the contribution of stored assimilates for grain filling could be 75-100% depending

on the degree of impairment of current assimilation by drought (Gavuzzi *et al*., 1997). The persistence of the antitranspirant, di-1-p-menthene, on the leaf in summer is 15-30 days, depending on the environmental conditions (Solarova *et al*., 1981). As described in the section 1.1.5, drought stress affects crop biology and physiology in many ways, and the response and the resistance of plants to drought stress are complex biological processes which have not been understood fully (Harb *et al*., 2010). Hence, the conservation of water by the antitranspirant film may contribute in many unknown ways to ameliorate the effect of drought on the crop. The contribution of the advantageous effects of the antitranspirant treatments at GS41 and GS59 towards yield formation might have counteracted that of the disadvantageous effect of the film, which is mainly, the impairment of photosynthesis during the period at which the film persisted on the leaf. This may be the reason why reduced photosynthesis has not shown up as a reduction in yield.

As described in section 1.2.2.4, drought stress at early grain development stages leads to decreased grain sink potential as well as kernel abortion. It was postulated that the high drought stress level occurred at later stages of grain development might have hidden the beneficial effects of antitranspirant treatments on yield to some extent. This is the reason why it was decided to irrigate the whole experiments in the subsequent years to field capacity when the crop reached GS69.

From the four antitranspirant treatments in 2008/2009, the antitranspirant treatment at GS59 possessed the lowest mean yield, therefore, it was decided to remove the antitranspirant spray application at GS59 from the subsequent experiments.

2.4.3.2 In 2009/2010

In 2009/2010, antitranspirant/control treatment was not significant for yield or any of the yield components. The means of the two SMD regimes for yield and grains ear^{-1} of all the antitranspirant treatments except the di-1-p-menthene treatment at GS41 were higher than those of the unsprayed control. The yield and the grains ear^{-1} of the antitranspirant,

di-1-p-menthene, treatment at GS41 in the high SMD regime were lower than those of the unsprayed control in the same SMD regime, making the means of the two SMD regimes for the two parameters at di-1-p-menthene treatment at GS41 lower than those at unsprayed control. In contrast, as described above, in 2008/2009, yield and yield components of all the antitranspirant treatments including the antitranspirant treatments at GS41 and GS59 were higher than that of unsprayed control, though the differences were not significant. The experiments previously carried out at Harper Adams University College showed a significant increase in yield by the antitranspirant, di-1-p-menthene, treatment at GS 41 (Kettlewell *et al*., 2010). Furthermore, the increase of yield by the antitranspirant, di-1-p-menthene was high when the SMD was high. The highest response for the antitranspirant was under the highest SMD tested which was 118 mm (Kettlewell *et al*., 2010). The difference in the behaviour of antitranspirant treatment at GS41 between the two years might not be due to an effect of the SMD at spraying, since, the SMD at antitranspirant treatment (GS41) in the high SMD regime, which was 102.6 mm, was not much different to the SMDs at GS41 and GS59 in 2008/2009, which were 91.7 mm and 108.4 mm respectively. Furthermore, the antitranspirant, latex, treatment at GS41, which was at the same SMD at spraying as the di-1-p-menthene treatment at GS41, did not show any reduction in yield or any of the yield components compared to the unsprayed control, and the experiments in 2010/2011 showed no significant difference between the two antitranspirants in terms of the effect on yield and yield components. However, the above discussed difference in yield and grains ear^{-1} between the antitranspirant, di-1-p-menthene, treatment at GS41 and the unsprayed control in 2009/2010 was not significant. Therefore, low yield and grains ear^{-1} obtained for the di-1-p-menthene treatment at GS41 compared to the unsprayed control might be an incident occurred by chance.

Showing a similarity to the experiment in 2008/2009, in 2009/2010 the means of the two SMD regimes for ears m^{-2} of all the antitranspirant treatments were higher than that of the unsprayed control, and the means of the two SMD regimes for TGW of all the antitranspirant treatments were lower than that of the unsprayed control. But these differences were not significant.

In contrast to 2008/2009, regression analyses between grains ear^{-1} and TGW, grains ear^{-1} and ears m^{-2} or ears m^{-2} and TGW did not show any compensatory effect between yield components. One could argue that the experiment in 2009/2010 was irrigated to field capacity after GS69 whereas the experiment in 2008/2009 was without irrigation until harvest and that difference might be the reason for the difference in compensatory effects between the yield components in the two years. However, Experiment 1 in 2010/2011, which was also irrigated to field capacity after GS69 showed a compensatory effect between grains ear^{-1} and TGW and ears m^{-2} and TGW. The low SMD regime might have hidden any compensatory effect shown by the high SMD regime when the results from the two SMD regimes were analysed together. However, regression analyses between the yield components, in which two SMD regimes were used as groups, did not show any significant difference in slopes of regression lines obtained for the two SMD regimes (analyses are not shown), which means there was no significant difference between the two SMD regimes in the extent of correlation of each yield component pair analysed.

There was no significant difference shown by the analysis between the irrigated sprayed plots and the irrigated unsprayed control in yield or any of the yield components. However, due to lack of replication and poor experimental design which ignores randomisation rules, the study is not powerful enough to obtain an in depth understanding of the effect of antitranspirant spray at GS33 on the crop under no drought stress. Some of the previous studies (Kettlewell *et al*., 2010; Amor and Rubio, 2009) have shown that film antitranspirants reduce yield under conditions where there was no water deficit.

2.4.3.3 In 2010/2011

The results from the experiments in 2008/2009 and 2009/2010 hinted that if variation with in treatments could be reduced, more clear results showing the effect of antitranspirants on yield and yield components at a specific growth stage may be obtained. For the experiment in 2010/2011 it was decided to study the response of the crop to a film antitranspirant sprayed at only one growth stage, selected according to the results from the experiments in 2008/2009 and 2009/2010, so that more space within the polytunnels

was available to increase the number of replicates of individual treatments. GS33 seemed to be the most effective growth stage to receive an antitranspirant treatment when yield and yield component results in variety Claire from the past two years were carefully studied. These are the reasons for choosing only one growth stage and particularly GS33 for the experiments in 2010/2011.

The reason for carrying out two experiments inside polytunnels in 2010/2011, one which was irrigated to field capacity after GS69 (Experiment 1) and one without irrigation until harvest (Experiment 2), was because the experiment in 2008/2009 which was without irrigation until harvest and the experiment in 2009/2010 which was irrigated after GS69 to field capacity showed some differences in results as described above.

In the occasions where the CV within treatments was low, both Experiment 1 and Experiment 2 showed that a film antitranspirant treatment at GS33 increased yield significantly compared to an unsprayed control under drought conditions. The difference between the mean yield of the two antitranspirant treatments and the unsprayed control in Experiment 1 was borderline significant ($p = 0.055$) as shown by the results from hand harvested crop at a CV of 10.7%, but at a CV of 11.7% the results from combine harvested crop did not show a significance ($p = 0.070$) in the difference. In Experiment 2, even though the results from the hand harvested crop did not show a significant difference in yield between the antitranspirant treatments and the unsprayed control at a CV of 9.7%, the results from the combine harvested crop showed a significant difference between the mean yield of the two antitranspirant treatments and yield of the unsprayed control at a CV of 6.9%.

When Experiment 1 and Experiment 2 were combined for analysis, the residual degree of freedom increased to 64, and the analysis clearly showed that the antitranspirant treatments at GS33 significantly increased yield compared to unsprayed controls.

Since both Experiment 1 and Experiment 2 showed significant increases in yield by antitranspirant treatments at GS33 compared to unsprayed controls, the fact that whether the crop suffered drought stress after GS69 or not seems not to influence the ability of a

film antitranspirant treatment at GS33 to increase yield compared to an unsprayed control, when the treatment is made under drought conditions.

In Experiment 1, regression analysis between ears m^{-2} and grains ear^{-1} revealed a positive correlation between the two yield components within the antitranspirant/control treatments. Therefore, it can be presumed that when the crop suffers no drought stress after GS69, although it was under drought stress until GS69, grains ear^{-1} is not necessarily compensated by ears m^{-2}. An experiment (Heping *et al*., 2010) which attempted to study the relationship between yield and yield components in low-, medium-, and high-rainfall zones of South-western Australia showed no negative association between ear m^{-2} and grains ear^{-1} when data from all the rainfall zones were pooled for analysis. However, in Experiment 1, TGW was compensated by both ears m^{-2} and grains ear^{-1}. Whereas in Experiment 2, there was no significant relationship between ears m^{-2} and TGW or grains ear^{-1} and TGW. Experiment 2 showed a compensatory effect between ears m^{-2} and grains ear^{-1}, in contrast to Experiment 1, but the same as the experiment inside polytunnels in 2008/2009 which was not irrigated until harvest as for Experiment 2. Although the only compensatory effect shown by Experiment 2 is the compensation between ears m^{-2} and grains ear^{-1}, the experiment in 2008/2009, however, also showed a significant negative relationship between TGW and ears m^{-2}. In the process of yield formation, a sink is created first and subsequently it is filled with the source. Both sink and source are impaired by drought stress depending on the timing and the severity of stress in relation to plant phenology (Saini, 1997; Blum, 1996). Yield component compensation is a vital developmental mechanism for reforming yield, at least to a certain extent, under or upon recovery from stress, and some yield components are developmentally correlated. When a plant senses drought stress at a stage where the plant is still affecting reproduction, the reproductive demand for carbon is decreased by reducing the number or size of sink. If the number of sink is affected the size may not be affected and vice versa (Blum, 1996).

In any of the regression analyses between yield components, the slope of the regression line of the unsprayed control was not significantly different from that of any of the

antitranspirant treatments either in Experiment 1 or in Experiment 2, which means the antitranspirant treatments have not interacted or contributed to the compensation between yield components.

2.4.4 The yield component which was responsible for the yield increase by an antitranspirant treatment

Linear regression analysis between yield and yield components is a way to detect the association of yield components with yield (Egli, 1998). There had been compensatory effects between yield components within individual plots in 2008/2009 and in both Experiment 1 and Experiment 2 in 2010/2011. Therefore, correlation between yield and each yield component, as shown by linear regression analysis, might not be a true indicator of which yield component is most responsible for the increase in yield in antitranspirant treatments compared to the unsprayed control.

In Experiment 1, from the three yield components, ears m^{-2} holds the lowest p-value ($p = 0.122$) for the ANOVA contrast analysis between the unsprayed control and the two antitranspirant treatments. Therefore, the yield component which was responsible in the nearly significant difference between the yield of the unsprayed control and the mean yield of the two antitranspirant treatments as shown by the hand harvested crop results might be ears m^{-2}. Whereas, in Experiment 2, grains ear^{-1} was the only yield component which showed a significant difference ($p = 0.046$) between the mean value of the two antitranspirant treatments and the value of the unsprayed control. Therefore, the yield increase shown by the antitranspirant treatments at GS33 compared to the unsprayed control might be from the increase in grains ear^{-1}. A similar result was shown by the experiment inside polytunnels in 2008/2009, which was also without irrigation until harvest as Experiment 2 in 2010/2011. This indicates that when the crop suffers drought stress, which continues after GS69 until harvest, the yield component which is responsible for the yield increase by an antitranspirant treatment is grains ear^{-1}, and, in contrast, when there is no drought stress after GS69, it is ears m^{-2}. However, it was not clear whether the increase in ears m^{-2} was because of an increase in tiller survival (due to conserved

moisture in the soil) or because of an establishment of at least one grain in tillers which otherwise would not bear grains. If the reason was the later, it is not actually an increase in ears m^{-2}, but an increase in grain number. Furthermore, the few grains established by the antitranspirants in tillers which otherwise would not bear grains, might have lowered the mean number of grains per ear, which is grains ear^{-1}, creating a false interpretation of the effect of film antitranspirants on the yield component. It has been recorded that drought stress at early grain development stages leads to kernel abortion (Saini and Westgate, 2000), and it could also be possible that, in the experiments which were not irrigated after GS69, the few grains established by the antitranspirants in tillers which otherwise would not bear grains, had been aborted owing to drought stress at later stages. Irrigation after GS69 might have avoided kernel abortion in these tillers, and that might be the reason for higher ears m^{-2} in antitranspirant treatments compared to the unsprayed control in the experiments irrigated after GS69. The two yield components, grains ear^{1} and ears m^{-2}, are interrelated in the above described manner, and therefore, it is not possible to decide precisely which of these two yield components are contributing to the observed yield increases. According to Egli (1998), in most crops, the yield component grains ear^{1}, could be population sensitive and varies inversely with plants m^{-2} over a wide range in population, so that grains m^{-2} remains constant. It has been suggested that for most of the crops, grains $area^{-1}$, which is the combination of the two yield components, ears $area^{-1}$ and grains ear^{-1} (or pods $area^{-1}$ and grains pod^{-1}) is better correlated with yield than the two components of it. Because of this reason it was decided to analyse the effect of treatments on grains m^{-2} as well. In Experiment 1, as for yield, the difference between the unsprayed control and the mean of the two antitranspirant treatments was nearly significant for grains m^{-2}. When Experiment 1 and Experiment 2 were combined for analysis, the difference between the unsprayed control and the mean of the two antitranspirant treatments was significant for grains m^{-2} as well as for yield. It was decided to report that yield increase by the antitranspirant treatments is from an increase in grains m^{-2}.

As discussed in chapter 3, in the variety Claire, meiosis in pollen mother cells occurs at early GS41. Therefore, the yield increase by the antitranspirant treatments at GS33 can be attributed to alleviation of the effect of drought stress on the crop during meiosis in pollen mother cells as hypothesised. However, as explained above the increase in yield could be partially because of an increase in tiller survival resulted from moisture conservation in soil by the antitranspirant treatments.

2.4.4.1 The combination of the four experiments inside polytunnels for analysis

When the data from the four experiments for the antitranspirant treatment at GS33 and the unsprayed control were combined for analysis, clear and significant increases in yield, grains ear^{-1}, ears m^{-2} and grains m^{-2} were shown in antitranspirant treatment at GS33 compared to the unsprayed control. It was clear from this analysis that the yield increase by the antitranspirant treatment was from an increase in grains m^{-2}.

The yield increase by film antitranspirant treatments at GS33 is well in agreement with Kettlewell *et al*. (2010) that indicated film antitranspirants increase yield in droughted wheat when sprayed prior to meiosis in pollen mother cells. Section 1.4.2.2 reviews some of the other studies which reported yield improvements from film antitranspirants in other seed crops including corn (Fuehring and Finckner, 1983), sorghum (Fuehring, 1973) and rapeseed (Patil and De, 1978). These studies, however, have not attempted to explore the effects of film antitranspirants on yield components.

2.5 Conclusion

This chapter included the studies carried out with the main objective of exploring the effect of film antitranspirants at different growth stages in relation to meiosis in pollen mother cells on yield and yield components of droughted wheat, with the purpose of determining the most effective growth stage to receive a film antitranspirant application targeted to increase yield under drought conditions. This objective was tested under three hypotheses, which were: Film antitranspirants increase yield of droughted wheat when applied before meiosis in pollen mother cells (GS41), which is the most sensitive stage of wheat yield formation to drought stress; the most effective growth stage to apply a film antitranspirant to increase yield under drought conditions may be GS31, GS33, GS39 or GS41; the increase of yield is by an increase in the number of grains. It can be concluded that, showing the most effective growth stage to apply a film antitranspirant to increase yield under drought conditions is GS33 (from the stages tested), two film antitranspirants used in the study, di-1-p-menthene and latex, increased yield by approximately 0.5 t/ha when sprayed at GS33. The yield increase by antitranspirant treatments was from an increase in grains m^{-2}. These results indicate that the three hypotheses tested were true. As discussed in chapter 3, in the variety Claire, meiosis in pollen mother cells occurs at early GS41. Therefore, the yield increase by the antitranspirant treatments at GS33 can be attributed to alleviation of the effect of drought stress on the crop during meiosis in pollen mother cells. However, the increase in yield could be partially because of an increase in tiller survival resulted from moisture conservation in the soil by the antitranspirant treatments.

As described in section 2.1, one of the objectives of the experiments in 2008/2009 was to compare two winter wheat varieties, Claire and Einstein, to investigate possible differences in their response in yield and yield components to the antitranspirant/control treatments. It was hypothesised that the two varieties, Claire and Einstein are different in their response in yield and yield components to the antitranspirant treatments made around meiosis in pollen mother cells. Rejecting the hypothesis it was indicated that there

was no significant difference between the two varieties in their response to antitranspirant/control treatments when yield and yield components were considered. It was postulated that there was little difference in the sensitivity of the two varieties to drought stress at the growth stages which are most sensitive to drought stress.

As described in section 2.1, one of the objectives of the experiments in 2009/2010 was to investigate possible differences in the effect of antitranspirant treatments made around meiosis in pollen mother cells on yield and yield components under two SMD regimes. It was hypothesised that the responses in yield and yield components to antitranspirant treatments made under different SMDs at application are different in winter wheat (variety Claire). Rejecting the hypothesis it was indicated that there was no significant difference between the two SMD regimes, in their response to antitranspirant/control treatments when yield and yield components were considered. The two SMD regimes were maintained from GS37 to GS69, and the difference in SMD in the two SMD regimes during that period might not be high enough to create a significant difference in yield or yield components between the two SMD regimes.

The key findings of the experiments explained in this chapter are summarised below.

1. The two film antitranspirants used in the study, di-1-p-menthene and latex, increased yield by approximately 0.5 t/ha when sprayed at GS33, under the conditions of this study (at SMDs above 66 mm).
2. The yield increase by antitranspirant treatments was from an increase in grains m^{-2}.
3. There was no significant difference between the two varieties in their response to antitranspirant/control treatments when yield and yield components were considered.

3 Physiological mechanisms underlying the yield increase of droughted wheat by film antitranspirants

3.1 Introduction to the chapter 3

The objective 2 of the project: the exploration of the underlying mechanism, by which film antitranspirants increase yield, was performed by exploring the physiological effects of antitranspirants on transpiration, stomatal conductance, leaf water status, CO_2 intake, photosynthesis and pollen development.

According to the literature review of section 2.3.3, the effect of film antitranspirants on leaf temperature is a topic of debate, therefore the effect of film antitranspirants on leaf temperature was also studied.

The effect of film antitranspirants on pollen development was assessed in terms of the differences in the starch content inside the pollen at anther dehiscence. As explained in chapter 2.2.2, the stage of meiosis in pollen mother cells is identified as the most sensitive stage to drought stress in the life cycle of wheat. During normal development, pollen grains accumulate starch, which is a source necessary for subsequent pollen germination and pollen-tube growth. Pollen grains affected by drought stress at pollen mother cell meiosis, fail to accumulate starch, and light microscopy of the anthers has shown a nearly complete lack of starch within sterile pollen grains (Saini *et al*., 1984; Dorion *et al*., 1996; Saini, 1997; Lalonde *et al*., 1997). In the presence of KI/I_2 starch turns to a dark blue/black colour, therefore KI/I_2 stains normal pollen grains filled with starch granules a dark blue/black colour and does not stain sterile pollen grains without accumulated starch (Nelson, 1968). In our study Lugol's solution (Sigma-Aldrich, Dorset, UK) which contains KI/I_2 was used to differentiate drought stress affected pollen grains from unaffected pollen grains under the light microscope depending on the presence or absence of starch within pollen grains.

All the above mentioned studies were performed on the field experiments carried out in the three years of 2008/2009, 2009/2010 and 2010/2011, of which experimental designs are described in chapter 2.2. Since antitranspirants perform better under drought conditions (Kettlewell *et al*., 2010), for most of the studies only the experiments under

polytunnels were used due to time constraints and to collect more useful data within the available time.

The above mentioned studies were conducted under the following hypotheses:

1. Film antitranspirants reduce transpiration and photosynthesis
2. The decrease in transpiration by the antitranspirant treatments is from increased resistance to diffusion of water vapour from stomata
3. The decrease in photosynthesis by the antitranspirant treatments is from decreased internal CO_2 concentration
4. Reduced transpiration increases leaf water potential and alleviates the effect of drought on pollen viability at the stage of meiosis in pollen mother cells irrespective of reduced photosynthesis
5. Film antitranspirants do not increase leaf temperature significantly

Apart from the main studies mentioned above another two studies, important in understanding and elaborating on the results of the main studies, were also carried out. The spray distribution pattern, leaf coverage and stomatal coverage of the most frequently used film antitranspirant in the research, di-1-p-menthene, on wheat leaves was assessed by image analysis and electron microscopy. Furthermore, studies were carried out to identify the growth stage and the external tiller morphology of winter wheat variety Claire at the time of meiosis in pollen mother cells.

3.1.1 The exploration of the distribution pattern, leaf coverage and stomatal coverage of di-1-p-menthene spray on wheat leaves

The aim of this study was to explore how much of a leaf surface is covered by the antitranspirant film and whether the film is evenly distributed or occurs as patches covering some stomata while leaving other stomata uncovered.

The distribution pattern and leaf coverage of antitranspirant spray droplets on leaves was explored by means of image analysis. The antitranspirant sprayed onto leaves is

colourless and could not be seen without creating contrast. Therefore the antitranspirant spray solution was mixed with titanium dioxide powder (TiO_2; Promega, Southampton, UK) which gives a white colour to the solution. Titanium dioxide is a non-toxic, white pigment, which is used in the production of paints, cosmetics, ink, paper, rubber, textile, plastics, leather and ceramics. Titanium dioxide is also permitted for use in food products. Furthermore, titanium dioxide serves as adjuvants and additives in commercial formulations (Owolade and Ogunleti, 2008). However, no information could be found from literature on studies related to titanium dioxide usage as a tracer in spray distribution analysis on leaves. The decision of using titanium dioxide for this purpose was made after testing a number of other dyes as tracers. These dyes included inorganic stains as well as fluorescent dyes, none of which provided enough contrast in scanned images to allow spray droplets to be distinguished from the background leaf blade, especially in the areas of grooves on the leaf blade.

Stomatal coverage by the film antitranspirant was explored by means of scanning electron microscopy. The conventional method of preparing samples for scanning electron microscopy involves dehydration with organic solvents followed by critical point drying and coating with a layer of heavy metal (Peacock *et al*., 1998). The strong organic solvents used in this process such as ethanol and acetone inevitably cause shrinkage and withdrawal of cellular constituents and may destroy the epicuticular wax layer (Ensikat and Barthlott, 1993; Peacock *et al*., 1998). Specimen shrinkage and any destruction caused to the epicuticular wax layer may in turn alter the pattern of antitranspirant spray deposits on the leaf and/or harm the antitranspirant deposits on the wax layer. Furthermore, the antitranspirant deposits may dissolve in the solvents and degrade during critical point drying. However, no evidence supporting that has been reported in the literature. Coating the samples with a layer of heavy metals, which serves as a way of increasing the resolution, may, however, change the surface characteristics of the samples (Ensikat and Barthlott, 1993; Peacock *et al*., 1998). Irrespective of these issues the conventional method of preparing samples for scanning electron microscopy has been used in the only study (Davies and Kozlowski, 1974), which could be found in the literature, attempting to

explore film antitranspirant spray deposits on leaves. The conventional method of preparing samples was not followed in this study. Instead, an alternative, safe procedure (Ensikat and Barthlott, 1993; Peacock *et al*., 1998) was used, which excluded dehydration with organic solvents, critical point drying and the necessity of coating the samples with a layer of heavy metals. The procedure involves treating the samples with a dilution series of a liquid, such as triethylene glycol or glycerol, which has a very low vapour pressure, without the surface to be studied being contaminated. The very low vapour pressure of these liquids allows them to infiltrate biological samples substituting the water in the samples so that the samples can be observed in the scanning electron microscope without drying. Furthermore, the added conductive properties of such liquids allow samples to be observed either uncoated or with very thin coatings. This method allows the retention of surface contaminants, waxes, lipids and loose particles on the sample surface and limits the artefacts imposed by the conventional preparation methods for scanning electron microscopy (Ensikat and Barthlott, 1993; Peacock *et al*., 1998). This method has been widely used in subsequent studies (Burkhardt *et al*., 2001; Koch *et al*., 2006; Zinsou *et al*., 2006; Kim, 2008) related to scanning electron microscopy of plant surfaces.

The knowledge about the distribution pattern of the antitranspirant film on the leaf is helpful in understanding the results obtained from the main studies, therefore, the study was included in this main chapter.

3.1.2 The exploration of the growth stage at which meiosis occurs in pollen mother cells

It was important to explore the external morphology of the shoot and the growth stage at which meiosis occurs in pollen mother cells of the variety Claire as different studies have identified different growth stages as the stage at which meiosis occurs in pollen mother cells. This difference might be due to differences in the varieties used in those studies. Some studies have mentioned the variety used whereas some studies have not. In the variety Chinese Spring, when meiosis in pollen mother cells is taking place, the shoot is at GS41 and the sheath of the flag leaf is extended 1-2 cm above the auricles of the

penultimate leaf (Bennett *et al*., 1973). Another study, where variety/varieties used in the study are not mentioned, have reported that generally in wheat, meiosis in pollen mother cells occurs at the boot swollen stage (GS45) when the ear is about to emerge from the inflated flag leaf sheath (Kirby, 2002). Tottman (1987) has reported that generally in wheat when meiosis happens in pollen mother cells, the flag leaf is usually beginning to emerge and the third node is likely to be detectable (just before GS33).

This study was done before antitranspirant spray application times were decided, because antitranspirant treatments were designed in relation to the time at which meiosis occurs in pollen mother cells as rationalised in chapter 1.4. It is important to take into account the stage at which meiosis occurs in pollen mother cells in order to understand the reasons behind the effect of different antitranspirant treatments on pollen development, therefore, the study was included in this main chapter.

3.2 Materials and Methods

3.2.1 The exploration of the distribution pattern, leaf coverage and stomatal coverage of di-1-p-menthene spray on wheat leaves

The spray distribution pattern, the leaf coverage and stomatal coverage of di-1-p-menthene sprayed onto Claire wheat leaves were assessed by image analysis and scanning electron microscopy. The spray characteristics were kept similar to the spray characteristics followed when the experimental plots were sprayed with the antitranspirants; i.e., the antitranspirant was applied at 2.5 l ha^{-1} (1.25% v/v antitranspirant in water), at a sprayer pressure of 0.2 MPa and a sprayer speed of 1 ms^{-1} with Flat Fan nozzles (FF110 03) and at a water volume of 200l/ha. The height of the boom was maintained at 0.5 m above the crop canopy while spraying.

For the purpose of the study performed by means of Image analysis, 100 ml of antitranspirant spray solution was prepared as described above and 1.5 g of titanium dioxide (Promega, Southampton, UK) was added to the solution which was then mixed by shaking. The solution was sprayed with an automatic sprayer (Precision Pot Sprayer; custom-made) onto the adaxial side of 5 detached, glasshouse grown Claire flag leaves placed on a paper. The leaves were allowed to dry. Then, on the same day, the leaves were scanned by a scanner (Scanjet 8300; HP) and the scanned images were analysed with image analysis (Scanjet 8300; HP). No information could be found from the literature on studies related to titanium dioxide usage as a tracer in spray distribution analysis on leaves. Therefore, the amount of titanium dioxide that should be applied to the spray solution was decided after testing several amounts. The lowest amount, which seemed to be creating a contrast enough to identify almost all the dry spray droplets from the background leaf blade in the scanned images of leaves, was chosen as the amount of titanium dioxide that should be applied to the spray solution in the study.

For the purpose of scanning electron microscopy a discard plot in a field experiment (in 2009/2010) under polytunnels was sprayed with the antitranspirant, di-1-p-menthene, at

GS41, with a hand held sprayer (following the characteristics described above) as experimentally sprayed plots. The day after the spray application, epidermal scrapings, of approximately 5 mm^2 in size, were sampled with a razor blade from random locations of both adaxial and abaxial sides of leaves. About 10 flag leaves at random locations of the plot were used in the study, and the samples were collected without detaching the leaves in order to minimize water loss and shrinkage of leaf tissues. Care was taken to not damage the epidermis, cuticle, wax layer or the antitranspirant deposits on the surface while scrapping the epidermis off. Soon after scraping off from the leaves, the leaf samples were prepared for scanning electron microscopy as explained in Peacock *et al*. (1998). The samples were directly moved into the fixative which was 4% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). After fixing for 1 hour, the samples were washed three times in the buffer by leaving the samples in the buffer for 10 minutes each time. Then the water in the samples was substituted with triethylene glycol (Sigma-Aldrich, Dorset, UK), by floating the samples on a dilution series of triethylene glycol in water. The dilution series was from 10% to 100% concentration in 10% increments. The samples were left at each concentration in the series for 4 hours. During all of the steps described above, the outer surface of the samples was not submerged in liquids. The samples were floated on the liquids using curved forceps in a way that outer surface of the samples is not in contact with the liquids. Great care was taken to keep the surface dry at all times. After the final dehydration step in 100% triethylene glycol, the samples were placed on Whatmann filter papers to allow the drainage of excess triethylene glycol. The inner side of the samples was glued to the stub with a carbon adhesive. The samples were then applied with a very thin coating of gold before viewing with scanning electron microscope (Stereoscan S200, Cambridge Instruments, Cambridge, UK).

3.2.2 The exploration of the growth stage at which meiosis occurs in pollen mother cells

The correlation between the crop growth stage defined by the decimal code or the external morphology of the shoot and meiosis in pollen mother cells in winter wheat

variety Claire was explored using the plants collected from field experimental plots of irrigated, unsprayed controls which are free from drought stress and unsprayed with any of the antitranspirants. The developing spikes were carefully 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. The development stage of the shoots at the time of sampling was noted. 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 (Morgan 1980; Ji *et al*., 2010). Furthermore, photographs of the shoots were taken before dissecting so that the external appearance of the shoots could be correlated with the development stage of the anthers as identified after microscopic studies. 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*., 1971; 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 it 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 and covered with a coverslip, then 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 microscopy camera (Infinity-2, Lumenera Corporation, Ottawa ON, Canada) fixed to the microscope.

3.2.3 The effect of film antitranspirants on the rate of transpiration, stomatal conductance, photosynthesis and internal CO_2 concentration

The effect of film antitranspirants on stomatal conductance, the rate of transpiration, photosynthesis and internal CO_2 concentration was explored with the experiment under polytunnels in 2009/2010 and the experiment under polytunnels irrigated after GS69 (Experiment 1) in 2010/2011. The measurements were made with the TPS-2 Portable Photosynthesis System (PP Systems, Hertfordshire, UK) which measures stomatal conductance, the rate of transpiration, photosynthesis and leaf temperature at the same instance.

In 2009/2010, the study was first done with the antitranspirant treatment at GS33. The measurements were obtained from the unsprayed control and the antitranspirant treatment at GS33 before, the day after and three days after the antitranspirant, di-1-p-menthene application at GS33. Although there were two SMD regimes in 2009/2010, as described in section 2.2, the two SMD regimes were brought into operation from GS 37 onwards, when the SMD reached 90 mm. Therefore, at the time of the antitranspirant treatment at GS33, two SMD regimes did not exist.

The study was done for the second time in 2009/2010 with the antitranspirant treatments at GS41. As described in section 2.2, in contrast to the single antitranspirant treatment at GS33, at GS41, there were two antitranspirant treatments, namely, di-1-p-menthene treatment at GS41 and latex treatment at GS41. Furthermore, by the time of the antitranspirant treatments at GS41, the low SMD regime had been brought into operation, and, therefore the two SMD regimes existed. The measurements were obtained before, the day after and three days after the antitranspirant applications from the unsprayed control and the two antitranspirant treatments at GS41 in both the SMD regimes.

In 2010/2011, the measurements were obtained before, three days after and seven days after the antitranspirant applications from the unsprayed control and the two antitranspirant treatments, which were di-1-p-menthene and latex at GS33.

Table 3.2.1 summarises the antitranspirant/control treatments from which the measurements were obtained in the two years.

Table 3.2.1: The experiments (under polytunnels) and the antitranspirant/control treatments which were used to explore the effect of antitranspirants on the rate of transpiration, stomatal conductance, photosynthesis and internal CO_2 concentration

	At the spray application at:	Measurements were obtained from:
2009/2010: Experiment under the polytunnels	GS33	• The antitranspirant (di-1-p-menthene) treatment at GS33 • Unsprayed control (two SMD regimes did not exist at this stage)
	GS41	Both the low and high SMD regimes - • The two antitranspirant treatments at GS41 (di-1-p-menthene at GS 41 and latex at GS 41) • Unsprayed control
2010/2011: Experiment 1	GS 33	From all the treatments, i.e.: • di-1-p-menthene at GS 33 • latex at GS 33 • unsprayed control

Although, there were six replicates of antitranspirant/control treatments in each polytunnel/block in Experiment 1, measurements were obtained only from one of the replicates in each polytunnel because of time constraints (In 2009/2010 there was only one replicate in each polytunnel/block).

During the spray application time of GS33, in both the years, the measurements were obtained from the penultimate leaf, since the flag leaf was not yet fully emerged. During the spray application time of GS41 (in 2009/2010), the measurements were obtained from the flag leaf. On each occasion of data collection the readings were taken from five randomly selected leaves (one on each plant) per plot of antitranspirant/control treatments. The readings were taken from the middle of the leaf blade. The variability in the parameters, especially in the rate of transpiration and photosynthesis, within plots was high. Therefore, from a leaf, three readings were taken at the same position at 15 second intervals. Before analysing, the mean of the three readings was calculated and in the analysis, this mean was used as the reading from a leaf in order to minimize the coefficient of variation within plots. Readings obtained from each antitranspirant treatment were compared with those of the unsprayed control by subjecting to ANOVA. Since, the leaf cuvette window was not fully covered by most of the leaves it was essential to adjust the readings obtained to the actual leaf area which was in the leaf cuvette. Therefore, the width of each leaf at the position to where the leaf cuvette was fixed while obtaining the readings was measured, and the actual leaf areas were calculated. Data for stomatal conductance were not normally distributed, the data were converted to log base 10, and converted data showed a normal distribution.

The incident solar radiation and the air temperature at each time of data collection were also recorded by the equipment. If any of these two was significantly influencing a parameter tested (as shown by ANOVA) while linearly correlating with the parameter (as shown by regression analysis), that was used as a covariate in data analysis. In the occasions where both solar radiation and air temperature were significant as covariates in separate ANOVAs performed for a parameter, the covariate which shows the highest correlation with the parameter (as shown by a correlation test provided by GenStat) was used as the covariate in final ANOVA. Since air temperature is affected by incident solar radiation both solar radiation and air temperature were not used together as covariates at anytime. The data obtained were subjected to ANOVA using GenStat 13th edition (VSN International, Hemel Hempstead UK). Table 3.2.2 and Table 3.2.3 show the skeleton

ANOVAs of the analyses of the readings obtained for all the four parameters around the antitranspirant applications at GS33 and GS41 respectively in 2009/2010. The Table 3.2.4 shows the skeleton ANOVA of the analyses of the readings obtained for all the four parameters around the antitranspirant applications at GS33 in 2010/2011. In the occasions where none of the covariates were used (since, none of the covariates were influencing the parameter significantly), the degrees of freedom corresponding to residual value of each stratum is higher by 1 than the degree of freedom shown in the tables of skeleton ANOVA. With the data collected around the spray applications at GS41 in 2009/2010 and GS33 in 2010/2011, Tukey's test was performed along with each ANOVA, since there were three antitranspirant/control treatments. With the data collected around the spray applications at GS41 in 2009/2010, Tukey's HSD test was performed manually to find out significant differences in the antitranspirant/control treatments within each SMD regime (GenStat only provides Tukey's results for means of the two SMD regimes at each antitranspirant/control treatment).

Table 3.2.2: The skeleton ANOVA of the rate of transpiration, stomatal conductance, photosynthesis and the internal CO_2 concentration measurements obtained before, and after the spray application at GS33 in 2009/2010

Source of variation	Degree of freedom
Block stratum	
Covariate: incident solar radiation or air temperature	1
Residual	1
Block / AT-control treatment "units" stratum	
AT-control treatment	1
Covariate: incident solar radiation or air temperature	1
Residual	25
Total	29

Table 3.2.3: The skeleton ANOVA of the rate of transpiration, stomatal conductance, photosynthesis and the internal CO_2 concentration measurements obtained before, and after the spray applications at GS41 in 2009/2010

Source of variation	Degree of freedom
Block stratum	
Covariate: incident solar radiation or air temperature	1
Residual	1
Block / SMD regime stratum	
SMD regime	1
Covariate: incident solar radiation or air temperature	1
Residual	1
Block / SMD regime / AT-control treatment stratum	
AT-control treatment	2
SMD regime x AT-control treatment	2
Covariate: incident solar radiation or air temperature	1
Residual	7
Block / SMD regime / AT-control treatment "units" stratum	
Covariate: incident solar radiation or air temperature	1
Residual	71
Total	89

Table 3.2.4: The skeleton ANOVA of the rate of transpiration, stomatal conductance, photosynthesis and the internal CO_2 concentration measurements obtained before, and after the spray application at GS33 in 2010/2011

Source of variation	Degree of freedom
Block stratum	
Covariate: incident solar radiation or air temperature	1
Residual	1
Block / AT-control treatment "units" stratum	
AT-control treatment	2
Covariate: incident solar radiation or air temperature	1
Residual	39
Total	44

3.2.4 The effect of film antitranspirants on leaf water potential

The effect of film antitranspirants on leaf water potential was explored with the experiment under polytunnels in 2008/2009 and 2009/2010. The measurements were made with the Scholander pressure bomb (SKMP 1405/50, Skye Instruments Ltd).

Even though two varieties were used in 2008/2009, only the variety Claire was used in leaf water potential measurements due to time constraints. As described in section 2.2, in 2008/2009, the antitranspirant treatments in the experiment under the polytunnels were sprayed with di-1-p-menthene at GS 33, GS39, GS41 and GS59. Leaf water potential readings were obtained in relation to the first three antitranspirant treatments on the variety Claire. Measurements were obtained once before and thrice after the antitranspirant spray application at GS 33; once before and twice after the antitranspirant spray application at GS 39; once before and frequently after the antitranspirant spray application at GS 41.

In 2009/2010, leaf water potential readings were obtained only in relation to the first antitranspirant treatment which is the treatment of antitranspirant, di-1-p-menthene, at

GS31. Since the low SMD regime in 2009/2010, was only brought into operation from GS 37 onwards, at the time of the antitranspirant treatment at GS33, two SMD regimes did not exist. In each occasion of data collection, measurements were obtained alternatively from the plots designated to the two SMD regimes so that none of the SMD regimes was over exploited from this destructive sampling.

The table 3.2.5 summarises the antitranspirant/control treatments from which the measurements were obtained in the two years.

Table 3.2.5: The experiments (under polytunnels) and the antitranspirant/control treatments which were used to explore the effect of antitranspirants on the rate of transpiration, stomatal conductance, photosynthesis and internal CO_2 concentration

<table>
<tr><th></th><th>Around the spray application at:</th><th>Measurements were obtained from:</th></tr>
<tr><td rowspan="3">2008/2009</td><td>GS33</td><td rowspan="3">The variety Claire
• The antitranspirant (di-1-p-menthene) treatment at the respective growth stage
• Unsprayed control</td></tr>
<tr><td>GS39</td></tr>
<tr><td>GS41</td></tr>
<tr><td>2009/2010</td><td>GS33</td><td>• The antitranspirant (di-1-p-menthene) treatment at GS33
• Unsprayed control
(two SMD regimes did not exist at this stage)</td></tr>
</table>

On each occasion readings were obtained from five randomly selected shoots for each plot belong to the antitranspirant treatment at consideration and the two control treatments. Water potential measurements at the antitranspirant spray application time of GS33 and GS39 were mainly from the penultimate leaf. The flag leaf blade which was still not fully unrolled might also be contributed to the measurement. The measurements at the

antitranspirant spray application time of GS41 were from the flag leaf. Readings obtained from each antitranspirant treatment were compared with those of the unsprayed control by subjecting to ANOVA.

The skeleton ANOVA of leaf water potential measurements of the two years is shown in Table 3.2.6. GenStat 13th edition was used to perform ANOVAs. Leaf water potential measurements were not obtained from the experiments in 2010/2011. The size of the plots in 2010/2011 was half the size of the plots in the two previous years and there were not enough plants available for this destructive sampling when plants had to be shared with other destructive analyses including pollen viability studies and gene expression studies.

Table 3.2.6: The skeleton ANOVA of the leaf water potential measurements from the experiments inside polytunnels in 2008/2009 and 2009/2010

Source of variation	Degree of freedom
Block stratum	2
Block / AT-control treatment "units" stratum	
AT-control treatment	1
Residual	26
Total	29

3.2.5 The effect of film antitranspirants on leaf temperature

The effect of film antitranspirants on leaf temperature was explored with the experiments under polytunnels in all the three years, 2008/2009, 2009/2010 and 2010/2011.

Even though two varieties were used in 2008/2009, only the variety Claire was used in leaf temperature measurements due to time constraints. As described in section 2.2, in 2008/2009, the antitranspirant treatments in the experiment under the polytunnels were sprayed with di-1-p-menthene at GS 33, GS39, GS41 and GS59. Leaf temperature readings were obtained in relation to the last three antitranspirant treatments (summarised

in table 3.2.7) using an Infrared (IR) thermometer (Fluke 66, Fluke Corporation, WA, USA) in 10 randomly selected leaves per plot belongs to the antitranspirant treatment at consideration at the time and the two control treatments. While taking the readings the equipment was held 0.5 – 1 m above the leaf blade, and readings were taken from the adaxial side of the leaf blade at the middle region. The accuracy of the IR thermometer reading can potentially be reduced by the incident solar radiation on the leaf, since the reflected radiation from the leaf might affect the calculation of the temperature measurement made by the instrument. Furthermore, solar radiation affects canopy temperature by affecting transpiration (Leigh *et al*., 2006). Therefore, at each time of obtaining a leaf temperature measurement, the temperature of a blank paper (non-shiny) with a colour similar to a wheat leaf, and incident solar radiation (Wm^{-2}) were measured. If any of these two was significantly influencing leaf temperature measurements (as shown by ANOVA) while linearly correlating with leaf temperature measurements (as shown by regression analysis), that was used as a covariate in data analysis. In the occasions where both solar radiation and the temperature of the green paper were significant as covariates in separate ANOVAs, the covariate which shows the highest correlation with leaf temperature measurements (as shown by a correlation test provided by GenStat) was used as the covariate in final ANOVA. Since temperature of the green paper is affected by incident solar radiation both solar radiation and temperature of the green paper were not used together as covariates. A pyranometer (SKS 1110, Skye Instruments Ltd, Powys, UK) was used to measure solar radiation. Measurements were obtained once before and frequently after each antitranspirant treatment. The data obtained were subjected to ANOVA using GenStat 13th edition. The skeleton ANOVA of leaf temperature measurements of the experiment in 2008/2009 is shown in Table 3.2.8. On the occasions where none of the covariates were used (since, none of the covariates were influencing the parameter significantly), the degrees of freedom corresponding to residual value of each stratum is higher by 1 than the degree of freedom shown in the tables of skeleton ANOVA.

Table 3.2.7: The antitranspirant/control treatments in the experiment under the polytunnels in 2008/2009 which were used to explore the effect of antitranspirants on leaf temperature

Around the spray application at:	Measurements were obtained from:
GS39	The variety Claire • The antitranspirant (di-1-p-menthene) treatment at the respective growth stage • Unsprayed control
GS41	
GS59	

Table 3.2.8: The skeleton ANOVA of the leaf temperature measurements from the experiments inside polytunnels in 2008/2009

Source of variation	Degree of freedom
Block stratum	
Covariate: incident solar radiation or temperature of none-shiny paper with a colour similar to wheat leaf	1
Residual	1
Block / AT-control treatment "units" stratum	
AT-control treatment	2
Covariate: incident solar radiation or temperature of none-shiny paper with a colour similar to wheat leaf	1
Residual	55
Total	59

In 2009/2010 and 2010/2011, leaf temperature was measured with TPS-2 Portable Photosynthesis System (PP Systems, MA, USA) at the same instances that the other parameters (stomatal conductance, the rate of transpiration and photosynthesis) were measured by the equipment. Therefore, the antitranspirant/control treatments from which the measurements were obtained in the two years are same as the antitranspirant/control

treatments summarised in Table 3.2.1, i.e. those which were used to study the effect of antitranspirants on the rate of transpiration, stomatal conductance, photosynthesis and internal CO_2 concentration. Measurements were obtained once before and twice after the antitranspirant treatments as described in section 3.2.3. Skeleton ANOVAs are also as given in section 3.2.3.

3.2.6 The effect of film antitranspirants on pollen fertility

The effect of film antitranspirants on pollen fertility was explored in terms of the presence or absence of accumulated starch in the experiment under polytunnels in 2009/2010 and the experiment under polytunnels, irrigated after GS 69 in 2010/2011. Treatments used were not shown in a separate table here, since all the treatments in both the experiments, which were explained in section 2.2, were used in the study. Pollen grains were sampled from the two experiments as follows.

From each plot 10 anthers which had just dehisced were excised and transferred into an Eppendorf tube with 1.5 ml of Lugol's solution (Sigma-Aldrich, Dorset, UK). The tube was closed and shaken gently so that the pollen grains were released into the solution. Only one anther from one spike was used, and the 10 spikes were selected from random locations. All the time, anthers were excised from a first floret in the middle of the spike. 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 4 ºC in the dark.

Within a week of collection, each pollen sample was subjected to light microscopy as follows. Each eppendorf tube was shaken gently so that the pollen grains distributed evenly within the solution, and an aliquot of 1 ml of the sample was transferred into 2.5 ml of distilled water in a watch-glass, so that the pollen sample was diluted. 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 in a dark blue/black colour were considered fertile and pollen grains which were unstained and partially stained were considered sterile (Nelson, 1968). In 2009/2010, 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 2010/2011, the total number of pollen grains and the number of fertile pollen grains within 10 randomly selected grid cells were counted and the percentage of fertile pollen per grid cell was calculated. As in 2009/2010, in 2010/2011 from each diluted sample, three replicates of pollen samples were observed. The data obtained were subjected to ANOVA using GenStat 13th edition. Table 3.2.9 and Table 3.2.10 show the skeleton ANOVAs of the analyses of the data respectively in 2009/2010 and 2010/2011.

Table 3.2.9: The skeleton ANOVA of pollen fertility data from the experiment in 2009/2010

Source of variation	Degree of freedom
Block stratum	2
Block / Common control stratum	
Common control vs. treatments	1
Residual	2
Block / Common control / SMD regime stratum	
SMD regime	1
Residual	2
Block / Common control / (SMD regime / AT-control treatment) stratum	
AT-control treatment	4
Common control x SMD regime x AT-control treatment	4
Residual	16
Block / Common control / (SMD regime / AT-control treatment "units") stratum	
Residual	66
Total	98

Table 3.2.10: The skeleton ANOVA of pollen fertility data from the experiment in 2010/2011

Source of variation	Degree of freedom
Block stratum	2
Block / AT-control treatment "units" stratum	
AT-control treatment	2
Residual	793
Total	797

3.3 Results

3.3.1 The distribution pattern and leaf coverage of di-1-p-menthene spray on wheat leaves

The spray distribution pattern, the leaf coverage and stomatal coverage of di-1-p-menthene sprayed on to Claire wheat leaves were assessed by image analysis and electron microscopy as described in chapter 3.2.

Figure 3.3.1 shows adaxial side of a Claire leaf sprayed with di-1-p-menthene mixed with titanium dioxide, following the same spray characteristics (application details) which were followed when experimental plots were sprayed with film antitranspirants. The mean percentage leaf coverage by the antitranspirant spray was 15.80%. This result was the mean of 15 measurements taken from tip, middle and base areas of five leaves (Table 3.3.1). All the five leaves were sprayed at the same time, therefore no other statistical parameters were analysed.

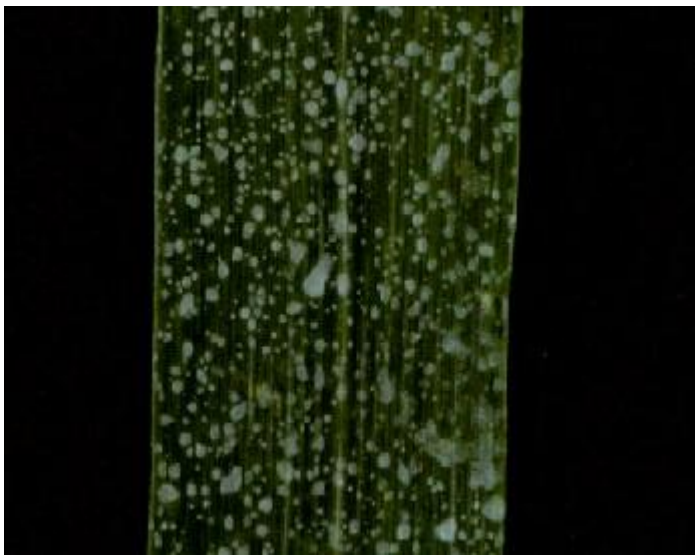


Figure 3.3.1: Adaxial side of a leaf sprayed with di-1-p-menthene mixed with titanium dioxide

Table 3.3.1: The percentage leaf coverage (%) by the antitranspirant spray

	Leaf 1	Leaf 2	Leaf 3	Leaf 4	Leaf 5	Mean
Tip	14.3	16.6	15.7	15.2	16.8	
Middle	16.7	16.7	16.4	14.9	15.8	
Base	17.5	14.5	14.6	15.4	16.1	
Mean						15.8

Note that all the five leaves were sprayed at the same time, therefore no statistical parameters were analysed apart from mean.

The images from scanning electron microscopy revealed the way stomata were covered by antitranspirant spray deposits. Figure 3.3.2 contains images from scanning electron microscopy showing the adaxial surface of Claire wheat leaves which were not sprayed with the antitranspirant. Figure 3.3.3 and Figure 3.3.4 contain images from scanning electron microscopy showing adaxial surface of Claire leaves which were sprayed with the antitranspirant, di-1-p-menthene, following the same spray characteristics which were followed when experimental plots were sprayed. Figure 3.3.3 contains images providing a closer look of covered stomata while Figure 3.3.4 a more distal look. It is clear from Figures 3.3.4 b) and c) that there were stomata which were not covered by antitranspirant deposits as well as ones which were covered. Figure 3.3.4 b) shows how a spray deposit occured covering some of the stomata from a row of stomata, Figure 3.3.4 c), which is a closer look of Figure 3.3.4 b) further clarifies the situation. It is clear from these images that under the used spray characteristics di-1-p-menthene spray deposits occur as patches of film rather than an evenly spread film on the leaf surface.

Figure 3.3.5 a) and b) are images from scanning electron microscopy of an abaxial surface of a Claire leaf which was not sprayed with the antitranspirant. Figure 3.3.5 c) is an abaxial surface of a Claire leaf on which there are di-1-p-menthene deposits covering stomata. Spray deposits may occur on abaxial surfaces which are facing upwards (due to leaf rolling/bending) while spraying.

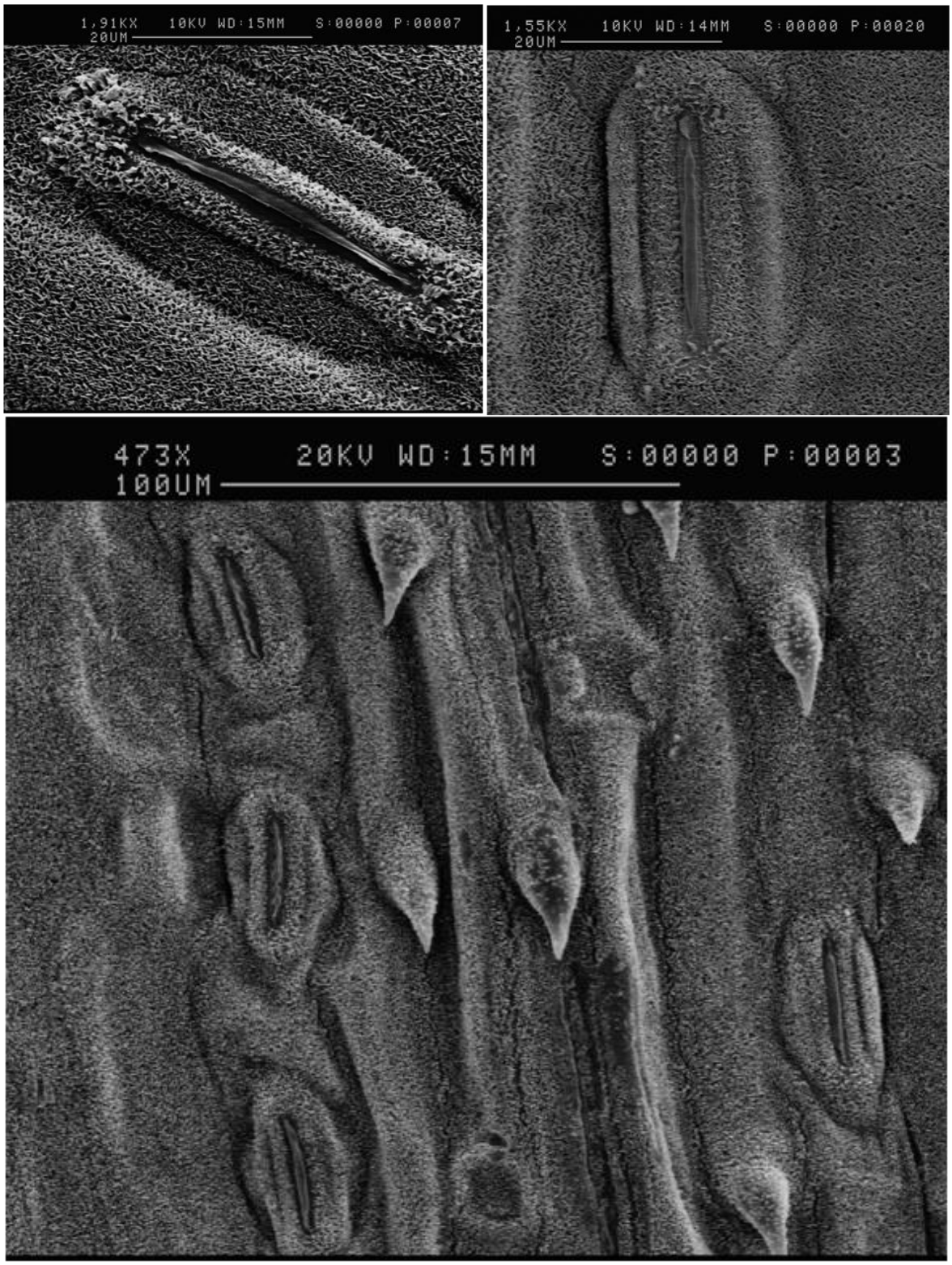


Figure 3.3.2: Images from scanning electron microscopy showing the adaxial surface of Claire leaves which were not sprayed with antitranspirants

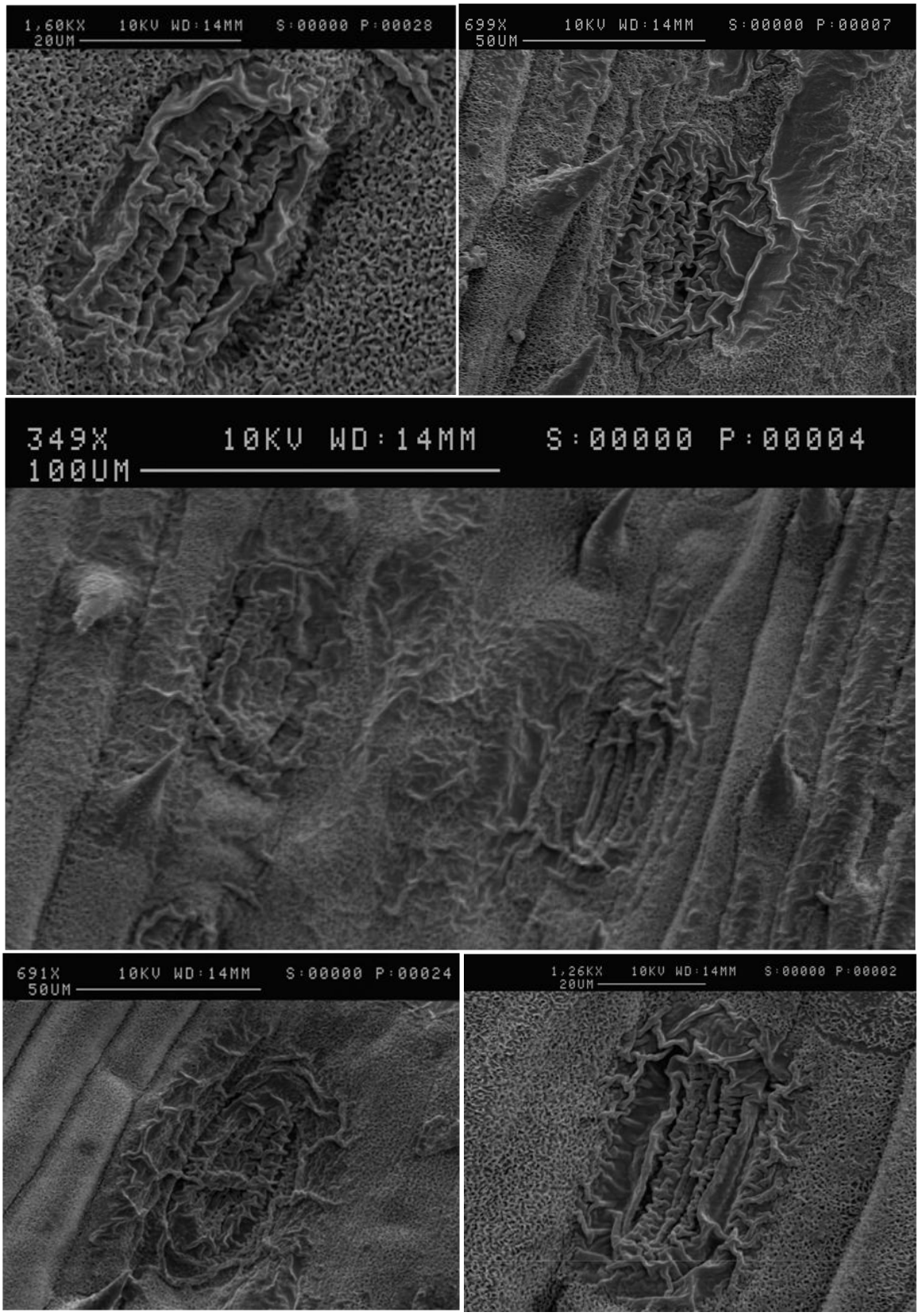


Figure 3.3.3: Images from scanning electron microscopy showing stomata on the adaxial surface of Claire leaves which were sprayed with the antitranspirant, di-1-p-menthene

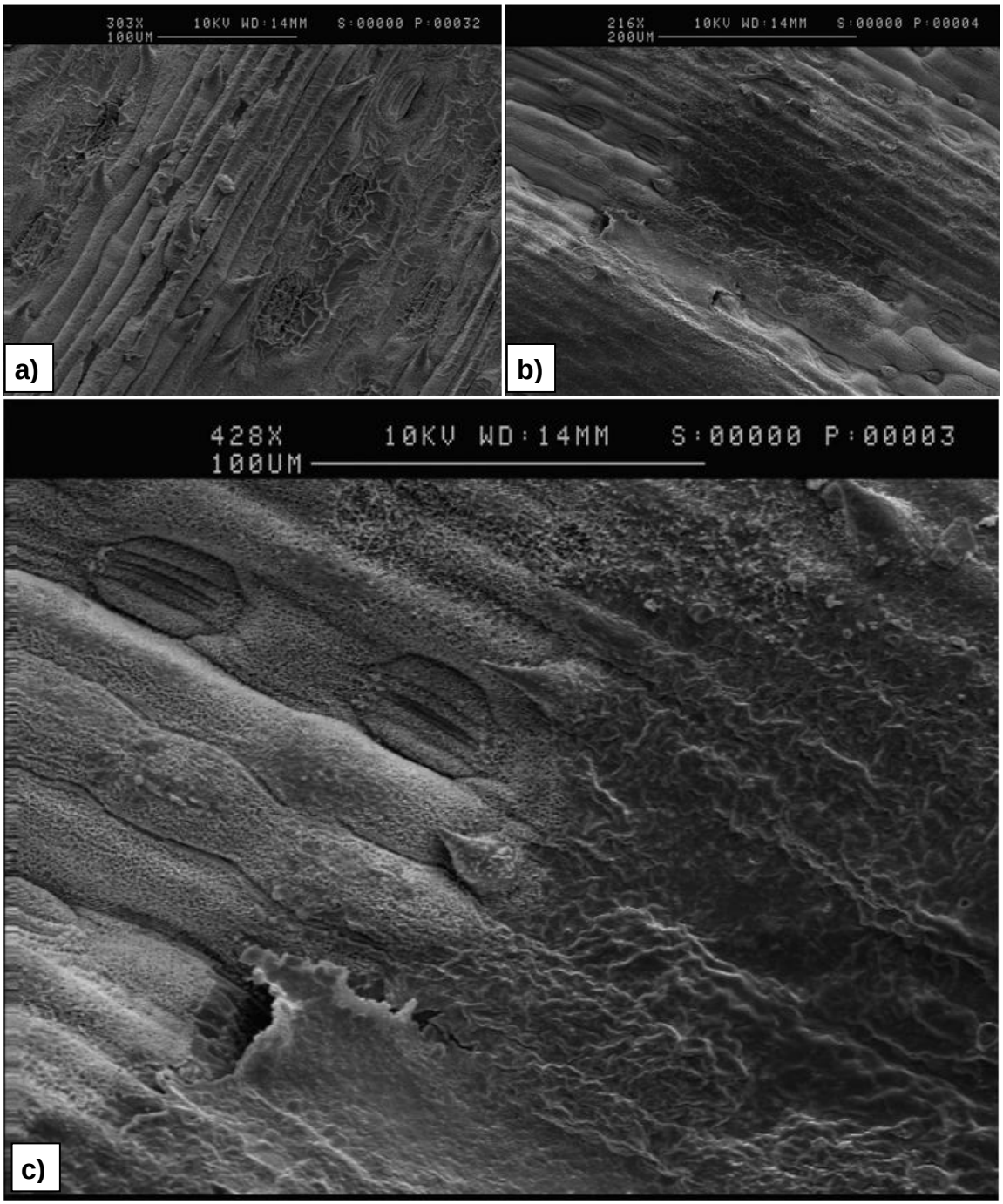


Figure 3.3.4: Images from scanning electron microscopy showing stomata on the adaxial surface of Claire leaves which were sprayed with the antitranspirant, di-1-p-menthene. **a)** a distal look of a leaf surface covered by spray deposits **b)** an occurrence of a spray deposit covering only some of the stomata from a row of stomata **c)** a closer look of Figure b)

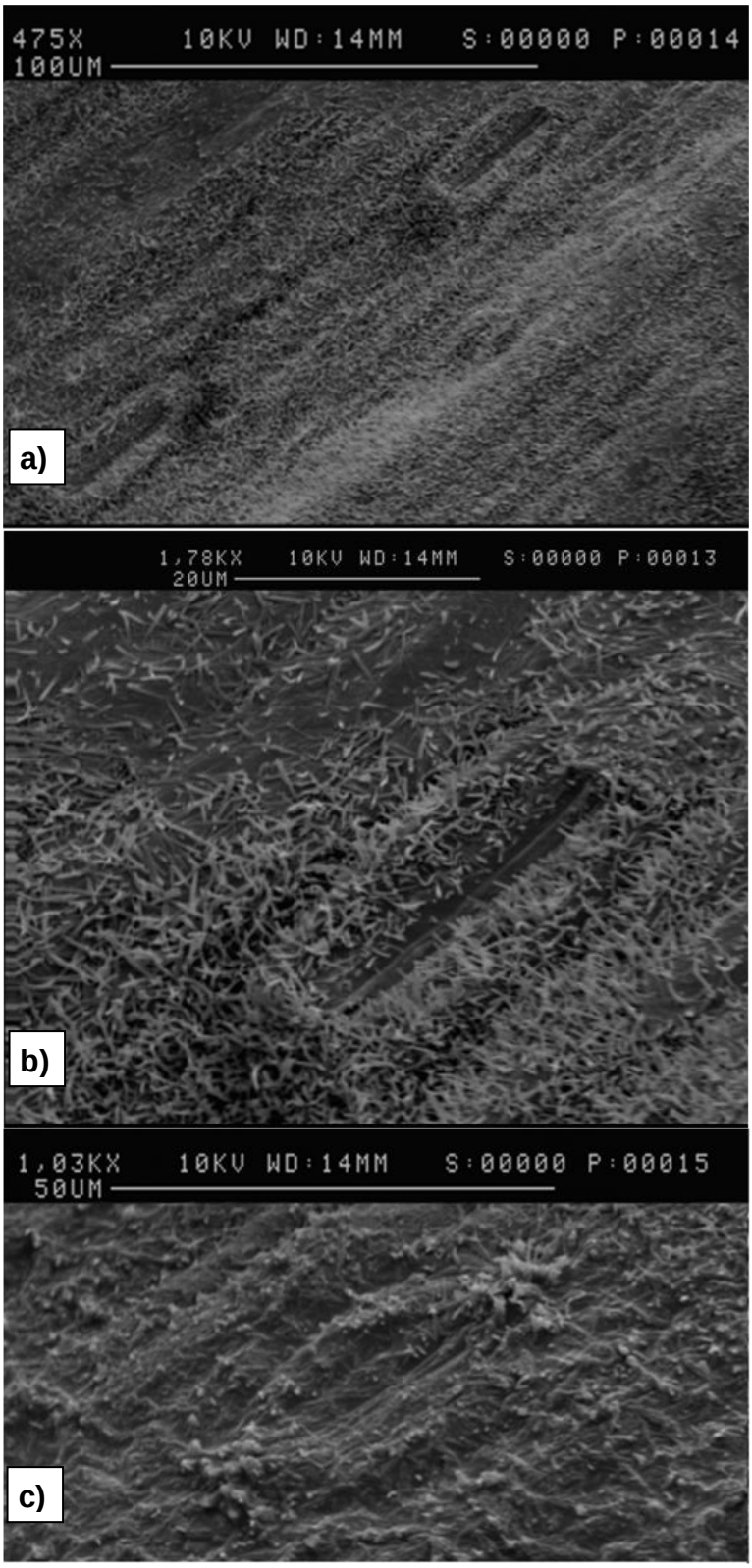


Figure 3.3.5: Images from scanning electron microscopy showing stomata on the abaxial surface of Claire leaves **a-b)** not sprayed with the antitranspirant **c)** sprayed with the antitranspirant

3.3.2 The growth stage at which meiosis occurs in pollen mother cells

As explained in the materials and methods of section 3.2.2, anthers from the spikelets (only from 1st and 2nd floret) in the middle of the spike which are most advanced in development (Kirby, 2002) were used in the study, and from all the anthers observed, anthers which were at meiotic stages were from shoots at early GS41. From the shoots observed, there were about 40 shoots below GS39 (Figure 3.3.6 a), and none of those shoots showed anthers at meiotic stages. Cells at meiotic stages were also not observed in any of the anthers collected from the 20 shoots of which the sheath of the flag leaf had extended 10 cm above the auricles of the penultimate leaf (Figure 3.3.6 b). The length of the flag leaf sheath above the auricles of the penultimate leaf was about 15 cm when the flag leaf sheath started to swell. Therefore, at the time that the sheath of the flag leaf was 10 cm above the auricles of the penultimate leaf, the flag leaf sheath was not yet fully extended. From the shoots which were used in the study, 30 were in between the two stages described above, i. e. in between GS39 and the stage at which flag leaf sheath extended 10 cm above the auricles of the penultimate leaf; and, anthers at meiotic stages were found in 18 of the 30 shoots. The remaining 12 shoots had anthers in which meiosis had not yet started or had just been completed. The mean length between the auricles of the flag leaf and the penultimate leaf, of the 18 shoots which were bearing anthers at meiotic stages, was 4.5 cm (Figure 3.3.7 a), and the mean length of the spikes which were bearing anthers at meiotic stages in the 1st and 2nd florets of the spikelets at the middle of the spike was 6 cm (Figure 3.3.7 b). The above described results of the experiment are summarised in Table 3.3.2.

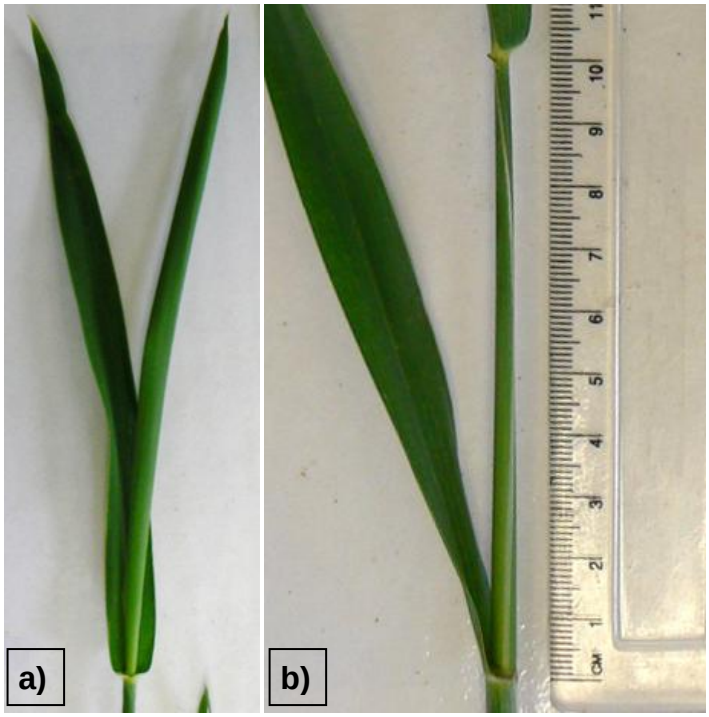


Figure 3.3.6: a) A Claire shoot at GS39 **b)** A Claire shoot at the stage at which the sheath of the flag leaf had extended 10 cm above the auricles of the penultimate leaf

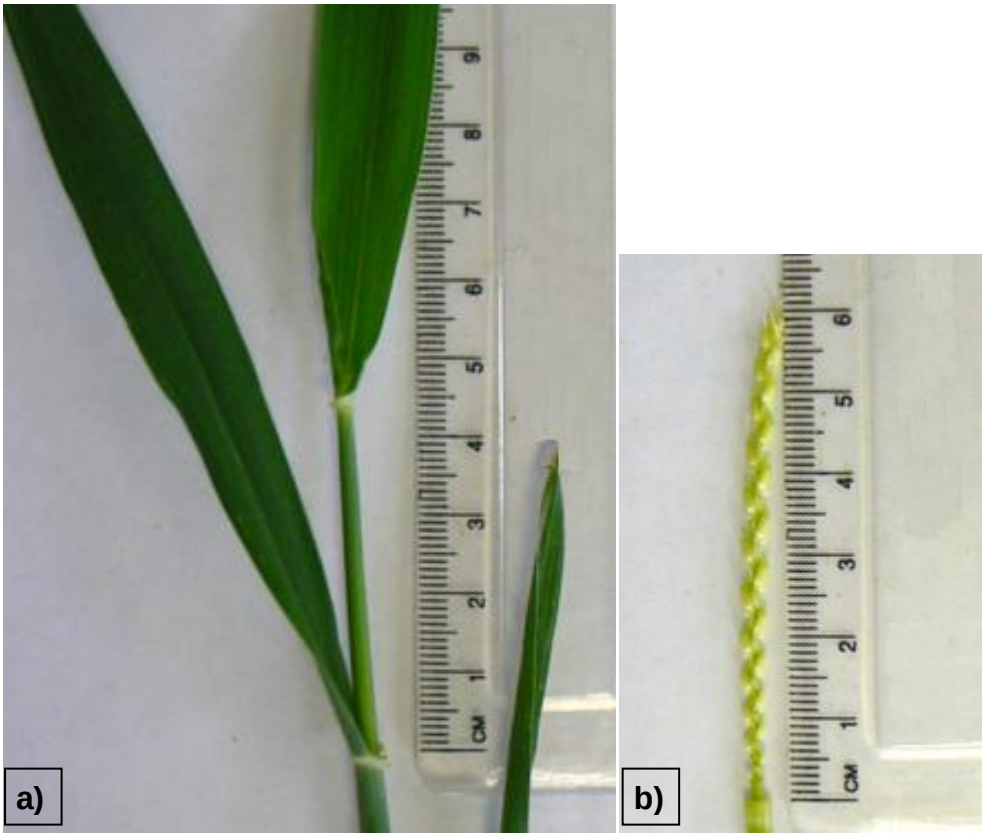


Figure 3.3.7: a) A Claire shoot at early GS41, the stage at which meiosis occurs in pollen mother cells in most of the Claire shoots **b)** A Claire spike bearing cells at meiotic stages in the 1st and 2nd florets of the spikelets at the middle of the spike

Table 3.3.2: Summary of the results of the experiment on developmental timing of meiosis

	At a stage below GS39	At a stage between GS39 and GS41 - flag leaf sheath longer than 10 cm	At a stage above GS41 - flag leaf sheath longer than 10 cm
Number of shoots	40	30	20
Number of shoots bearing anthers at meiotic stages	0	18 (mean length of the spikes = 6 cm)	0

Note that there were no statistical analyses involved with this study

Some of the stages of meiosis, interesting structures and cells observed are described in the following text. The stages of meiosis, cells and structures observed were identified and described with the help of published literature on wheat (Bennett *et al*., 1973; El-Ghazaly and Jensen, 1986) and generally on plant meiosis (McCormick, 1993; McCormick, 2004). The observations were made at the magnification of 40 x 40, but the digital camera further increased the magnification up to an unknown level.

From the meiotic stages observed, a few stages with clear photographs are shown in Figure 3.3.8, and described briefly. By the time that meiosis initiates, pollen mother cells are covered with a callose wall. All the meiocites including the finally formed tetrad are covered with a callose wall (c.w), which is visible in all the pictures in Figure 3.3.8. Meiosis consists with two steps, meiosis I and meiosis II. During meiosis I, the pollen mother cell, which is diploid, divides into two haploid daughter cells, and during meiosis II each of the two haploid daughter cells undergo equational division similar to mitosis ultimately yielding 4 haploid cells at the end of the process of meiosis. Meiosis I consist of 4 steps which are prophase I, metaphase I, anaphase I and telophase I. During prophase I, homologous chromosomes (each made up of two sister chromatids) pairs to form bivalents and undergo homologous recombination; the centrosome divides and a spindle forms between the two centrosomes now located at opposite poles of the cell. During metaphase I, the bivalents arrange in an equatorial plane that bisects the spindle in a way that the

centromeres of the homologous chromosomes in a bivalent are attached to spindle fibres connecting to opposite poles of the spindle. During anaphase 1 (Figure 3.3.8 a), the separation of homologues chromosomes of the bivalents occurs when the spindle fibres shortens pulling the 2 chromosomes in each of the bivalent to the opposite poles. In the Figure 3.3.8 a, the two bunches of chromosomes being pulled to opposite directions are visualise as strings stretched along the cell, even though chromosomes cannot be distinguish from one another and the spindle is not clear. During telophase I, the chromosomes, which are separated, form two daughter nuclei in the pole regions of the meiosis I spindle. A cell wall forms separating the two nuclei creating a dyad (Figure 3.3.8 b). The two nuclei in the dyad are typically elongated.

Meiosis II consists of 4 steps which are prophase I, metaphase I, anaphase I and telophase I. During prophase II, the envelops of the two dyad nuclei disappear, chromatids become shorten and thicken and the formation of meiosis II spindles within each cell of the dyad occurs. During metaphase II, the chromosomes in each cell of the dyad arrange in an equatorial plane that bisects the spindle in a way that the centromeres of each chromosome is attached to spindle fibres from both the opposite poles. During anaphase II, the centromeres split upon shortening of the spindle fibres and two chromatids of each chromosome are pulled to opposite directions. The cell in the Figure 3.3.8 c is at just after metaphase II or at the beginning of anaphase II, since although the chromatids are so close to the middle of the cell, they are not in a one line, but in two lines. The spindle fibres (s.f.) can be seen clearly, in the figure. The Figure 3.3.8 d shows an early stage of anaphase II where chromatids are more close to the equatorial plane of the spindles, whereas Figure 3.3.8 e) shows a late stage at which chromatids are closer to the poles. In Figures 3.3.8 a, 3.3.8 d and 3.3.8 e chromatids being pulled to opposite directions are visable as strings even though chromatids cannot be distinguished from one another and the spindle is not clear. During telophase II, the chromatids, which are separated, form nuclei in the pole regions of each of the meiosis II spindles. Cell walls form separating the two nuclei within a cell of the dyad creating a tetrad (Figure 3.3.8 b and Figure 3.3.10 a). Figure 3.3.9 shows tetrad walls of the tetrads through which the

cytoplasm leaked out during the staining process. The shape or the 3d nature of the tetrads is clearly shown by the pictures of these cell walls.

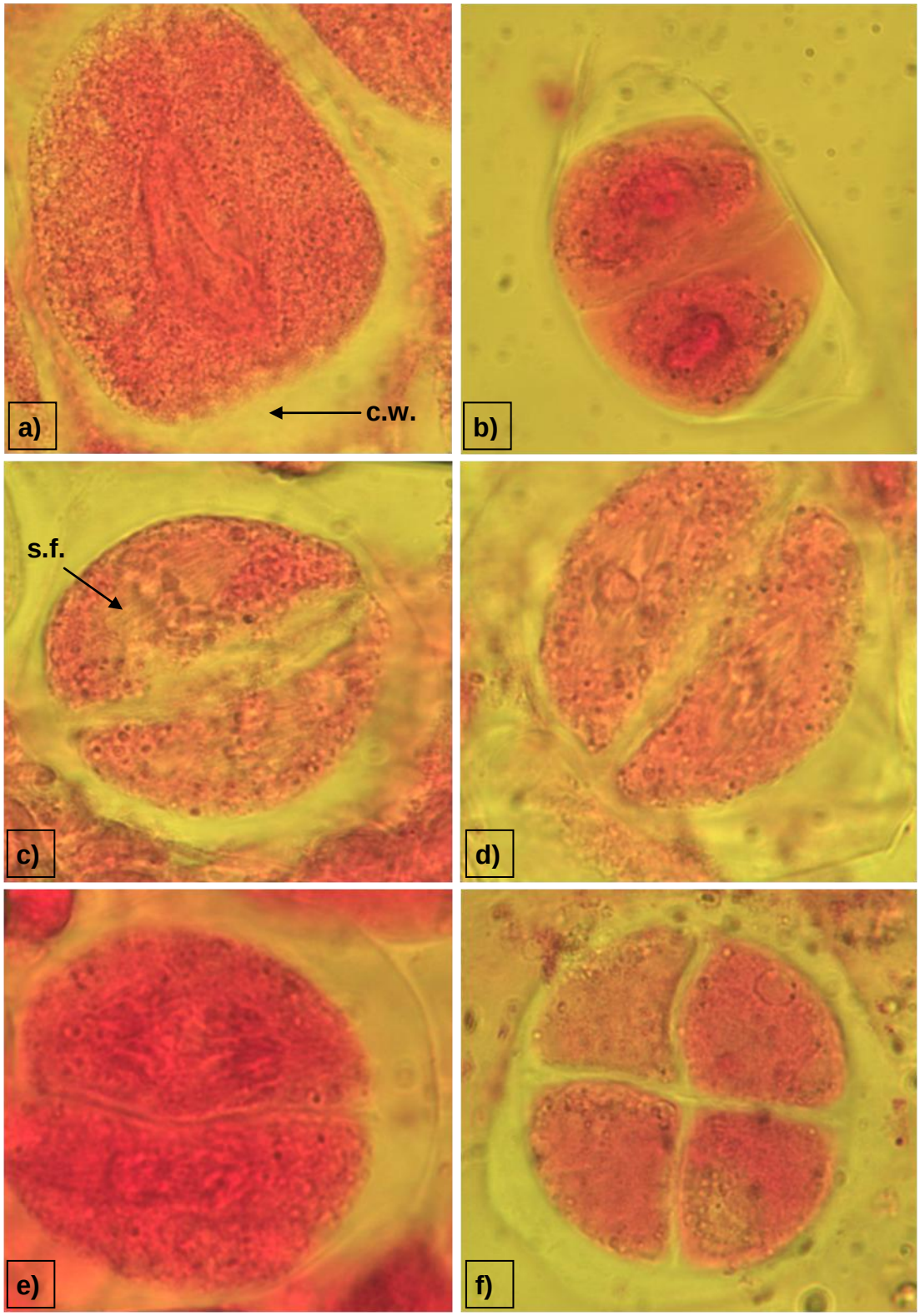


Figure 3.3.8: Nuclei of pollen mother cells of the variety Claire at **a)** anaphase I (late) **b)** dyad stage (first telophase) **c)** just after metaphase II **d)** anaphase II (early) **e)** anaphase II (late) **f)** tetrad stage; c.w. = callose wall; s.f. = spindle fibres

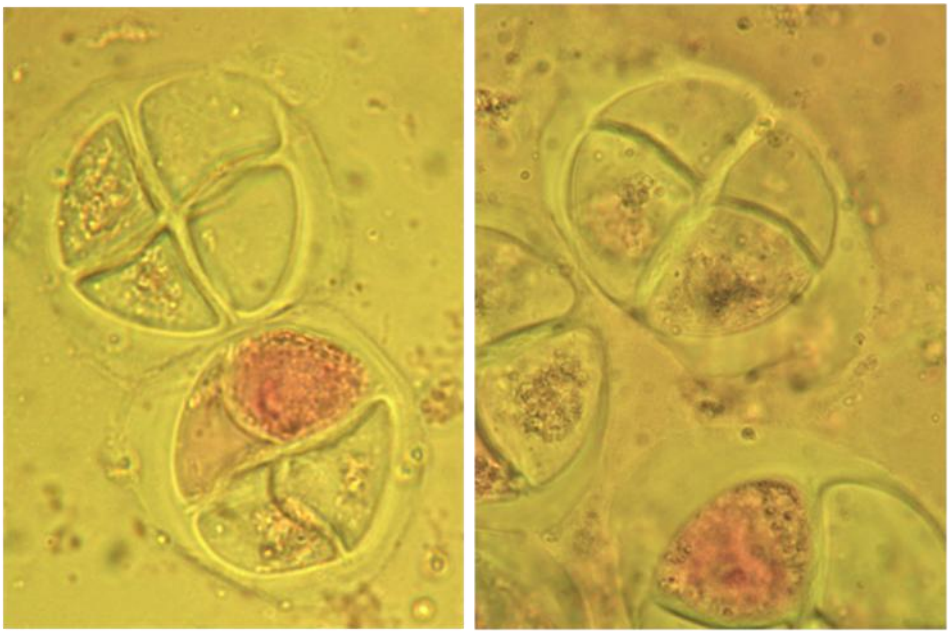


Figure 3.3.9: Tetrad walls showing the 3d nature of the tetrads (cytoplasms leaked out during the staining process).

The Figure 3.3.10 shows some steps occur just after the completion of meiosis in pollen mother cells. The callose wall of the tetrad degenerates. At the stage shown in the Figure 3.3.10 b, callose wall was still not fully degenerated. When the callose wall is degenerated young haploid, uninucleate microspores (Figure 3.3.10 c) are released. These microspores later develop a germ pore (Figure 3.3.10 d). After the germ pore is well formed a single obvious vacuole is developed. At the stage shown in the Figure 3.3.10 e, the vacuole has started to develop, and by the stage shown in the Figure 3.3.10 f the vacuole is fully developed. The young microspore undergoes several other developments before anther dehiscence, at which the microspore is considered as fully matured. But those stages are not described here. The time taken from the breakdown of the tetrad wall to the young microspore stage shown in the Figure 3.3.10 e is about 18 hours for the variety, Chinese Spring at 20°C (Bennett *et al*., 1973).

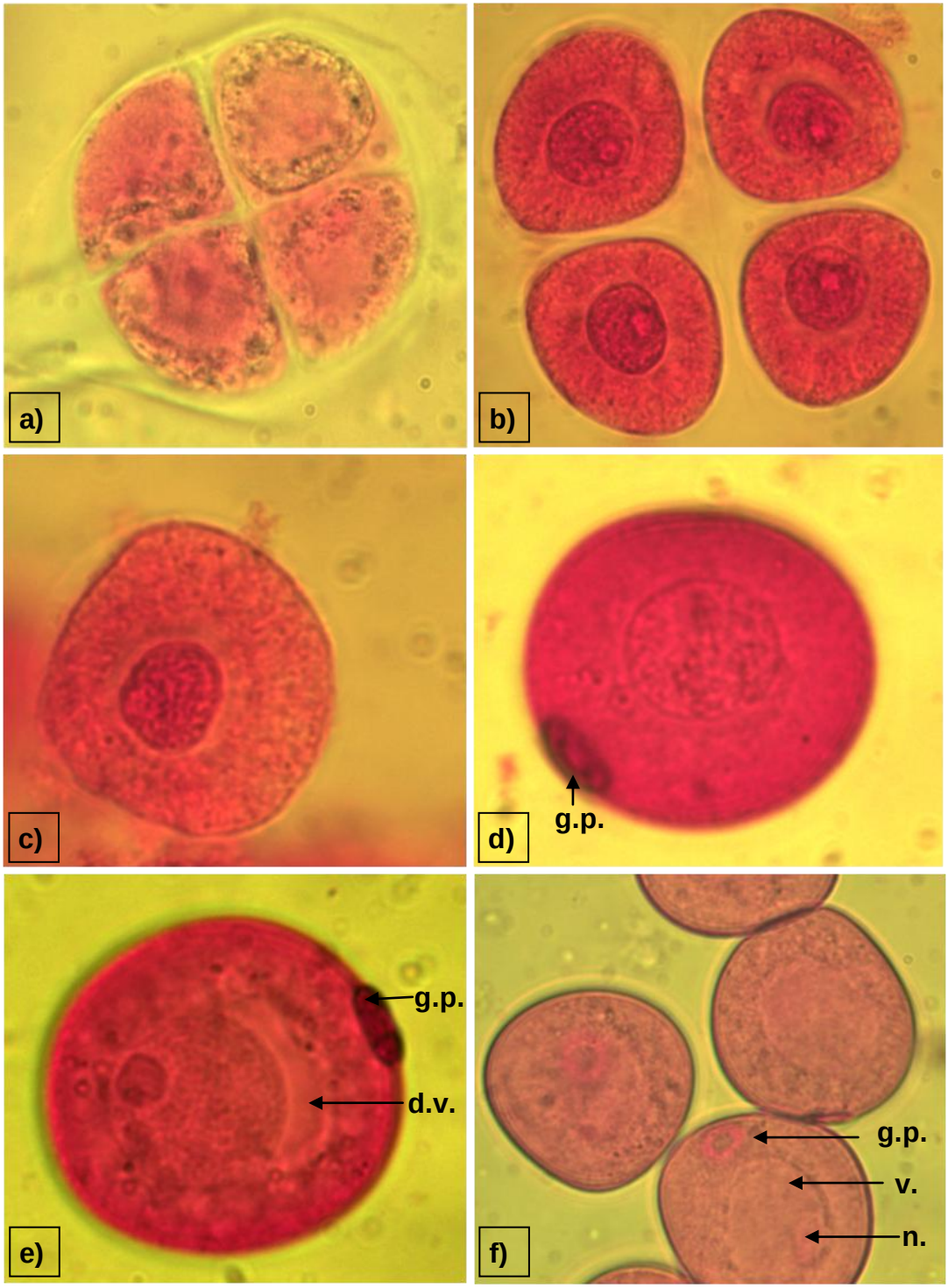


Figure 3.3.10: Pollen development in the variety Claire **a)** tetrad stage **b)** late tetrad stage **c)** young pollen without a germ pore **d)** young pollen with germ pore just visible **e)** young pollen with well formed germ pore and a vacuole still developing **f)** young pollen with a well formed germ pore and a single obvious vacuole; g.p. = germ pore; d.v. = developing vacuole; v. = vacuole; n. = nucleus

The 1st florets were all the time advanced in development compared to the 2nd florets. There were occasions where there were young microspores in the 1st floret and dyads in the 2nd florets, and microspores with a developed single vacuole and germ pore in the 1st florets and tetrads in the 2nd florets. An apparent difference in development was not detected between the three anthers in a floret.

3.3.3 The effect of film antitranspirants on the rate of transpiration, stomatal conductance, the rate of photosynthesis and internal CO_2 concentration

Table 3.3.1 shows the results obtained for the rate of transpiration, stomatal conductance, the rate of photosynthesis and the internal CO_2 concentration before, the day after and three days after the antitranspirant, di-1-p-menthene, application at GS33 in 2009/2010.

Before the antitranspirant spray application there was no significant difference in any of the parameters between the unsprayed control and the antitranspirant treatment.

The day after and three days after the spray application, the rate of transpiration of the antitranspirant treatment was significantly ($p < 0.001$) lower than that of the unsprayed control. The stomatal conductance of the antitranspirant treatment was also significantly lower compared to the unsprayed control both the day after ($p = 0.022$) and three days after (0.005) the spray application.

There was no significant difference in the rate of photosynthesis between the unsprayed control and the antitranspirant treatment before, one day after or three days after the spray application. One day after the spray application, the difference in the rate of photosynthesis between the unsprayed control and the antitranspirant treatment was, however, close to being significant ($p = 0.066$).

Although there was no significant difference in internal CO_2 concentration between the two antitranspirant/control treatments before the antitranspirant spray application, the internal CO_2 concentration of the antitranspirant treatment was significantly lower

compared to the unsprayed control both the day after ($p = <0.001$) and three days after (0.019) the spray application.

The mean values (out of 10 random measurements) for the rate of transpiration, stomatal conductance (log 10 transformed), the rate of photosynthesis and the internal CO_2 concentration of the irrigated unsprayed control one day after the spray application were 8.57 $mmolm^{-2}s^{-1}$, 3.50 $log(mmolm^{-2}s^{-1})$, 18.26 $\mu molm^{-2}s^{-1}$ and 320.26 ppm respectively.

The mean values (out of 10 random measurements) for the rate of transpiration, stomatal conductance (log 10 transformed), the rate of photosynthesis and the internal CO_2 concentration of the irrigated unsprayed control three days after the spray application were 4.60 $mmolm^{-2}s^{-1}$, 3.57 $log(mmolm^{-2}s^{-1})$, 9.08 $\mu molm^{-2}s^{-1}$ and 350 ppm respectively.

Table 3.3.3: The rate of transpiration, the stomatal conductance (log 10 transformed), the rate of photosynthesis and the internal CO_2 concentration before, the day after and three days after the antitranspirant application at GS33 in 2009/2010

	Parameter	AT/control treatment		P	S.E.M	CV% (df)
		UC	**di-GS33**			
Before spray application	**Transpiration** ($mmolm^{-2}s^{-1}$)	4.47	4.19	0.573	0.336	30.1 (26)
	Log st. co. [$log(mmolm^{-2}s^{-1})$]	2.823	2.843	0.874	0.0865	11.8 (26)
	Photosynthesis ($\mu molm^{-2}s^{-1}$)	12.52	12.63	0.933	0.894	27.6 (26)
	Internal CO_2 con. (ppm)	306	308.9	0.846	10.52	13.3 (26)
1 day after spray application	**Transpiration** ($mmolm^{-2}s^{-1}$)	5.83	3.28	<.001	0.349	29.7 (26)
	Log st. co. [$log(mmolm^{-2}s^{-1})$]	2.679	2.426	0.022	0.0729	9.7 (25)
	Photosynthesis ($\mu molm^{-2}s^{-1}$)	18.77	16.07	0.066	0.994	19.4 (25)
	Internal CO_2 con. (ppm)	271.5	214.4	<0.001	10.13	14.1 (25)
3 days after spray application	**Transpiration** ($mmolm^{-2}s^{-1}$)	4.44	3.25	<0.001	0.202	19.4 (25)
	Log st. co. [$log(mmolm^{-2}s^{-1})$]	3.228	2.896	0.005	0.0757	9.6 (26)
	Photosynthesis ($\mu molm^{-2}s^{-1}$)	12.24	12.17	0.933	0.608	16.9 (25)
	Internal CO_2 con. (ppm)	345.4	319.1	0.019	7.42	8.6 (26)

AT = antitranspirant; UC = unsprayed control; di-GS33 = di-1-p-menthene treatment at GS33; st. co. = Stomatal conductance; Internal CO_2 con. = Internal CO_2 concentration; df = residual df

Table 3.3.2 shows the rate of transpiration and stomatal conductance in the unsprayed control and the antitranspirant treatments before, the day after and three days after the antitranspirants, di-1-p-menthene and latex applications at GS41 in both the SMD regimes.

The mean rate of transpiration and stomatal conductance of the Low SMD regime was significantly higher than those of the high SMD regime before (p, transpiration rate = 0.012; p, stomatal conductance = 0.007), the day after (p, transpiration rate = 0.003; p, stomatal conductance = 0.002) and three days after (p, transpiration rate = 0.007; p, stomatal conductance = 0.001) the spray applications.

Before the spray applications, there was no significant difference in mean values of the two SMD regimes for the rate of transpiration or stomatal conductance between the antitranspirant/control treatments. Furthermore, there was no significant interaction between the factor, SMD regime and the factor, antitranspirant/control treatment, when the two parameters are considered. Tukey's HSD test did not reveal any significant differences in the rate of transpiration or stomatal conductance between the antitranspirant/control treatments within each SMD regime.

Both the day after and three days after the spray applications, there was no significant difference in the rate of transpiration or stomatal conductance between antitranspirant/control treatments when mean values of the two SMD regimes were considered.

The interactive effect of the factor, SMD regime and the factor, antitranspirant/control treatment on the rate of transpiration was significant ($p < 0.001$) during the day after the spray applications. According to Tukey's HSD test, in the high SMD regime, the rate of transpiration of the latex treatment was significantly ($p < 0.05$) lower than that of the other two treatments, whereas, in the low SMD regime, the rate of transpiration in the latex treatment was significantly ($p < 0.05$) higher than that of the other two treatments. The low SMD regime was irrigated to maintain the SMD level using trickle tape irrigation system, and the irrigation to the low SMD regime was started few days before the spray applications at GS41. When interpreting the results obtained for the low SMD regime it should be considered that there might be a variation in the amount of irrigation with in the low SMD regime, which might have affected the results. The interaction between the two factors was not significant on stomatal conductance during the day after the spray

applications. There was no significant difference in stomatal conductance between the treatments in the low SMD regime, whereas in the high SMD regime the stomatal conductance in the latex treatment was significantly lower than that of the unsprayed control but not significantly different to the di-1-p-menthene treatment.

Three days after spray application, there was no significant interaction between the two factors on the rate of transpiration. However, Tukey's HSD test showed that in the low SMD regime, the rate of transpiration of the latex treatment was significantly lower compared to the unsprayed control, and there was no significant difference in the rate of transpiration between the latex treatment and the di-1-p-menthene treatment or the di-1-p-menthene treatment and the unsprayed control. There was no significant difference in the rate of transpiration between the antitranspirant/control treatments in the high SMD regime. The interaction between the two factors was not significant also on stomatal conductance. Showing the same pattern as the rate of transpiration, in the low SMD regime, the stomatal conductance of the latex treatment was significantly lower than that of the unsprayed control with no significant difference with that of the di-1-p-menthene treatment, and there was no significant difference between the unsprayed control and the di-1-p-menthene treatment in stomatal conductance. In the high SMD regime, there was no significant difference in stomatal conductance between the antitranspirant/control treatments.

The mean values (out of 10 random measurements) for the rate of transpiration and stomatal conductance (log 10 transformed) of the irrigated unsprayed control one day after the spray application were 2.28 $mmolm^{-2}s^{-1}$ and 2.66 log ($mmolm^{-2}s^{-1}$) respectively.

The mean values (out of 10 random measurements) for the rate of transpiration and stomatal conductance (log 10 transformed) of the irrigated unsprayed control three days after the spray application were 0.71 $mmolm^{-2}s^{-1}$ and 2.68 log ($mmolm^{-2}s^{-1}$) respectively.

Table 3.3.4: The rate of transpiration and the stomatal conductance (log 10 transformed) of the antitranspirant/control treatments of the two SMD regimes before, the day after and three days after the antitranspirant applications at GS41 in 2009/2010

				AT/control Treatment				P: SMD	P: AT/con.	P: SMD x AT/con.	SEM: SMD x AT/con.	CV%: SMD x AT/con.(df)
				UC	**di-GS41**	**la-GS41**	Mean					
Before spray application	**Transpiration** (mmolm^{-2}s^{-1})	**SMD**	**L**	3.99 (a)	3.89 (a)	4.12 (a)	4.00	0.012	0.677	0.962	0.328	35.2 (72)
			H	2.30 (a)	2.05 (a)	2.48 (a)	2.27					
		Mean		3.14 (a)	2.97 (a)	3.30 (a)						
	Log st. co. [log(mmolm^{-2}s^{-1})]	**SMD**	**L**	3.017 (a)	3.021 (a)	3.202 (a)	3.080	0.007	0.427	0.623	0.1229	23.6 (72)
			H	2.585 (a)	2.326 (a)	2.536 (a)	2.482					
		Mean		2.801 (a)	2.673 (a)	2.869 (a)						
1 day after spray application	**Transpiration** (mmolm^{-2}s^{-1})	**SMD**	**L**	3.31 (a)	3.37 (a)	3.89 (b)	3.523	0.003	0.413	<.001	0.1180	32.4 (72)
			H	2.08 (b)	1.88 (b)	1.16 (a)	1.705					
		Mean		2.69 (a)	2.62 (a)	2.52 (a)						
	Log st. co. [log(mmolm^{-2}s^{-1})]	**SMD**	**L**	2.804 (a)	2.814 (a)	2.912 (a)	2.843	0.002	0.230	0.062	0.0914	19.6 (72)
			H	2.498 (b)	2.216 (ab)	1.986 (a)	2.233					
		Mean		2.651 (a)	2.515 (a)	2.449 (a)						
3 days after spray application	**Transpiration** (mmolm^{-2}s^{-1})	**SMD**	**L**	0.90 (b)	0.65 (ab)	0.60 (a)	0.718	0.007	0.191	0.076	0.0610	44.8 (72)
			H	0.27 (a)	0.27 (a)	0.33 (a)	0.290					
		Mean		0.58 (a)	0.46 (a)	0.47 (a)						
	Log st. co. [log(mmolm^{-2}s^{-1})]	**SMD**	**L**	2.345 (b)	2.157 (ab)	1.901 (a)	2.134	0.001	0.106	0.298	0.0939	32.9 (72)
			H	1.501 (a)	1.342 (a)	1.399 (a)	1.414					
		Mean		1.923 (a)	1.749 (a)	1.650 (a)						

L = low SMD regime; H = high SMD regime; UC = unsprayed control; di-GS41 = di-1-p-menthene treatment at GS41; la-GS41 = latex treatment at GS41; st.co. = stomatal conductance; AT/con. = antitranspirant/control treatment; df = residual df; data within rows accompanied by the same letter are not significantly different at p = 0.05

Table 3.3.3 shows the rate of photosynthesis and internal CO_2 concentration in the unsprayed control and the antitranspirant treatments before, the day after and three days after the antitranspirants, di-1-p-menthene and latex applications at GS41 in both the SMD regimes.

There was no significant difference in the rate of photosynthesis or internal CO_2 concentration between the two SMD regimes on any of the three occasions of data collection. In all the occasions the mean photosynthesis rate and the mean internal CO_2 concentration of the low SMD regime was higher than those of the high SMD regime from about 10% - 30% and 10% - 20% respectively. The day after the spray application, the factor, SMD regime was very close to being significant ($p = 0.058$) for internal CO_2 concentration.

Before the spray applications, there was no significant difference in mean values of the two SMD regimes for photosynthesis rate or internal CO_2 concentration between the antitranspirant/control treatments. Moreover, there was no significant interaction between the factor, SMD regime and the factor, antitranspirant/control treatment for both the parameters. Tukey's HSD test did not reveal any significant difference in the rate of transpiration or stomatal conductance between the antitranspirant/control treatments within each SMD regime.

Both the day after and three days after the spray applications there was no significant difference in the rate of photosynthesis between the antitranspirant/control treatments, when the mean values of the two SMD regimes for photosynthesis rate at each antitranspirant/control treatment were considered. A significant difference was not shown in the rate of photosynthesis between the antitranspirant/control treatments either within the two SMD regimes. Furthermore, the interaction between the factor, SMD regime and the factor, antitranspirant/control treatment, was also not significant for photosynthesis rate.

On the day after the spray applications, although, according to Tukey's test there was no significant difference between mean internal CO_2 concentrations of the two SMD regimes

for the antitranspirant/control treatments, according to ANOVA, the factor, antitranspirant/control treatment was significant ($p = 0.031$) for the parameter when the means of the two SMD regimes were considered. On the day, there was a significant interaction between the factor, SMD regime and the factor antitranspirant/control treatment for internal CO_2 concentration. The internal CO_2 concentration of latex treatment in the high SMD regime was significantly ($p < 0.05$) lower than that of the unsprayed control in the same SMD regime. There was no significant difference either between the latex treatment and the unsprayed control or the latex treatment and the di-1-p-menthene treatment in internal CO_2 concentration. The internal CO_2 concentrations of the antitranspirant/control treatments in the low SMD regime were not significantly different from each other.

Three days after the spray application, there was no significant difference in internal CO_2 concentration between antitranspirant/control treatments either within each SMD regime or when mean values of the two SMD regimes for the antitranspirant/control treatments were considered. The interaction between the two factors was also not significant for the parameter.

The mean values (out of 10 random measurements) for the rate of photosynthesis and the internal CO_2 concentration of the irrigated unsprayed control one day after the spray application were 13.18 $\mu molm^{-2}s^{-1}$ and 266.08 ppm respectively.

The mean values (out of 10 random measurements) for the rate of photosynthesis and the internal CO_2 concentration of the irrigated unsprayed control three days after the spray application were 9.9 $\mu molm^{-2}s^{-1}$ and 256 ppm respectively.

Table 3.3.5: The rate of photosynthesis and the internal CO_2 concentration of the antitranspirant/control treatments of the two SMD regimes before, the day after and three days after the antitranspirant applications at GS41 in 2009/2010

				AT/control treatment				P: SMD	P: AT/con.	P: SMD x AT/con.	SEM: SMD x AT/con.	CV%: SMD x AT/con.(df)
				UC	**di-GS41**	**la-GS41**	Mean					
Before spray application	**Photosyn.** ($\mu molm^{-2}s^{-1}$)	**SMD**	**L**	16.36	15.36	15.34	15.69	0.395	0.801	0.740	1.245	18.1 (71)
			H	13.74	14.04	13.81	13.86					
		Mean		15.05 (a)	14.70 (a)	14.58 (a)						
	Internal CO_2 con. (ppm)	**SMD**	**L**	330.1	316.7	353.1	333.3	0.202	0.297	0.446	22.34	19.1 (72)
			H	265.6	297.9	301.0	288.2					
		Mean		297.8 (a)	307.3 (a)	327.1 (a)						
1 day after spray application	**Photosyn.** ($\mu molm^{-2}s^{-1}$)	**SMD**	**L**	16.04	14.82	14.86	15.24	0.260	0.141	0.726	0.654	10.3 (71)
			H	14.14	13.62	13.55	13.77					
		Mean		15.09 (a)	14.22 (a)	14.20 (a)						
	Internal CO_2 con. (ppm)	**SMD**	**L**	266.7	267.2	289.0	274.3	0.058	0.031	0.035	12.42	18.6 (71)
			H	256.0	238.1	193.1	229.1					
		Mean		261.3 (a)	252.7 (a)	241.1 (a)						
3 days after spray application	**Photosyn.** ($\mu molm^{-2}s^{-1}$)	**SMD**	**L**	7.32	7.10	6.90	7.10	0.525	0.984	0.329	0.802	16.6 (71)
			H	5.36	5.65	5.68	5.56					
		Mean		6.34 (a)	6.38 (a)	6.29 (a)						
	Internal CO_2 con. (ppm)	**SMD**	**L**	337.4	321.2	302.7	320.4	0.081	0.160	0.964	13.80	19.2 (79)
			H	289.8	272	299.6	287.1					
		Mean		305.5 (a)	287.4 (a)	318.5 (a)						

L = low SMD regime; H = high SMD regime; UC = unsprayed control; di-GS41 = di-1-p-menthene treatment at GS41; la-GS41 = latex treatment at GS41; Photosyn. = photosynthesis rate; AT/con. = antitranspirant/control treatment; df = residual df; data within rows accompanied by the same letter are not significantly different at p = 0.05

Table 3.3.4 shows the rate of transpiration, stomatal conductance the rate of photosynthesis and internal CO_2 concentration in the antitranspirant/control treatments before, the day after and three days after the antitranspirant applications (di-1-p-menthene and latex applications at GS33) in the Experiment 1 in 2010/2011.

Before the spray applications there was no significant difference in any of the four parameters between the antitranspirant/control treatments. There was no significant difference in the rate of transpiration, stomatal conductance and internal CO_2 concentration between the treatments, but the rate of photosynthesis in the latex treatment was significantly ($p = 0.010$) lower compared to the unsprayed control. There was no significant difference in the rate of photosynthesis between the latex treatment and the di-1-p-menthene treatment or the di-1-p-menthene treatment and the unsprayed control. The antitranspirant/control treatment was not significant for any of the parameters three days after the spray application.

The mean values (out of 10 random measurements) for the rate of transpiration, stomatal conductance (log 10 transformed), the rate of photosynthesis and the internal CO_2 concentration of the irrigated unsprayed control one day after the spray application were 3.60 $mmolm^{-2}s^{-1}$, 2.37 $log(mmolm^{-2}s^{-1})$, 10.08 $\mu molm^{-2}s^{-1}$ and 280 ppm respectively.

The mean values (out of 10 random measurements) for the rate of transpiration, stomatal conductance (log 10 transformed), the rate of photosynthesis and the internal CO_2 concentration of the irrigated unsprayed control three days after the spray application 7.57 $mmolm^{-2}s^{-1}$, 3.40 $log(mmolm^{-2}s^{-1})$, 18.56 $\mu molm^{-2}s^{-1}$ and 335.26 ppm respectively.

Table 3.3.6: The rate of transpiration, the stomatal conductance (log 10 transformed), the photosynthesis rate and the internal CO_2 concentration before, three days after and seven days after the antitranspirant application at GS33 in 2010/2011

	Parameter	AT/control treatment			P	S.E.M	CV% (res. DF)
		UC	**di-GS33**	**la-GS33**			
Before spray application	**Transpiration** ($mmolm^{-2}s^{-1}$)	2.51 (a)	2.21 (a)	2.70 (a)	0.316	0.222	32.8 (39)
	Log stom. con. [$log(mmolm^{-2}s^{-1})$]	2.585 (a)	2.519 (a)	2.625 (a)	0.922	0.188	28.3 (40)
	Photosyn. ($\mu molm^{-2}s^{-1}$)	8.66 (a)	8.53 (a)	9.13 (a)	0.513	0.385	16.8 (39)
	Internal CO_2 con. (ppm)	281.6 (a)	273.4 (a)	275.1 (a)	0.965	22.6	31.6 (40)
3 days after spray application	**Transpiration** ($mmolm^{-2}s^{-1}$)	1.43 (a)	1.31 (a)	1.39 (a)	0.706	0.1018	28.6 (40)
	Log stom. con. [$log(mmolm^{-2}s^{-1})$]	2.457 (a)	2.556 (a)	2.529 (a)	0.607	0.0739	10.9 (39)
	Photosyn. ($\mu molm^{-2}s^{-1}$)	12.16 (a)	10.33 (a)	11.03 (a)	0.510	0.618	19.9 (39)
	Internal CO_2 con. (ppm)	256.0 (a)	244.5 (a)	247.6 (a)	0.803	13.71	19.1 (39)
7 days after spray application	**Transpiration** ($mmolm^{-2}s^{-1}$)	5.80 (a)	5.37 (a)	5.35 (a)	0.733	0.456	28.1 (39)
	Log stom. con. [$log(mmolm^{-2}s^{-1})$]	2.788 (a)	2.693 (a)	2.596 (a)	0.255	0.0813	11.3 (39)
	Photosyn. ($\mu molm^{-2}s^{-1}$)	11.24 (a)	9.94 (a)	11.78 (a)	0.265	0.825	28.1 (39)
	Internal CO_2 con. (ppm)	327.0 (a)	338.5 (a)	306.0 (a)	0.428	18.3	21.1 (39)

AT = antitranspirant; UC = unsprayed control; di-GS33 = di-1-p-menthene treatment at GS33; la-GS33 = latex treatment at GS33; stom. con. = Stomatal conductance; Photosyn. = Photosynthesis rate; Internal CO_2 con. = Internal CO_2 concentration; df = residual df; data within rows accompanied by the same letter are not significantly different at p = 0.05

3.3.3.1 The correlation between parameters

The correlation between the rate of transpiration and stomatal conductance, photosynthesis rate and internal CO_2 concentration and internal CO_2 concentration and stomatal conductance was explored with regression analysis in groups in which the

antitranspirant/control treatments were used as groups. The analyses were performed with each set of data following ANOVA as presented above. The results of each regression analysis are not presented separately with graphs. There was little difference between different sets of data in the way the parameters were correlated. Therefore, results are described in general for each set of data.

Irrespective of the fact that the crop had been sprayed with the antitranspirant or not, there was a significant positive correlation ($p < 0.05$) between the rate of transpiration and stomatal conductance and also between internal CO_2 concentration and stomatal conductance. There was no significant difference between the slopes of the regression lines of unsprayed control and antitranspirant treatment at any of the occasions, for the regression between the rate of transpiration and stomatal conductance or internal CO_2 concentration and stomatal conductance.

Regression analysis between internal CO_2 concentration and the rate of photosynthesis did not indicate a significant relationship between the two parameters.

3.3.4 The effect of film antitranspirants on leaf water potential

The results from the analyses of leaf water potential measurements from the antitranspirant/control treatments before and after the antitranspirant spray applications at GS33, GS39 and GS41 in 2008/2009 are shown respectively in Table 3.3.5, 3.3.6 and 3.3.7.

There was no significant difference in leaf water potential between the unsprayed control and the antitranspirant treatment before any of the three antitranspirant spray applications.

The leaf water potential of the antitranspirant treatment was significantly lower than that of the unsprayed control the day after ($P = 0.008$) and three days after ($p = 0.010$) the spray application at GS33 (Table 3.3.5). Still the leaf water potential of the antitranspirant

treatment was lower than that of the unsprayed control nine days after the spray application and the difference was close to significance ($p = 0.060$).

Showing the same trend as the antitranspirant spray application at GS33, the leaf water potential of the antitranspirant treatment was significantly lower than that of the unsprayed control both the day after ($p = 0.022$) and three days after ($p = 0.013$) the antitranspirant spray application at GS39 (Table 3.3.6).

When the antitranspirant spray application at GS41 is considered (Table 3.3.7), although the leaf water potential of the antitranspirant treatment was significantly lower ($p = 0.045$) than that of the unsprayed control one day after the spray application, the difference between the two antitranspirant/control treatments was not significant three, five, seven and 17 days after the spray application. However, the leaf water potential of the antitranspirant treatment was lower than that of the unsprayed control by about 0.1 Mpa, three and five days after the spray application and by 0.21 Mpa, 17 days after the spray application. The leaf water potential of the antitranspirant treatment was slightly higher (0.045 MPa) compared to the unsprayed control seven days after the spray application. However, the measurements obtained 23 days after the spray application surprisingly indicates a significantly lower ($p = 0.011$) leaf water potential in the antitranspirant treatment compared to the unsprayed control.

The results from the analyses of leaf water potential measurements from the antitranspirant/control treatments before and after the antitranspirant spray application at GS33 in 2009/2010 are shown in Table 3.3.8. There was no significant difference in leaf water potential between the unsprayed control and the antitranspirant treatment before the spray application. The leaf water potential of the antitranspirant treatment was significantly lower than that of the unsprayed control both the day after ($P < 0.001$) and three days after ($p = 0.002$) the spray application.

Table 3.3.7: The leaf water potential (MPa) of the antitranspirant/control treatments before and after the antitranspirant spray application at GS33 in the experiment in 2008/2009

Days from spray application	AT/control treatments		P	S.E.M	CV% (res. df)
	UC	**di-GS33**			
Day before	- 0.228	- 0.244	0.682	0.0273	44.8 (26)
Day after	- 0.225	- 0.137	0.008	0.0214	45.8 (26)
3 days after	- 0.726	- 0.564	0.010	0.0414	24.9 (26)
9 days after	- 1.024	- 0.869	0.060	0.0558	22.8 (26)

AT = antitranspirant; UC = unsprayed control; di-GS33 = di-1-p-menthene treatment at GS33; res. df = residual df

Table 3.3.8: The leaf water potential (MPa) of the antitranspirant/control treatments before and after the antitranspirant spray application at GS39 in the experiment in 2008/2009

Days from spray application	AT/control treatments		P	S.E.M	CV% (res. df)
	UC	**di-GS39**			
Day before	- 0.841	- 0.865	0.755	0.0538	24.4 (26)
Day after	- 0.998	- 0.812	0.022	0.0542	23.2 (26)
3 days after	-1.024	-0.784	0.013	0.0635	27.2 (26)

AT = antitranspirant; UC = unsprayed control; di-GS39 = di-1-p-menthene treatment at GS39; res. df = residual df

Table 3.3.9: The leaf water potential (MPa) of the antitranspirant/control treatments before and after the antitranspirant spray application at GS41 in the experiment in 2008/2009

Days from spray application	AT/control treatments		P	S.E.M	CV% (res. df)
	UC	**di-GS41**			
Day before	- 1.098	- 1.023	0.133	0.0340	12.4 (26)
Day after	- 1.360	- 1.242	0.045	0.0396	11.8 (26)
3 days after	- 1.453	- 1.358	0.099	0.0395	10.9 (26)
5 days after	- 0.942	- 0.816	0.195	0.0670	29.5 (26)
7 days after	- 1.147	- 1.192	0.487	0.0455	15.1 (26)
17 days after	- 1.021	- 0.808	0.094	0.0868	36.8 (26)
23 days after	- 1.352	- 1.151	0.011	0.0519	16.1 (26)

AT = antitranspirant; UC = unsprayed control; di-GS41 = di-1-p-menthene treatment at GS41; res. df = residual df

Table 3.3.10: The leaf water potential (MPa) of the antitranspirant/control treatments before and after the antitranspirant spray application at GS33 in the experiment in 2009/2010

Days from spray application	AT/control treatments		P	S.E.M	CV% (res. DF)
	UC	**di-GS33**			
Day before	- 1.092	- 1.066	0.683	0.0445	16.0 (26)
Day after	- 1.088	- 0.847	<.001	0.0439	17.6 (26)
3 days after	- 1.026	- 0.812	0.002	0.0431	18.2 (26)

AT = antitranspirant; UC = unsprayed control; di-GS33 = di-1-p-menthene treatment at GS33; res. df = residual df

3.3.5 The effect of film antitranspirants on leaf temperature

Tables 3.3.9, 3.3.10 and 3.3.11 show the results of the analyses of leaf temperature respectively around the antitranspirant application times of GS39, GS41 and GS59 in the experiment inside polytunnels in 2008/2009.

The difference in temperature between the unsprayed control and the antitranspirant treatment at consideration was less than 1°C all the time. Most of the time, the temperature of the antitranspirant treatment was lower than that of the unsprayed control, but on a few occasions the difference was significant. The occasions where the difference was significant were, two days ($p < 0.001$) after the antitranspirant application at GS39, four ($p = 0.003$) and seven ($p < 0.001$) days after the antitranspirant application at GS39 and seven days ($p = 0.043$) after the antitranspirant application at GS59. There were few occasions where the temperature of the antitranspirant treatment was higher than that of the unsprayed control, but the difference was not significant at any of those occasions. The results of the analyses of leaf temperature from the experiments in 2009/2010 and 2010/2011 were not different to that of 2008/2009, and are presented in Appendix VIII.

Table 3.3.11: The leaf temperature (°C) of the antitranspirant/control treatments after the antitranspirant spray application at GS39 in the experiment in 2008/2009

Days from spray application	**AT/control treatments**		P	S.E.M	CV% (res. df)
	UC	**di-GS39**			
2 days after	13.70	13.52	<.001	0.0311	1.3 (56)
4 days after	20.65	20.48	0.701	0.329	7.8 (55)
7 days after	17.14	16.77	0.106	0.163	5.1 (55)
11 days after	20.0	19.98	0.930	0.1336	3.7 (56)

AT = antitranspirant; UC = unsprayed control; di-GS39 = di-1-p-menthene treatment at GS39; res. df = residual df

Table 3.3.12: The leaf temperature (°C) of the antitranspirant/control treatments before and after the antitranspirant spray application at GS41 in the experiment in 2008/2009

Days from spray application	AT/control treatments		P	S.E.M	CV% (res. df)
	UC	**di-GS41**			
2 days before	17.16	17.18	0.922	0.174	5.3 (55)
2 days after	20.00	19.98	0.906	0.1198	3.3 (56)
4 days after	19.34	19.03	0.003	0.0714	2.0 (56)
7 days after	14.53	13.61	<.001	0.176	6.9 (56)
11 days after	15.15	15.24	0.817	0.272	7.1 (55)
17 days after	16.10	15.58	0.092	0.213	6.9 (55)
20 days after	15.44	15.43	0.961	0.191	6.8 (56)
28 days after	18.37	18.45	0.638	0.1190	2.6 (55)

AT = antitranspirant; UC = unsprayed control; di-GS41 = di-1-p-menthene treatment at GS41; res. df = residual df

Table 3.3.13: The leaf temperature (°C) of the antitranspirant/control treatments before and after the antitranspirant spray application at GS59 in the experiment in 2008/2009

Days from spray application	AT/control treatments		P	S.E.M	CV% (res. df)
	UC	**di-GS59**			
2 days before	15.07	14.78	0.366	0.222	6.9 (55)
4 days after	16.41	16.07	0.266	0.214	7.1 (56)
7 days after	15.58	15.14	0.043	0.151	5.2 (55)
15 days after	18.23	18.22	0.965	0.100	3.0 (55)

AT = antitranspirant; UC = unsprayed control; di-GS59 = di-1-p-menthene treatment at GS59; res. df = residual df

3.3.6 The effect of film antitranspirants on pollen fertility

As described in section 3.2, pollen grains collected from the experiments were stained by Lugol's solution, and the percentage number of fertile pollen grains (pollen grains stained fully in a dark blue/black colour) was calculated after obtaining the necessary measurements while observing the samples under light microscopy. Figure 3.3.11 shows fertile pollen grains, which are stained fully and sterile pollen grains, which are not stained (hence yellow colour) or partially stained, as seen under the light microscope. The observations were made at the magnification of 40 x 10, but the digital camera further increased the magnification up to an unknown level.

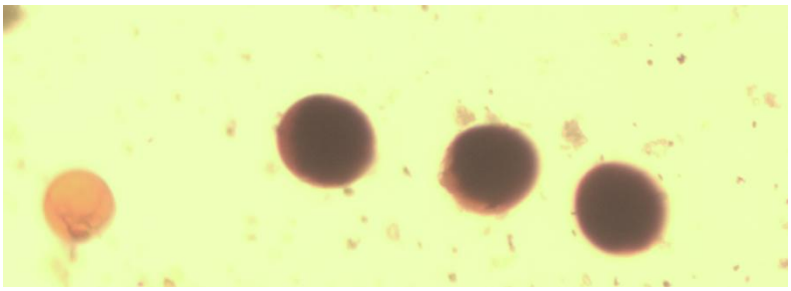


Figure 3.3.11: Fertile (dark blue/black colour) and sterile (yellow colour) pollen grains stained with Lugol's solution

Table 3.3.12 and Table 3.3.13 show the results of percentage pollen fertility data analyses respectively for the experiments in 2009/2010 and 2010/2011.

In 2009/2010, the mean percentage pollen fertility of the low SMD regime was significantly higher ($p = 0.009$) than that of the high SMD regime. The factor, antitranspirant/control treatment was significant ($p < 0.001$) on percentage pollen fertility. In both the SMD regimes, the percentage pollen fertility of di-1-p-menthene treatments at GS31 and GS33 were significantly higher ($p < 0.05$) than that of all the other treatments in the same SMD regime. The same result was shown by the mean percentage pollen fertility values of the two SMD regimes at each antitranspirant/control treatments. In the low SMD regime, the percentage pollen fertility in the di-1-p-menthene treatment at GS33 was significantly higher ($P < 0.05$) than that of the di-1-p-menthene treatment GS31. There was no significant difference in percentage pollen fertility between the di-1-p-menthene treatments

at GS31 and GS33 in the high SMD regime. This difference between the two SMD regimes might be the reason for the significant ($p = 0.001$) interaction between the factor, SMD regime and the factor, antitranspirant/control treatment on percentage pollen fertility. In both the SMD regimes, there was no significant difference in percentage pollen fertility between the unsprayed control, the di-1-p-menthene treatment GS41 and the latex treatment at GS41.

In 2010/2011, the percentage pollen fertility in the di-1-p-menthene treatment at GS33 was significantly higher ($p<0.05$) than that of the unsprayed control. There was no significant difference in percentage pollen fertility between the latex treatment at GS33 and the unsprayed control or between the di-1-p-menthene treatment at GS33 and the latex treatment at GS33.

The percentage pollen viabilities in the treatments in the experiment in 2010/2011 are conspicuously higher than those of in 2009/2010. Possible reasons for this are discussed in section 3.4.6.

Table 3.3.14: The results for pollen fertility (%) of the antitranspirant/control treatments from the experiment in 2009/2010

		AT/control treatments						Mean (without IUC)	P - SMD	P – AT/con	P – SMD- AT/con	S.E.M. -SMD-AT/con	CV % - SMD-AT/con (res. df)
		IUC (Common control)	**UC**	**di-GS31**	**di-GS33**	**di-GS41**	**la-GS41**						
SMD	**L**	94.19	70.77 (a)	80.67 (b)	85.84 (c)	72.86 (a)	70.52 (a)	76.13	0.009	<.001	0.001	0.772	5.6 (66)
	H		67.55 (a)	79.83 (b)	83.40 (b)	66.97 (a)	68.71 (a)	73.29					
Mean		94.19	69.16 (a)	80.25 (b)	84.62 (b)	69.92 (a)	69.62 (a)						

L = low SMD regime; H = high SMD regime; IUC = irrigated unsprayed control; UC = unsprayed control; di-GS31, di-GS33 and di-GS41 = di-1-p-menthene treatment at respective growth stages; la-GS41 = latex treatment at GS41; AT/con = AT/control treatment; res. df = residual df; data within rows accompanied by the same letter are not significantly different at p = 0.05

Table 3.3.15: The results for pollen fertility (%) of the antitranspirant/control treatments from Experiment 1 in 2010/2011

AT/control treatments			P	S.E.M	CV% (res. df)
UC	**di-GS33**	**la-GS33**			
92.61 (a)	93.85 (b)	93.06 (ab)	0.001	0.240	4.2 (793)

AT = antitranspirant; UC = unsprayed control; di-GS33 = di-1-p-menthene treatment at GS33; la-GS33 = latex treatment at GS33; res. df = residual df; data within rows accompanied by the same letter are not significantly different at p = 0.05

3.4 Discussion

3.4.1 The distribution pattern and leaf coverage of di-1-p-menthene spray on wheat leaves

The percentage of a leaf surface covered by the antitranspirant film and whether the film is evenly distributed or occur as patches covering some stomata while leaving some stomata uncovered, might be helpful in understanding the influence of film antitranspirant treatments on stomatal conductance, leaf gas exchange and photosynthesis, and in turn in yield and yield components. The antitranspirant, di-1-p-menthene is the film antitranspirant used most frequently in the experiments over the three years. As explained in section 3.1, the objective of this study was to explore the distribution pattern and the leaf coverage of di-1-p-menthene applied on to Claire wheat leaves following the same spray characteristics (explained in section 2.2) which were used to spray antitranspirants to experimental plots.

Measurement of leaf coverage of spray liquids is usually achieved with image analysis techniques (O'Sullivan *et al*., 2011). However, standard photographic and image analysis techniques are likely to underestimate spray coverage, particularly when spray coverage on actual plant leaves needs to be determined. This is because a good contrast between leaf surface and deposits can be difficult to achieve and, therefore, the smallest droplets cannot be detected (O'Sullivan *et al*., 2011). When the software settings were adjusted so that almost all the droplets in the scanned image could be visualised, the software could not distinguish between spray droplets and shiny spots (created by lighting effects) on the leaf surface. The percentage leaf coverage by the antitranspirant spray, according to the results of the study, was 15.80%. Generally the agrochemical leaf coverages reported in the literature are above 30% (O'Sullivan *et al*., 2011; Lardoux *et al*., 2007) and the value obtained in this study might be an underestimation of the actual leaf coverage. Although, if an artificial target had been used, the image analysis technique may have determined the coverage by the antitranspirant spray much more accurately, however the coverage on an artificial target would probably not be the same as on a leaf of the variety in interest

(O'Sullivan *et al*., 2011). In agrochemical applications, the coverage of the target surface by a spray application is decided mainly by droplet size and their impact, adhesion, spreading and retention (Knoche, 1994; Basu *et al*., 2002). These parameters are influenced by the application technique/system which decides droplet size, velocity distributions and application volume (Knoche, 1994; Cooke *et al*., 1986). The adhesion, spreading and retention of spray droplets depends not only on application technique but also on leaf surface properties (Chachalis, 2001), leaf angle and properties of the spray solution (Basu *et al*., 2002), which influence the static contact angle of spray droplets on a target. Static contact angle is a measure of adhesion and spreading (Basu *et al*., 2002). The smaller the static contact angle the better the surface coverage (Basu *et al*., 2002). The leaf surface properties, which affect the static contact angle of spray droplets are, the structure of the wax layer, nature and abundance of leaf hairs and topographical features of the surface (occurrence of grooves and ridges) (Chachalis, 2001), which would be expected to be dependent on variety and growing conditions of the plant (Ellis *et al*., 2004). It is difficult to find or make an artificial target that is identical to a leaf surface of a particular variety and also in ensuring that the tracer dye or chemical do not bind or absorbed to the target (Sullivan *et al*., 2011). Because of this reason actual leaves were used in the study instead of artificial targets.

As described above adhesion, spreading and retention of spray liquid depend also on properties of the spray solution. The properties of the liquid such as dynamic surface tension and viscosity influence the static contact angle of spray droplets on the leaf surface (Spillman, 1984). The tracer dye, titanium dioxide, is completely insoluble in water and might have changed dynamic surface tension and/or viscosity affecting the static contact angle of spray droplets on the leaf surface ultimately affecting spray distribution and percentage leaf coverage of di-1-p-menthene spray on wheat leaves. Nevertheless, if a water soluble tracer dye had been used it cannot be ensured that the dye would have not changed the properties of the spray solution. The decision to use titanium dioxide for this purpose was made after testing a number of other dyes as tracers. These dyes included inorganic stains as well as fluorescent dyes, none of which provided an enough

contrast to spray droplets in scanned images to be distinguished from the background leaf blade, especially in the areas of grooves on the leaf blade. Although, titanium dioxide, is completely insoluble in water and makes a suspension, the suspension is well re-dispersible (for the concentration of titanium dioxide used) upon shaking, and takes a while to suspend back, during which spray application can be accomplished, so that titanium dioxide is evenly distributed in the spray solution while spraying.

In this study the solution was sprayed onto detached leaves which were laid down on a flat surface. On plants, generally, leaves occur in an angle, and distribution and coverage of a spray application on leaves, laid horizontally, might be different from those on leaves occur in an angle (Ellis *et al*., 2004; Basu *et al*., 2002). Surface coverage decreases with the increase in tilt angle of a leaf (Basu *et al*., 2002). Therefore, the coverage on leaves laid horizontally is the maximum coverage of a spray application that can have on a leaf in a canopy.

As described in the section of introduction of 3.1.1, the method used to prepare samples for scanning electron microscopy did not involve dehydration with organic solvents followed by critical point drying, which can cause shrinkage and withdrawal of cellular constituents and may destroy the epicuticular wax layer and antitranspirant deposits. Therefore, results obtained from the scanning electron microscopy are reliable. Scanning electron microscopy clearly showed that the antitranspirant deposits occurred as patches covering some stomata while leaving some stomata uncovered.

3.4.2 The growth stage at which meiosis occurs in pollen mother cells

As explained in the section of results (3.3.2), in the variety Claire meiosis occurs in pollen mother cells between GS39 and the stage at which flag leaf sheath extended 10 cm above the auricles of the penultimate leaf; and, anthers at meiotic stages were found in 18 of the 30 shoots in between these two stages. The rest of the 12 shoots had anthers in which meiosis was not yet started or just had been completed. The duration of meiosis in the variety, Chinese Spring was about 42 hours at 15 °C, 24 hours at 20 °C and 18 hours

at 25 °C. T. aestivum has one of the shortest meiotic divisions known in higher plants (Bennett *et al*., 1973). Therefore, there is a high possibility of not detecting any cell at a stage of meiosis with in anthers (of 1st or 2nd florets of the middle spikelets) upon examination of a shoot even at a stage in between the above defined two stages. Since, the mean length between auricles of the penultimate leaf and the flag leaf of the shoots, which bared anthers at meiosis, was 4.5 cm, under conditions where the flag leaf sheath could extend up to a maximum of 15 cm above the auricles of the penultimate leaf, it can be simplified that meiosis in the variety Claire occurred at early GS41.

From the three studies mentioned in the introduction of section 3.1.2, which have explored the growth stage of wheat at which meiosis occurs in pollen mother cells, the only study which showed a similar result to this study is the one in Bennett *et al*., (1973), which explained that in the variety of Chinese Spring, meiosis in pollen mother cells occurred when the shoot was at GS41 and the sheath of the flag leaf was extended 1-2 cm above the auricles of the penultimate leaf. And the result of this study is completely different to Kirby, (2002) and Tottman, (1987) who reported that the growth stage that coincided with meiosis in pollen mother cells was GS45 and GS33 respectively. The growth stage at which meiosis occurs in pollen mother cells might be different from variety to variety. Showing a similarity to the variety Claire, it is reported in one of the rice varieties, meiosis begins when the flag leaf auricles are 3 cm below the auricles of the penultimate leaf and ends before the flag leaf auricles reach more than 10 cm above the auricles of the penultimate leaf (Moldenhauer and Gibbons, 2003).

According to the results, the first florets were always more advanced in development compared to second florets. This observation is in agreement with Bennett *et al*. (1973) who reported that the first floret is the most developed floret in a spikelet, and in the variety of Chinese Spring, the second floret reached late meiotic stages 12 ± 2 hours after the first floret at 20 °C. The time taken by the third floret to reach a level of development shown by the second floret was about 26 ± 6 hours at 20 °C.

An apparent difference in development was not detected between the three anthers in a floret, and it is reported that the pollen mother cells of the three anthers within individual florets are approximately synchronised in meiotic development (Bennett *et al*., 1971; Bennett *et al*., 1973). Although, it was difficult to identify a developmental gradient in the cells of the archesporium of an anther in this study, it is reported that, in the variety of Chinese Spring, that there was a developmental gradient in the cells of the anther archesporium corresponding to development lasting about 1 to 2 hours at 20 °C from tip to base of the anther locus and the anther tip contained the cells which were most advanced in development (Bennett *et al*., 1973).

3.4.3 The effect of film antitranspirants on the rate of transpiration, stomatal conductance, photosynthesis and internal CO_2 concentration

In total there are six instances of results (from the day after and three days after the antitranspirant treatment at GS33 in 2009/2010, from the day after and three days after the antitranspirant treatment at GS41 in 2009/2010 and from three days after and seven days after the antitranspirant treatment at GS33 in 2010/2011) to understand the effect of film antitranspirants on the rate of transpiration, stomatal conductance, rate of photosynthesis and internal CO_2 concentration. The results obtained from the low SMD regime in 2009/2010 around the spray application at GS41, however, are not used in the understanding of the effect of the antitranspirants on the four parameters. The reason for this is, the low SMD regime was irrigated to maintain the SMD level using trickle tape irrigation system, and the irrigation to the low SMD regime was started few days before the spray applications at GS41. There might be a variation in the amount of irrigation with in the low SMD regime, which might have affected the results from the antitranspirant/control treatments of the regime. Only the mean value for each parameter of the low SMD regime was compared with that of the high SMD regime to understand the effect of the factor, SMD regime on antitranspirant/control treatments. Most of the occasions the CV of the four parameters is high (higher than 15%). The variation in SMD within plots might have contributed to this high CV, especially in 2009/2010 at the

antitranspirant application time around GS41, where the low SMD was irrigated with trickle tapes. Furthermore, as explained in section 3.4.1, leaf coverage by antitranspirants may vary with tilt angle of the leaves and this might also have accounted for the high CV in the parameters.

3.4.3.1 The rate of transpiration and stomatal conductance

First, the effect of film antitranspirants on the rate of transpiration on the day after and three days after a spray application is discussed. As shown by the results obtained, the day after and three days after the antitranspirant treatment at GS33 in 2010/2011, the rate of transpiration was significantly decreased by di-1-p-menthene. A similar result was observed the day after the antitranspirant treatment at GS41, in the same year, from the latex treatment in the high SMD regime. However, three days after the spray application, there was no significant difference in the rate of transpiration between antitranspirant/control treatments in high SMD regime. Three days after the antitranspirant treatment at GS33 in 2010/2011, also, there was no significant difference in the parameter between antitranspirant/control treatments. When the results were studied it can be seen that in the occasions where the difference between antitranspirant treatment and unsprayed control in the rate of transpiration was significant, the transpiration rate of the unsprayed control was at least above 2 mmol $m^{-2}s^{-1}$. Both the, above stated occasions, of which the difference in the parameter between antitranspirant treatment/treatments and unsprayed control was not significant, the transpiration rate of the unsprayed control was below 1.5 mmol $m^{-2}s^{-1}$. This implies that a significant decrease in the rate of transpiration in an antitranspirant treatment, compared to an unsprayed control occurs when the prevailing weather and the crops response to the weather (by stomatal behaviour) encourage transpiration. Whereas, when transpiration is not encouraged/or discouraged naturally there may no significant difference in transpiration between an antitranspirant treatment and an unsprayed control, since, when the rate of transpiration is naturally lowered there is no much scope to an antitranspirant to lower the rate of transpiration more.

Transpiration occurs mainly via stomata and to a lesser extent from the cuticle (Turner, 1991). When taken as a whole, the rate of transpiration from epidermis is dependent on stomatal, conductance, boundary layer conductance (conductance of air layer near leaf surface), cuticular conductance (Turner, 1991; Jones, 1998; Chaves *et al*., 2003), and hydraulic conductance of inner walls of the epidermis (Sheriff, 1984). The factors controlling transpiration include, SMD, intensity of solar radiation, air temperature, relative humidity and wind speed. These factors control transpiration by influencing the degree of stomatal opening, i.e., stomatal conductance (Turner, 1991; Jones, 1998; Chaves *et al*., 2003; Pallardy and Kozlowski, 1979). The factors, air temperature, relative humidity and wind speed effect transpiration also by influencing boundary layer conductance (Sheriff, 1984; Jones, 1998). The underlying mechanisms involved in regulation of transpiration upon interaction of all the factors affecting transpiration are complex and are not entirely certain (Jones, 1998; Chaves *et al*., 2003). Near complete or complete Stomatal closure occurs under high light intensity, low relative humidity and high temperature, especially under drought conditions, (Pallardy and Kozlowski, 1979; Kudoyarova *et al*., 2007; Turner, 1991; Chaves *et al*., 2003). However, near completely stomatal closure occurs under low light intensity as well indicating adaptation for increased water use efficiency under conditions which are not favorable to photosynthesis relative to transpiration (Pallardy and Kozlowski, 1979).

The reason for low transpiration, on the two dates that no significant reduction in transpiration was gained by antitranspirant applications, might be stomatal closure due to low light intensity or high boundary layer resistance due low air temperature and high relative humidity occurred as a result. The equipment measured solar radiation and air temperature at each datum of transpiration rate recorded, and the mean solar radiation and air temperature during the period of data recording on the two dates are as follows; three days after antitranspirant application at GS41 in 2009/2010: 636.2 $\mu molm^{-2}s^{-1}$ and 13.54℃; three days after the antitranspirant applications at GS33 in 2010/2011: 1324.7 $\mu molm^{-2}s^{-1}$ and 15.04℃. On the dates which transpiration was high and significant

reductions of transpiration rate was gained by antitranspirant applications, the mean solar radiation was above 1500 $\mu molm^{-2}s^{-1}$ and air temperature was above 20 ℃.

Film antitranspirant treatments might not provoke a significant reduction in transpiration also in a situation where transpiration is naturally low due to stomatal closure/low stomatal conductance resulted by high light intensity, low relative humidity and/or high temperature. However, such a situation was not encountered on any of the dates of data collection. These findings and arguments suggest that film antitranspirants decrease transpiration when it is most wanted to do so; i.e., when transpiration is not decreased by any natural means. Furthermore, it is not only the transpiration occurred from stomata is decreased by film antitranspirants, but cuticular transpiration is also decreased. Figure 3.3.3 and 3.3.4 explain how antitranspirant deposits occur on leaves covering stomata as well as cuticle. Cuticular transpiration is inversely related to stomatal conductance (Sheriff, 1984); i.e., although plants can control stomatal transpiration by lowering stomatal conductance in adverse situations, cuticular transpiration increases. Decrease in cuticular transpiration provided by a film antitranspirant might be advantageous in such situation.

The only occasion at which the rate of transpiration (and the other three parameters) is measured seven days after an antitranspirant spray application is after the antitranspirant treatment at GS33 in 2010/2011, and that is the only situation, where the difference in the rate of transpiration between the two treatments was not significant and the rate of transpiration was above 2 $mmolm^{-2}s^{-1}$. The weather conditions prevailed during the seven days after this spray application was severe (Appendix III) i.e., temperature was high and intensity of solar radiation was also high. Antitranspirant deposits on the leaves might have been degraded to some extent by that severe weather. Furthermore, the growth of the leaves that might have occurred during the seven days might have intensified distance between the spray droplets increasing the number of stomata which are not under a patch of film of the antitranspirant. The higher the prevailing temperature the higher the leaf growth rate (McMaster *et al*., 1992). The reason for none significance of the difference in the rate of transpiration in this occasion might be the decrease in proportion area covered by the antitranspirant due to one or both of the above described reasons.

As explained in the literature review of section 1.4.1, it has been clearly demonstrated that film antitranspirants decrease the rate of transpiration (Davenport *et al*., 1972; Davenport *et al*., 1974; Colombo and Odlum, 1987; Mokhtari *et al*., 2006; Moftah and Al-Humaid, 2005; Win *et al*., 1991). However, the effect of evaporative demand on the performance of film antitranspirants is not demonstrated in the literature.

When the rate of transpiration is significantly decreased by an antitranspirant treatment compared to the corresponding unsprayed control, log stomatal conductance was also significantly decreased. The only occasion, at which the similarity in the response of the two parameters to the treatments was slightly deviated, is one day after the antitranspirant treatments at GS41 in the low SMD regime in 2009/2010. However, as explained above this might be from high variation in the water quantity in the low SMD regime. Regression analyses showed a significant positive correlation between the rate of transpiration and log stomatal conductance; all of which suggest that the film antitranspirants decreased the rate of transpiration by decreasing stomatal conductance. However, it should be considered that the stomatal conductance calculated by the equipment is not really "stomatal conductance", but a value which represents the conductance to diffusion of water vapour from the leaf surface, which is influenced by stomatal functioning as well as antitranspirant deposits (and also by cuticular conductance, hydraulic conductance of inner walls of the epidermis and boundary layer conductance). Therefore, it can be concluded that antitranspirant treatments increased the resistance to diffusion of water vapour from leaf surface.

As explained in the literature review of section 1.4.1, it has been widely accepted in the literature that, decrease in the rate of transpiration by film antitranspirants is via increased resistance to diffusion of water vapour from leaf surface (Davenport *et al*., 1971; Solarova *et al*., 1981). Although, Davenport *et al*. (1971) agreed with this statement, it was reported that decrease in the rate of transpiration increases leaf water potential and as a result widens stomatal pores immediately under the film, as well as on the parts of the leaf which are not covered by the film. However, this is not a matter creating a disadvantage as long as collective functioning of stomata and antitranspirant deposits reduce transpiration rate.

If the increase in the rate of transpiration from so called wider stomatal pores occur on the parts of the leaf which are not covered by the deposits counteracts the decrease in the rate of transpiration from parts of the leaf covered by the deposits, the results from this study as well from numerous studies published including Davenport *et al*. (1971) itself, would not show a significant reduction either in the rate of transpiration or stomatal conductance in antitranspirant treatments compared to unsprayed controls.

In 2009/2010, both the rate of transpiration and stomatal conductance of high SMD regime were significantly lower than those of low SMD regime. It is well documented that the rate of transpiration and stomatal conductance (Turner, 1991; Jones, 1998; Chaves *et al*., 2003) are decreased by drought.

3.4.3.2 Photosynthesis and intercellular CO_2 content

As explained in the literature review of section 1.3, it has been demonstrated that film antitranspirants decrease photosynthesis. Although, the results of this study have not proven that the antitranspirant treatments decrease photosynthesis compared to unsprayed controls, there is some indication of a possibility. Although the differences were not significant, in all the occasions photosynthesis in antitranspirant treatments was lower than that of unsprayed controls. The day after the antitranspirant application at GS33 in 2009/2010, the difference in photosynthesis between the antitranspirant treatment and the unsprayed control was border line significant ($p = 0.066$).

According to literature, the reason why film antitranspirants decrease photosynthesis is, antitranspirants are of low permeability to CO_2 entering the leaves, not only to water vapour leaving the leaves (Davenport *et al*., 1971; Solarova *et al*., 1981). According to the results, in the occasions where log stomatal conductance was significantly decreased by antitranspirant treatments, internal CO_2 concentration was also decreased, and except in one occasion, the decrease in internal CO_2 concentration was also significant. As shown by regression analyses the correlation between log stomatal conductance and internal CO_2 concentration was significantly positive in all the occasions. Therefore, from the

results of this study as well, it is clear that antitranspirant films decrease amount of CO_2 entering the leaves.

However, decrease in internal CO_2 concentration by antitranspirant treatments had not significantly decrease photosynthesis. One of the possible reasons for this non significance in the difference of photosynthesis is that CV might not be low enough to show the differences between the treatments as significant. The other possible reason is, decrease in internal CO_2 concentration might not have incurred a decrease in photosynthesis of a similar or a higher magnitude but of a lower magnitude, i.e., photosynthesis might have been impaired to a lesser extent compared to internal CO_2 concentration. The relationship between the rate of photosynthesis and internal CO_2 concentration at different intensities of PAR has been explored (von Caemmerer and Farquhar, 1981; Davis *et al*., 1987). It has been shown that, under a particular level of PAR, rate of photosynthesis rises linearly with rising intercellular CO_2 concentration up to a certain level of internal CO_2 concentration and then with further increases in internal CO_2 concentration the change in the rate of photosynthesis levels off ultimately reaching a plateau, at which photosynthesis is limited by PAR. Furthermore, at low levels of PAR the slope of the linear phase is lower than that of high levels of PAR, i.e., the lower the PAR, the lesser the change in the rate of photosynthesis for a change in internal CO_2 concentration. It has also been shown that, the lower the PAR the higher the concentration of internal CO_2, at which the curve reaches to a plateau. These findings show a number of possible occasions where a comparably less change in photosynthesis occurs for a change in internal CO_2 concentration. The results showed no significant correlation between photosynthesis and internal CO_2 concentration in any of the occasions. This implies that in individual leaves decrease in internal CO_2 concentration had not affected photosynthesis significantly, which further clarifies that at the occasions of data collection the interaction between PAR intensity and internal CO_2 concentration might have maintained the relationship between photosynthesis and internal CO_2 concentration at plateau region in the curve or at a region where there is only a small change in photosynthesis for a considerable change in internal CO_2 concentration.

In 2009/2010, there was no significant difference either in internal CO_2 concentration or in the rate of photosynthesis between the two SMD regimes. However, both the rate of photosynthesis and internal CO_2 concentration of high SMD regime were lower than those of low SMD regime. The high CVs might have prevented showing up the differences as significant differences. It is well documented that the rate of photosynthesis and intercellular CO_2 concentration (Loggini *et al*., 1999; Siddique *et al*., 1999; Tambussi *et al*., 2000) are decreased by drought. Internal CO_2 concentration is decreased by drought induced stomatal closure (Siddique *et al*., 1999; Tambussi *et al*., 2000), and rate of photosynthesis is decreased with the lack of internal CO_2 as well as by none-stomatal related factors as described in section 1.3. In agreement with the results of this study, Changhai *et al*. (2010), reported that the rate of transpiration was reduced more strongly than the rate of photosynthesis by drought.

3.4.4 The effect of film antitranspirants on leaf water potential

In general it can be concluded that the antitranspirant, di-1-pmenthene increase leaf water potential significantly (under the conditions in this study). The day after and three days after the antitranspirant treatments at GS33 and GS39 in 2008/2009, and GS33 in 2009/2010 leaf water potential was significantly increased by di-1-p-menthene. Furthermore, nine days after the antitranspirant treatments at GS33 in 2008/2009, leaf water potential in the antitranspirant treatment was higher than that of the unsprayed control and the difference was border line significant.

For how long an antitranspirant treatment increase leaf water potential compared to an unsprayed control may depend on prevailing weather conditions and SMD. Leaf water potential measurements were obtained frequently after the antitranspirant treatment at GS41 in 2008/2009 up to 23 days (and this is the only occasion leaf water potential measurements were obtained up to this long after an antitranspirant treatment), and the leaf water potential in the antitranspirant treatment was lower than that of the unsprayed control the day after and also 23 days after the treatment. However, the difference in leaf water potential between the two treatments was not significant at the occasions between

these two. High variation within treatments might have prevented showing up the differences as significant.

Two different stomatal behaviours have been identified in plant species. The leaf water potential in plant species with anisohydric behaviour changes according to the changes in the rate of transpiration (decreases with increasing transpiration rate and increases with decreasing transpiration rate) during the day, and is lower in the plants under high SMDs compared to plants in relatively lower SMDs. In contrast, the leaf water potential in plant species with isohydric behaviour remains nearly constant during the day, upon the changes in the rate of transpiration, and does not depend on SMD until plants are close to death (Tardieu and Simonneau, 1998; Jones, 1998). Wheat is of anisohydric behaviour (Henson *et al*., 1989; Tardieu and Simonneau, 1998), and the increase in leaf water potential might be from the water conserved within the plant/soil as a result of reduced transpiration by the antitranspirant. Moftah and Al-Humaid (2005), Win *et al*. (1991), Patil and De (1976) and Devenport *et al*. (1972) are some from several publications which demonstrated that leaf water potential is increased as a result of reduced transpiration by film antitranspirants. Relevant results from these publications are given in section 1.4.1. The significant difference in leaf water potential between the antitranspirant treatment and the unsprayed control 23 days after the spray application provides evidence that the effect of the antitranspirant treatment on leaf water potential lasted more than three weeks. This might be from persisted spray deposits on the leaves. Alternatively, the spray deposits might have degraded by this time, but moisture conserved in the soil by reduced transpiration during the time period where the antitranspirant persisted on leaves might have slowed the development in SMD in the soil, and the high leaf water potential in antitranspirant treatment compared to the unsprayed control might be from low SMD in antitranspirant treatment compared to the unsprayed control. It has been reported that water uptake was reduced significantly by a di-1-p-menthene application on sweet pepper because of reduced transpiration (Amor and Rubio, 2009). Poljakoff-Mayber and Gale (1972) reported that timely application of an antitranspirant can delay the development of SMD, and as a result transpiration and photosynthesis in antitranspirant treated plants

may reach to the same level or even to a higher level of those of control plants at a later stage when spray deposits disappears. However, the authors and lately Solarova *et al*. (1981) have pointed out this as a negative effect of antitranspirants. But the occurrence of this increase in transpiration and photosynthesis is because, SMD and leaf water potential in antitranspirant treatments are still higher than those of control treatments, therefore, this cannot be seen actually as a negative effect of film antitranspirants.

Under the similar application conditions other anisohydric plants such as Barley, Soy bean and Sun flower (Tardieu and Simonneau, 1998) may also demonstrate higher leaf water potentials upon decreases in the rate of transpiration by film antitranspirants. Although, isohydric plants such as Maize, Pea and Sugarcane (Tardieu and Simonneau, 1998) will not change leaf water potential upon decreases in the rate of transpiration by film antitranspirants, decreases in the rate of transpiration may slow down the development of SMD and in turn may bring about beneficial outcomes.

The other film antitranspirant used in the experiments, latex, was not used in this study, however, since an apparent difference in the effect of the two antitranspirants on yield and yield components and gas exchange properties has not been indicated, it can be assumed that latex may also show similar effects on leaf water potential as di-1-p-menthene.

3.4.5 The effect of film antitranspirants on leaf temperature

As discussed in the literature review of section 1.4.3, the effect of film antitranspirants on leaf temperature is a topic of controversy. Some debate that as absorbed net energy removed from plants via transpiration is high, curtailing transpiration by an antitranspirant may increase leaf temperature to a point which is threat full for physiological/biochemical processes of the plant (Gates, 1968; Solarova *et al*., 1981). Some debate that since, reduction in transpiration will not cause a proportional rise in leaf temperature, leaf temperature may not rise to a point which is a threat to the plant (Gale and Hagan, 1966). Both the above debates are based on energy balance calculations from different

equations. As explained in the literature review of section 1.4.3, however, research has indicated that transpiration could not lower leaf temperatures by more than about 5℃. As stated in the introduction of section 3.4.1, this study was performed with the objective of exploring the effect of antitranspirant treatments on leaf temperature, since it is a topic of controversy.

According to the results of this study, the difference in temperature between the unsprayed control and the antitranspirant treatment at consideration was less than 1℃ all the time. Most of the time, the temperature of the antitranspirant treatment was lower than that of the unsprayed control, and in very few occasions the difference was significant. At the same time, there are studies which showed transpiration curtail by antitranspirants did not affect leaf temperature (Gale and Poljakoff-Mayber, 1966). Moreover, there are studies which claim that both transpiration and leaf temperature are decreased by antitranspirants which are not of reflective type, known to reduce leaf temperature by reflecting solar radiation away (Prakash *et al*., 1992). However, it is not clear how leaf temperature can be decreased when transpiration is decreased by a film antitranspirant. In the results there were few occasions where the temperature of the antitranspirant treatment was higher than that of the unsprayed control, but the difference was not significant at any of those occasions. As explained in literature review (section 1.4.3) there are a number of studies which showed that leaf temperature is significantly increased by antitranspirants (Gale and Hagan, 1966; Han, 1990; Irmak and Jones, 2000; Maftah and Al-Humaid, 2005).

3.4.6 The effect of film antitranspirants on pollen fertility

The effect of film antitranspirants on pollen fertility has not been studied before. As discussed in the literature review of section 1.2.2.2, the effect of drought on pollen development has been explored. In fact it has been explored that, the most sensitive stage towards drought in most cereal crops is the stage of meiosis in pollen mother cells. Drought stress during meiosis in pollen mother cells leads to pollen sterility. Drought

stress affected pollen grains at pollen mother cell meiosis, failing to accumulate starch, and light microscopy of the anthers had shown a nearly complete lack of starch within sterile pollen grains (Saini *et al.*, 1984; Dorion *et al*., 1996; Saini, 1997; Lalonde *et al*., 1997). In our study KI/I_2 was used to differentiate drought stress affected pollen grains from unaffected pollen grains under the light microscope depending on the presence or absence of starch within pollen grains. As stated in the general introduction of section 4.1, it was hypothesised that reduced transpiration by a film antitranspirant, alleviates the effect of drought on pollen viability at the stage of meiosis in pollen mother cells irrespective of reduced photosynthesis.

According to the study of the growth stage at which meiosis occurs in pollen mother cells (section 3.1.2, 3.2.2, 3.3.2 and 3.4.2), in the variety Claire meiosis occurs in early GS41. The decreased transpiration by antitranspirant treatments at GS31 and GS33 might have ameliorated the effect of drought on pollen fertility during meiosis in pollen mother cells. This might be the reason for the significantly high percentage pollen fertility in di-1-p-menthene treatments at GS31 and GS33 compared to other treatments in 2009/2010 and for the significantly high percentage pollen fertility in di-1-p-menthene treatment at GS33 compared to the unsprayed control in 2010/2011. Wheat floral organs maintain high internal water status even in the period of substantial leaf drying during drought stress, therefore, It is believed that, inhibition of pollen development under drought stress triggers from a yet undefined signal from the vegetative organs affected by drought stress (Saini and Aspinall, 1981; Saini and Westgate, 2000). As explained in the discussion of the effect of film antitranspirants on leaf water potential, decreased transpiration by antitranspirant treatments increases leaf water potential and also possibly results in a lower SMD in antitranspirant treated plots compared to unsprayed control plots. The higher leaf water potential (possibly higher water potential in a vegetative organ other than leaves) and/or lower SMD in antitranspirant treated plots compared to unsprayed control plots might be the reason for this increase in pollen viability by antitranspirant treatments at GS31 and GS33. The information on signal transduction related to drought stress response in plants is still not fully revealed (Ha *et al*., 2012; Huang *et al*., 2012). It is

believed that abscisic acid (Saini, 1997; Saini and Westgate, 2000; Ha *et al*., 2012; Huang *et al*., 2012), cytokinins (Ha *et al*., 2012), reactive oxygen species and various other molecules (Huang *et al*., 2012) act as sporocidal signals in triggering drought stress response reactions in plants; nevertheless, exact roles of these chemical signals remains inconclusive. It has also been suggested that sugar could be involved in this signalling, since, sugar availability in anthers can be decreased upon inhibition of photosynthesis under drought stress (Boyer and McPherson, 1975). However, if this is the case it is inconclusive how a film antitranspirant, which further retards photosynthesis, increases pollen viability compared to an unsprayed control.

The antitranspirant treatments at GS41 might be too late to ameliorate the effect of drought on pollen viability during meiosis, since meiosis also occurs in early GS41. Possibly the stage of meiosis might have passed in most of the plants by the time of antitranspirant spray application. The significant difference in mean percentage pollen fertility between the two SMD regimes can be attributed to the difference in the amount of moisture in the two SMD regimes during the time of meiosis in pollen mother cells.

The percentage pollen viabilities in the treatments in the experiment in 2010/2011 were conspicuously higher than those of in 2009/2010. Drought stress induced sterile pollen grains do not always completely lack starch, and there are pollen grains lacking starch partially. When a pollen grain with partial lack of starch is stained with the Lugol's solution and subjected to microscopy, if the side of the pollen grain with starch granules face upwards, that pollen grain may be counted as a viable pollen grain, since the side of the pollen grain without starch could not be seen. After anther dehiscence, as time passes starch in sterile pollen grains with partial lack of starch may disappear (however, no information could be found from literature to support this statement). When pollen samples were collecting for the study in 2009/2010, the majority of anthers were dehisced. Although pollen grains were collected from newly dehisced anthers, identified from the appearance, there was no way to know how long before exactly the anthers had dehisced. Therefore, in 2009/2010 by the time of observation through microscope, starch in most of the pollen grains with partial lack of starch might have disappeared, and

therefore might have counted as dead pollen without mistakenly counting as viable pollen. In contrast, in 2010/2011 pollen samples were collected from anthers which had still not completely dehisced, but had partially split opened, and these pollen grains were stained and observed through the microscope straight away without a delay. Therefore, there might be a higher number of sterile pollen but with partial lack of starch compared to the samples in 2009/2010. Some of these pollen grains might have mistakenly counted as viable pollen. This might be the reason for the conspicuously high percentage pollen viabilities in the treatments of the experiment in 2010/2011 compared to those of the experiment in 2009/2010. The rate of development of the plants in the irrigated unsprayed control/common control in the experiment in 2009/2010 were lower than that of the other treatments and at the time of pollen sample collection in 2009/2010 anthers in irrigated control were just dehisced as in 2010/2011, and viability of pollen in irrigated unsprayed control in 2009/2010 was close to pollen viabilities demonstrated by treatments in 2010/2011. Apart from the effect of high soil moisture, the above explained fact might also have contributed to the significantly high pollen viability in irrigated unsprayed control compared to that of other antitranspirant/control treatments in 2009/2010.

3.5 Conclusion

The chapter included the studies carried out with the objective of exploring the underling physiological mechanism by which film antitranspirants increase yield. It was hypothesised that film antitranspirants reduce transpiration and photosynthesis; the decrease in transpiration by the antitranspirant treatments is from increased resistance to diffusion of water vapour from stomata; the decrease in photosynthesis by the antitranspirant treatments is from decreased internal CO_2 concentration; reduced transpiration increases leaf water potential and alleviates the effect of drought on pollen viability at the stage of meiosis in pollen mother cells irrespective of reduced photosynthesis; film antitranspirants do not increase leaf temperature significantly.

It can be concluded that as hypothesised, the two film antitranspirants used in the study decreased the rate of transpiration under the spray characteristics of the study. A significant decrease in the rate of transpiration in an antitranspirant treatment, compared to an unsprayed control may occur when the prevailing weather and the crops response to the weather (by stomatal behaviour) encourage transpiration. Whereas, when transpiration is not encouraged/or discouraged naturally there may no significant difference in the rate of transpiration between an antitranspirant treatment and an unsprayed control, since, when the rate of transpiration is naturally lowered there is no much scope to an antitranspirant to lower the rate of transpiration more. The decrease in the rate of transpiration by the antitranspirant treatments is from increased resistance to diffusion of water vapour from stomata. At the occasions when transpiration was decreased, antitranspirant treatments significantly decreased internal CO_2 concentration as well. However, decrease in internal CO_2 concentration by the antitranspirant treatments did not significantly decrease the rate of photosynthesis. The antitranspirant, di-1-pmenthene increased leaf water potential significantly. The increase in leaf water potential in an antitranspirant treatment compared to an unsprayed control might be from direct conservation of water with in the leaves as a result of reduced transpiration and/or from delayed development in SMD in antitranspirant treatments compared to unsprayed

controls as a result of reduced transpiration. The difference in temperature between antitranspirant treatments and unsprayed controls was less than 1 ℃, and was not significant at most of the occasions. Therefore, curtailed transpiration by antitranspirants did not increase leaf temperatures to a point which is threat full for plants as suggested by some publications. In the variety Claire meiosis in pollen mother cells occurs at early GS41. The percentage pollen viability in di-1-p-menthene treatments at GS33 was significantly higher than that of unsprayed controls. As hypothesised, decreased transpiration by antitranspirant treatments at GS33 might have ameliorated the effect of drought on pollen fertility during meiosis in pollen mother cells.

The key findings of the experiments explained in this chapter are summarised below.

1. The antitranspirant treatments significantly decreased transpiration and stomatal conductance to water vapour.
2. The antitranspirant treatments decreased internal CO_2 concentration and indicated a reduction in photosynthesis which was not significant.
3. The antitranspirant treatments significantly increased leaf water potential.
4. The antitranspirant treatments at GS33 and GS31 increased pollen fertility.
5. The antitranspirant treatments did not increase leaf temperature significantly.

Apart from the main studies mentioned above another two studies, important in understanding and elaborating on the results of the main studies, were also carried out. The spray distribution pattern, leaf coverage and stomatal coverage of the most frequently used film antitranspirant in the research, di-1-p-menthene, on wheat leaves was assessed by image analysis and electron microscopy. The leaf coverage by the antitranspirant was 15.80 %. Electron microscopic studies revealed that spray droplets occur as patches on the leaf (not as a continuous film). Studies were carried out to identify the growth stage and the external tiller morphology of winter wheat at the time of meiosis in pollen mother cells. The study showed meiosis occurs at pollen mother cells at early GS41.

4 Molecular mechanisms underlying the yield increase of droughted wheat by film antitranspirants

4.1 Introduction to the chapter 4

The effect of film antitranspirants on pollen development was assessed in terms of the differences in the expression of the genes related to drought induced pollen sterility with the objective of understanding the molecular mechanisms underlying yield increase by antitranspirants.

As explained in section 2.2.2, the most prominent sign of metabolic failure in drought-stress-affected wheat pollen grains is their failure to accumulate starch (Dorion *et al*., 1996). During normal development, pollen grains accumulate starch, which serves as the energy source for subsequent pollen germination and pollen-tube growth (Dorion *et al*., 1996; Saini, 1997; Lalonde *et al*., 1997). The pattern of distribution of starch in anthers is also changed by drought stress (Saini, 1997). It has been shown that instead of an impairment of enzymes directly involved in starch biosynthesis or a restriction of sugar import into anthers, an inability to metabolize incoming sucrose to hexoses, due to an impairment of invertase under drought stress, may be involved in this reproductive failure (Dorion *et al*., 1996; Koonjul *et al*., 2005).

Sucrose is the principal sugar imported into sinks in wheat. Prior to the utilization in physiological or metabolic processes, sucrose is generally converted to hexoses by invertase and/or sucrose synthase. The resulting hexoses are channelled into several important metabolic routes, including starch synthesis (Dorian *et al*., 1996). Therefore, an inhibition of any of the steps in sucrose metabolism could limit starch accumulation in pollen (Dorian *et al*., 1996). Invertase is the main enzyme of sucrose cleavage in pollens and anthers of wheat and several other species (Koonjul *et al*., 2005). In wheat and rice anthers affected by meiotic stage drought, a significant and immediate decline in the activity of vacuolar (Dorion *et al*., 1996; Koonjul *et al*., 2005) and cell-wall bound invertase (Koonjul *et al*., 2005) precedes any other sign of developmental failure.

Koonjul *et al*. (2005) have identified two invertase genes, *Ivr1* and *Ivr5*, which are down regulated only when drought stress occurs during meiosis in pollen mother cells. The expression of the two genes does not recover even though the drought stress disappears

in a subsequent stage. *Ivr1* and *Ivr5* encode cell wall and vacuolar invertase isoforms respectively, and both the genes are expressed preferentially, though not solely, in anthers. In our study, the ability of the antitranspirant treatments to ameliorate the effect of drought on pollen development was assessed by comparing the differences in the expression profile of the drought stress sensitive invertase gene, *Ivr5,* in anthers from different antitranspirant treatments and controls. The semi quantitative Reverse-Transcription PCR (RT-PCR) technique was used to compare the gene expression levels.

The study was conducted under the following hypothesis:

- Film antitranspirant increases pollen viability by alleviating the effect of drought stress on the expression of drought stress sensitive invertase genes, which are down regulated under drought stress leading to pollen sterility

4.2 Materials and Methods

4.2.1 Collection of experimental material

The effect of film antitranspirants on the expression of the invertase gene, *Ivr5*, related to drought stress induced pollen sterility (Koonjul *et al*., 2005) was explored from the anthers collected from the experiments and the treatments shown in Table 3.2.11. During the procedure of removing anthers from florets, there is a possibility of losing moisture from anthers and substantial changes to the expression profile of the genes in interest occurring. Therefore, It was not anthers which were collected from the plots, but whole spikes. Spikes were collected at GS 59 (ear completely emerged above flag leaf). Three spikes from randomly selected locations per plot were excised. Immediately after the excision each spike was immersed in liquid N_2, so that the gene expression profile at the time of excision was preserved. All the three spikes from a plot were inserted into a labelled 15 ml collection tube and stored in dry ice until it was transferred to the laboratory. The tubes were stored at -80ºC in a freezer.

Table 4.2.1: The experiments (under polytunnels) and the treatments from which anthers were collected to explore the effect of film antitranspirants on the expression of *Ivr5*

Experiment	Treatments
2008/2009	Claire: di-1-p-menthene at GS33 Claire: unsprayed control
2009/2010	High SMD regime: di-1-p-menthene at GS33 High SMD regime: unsprayed control
2010/2011: Experiment 1	From all the treatments, i.e.: di-1-p-menthene at GS33 latex at GS33 unsprayed control

Anthers were collected only from the first and the second florets of the spikelets occur in the middle of the spike, which are most advanced in development (Bennett *et al*., 1971;

Bennett *et al*., 1973). Anthers were excised from the florets while the florets were in contact with liquid N_2. This was facilitated by a small apparatus (Figure 3.2.1) prepared in the laboratory. A circular area the size of a Petri dish was removed from a lid of a small polystyrene box and a Petri dish was fixed tightly into the area in such a way that the bottom of the Petri dish was immersed in liquid within the box when the box lid was. The box was filled with liquid N_2, and the lid closed. The anther separation from the florets was carried out in the Petri dish. Liquid N_2 poured onto the florets in the Petri dish lasted longer without evaporating, than otherwise it would have, since the Petri dish was surrounded by the liquid N_2 inside the box. First, the florets which were to be used were separated from the rest using two pairs of forceps. Then the florets were carefully crushed to locate the anthers. The anthers were removed into an eppendorf tube, using a pair of forceps, which was placed in a corner of the same box with the bottom immersed in liquid N_2. The anthers were not allowed to thaw while, before or after they had been excised from the florets. The eppendorf tubes containing the anthers were stored in at -80ºC in a freezer.

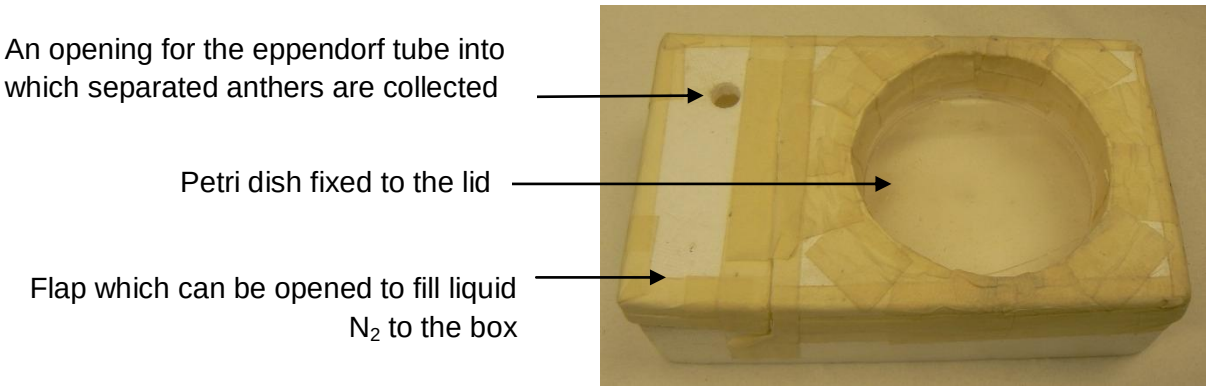


Figure 4.2.1: Apparatus used to separate anthers from florets

4.2.2 Procedure of total RNA extraction from the anthers

The eppendorf tubes containing anthers were transferred to a container of liquid N_2, immediately after the tubes had been taken out from the -80ºC freezer. Total RNA extraction from anthers was performed with a RNA isolation kit, SV Total RNA Isolation

System (Promega, Southampton, UK) according to the manufacturer's instructions as follows.

First, the RNA Lysis Buffer, the RNA Wash Solution and the DNase Stop Solution were diluted by adding respectively 50 ml, 100 ml and 8 ml of 95% ethanol to the provided concentrated solutions with the RNA isolation kit in 50 preps size. Approximately 30 mg of each anther sample was transferred in to a new eppendorf tube of which the lower half was immersed in dry ice, and the anthers were ground into a powder using a pestle. The eppendorf tube was taken out from dry ice and 175 µl of RNA Lysis Buffer was added onto the ground tissue sample. Then 350 µl of RNA Dilution Buffer was added to the sample, mixed by inversion and centrifuged at 12,000 g for 10 minutes. After that, 200 µl of 95% ethanol was added to the clear lysate and mixed by pipetting 3-4 times. This mixture was transferred to the Spin Column Assembly and centrifuged at 12,000 g for one minute. The liquid in the collection tube was discarded and 600 µl of RNA wash solution was added to the Spin Column Assembly. Then the Spin Column Assembly was centrifuged at 12,000 g for 1 minute. The DNase incubation mix was prepared by combining 40 µl Yellow Core Buffer, 5 µl 0.09M $MnCl_2$ and 5 µl of DNase I enzyme per sample, in the given order, in a sterile tube. The components were mixed by gentle pipetting. An amount of 50 µl from the freshly prepared DNase incubation mix was applied directly to the membrane inside the Spin Basket, in such a way that the membrane was thoroughly covered by the mix. Then it was incubated for 15 minutes at 20-25ºC. After this incubation, 200 µl of DNase Stop Solution was added to the Spin Basket and centrifuged at 12,000 g for 1 minute. An amount of 600 µl of RNA Wash Solution was added to the spin basket and centrifuged at 12,000 g for 1 minute. After the collection tube was emptied 250 µl of RNA Wash Solution was added to the Spin Basket and centrifuged at 12,000 g for 2 minutes. The Spin Basket was transferred from the Collection Tube to a 1.5 ml Elution Tube, after the cap of the Spin Basket was removed. Then 100 µl of Nuclease-Free Water was added to the membrane of the Spin Basket and centrifuged at 12,000 g for 1 minute. The Spin Basket was discarded and the Elution Tube containing purified RNA was stored at -80ºC in a freezer.

4.2.3 The Estimation of RNA concentration and quality

The concentration (ng/µl) and the purity of the extracted RNA were estimated by the NanoDrop system (ND-1000, Labtech, East Sussex, UK) using 1.5 µl from each of the total RNA extracts. The A260/A280 absorbance ratio indicates the purity of RNA samples by providing an estimation of contamination mainly by proteins and phenol (Portillo *et al*., 2006). An RNA sample can be assumed to have a low contamination by proteins and phenols if the A260/A280 absorbance ratio is 1.7 or high (Sambrook and Russel, 2006). The A260/A280 absorbance ratio of all the samples was around 2, indicating that the purity of the RNA samples was high. The intactness of the RNA samples was explored by gel electrophoresis to see whether there are well-defined 18S and 28S ribosomal RNA bands (Sambrook and Russel, 2006). For that, a 1% (W/V) Agarose/ TAE (Tris-acetate-EDTA) gel was prepared in to which GelRed (Promega, Southampton, UK) was added as the nucleic acid stain. A volume of 0.5 µl of each RNA sample mixed with 4.5 µl of RNase free water and 1 µl 6 x loading buffer (Promega, Southampton, UK) was loaded into the wells. A standard 2-log ladder (New England Biolabs, Herts, UK) was used for nucleic acid size comparison. The gel electrophoresis was carried out at 80 V until the fastest band has moved 2/3 of the gel length and the bands were observed under UV light.

4.2.4 Semi-quantitative Reverse Transcriptase PCR (RT-PCR)

Semi-quantitative RT-PCRs were carried out by reverse transcription of 0.5 µg of total anther RNA using the one-step RT-PCR kit (Qiagen, West Sussex, UK). The primer sequences, for *Ivr5* were from Koonjul *et al*. (2005). The internal standard used was 18s rRNA, and the primer sequences were from Wang *et al*. (2011). The primer pairs used to amplify *Ivr5* and the internal standard are given in the table 4.2.3.

Table 4.2.2: The primer pairs used to amplify Ivr5 and the internal standard

gene	Primer pairs
Ivr5	F (5'-CAACGACTCCCTCCTCCGCAACT-3')
	R (5'-TTCTCGTCCAGCTCCACCGTCCTC-3')
18s rRNA	F (5'-TTCATATCACGTGCTGCATGG-3')
	R (5'-AGACGACTTCGGTGAGACG-3')

The master mix was prepared by mixing the components in their quantities necessary (according to the instruction manual of the RT-PCR kit) for the total number of reactions and one more. The volumes given in Table 4.2.4 are the volumes per reaction. A volume of 48 µl of the master mix was dispensed per well in PCR plate. A volume of 2 µl of each template RNA containing 400 ng of total RNA was added to the corresponding well. A volume of 2 µl of RNase-free water was added to the well of negative control.

The contents were mixed gently and subjected to the steps/conditions given below in a PCR machine (GeneAmp PCR system, Applied Biosystems, California, USA). The steps/conditions were the same as that described in Koonjul *et al*. (2005), except the annealing temperature, number of cycles and addition of $MgCl_2$. The internal standard used in this study was different from that of Koonjul *et al*. (2005) and these changes were necessary to optimise the PCR.

I. Reverse transcription: at 50ºC for 30 minutes
II. Initial PCR activation step: at 95 ºC for 15 minutes
III. 30 cycles of the following
    a. 30 seconds at 94 ºC
    b. 30 seconds at 57 ºC
    c. 1 minute at 72 ºC
IV. Final extension at 72 ºC for 10 minutes

All the RNA samples were amplified at once in one PCR machine to avoid any differences occur during the process which will affect the comparison of the samples.

Table 4.2.3: Components and volume parts of RT-PCR reaction mixture per reaction

Component	Volume/µl (per 50 µl reaction)	Final concentration/µM
RNase-free water	26.0	-
5x Qiagen OneStep RT-PCR Buffer	10.0	Not known
dNTP Mix (containing 10mM of each dNTP)	1.0	200 µM of each dNTP
Forward primer – Ivr 5	2.0	0.4
Reverse primer – Ivr 5	2.0	0.4
Forward primer – 18s rRNA	2.0	0.4
Reverse primer – 18s rRNS	2.0	0.4
Qiagen OneStep RT-PCR Enzyme Mix	2	-
$MgCl_2$	1	5

4.2.5 Gel electrophoresis and quantification of the bands

A 2% (W/V) Agarose/ TAE gel was prepared in to which GelRed (Promega, Southampton, UK) was added as the nucleic acid stain. A volume of 5 µl of each RT-PCR product and the negative control mixed with 1 µl 6 x loading buffer (Promega, Southampton, UK) was loaded into the wells. A standard 2-log ladder (New England Biolabs, Herts, UK) was used for nucleic acid size comparison. The gel electrophoresis was carried out and the bands were observed under UV light. The intensity of each cDNA band was quantified by an image analysis system (Gel Logic 212 PRO, Carestream, NY, USA). The relative level of expression of the gene in each sample was determined by comparing the band intensity of gene cDNA to that of internal standard cDNA.

The data obtained (relative *Ivr5* gene expression) were subjected to ANOVA using GenStat 13th edition. Table 4.2.5 shows the skeleton ANOVA of the analyses of the data in the experiments in 2008/2009 and 2009/2010. Table 4.2.6 shows the skeleton ANOVA

of the analysis of the data in 2010/2011. The skeleton ANOVA of the analysis for which replicate data from experiments in the three years were combined is shown in Table 4.2.7. When combining the experiments for analysis, the data from the latex treatment at GS33 in 2010/2011 were not taken into account (since a latex treatment at GS33 was absent in the experiments in 2008/2009 and 2009/2010.)

Table 4.2.4: The skeleton ANOVA of *Ivr5* gene expression data from the experiments in 2008/2009 and 2009/2010

Source of variation	Degree of freedom
Block stratum	2
Block / AT-control treatment "units" stratum	
AT-control treatment	1
Residual	2
Total	5

Table 4.2.5: The skeleton ANOVA of *Ivr5* gene expression data from the experiment in 2010/2011

Source of variation	Degree of freedom
Block stratum	2
Block / AT-control treatment "units" stratum	
AT-control treatment	2
Residual	49
Total	53

Table 4.2.6: The skeleton ANOVA of the analysis for which the *Ivr5* gene expression data of the three experiments were combined

Source of variation	Degree of freedom
Experiment stratum	2
Experiment / polytunnel stratum	6
Experiment / polytunnel "units" stratum	
AT-control treatment	1
Residual	38
Total	47

4.3 Results

4.3.1 The effect of film antitranspirants on the expression of genes related to drought stress induced pollen sterility

As described in section 3.2.7, the effect of film antitranspirants on the expression of the invertase gene, *Ivr5*, related to drought stress induced pollen sterility (Koonjul *et al*., 2005) in the anthers in shoots at GS59 was explored. The data subjected to ANOVA are relative expression levels of the *Ivr5* gene in PCR wells which were determined by comparing the level of expression of *Ivr5* with the level of expression of 18s rRNA (internal standard) in each PCR well, and hence there is no unit to the values/results presented.

Table 4.3.1 shows the results from the analyses of relative *Ivr5* gene expression data gathered from the experiments in 2008/2009, 2009/2010 and the analysis for which replicate data from experiments in the three years are combined. Note that when combining the experiments for analysis, the data from the latex treatment at GS33 in 2010/2011 were excluded, since a latex treatment at GS33 was absent in the experiments in 2008/2009 and 2009/2010. The relative *Ivr5* gene expression in the anthers collected from di-1-p-menthene treatments at GS33 was higher than that of the anthers collected from unsprayed controls in the experiments in 2008/2009 and 2009/2010, but the difference was not significant. The difference was greater in the results from the analysis in which data from the experiments in the three years were combined but still the difference was not significant.

Table 4.3.1 shows the results for the analysis of relative *Ivr5* gene expression data from the experiments in 2010/2011. There was also no significant difference in relative *Ivr5* gene expression between AT/control treatments in the experiment in 2010/2011.

Figure 4.3.1 shows bands obtained from gel electrophoresis of the RT-PCR products. This is one of the gels which were used in the calculations of relative *Ivr5* band intensity in each PCR well or in samples collected from each plot (as explained in the materials and methods of section 3.2.7, mean values of relative *Ivr5* band intensities obtained from two

gel electrophoresis products were used in ANOVA). The bands at 597 bp and 256 bp corresponds to *Ivr5* gene and the internal standard, 18s rRNA.

Table 4.3.1: The results for relative gene expression of *Ivr5* gene (compared to 18s rRNA /internal standard) from the experiments in 2008/2009, 2009/2010, 2010/2011 (Experiment 1) and from the analysis in which the experiments in 2008/2009 and 2009/2010 and 2010/2011 (Experiment 1) are combined

Year/ Experiment	**AT/control treatments**			P	S.E.M	CV% (res. df)
	UC	**di-GS33**	**la-GS33**			
2008/2009	0.431	0.451	-	0.785	0.0463	18.2 (2)
2009/2010	0.392	0.425	-	0.761	0.0675	28.6 (2)
2010/2011	0.500 (a)	0.548 (a)	0.469 (a)	0.269	0.0345	29.0 (49)
three years combined	0.477	0.521	-	0.295	0.0288	28.3 (38)

AT = antitranspirant; UC = unsprayed control; di-GS33 = di-1-p-menthene treatment at GS33; la-GS33 = latex treatment at GS33; res. df = residual df; data within rows accompanied by the same letter are not significantly different at p = 0.05

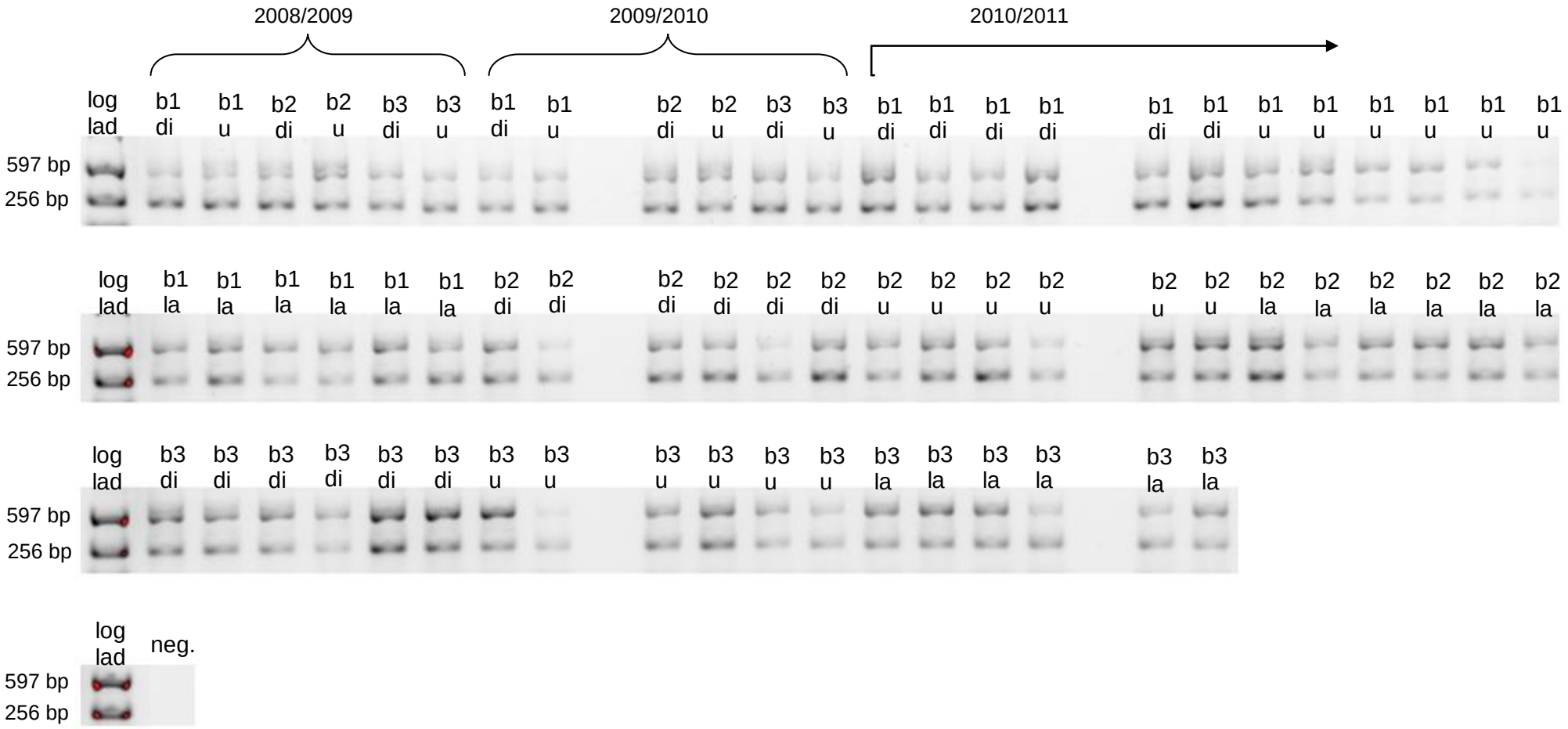


Figure 4.3.1: The bands obtained from gel electrophoresis of the RT-PCR products; the upper band and the lower band of the log ladder (log lad) are respectively 500 bp and 200 bp; The bands at 597 bp and 256 bp corresponds to *Ivr5* gene and 18s rRNA; b = block; la = latex; u = unsprayed control; di = di-1-p-menthene; neg = negative control in RT-PCR

4.4 Discussion

As explained in the materials and methods of section 3.2.7, the effect of film antitranspirants on the expression of genes related to drought stress induced pollen sterility was explored by studying the expression of the invertase gene, *Ivr5* (Koonjul *et al*., 2005). As explained in the literature review of section 1.3, it has been identified that the cause of the inhibition of starch accumulation in pollen is an impairment in invertase production induced by drought stress coincided with meiosis in pollen mother cells (Dorion *et al*., 1996; Koonjul *et al*., 2005). The invertase gene, *Ivr5*, has been identified as one of the genes down regulated under drought stress coincide with meiosis in pollen mother cells (Koonjul *et al*., 2005). Since, as explained above, pollen viability of di-1-p-menthene treatments at GS33 is higher than that of unsprayed controls in the experiments in both 2009/2010 and 2010/2011, it was expected to see a higher level of expression in ivr5 gene in antitranspirant treatments at GS33 compared to respective unsprayed controls. However, according to the results, although, *Ivr5* gene expression in di-1-p-menthene treatments at GS33 was higher than that of unsprayed controls in the experiments in 2008/2009 and 2009/2010, the difference was not significant. The difference was greater in the results from the analysis in which data from the experiments in the three years are combined but still the difference was not significant. When combining the data from the three years the data from the latex treatment at GS33 in 2010/2011 were excluded (since a latex treatment at GS33 was absent in the experiments in 2008/2009 and 2009/2010). However, according to the analysis of data from 2010/2011 alone, there was no significant difference in between the di-1-p-menthene treatment at GS33 and the unsprayed control, as well as the latex treatment at GS33 and the unsprayed control.

As shown by the correlation matrix (Table 4.4.1) relative gene expression was not tightly correlated with pollen fertility, grains m^{-2} or yield. The only parameters which were highly correlated were yield and grains m^{-2}.

Table 4.4.1: The correlation matrix showing the correlation of relative gene expression with pollen fertility, grains m^{-2} and yield

	Relative gene expression	Pollen fertility (%)	Grains m^{-2}	Yield (t/ha)
Yield (t/ha)	0.1609	0.1089	0.9755	-
Grains m^{-2}	0.0825	0.2114	-	-
Pollen fertility (%)	0.3885	-	-	-
Relative gene expression	-	-	-	-

As explained in the materials and methods of section 3.2.7, anthers were collected only from three spikes per plot, since plants had to be carefully shared among all the analyses. From the pool of anthers of these 3 spikes an amount of about 30 mg was used in RNA extraction (as instructed by the procedure for RNA extraction). When the variations in soil moisture with in plots are considered (as discussed in Chapter 2) anthers from only three spikes might not be enough to represent a plot and collectively samples from all the plots might not have represented a treatment. The sensitivity of the analyses could have been increased by extracting RNA from anthers from a larger number of spikes per plot, and by mixing couple of RNA extractions to prepare a representative sample for each plot. However, with the constraints of time, money and number of plants, this was not possible. Dorion *et al*. (1996), Lalonde *et al*. (1997) and Koonjul *et al*. (2005) have performed similar studies to explore the effect of drought stress on gene expression in anthers, and have stated that RNA was extracted from a large pool of anthers (although, how large were the anther pools was not clearly stated). There is no information in literature on studies which have explored the effect of film antitranspirants on plants at gene expression level. This study is the first to explore the effect of film antitranspirants on gene expression.

4.5 Conclusion

It was attempted to explore the effect of antitranspirants on one of the drought stress responsive invertase genes expressed in anthers. The results did not show a significant difference in the expression level of the gene between antitranspirant/unsprayed control treatments. If the sensitivity of the study is increased by increasing the size of the pollen pool sampled from each plot and by increasing the number of replication, the results may show a significant difference between antitranspirant/unsprayed control treatments.

It can be concluded that the results did not show evidence to support the hypothesis that film antitranspirants increase pollen viability by alleviating the effect of drought stress on the expression of drought stress sensitive invertase genes, which are down regulated under drought stress leading to pollen sterility.

5 General discussion and further studies

5.1 General discussion

As explained in the general introduction of chapter 1.1, the first objective of the study was to determine the effect of film antitranspirants at different growth stages in relation to meiosis in pollen mother cells on yield and yield components of droughted wheat, with the purpose of determining the most effective growth stage to receive a film antitranspirant application targeted to increase yield under drought conditions. The experiments related to the first objective of the study are included in chapter 2, and as discussed there, the experiments in 2008/2009 and 2009/2010 indicated that, among the growth stages included into the experiments, GS33 is the most effective growth stage to apply film antitranspirants in order to increase yield of droughted wheat. The experiments in 2010/2011, further confirmed the fact. The yield increase by film antitranspirants, when sprayed at GS33, at SMDs above 66, mm was approximately 0.5 t/ha under the conditions of this study. When the effect of antitranspirant treatments on yield components is considered, it is evident that the yield increase by antitranspirant treatments was from an increase in grains m^{-2}. As discussed in chapter 3, in the variety Claire, meiosis in pollen mother cells occurs at early GS41. Therefore, the yield increase by the antitranspirant treatments at GS33 can be attributed to alleviation of the effect of drought stress on the crop during meiosis in pollen mother cells as hypothesised. However, the increase in yield could be partially because of an increase in tiller survival resulted from moisture conservation in soil by the antitranspirant treatments.

The second objective of the study was to determine the underlying physiological mechanism by which antitranspirants increase yield by exploring the physiological effects of antitranspirants on gas exchange, plant water status and pollen development. The experiments related to the second objective of the study are included into chapter 3, and as discussed there, the antitranspirant treatments significantly decreased transpiration, significantly increased leaf water potential and indicated a reduction in photosynthesis which was not significant. The antitranspirant treatments at GS33 and GS31 increased pollen viability, and this increase can be attributed to an alleviation of the effect of drought

stress on the crop during meiosis in pollen mother cells, which occurs in early GS41 in the variety Claire. The alleviation of the effect of drought stress on the crop by film antitranspirants might be from reduced transpiration followed by increased leaf water potential and/or conserved soil moisture. The information on signal transduction related to drought stress response in plants is still not fully revealed (Ha *et al*., 2012; Huang *et al*., 2012), therefore, underlying mechanisms of yield increase in droughted wheat by film antitranspirants at the level of signal perception and transduction cannot be explored. However, it was attempted to explore the underlying mechanisms of this yield increase at the molecular level by exploring the effect of antitranspirant treatments at GS33 on a drought stress sensitive invertase gene, *Ivr5*, which down regulates under drought stress leading to pollen sterility (Koonjul *et al*., 2005). The experiment on this is included into chapter 4, and the experiment did not show a significant difference in the level of expression of the gene between antitranspirant/control treatments. More promising results would be indicated if the sensitivity of the test is increased by increasing the number of ears, used in anther collection, per plot per treatment.

When the results across the three experimental chapters are studied a clear relationship between the effects of film antitranspirants on yield, yield components, pollen fertility, leaf water potential and leaf gas exchange, particularly the rate of transpiration can be seen. The antitranspirant treatment at GS33 showed a significant mean yield increase of about 0.5 t/ha (8.5 %) compared to the unsprayed control. Providing evidence for that this yield increase from the antitranspirant treatment at GS33 was from an increase in grains m^{-2}, the mean grains m^{-2} of the antitranspirant treatment was 1255 (11.5%) higher than that of the unsprayed control and the difference was significant. The significantly high pollen fertility of the antitranspirant treatment at GS33 compared to the unsprayed control implies that the antitranspirant treatment increased grains m^{-2} by increasing pollen fertility. The difference in pollen fertility between the two treatments was 15.46 % in 2009/2010, which is 22.35% from the pollen fertility of the unsprayed control. The results obtained for the effect of film antitranspirants on the rate of transpiration and leaf water potential explains the underlying physiological mechanisms which brought about the above mentioned

increases by the antitranspirant. The antitranspirant treatment at GS33 significantly decreased the rate of transpiration by a mean value of 1.87 mmolm-^{2}s^{-1} (36.4%) and significantly increased the leaf water potential by a mean value of 2.27 MPa (21%) compared to the unsprayed control the day after and three days after the treatment in 2009/2010. According to the results it is clear that the decreased transpiration by the antitranspirant increased leaf water potential which led to an increase in pollen fertility and then grains m^{-2} and yield in turn. The decreased transpiration by the antitranspirant might have increased the SMD. When the transpiration was decreased by 1.87 mmolm^{-2}s^{-1} it stopped an amount of 1.87 mmol of water loosing from soil per m^{2} per second, which is 6.73 mol per hour. The molar volume of liquid water is 18.016 ml (at 277 K). Therefore the volume of 6.73 mol of water is 121.25 ml. This amount of water in 1 m depth soil gives 0.12 water per mm. Therefore the potential scale of SMD adjustment through antitranspirants is 0.12 mm per hour. The attempt to understand the underlying molecular mechanism of the effect of antitranspirants was, however, unsuccessful since the difference in invertase gene expression between the antitranspirant treatment at GS33 and the unsprayed control was not significant.

The yield increase by film antitranspirant treatments at GS33 is well in agreement with Kettlewell *et al*. (2010) that indicated film antitranspirants increase yield in droughted wheat when sprayed prior to meiosis in pollen mother cells. Section 1.4.2.2 reviews some of the other studies which reported yield improvements from film antitranspirants in other seed crops including corn (Fuehring and Finckner, 1983), sorghum (Fuehring, 1973) and rapeseed (Patil and De, 1978). These studies, however, have not attempted to explore the effects of film antitranspirants on yield components or the underlying mechanisms of the yield increase by antitranspirants. Our study is an eye-opener which points out the prospects for antitranspirants in food security in the future world especially in semi arid and aid areas. The prospects for antitranspirants are further discussed in the section of further studies (section 5.3) pointing out research aspects which can be useful to improve the application of antitranspirants in future food production.

In this study, the yield increased by film antitranspirants was about 0.5 t/ha. According to Kettlewell (2011), the increase in yield by a film antitranspirant is linearly related to SMD, and an economic threshold SMD above which an economic yield response should be obtained can be calculated based on the linear relationship between spray cost and grain price. For a wheat grain price of £200 per tonne, a minimum yield response of 0.3 t/ha to film antitranspirant is necessary to cover the total application cost. However, if the antitranspirant is tank mixed with a fungicide, which is normally sprayed around GS33, the cost of spraying the antitranspirant could be reduced (Kettlewell, 2011).

It is explained in the general introduction of section 1.1 that the phenomenon, which has held back research in the area of film antitranspirants on food crops, is that the well established idea that film antitranspirants decrease photosynthesis and decreased photosynthesis may ultimately decrease yield. Kettlewell *et al*. (2010) proposed that most sensitive stages in crop yield formation to drought stress may respond positively to film antitranspirant applications irrespective of reduced photosynthesis, and their research indicated that film antitranspirant application around the time of meiosis increased yield in droughted wheat. The results from this study confirm this.

An argument may be raised that possible reduction in photosynthate by a film antitranspirant treatment at GS33 may limit pre anthesis biomass partitioning to the developing ear resulting in low numbers of floral primodia/florets followed by low grain numbers. According to Fischer and Stockman (1980), as responses to pre anthesis shading both grains ear^{-1} and grains m^{-2} were significantly decreased. These decreases were associated with significant decreases in well developed florets. The fertility of well developed florets was, however, unaffected. It is reported that reduced assimilate supply to the spike is probably the cause of these responses. These findings suggest that a possible reduction in assimilate supply by an antitranspirant may reduce the number of well developed florets. But, as shown by this study, under drought conditions, antitranspirant treatments at GS33 increase pollen fertility and hence floret fertility. Whereas, in unsprayed controls both the number of well developed florets and floret fertility are decreased because of drought induced reductions in assimilate supply to the

developing ear and drought stress effects on floret fertility. Although, reductions in developed florets might be high in the antitranspirant treatment than in the unsprayed control, the increases in grains m^{-2} in antitranspirant treatment may be from increased floret fertility which counteracted the effect of reduced developed florets on grains m^{-2}.

A possible reduction in photosynthesis by an antitranspirant treatment at any growth stage may reduce net amount of source available for grain filling. However, the purpose of an antitranspirant treatment prior to the most drought stress sensitive stage, which is meiosis in pollen mother cells, is to conserve water when conserving water is more important towards yield production than photosynthesis. Yield of modern wheat is more sink limited than source limited during grain filling (Foulkes *et al*., 2011; Reynolds *et al*., 2009), and It has been suggested that sink strength during grain filling is the main factor limiting yield potential in wheat (Reynolds *et al*., 2009). This might be one of the reasons why an increase in sink even with a small expense in source as serve by an antitranspirant application may ultimately bring about yield increases.

In order to satisfy the expected demand with increasing population, the global cereal production should increase by 50% within the first half of the 21st century (Rosegrant and Cline, 2003). At present, 45% of cereals used as food for human consumption is wheat (Chand, 2009). With limited cropping area and increasing water scarcity, the aim should be to achieve further production in wheat by increasing yield per unit area with a minimal amount of water (Condon *et al*., 2004; Foulkes *et al*., 2011; Reynolds *et al*., 2009). Introduction of cultivars with higher yield potential under drought conditions is one way of achieving this task (Foulkes *et al*., 2011; Reynolds *et al*., 2009). Wheat yield increases associated with the Green Revolution of the 1960s and 1970s were from the introduction of semidwarf cultivars with higher yield potential (Foulkes *et al*., 2011). However, the genetic gain in yield potential since the Green Revolution has been around 1% per year (Zhou *et al*., 2007), which, if continued, will not be enough to produce yield to meet the predicted demand (Reynolds *et al*., 2009). Raising yield potential further does not seem to be an easy task. Developing varieties of high water use efficiency with conventional breeding or genetic engineering techniques is a complex time-taking task, mainly since

the traits related to water use efficiency are controlled by many genes of which the contribution of each gene to the trait is low (Mayes *et al*., 2005). Therefore, increasing crop yield under drought conditions by agronomic practices can be regarded as more fruitful in the short term (Kettlewell *et al*., 2010). The influence in agronomic practices in increasing wheat water use efficiency/yield under drought conditions should not be underestimated. About half of the increase in wheat yield over the past 12 decades, up to the early 1980's was from the introduction of new cultivars and half from improved agronomy (Turner, 2004). Wheat yield increases associated with the Green Revolution and as well as yield increases achieved thereafter have been from a combination of improved agronomy coupled with suitable varieties (Turner, 2004).

Indeed, irrigation is a promising method to gain yield increases under drought conditions and irrigation management strategies can benefit water use efficiency in water limited environments (Howell, 2001). But irrigation can be expensive (Howell, 2001) and potential for irrigation is decreasing with increasing competition for water from urban and industrial needs and with the increasing need for conserving water in order to maintain environmental flows (Howell, 2001; Turner, 2004).

There are a number of agronomic practices that have the potential to increase wheat yield and water/rainfall use efficiency other than irrigation management strategies. One of the strategies is to increase the depth of rooting by eliminating physical constraints (e.g. compacted sub soils), ameliorating chemical constraints (e.g. soil acidity and alkalinity) and controlling biological constraints (e.g. root diseases and nematodes) so that water in deep down the soil profile can be exploited. Agronomic options for decreasing moisture/water losses from the soil and weeds are also important in increasing water use efficiency. These include weed control and minimisation of soil evaporation, deep drainage, surface runoff and lateral throughflow. Furthermore, practices such as crop rotation, timely use of fertilizer and timely planting provide opportunities to increase water use by crops (Turner, 2004). Although the importance of the use of film antitranspirants is currently underestimated, the results from this research may be helpful in the recognition

of the possible addition of film antitranspirants to the above agronomic practices for improving global crop production.

5.2 General conclusion

The chapter 2 included the studies carried out with the main objective of exploring the effect of film antitranspirants at different growth stages in relation to meiosis in pollen mother cells on yield and yield components of droughted wheat, with the purpose of determining the most effective growth stage to receive a film antitranspirant application targeted to increase yield under drought conditions. This objective was tested under three hypotheses, which were: Film antitranspirants increase yield of droughted wheat when applied before meiosis in pollen mother cells (GS41), which is the most sensitive stage of wheat yield formation to drought stress; the most effective growth stage to apply a film antitranspirant to increase yield under drought conditions may be GS31, GS33, GS39 or GS41; the increase of yield is by an increase in the number of grains. It can be concluded that, showing the most effective growth stage to apply a film antitranspirant to increase yield under drought conditions is GS33 (from the stages tested), two film antitranspirants used in the study, di-1-p-menthene and latex, increased yield by approximately 0.5 t/ha when sprayed at GS33. The yield increase by antitranspirant treatments was from an increase in grains m^{-2}. These results indicate that the three hypotheses tested were true.

One of the objectives of the experiments in 2008/2009 was to compare two winter wheat varieties, Claire and Einstein, to investigate possible differences in their response in yield and yield components to the antitranspirant/control treatments. It was hypothesised that the two varieties, Claire and Einstein are different in their response in yield and yield components to the antitranspirant treatments made around meiosis in pollen mother cells. Rejecting the hypothesis it was indicated that there was no significant difference between the two varieties in their response to antitranspirant/control treatments when yield and yield components were considered. It was postulated that there was little difference in the sensitivity of the two varieties to drought stress at the growth stages which are most sensitive to drought stress.

One of the objectives of the experiments in 2009/2010 was to investigate possible differences in the effect of antitranspirant treatments made around meiosis in pollen

mother cells on yield and yield components under two SMD regimes. It was hypothesised that the responses in yield and yield components to antitranspirant treatments made under different SMDs at application are different in winter wheat (variety Claire). Rejecting the hypothesis it was indicated that there was no significant difference between the two SMD regimes, in their response to antitranspirant/control treatments when yield and yield components were considered. The two SMD regimes were maintained from GS37 to GS69, and the difference in SMD in the two SMD regimes during that period might not be high enough to create a significant difference in yield or yield components between the two SMD regimes.

The chapter 3 included the studies carried out with the objective of exploring the underlying physiological mechanism by which film antitranspirants increase yield. It was hypothesised that film antitranspirants reduce transpiration and photosynthesis; the decrease in transpiration by the antitranspirant treatments is from increased resistance to diffusion of water vapour from stomata; the decrease in photosynthesis by the antitranspirant treatments is from decreased internal CO_2 concentration; reduced transpiration increases leaf water potential and alleviates the effect of drought on pollen viability at the stage of meiosis in pollen mother cells irrespective of reduced photosynthesis; film antitranspirants do not increase leaf temperature significantly.

It can be concluded that as hypothesised, the two film antitranspirants used in the study decreased the rate of transpiration under the spray characteristics of the study. At the occasions when transpiration was decreased, antitranspirant treatments significantly decreased internal CO_2 concentration as well. However, decrease in internal CO_2 concentration by the antitranspirant treatments did not significantly decrease the rate of photosynthesis. The antitranspirant, di-1-pmenthene increased leaf water potential significantly. The increase in leaf water potential in an antitranspirant treatment compared to an unsprayed control might be from direct conservation of water with in the leaves as a result of reduced transpiration and/or from delayed development in SMD in antitranspirant treatments compared to unsprayed controls as a result of reduced transpiration. The difference in temperature between antitranspirant treatments and unsprayed controls was

less than 1 ℃, and was not significant at most of the occasions. Therefore, curtailed transpiration by antitranspirants did not increase leaf temperatures to a point which is threat full for plants as suggested by some publications. In the variety Claire meiosis in pollen mother cells occurs at early GS41. The percentage pollen viability in di-1-p-menthene treatments at GS33 was significantly higher than that of unsprayed controls. As hypothesised, decreased transpiration by antitranspirant treatments at GS33 might have ameliorated the effect of drought on pollen fertility during meiosis in pollen mother cells.

Apart from the main studies mentioned above the spray distribution pattern, leaf coverage and stomatal coverage of the most frequently used film antitranspirant (di-1-p-menthene), on wheat leaves was assessed by image analysis and electron microscopy, and studies were carried out to identify the growth stage of winter wheat at the time of meiosis in pollen mother cells. The leaf coverage by the antitranspirant was 15.80 %. The electron microscopic studies revealed that spray droplets occur as patches on the leaf (not as a continuous film). The study focused to determine the stage of meiosis in pollen mother cells showed that pollen mother cell meiosis occurs at early GS41.

The studies included in chapter 4 attempted to explore the effect of antitranspirants on one of the drought stress responsive invertase genes expressed in anthers. It was hypothesised that film antitranspirants increase pollen viability by alleviating the effect of drought stress on the expression of drought stress sensitive invertase genes, which are down regulated under drought stress leading to pollen sterility. The results did not show a significant difference in the expression level of the gene between antitranspirant/unsprayed control treatments. If the sensitivity of the study is increased by increasing the size of the pollen pool sampled from each plot and by increasing the number of replication, the results may show a significant difference between antitranspirant/unsprayed control treatments. It can be concluded that the results did not show evidence to support the hypothesis.

When the results across the three experimental chapters are studied a clear relationship between the effects of film antitranspirants on yield, yield components, pollen fertility, leaf

water potential and leaf gas exchange, particularly the rate of transpiration can be seen. It is clear that the decreased transpiration by the antitranspirant increased leaf water potential which led to an increase in pollen fertility and then grains m^{-2} and yield in turn. This study is an eye-opener which points out the prospects for antitranspirants in food security in the future world especially in semi arid and aid areas.

5.3 Further studies

There are a number of further studies which can be performed to understand better the effect of film antitranspirants on food crops and to optimise the use of film antitranspirants on wheat, as well as on other food crops.

The leaf coverage obtained under the spray characteristics used in this study are discussed in chapter 3, and it was evident that less than 80% of the leaf is covered by spray deposits of di-1-p-menthene under the spray characteristics used. However, this figure might be an underestimation of the actual leaf coverage as discussed, especially because of any alterations done to the static contact angle of the spray droplets on the leaf by the tracer dye used. Therefore, further studies should be performed with different tracer dyes to determine the leaf coverage of di-1-p-menthene, as well as, the latex antitranspirant, under the spray characteristics used in this study. Determining the leaf coverage precisely is important, because, then the leaf coverage under different spray characteristics can be compared. Thereafter, the relationship between the leaf coverage and the performance of the film antitranspirant in terms of the effect on pollen viability, yield and yield components can be explored in order to find out the optimum leaf coverage. The spray characteristics related to that optimum leaf coverage can be used in future commercial and research based application of that particular film antitranspirant on the crop, which has been used in the optimisation studies. The optimum leaf coverage/spray characteristics may differ from film antitranspirant to film antitranspirant with the difference in physical properties of the film antitranspirants and also from crop to crop with the differences in leaf surface properties (as discussed in section 3.4.1). Therefore, optimum spray characteristics may have to be determined for each film antitranspirant and also for each crop on which the film antitranspirant to be used.

The exploration of the temporal changes of film antitranspirant deposits on leaves at different environmental conditions would be helpful to find out the duration and degradation patterns of antitranspirant deposits on leaves under different environmental conditions.

In this study the effect of antitranspirant treatments made once in crop life time was explored. It will be interesting to explore the effect of film antitranspirants treated twice in crop lifetime, for example at GS33 and GS41. However, antitranspirant treatments made twice on a crop may decrease assimilate supply to developing ear affecting crop yield in a way which cannot be counteracted by the advantageous effects of film antitranspirants. Furthermore, if there is any yield increase by applying antitranspirants twice, that may not commercially advantageous over the cost of spraying antitranspirant twice.

It would be important to explore the performance of film antitranspirants under natural drought conditions in different agro-ecosystems, where wheat is grown in the world. The persistence of antitranspirant spray droplets on leaves may be different in different environmental conditions. Furthermore, the extent to which film antitranspirants conserve moisture in the soil may be different from soil to soil and environment to environment. The experiments in this study were performed under polytunnels in artificial drought conditions, which were designed to resemble natural drought years in UK, where there was no rainfall throughout late tillering, stem elongation and anthesis stages but pouring from about GS69 to harvest time. However, the environmental conditions other than SMD might be different in side polytunnels compared to a natural environment in drought seasons.

Research is needed to explore whether drought stress sensitive stages of other food crops are also responding positively to film antitranspirant application. Optimising the use of film antitranspirants on food crops will be beneficial for food security and sustainability with the predicted climate changes.

In this study the effect of film antitranspirants on quality characteristics of the wheat grain was not explored. It has been reported that drought stress during tillering reduces lipid content in the grain (Singh *et al*., 1971). Furthermore, Grain protein content was increased by drought stress at anthesis (Jamal *et al*., 1996). In general, the proteins are synthesized essentially from photosynthate produced before grain filling, whereas starch is produced from the net assimilation of CO_2 after anthesis. Therefore, protein and starch deposition in the process of grain filling do not proceed simultaneously, that is, the rate of protein

accumulation may reach a peak before that of starch. Hence, drought stress during the grain filling period mainly affects starch accumulation, and has little effect on nitrogen/protein accumulation (Herzog and Stamp, 1983). Therefore, drought during grain filling alters grain protein/starch ratio affecting the grain quality (Herzog and Stamp, 1983; Beltrano *et al*., 2006; Ozturk and Aydin, 2004). It is useful to know how film antitranspirant applications alter the effect of drought at these stages on quality characteristics of the grain.

More research should be performed to understand the effect of film antitranspirants on physiological processes affected by drought stress. Photosynthesis is impaired under mild drought stress mainly due to stomatal closure and decreased CO_2 entering the leaf, but under severe drought stress due to metabolic impairments (reviewed in section 1.3.1). It is established that film antitranspirants decreases photosynthesis (section 1.4.2) by decreasing CO_2 entering the leaf, however, film antitranspirants may favour photosynthesis under severe drought stress conditions because of stress alleviating effects of the antitranspirants. This idea can be researched by exploring the effects of film antitranspirants on RuBP generation, rubisco activity and reactive oxygen species under severe drought conditions.

More research should be performed to understand the molecular basis of the underlying mechanisms by which film antitranspirants increase yield. The study performed to understand the effect of film antitranspirants on the expression of one of the drought stress sensitive invertase genes, down regulated under drought stress, did not show any significant difference in the level of gene expression between antitranspirant/control treatments. If the number of ears, used in anther collection, per each treatment is increased, the sensitivity of the test can be increased. Understanding the effect of film antitranspirants on the expression of drought stress responsive genes encoding enzymes hormones and other compounds may be helpful to understand the molecular basis of the underlying mechanisms by which film antitranspirants increase yield. The impotence in such studies is that, the knowledge obtained by these studies can also be applied to other

research disciplinary within the area of enhancing crop performance under drought stress including by genetic manipulation.

Research is needed in exploring plant extracts which can be used as film antitranspirants. For the plant extracts, which are identified as of with film antitranspirant properties, cost effective processes of extraction and production should be developed.

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Appendices

Appendix I. a: The crop management inputs for the experiments in 2008/2009 for which seeds were sown on 26.11.08.

Date	Input	Rate of application
11.03.2009	Herbicide: Ipu	2 (l/ha)
	Herbicide: Stomp	2 (l/ha)
	Herbicide: Vixen	40 g/ha
	Pesticide: Permaset	0.25 (l/ha)
13.03.2009	Fertilizer: Doubletop (27% N, 30% SO_3)	150 kg/ha
09.04.2009	Fertilizer: Nitropril (34.5% N)	130 kg/h
12.05.2009	Fungicide: Tracker	1 (l/ha)
	Fungicide: Attenzo	0.2 (l/ha)
24.05.2009	Fungicide: Tracker	1.25 (l/ha)
	Fungicide: Ceando	0.5 (l/ha)
	Herbicide: Tomahawk	0.75 (l/ha)
	Herbicide: Ally	20 (g/ha)
06.07.2009	Pesticide: Aphox	280 (g/ha)

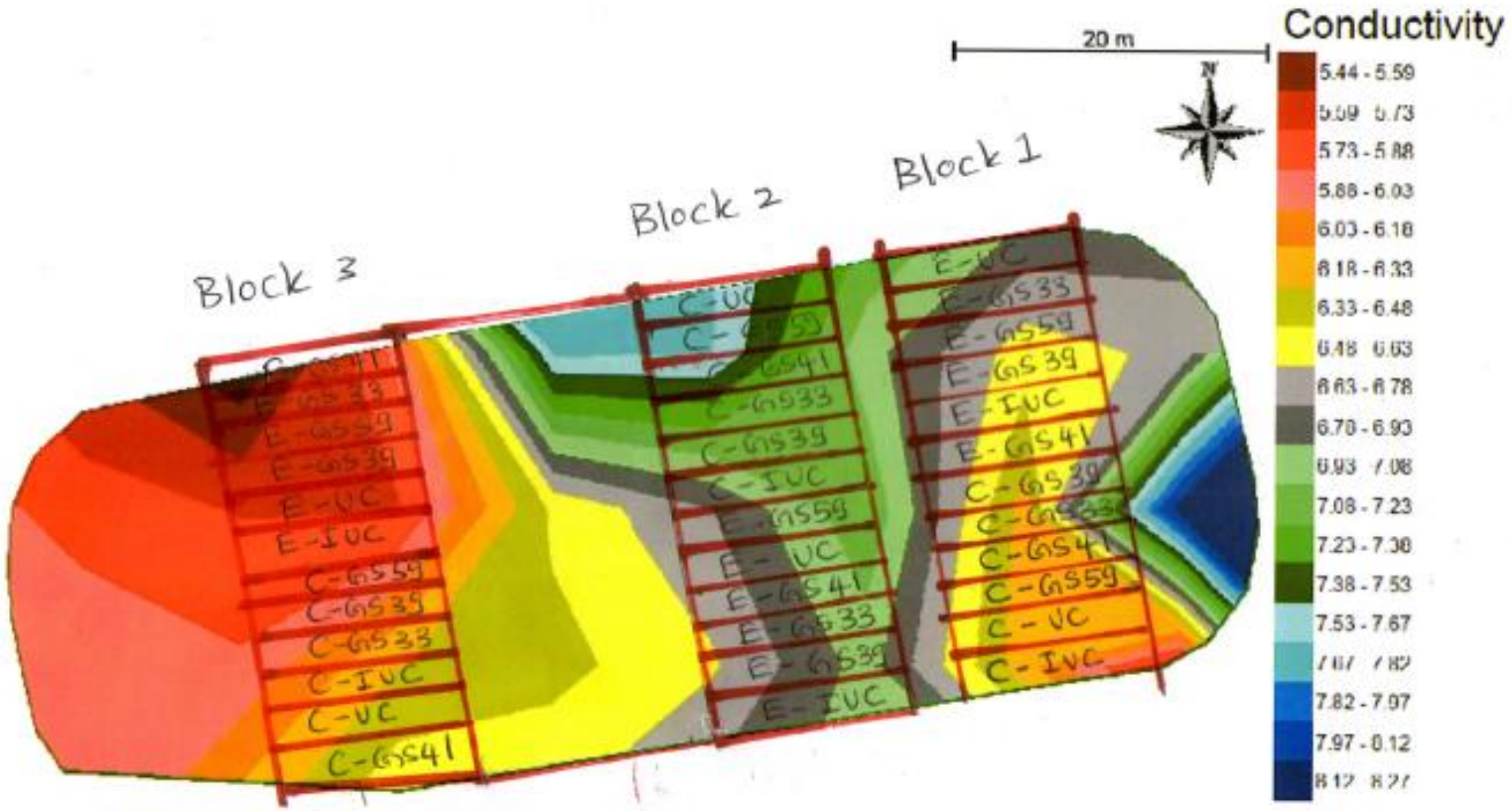
Appendix I. b: The crop management inputs for the experiments in 2009/2010 for which seeds were sown on 19.10.2009.

Date	Input	Rate of application
10.12.2009	Fertilizer: Gradnate (mainly N)	0.025 (l/ha)
	Fertilizer: Hallmark (mainly P)	0.05 (l/ha)
16.04.2010	Fungicide: Jaunt	0.6 (l/ha)
	Fungicide: CCC	2 (l/ha)
	Fungicide: Flexity	0.2 (l/ha)
	PGR: Moddus	0.2 (l/ha)
14.05.2010	Fungicide: Jaunt	0.6 (l/ha)
	Fungicide: Flexity	0.2 (l/ha)
	Herbicide: Finish sx	0.06 (kg/ha)

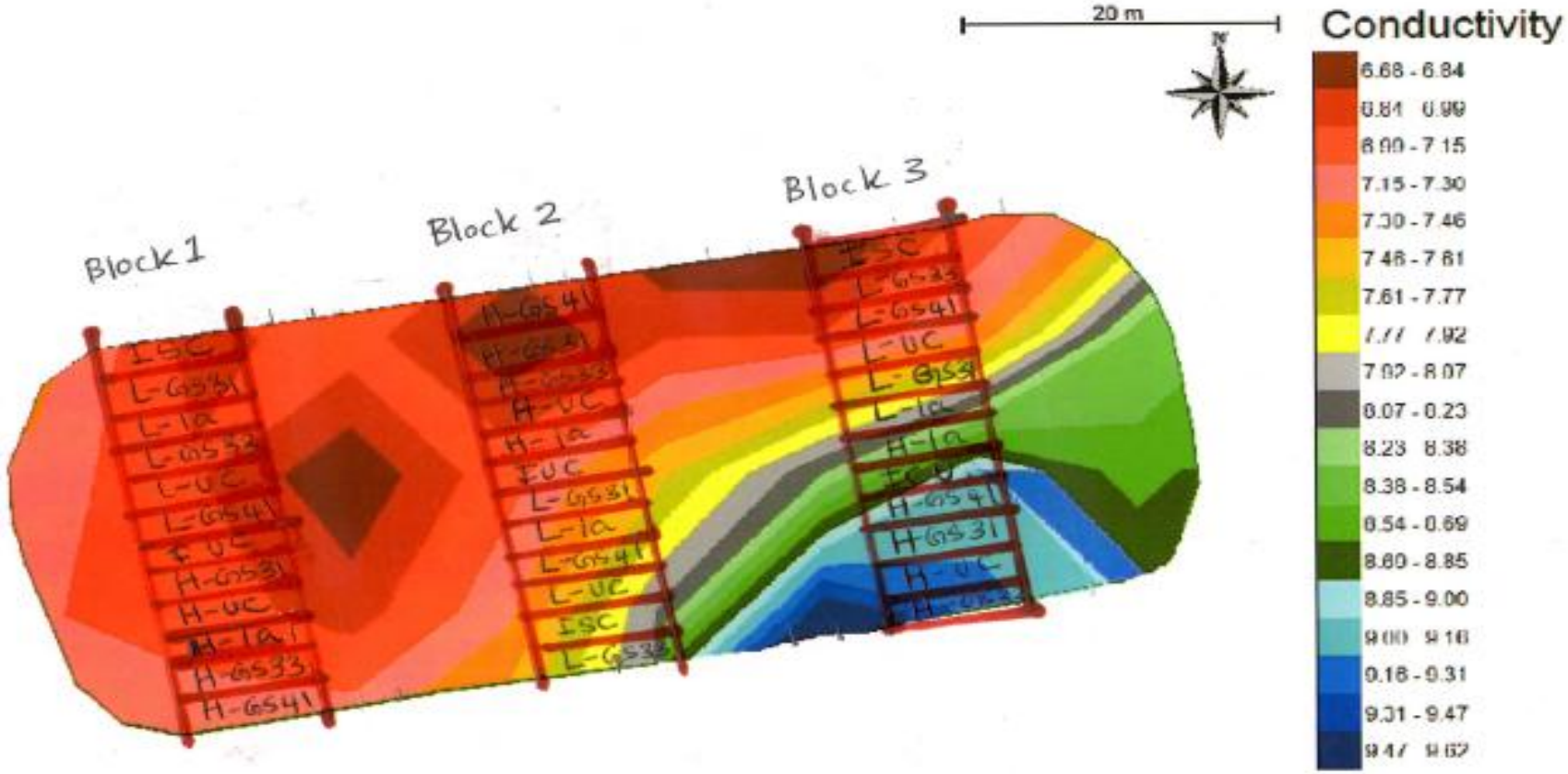
Appendix I. c: The crop management inputs for the experiments in 2010/2011 for which seeds were sown on 18.01.2011.

Date	Input	Rate of application
01.04.2011	Herbicide: Harmony	125 (g/ha)
	Herbicide: Compitox Plus	1.25 (l/ha)
	Herbicide: Magonese	1 (l/ha)
20.04.2011	Fertilizer: Gradnate (mainly N)	0.025 (l/ha)
	Fertilizer: Hallmark (mainly P)	0.05 (l/ha)
26.04.2011	Fungicide: Opus	0.75 (l/ha)
	Fungicide: Bravo	1 (l/ha)
	PGR: Agrovista3	1 (l/ha)
17.06.2011	Fungicide: Corbel	0.5 (l/ha)
24.06.2011	Pesticide: Standon	250 (g/ha)

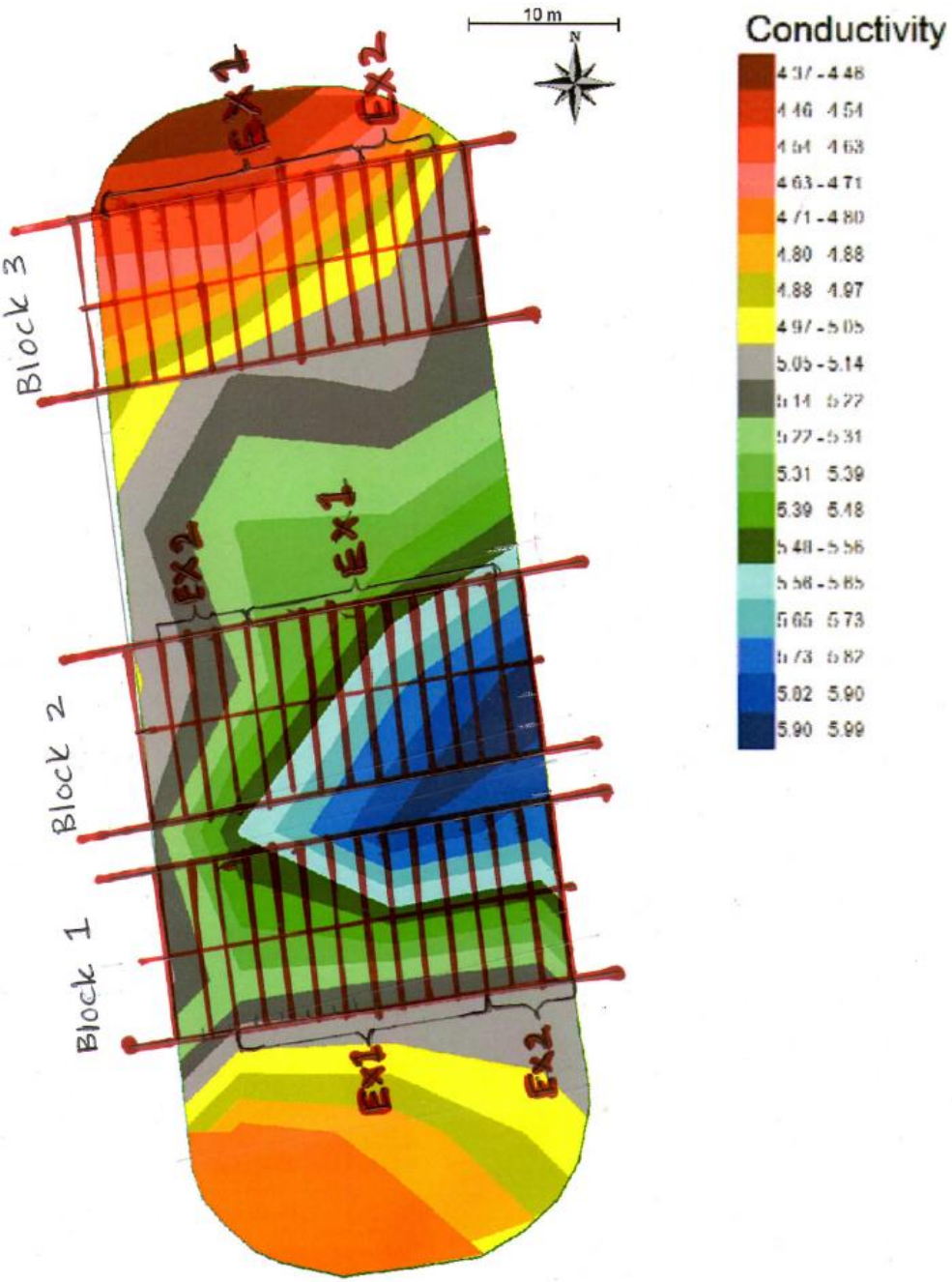
Appendix I. a: The soil electric conductivity map for the experiments in 2008/2009; labels are same as the labels presented in the layout of the experiment in section 2.2.3.1.



Appendix I. b: The soil electric conductivity map for the experiments in 2009/2010; labels are same as the labels presented in the layout of the experiment in section 2.2.3.2.



Appendix I. c: The soil electric conductivity map for the experiments in 2010/2011; labels are same as the labels presented in the layout of the experiment in section 2.2.3.3.



Appendix III: Daily weather data for each growing season of field experimentation (2008/2009, 2009/2010 and 2010/2011)

2008 - October					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	4.5	13.8	9.5	74.8	4.6
2	1.6	13.7	5.9	99.1	3.6
3	3.3	11.7	5.6	80.4	0.0
4	4.2	14.7	2.3	99.8	31.8
5	2.3	12.4	6.7	95.0	2.2
6	3.2	16.0	1.9	87.2	1.4
7	5.8	18.3	9.4	98.2	5.2
8	1.1	17.2	5.0	90.4	0.0
9	1.0	17.6	3.5	96.6	0.0
10	3.0	17.4	10.8	69.6	0.0
11	1.0	15.5	12.9	87.5	8.2
12	1.0	20.2	9.3	93.0	0.0
13	1.4	18.2	12.2	93.3	0.0
14	1.8	15.9	11.5	91.4	6.2
15	0.6	13.5	10.7	95.0	0.6
16	1.5	13.5	4.2	84.5	0.0
17	0.0	15.9	3.6	85.4	0.0
18	1.5	15.7	6.2	83.2	0.4
19	2.8	15.3	10.5	88.6	1.4
20	4.2	16.2	12.2	92.9	2.2
21	2.2	12.3	4.5	76.7	0.0
22	1.0	14.8	2.9	83.7	0.0
23	2.8	14.3	5.6	85.6	1.2
24	0.0	13.4	5.5	94.0	0.0
25	3.2	13.7	3.9	97.3	16.2
26	0.0	14.5	7.8	96.0	0.2
27	1.2	10.2	2.2	89.4	3.8
28	0.0	5.5	0.5	94.0	6.2
29	0.0	6.3	-3.2	67.8	7.4
30	3.3	6.3	1.4	88.3	0.0
31	3.9	8.0	0.9	89.2	0.0
Mean		13.9	6.0		

2008 - November					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	2.3	9.2	-3.6	68.3	6.2
2	4.9	8.2	2.4	93.4	0.0
3	0.0	12.2	3.3	97.0	0.2
4	2.7	10.5	6.2	98.0	0.2
5	1.5	10.2	9.1	98.0	0.0
6	0.0	9.9	7.9	96.0	2.2
7	1.2	12.6	6.5	96.1	1.2
8	3.7	14.0	7.0	95.0	3.4
9	3.0	9.5	3.8	85.2	14.0
10	3.6	7.7	4.6	85.5	2.4
11	2.6	10.5	5.7	93.6	0.4
12	0.6	11.7	5.7	96.0	1.0
13	2.1	12.1	2.4	96.0	3.4
14	1.1	14.1	7.7	98.8	0.0
15	2.7	15.1	10.6	91.5	3.8
16	2.5	10.5	8.9	9.3	2.2
17	4.0	10.3	1.5	9.4	2.2
18	5.2	11.0	5.6	94.3	0.0
19	0.9	13.0	6.6	91.3	0.0
20	4.6	12.0	7.9	86.4	1.0
21	6.1	8.9	7.4	90.1	2.0
22	1.1	6.1	3.4	83.5	5.6
23	1.4	8.3	-1.9	94.0	7.6
24	4.8	7.7	3.5	83.2	0.2
25	4.5	7.4	1.6	80.4	0.0
26	0.3	12.9	-0.9	91.0	0.0
27	5.3	11.8	7.4	86.4	1.6
28	0.9	3.6	-0.1	86.0	0.0
29	0.7	0.5	0.4	96.0	0.0
30	2.4	2.5	-1.4	100.0	0.0
31					
Mean		9.8	4.3		

2008 - December					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	0.0	3.9	-5.7	82.0	0.8
2	0.0	5.9	-3.2	95.0	0.4
3	0.0	6.1	-3.1	96.0	3.2
4	3.6	8.0	-1.5	88.0	0.4
5	0.0	6.3	0.3	95.0	8.2
6	0.0	6.5	-0.7	96.0	0.0
7	0.0	7.0	-4.7	96.0	0.0
8	2.2	5.5	-3.3	89.0	5.6
9	0.0	5.2	-0.9	95.0	2.6
10	0.0	5.1	-3.3	96.0	0.0
11	0.0	4.1	-3.0	67.0	0.6
12	3.1	8.6	-1.6	95.0	15.4
13	1.1	5.8	1.3	92.0	4.0
14	0.0	4.2	0.4	95.0	0.0
15	0.0	3.7	-2.3	94.0	0.0
16	2.3	7.9	-1.2	92.0	1.8
17	0.0	10.5	-0.6	91.0	0.0
18	4.2	12.3	-0.3	94.0	0.4
19	0.2	11.8	0.5	91.0	2.0
20	0.8	12.1	0.9	94.0	0.2
21	2.4	12.6	8.3	92.5	0.2
22	0.0	12.8	9.1	98.0	0.0
23	1.3	7.4	6.4	95.0	0.0
24	0.0	8.4	6.0	96.0	0.0
25	1.1	7.2	5.4	96.0	0.0
26	2.6	4.6	2.6	96.5	0.0
27	3.2	3.4	0.6	94.3	0.0
28	1.6	4.6	2.0	89.7	0.0
29	3.0	1.8	0.5	83.5	0.0
30	1.7	0.6	-1.0	90.9	0.0
31	0.0	-2.4	-4.9	96.0	0.0
Mean		6.5	0.1		

2009 - January					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	0.9	-0.4	-3.5	84.0	0.0
2	0.0	4.0	-2.7	96.0	0.0
3	0.0	-2.2	-6.5	95.0	0.0
4	0.0	0.6	-6.7	92.0	1.8
5	0.7	2.6	-4.6	80.0	0.0
6	0.0	0.5	-9.2	91.0	0.0
7	0.0	1.6	-9.0	84.0	0.8
8	0.0	5.7	-0.4	97.0	0.0
9	0.0	3.9	-0.4	95.0	0.0
10	3.4	5.8	-3.8	96.0	0.0
11	5.8	9.8	-2.4	94.0	7.0
12	4.5	10.0	5.1	89.0	10.2
13	0.5	9.5	4.8	97.0	1.6
14	0.0	7.7	-2.0	94.0	0.0
15	5.1	8.7	-0.5	96.0	0.0
16	3.6	9.8	5.4	95.0	4.6
17	3.7	9.0	5.2	92.7	11.8
18	1.6	7.1	0.8	97.0	0.0
19	1.5	6.8	2.0	94.0	1.6
20	2.8	7.0	0.5	95.0	0.0
21	1.5	9.8	-1.0	95.0	3.8
22	2.9	8.6	1.1	96.0	4.2
23	4.3	7.9	2.7	91.0	0.0
24	0.0	7.7	-1.5	95.0	2.2
25	3.7	7.8	0.3	94.0	0.0
26	1.7	8.2	2.6	94.0	0.0
27	1.0	6.9	-0.7	95.0	19.0
28	2.0	6.8	0.9	96.0	0.2
29	3.7	6.2	4.6	93.0	0.4
30	2.9	6.1	2.1	94.0	0.4
31	3.4	7.1	2.8	96.8	0.0
Mean		6.2	-0.5		

2009 - February					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	4.8	3.1	0.9	86.6	0.0
2	1.8	1.0	-1.9	94.0	4.0
3	1.9	2.7	-2.6	81.0	0.0
4	1.3	5.8	-2.2	97.0	2.8
5	0.7	1.2	-1.0	89.0	2.6
6	2.6	3.3	-0.6	86.0	0.2
7	0.5	4.9	-3.1	99.4	0.0
8	0.0	3.8	-3.1	87.0	1.2
9	0.6	3.5	-1.7	97.0	5.8
10	4.2	5.6	0.6	82.0	0.0
11	0.0	6.7	-2.0	71.0	2.6
12	0.2	7.4	-4.4	80.0	2.0
13	0.0	7.6	-1.4	81.0	0.0
14	1.6	6.4	1.7	89.0	0.0
15	2.5	10.1	3.2	95.0	0.0
16	1.1	11.7	3.2	91.0	0.0
17	2.4	9.7	5.9	86.0	1.2
18	0.0	10.3	6.6	88.0	0.0
19	0.0	10.9	2.5	83.0	0.2
20	0.0	9.9	-2.2	93.5	0.0
21	1.1	12.3	1.6	99.5	0.0
22	4.1	11.1	5.0	91.1	0.0
23	2.8	10.7	7.7	94.5	0.0
24	1.1	11.3	7.3	56.0	0.0
25	3.2	12.2	7.2	96.0	0.0
26	2.4	10.7	3.1	95.0	0.0
27	2.5	12.0	6.2	96.4	0.4
28	1.1	10.9	5.6	86.0	0.0
29					
30					
31					
Mean		7.7	1.5		

2009 - March					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	1.8	12.2	3.9	96.0	0.0
2	2.8	10.8	-0.5	99.0	0.6
3	3.1	8.3	3.9	91.0	7.4
4	1.4	8.3	-1.3	91.8	0.0
5	1.3	8.3	-2.1	95.0	0.2
6	0.0	10.2	-3.1	78.1	0.2
7	3.8	11.7	2.9	97.8	0.0
8	5.2	7.7	3.7	81.8	3.4
9	4.8	10.0	2.7	85.1	5.2
10	1.9	10.0	5.3	92.0	0.2
11	0.4	13.1	-1.1	93.0	0.0
12	3.3	14.7	5.5	99.0	0.0
13	2.2	10.8	4.9	90.0	0.0
14	5.8	12.8	7.4	77.5	0.0
15	1.8	14.7	4.6	87.1	0.0
16	0.0	16.0	2.9	90.8	0.0
17	1.1	10.0	1.7	90.0	0.0
18	2.1	13.1	4.7	88.0	0.0
19	0.2	14.5	0.3	84.0	0.0
20	2.8	13.0	3.5	99.0	0.0
21	1.0	13.4	-1.2	89.9	0.0
22	2.3	13.7	1.6	92.8	0.0
23	6.9	11.8	5.0	82.5	2.2
24	4.1	12.4	2.9	91.8	1.8
25	6.7	11.2	5.9	94.1	0.8
26	2.8	12.5	5.0	74.0	0.2
27	4.5	10.4	3.7	78.0	1.0
28	6.0	8.8	4.0	89.3	3.0
29	1.2	10.7	-2.8	79.7	0.0
30	1.8	12.7	0.6	96.4	0.0
31	0.0	15.0	6.4	84.4	0.0
Mean		11.7	2.6		

2009 - April					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	0.0	16.7	4.7	87.0	0.0
2	0.0	13.9	5.9	95.4	0.0
3	1.2	14.4	4.8	96.0	1.6
4	3.2	14.2	5.3	83.5	0.0
5	2.3	14.5	0.0	81.3	0.0
6	2.1	13.8	5.2	91.2	1.0
7	3.5	14.2	4.3	85.7	1.2
8	4.0	14.6	6.9	57.0	1.8
9	3.5	15.1	4.5	83.0	1.0
10	2.9	13.4	9.7	94.1	2.2
11	1.7	15.8	4.6	88.4	0.0
12	0.0	15.2	0.8	92.8	0.0
13	1.7	16.3	3.6	98.0	0.0
14	2.6	15.4	6.2	90.1	4.2
15	4.0	16.6	8.7	97.9	0.2
16	3.4	11.4	9.1	97.7	5.0
17	1.1	15.0	7.5	96.0	0.0
18	2.8	14.1	0.7	93.1	0.0
19	0.6	15.9	4.3	97.0	0.0
20	1.0	20.0	1.7	81.7	0.0
21	2.0	18.2	1.9	86.7	0.0
22	0.1	17.0	3.0	98.0	0.0
23	2.4	17.2	5.2	90.1	0.0
24	3.4	18.4	9.2	72.7	5.2
25	2.2	16.2	8.6	96.0	0.2
26	1.9	16.6	2.5	78.6	8.8
27	0.7	11.7	5.8	95.0	2.4
28	1.4	12.1	3.8	87.0	4.2
29	3.5	17.3	3.3	89.8	0.0
30	3.7	15.1	9.7	88.0	2.0
31					
Mean		15.3	5.1		

2009 - May					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	1.4	17.4	10.5	82.1	0.6
2	0.7	16.8	5.4	73.1	0.2
3	3.3	14.0	5.8	77.7	0.8
4	1.1	15.1	2.5	97.0	1.8
5	1.7	16.1	8.9	96.0	0.2
6	2.4	17.9	11.4	84.8	0.0
7	2.9	17.6	6.6	69.5	1.0
8	3.3	15.1	5.6	72.7	0.2
9	2.0	15.7	6.5	80.1	0.0
10	0.0	16.9	4.4	80.4	0.0
11	5.0	15.3	5.8	91.1	0.0
12	5.0	16.0	5.8	65.9	0.0
13	3.2	13.3	6.5	95.5	0.0
14	2.1	12.8	9.9	84.0	16.4
15	0.6	17.0	9.5	95.0	4.6
16	4.2	15.7	5.4	89.1	0.8
17	4.3	14.0	5.1	86.4	9.4
18	3.9	15.2	7.6	95.0	0.8
19	2.1	16.6	7.6	93.0	2.0
20	1.8	17.6	5.8	85.6	7.2
21	2.5	17.3	7.4	87.9	0.2
22	0.7	16.2	6.8	94.8	0.0
23	0.7	20.9	9.8	83.9	0.0
24	1.0	21.8	3.9	74.5	0.0
25	2.5	20.6	6.4	88.5	4.0
26	4.8	16.4	10.0	65.0	0.0
27	1.8	18.8	7.1	93.9	0.0
28	0.6	23.8	11.0	89.0	0.0
29	1.7	24.8	8.6	77.3	0.0
30	2.1	22.8	8.1	70.0	0.0
31	2.0	23.7	7.5	72.0	0.0
Mean		17.5	7.2		

2009 - June					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	2.6	24.7	7.0	42.0	0.0
2	0.0	27.0	7.6	45.9	0.0
3	2.2	16.1	11.2	87.8	0.0
4	0.0	19.2	5.1	58.0	0.0
5	0.9	18.1	7.0	63.4	14.2
6	2.3	11.6	8.9	98.0	8.8
7	4.3	13.5	9.1	96.4	14.8
8	4.1	16.8	8.5	65.0	0.0
9	2.7	16.5	7.6	71.2	5.8
10	1.4	16.8	9.3	94.1	17.4
11	1.8	16.7	5.9	80.4	0.0
12	0.9	18.9	1.9	64.7	0.0
13	1.1	23.6	11.3	68.2	0.0
14	0.9	23.1	9.2	69.6	0.0
15	1.0	21.4	8.5	69.8	1.8
16	0.2	22.2	6.5	74.1	0.0
17	1.9	16.4	11.1	91.3	5.0
18	0.6	17.8	5.9	87.6	0.0
19	2.6	16.9	7.8	77.0	2.0
20	1.2	18.8	9.8	89.0	0.0
21	1.9	17.6	10.7	88.2	0.0
22	0.4	22.0	12.8	89.0	0.0
23	0.7	25.4	10.3	84.0	0.0
24	2.4	23.1	10.1	66.8	0.0
25	2.4	23.6	10.4	84.8	0.0
26	1.4	22.3	11.8	95.4	20.8
27	0.0	23.3	16.2	95.0	0.4
28	1.7	24.5	16.2	82.7	0.0
29	2.1	27.8	15.9	83.5	1.2
30	1.0	25.2	16.8	91.1	0.0
31					
Mean		20.4	9.7		

2009 - July					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	0.0	28.8	18.6	68.9	0.0
2	2.2	28.7	17.5	65.7	0.0
3	0.1	23.1	17.5	96.4	0.4
4	1.7	23.9	11.0	84.6	missing data
5	0.0	23.3	15.3	76.2	missing data
6	1.0	19.0	13.1	86.1	missing data
7	1.3	19.7	10.0	96.0	6.6
8	0.6	19.8	10.8	85.7	0.0
9	2.9	19.2	6.6	74.1	0.0
10	3.5	20.7	5.5	56.8	missing data
11	0.0	22.7	13.3	99.5	missing data
12	1.5	21.5	13.5	79.7	6.5
13	1.6	22.4	9.3	75.5	2.6
14	1.6	21.3	8.9	89.0	3.0
15	1.2	22.8	13.9	84.0	1.2
16	0.9	22.3	9.7	73.2	8.6
17	3.1	16.2	13.5	98.4	7.4
18	2.4	20.6	13.1	77.9	2.6
19	0.0	19.6	11.7	93.6	9.0
20	1.6	21.6	8.3	73.7	3.0
21	2.7	18.9	10.7	96.0	3.8
22	2.4	19.9	12.0	92.0	1.0
23	1.7	21.6	12.3	70.5	2.4
24	1.3	19.2	10.5	79.5	missing data
25	1.2	22.6	7.8	70.7	missing data
26	2.9	17.7	11.9	97.0	25.9
27	0.7	20.3	11.7	94.1	1.8
28	1.5	19.8	9.7	81.6	4.6
29	2.2	16.4	13.3	97.0	12.6
30	1.8	18.0	8.6	76.3	1.8
31	2.2	20.0	8.0	69.7	5.8
Mean		21.0	11.5		

2009 - August					
DATE	**Wind sp m/s**	**Max temp ºC**	**Min temp 24 hrs ºC**	**RH %**	**Total rain 24 hrs mm**
1	0.8	17.9	12.5	98.0	1.8
2	0.8	20.8	8.4	66.1	0.0
3	3.1	21.3	11.3	83.2	1.2
4	2.7	22.0	15.9	98.6	2.8
5	0.5	23.8	14.7	85.7	0.2
6	0.7	21.8	10.4	74.0	0.0
7	1.7	23.5	10.7	75.1	0.0
8	0.8	22.5	7.9	74.3	0.0
9	0.0	24.4	8.0	72.5	1.0
10	1.5	21.6	13.5	89.5	0.0
11	1.0	25.3	12.9	77.2	9.2
12	0.8	21.8	15.7	93.0	2.0
13	2.4	20.6	11.3	83.5	0.0
14	0.0	21.8	9.9	74.1	0.0
15	1.9	24.1	16.7	81.6	0.0
16	2.0	20.6	10.8	72.8	0.6
17	2.0	22.0	12.6	77.4	0.0
18	2.0	22.2	10.7	84.9	0.0
19	2.3	25.8	14.7	81.6	0.0
20	1.9	21.8	17.3	81.2	0.2
21	0.9	19.0	10.4	77.5	2.8
22	0.0	23.4	7.8	73.9	0.0
23	3.0	21.9	14.0	80.6	0.6
24	0.0	18.9	13.8	97.3	3.0
25	1.3	21.5	9.4	74.4	5.6
26	4.5	19.4	10.5	97.4	3.4
27	0.8	22.4	12.6	78.2	0.6
28	1.7	16.9	8.8	98.3	1.6
29	2.2	19.3	7.3	70.0	0.0
30	0.0	21.0	9.7	87.6	0.6
31	2.5	21.9	15.1	94.7	0.6
Mean		21.7	11.8		

2009 - October					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	2.3	16.3	11.6	78.3	0.0
2	1.3	16.7	8.9	70.5	0.0
3	3.0	16.3	12.8	86.6	0.2
4	0.6	17.2	5.1	75.2	0.0
5	0.5	15.8	3.4	88.7	7.8
6	1.5	20.8	8.3	97.0	14.8
7	0.6	12.4	4.5	76.1	1.2
8	0.0	14.5	1.4	96.0	0.0
9	1.7	14.2	3.8	83.3	0.8
10	0.0	18.3	5.8	97.0	0.0
11	0.8	17.1	11.6	95.4	0.0
12	0.0	15.7	3.1	91.1	0.0
13	0.0	16.4	0.8	96.3	0.0
14	0.0	17.1	7.3	95.7	0.0
15	0.0	15.9	11.7	93.0	0.0
16	2.3	14.8	11.6	75.7	0.0
17	0.0	13.2	1.9	97.1	0.0
18	0.2	13.5	1.6	80.7	0.0
19	1.5	15.3	6.9	90.0	0.0
20	3.1	12.9	6.3	87.0	2.2
21	1.2	15.1	8.8	89.0	0.2
22	1.9	15.2	10.0	97.5	3.0
23	1.6	17.0	9.7	98.0	1.4
24	2.6	18.3	11.0	96.0	0.8
25	2.6	15.7	12.0	93.0	3.2
26	0.6	16.7	11.4	76.0	1.2
27	1.2	16.6	10.6	90.0	0.0
28	0.0	18.8	9.8	90.2	0.0
29	0.5	16.6	6.3	94.0	0.0
30	2.0	15.3	11.3	96.5	3.6
31	0.0	17.9	12.2	95.0	13.0
Mean		16.0	7.8		

2009 - November					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	5.3	16.8	10.2	96.0	2.8
2	1.3	11.9	5.4	82.8	6.2
3	2.1	13.7	7.7	98.0	0.6
4	1.9	12.1	2.8	88.5	11.8
5	2.7	12.8	6.0	99.5	4.2
6	1.2	10.7	3.9	89.0	4.6
7	0.3	10.2	0.5	99.2	0.2
8	1.2	9.5	4.9	99.9	0.0
9	0.0	6.5	1.5	98.0	1.2
10	0.6	8.2	2.0	99.0	0.8
11	1.2	8.7	5.8	92.0	1.0
12	1.2	12.7	4.7	90.0	3.8
13	0.0	14.1	6.9	95.0	13.2
14	5.6	12.4	7.3	89.0	0.6
15	2.2	12.7	4.9	89.0	9.2
16	2.4	13.4	6.1	90.0	1.8
17	3.1	13.4	4.9	95.1	6.4
18	5.6	14.5	9.0	94.8	0.6
19	3.9	15.6	11.1	78.4	7.8
20	3.6	14.4	12.1	89.3	0.0
21	3.2	14.1	5.0	94.0	10.0
22	1.9	9.8	6.6	94.0	4.2
23	1.6	13.5	7.3	96.0	8.6
24	4.1	13.9	8.4	92.1	11.2
25	5.3	10.7	6.5	88.0	4.4
26	3.9	9.4	5.3	93.4	1.2
27	1.8	9.2	2.6	94.9	3.4
28	1.3	6.1	1.6	95.0	3.4
29	2.3	8.3	2.9	99.0	0.2
30	3.1	5.6	1.9	96.4	0.0
31					
Mean		11.5	5.5		

2009 - December					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	0.0	6.0	-3.3	87.0	2.4
2	2.8	8.6	-0.7	96.0	1.4
3	3.7	6.7	3.9	80.0	0.4
4	0.0	8.0	0.0	92.0	2.2
5	1.7	12.3	1.3	94.0	7.6
6	3.6	10.1	6.4	96.4	0.2
7	3.4	9.7	5.7	85.0	1.8
8	0.0	9.8	2.4	83.0	0.0
9	0.0	10.9	4.1	96.0	5.4
10	0.0	9.6	2.5	95.0	0.0
11	1.0	4.6	-1.9	93.0	0.0
12	0.0	7.5	-1.1	85.0	0.0
13	0.0	5.6	-3.0	98.0	0.0
14	0.0	5.9	-1.9	92.0	6.8
15	1.7	5.6	2.7	78.0	0.0
16	0.8	6.4	2.3	98.0	1.2
17	2.8	4.2	0.2	87.0	0.0
18	3.5	0.8	-2.4	85.0	0.0
19	0.0	3.1	-7.5	81.0	1.8
20	0.9	3.3	-6.4	98.0	0.0
21	1.5	3.6	-2.8	71.0	0.0
22	0.0	3.5	-6.3	86.0	0.0
23	0.9	1.5	-5.8	98.0	2.8
24	1.3	2.0	-2.3	98.0	0.0
25	0.0	5.4	-0.4	85.0	2.2
26	1.0	7.6	-0.1	81.0	1.4
27	1.3	6.7	2.1	87.0	0.0
28	0.0	3.6	-2.9	73.0	0.0
29	4.0	4.0	-2.8	86.0	10.0
30	4.6	4.1	1.4	82.0	1.4
31	3.3	3.7	1.8	98.0	0.0
Mean		5.9	-0.5		

2010 - January					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	0.0	2.0	-6.2	71.0	0.2
2	0.4	5.3	-5.9	85.0	4.0
3	0.0	1.6	-3.2	76.0	0.0
4	0.0	1.8	-7.6	98.0	0.0
5	0.6	1.4	-7.1	86.0	2.8
6	3.0	2.3	-0.8	93.0	0.0
7	0.0	-0.7	-12.3	92.0	0.0
8	0.0	-0.3	-12.7	92.0	0.0
9	0.0	1.8	-11.9	93.0	0.0
10	3.3	2.5	-6.8	81.0	2.0
11	2.4	1.2	0.4	91.0	0.0
12	3.2	1.9	-0.6	91.0	3.0
13	1.6	0.9	-0.1	91.0	1.0
14	0.0	3.0	-0.1	72.0	3.6
15	2.3	6.6	-0.3	73.0	9.0
16	4.1	6.4	2.1	79.0	1.0
17	0.0	9.2	0.8	73.0	0.0
18	1.4	9.6	0.6	89.0	0.0
19	2.5	7.1	2.0	92.0	1.8
20	3.2	3.9	3.2	89.0	1.6
21	2.0	8.1	-0.5	98.0	3.8
22	0.4	8.4	0.2	96.0	7.2
23	2.7	5.3	4.2	missing data	0.0
24	0.3	7.1	0.4	missing data	2.4
25	1.7	4.0	1.3	84.0	0.0
26	1.1	3.5	1.4	82.0	0.0
27	1.5	8.4	0.2	85.0	0.2
28	2.1	8.2	3.0	88.0	6.2
29	3.0	4.8	3.3	88.0	0.0
30	0.8	4.0	-2.4	missing data	0.0
31	0.2	3.5	-2.9	missing data	2.4
Mean		4.3	-1.9		

2010 - February					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	0.5	6.0	-0.9	77.0	0.8
2	1.3	10.3	-1.2	92.0	3.8
3	0.4	3.4	-1.8	74.0	1.6
4	1.5	8.5	0.2	97.0	0.4
5	0.8	10.1	3.2	97.0	0.0
6	0.0	7.1	-2.0	77.0	0.0
7	0.5	4.8	-0.2	82.0	0.0
8	3.4	3.3	0.1	75.0	0.0
9	3.5	5.5	1.3	78.0	0.0
10	2.8	4.7	-1.1	79.0	0.2
11	2.3	4.8	-2.6	70.0	0.0
12	3.5	6.7	-3.4	79.0	0.0
13	0.4	5.3	-0.4	74.0	0.0
14	0.0	7.3	-1.2	77.0	0.2
15	0.2	7.5	0.6	82.0	0.8
16	0.0	7.7	-1.0	80.0	0.4
17	0.6	2.3	-2.8	76.0	1.2
18	2.1	3.8	-0.5	92.0	2.6
19	0.9	5.5	-0.6	91.0	0.0
20	0.3	6.2	-2.5	77.0	3.4
21	0.5	5.8	-1.6	77.0	0.2
22	2.6	4.4	-4.2	75.0	0.0
23	3.2	4.6	0.9	72.0	1.2
24	0.0	10.3	0.7	97.0	0.0
25	1.8	8.7	4.4	95.0	7.6
26	4.4	6.7	3.7	80.0	1.4
27	1.3	6.8	-0.2	78.0	2.0
28	3.0	6.0	2.6	82.0	0.0
29					
30					
31					
Mean		6.2	-0.4		

2010 - March					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	1.9	9.3	-1.8	94.5	0.0
2	0.0	9.9	-4.9	84.7	0.0
3	0.9	6.4	0.2	70.2	0.0
4	0.0	8.1	-1.0	73.4	0.0
5	0.0	8.4	-5.4	82.5	0.0
6	1.6	7.7	-0.3	84.8	0.0
7	0.0	5.6	-5.9	97.5	0.0
8	0.0	8.5	-7.9	80.9	0.0
9	0.0	9.4	-3.5	60.4	0.0
10	3.2	8.1	3.5	99.1	0.0
11	0.0	9.1	-4.7	66.4	0.4
12	2.9	10.2	-1.4	85.2	0.0
13	1.6	9.5	-2.7	79.1	0.0
14	4.7	12.0	1.1	93.2	0.0
15	3.3	11.1	-0.3	76.0	0.0
16	0.0	12.3	-0.7	57.0	0.0
17	5.2	15.0	5.5	95.3	0.0
18	3.7	14.9	7.8	88.4	0.0
19	3.8	12.7	6.2	78.9	4.0
20	2.8	13.8	9.0	89.9	3.6
21	2.7	14.4	-0.1	91.8	0.4
22	6.1	11.9	6.0	97.2	1.6
23	3.0	10.6	0.4	88.0	2.0
24	5.2	13.8	3.8	84.5	2.0
25	7.8	13.5	9.7	93.4	10.6
26	5.8	12.9	6.3	92.5	0.4
27	2.7	12.9	5.5	95.7	0.0
28	4.8	12.5	3.0	86.9	4.2
29	3.5	11.8	4.3	91.4	1.8
30	5.8	12.8	5.2	97.5	6.6
31	7.0	6.1	1.5	93.7	5.4
Mean		10.8	1.2		

2010 - April					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	6.1	7.0	0.3	82.9	2.6
2	2.5	9.3	2.8	90.6	3.4
3	3.6	9.6	1.3	93.5	1.0
4	3.7	11.2	3.6	93.5	0.4
5	10.8	13.4	1.0	90.1	0.0
6	7.3	15.4	7.3	90.1	1.8
7	3.1	13.2	6.8	97.2	0.6
8	3.1	16.5	-0.7	83.1	0.0
9	1.1	17.5	2.7	86.5	0.0
10	0.0	18.9	2.7	79.2	0.0
11	2.2	14.5	6.1	75.7	0.0
12	2.6	13.6	4.6	85.5	0.0
13	2.5	15.5	-1.2	71.5	0.0
14	4.3	13.2	6.3	90.4	0.0
15	2.0	11.4	0.2	88.0	0.0
16	3.8	15.0	-0.2	67.3	0.0
17	1.7	18.5	0.0	82.6	0.0
18	0.4	16.9	0.2	86.3	0.0
19	3.7	12.5	7.3	94.5	0.0
20	4.9	11.5	-1.6	69.3	0.0
21	3.8	12.2	-1.1	70.5	0.0
22	0.8	15.3	-2.3	74.7	0.0
23	3.1	17.6	-1.5	62.1	0.0
24	4.0	19.2	3.3	52.3	0.0
25	4.9	18.0	10.4	82.5	0.2
26	2.7	18.0	7.5	81.9	0.0
27	2.5	19.6	3.8	77.2	0.4
28	5.5	19.4	9.8	78.8	0.0
29	1.5	14.8	11.5	85.3	8.2
30	3.8	13.7	5.1	85.5	8.2
31					
Mean		14.7	3.2		

2010 - May					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	1.2	15.3	5.3	84.6	2.0
2	5.1	9.7	6.3	75.7	1.6
3	3.1	12.3	2.2	72.0	2.6
4	2.2	14.5	0.2	66.2	0.0
5	2.4	13.1	3.7	95.9	1.4
6	1.4	13.4	9.5	91.6	2.4
7	3.6	13.5	4.7	84.7	2.2
8	4.7	11.0	6.6	82.3	0.6
9	2.6	14.7	2.3	64.1	0.0
10	1.4	11.1	5.9	80.4	1.8
11	1.3	11.6	3.2	69.5	0.0
12	2.4	11.6	-0.3	86.0	0.0
13	2.8	14.1	1.4	72.0	0.4
14	4.2	12.9	3.8	86.3	0.0
15	2.4	16.6	1.2	73.4	0.6
16	3.6	16.4	3.3	60.5	0.0
17	3.2	17.5	3.5	65.9	0.0
18	0.0	19.8	1.9	68.0	0.4
19	4.2	18.9	10.3	75.3	0.0
20	3.1	24.3	12.3	81.2	0.0
21	0.0	26.3	9.5	81.5	0.0
22	1.1	25.8	13.3	58.0	0.0
23	1.8	28.4	9.8	62.8	0.0
24	3.2	22.1	9.5	71.6	0.0
25	1.9	18.8	4.4	82.6	0.0
26	2.3	15.7	9.0	82.0	1.8
27	2.8	15.9	2.7	86.3	0.2
28	3.3	17.8	6.6	60.8	4.6
29	1.6	15.8	8.3	79.2	2.6
30	6.4	16.1	9.1	92.6	0.0
31	1.0	18.1	5.6	83.4	2.2
Mean		16.6	5.6		

2010 - June					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	2.2	15.2	11.6	93.1	0.6
2	0.6	21.0	8.5	88.0	0.0
3	1.7	23.4	6.6	62.7	0.0
4	2.5	25.4	8.6	66.1	0.0
5	1.3	24.3	11.1	65.5	5.0
6	2.2	21.3	13.3	96.7	0.4
7	1.5	17.6	9.8	79.2	12.0
8	1.1	17.6	12.1	93.4	7.8
9	1.3	19.6	12.7	90.7	0.0
10	3.4	16.5	12.8	90.8	0.0
11	1.5	19.0	10.1	87.8	0.0
12	4.9	18.9	10.8	64.5	0.0
13	2.4	19.6	9.2	68.8	26.0
14	2.9	16.1	11.5	79.3	0.0
15	2.4	18.9	4.3	59.6	0.0
16	2.2	22.3	6.3	67.4	0.0
17	1.4	23.3	8.6	68.9	0.0
18	2.4	19.6	9.2	82.5	6.6
19	2.7	16.8	8.6	69.6	0.0
20	2.4	20.7	5.7	60.6	0.0
21	1.9	24.2	7.0	61.9	0.0
22	1.8	24.5	9.5	72.0	0.0
23	2.1	24.2	9.1	72.6	0.0
24	1.9	22.5	12.9	86.9	0.0
25	1.1	24.4	6.9	62.9	0.0
26	1.5	27.0	12.0	72.2	0.0
27	1.9	26.6	12.7	66.0	0.0
28	3.2	26.0	10.4	69.2	1.0
29	2.1	25.1	14.8	74.1	0.0
30	0.4	24.4	10.3	66.4	0.0
31					
Mean		21.5	9.9		

2010 - July					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	5.3	24.6	13.6	70.7	3.6
2	3.4	23.9	13.5	76.5	0.0
3	3.2	21.8	10.4	58.1	0.0
4	7.2	22.6	9.5	75.9	0.0
5	3.5	21.3	10.2	64.0	0.0
6	1.3	21.8	8.3	70.6	0.0
7	4.9	21.0	15.1	77.8	1.6
8	5.0	22.5	13.2	64.8	0.4
9	3.0	missing data	13.4	68.2	0.0
10	missing data	missing data	missing data	missing data	0.0
11	missing data	missing data	missing data	missing data	1.9
12	0.0	missing data	missing data	91.4	0.0
13	missing data	missing data	missing data	missing data	0.0
14	missing data	missing data	missing data	missing data	8.6
15	missing data	22.0	missing data	missing data	3.2
16	missing data	missing data	12.0	76.8	0.0
17	missing data	missing data	missing data	missing data	0.0
18	missing data	missing data	missing data	missing data	3.7
19	missing data	missing data	missing data	70.1	0.0
20	missing data	18.6	missing data	missing data	2.6
21	2.0	21.3	12.1	86.1	0.0
22	0.5	19.3	12.1	92.0	8.0
23	0.0	20.1	11.9	87.2	0.0
24	2.4	19.7	8.3	78.3	0.2
25	1.4	22.3	14.1	82.4	3.6
26	1.3	22.6	15.8	99.7	0.2
27	2.4	22.3	16.1	87.3	0.0
28	2.3	19.7	11.1	83.0	0.4
29	1.7	17.8	13.3	88.1	0.0
30	2.3	18.9	13.1	87.9	3.2
31	0.0	20.6	14.6	93.1	0.0
Mean		21.2	12.5		

2010 - August					
DATE	**Wind sp m/s**	**Max temp ºC**	**Min temp 24 hrs ºC**	**RH %**	**Total rain 24 hrs mm**
1	0.3	19.7	13.4	92.3	0.0
2	0.7	19.3	13.0	94.1	0.0
3	2.3	23.5	8.3	95.1	8.2
4	0.4	17.6	12.0	96.6	1.0
5	2.2	18.4	10.1	96.1	0.0
6	4.1	19.9	11.6	95.1	1.4
7	2.3	21.5	14.1	91.9	0.6
8	1.1	20.1	13.7	82.7	0.0
9	3.2	21.0	9.4	75.0	0.0
10	3.8	21.4	10.5	70.2	0.0
11	2.5	20.7	7.6	67.2	0.4
12	1.6	20.8	13.0	97.0	3.2
13	1.4	17.8	11.8	97.8	3.0
14	1.9	20.2	11.8	96.5	0.4
15	1.9	22.2	11.1	81.3	0.0
16	1.6	23.1	8.9	81.5	1.0
17	2.1	22.0	13.6	90.9	0.4
18	2.9	20.4	9.4	74.7	2.0
19	3.8	19.6	10.8	78.5	8.2
20	3.1	23.0	14.5	92.8	0.8
21	5.0	21.6	14.7	78.0	2.6
22	3.9	21.8	12.0	77.2	4.8
23	2.0	18.0	12.5	98.5	1.8
24	8.9	17.8	10.2	70.5	0.2
25	0.0	16.8	9.3	88.6	3.8
26	2.3	14.7	12.0	97.3	1.4
27	1.0	19.1	7.7	85.7	0.0
28	3.8	18.7	8.1	72.2	0.8
29	3.5	17.1	11.3	92.3	0.4
30	1.6	18.9	2.9	71.3	0.0
31	2.2	20.4	3.7	82.2	0.0
Mean		19.9	10.7		

2010 - October					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	5.4	15.5	11.6	88.8	12.2
2	2.4	15.4	7.2	98.1	15.4
3	3.5	15.6	10.9	79.9	11.6
4	0.0	17.9	5.5	97.7	0.6
5	3.6	18.1	11.3	83.9	1.8
6	5.5	16.7	9.6	90.5	0.2
7	2.1	16.9	7.7	69.0	0.0
8	4.7	18.5	10.6	82.3	0.0
9	5.5	16.8	14.1	88.8	0.0
10	4.3	18.8	12.5	89.2	0.0
11	0.0	16.0	1.7	93.5	0.0
12	2.4	12.5	7.5	87.7	0.0
13	0.6	12.1	0.6	97.3	0.0
14	0.0	10.3	3.3	96.2	0.0
15	0.9	12.9	8.1	96.3	0.4
16	1.0	13.2	1.9	97.0	0.0
17	0.0	13.4	0.4	86.6	0.0
18	3.9	14.6	3.4	92.3	2.4
19	2.6	12.0	5.3	93.7	2.4
20	1.2	9.8	-0.4	85.7	0.0
21	2.4	13.0	-1.2	91.4	0.0
22	4.3	13.5	5.7	87.1	1.6
23	2.0	12.5	6.3	97.4	0.4
24	0.0	10.3	-1.3	97.0	0.2
25	0.0	10.0	-3.5	98.7	4.0
26	3.6	15.2	1.0	98.7	3.8
27	3.2	15.3	8.7	90.3	0.4
28	1.8	15.0	9.2	89.4	0.4
29	4.5	14.9	11.4	99.5	0.0
30	2.6	13.5	5.8	93.2	0.0
31	4.2	12.3	8.2	97.5	0.0
Mean		14.3	5.9		

2010 - November					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	0.3	13.4	6.6	93.8	0.6
2	5.1	14.7	8.5	79.6	3.8
3	4.3	16.0	7.4	86.2	0.6
4	9.1	17.4	10.6	91.6	0.6
5	3.3	13.4	12.0	85.1	4.2
6	0.2	11.5	5.3	93.3	2.2
7	1.3	8.2	1.6	98.5	7.8
8	5.5	6.8	3.7	98.5	4.8
9	3.1	9.0	3.6	88.4	0.0
10	0.4	10.5	-1.4	86.4	1.6
11	3.9	14.0	0.7	97.6	0.2
12	6.6	11.1	9.0	78.3	0.0
13	4.2	10.9	3.8	86.9	1.8
14	0.2	7.3	0.2	87.2	0.0
15	0.1	10.4	-2.4	92.9	0.0
16	0.7	8.1	-2.1	87.4	2.2
17	7.7	6.8	-0.5	97.3	5.0
18	4.2	9.8	5.0	91.9	0.4
19	2.4	6.8	1.8	76.6	0.0
20	3.0	6.5	2.7	94.3	0.6
21	1.0	6.1	3.8	95.5	0.6
22	0.0	7.4	2.2	89.4	0.2
23	1.1	8.0	3.1	98.4	0.0
24	0.3	5.3	-2.7	87.1	0.0
25	0.8	3.0	-3.8	98.1	0.0
26	0.2	1.1	-5.6	87.9	0.6
27	1.4	1.6	-4.1	90.3	0.6
28	0.0	0.2	-11.7	88.2	0.0
29	1.6	1.1	-9.5	72.7	0.6
30	2.7	1.4	-8.5	74.3	0.8
31					
Mean		8.3	1.3		

2010 - December					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	1.2	0.3	-3.4	94.9	1.0
2	3.4	0.7	-2.2	86.7	0.0
3	0.2	1.4	-13.5	96.2	0.0
4	0.9	2.2	-12.5	92.7	4.0
5	0.1	2.0	-3.4	98.8	0.0
6	0.7	-3.0	-5.0	98.3	0.0
7	0.2	-2.5	-9.9	93.1	0.0
8	0.1	-0.7	-9.4	94.9	0.0
9	0.0	4.4	-8.6	96.6	0.4
10	1.1	7.1	-1.0	95.7	0.0
11	2.5	8.4	3.2	90.2	0.0
12	0.9	1.5	-1.4	99.8	0.0
13	0.0	3.4	-1.7	100.0	0.0
14	0.6	4.0	-0.9	99.0	0.0
15	0.0	7.0	-1.1	99.6	0.6
16	6.1	7.2	-0.6	91.2	6.0
17	3.4	0.6	-4.9	89.0	0.2
18	3.3	-1.4	-2.8	95.2	1.2
19	0.0	-7.3	-14.5	88.8	0.2
20	0.0	-4.2	-14.4	91.5	0.2
21	0.0	-0.2	-11.1	97.4	0.6
22	1.5	-0.9	-4.7	92.8	0.8
23	2.1	-1.0	-11.9	95.7	0.0
24	0.2	-1.0	-10.2	93.4	0.0
25	0.0	-3.7	-13.9	89.4	0.0
26	0.4	2.4	-13.6	91.7	0.0
27	1.7	2.7	-11.6	87.5	8.4
28	2.3	5.6	1.4	100.0	0.2
29	1.8	6.3	2.5	100.0	0.0
30	2.9	5.9	4.5	100.0	0.0
31	0.0	5.6	3.9	100.0	0.0
Mean		1.7	-5.6		

2011 - January					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	0.8	7.8	3.9	100.0	1.6
2	0.0	3.1	0.2	85.4	0.0
3	0.0	2.1	-1.9	91.3	0.0
4	1.3	6.5	-4.8	93.4	5.0
5	2.5	5.7	0.3	95.7	4.4
6	0.6	3.6	-1.3	100.0	0.8
7	2.0	8.3	-2.1	100.0	5.6
8	3.6	5.5	0.3	90.4	0.0
9	5.3	6.6	0.5	82.2	0.0
10	2.2	8.2	-0.6	93.7	1.6
11	4.6	8.3	3.3	85.3	2.8
12	0.0	12.2	-0.1	98.4	2.4
13	3.4	12.7	7.8	92.1	5.2
14	6.6	11.8	9.6	82.8	1.4
15	7.5	12.7	4.5	80.7	0.0
16	10.4	12.6	10.9	74.0	6.2
17	0.0	8.6	3.2	98.6	0.0
18	0.0	8.2	-0.3	98.6	0.0
19	0.0	7.0	-2.4	100.0	0.0
20	0.0	0.5	-2.4	98.5	0.0
21	0.0	3.2	-1.6	99.3	0.0
22	1.4	4.0	-3.3	100.0	0.0
23	1.5	6.4	-1.9	93.9	0.0
24	1.2	7.6	2.6	88.1	1.2
25	2.8	8.2	3.8	99.3	5.2
26	3.1	6.1	5.1	84.5	0.0
27	3.5	2.3	0.8	68.2	0.0
28	0.6	2.1	-0.6	74.4	0.0
29	0.9	1.5	-4.0	78.8	0.0
30	0.0	3.4	-3.1	95.0	0.0
31	0.0	5.3	-7.1	97.8	0.8
Mean		6.5	0.6		

2011 - February					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	0.0	11.9	-4.9	98.6	0.0
2	4.2	11.4	2.4	80.7	0.4
3	2.3	11.8	3.0	81.5	0.0
4	13.1	13.2	4.6	79.1	0.0
5	7.6	12.9	11.4	77.6	3.2
6	9.4	12.5	11.1	79.6	0.0
7	8.7	12.8	9.2	81.3	0.4
8	0.0	8.9	-1.5	100.0	1.2
9	2.7	10.4	1.1	94.1	2.8
10	1.2	9.2	7.4	98.6	0.0
11	0.0	11.6	6.0	95.3	1.4
12	1.0	10.5	5.1	98.6	0.2
13	7.5	9.0	4.3	92.8	7.6
14	4.6	7.9	2.6	93.8	0.2
15	6.8	6.4	0.4	91.9	2.2
16	0.0	7.6	0.4	98.6	0.0
17	2.6	7.5	3.2	97.9	0.0
18	4.5	6.4	4.5	97.2	5.8
19	3.7	5.3	2.7	99.3	0.2
20	1.7	5.2	3.1	97.9	0.0
21	4.4	5.4	3.1	99.3	0.0
22	1.4	8.7	3.2	98.0	3.6
23	2.9	12.7	5.3	98.0	0.0
24	3.1	13.4	7.6	88.5	7.8
25	4.9	13.0	8.4	85.8	14.8
26	2.3	10.3	6.3	98.6	0.8
27	3.1	8.4	0.9	93.9	1.8
28	1.0	5.9	1.1	88.7	0.0
29					
30					
31					
Mean		9.7	4.0		

2011 - March					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	0.0	9.0	2.4	83.1	0.0
2	2.7	6.6	2.1	76.7	0.0
3	1.8	5.2	-3.3	97.9	0.0
4	2.6	4.7	1.0	87.4	0.0
5	1.3	6.5	-3.2	99.3	0.8
6	3.8	6.8	-1.3	72.9	0.0
7	0.0	9.4	-1.9	84.8	0.0
8	0.0	10.1	-4.6	90.4	0.2
9	6.9	10.2	1.3	72.4	0.2
10	6.7	12.0	6.8	75.6	0.4
11	6.9	10.5	1.7	76.2	0.0
12	2.1	12.2	6.8	92.1	4.8
13	1.3	10.3	3.9	99.3	0.0
14	1.5	10.8	-2.5	82.6	0.0
15	3.3	10.2	4.1	99.3	0.0
16	0.6	9.3	5.7	99.3	0.2
17	0.0	11.4	4.5	99.3	0.0
18	1.1	10.9	0.2	98.6	0.0
19	0.8	13.7	-2.8	70.9	0.0
20	5.5	12.4	6.0	62.7	0.2
21	2.8	15.8	4.6	88.0	0.0
22	1.3	16.4	2.1	94.7	0.0
23	0.0	17.0	-0.2	90.9	0.0
24	0.0	16.9	-0.3	100.0	0.0
25	0.0	17.3	1.6	99.3	0.0
26	0.0	9.1	1.9	99.3	0.0
27	2.9	9.9	4.7	90.6	0.0
28	0.0	13.7	0.5	100.0	0.0
29	2.4	13.9	3.5	89.6	0.2
30	2.7	13.9	7.1	96.1	4.8
31	11.1	16.2	7.9	79.9	0.0
Mean		11.4	1.9		

2011 - April					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	0.0	11.9	-4.9	98.6	0.0
2	4.2	11.4	2.4	80.7	0.4
3	2.3	11.8	3.0	81.5	0.0
4	13.1	13.2	4.6	79.1	0.0
5	7.6	12.9	11.4	77.6	3.2
6	9.4	12.5	11.1	79.6	0.0
7	8.7	12.8	9.2	81.3	0.4
8	0.0	8.9	-1.5	100.0	1.2
9	2.7	10.4	1.1	94.1	2.8
10	1.2	9.2	7.4	98.6	0.0
11	0.0	11.6	6.0	95.3	1.4
12	1.0	10.5	5.1	98.6	0.2
13	7.5	9.0	4.3	92.8	7.6
14	4.6	7.9	2.6	93.8	0.2
15	6.8	6.4	0.4	91.9	2.2
16	0.0	7.6	0.4	98.6	0.0
17	2.6	7.5	3.2	97.9	0.0
18	4.5	6.4	4.5	97.2	5.8
19	3.7	5.3	2.7	99.3	0.2
20	1.7	5.2	3.1	97.9	0.0
21	4.4	5.4	3.1	99.3	0.0
22	1.4	8.7	3.2	98.0	3.6
23	2.9	12.7	5.3	98.0	0.0
24	3.1	13.4	7.6	88.5	7.8
25	4.9	13.0	8.4	85.8	14.8
26	2.3	10.3	6.3	98.6	0.8
27	3.1	8.4	0.9	93.9	1.8
28	1.0	5.9	1.1	88.7	0.0
29					
30					
31					
Mean		9.7	4.0		

2011 – May					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	0.0	9.0	2.4	83.1	0.0
2	2.7	6.6	2.1	76.7	0.0
3	1.8	5.2	-3.3	97.9	0.0
4	2.6	4.7	1.0	87.4	0.0
5	1.3	6.5	-3.2	99.3	0.8
6	3.8	6.8	-1.3	72.9	0.0
7	0.0	9.4	-1.9	84.8	0.0
8	0.0	10.1	-4.6	90.4	0.2
9	6.9	10.2	1.3	72.4	0.2
10	6.7	12.0	6.8	75.6	0.4
11	6.9	10.5	1.7	76.2	0.0
12	2.1	12.2	6.8	92.1	4.8
13	1.3	10.3	3.9	99.3	0.0
14	1.5	10.8	-2.5	82.6	0.0
15	3.3	10.2	4.1	99.3	0.0
16	0.6	9.3	5.7	99.3	0.2
17	0.0	11.4	4.5	99.3	0.0
18	1.1	10.9	0.2	98.6	0.0
19	0.8	13.7	-2.8	70.9	0.0
20	5.5	12.4	6.0	62.7	0.2
21	2.8	15.8	4.6	88.0	0.0
22	1.3	16.4	2.1	94.7	0.0
23	0.0	17.0	-0.2	90.9	0.0
24	0.0	16.9	-0.3	100.0	0.0
25	0.0	17.3	1.6	99.3	0.0
26	0.0	9.1	1.9	99.3	0.0
27	2.9	9.9	4.7	90.6	0.0
28	0.0	13.7	0.5	100.0	0.0
29	2.4	13.9	3.5	89.6	0.2
30	2.7	13.9	7.1	96.1	4.8
31	11.1	16.2	7.9	79.9	0.0
Mean		11.4	1.9		

2011 - June					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	4.0	18.8	4.8	74.4	0.0
2	0.3	21.4	9.4	81.4	0.0
3	0.0	24.3	7.8	74.2	0.0
4	2.8	22.9	8.3	64.5	2.6
5	3.1	15.7	9.9	75.9	0.8
6	1.3	17.8	7.8	69.6	0.0
7	3.1	17.3	6.3	80.4	1.2
8	3.6	16.9	8.8	68.3	4.6
9	3.0	17.2	9.5	60.0	0.0
10	2.1	16.4	4.9	61.4	0.4
11	2.1	15.6	2.8	60.2	2.0
12	2.0	16.2	1.4	89.2	11.2
13	4.2	17.6	9.2	77.0	0.0
14	1.1	20.9	5.1	70.2	0.0
15	2.5	20.0	13.5	74.7	0.8
16	4.2	18.4	7.8	80.7	0.6
17	2.6	16.9	9.3	78.4	3.4
18	4.2	17.2	9.1	71.5	1.8
19	2.8	18.3	9.1	67.3	0.0
20	0.8	21.1	4.3	87.9	8.4
21	4.0	19.8	13.5	78.9	1.8
22	5.1	19.4	12.3	92.9	0.2
23	3.5	16.6	9.8	77.0	0.6
24	1.6	16.8	7.7	69.9	10.4
25	3.8	20.4	10.4	97.8	0.0
26	2.5	27.4	15.0	76.5	0.0
27	3.0	26.9	15.2	72.0	0.8
28	1.7	19.1	8.2	56.4	0.0
29	1.7	19.4	5.1	62.0	0.0
30	1.1	19.3	4.9	72.4	0.0
31					
Mean		19.2	8.4		

2011 – July					
DATE	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
1	1.4	19.2	7.1	62.6	0.0
2	19.2	22.2	5.3	71.1	0.0
3	22.2	23.4	9.0	66.4	0.0
4	0.0	25.0	13.0	59.3	0.0
5	4.8	21.1	14.1	79.6	3.2
6	3.3	19.6	12.3	89.3	2.4
7	4.6	20.3	12.1	84.9	1.2
8	3.9	18.4	11.5	84.2	6.4
9	3.6	22.6	11.2	91.1	0.0
10	1.1	20.5	8.4	75.4	1.2
11	1.6	22.6	7.4	79.2	0.0
12	3.0	20.0	10.7	82.3	0.0
13	1.1	19.0	11.4	84.5	0.0
14	0.9	23.2	6.4	71.2	0.0
15	1.0	21.9	8.4	64.8	5.6
16	3.2	19.5	14.0	95.0	4.9
17	3.3	17.9	11.8	87.1	7.6
18	4.1	16.9	12.8	85.7	13.5
19	1.0	18.3	11.5	92.5	0.0
20	0.0	19.0	12.0	82.0	0.5
21	1.5	19.8	12.6	82.3	0.7
22	1.7	18.6	11.6	82.0	9.8
23	1.1	19.6	6.4	90.1	0.0
24	1.6	21.2	6.5	78.3	0.0
25	1.4	22.4	5.7	78.4	0.0
26	1.5	23.2	10.7	89.7	0.0
27	1.8	22.2	12.1	69.6	0.0
28	0.0	22.0	10.6	72.2	0.0
29	3.2	18.3	14.1	73.2	0.0
30	2.8	22.6	8.7	60.3	0.0
31	2.9	24.2	14.4	74.3	0.0
Mean		20.8	10.4		

2011 - August					
DATE	**Wind sp m/s**	**Max temp ºC**	**Min temp 24 hrs ºC**	**RH %**	**Total rain 24 hrs mm**
1	1.9	23.8	17.4	75.8	0.0
2	2.2	24.8	15.0	78.6	0.0
3	0.7	27.0	12.8	78.9	1.0
4	2.3	22.5	14.3	91.1	0.7
5	1.9	22.6	13.4	73.2	0.0
6	1.2	20.7	10.6	75.6	0.5
7	5.2	20.4	9.7	60.2	7.1
8	2.3	17.9	8.3	84.8	0.5
9	1.8	19.5	10.1	69.5	0.0
10	6.1	20.9	9.7	66.0	4.2
11	6.4	20.5	14.7	82.4	0.5
12	0.0	21.0	15.4	90.2	1.7
13	3.1	21.1	15.0	78.5	0.0
14	2.2	20.8	11.9	75.3	0.0
15	5.4	21.8	7.5	64.5	0.5
16	2.7	22.0	13.1	76.9	0.2
17	0.0	19.9	6.7	81.9	0.0
18	0.0	19.4	5.6	85.2	0.2
19	2.0	22.1	6.9	88.9	0.0
20	1.9	21.0	11.9	76.7	1.0
21	5.0	23.4	14.0	75.8	0.0
22	0.0	22.8	10.6	81.8	0.0
23	3.7	21.0	13.7	85.6	0.0
24	2.9	21.4	10.3	83.6	6.4
25	2.6	19.9	8.6	84.2	2.0
26	0.7	18.7	9.4	90.8	4.2
27	2.9	18.1	10.2	80.4	0.2
28	3.0	19.5	10.2	88.5	1.0
29	3.1	15.9	9.7	89.1	0.0
30	1.6	16.0	11.1	83.0	0.2
31	0.0	20.1	8.5	91.8	0.0
Mean		20.8	11.2		

Appendix IV. a: Monthly weather data for each growing season of field experimentation (2008/2009, 2009/2010 and 2010/2011)

Growing season	Month	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
2008/2009	October	2.0	13.9	6.0	88.6	3.3
	November	2.6	9.8	4.3	85.7	2.0
	December	1.1	6.5	0.1	92.3	1.5
	January	2.0	6.1	-0.5	93.3	2.2
	February	1.6	7.7	1.5	88.3	0.8
	March	2.7	11.7	2.6	89.0	0.8
	April	2.1	15.3	5.1	89.0	1.4
	May	2.3	17.5	7.2	83.6	1.6
	June	1.6	20.4	9.7	78.5	3.1
	July	1.5	21.0	11.5	82.6	4.6
	August	1.5	21.7	11.8	82.4	1.2
2009/2010	October	1.1	16.1	7.8	89.2	1.7
	November	2.5	11.5	5.5	93.2	4.1
	December	1.4	5.9	-0.5	88.5	1.6
	January	1.4	4.3	-1.9	86.2	1.7
	February	1.5	6.2	-0.4	81.5	1.0
	March	2.9	10.8	1.2	85.5	1.4
	April	3.4	14.7	3.2	81.6	0.9
	May	2.6	16.6	5.6	76.7	0.9
	June	2.0	21.5	9.9	74.6	2.0
	July	2.5	21.2	12.5	79.6	1.3
	August	2.5	19.9	10.7	85.8	1.5
	October	2.5	14.3	5.9	91.4	1.9
2010/2011	November	2.5	8.3	1.3	89.1	1.3
	December	1.2	1.7	-5.6	94.8	0.8
	January	2.1	6.5	0.6	91.0	1.4
	February	3.7	9.7	4.0	92.3	1.9
	March	2.3	11.4	1.9	88.7	0.4
	April	3.7	9.7	4.0	92.3	1.9
	May	2.3	11.4	1.9	88.7	0.4
	June	2.6	19.2	8.4	74.1	1.7
	July	3.3	20.8	10.4	78.7	1.8
	August	2.4	20.9	11.2	80.3	1.0

Appendix IV. b: Average weather data of each growing season of field experimentation (2008/2009, 2009/2010 and 2010/2011)

Growing season	Wind sp m/s	Max temp ºC	Min temp 24 hrs ºC	RH %	Total rain 24 hrs mm
2008/2009	1.9	13.8	5.4	86.6	2.1
2009/2010	2.2	13.5	4.9	83.9	1.6
2010/2011	2.6	12.1	4.0	87.4	1.3

Appendix V: The results for yield and yield components from the experiment in natural environment in 2008/2009, 2009/2010 and 2010/2011

Appendix V. a: The results for yield and yield components from the experiment in natural environment in 2008/2009 (from hand harvested crop).

		Antitranspirant/control treatment						Mean	*P* -Var	*P* -Treat	*P* - Var-Treat	S.E.M. - Var-Treat	CV % - Var-Treat (DF)
		IUC	**UC**	**GS32**	**GS37**	**GS39**	**GS55**						
Yield (t/ha)	**E**	10.30	10.30	10.34	10.18	10.48	10.94	10.42	0.695	0.788	0.374	0.410	6.7 (20)
	C	10.48	11.14	11.01	10.32	10.09	10.16	10.53					
	Mean	10.39 (a)	10.72 (a)	10.67 (a)	10.25 (a)	10.28 (a)	10.55 (a)						
TGW (g)	**E**	55.29	54.95	54.74	55.11	53.29	55.64	54.84	0.005	0.956	0.429	0.967	3.4 (20)
	C	48.32	49.66	49.60	49.20	49.61	47.88	49.05					
	Mean	51.80 (a)	52.30 (a)	52.17 (a)	52.16 (a)	51.45 (a)	51.76 (a)						
Grains ear^{-1}	**E**	45.94	50.22	47.66	48.76	50.90	49.02	48.75	0.161	0.569	0.622	1.713	6.2 (20)
	C	45.30	45.21	47.60	47.64	46.55	47.14	46.57					
	Mean	45.62 (a)	47.72 (a)	47.63 (a)	48.20 (a)	48.73 (a)	48.08 (a)						
Ears m^{-2}	**E**	402.3	370.7	393.7	374.7	383.7	401.3	387.7	0.002	0.446	0.410	16.99	7.6 (20)
	C	477.0	492.7	465.0	438.7	435.0	448.3	459.4					
	Mean	439.7 (a)	431.7 (a)	429.3 (a)	406.7 (a)	409.3 (a)	424.8 (a)						

E = Einstein; C = Claire; IUC = irrigated unsprayed control; UC = unsprayed control; GS33, GS39, GS41 and GS59 = antitranspirant (di-1-p-menthene) treatment at respective growth stages; Var = Variety; Treat = Treatment; res. DF = residual DF; data within rows accompanied by the same letter are not significantly different at p = 0.05.

Appendix V. b: The results for yield (t/ha) from the experiment in natural environment in 2008/2009 (from combine harvested crop).

	Antitranspirant/control treatment						Mean	*P* -Var	*P* -Treat	*P* - Var-Treat	S.E.M. - Var-Treat	CV % - Var-Treat (DF)
	IUC	**UC**	**GS32**	**GS37**	**GS39**	**GS55**						
E	8.26	9.09	8.75	9.29	9.70	9.62	9.12	0.002	0.708	0.395	0.512	10.4 (20)
C	9.75	9.53	9.90	8.59	9.44	9.81	9.50					
Mean	9.00 (a)	9.31 (a)	9.32 (a)	8.94 (a)	9.57 (a)	9.71 (a)						

E = Einstein; C = Claire; IUC = irrigated unsprayed control; UC = unsprayed control; GS33, GS39, GS41 and GS59 = antitranspirant (di-1-p-menthene) treatment at respective growth stages; Var = Variety; Treat = Treatment; res. DF = residual DF; data within rows accompanied by the same letter are not significantly different at p = 0.05.

Appendix V. c: The results for yield and yield components from the experiment in natural environment in 2009/2010 (from hand harvested crop).

		Antitranspirant/control treatment						Mean (without IUC)	P - SMD	P - Treat	P – SMD-Treat	S.E.M - SMD-Treat	CV % - SMD-Treat (DF)
		IUC (Common control)	UC	di-GS31	di-GS33	di-GS41	la-GS41						
Yield (t/ha)	**L**	10.38	8.87	9.64	9.48	9.14	9.60	9.34	0.922	0.875	0.916	0.477	8.8 (16)
	H		9.21	9.16	9.41	9.33	9.38	9.30					
	Mean	10.38	9.04	9.40	9.45	9.24	9.49						
TGW (g)	**L**	48.65	51.37	51.49	50.73	51.48	51.02	51.22	0.797	0.869	0.613	0.652	1.5 (16)
	H		51.69	51.46	51.79	51.17	51.17	51.46					
	Mean	48.65	51.53	51.48	51.26	51.33	51.10						
Grains ear^{-1}	**L**	45.99	43.68	46.02	44.18	44.75	44.05	44.54	0.815	0.706	0.956	1.710	6.6 (16)
	H		43.18	44.62	44.81	45.24	42.78	44.13					
	Mean	45.99	43.43	45.32	44.49	44.99	43.42						
Ears m^{-2}	**L**	464.3	395.7	406.3	425.3	398.0	426.7	410.4	0.962	0.521	0.846	16.84	7.2 (16)
	H		414.0	398.3	405.3	403.0	428.0	409.7					
	Mean	464.3	404.8	402.3	415.3	400.5	427.3						

L = low SMD regime; H = high SMD regime; IUC = irrigated unsprayed control; UC = unsprayed control; di-GS31, di-GS33 and di-GS41 = the antitranspirant, di-1-p-menthene, treatment at respective growth stages; la-GS41 the antitranspirant, latex, treatment at GS41; Treat = Treatment; res. DF = residual DF; data within rows accompanied by the same letter are not significantly different at p = 0.05.

Appendix V. d: The results for yield (t/ha) from the experiment in natural environment in 2009/2010 (from combine harvested crop).

	Antitranspirant/control treatment						Mean (without IUC)	P - SMD	P - Treat	P – SMD-Treat	S.E.M. - SMD-Treat	CV% - SMD-Treat (DF)
	IUC (Common control)	**UC**	**di-GS31**	**di-GS33**	**di-GS41**	**la-GS41**						
L	10.36	8.63	9.35	9.43	9.30	8.53	9.05	0.572	0.632	0.639	0.478	9.5 (16)
H		9.16	8.83	9.17	8.34	8.56	8.81					
Mean	10.36	8.89	9.09	9.30	8.82	8.55						

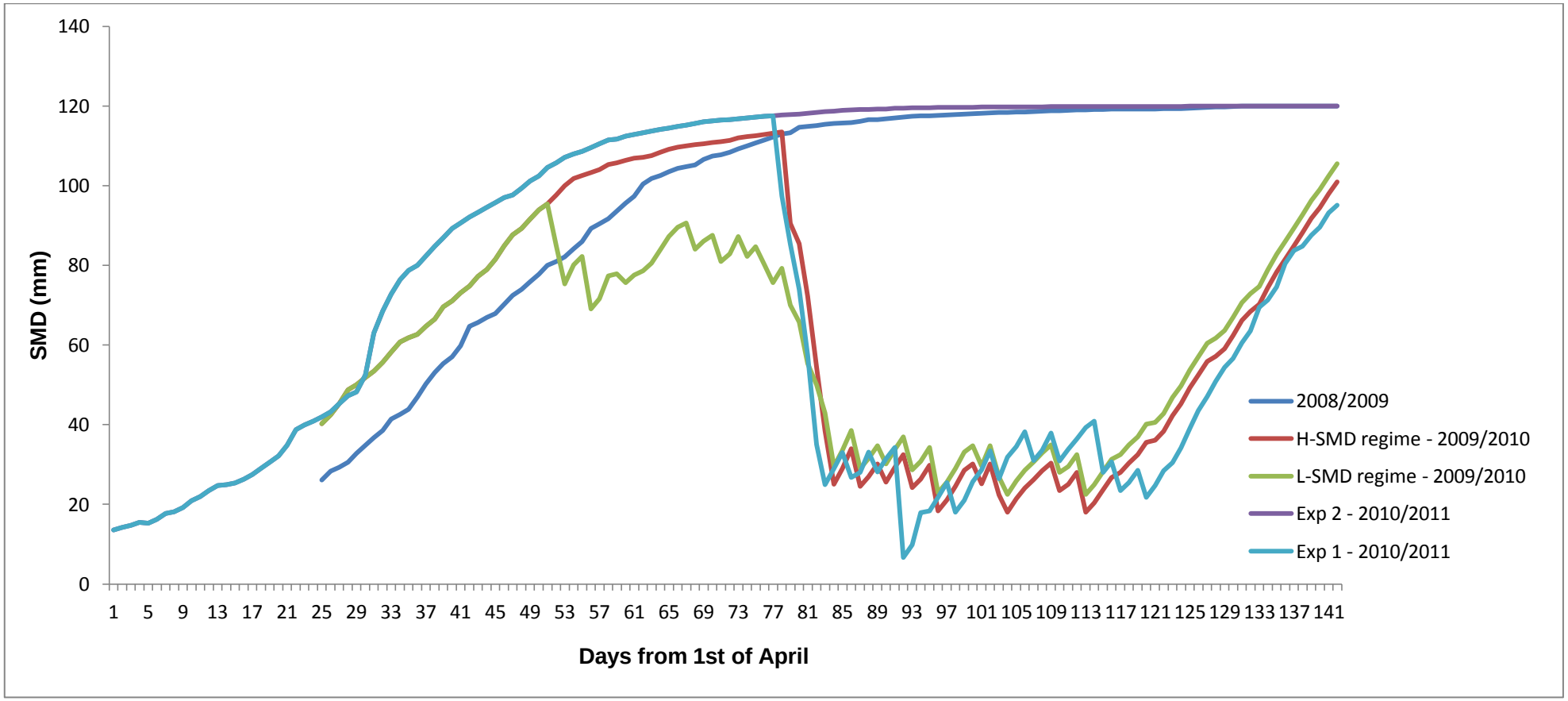
L = low SMD regime; H = high SMD regime; IUC = irrigated unsprayed control; UC = unsprayed control; di-GS31, di-GS33 and di-GS41 = the antitranspirant, di-1-p-menthene treatment at respective growth stages; la-GS41 the antitranspirant, latex treatment at GS41; Treat = Treatment; res. DF = residual DF; data within rows accompanied by the same letter are not significantly different at p = 0.05.

Appendix V. e: The results for yield and yield components from the experiment in natural environment in 2010/2011 (from both hand and combine harvested crop).

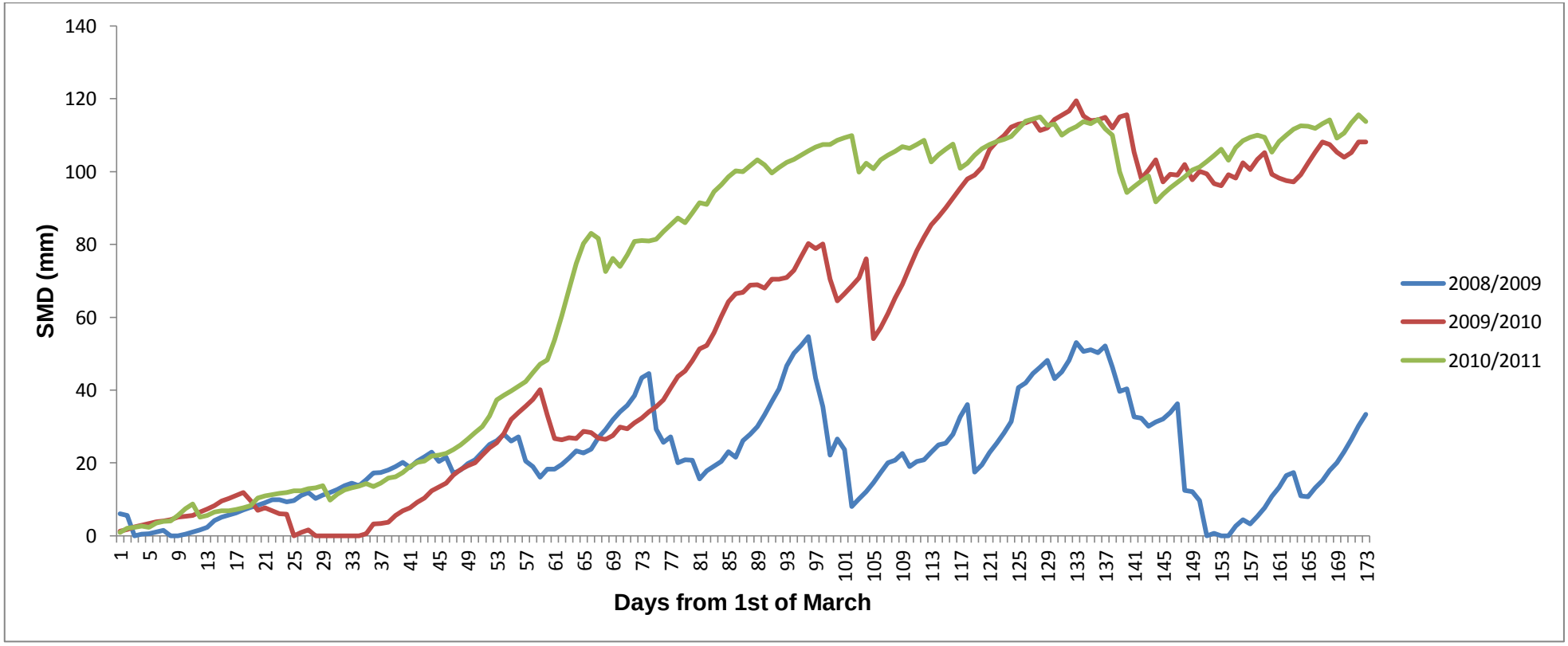
	IUP	Antitranspirant/control treatment			p	S.E.M	CV (DF)
		UC	di-GS33	la-GS33			
Yield (t/ha) (Hand harvested)	9.32	6.605 (a)	6.553 (a)	6.163 (a)	0.187	0.179	8.4
TGW (g)	48	48.30 (a)	47.69 (a)	47.92 (a)	0.637	0.457	2.9
Grains ear^{-1}	43.85	41.71 (a)	39.38 (a)	40.02 (a)	0.311	1.086	8.1
Ears m^{-2}	443	328.1 (a)	349.9 (a)	324.6 (a)	0.162	9.75	8.8
Yield (t/ha) (Combine harvested)	9.78	7.101 (a)	6.781 (a)	6.593 (a)	0.117	0.1719	10.7

IUP = Irrigated unsprayed plots; UC = unsprayed control; di-GS33 = di-1-p-menthene treatment at GS33; la-GS33 = latex treatment at GS33; DF = residual DF; data within rows accompanied by the same letter are not significantly different at p = 0.05.

Appendix VI: The changes in SMD with time inside the polytunnels in 2008/2009, 2009/2010 and 2010/2011 from the dates polytunnels were installed to 20th of August, the date by which harvesting was finished in all the experiments. H-SMD regime = high SMD regime; L-SMD regime = low SMD regime; Exp 1 = Experiment 1; Exp 2 = Experiment 2; Note that: for the experiments in 2008/2009 and 2009/2010, polytunnels were installed on 25th of April, and for the experiments in 2010/2011, polytunnels were installed on 1st of April



Appendix VII: The changes in SMD with time in natural environment in 2008/2009, 2009/2010 and 2010/2011 from 1st of March to 20th of August, the date by which harvesting was finished in all the experiments.



Appendix VIII: The results of the analyses of leaf temperature from the experiments inside polytunnels in 2009/2010 and 2010/2011.

Appendix VIII. a: The leaf temperature (℃) of the antitranspirant (AT)/control treatments after the antitranspirant spray application at GS33 in the experiment inside polytunnels in 2009/2010

Days from spray application	**AT/control treatments**		P	S.E.M	CV% (res. DF)
	UC	**di-GS33**			
Day before	21.72	21.70	0.964	0.378	5.7 (25)
Day after	24.96	24.83	0.786	0.340	4.2 (25)
3 days after	21.308	21.321	0.944	0.1313	2.3 (25)

UC = unsprayed control; di-GS39 = di-1-p-menthene treatment at GS39; res. df = residual df

Appendix VIII. b: The leaf temperature (℃) of the antitranspirant (AT)/control treatments after the antitranspirant spray applications at GS41 in the experiment inside polytunnels in 2009/2010

			AT/control treatments				P - SMD	P - Treat	P - SMD -Treat	S.E.M. - SMD - Treat	CV% - SMD - Treat (DF)
			UC	**di-GS41**	**la-GS41**	Mean					
Before spray application	**SMD**	**L**	17.31	17.25	17.26	17.27	0.507	0.533	0.704	1.089	8.6 (71)
		H	17.84	18.82	18.81	18.49					
	Mean		17.57 (a)	18.04 (a)	18.03 (a)						
1 day after spray application	**SMD**	**L**	17.92	18.03	17.38	17.78	0.718	0.444	0.614	1.9374	4.1 (71)
		H	19.36	19.62	20.32	19.76					
	Mean		18.64 (a)	18.83 (a)	18.85 (a)						
3 days after spray application	**SMD**	**L**	14.17	13.73	13.58	13.83	0.301	0.279	0.052	0.3179	4.5 (72)
		H	14.24	14.62	14.35	14.40					
	Mean		14.20 (a)	14.17 (a)	13.97 (a)						

L = low SMD regime; H = high SMD regime; UC = unsprayed control; di-GS41 = the antitranspirant, di-1-p-menthene treatment at GS41; la-GS41 the antitranspirant, latex treatment at GS41; Treat = Treatment; res. DF = residual DF; data within rows accompanied by the same letter are not significantly different at p = 0.05.

Appendix VIII. c: The leaf temperature (℃) of the antitranspirant (AT)/control treatments after the antitranspirant spray applications at GS33 in the experiment inside polytunnels in 2010/2011

Days from spray application	**AT/control treatments**			P	S.E.M	CV% (res. DF)
	UC	**di-GS33**	**la-GS33**			
Day before	14.07 (a)	13.99 (a)	14.04 (a)	0.974	0.252	6.5 (39)
Day after	16.27 (ab)	15.99 (a)	16.47 (b)	0.003	0.0888	2 (39)
3 days after	24.52 (a)	23.94 (a)	24.90 (a)	0.212	0.390	5.4 (39)

UC = unsprayed control; di-GS33 = the antitranspirant, di-1-p-menthene treatment at GS33; la-GS33 the antitranspirant, latex treatment at GS41; res. DF = residual DF; data within rows accompanied by the same letter are not significantly different at p = 0.05.