Mitigation of drought damage to rapeseed (*Brassica napus* L.) from sprays of film antitranspirants at different concentrations

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Abstract

Drought causes massive yield losses in rapeseed (*Brassica napus*), particularly during flowering when crops are most sensitive to water stress. It has been shown that film antitranspirant (AT) can effectively improve the yield of droughted rapeseed if applied at the flowering stage, yet the mechanism by which film AT mitigates drought damage and the concentration-response relationship between AT and crop yield remain unclear. To understand the underlying physiological mechanism and determine the optimum concentration of AT for rapeseed, the following experiments were conducted: five experiments measuring leaf coverage; three experiments imposing a short-term controlled drought during the flowering stage in the glasshouse; two experiments imposing a terminal drought in the field under polytunnels. Results showed that AT application at the early flowering stage decreased stomatal conductance more than photosynthesis of rapeseed, which improved intrinsic water use efficiency under both drought conditions. This suppression of gas exchange was positively correlated with AT concentrations due to changes in leaf coverage. However, yield responses to AT were only observed when rapeseed was under terminal drought, with increasing AT concentrations resulting in higher yields. Across concentrations and two sites, AT increased seed yield by 24%, predominantly from pod number increases compared to droughted control. Rapeseed treated with 3% AT produced the highest seed yield by 37% over unsprayed droughted plots, with about 31% adaxial leaf coverage. Pod number and leaf relative water content were positively correlated. It was concluded that yield benefits from AT application might be mediated by improving leaf water status to sustain rapeseed pod formation, and a greater yield can be achieved by increasing AT concentrations with conventional spraying on the adaxial surface. Therefore, the interactive effects of drought with AT concentrations and developing a cost-effective spraying method by also covering the abaxial leaf surface would merit further investigation.

Declaration

I declare that this thesis has been written by myself and describes the work carried out by me unless otherwise stated. Information from other sources has been fully acknowledged and referenced in the text. The work contained herein has not been submitted for any other degree or professional qualification. Part of this work has been published in *Crop Protection* and *Agricultural Water Management*.

Jie Xiang September 2022

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You can do it, Jie!

Keep up the good work!

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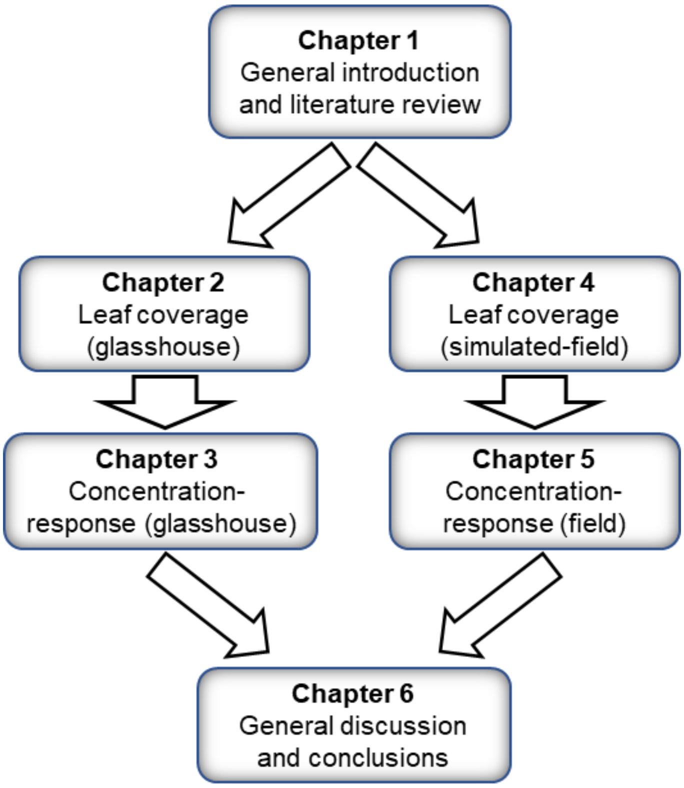
The flow diagram of the thesis



Chapter 1 General Introduction and literature review

1.1 General introduction

Rapeseed is the third most important oil crop for edible oil, fodder, and biofuel production around the world, and rapeseed oil accounts for about 12% of global oil production after palm (40%) and soybean (29%) (FAOSTAT). Drought has become the most devastating abiotic stress limiting crop growth worldwide, particularly in arid and semi-arid regions. It can cause massive yield losses of rapeseed when drought occurs during the flowering stage. As reported by previous studies, film antitranspirants are water-emulsifiable polymers that form a physically waterproof film to block stomata, which have shown great potential to mitigate drought damage and improve the yield of rapeseed and other crops such as wheat and soybean. However, the underlying physiological mechanism has not well understood. Further, there is little information about the concentration-response of crops, which is crucial for the commercial use of film antitranspirant while achieving comparable yield benefits at the lowest cost.

1.2 About rapeseed (*Brassica napus* L.)

1.2.1 Species, distribution, and production

Brassica oilseed crops comprise several species grown in several producing areas of the world. *Brassica rapa* (Turnip rape) is more resistant to the cold than other *Brassica* species, mainly grown in western Canada as it matures early. *Brassica juncea* (Indian mustard) is formed by the hybridisation between *Brassica rapa* and *Brassica nigra* (Yang et al., 2016), which is well-adapted to low rainfall environments. It is predominantly cultivated throughout India and, to a limited extent, in central Africa, southern Russia, the Caspian steppes and China (Sinha et al., 2007). *Brassica napus* (Swede rape) is the most grown species in temperate regions, including Europe, Canada, and Northern China. *Brassica carinata* (Ethiopian mustard) is less widely grown than any other species, and it is restricted to certain areas such as Ethiopia and the surrounding countries in North East Africa (Booth and Gunstone, 2004). *Brassica oleracea* comprises many important vegetable crops, such as broccoli and cabbages, which are grown worldwide for their leaves, flowers and stems (Liu et al., 2014). The evolutionary relationships among these species are depicted by the "triangle of U" model shown in Figure 1.2.1 (Nagaharu, 1935).

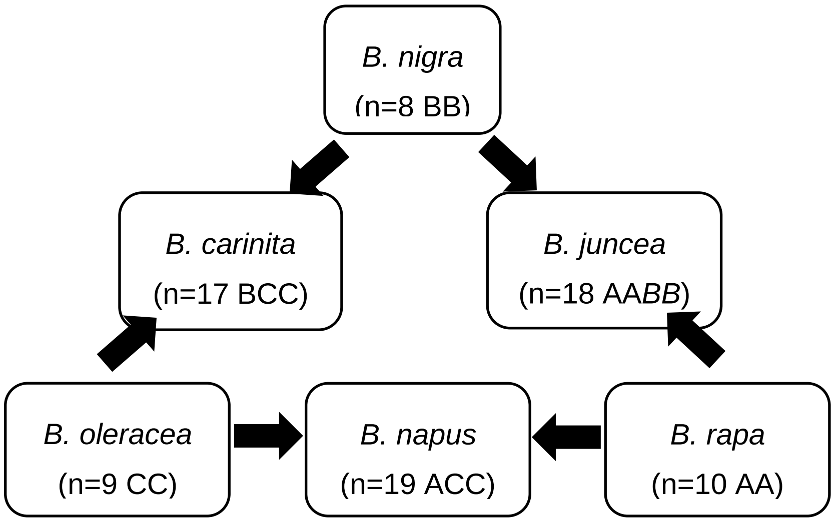


Figure 1.2.1 The “triangle of U “model: the genomic relationships among *Brassica* species. (n represents haploid chromosome number, and A, B, and C represent different genomes, respectively) (Nagaharu, 1935).

Rapeseed (*Brassica napus* L.) is extensively produced in many regions worldwide, such as Europe, Asia, Canada, Australia, and part of the United States. Winter-sown *B*. *napus* is the primary oilseed rape crop in most of Europe and Northern China, while spring-sown *B. napus* is mainly produced in Canada, Northern Europe, and parts of China. In Australia and the northern plains of the US, where winters are mild enough whilst water is limited, spring type *B. napus* can be planted in autumn as a winter crop.

The last twenty years have witnessed a significant increase in global rapeseed production with some fluctuations and a steady growth in oil production (Figure 1.2.2). In 2020, China and Canada ranked top in worldwide rapeseed production, a total of which account for about 51% of the world production; while China ranked first in oil production (27%), followed by Germany and Canada (16% and 14%, respectively) (Figure 1.2.3).

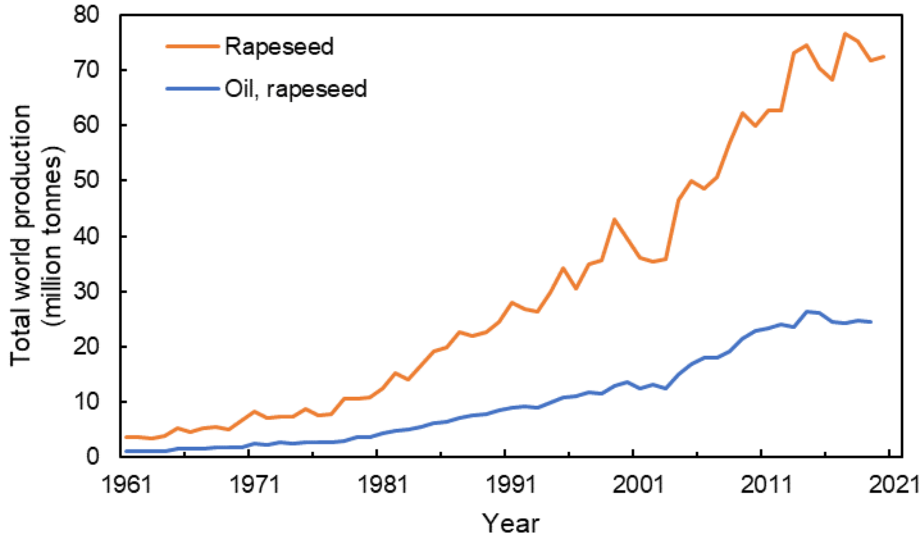
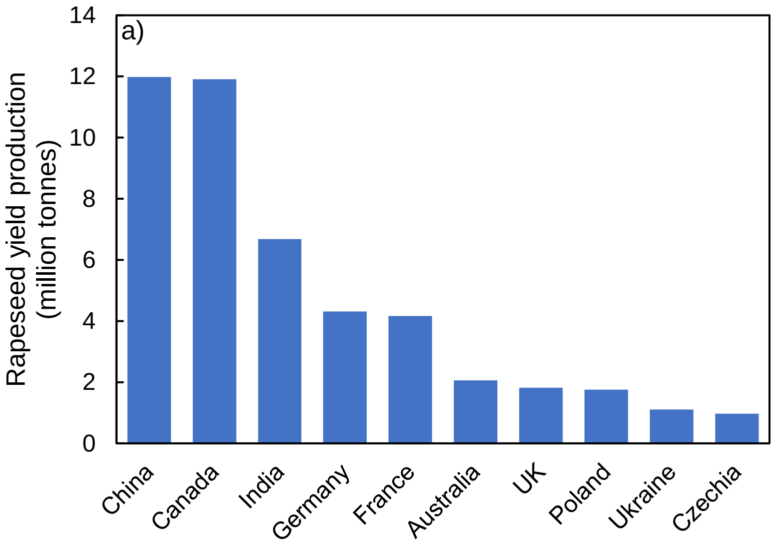


Figure 1.2.2 The worldwide production of rapeseed and rapeseed oil from 1961-2020 (accessed on 27022022 in https://www.fao.org/faostat/en/#data/QCL).



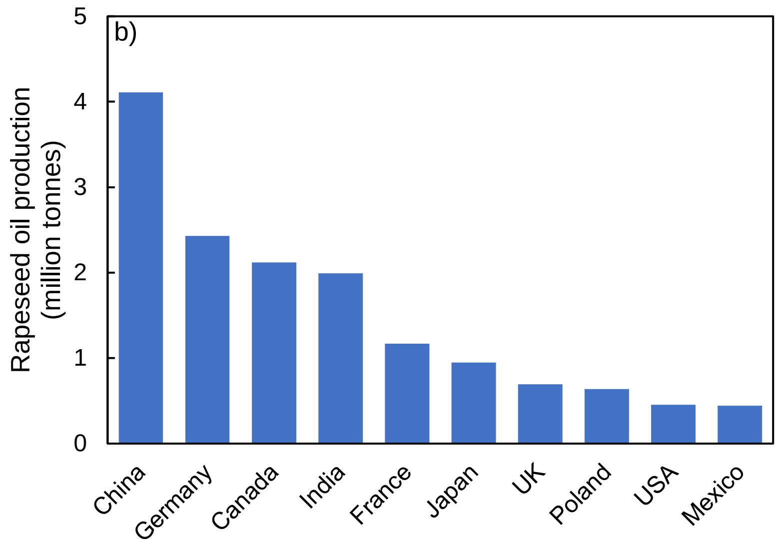


Figure 1.2.3 Yield (a) and oil (b) production of rapeseed averaged from 1961-2020 in the top 10 countries around the world (accessed on 27022022 in https://www.fao.org/faostat/en/#data/QCL).

1.2.2 Agronomic Characteristics

Phenology

Rapeseed is one of the most important oil crops with several agronomic features. Its life cycle can be divided into nine sequential stages, as shown in Table 1.2.1 (Meier, 1997). Rapeseed is a "long-day" species, which requires 16h-18h of the day length to the minimum photoperiod, thus initiating reproductive progress (Major, 1980). The temperature primarily determines the duration of the phenological development (Habekotte, 1997; Weymann et al., 2015). According to Morrison et al. (1992), the leaf expansion rate increases to a maximum value when the maximum individual leaf area is achieved by changing leaf types from large rosette leaves to cauline leaves. The longer the basic vegetative period is, the more rosette leaves are produced (Kirkegaard et al., 2012).

Table 1.2.1 Physiological growth stages and BBCH-identification keys of rapeseed (adapted from Meier, 1997).

Principal growth stage	Code	Description
Germination	0.0-0.9	Dry seed to emergence: cotyledons emerge through the soil surface
Leaf development	1.0-1.9	Cotyledons completely unfolded to 9 or more leaves unfolded
Formation of side shoots	2.0-2.9	No side shoots to end of side shoot development: 9 or more side shoots detectable
Stem elongation	3.0-3.9	Beginning of stem elongation: no internodes ("rosette") to 9 or more visibly extended internodes
Inflorescence emergence	5.0-5.9	Flower buds present, still enclosed by leaves to First petals visible, flower buds still closed ("yellow bud")
Flowering	6.0-6.9	First flowers open to the end of flowering
Development of fruit	7.1-7.9	10% of pods have reached a final size to nearly all pods have reached final size
Ripening	8.0-8.9	Beginning of ripening: seed green, filling pod cavity to fully ripe: nearly all pods ripe, seeds dark and hard
Senescence	9.7-9.9	Plant dead and dry to harvested product

Environmental conditions like precipitation have profound effects on the expression of the plasticity of the development of spring rapeseed (Franks, 2011). Angadi (2003) reported that spring rapeseed could sustain comparable yield levels across a range of populations (20 – 80 plants/m^2) under above-normal growing season precipitation. However, rapeseed plants may adjust the number of branches and pods to compensate for precipitation-induced reductions in plant populations when the reverse scenario happens. Weymann et al. (2015) conducted field experiments over five years, showing that seed yield and oil yield were affected substantially by weather conditions with different predominant factors limiting rapeseed growth. For example, during the early reproductive stage, many factors such as light radiation, temperature, water stress and interactions between them could affect the yield formation, while only temperature influenced duration and photosynthesis rate over the seed filling phase.

The yield formation of grain crops can be primarily divided into the following three phases, vegetative growth to form organs for nutrient absorption and photosynthesis (Stage one), the period when seed number is determined (Stage two), and finally, the seed-filling period when the yield is produced (Stage three) (Murata, 1969; Egli, 2017). In rapeseed, Kirkegaard et al. (2018) reported that the critical period of rapeseed could be extended from 100 to 500 °Cd (thermal time described as the sum of temperature in degree day) after the start of the flowering stage (BBCH 6.0), and centred at 300 °Cd when 50% of plants had one opening flower. The change of annual photoperiod and the period of vernalisation are considered the primary environmental factors that induce changes in the reproductive stage of winter rapeseed (Filek et al., 2007). In addition, compared to other crops such as wheat (Hess et al., 2015) and barley (Dreccer et al., 2018), rapeseed is more sensitive to water stress, particularly at reproductive stages (Istanbulluoglu et al., 2010). The duration from flowering to maturity is positively correlated to grain yield as a longer grain-filling period allows more time for rapeseed to accumulate biomass and thereby increase the yield (given that sufficient pre-flowering reserves are provided) (Riffkin et al., 2016). Leaf defoliation is generally thought to reduce yield production due to restricted CO_2 assimilate capacity, resulting in large yield losses. However, this yield loss can be possibly minimised if sufficient time is given for biomass recovery before flowering stage by producing larger rosette leaves to fulfil seed yield potential under favourable stress-free conditions (Kirkegaard et al., 2012).

Yield and yield components

Yield can be defined as the weight of seeds, which is the final product of multiple environmentally sensitive morphological and physiological processes integrated throughout the life cycle of rapeseed (Berry and Spink, 2006). Dividing yield into separate components is important to understand the process involved in yield production (Egli, 2017). In general, the yield is determined by combining many factors with emphasis on the primary components, including the number of seeds per unit area and the size (weight) of the individual seed. Therefore, an equation raised by Egli (2017) has been applied to all grain crop species, with emphasis on the consistency of the yield production processes, regardless of their different growth habits:

Yield (weight/area) = (seeds/area) / (weight/seed).

The seed yield of rapeseed has complex characteristics comprising the primary and secondary components, as shown in Figure 1.2.4 (Diepenbrock, 2000). Berry and Spink (2006) summarised rapeseed yield into two main components: seed number/m^2 and individual seed weight, which is also consistent with the abovementioned equation.

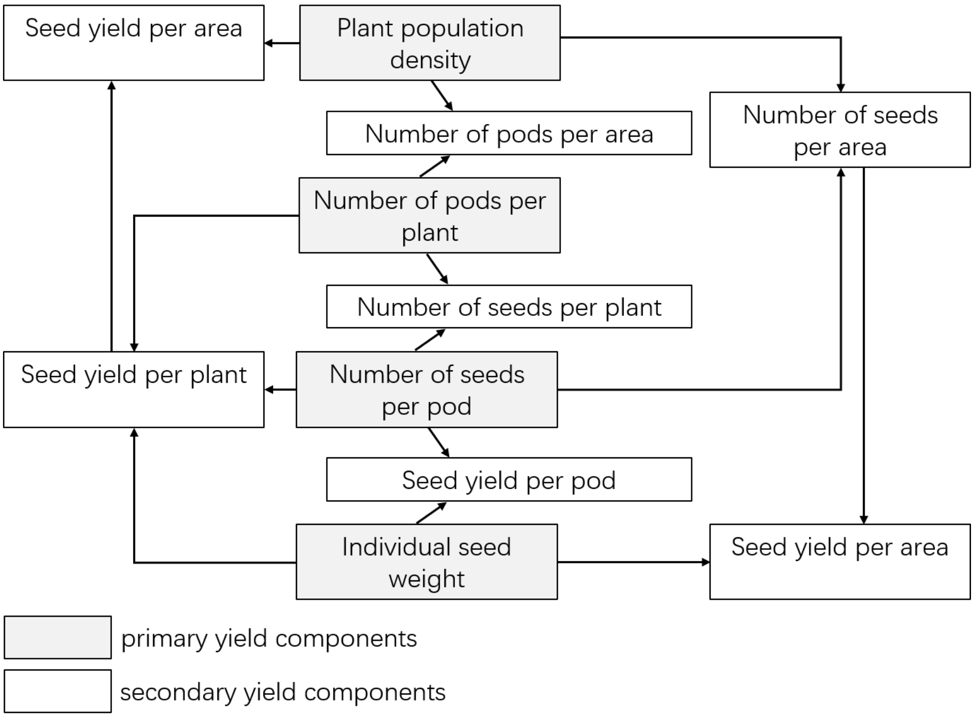


Figure 1.2.4 The yield structure of rapeseed (adapted from Diepenbrock 2000).

Plant density

Plant density plays an important role in rapeseed growth, yield and yield components (Diepenbrock, 2000). The optimum density of rapeseed varies in different regions and cultivars. In the UK, the target plant population of established winter rapeseed is recommended 25-40 plants/m^2 while at least 45-50 plants/m^2 is required for spring rapeseed (AHDB - Oilseed rape growth guide). In Asian countries such as China, the plant density of winter rapeseed ranges from 100 to 140 plants/m^2 (Wang et al., 2014). In fact, yield stability can be achieved within a wide range of plant densities by altering the distribution of pods within the canopy. At high densities the increase in the number of plants/m^2 can be counteracted by plants producing fewer and smaller pods. In contrast, at low densities plant produce more pod-bearing branches, thus extending the development of seeds and maintaining yield production (Leach et al., 1999). This compensation is also reported by Momoh and Zhou (2001) that the decrease in seed number during the flowering stage could be fully compensated by the single-seed weight, while oil contents remained almost stable. However, yields of winter rapeseed decrease as the density increases, especially at very high density (>150 plants/m^2). It might have resulted from a greater inter- or intra-plant competition for nutrients and solar radiation or increased pests and diseases (Leach et al., 1999). The terminal raceme is least affected in reducing branches and pods on all the branches at very high density (Diepenbrock, 2000).

Seed number per area

Seed number per area determines the size of the sink (seeds) during the critical stage lasting about 300 °Cd after mid-flowering (Mendham et al., 1981), which is recognised to be the most important yield component for the final seed yield (Berry and Spink, 2006)**.** It decreases from the terminal raceme to the lower branches (Diepenbrock, 2000). During this phase, the amount of radiation intercepted by photosynthetic tissues like leaves and pods is closely related to the development of pods and seeds, thus the final yields (Tesfamariam et al., 2010). Flowers at the top of plants can reflect up to about 60% of light radiation, which induces more intense competition between seeds for a limited supply of CO_2 assimilates. So the maximum number of seeds per area can be achieved by reducing the quantity of light reflected by the flowering layers whilst not leading to the reduction of leaf areas (Mendham et al., 1981; Ystes and Steven, 1987; Berry and Spink, 2006). It has been demonstrated that when the proportion of flower cover reduced from 0.50 of a typical canopy to 0.38, the amount of radiation reaching green tissues can be increased by 25% (Berry and Spink, 2006).

According to Lunn et al. (2001), seed number per area, pod number per area and seed number per pod are negatively related. The optimum fertile pod number can be achieved to 8000 pods/m^2. Smaller canopies trap less active radiation, resulting in fewer seeds per area (e.g., less than 6000 pods/m^2). In contrast, bigger canopies could decrease the amount of light radiation absorbed by the photosynthetic tissues, thus reducing the number of seeds per pod/area (Berry and Spink, 2006). The determination of seed number is adjusted downward. For example, crops respond to a favourable environment by producing more seed-bearing structures during the flowering stage, such as branches, buds and flowers (Egli, 2017). The actual seed number in rapeseed is normally smaller than the potential seed number due to abortion of pods and seeds over the growth stage (Egli, 2017). The selective abortion of pollinated flowers, pods and seeds depends on multiple factors such as the order of pollination, maternal sources, the number of developing seeds, weather conditions etc., which can minimise the amount of resources wasted by abortion and conserve resources for the remaining pods and seeds, and other growth process (Stephenson, 1981).

Seed size

Seed size (seed weight) of rapeseed is determined during the period from the mid-flowering stage to the physiological maturity. It overlaps with the determination period of seed number (Mendham et al., 1981). Besides genetic differences, the assimilate availability and environmental conditions also affect the seed weight. However, they contribute less to final yield variation after seed number is fixed during the critical stage (i.e., Stage Two) (Egli, 2017). Therefore, seed weight indicates how well the yield container is filled. It depends upon the capacity to accommodate the photosynthesis assimilates, i.e., seed growth rate and the length of the seed-filling period.

The leaf area decreases rapidly during the seed determination as the pod area increases. At the end of seed filling, the leaf area will decrease to zero leading to a substantial drop in the leaf area index (LAI) but a gradual rise in the pod area index (PAI) (Diepenbrock, 2000). Generally, the maximum rate of CO_2 assimilation per unit area in leaves is approximately three-fold higher than that in pods. Nevertheless, pods are still crucial for rapeseed development, particularly during the seed filling stage (Gammelvind et al., 1996).

Source-sink limitations of yield

The seed yield of grain crops can also be recognised as the result of the balance between the supply of carbohydrates (source) and the capacity of seeds to accumulate available carbohydrates (sink) (Zhang and Flottmann, 2018). It is important to understand the sensitivity of crops to source and sink manipulation, which can help achieve higher yields through efficient management practices (Zhang and Flottmann, 2018). For rapeseed, there is a strong overlap of stem and branch growth, flowering, pod development and seed filling stage, as mentioned before. It can lead to a great inter- and intra-plant competition for the supply of assimilates by different organs and substantially affect seed number per pod at early seed filling stage (Tayo and Morgan,1979; Iglesias and Miralles, 2014). Furthermore, most flowers appear on the terminal raceme and the upper three nodes on the basal- and middle-axillary inflorescences (Tayo and Morgan, 1979). From the experiments carried out by Tayo and Morgan (1979) to investigate the factors that affected the development of flowers and pods, they found that the decreased supply of carbon assimilates resulted in fewer flowers and pods from both terminal and axillary racemes by applying shade treatments and removing leaves during the flowering stage.

Additionally, removing the basal flowers or pods induced more pods to form in the terminal inflorescence. An initial conclusion can be made that the competition for the supply of sources is crucial in determining the number and size of rapeseed pods and seeds; rapeseed is source-limited during the seed filling stage. This feature is also in accordance with Fortescue and Turner (2007), that the increase of source-sink ratio by pruning axillary branches produced heavier seeds, while seed number per pod was reduced by excluding light significantly.

Rapeseed is known to have the plasticity to counteract the negative effects of reduced sink size through other yield components (Kirkegaard et al., 2012). Kirkegaard et al. (2012) found that seed yields of winter rapeseed could be recovered in Australia when defoliation by sheep grazing occurred before the budding stage. Reductions in pod number in the defoliated treatment were partially compensated by the seed number per pod and seed weight. However, when defoliation occurred at the bolting stage, seed yield reduced by 23% compared to control. It was mainly due to too limited time for plants to recover by increases in leaf area and assimilation capacity. Zhang and Flottmann (2018) conducted a series of experiments on spring rapeseed to manipulate source-sink relationships and found that mean seed weight decreased when source availability was downward manipulated during the seed filling stage, suggesting that yield is source-limited by source availability during the seed filling stage. Furthermore, rapeseed yield can also be limited by the established sink size during the flowering because either shading or defoliation reduced the number of pods and seeds (sink size) resulted from restricted assimilate supply to form viable pods and seeds, thus leading to final yield losses. On the contrary, Labra et al. (2017) reported that the decrease of the source-sink ratio by shading application at the flowering stage led to fewer seed number per area, which could be fully compensated by the rise of the single-seed weight by 47%-61%. The difference in the capacity of compensation between studies might be associated with growth conditions other than genetic variances. Therefore, agronomic management should be not only directed to increase assimilate supply available to rapeseed during the reproductive stage, but also adaptable according to specific environmental conditions in conjunction with appropriate varieties selection to fulfil yield potential as possible.

Agronomy

Rapeseed consumes the highest amount of water during the flowering stage (Tesfamariam et al., 2010; Istanbulluoglu et al., 2010). Rapeseed usually yields more as a break crop if grown after other species than monoculture or continuous rapeseed (Hegewald et al., 2018). The increased yield can be attributed to improving all crops and eliminating soil-borne cereal pathogens and root maggot damages (Koffi et al., 2018). Sowing time also impacts the growth and yield of rapeseed, that earlier sowing can provide advantages of greater yield and oil content. On one hand, earlier seeding can result in earlier flowering and extended reproductive growth duration, whereby the plant has enough time for foliage development and dry matter accumulation, resulting in heavier seed weight (Ma et al., 2016). Further, earlier seeding can help plants avoid detrimental effects from abiotic stresses like heat and water especially during the flowering stage, which benefit for yield development and oil concentration (Kirkland and Johnson, 2000). As reported by Kaur et al. (2018), late-sown winter rapeseed further to November resulted in substantial yield losses compared to those sown in early October because of delaying duration of emergency and flowering while hastening maturity. However, the optimum sowing dates may vary depending on the compound effects of multiple factors from local environmental conditions such as cultivar, water, temperature etc. (Faraji et al., 2009). Sieling et al. (2017) reported that aboveground dry matter (DW) decreased substantially from 190 g/m^2 when sown in early August to only about 6 g/m^2 when sown at the end of September. At the same time, tap root DW was also reduced from 80 g/m^2 to 1-0.6 g/m^2. Although nitrogen fertilisation could compensate for the decrease in plant density by promoting individual plant growth and increasing the number of pods per plant, yield losses resulting from a delayed sowing date could only be partially compensated by additional N application. On the other hand, modified sowing date can also control the damage from insects such as cabbage stem flee beetle (CSFB) feeding on mature leaves, stems and pods of rapeseed (reviewed by Hoarau et al., 2022). That is, too early or late sown rapeseed can be damaged by CSFB severely for winter and spring varieties. Also, Aubertot et al., (2004) observed that earlier sowing at the beginning of August reduced crown canker severity in winter rapeseed than later at early September. Furthermore, there was an interaction between susceptibility and cultural practices including sowing dates, nitrogen application and cultivars, indicating that the benefit from early sowing date on phoma canker development might be less or even opposite depending on specific agronomic practices conducted and crop cultivars.

1.3 Soil and plant water

1.3.1 Soil water relations

Soil physical characteristics

Soil physical characteristics mainly refer to the texture and structure of the soil. According to the USDA (US Department of Agriculture), soil particles are classified into three groups according to their particle size: sand, silt, and clay. Sand particles are the largest (2-0.05 mm), while clay particles are the smallest (< 0.002 mm). Soil texture is defined as the size distribution of those primary mineral particles in the soil, that is, the relative percentage of sand, silt, and clay. The combination of these particles determines soil water status (Dexter, 2004). Soil structure is defined as "the spatial heterogeneity of the different components or properties of the soil" (Dexter, 1988). It is how individual particles (sand, silt, and clay) are assembled. The assemblage of single particles can form large particles (i.e., aggregates).

Texture influences the amount of air and water the soil holds, as well as the ease with which it can be worked and the rate of water moving out of and into the soil (Dexter, 2004). There are three types of soil pores: macropores, mesopores, and micropores. Macropores allow rapid movement of water. All the macropores in the soil are filled with air at field capacity. Mesopores store water for the plant, whereas water in micropores is held so firmly that it is unavailable for plant use (Gerrard, 2000).

Soil water content

Soil water plays an essential role in the terrestrial water cycle, although it only accounts for a minimal part of the total water on earth (Daly et al., 2005). When the soil is saturated from irrigation or natural rain, all the pores are full of water. The excess water is then drained due to gravity, leading to a thin film around solid particles in macropores while others are still filled with water. This soil water status is also known as the field capacity, under which air is occupied in macropores and water is occupied in mesopores and micropores.

Holding water in the soil is not only determined by the size distribution of individual pores but also by the percentage of micropores. Soil water content is usually described as either gravimetric water content or volumetric water content (Gerrard, 2000). Mass or gravimetric soil water content is related to the mass of oven-dry soil, and volumetric water content refers to the volume of water per unit volume of soil, which can be expressed as follows (Warrick, 2001):

Gravimetric water content = (mass of water/mass of dry soil)

Volumetric water content = (volume of water/ bulk volume of soil)

Apart from the most basic measurement of soil water content, i.e., gravimetric method, a wide range of techniques, including capacitance and electromagnetic sensors and neutron probes, have been developed for measuring soil water content (Topp and Davis, 1985; Noborio, 2001; Jones, 2007).

Soil water potential

The water potential is related to how much work to do at the energy level of water (Tan, 2009). It is the sum of all the individual potentials determining the amount of work that must be expended to remove water from the soil (Hassett and Banwart, 1992). The driving force comes from the difference in water potential between places (Tan, 2009). Water potential is usually expressed in units of pressure and can be described as follows:

$Y_w = y_g + y_m + y_o$

Where y_g means the gravitational potential, y_m and y_o represent the matric potential and osmotic potential, respectively (Hassett and Banwart, 1992). Gravitational potential is due to gravity, and the attraction between soil particles and water is matric potential, while the osmotic potential is the attraction between dissolved ions and water (Schaetzl and Anderson, 2005). Generally, soil water potential has a negative value resulting from the reduction of the free energy of soil water by the matric and osmotic forces shown in Figure 1.3.1(Tan, 2009). However, specific water potentials need to be considered, such as the pressure potential in some cases where the soil pressure is higher than the atmospheric pressure or where the overlying soil exerts pressure on the soil water (Schaetzl and Anderson, 2005; Tan, 2009).

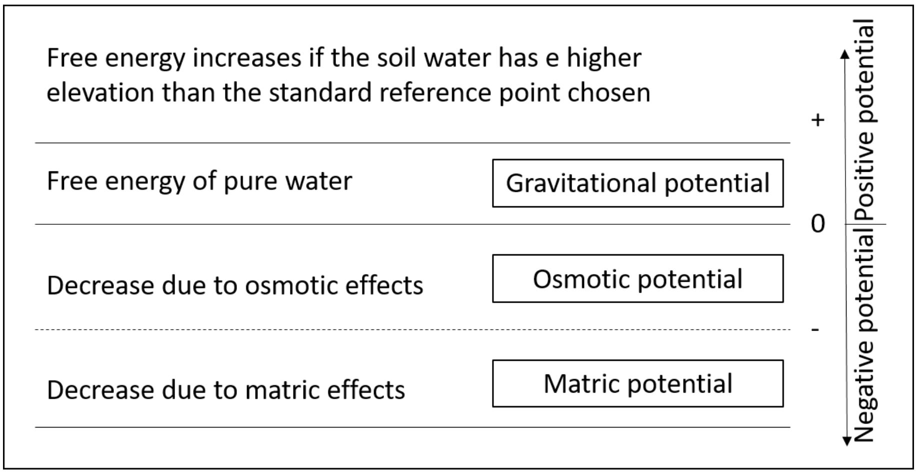


Figure 1.3.1 Representation of water potential with free and pure water having a potential zero (Adapted from Weil and Brady, 2016).

Soil water retention curve

Water retention in the soil is mainly attributed to two reactions: adhesion and cohesion. Solid particles make it possible by the adhesion of water with hydrogen bonding. Most water is retained around solid particles, the surface area of which plays a vital role in water retention. On the other hand, water can be retained by the surface tension in the capillaries (i.e., cohesion, the mutual attraction of water molecules) (Tan, 2009).

The amount of water held in the soil, particularly at low soil water potential, mainly depends on soil texture and the capillary effect (Hillel, 2008). As the soil dries out, water absorption becomes more important in response to a more negative soil water potential (Rawls et al., 1991; Hillel, 2008). The amount of water remaining in the soil at equilibrium is a function of the matric potential. Thus, the function is plotted by the soil water retention curve, also described as the soil water characteristic curve (Hillel, 2008). The shape of the water retention curve is affected by the soil structure and texture, primarily at low water potentials (Hopmans and Bristow, 2002). The slope of the soil water retention curve is termed specific water capacity, which means the change of water content per unit change of water potential.

It is an important property that influences the amount of water stored in the soil and is available for plants as it depends on the matric potential (Hillel, 2008).

Field capacity and permanent wilting point

There is a constant balance between the water held in the soil and forces trying to remove it downwards by gravity and upwards by the evapotranspiration of plants and the soil, which will be described in the following sections (Tan, 2009). The water retention curve for three types of soil, such as sand, loam, and clay soils, is shown in Figure 1.3.2. Field capacity has been discussed above, which is the amount of water held in the soil after all the excess water is drained away by gravity (Tan, 2009). The water potential measured is typically about -0.3 bar. Another important water potential benchmark is the permanent wilting point, where the plant cannot exert sufficient suction to remove water from the soil. For most of the plants, it is about -15 bar (Schaetzl and Anderson, 2005). Therefore, the process of utilisation of water in the soil by plants can only occur on the condition that the tension is above -15 bar. The amount of the water between the field capacity and the permanent wilting point is available for plants, also described as "available water".

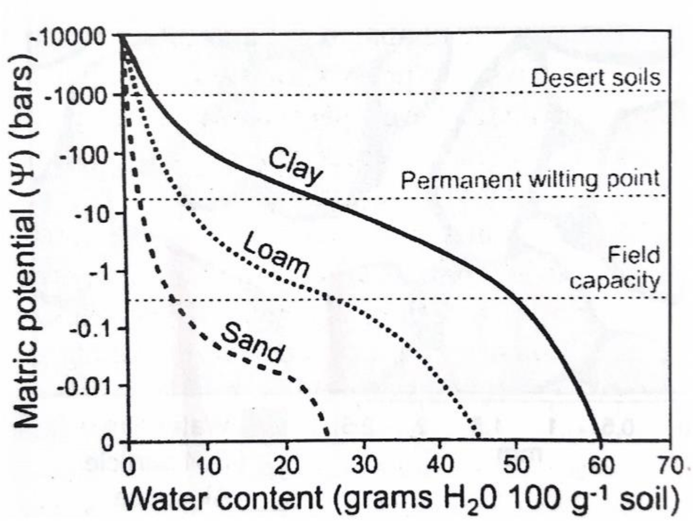


Figure 1.3.2 Water retention curve for sand, loam, and clay soils (Adapted from Weil and Brady, 2016).

1.3.2 Plant water relations

It is well established that measuring plant water status is crucial to understanding the effect of different water supplies in different experiments, indicating that plant water status controls crop performance when exposed to drought conditions. The available measures of plant water status can be divided into energy status and water amount (Jones, 2007). Among these measurements, it is widely accepted that leaf water potential and relative water content are usually used to evaluate drought severity (Flexas and Medrano, 2002).

Plant water potential

Plant water potential has a profound effect on crop productivity. It measures the free energy of water per unit volume relative to pure water under the same temperature and pressure conditions. The commonly accepted measurement unit for the water potential is the pascal (Pa), also expressed as kPa or MPa. It should be noted that water always moves in response to a water potential gradient from higher to lower potential (Taiz et al., 2018). The water potential is primarily composed of three components, including solute potential (Ψ_s), pressure potential (Ψ_p) and gravitational potential (Ψ_g). The gravitational potential is usually negligible compared with the solute potential and pressure, so the water potential can be simplified as follows,

$$\Psi_w = \Psi_s + \Psi_p$$

Solute potential (also known as osmotic potential) represents the effect of dissolved solutes in water potential. It is always negative as solutes reduce water's free energy by diluting it (Hsiao, 1973). Pressure potential measures the impact on the free energy of water from the hydrostatic pressure. The positive hydrostatic pressure within cells is called turgor pressure (Taiz et al., 2018). It plays a crucial role in the expansion and growth of cells (Prichard, 2001). Tension is frequently developed in xylem conduits as negative hydrostatic pressures, which is crucial in the long-distance movement of water through the plant (Taiz et al., 2018).

There are two principal methods of measuring water potential: the psychrometer and the pressure chamber. The former is based on the large latent heat of vaporisation. The latter determines leaf water potential by applying external gas pressure to an excised leaf until water is forced out of the stem (Taiz et al., 2018).

Leaf relative water content

Relative water content (RWC) is another important character that indicates the leaf water status. RWC is the ratio of the amount of water in a plant tissue relative to that present at full turgor, expressed as RWC (%) = (fresh weight- dry weight) / (turgid weight-dry weight) *100 (Barrs and Weatherley, 1962). RWC, as the metabolically available water, could reflect the metabolic activity (e.g., transpiration and photosynthesis) in plant tissues (Yan et al., 2016; Raza et al., 2017).

1.3.3 Soil-plant relations

Movement of water in the soil

The bulk flow determines the movement of water in the soil. It is the concerted movement of molecules and masses (Taiz et al., 2018). Water moves to and from the soil in specific ways, such as precipitation and transpiration. Water can move in any direction in the soil by capillarity but only downwards by gravity (Simpson, 1983). The water potential difference forces water to flow into the soil. An equilibrium can be approached when the difference in water potential between two regions is zero. It is widely accepted that there are two main kinds of water flow in the soil: liquid water and water vapour (Tan, 2009).

Generally, in unsaturated soil, macropores are filled with air. As a result, water flows downwards due to the water potential difference and the gravitational potential and upwards caused by the capillary rise. The capillary flow of water greatly affects the growth of plants, as it provides water available for the root, especially in dry soil. It occurs when the subsoil is wet while the surface of the soil is dry. On the one hand, adhesion (hydrogen bonding) leads to the attraction between the capillaries and water molecules. Those adsorbed water molecules, in turn, attract other water molecules through cohesion. In wet (saturated) soil, all the pores are full of water. Water only flows downwards in the soil by percolation, the driving force that comes from the combination of water and gravitational potential (Tan, 2009).

Water vapour is another type of water moving in the soil, and it is related to the partial pressure, which determines the relative humidity of soil air. The movement of water vapour occurs spontaneously from higher to lower concentrations to attain an equilibrium status. Water vapour can move from dry to wet soil because of differences in their water vapour concentrations. In addition, temperature and barometric pressure also affect water vapour transport in the soil (Tan, 2009).

The flow rate of water moving in the soil is mainly determined by two factors. One is the degree of the pressure gradient through the soil, and the other is hydraulic conductivity. Soil hydraulic conductivity refers to the ease of water moving in the soil. It differs in different types of soil and with different water content (Taiz et al., 2018). For example, sandy soils have larger hydraulic conductivity than clay soil under saturated conditions because of more space between sandy particles (Taiz et al., 2018).

Removal of water in the soil

Other than water movement in the soil, water removal from the soil often occurs in several ways. It can be lost as liquid water from the soil through runoff, percolation, and leaching. More importantly, water loss in the form of water vapour (i.e., evapotranspiration) is the combined effect of evaporation and transpiration. Evaporation is a passive process that water is transformed from the solid and liquid phases into vapour; transpiration is the loss of water from the root to the stem to the leaf and finally to the atmosphere. Water from the roots is pulled up by the tension, i.e., negative potential caused by the evaporation of water at the leaf-atmosphere interface. This gradient of water potential between the soil and the atmosphere drives water to move toward areas with less water. The loss of water through transpiration causes high surface tension and negative turgor pressure in the xylem, which enables water from the roots then up to the apex of the plant (Tan, 2009). The first resistance to transpirational flow of water from the soil hydraulic conductivity determines the velocity of water absorbed by the root surface from the soil (Bodner et al., 2015). Accordingly, the structure of plant roots can be adjusted to access more water from the soil by the increase in the surface area of roots. For example, the increase in root density can decrease the distance of water movement between roots and facilitate the process of water uptake by roots (Gardner, 1960). Improving rhizosphere hydraulic properties, such as the accumulation of organic compounds, can also enhance water availability for roots (Carminati et al., 2011).

Water transport through the plant

Water uptake in the plant occurs on the surface area between roots and soils. Namely, root hairs can provide enough surface area to absorb water and ions from the soil, which are filamentous and made up of epidermal cells outgrowing roots (Taiz et al., 2018). As Steudle (2000) proposed a composite transport model, there are primarily two pathways accounting for water movement through the roots (Figure 1.3.3). One is the apoplast route, in that water flows through the cell walls without crossing any membrane. There is only hydraulic water flow, and it moves in nature. The cell-to-cell pathway is another route, including both hydraulic and osmotic flow. This route can also be split into the symplastic and the transmembrane pathway. The former refers to water that travels across the plasmodesmata within the symplast; while the latter is defined as water that crosses the plasma membrane of each cell twice (two membranes per cell layer), including entering on one side and exiting on the other side (Taiz et al., 2018).

Apoplast and cell-to-cell pathways can be switched depending on the absence/presence of transpiration (Steudle, 2000). For example, in the presence of transpiration, both the apoplastic and cell-to-cell routes occur, driven by the difference in the hydrostatic pressure between soil and xylem. In the absence of transpiration, only the cell-to-cell pathway is dominated, whilst it has a relatively higher hydraulic resistance because of apoplastic barriers (Casparian bands and suberin lamellae). When soil water deficit occurs, the hydraulic resistance of roots can be increased due to development of apoplastic barriers for water and ion flow, preventing plants from excessive water loss (Steudle, 2000).

The pathway provided by the xylem has low resistance for water transport in the plant (Nobel, 2005). The tension can be increased to pull water from the soil through the xylem due to plant transpiration, also known as cohesion-tension theory (Taiz et al., 2018). It is the primary driving force for water moving through xylem vessels, which also impacts the water uptake by roots under water-stressed conditions (Steudle, 2000).

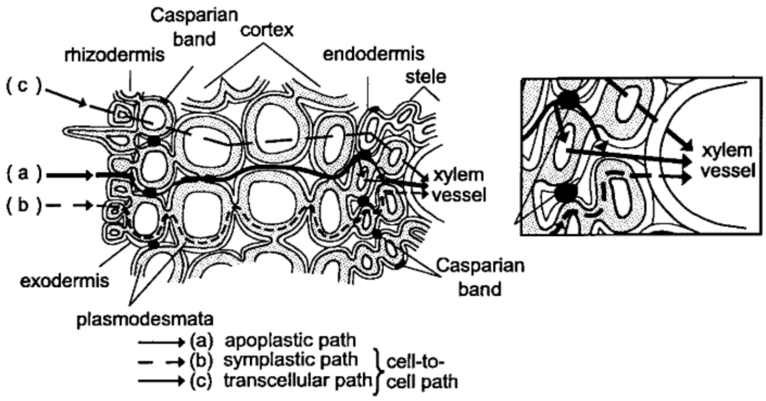


Figure 1.3.3 Three transport pathways in the root (adjusted from Steudle, 2000).

1.4 Effects of drought on rapeseed

1.4.1 What is drought?

Drought can be grouped by type of disciplinary perspective according to three primary characteristics: intensity, duration and spatial coverage, and their relationships are displayed in Figure 1.4.1 (Wilhite, 2000). Climate change is projected to intensify water scarcity and the frequency of drought events in the foreseeable future (Pachauri et al., 2014). Agricultural drought refers to environmental conditions that adversely affect cellular homeostasis, impair crop growth, and suppress yield production (Wu et al., 2018). Empirical studies have reported that drought is the most devastating abiotic stress that has detrimental effects on crop growth, thus leading to considerable yield losses of crops, particularly in arid and semi-arid regions (Kebede et al., 2019). Crop responses to drought involve with complex processes. At the physiological level, drought influences stomatal conductance by changes in leaf turgor and osmotic adjustment, thereby reducing transpiration and photosynthesis. Accumulating antioxidants and plant growth substances such as abscisic acid can also help crops adapt to drought conditions (Farooq et al., 2009; Kuromori et al., 2018). An overview of the current work on crop responses to drought is elaborated below, emphasising rapeseed in aspects of yield, morphological and physiological processes.

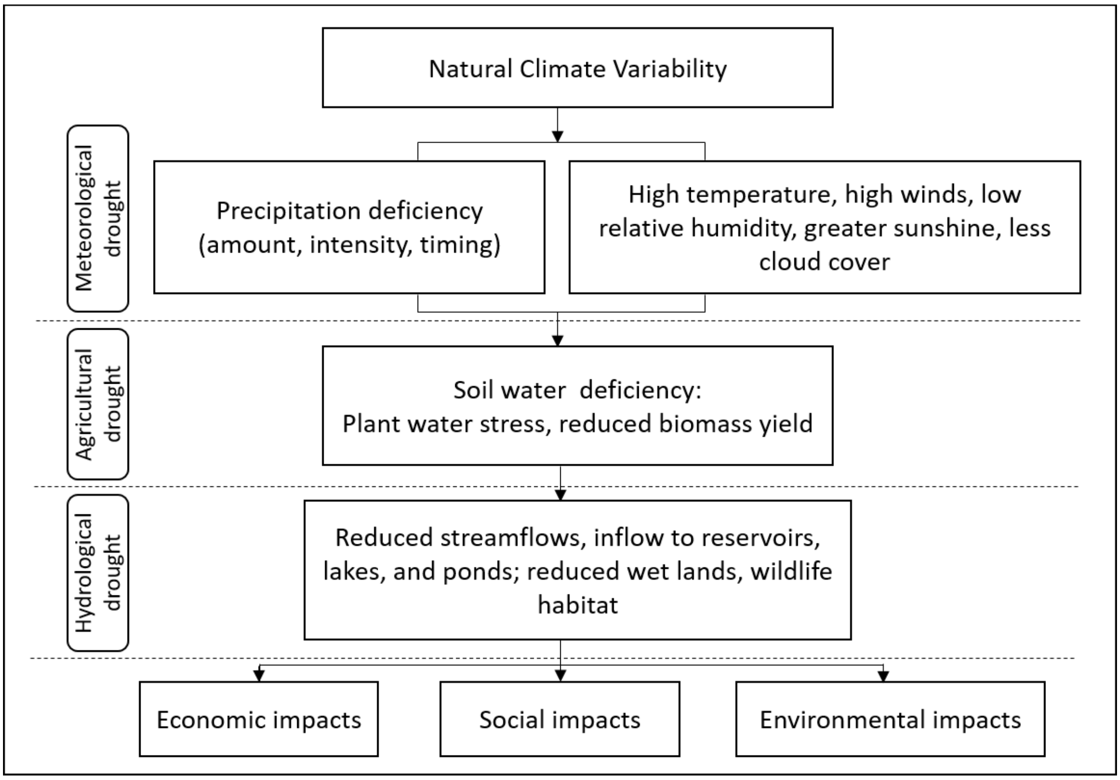


Figure 1.4.1 The relationship between various types of drought (adapted from Wilhite, 2000)

1.4.2 Effects of drought on rapeseed

Drought resistance and crop phenology

Drought resistance can be categorised into three drought response strategies as drought avoidance (e.g., reduction in water loss or increase in water uptake by roots), drought escape (shortened growing season or life cycle such as early maturity) and drought tolerance (favouring survival mechanisms) (Levitt, 1980). Rapeseed plants tend to cope with drought through avoidance by conserving water to improve water use efficiency (Jensen et al., 1996). Nonetheless, there are likely trade-offs between avoidance and escape that greater avoidance (such as high WUE) may reduce the rate of growth and development (Franks, 2011).

Appropriate phenology plays a crucial role in affecting crop adaptation to variable conditions as phenological events such as the flowering impact the allocation of carbohydrates to different plant organs, which are highly associated with yield production (Richards, 1991). Numerous studies have shown that water stress could accelerate crop phenology by initiating flowering earlier and shortening the number of days to maturity (Papantoniou et al., 2013). Zhang et al. (2013) evaluated the interaction between genotypes of rapeseed with wide-ranging phenology and environmental conditions for seed yield, showing that higher temperature and relative drought conditions (< 300 mm rainfall) resulted in a short growing season and thus leading to a lower seed yield. Under such conditions, early flowering genotypes produced higher seed yields than medium-flowering ones, emphasising the importance of phenology in adapting rapeseed plants to a specific environment.

Plant water status

Several important characteristics can impact plant water status, such as relative water content (RWC), leaf water potential (LWP), stomata resistance, leaf temperature and canopy temperature (Farooq et al., 2009). RWC and LWP, as drought stressors, decrease in response to drought at different intensities. Also, LWP responses may vary in different species depending on the drought resistance (Yan et al., 2016). As rapeseed can adopt a "conservational" strategy to stabilise plant water status through stomata closure under mild drought conditions, the greater reductions are pronounced when drought develops more severely to a certain degree (Jones, 2007). For example, Hosseini et al. (2015) reported that drought stress reduced the RWC of two different spring canola cultivars (sensitive cultivar and tolerant cultivar) significantly under either moderate drought conditions (50% AWC) or high-stress conditions (25% AWC). Compared to control treatments (80% AWC), a greater reduction was observed in the sensitive cultivar than in the tolerant one.

Osmotic adjustment

Osmotic adjustment (OA) is defined as the capacity of plants to accumulate solutes and sustain the metabolic activity in plant cells in response to water deficit (Munns, 1988). It is usually expressed as the change of the osmotic potential (Ψ_s). Leaf Ψ_s decreases when plants are exposed to drought relative to well-watered controls due to the accumulation of osmotically active solutes, i.e., osmolytes (Ashraf and Mehmood, 1990). The rise of osmolality (the concentration of osmolytes) in stressed plants is not only due to the accumulation of osmolytes but also it is attributed to the water deficit of leaves (shown in the reduction of leaf RWC) (Müller et al., 2010). As soil moisture deficit develops, rapeseed reacts first with OA, which favours turgor maintenance, thus sustaining tissue metabolic functions (Müller et al., 2010; Tuberosa, 2012).

OA has been considered a primary regulator of turgor and stomatal conductance (Blum, 2017). The turgor in the root or shoot can be maintained via OA, and the capture of soil water can also be promoted to improve the yield when plants are exposed to drought (Kusaka et al., 2005; Velázquez-Márquez et al., 2015). Different genotypes vary in the ability of OA from *Brassica* species. High-osmotic adjustment rapeseed genotypes can have a higher drought resistance as they can maintain relatively high RWC via great reductions in the osmotic potential while maintaining turgor pressure and subsequently turgor-related processes such as stomatal movement and photosynthesis (Kumar and Singh, 1998). Moreover, OA is positively associated with yield under water stress (Norouzi et al., 2008; Blum, 2017). Niknam et al*.* (2003) reported *Brassica* genotypes experienced a larger reduction in the yield up to 40% with low OA than those with high OA (0-10%) under rain-fed conditions. Ma et al. (2006) also reported fewer yield reductions in droughted *Brassica* species with high OA.

Stomatal movement

Stomata are the pores accompanied by a pair of specialised guard cells on the surface of leaves, regulating the flow of gases in and out of leaves (Hetherington and Woodward, 2003). The size of the stomatal aperture is controlled by changes in the guard cell turgor and neighbouring epidermal cells that counteract the bending of guard cells. That is, higher turgor in guard cells than in epidermal cells provokes stomata opening and vice versa (Kollist et al., 2014). The responsiveness of stomatal movement is highly associated with its morphological traits such as stomatal density (stomatal number per unit area), stomatal size (stomatal width and length), the shape of guard cells and the absence/presence of subsidiary cells (Bertolino et al., 2019). From a long-term perspective in morphology, stomatal size and density can be modified in response to environmental conditions, thus altering maximum stomatal conductance to water vapour that represents the maximal gas exchange when all stomata are fully open. Over the short term, dynamic adjustments to the opening degree of stomatal pores are responsible for regulating stomatal conductance, allowing plants to quickly reduce water loss according to changing environmental conditions (Dow et al*.*, 2014; Bertolino et al., 2019). As stomatal size and density are negatively correlated, an efficient and faster stomatal response can be achieved by decreasing stomatal size while increasing stomatal density. This is mainly due to a high ratio of membrane surface area to volume, with a short diffusion path provided by a small pore depth (Bertolino et al., 2019). In eudicots such as *Brassica napus*, two kidney-shaped guard cells form on the epidermal cells. Unequal distribution of stomata with more on the abaxial surface than on the adaxial is beneficiary for water conservation when rapeseed is under drought.

The Opening/closure of stomata pores is attributed to the increase/reduction of guard cell turgor pressure, which influences the rate of CO_2 uptake and water loss by transpiration (Bertolino et al., 2019). According to genetically molecular and physiological attributes of crops in response to water stress, there are two types of stomatal behaviours, isohydric and anisohydric (Tardieu and Simonneau, 1998; Martínez-Vilalta and Garcia-Forner, 2017). Isohydric plants could maintain a constant leaf water potential and relative water content by reducing g_s and transpiration within a certain range to avoid damage from water stress. This response is mainly triggered by the interaction between hydraulic and chemical signals. In contrast, leaf water potential in anisohydric plants continuously declines as the soil dries with the evapotranspiration demand (Tardieu and Simonneau, 1998). Rapeseed tends to adjust their stomata closing in an "isohydric" manner to maintain leaf water status relatively stable under mild or moderate drought conditions, until the drought becomes more severe to the point where there are obvious decreases in leaf water potential (Faralli et al., 2017).

Stomatal conductance

One of the conventional approaches for estimating the relationship between net CO_2 assimilation rate (*A*) and stomatal conductance (g_s) was initially raised by Ball Berry, thereby called the Ball-Berry (BB) model (Miner et al., 2017). It can be described as $g_s = m*A\frac{Hs}{Cs} + g_0$, where H_s is the relative humidity at the leaf surface, C_s is the concentration of CO_2 at the surface of leaves, and m and g_0 are the slope and intercept of the regression, respectively. It indicates that stomatal responses can be affected by environmental factors such as CO_2, light, and temperature (Ball, 1988).

Efficient and fast stomatal movement plays a role in crops adapting to drought stress (Franks et al., 2009). Stomatal closure induced by drought can help plants survive by minimising water loss by the reduction in transpiration from the surface of leaves, achieving optimal water use efficiency despite the limitation in photosynthesis (Buckley, 2005). Empirical studies have shown that drought can reduce g_s of rapeseed plants due to stomatal closure (Hess et al., 2015). Elferjani and Soolanayakanahally (2018) reported that g_s dropped nearly to one-fourth of the well-watered control treatment. Likewise, Hosseini et al. (2015) also found negative effects from drought on g_s. However, appropriate cultivars may counteract the adverse impact of drought to a certain degree. In drought-tolerant varieties, g_s decreases more than other physiological parameters under different drought intensities. Furthermore, reductions in g_s account for the reduction of transpiration more than photosynthesis (Yan et al., 2016).

Photosynthesis

Photosynthesis is the primary source of carbon input in the plant, during which light energy is used to synthesise carbohydrates and generate oxygen from carbon dioxide and water (Taiz et al., 2016). The capacity of plant photosynthesis is mainly dependent on two components: CO_2 diffusion across the air-leaf path and the biochemical traits such as enzymes related to photosynthesis and light-harvesting complexes (Nadal and Flexas, 2018). In rapeseed, photosynthetic capacity and some photosynthesis-related chemicals in leaves such as chlorophyll *a*, *b* and carotenoid contents are substantially higher than in the pods. The photosynthesis rate (*A*) of leaves is similar from the vegetative to the mid-flowering stage, and it drops by the end of flowering (Lu et al., 2017; Tsialtas et al., 2017). Meanwhile, the surface area and photosynthesis of leaves affect the development of pods during the flowering stage and, thus, the final seed yield. At the pod-forming and seed filling stage, CO_2 assimilation mainly depends on pods because leaf senescence affects seed yield and oil content (Wang et al., 2016). In addition, photosynthesis occurring in the chloroplasts from the outside pod wall may contribute 70-100% to seed growth (Jensen et al., 1998).

The diffusion of CO_2 into the leaf through photosynthesis and water loss through transpiration is controlled by stomatal conductance (Hetherington and Woodward, 2003). It has been reported that the closing of stomata contributes most to the decline of leaf photosynthesis during mild drought conditions (Cornic, 2000). The stomata control about 95% of all gaseous fluxes between leaves and the atmosphere with similar pathways for CO_2 and water. Two diffusional resistances in sequence determine the pathway for CO_2 uptake from the air to the site where photosythsis occurs. Apart from the dominant resistance to CO_2 flux from the atmosphere to the leaves provided by stomata, the second resistance is the mesophyll after CO_2 passes through the aqueous and lipid boundaries. On the other hand, water leaves the leaf through the same pathway reversely without resistance from the mesophyll (Lawson and Blatt, 2014).

Signalling system

There are two principal types of signals in plants which can respond to drought, hydraulic and chemical signals. Hydraulic signals are internal changes related to water status in plants, which propagate from one cell/organ to another. Reduced leaf water potential leads to stomatal closure by increasing the stomata sensitivity to the plant hormone abscisic acid (ABA) (Boyle et al., 2016). Chemical signals are related to the synthesis and transport of hormones and other chemical species in droughted plants (Wilkinson and Davies, 2010). Water stress can be detected first in the root tissues and leads to the responses of hydraulic and non-hydraulic signals between roots and shoots (Jiang and Hartung, 2008).

ABA is a plant hormone that triggers plant adaptation to water stress by controlling stomatal closure. It is well established that the accumulation of ABA in guard cells triggers stomatal closure, thereby avoiding an excessive water loss of plants under drought conditions (Kollist et al., 2014). Accumulated ABA induces rapid Ca^{2+} influx in the guard cells, and the increase of Ca^{2+} concentration in the cytosol inhibits the membrane proton pumps and K^+ inward channels while activating the anion and K^+ outward channels in the plasma membrane. This rapid efflux of anions and cytosolic solutes can result in membrane depolarisation, leading to loss of guard cell turgor and eventual stomatal closure (Wan et al., 2009). It is synthesised in root tissues under drought conditions and then released to the xylem vessels. Stomatal closure occurs after ABA is transported to the shoot, thus limiting transpiration to minimise water loss (Jiang and Hartung, 2008; Sirichandra et al., 2009).

Two types of ABA from internal and external resources in the plant need to be considered. Internal ABA is dominantly synthesised by the root, and part of it is from the import of phloem into the root, which previously has been synthesised in the shoot, while external ABA mainly comes from root exudation and individual ABA-producing soil organisms such as fungus (Jiang and Hartung, 2008). The amount of ABA increases in the xylem sap when soil drying occurs. It can be carried with a transpiration stream entering the leaf in a symplastic way and before it reaches the guard cells (target cells), mesophyll normally removes (by metabolism and sequestration) much of the ABA passing through it. Increased concentration of ABA in the guard cells results in increased amount of external and internal cytoplasmic calcium, thereby reducing osmotic potential via loss of K^+ and Cl^-. Consequently, it induces the closure of stomata (Wilkinson and Davies, 2002).

Under drought conditions, pH in the xylem sap is different in droughted plants compared to well-watered control, which is about pH 7.0 and 6.0, respectively (Wilkinson, 1999). Generally, the leaf has a sizable reservoir (i.e., mesophyll cells) to sequester and catabolise ABA. It can prevent ABA from entering the symplast, so a larger amount of ABA can be observed in the xylem as more synthesised ABA enters the apoplast (Wilkinson, 1999). As such, increases in the pH of the xylem sap weaken the leaves' ability to sequestrate ABA into the symplastic reservoir, leading to more of the ABA entering the leaf. It may give a rise in the ABA concentration of the stomatal guard cells, despite much of the ABA being removed by the mesophyll cells before it reaches the guard cells through sequestration and catabolism (Wilkinson and Davies, 2002). Therefore, ABA plays a crucial role in regulating stomatal behaviour and gas exchange under drought conditions (Ashraf and Harris, 2013).

Water-use efficiency (WUE)

Water is highly important for crops to maintain the growth and expansion of tissues. Nevertheless, more than 90% of the water absorbed by the plant will be transpired into the air (Morison et al., 2007). Regardless of availability, water use characteristics vary in different crops (Gan et al., 2009). For instance, Johnston et al. (2002) found that the seed yield of rapeseed increased at a rate of 7.2 - 7.7 kg ha^{-1} per mm of water based on the minimum amount of water use. It is consistent with Tesfamariam et al. (2010), that rapeseed produced about 7.09 kg ha^{-1} per mm of water under no water-stressed conditions. Plants consumed the lowest amount of total water use, while the lowest water-use efficiency (WUE, 4.45 kg/mm) was also recorded when plants were exposed to water stress at the flowering stage. However, the improvement of WUE under drought conditions can be achieved if efficient cultivars are selected. It is mainly because consuming less water due to the stomatal closure, thus leading to less transpiration rate, whilst the dry matter of plants was still accumulated (Fahad et al., 2017). More than recently, how to improve the WUE of crops has been proposed as a primary goal in agriculture (Medrano et al., 2015).

From the physiological perspective, WUE is the ratio between net CO_2 assimilation and water loss to the air through transpiration (Li, 2013). At the level of plants, it can be defined as WUE=biomass/water transpired (Vadez et al., 2014); at a leaf level, it can be calculated with instantaneous ($WUE_{instantaneous}$) measurements, i.e., the ratio of net photosynthetic assimilation rate (*A*) to transpiration rate (*T*). It is also known as the agronomic water-use efficiency or transpiration efficiency, which is related to the harvested yield of crops achieved with the amount of available water provided by artificial irrigation or natural precipitation (Condon et al., 2004; Vadez et al., 2014).

Carbon isotope discrimination ($\Delta^{13}C$) provides an indirect and useful tool to measure the variation of A/T and is negatively related to WUE (Condon et al., 2004). It is the ratio of stable carbon isotopes ($^{13}C/^{12}C$) in the plant dry matter since C_3 species discriminate against ^{13}C during photosynthesis(Condon et al., 1990), primarily based on the carbon-fixing enzyme (Rubisco) with a higher affinity for ^{12}C than ^{13}C (Mir et al., 2012). The smaller $\Delta^{13}C$ indicates a higher WUE due to the well-established relationship between i$\Delta^{13}C$ and the ratio of intercellular to atmospheric partial pressures of CO_2 (Farquhar and Richards, 1984). Under drought conditions, $\Delta^{13}C$ increases because of reduced g_s that restricts CO2 diffusion. $\Delta^{13}C$ has been developed as one of the indirect selection criteria for C_3 species breeding programmes with an improved WUE (Farquhar and Richards 1984).

Intrinsic water-use efficiency (WUE_i) is another method to describe the status of water use instantly, which is the ratio of the net photosynthetic assimilation rate (*A*) by the stomatal conductance (g_s). It varies as the environmental conditions change. High yields can be achieved by increasing *A,* whereas increasing g_s can reduce WUE_i, especially at a high g_s (Morison et al., 2007). Generally, WUE_i in droughted plants is higher than that in well-watered plants as a larger reduction occurs in g_s than *A*. Elferjani and Soolanayakanahally (2018) reported that WUE_i increased significantly by about 173% when rapeseed was subjected to water stress.

Root system architecture

Root system architecture refers to the spatial configuration of a root system in the soil, including the shape and structure (de Dorlodot et al., 2007). Rapeseed has a typical taproot architecture with a large surface area for the nutrients and water uptake from the soil. The taproot only accounts for a small proportion of the total in the root length (≤ 1%), surface area (< 2%) and root volume (around 15%), while the remaining proportion is mainly occupied by the smallest roots (diameter within 0 - 1 mm) (Wu et al., 2017). In contrast with wheat that has the capacity to grow adventitious roots with aerenchyma to facilitate the diffusion of oxygen from the shoot into and along the roots, roots of rapeseed cannot develop aerenchyma under waterlogging conditions (Ploschuk et al., 2018).

Good root elongation is crucial for crops to survive under drought conditions, and the root elongation rate is influenced by both water stress and penetration resistance in the soil (Bengough et al., 2011). Further, limited access to water also impacts the uptake of many important nutrients by roots due to the restricted movement of these nutrients in the soil (Fahad et al., 2017). In rapeseed, the sensitivity to water stress was primarily from differences in the shoot, rather than root characteristics (Hess et al., 2015). Compared to aboveground biomass, root biomass is less influenced by water stress; consequently, the root/shoot ratio increases (Wu et al., 2018b). Qaderi et al. (2006) found that droughted rapeseed had shorter and thinner stems, smaller leaves, and lower yield production. In contrast, the root/shoot ratio of rapeseed increased significantly. Rapeseed roots can grow more than 1.5 m deep in the soil to extract water when suffering drought (Johnston et al., 2002).

1.4.3 Effects of drought on rapeseed yield

Yield and yield components

Previous studies have reported that rapeseed is the most susceptible to water stress over the flowering stage, during which a considerable reduction of leaf areas could inhibit the assimilate availability, thereby decreasing sink size (seed number) (Istanbulluoglu et al., 2010; Hess et al., 2015). Near-open buds and newly opened flowers are highly sensitive to assimilate supply for ovule development; thus, assimilation restrictions at flowering could result in pod abortion and impair the capacity for compensatory growth of surviving pods over the late reproductive stage (Kirkegaard et al., 2018). Compared to the vegetative and seed filling stages, rapeseed crops are more sensitive to water stress at flowering, and they can benefit from irrigation as possible in seed yield. Recent field studies about yield losses caused by drought at flowering are summarised in Table 1.4.1.

The development of pods in rapeseed plays a crucial role in enhancing seed yield and quality. Further, In Rood and Major (1984) showed that the growth of pods was more sensitive to irrigation than leaves. Norouzi et al. (2008) reported that pod number per plant was more influenced by water stress than other yield components during the reproductive stage. Similar conclusions have also been drawn by Ahmadi and Bahrani (2009), and in Müller et al. (2010), pod dry weight in drought-stressed treatments was, on average, reduced by 29% with respect to well-watered rapeseed. Hess et al. (2015) found that comparing the sensitivity to water stress between rapeseed and wheat, the former was more sensitive, particularly in the shoot, resulting in a greater biomass reduction by about 52% compared to its irrigated controls. Similar conclusions are made by Dreccer et al. (2018) that winter rapeseed was the most susceptible to water deficit among several crops, such as barley, chickpea and wheat.

Seed weight per pod is the product of individual seed weight and seed number per pod, i.e., seed weight × seed number (Diepenbrock, 2000). Raza et al. (2017) reviewed several experiments and summarised that seed yield, thousand-seed weight, and seed number per pod of rapeseed decreased under drought conditions by influencing the growth and uptake of plant nutrients. Moreover, the number of seeds per pod changes less than the pod number per plant (Zarei et al., 2010). It has also been reported that there was a positive and significant association between 1000-seed weight and oil contents ($r^2 = 0.762$) (Asrami et al., 2014). In Faralli et al. (2016), drought decreases the seed dry weight of rapeseed by 39% on average.

Selective abortion of pollinated flowers and fruits is beneficial for allocating resources to reproduction from an evolutionary perspective and is also a major limiting factor of yield under drought conditions (Stephenson, 1981). There are two causes of drought-induced pod and seed abortion: reduced turgor pressure leading to limited cell expansion during cell growth, and reduced carbon supply resulting from stomatal closure restricting photosynthesis (Turc and Tardieu, 2018). For example, aborted ovaries of maize are located in regions carrying the youngest ovaries, i.e., at the ear apex, when mild water deficit occurs at flowering time. Under such circumstances, water deficit does not change this pattern, but extends the region of shoot/inflorescence where ovary abortion occurs by reducing cell expansion of reproductive structures. Carbon deprivation appears to dominate only in severe post-flowering drought scenarios, which substantially decreases photosynthesis, and results in a random distribution abortion on the ear (Turc and Tardieu, 2018). In rapeseed as discussed above, water stress at flowering has the greatest negative impact on the number of pods and seeds (sink), resulting in final yield losses. The reasons for pod and seed abortion can be attributed to the development-based abortion that is reinforced by water stress limiting cell expansion, and more pronounced when water deficit is moderate. Also, as there is a strong overlap between stem and branch growth, flowering, pod setting and development, and seed filling, which creates a strong competition for sources by different plant organs, carbon-starvation-based abortion due to limited sources available can induce pod and seed abortion that occurs later, in particular when severe drought is imposed (Turc and Tardieu, 2018; Zhang and Flottman, 2018).

Table 1.4.1 Effects of drought over the reproductive stage on seed yield and oil content of rapeseed (*Brassica napus* L.) under field conditions.

Varieties	Drought		Reduction (%)		Sources
	Type	Timing	Seed yield	Oil content	
Spring *B. napus*	Rain-shelter	Flowering to maturity	20	-	(Nielsen, 1997)
	Rain-shelter	Throughout the season	68	-	(Angadi et al., 2008)
	Rain-fed	Flowering to maturity	23	3	(Shirani Rad and Zandi, 2012)
	Rain-fed	Flowering to maturity	23	3	(Zandi et al., 2012)
	Rain-fed	Flowering to maturity	51	11	(Katuwal et al., 2020)
Winter *B. napus*	Rain-fed	Flowering	16	1	(Istanbulluoglu et al., 2010)
	Rain-shelter	Flowering	39	8	(Tesfamariam et al., 2010b)
	Rain-fed	Flowering to maturity	19	1	(Rad et al., 2014)
	Rain-fed	Flowering to maturity	51	6	(Shirani Rad and Zandi, 2014)
	Rain-fed	Flowering to maturity	76	6	(Alipour and Zahedi, 2016)
	Rain-fed	Flowering to maturity	33	18	(Farahani et al., 2019)

Rain-fed	Flowering to maturity	29	-	(Shafighi et al., 2020)

Oil content

The oil, protein and glucosinolate contents are important quality attributes of rapeseed, and these attributes are highly affected by drought. The meal that remains after oil extraction has value as a source of protein for the livestock feed industry. Glucosinolates are produced as secondary metabolites from droughted tissues (Jensen et al., 1996; Robertson and Holland, 2004).The oil content of seeds is mainly influenced by the sowing date, locations and environmental factors such as temperature and water stress during the seed filling stage (Robertson and Holland, 2004). High temperature can adversely lower oil content and reduce fatty acid in the seed (Aslam et al., 2009). Safavi Fard et al*.* (2018) explored the effects of drought stress on some qualitative characteristics in several cultivars of winter rapeseed, and they found that oil content was decreased to 41.1% and 42.2%, respectively when the irrigation withheld after flowering and pod formation stage relative to normal irrigation control (43.4%), but there was no significant difference between two stages. Robertson and Holland (2004) reviewed multiple papers and found a 0.8% - 2.7% decrease in oil content in response to each 1 °C rise in various locations with different sowing dates.

Additionally, oil content is positively related to seed yield, but this relationship is not as strong as that with the sowing date. The intensity of the correlation between oil content and seed yield depends on the water supply. In other words, the wetter locations have a stronger correlation between seed yield and oil content, and vice versa (Robertson and Holland, 2004).

1.5 Application of antitranspirants on crops

1.5.1 General introduction to antitranspirants

Antitranspirants (AT) are mainly classified into three types according to their different roles: 1) reflecting materials (e.g., Kaolin) that reduce the absorption of radiation, followed by the decrease in the temperature and stomatal conductance of leaf (Glenn 2009; Boari et al*.*, 2015, 2016); 2) metabolic compounds (e.g., Chitosan; fulvic acid, FA; abscisic acid, ABA; phenylmercuric acerate, PMA) that affect the guard cells around stomatal pores to inhibit stomatal opening fully, and thereby decrease the loss of water vapour from the leaf (Iriti et al*.*, 2009); 3) film-forming polymers (e.g., di-1-*p*-menthene, Vapor Gard; poly-1-*p*-menthene, Nu-Film P) are generally wax and plastic-based emulsions on the surface of leaves that can create a waterproof layer to block stomata and thus improve plant water status (Gale and Poljakoff-Mayber, 1967; Kettlewell, 2014; Khan et al*.*, 2018).

Research into the film AT started from the 1950s to the 1970s on various plant species. Gale and Hagan (1966), Das Raghavendra (1979) and Solarova et al. (1981) reviewed a number of previous studies on film-forming AT to explore different physiological aspects of AT on plants. They made a consistent conclusion that although reduced water loss from leaves, photosynthesis was also restricted resulting from the low permeability of films to CO_2 entering the leaf, thus inhibiting plant growth. Therefore, film AT was mainly recommended for some species, with reduced water loss being more important than photosynthesis, such as woody plants (Davies and Kozlowski, 1974). Apart from that, empirical evidence has shown that film AT can inhibit fungal pathogens through physical barriers for crop protection (Han, 1990; Sutherland and Walters 2002; Percival and Boyle 2009). The film-forming polymers usually provide a physical barrier on the leaf to prevent spore adhesion, hydration and germination, and enable leaves to disguise the cues necessary for germ tube growth (Walters, 2006). For example, Ziv and Frederiksen (1987) reported that film AT could control the leaf rust and powdery mildew sufficiently on wheat if sprayed during the vegetative stage. Sutherland and Walters (2002) also reported that film AT could significantly reduce powdery mildew infection on barley under both glasshouse and field conditions.

Kettlewell et al. (2010) initially explored the effect of film AT application at different growth stages of wheat for three years and found that yield was increased when AT was applied just before the stage the most sensitive to drought. This discovery of the role of the crop growth stage in modulating AT efficacy in wheat highlights the potential of film AT to mitigate drought damage to crops. Thereafter, there is increasing research interest in the potential of film AT on a wider variety of crops, taking the spray timing into account for specific crops. Given that CO_2 encounters the second resistance between the sub-stomatal cavities and the chloroplast in addition to the stomatal resistance, film AT can potentially improve water use efficiency by reducing transpiration more than photosynthesis (Anderson and Kreith, 1978). The yield benefits from film AT have been reported on many crops such as sweet pepper (del Amor et al., 2010), pea (Aldasoro et al., 2019), wheat (Kettlewell, 2014; Abdullah et al., 2015; Weerasinghe et al., 2016) and rapeseed (Faralli et al., 2016, 2017b), when film AT is applied at the most drought-sensitive stage, during which reduced photosynthesis is less important than conserving water for yield production. Responses to film AT application on crops under drought will be elaborated in this section, with an emphasis on physiological processes and yield production, and rapeseed-related research will be mainly demonstrated in a separate section.

1.5.2 Application of film antitranspirant on crops

Stomatal aperture and leaf coverage

Given the fact that film AT acts as a physical barrier to water loss from leaves, stomatal aperture and coverage from the leaf can impact AT efficacy. One way is to collect epidermal impressions and observe them under a microscope. Gale and Poljakoff-Mayber (1967) collected silicone-rubber impressions from the leaf epidermis and showed that the films were of uneven thickness and contained many gaps and micropores. Faralli et al., (2016) used Dental putty for stomatal imprinting of rapeseed leaves, followed by re-produced impressions by nail-varnish peels for slides for stomatal aperture under the microscope. They observed application (1%) of Vapor Gard (VG) significantly increased adaxial aperture of stomata uncovered, when compared to control when rapeseed was under well-watered or water-stressed conditions. Alternatively, scanning electron microscopy (SEM) can be used for stomatal aperture, albeit with more complex preparation procedures than the impression method. Iriti et al. (2009) observed a slight increase in the abaxial stomatal opening when VG 2% was applied to the bean compared to the control under SEM.

In contrast, Fahey and Rogiers (2019) found no reduction in the stomatal aperture of grape leaves at 24 h after spraying VG 2% under SEM. The formed-film on the surface can result in a partial loss of topographic details (Iriti et al., 2009). Given the fact that the size of the area from the microscopic view might be limited, leaf coverage, related to the spraying method, may be useful to investigate the optimum concentration of film AT for specific crops. To our knowledge, however, there is little information about leaf coverage of crops after AT application from publications.

The spatial distribution of spray droplets discharging from the sprayer determines the canopy deposition and drift (Salyani et al., 2013). Generally, canopy deposition and spray losses depend on multiple factors, such as operational parameters (e.g., nozzle type and configuration, spray pressure, application volume, the distance between the nozzle and target area), and tank mix properties. Those factors, in turn, influence the droplet size and spatial distribution of spray droplets (Salyani et al., 2007; Ozkan et al., 2012). So, assessing the spray distribution of AT solutions is of great importance to understand the efficacy related to coverage from different concentrations (or dose rates) using quantitative methods.

A quantitative method of measuring spray distribution is using a tracer (De Moor et al., 2000). For instance, fluorescence dyes can be used to quantify spray droplets deposited on the target sites (Pergher and Gubiani, 1995; Khot et al., 2011). Holownicki et al. (2002) applied a water-soluble tracer called sodium salt of fluorescein to evaluate the spray deposition. However, it could only tell the concentration of chemicals deposited on the target, whereas the uniformity of the spray distribution on the target areas and overdosing of sprays were unknown (Holownicki et al., 2002).

Water-sensitive paper (WSP) is another conventional method of visualising and quantifying spray distribution. WSP has been applied in the assessment of spray qualities for more than 30 years in agriculture (Hill and Inaba, 1989). In general, WSP has a yellow surface, and an aqueous droplet can leave a dark blue stain on it (Salyani et al., 2013). This transition is primarily due to the reaction of Bromophenol Blue to a pH at a range of 2.8-4.6 (Turner and Huntington, 1970). Droplet spot analysis, such as spray coverage and the number of spots per unit area, are thus determined by image analysis techniques (Zhu et al., 2008). Fox et al. (2003) compared three methods of evaluating spot distributions on WSP and found that the imaging system could provide reasonably consistent droplet size and spray coverage measurements. Although WSP has weakness in assessing spray quality (such as spray uniformity and crop distribution) as the target and leaf surface vary in morphology and texture, reliable data about spray coverage can be accessed as a representative parameter extracted from WSP only if appropriate software is used to analyse scanned images (Panneton, 2002; Sánchez-Hermosilla and Medina, 2004).

Titanium dioxide (TiO_2) is extracted from various naturally occurring ores, and it is nontoxic and stable, mostly used as pigments in sunscreens, paints, and ointments (Chen and Mao, 2007). TiO_2 pigments have been widely applied for imparting brightness, whiteness and opacity within a range of applications as inorganic chemical products (Khataee and Kasiri, 2010). Potentially, TiO_2 can be the ideal material to estimate the deposit distribution where AT are on the leaf surface to visualise film AT sprayed on the leaf surface, which is not visible to naked eyes/scanners. Nonetheless, whether TiO_2 affects spray coverage and droplet size of AT solutions is yet to be available from publications and further investigations on TiO_2 as a marker for estimation of leaf coverage are required.

Plant water status – RWC and LWP

A physical barrier from film AT against water loss is expected to improve plant water status by showing increased leaf RWC and LWP, which are the two most common indicators as reported from empirical studies on numerous crops such as sweet corn ((Shekour et al., 1987), pepper (del Amor et al., 2010), wheat and barley (Ouerghi et al., 2014), pea (Aldasoro et al., 2019) and tomato (Abdallah, 2019). Responses to AT vary in different species, and for a specific plant, the improvement by AT on the water status is determined by the interaction of multiple factors such as soil water status, concentrations of AT (also dose rates) and the time of spraying (Davenport et al., 1974; Abdallah, 2019).

For example, under well-watered conditions, Shekour et al., (1987) sprayed VG 0.5% twice on sweet corn at 38 and 52 days after planting (only one study), and VG increased LWP.

Mikiciuk et al. (2015) sprayed 0.75% VG on strawberries before flowering, showing that VG significantly increased leaf RWC. However, AbdAllah et al. (2018) found that the improvement in RWC of tomato from AT (Emulsion Linus seed oil – ELO, 1% and 2%) application at the fruit stage was limited (without significant differences) as compared to unsprayed control. Similarly, del Amor et al. (2010) found that film AT 2.5% significantly increased the LWP of water-stressed sweet pepper while had no significant effects on well-watered plants.

When water stress occurs, the magnitude of the increase in plants' water status is closely associated with the intensity of water stress and concentrations. Abdallah (2019) applied VG (1%) on tomato plants under deficit irrigation over two seasons at fruit stage, showing that AT significantly improved leaf RWC of tomato plants subjected to moderate water stress (60% depletion of AWC) with respect to the unsprayed control plants, but this improvement was not observed under well-watered or severe water stress conditions (80% depletion of AWC). However, Ouerghi et al., (2014) investigated the effect of VG with high concentrations (5%, 7% and 10%) and water stress at different levels on potted wheat and barley. They found that VG application twice before anthesis could increase LWP positively related to the intensity of water stress (100%FC-25%FC). However, very high concentrations of VG, like 7% and 10%, may negatively affect LWP, compared to 5% VG.

The efficacy of film AT starts to diminish along with time, and the duration may be determined by many factors such as the structure of the leaf surface and thereby affecting the adherence of products on leaves, and leaf coverage, apart from species, concentrations and spraying method (Plaut et al., 2004). Differences in duration are expected from different species and compounds of film AT. For example, Faralli et al. (2016) found VG (di-1-*p*-menthene) may be efficient for 20-25 days while Nufilm-P (poly-1-*p*-menthene) for 7-10 days on winter rapeseed. Shekour et al., (1987) found VG 0.5% effects on LWP of sweet corn lasted for more than one month. Surprisingly, Aldasoro et al. (2019) found that VG 2.5% only lasted for less than one week. It was more than 60 days reported by Vitis et al. (2020) on grapevines when VG 2% was applied on both abaxial/adaxial surfaces. Therefore, the evidence has implications that research on the efficacy of film AT needs to be conducted for specific crops under specific environmental conditions with spraying methods concerned.

Gas exchange

Numerous studies have reported that the application of AT reduces water loss from the leaf by blocking stomata mechanically and concurrently inhibits the photosynthesis of crops like rapeseed (Faralli et al., 2017a), wheat (Abdullah et al., 2015), tomato (AbdAllah et al., 2018), grapevines (Silvestroni et al., 2020). As discussed above, the Gas exchange responses to AT are highly related to leaf water status, which depends on multiple factors. Anderson and

Kreith (1978) compared three types of film AT: 16.5% Mobileaf (petroleum-derived wax emulsion in water), 16.5% Wilt Pruf (Beta pinene emulsion oil in water) and 5% XEF (siloxane emulsion in water) on four herbaceous plant species, Mobileaf reduced transpiration significantly by 45-70% relative to control. Abdullah et al. (2015) sprayed VG at GS39 and Z69 on wheat; however, they observed increased photosynthesis in water-stressed plants following AT treatment as soil moisture deficit increased. This unexpected increase in photosynthesis might be attributed to improved plant water relations and leaf turgor due to the higher soil moisture conservation in AT-sprayed plants. Previously, the counteractive effects of film AT have also been reported by Davenport et al. (1972), showing wider apertures from AT-treated fava bean leaves, as mentioned above. However, the relationship between plants' water status and photosynthesis still needs further investigation in future studies.

In principle, a higher concentration of AT can lead to a further reduction in stomatal conductance and transpiration, accompanied by photosynthesis (Plaut et al., 2004). Plaut et al., (2004) found higher reductions in gas exchange from increased concentrations of AT from 2% to 8% on five of six horticultural crops. However, increasing AT concentrations may not effectively mitigate drought damage to the final yield, as Fahey and Rogiers (2019) reported on grapes and Kettlewell and Holloway (2010) on wheat, although the threshold of concentrations is not the same between studies. From an economic point of view, high concentrations will increase the cost of products. Therefore, the optimum concentration of AT requires further investigations concerning both cost and profit from specific crops.

Water use efficiency (WUE)

Following the application of film AT, transpiration is expected to be reduced more than photosynthesis because there is an additional resistance from the mesophyll between the sub-stomatal cavity of leaves and the chloroplast in addition to the stomatal and leaf boundary layer resistances to diffusion. Subsequently, instantaneous water use efficiency (iWUE = A/E) and intrinsic water use efficiency (WUEi = A/g_s) increase (Anderson and Kreith, 1978). Further, AT efficacy depends on the plant species/varieties and the drought intensity (AbdAllah et al., 2019). For example, AbdAllah et al. (2018) compared the effect of three different types of AT on well-irrigated tomato plants, biomass WUE was increased by 13% and 17% at the concentration of 1% and 2%, respectively, albeit by varying degrees. Similar improvements in WUEi has also been reported in droughted tomato (Abdallah, 2019) and wheat (Mphande et al., 2021). VG 3% at the pre-flowering stage of grapevines twice increased WUEi, but temporarily for about 10 days due to a larger reduction in stomatal conductance than photosynthesis (Palliotti et al., 2010). On the contrary, Brillante et al., (2016) found that 2% VG on grapevines did not affect WUE_i significantly during drought, limiting *A* more than g_s. This discrepancy might be possibly related to the different varieties or variations from different environmental conditions of taking measurements. In Plaut et al. (2004), a new acrylic film AT was applied on several horticultural crops under well-watered conditions, showing that g_s decreased more than *A,* leading to increased WUEi except for apple.

Canopy/leaf temperature

Blocked stomata by film AT physically can reduce cooling effects, thereby increasing leaf temperature, which is more prevalent at mid-day compared to morning and late afternoon (AbdAllah et al., 2018). Brillante et al. (2016) reported that VG 2% treatment had significantly increased leaf temperature of grapevines by an average of about 1.19 °C compared to unsprayed control during drought years. AbdAllah (2019) found an interaction between water stress and AT. Following VG (1%) application, canopy temperature of tomato plants at two days after spraying increased slightly (< 0.6 °C) but not significantly as compared to corresponding unsprayed control at all levels of water depletion. To a larger extent, AbdAllah et al. (2019) reported that VG 1% was more effective at mid-day when film AT increased leaf temperature of snap beans by about 2 °C at 5 days after spraying when seedlings were under a 5-day progressive drought. However, detrimental effects from increased leaf temperature can be outweighed by the benefit of improved water status, especially during which crops are most drought-sensitive, thereby increasing crop yield, as detailed below.

Crop yield

The research on film AT began very early in the 1970s, mainly on horticultural crops due to penalty on photosynthesis; however, studies on arable crops (such as wheat and rapeseed) have just become of interest for researchers during the past twenty years when spray timing is considered, that water loss reduced by film AT can provide yield benefits if AT is applied at the critical stage during which crops are most sensitive to drought, although at the expense of photosynthesis (Kettlewell et al., 2010). Kettlewell et al., (2010) emphasised the importance of spraying time in terms of wheat, showing that grain yield only increased when VG was applied at GS 37 and GS 39 before the boot stage, during which wheat was most sensitive to drought, whereas it decreased when VG was applied at GS 55 and GS 69. This is also consistent with later findings by Abdullah et al. (2015) on droughted wheat by withholding water during the boot stage for 10 days, that yield benefits from AT application were only observed at GS 39 instead of at GS 69. Similarly, Weerasinghe et al. (2016) found a grain yield benefit of 0.66 t ha^{-1} when 1.25% VG was applied at GS33, compared to unsprayed droughted plots. In contrast, AbdAllah et al., (2019) sprayed VG 1% on snap bean seedlings, and there were no significant effects from AT on shoot biomass under three 5-day irrigation/drying cycles, which might be mainly due to limited carbohydrate supply.

Without water stress, improved water status from AT could improve shoot growth, such as in olive trees (Cirillo et al., 2021), and accelerate berries ripening (Vitis et al., 2020). However, AbdAllah et al. (2018) found that one type of AT (ELO) decreased the yield of tomatoes in the field compared to control, without obvious effects on fruit qualities, when AT was applied at fruit stage where transpiration was the dominant component of the evapotranspiration.

1.5.3 Application of film antitranspirant on rapeseed

Patil and De (1976) firstly compared three types of AT on *Brassica campestris* under controlled drought in a single pot study. They found that film AT (Mobileaf) at 12.5% increased dry matter production due to improved leaf water status over well-watered control. Later, Patil and De (1978) further investigated the effects of AT along with nitrogen fertiliser and plant population density on *Brassica campestris* under dryland conditions, showing that all AT applied at early flowering stage improved leaf water relative content. Although oil decreased from AT application, 10% film AT (Mobileaf) significantly increased seed yield by 26%, while phenyl-mercuric acetate and Kaolin only by 11% and 17%, respectively, compared to unsprayed plots across two-year field experiments. More recently, Faralli et al. (2016) compared two types of film AT at 1% (i.e. poly-1-*p*-menthene and di-1-*p*-menthene) on rapeseed (*Brassica napus*) when subjected to drought during the flowering stage, showing that film AT significantly increased seed yield by 13% (poly-1-*p*-menthene) and 17% (di-1-*p*-menthene), on average, compared with the unsprayed droughted plants, as a result of a significant increase in pod number per plant, by 11% and 13%, respectively. This yield benefit was attributed to significant improvements in leaf and flower-pod water potential, and reduced endogenous ABA content in leaf and reproductive organs. To assess the interaction between film AT and drought intensity, Faralli et al. (2017a) conducted two pot experiments and found that sustained seed yield from AT was more effective on severe water-stressed rapeseed (10% and 20% AWC) in addition to improved water status and decreased ABA accumulation. In line with controlled drought, AT at 1, 2, and 4 L ha^{-1} also increased seed yield by an average of 22% across two years of field experiments when rapeseed plants were under terminal drought, but only occurred when AT was applied at the flowering stage (Faralli et al., 2017b).

1.6 Conclusions

Drought has multiple negative effects on rapeseed, leading to large yield losses. Numerous studies have shown that rapeseed, as one of the most important oil crops, is the most sensitive to water stress during the flowering stage. The improvement of yields under such conditions can be achieved if appropriate practices about crop management are conducted, such as the application of AT.

Film AT has shown great potential to reduce water loss via blocking part of stomata physically to improve water use efficiency of droughted plants. There is increasing evidence that droughted crops could benefit from the application of AT in yield. However, there is still limited knowledge about the physiological mechanism of film AT sprayed on rapeseed. Besides, the concentration-response of rapeseed to film AT in yield and yield components has not been investigated. Therefore, it is envisaged that the application of film AT may offer a novel approach for improving the yield of rapeseed under drought, but also, the optimal concentration of AT could help extend the commercial use of film with fewer compound inputs.

1.7 Central hypothesis and objectives of the study

Central hypothesis:

The main aim of this research project is to test the hypothesis that film antitranspirant has the potential to mitigate the drought damage to rapeseed at the flowering stage and increase the yield of rapeseed.

The primary objectives of the study are as follows:

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

(Published in *Crop Protection*)
https://www.sciencedirect.com/science/article/pii/S0261219421000016

The objective of **Chapter 2** was to evaluate the effect of TiO_2 on the spray characteristics and coverage of film AT (a.i., di-1-*p*-menthene) on water-sensitive papers and its subsequent use to estimate the relationship between dose rates of AT and leaf coverage of rapeseed when applied at the flowering stage through four glasshouse experiments. The null hypotheses tested are that TiO_2 has no effect on droplet size spectra and class size distribution, and spray coverage using water-sensitive papers is similar to spraying different dose rates of film AT with/without TiO_2 at two distances from nozzles to water-sensitive papers; and that there is no significant difference in leaf coverage of different dose rates of film AT with TiO_2 as spray marker when solutions were applied on rapeseed at flowering stage.

2. Evaluation of the concentration-response relationship between film antitranspirant and yield of rapeseed (*Brassica napus* L.) under drought

(Published in *Agricultural Water Management*)

https://www.sciencedirect.com/science/article/pii/S0378377422002797

The objective of **Chapter 3** was to investigate the effect of irrigation at two levels, well-watered and water-stressed, and film AT at five concentrations from 0% to 1% on rapeseed under controlled drought through three glasshouse experiments. The null hypothesis tested is that there is no concentration-response of rapeseed to film AT on gas exchange, seed yield and yield components when plants are subjected to drought during the flowering stage.

3. A preliminary study about leaf coverage and stomatal density of rapeseed under simulated field conditions in the glasshouse

The objective of **Chapter 4** was to estimate adaxial stomatal density and leaf coverage of rapeseed treated with different concentrations of film AT by simulating field-growing conditions in the glasshouse. The null hypothesis tested is that there is no significant difference in leaf coverage of film AT from 1% to 3% when applied at the flowering stage of rapeseed under field-simulated conditions.

4. Increasing the concentration of film antitranspirant enhances yield benefits on rapeseed (*Brassica napus* L.) under terminal drought

(Submitted to *Field Crops Research*)

The objective of **Chapter 5** was to investigate the effect of different concentrations of AT from 1% to 3% on yield and its components of rapeseed under terminal drought through two field experiments. Some physiological parameters were also determined to estimate plant water status, gas exchange and endogenous abscisic acid content in plant tissues. The null hypothesis is that there is no significant improvement in rapeseed yield when plants are applied with AT at increasing concentrations from 1% to 3% at the flowering stage.

Table 1.7.1 A summary of experiments reported in the thesis.

Chapters	Objective	Experiments	Experimental design	Type and duration of drought	Concentrations of AT_% (L ha^{-1})
Chapter 3	Spray coverage	Glasshouse Expt 1 (WSP[a])	CRD[b] (n = 3)	-	0, 0.5% (1), and 1% (2)
		Glasshouse Expt 2 (WSP[a])	CRD (n = 3)	-	0, 0.5% (1), 1% (2), and 1.5% (3)
	Leaf coverage	Glasshouse Expt 3	CRD (n = 3)	-	0, 0.25% (0.5), 0.5% (1), 0.75% (1.5), and 1% (2)
		Glasshouse Expt 4	CRD (n = 3)	-	0, 0.25% (0.5), 0.5% (1), and 1% (2)
Chapter 4	Concentration-response (pot)	Glasshouse Expt 1	2 × 5 factorial RCBD[c] (n = 8)	Controlled_20 days	0, 0.25% (0.5), 0.5% (1), 0.75% (1.5), and 1% (2)
		Glasshouse Expt 2	2 × 5 factorial RCBD (n = 8)	Controlled_20 days	0, 0.25% (0.5), 0.5% (1), 0.75% (1.5), and 1% (2)
		Glasshouse Expt 3	2 × 3 factorial RCBD (n = 8)	Controlled_25 days	0, 0.25% (0.5), 0.5% (1), and 1% (2)

Chapter 5	Leaf coverage (field-simulation)	Glasshouse Expt 1	CRD (n = 4)	-	0, 0.5% (1), 1% (2), 1.5% (3), 2% (4), and 3% (6)
Chapter 6	Concentration-response (field)	Polytunnel Expt 1	One-way RCBD (n = 8)	Terminal	0, 1% (2), and 3% (6)
		Polytunnel Expt 2	One-way RCBD (n = 4)	Terminal	0, 1% (2), 1.5% (3), 2% (4), and 3% (6)

[a] – water-sensitive paper; [b] – completely randomised design; [c] – randomised complete block design.

Chapter 2 Estimation of film antitranspirant spray coverage on rapeseed (*Brassica napus* L.) leaves using titanium dioxide

(Published in *Crop Protection*)

2.1 Introduction to Chapter 2

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

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

In practice, methods of estimating deposition of sprays are mainly categorized into two groups with their limitation: dye tracers mixed with spray liquid that visualize the liquid; and sensitive papers or cards, which detect spray droplets with a colour change (Jaeken et al., 2000). Dye tracers are commonly used to determine spray retention (i.e., total mass retained per leaf area or plant area) as they can provide clear contrast between spray deposits and the background (Nairn and Forster, 2019), such as fluorescence dyes like Rhodamine (Bueno et al., 2017). They are less useful, however, in determining the distribution of spray droplets on the target areas.

Water-sensitive paper (WSP), which has been used for more than 30 years to assess spray qualities in agriculture, is another conventional method of visualizing and quantifying the distribution of deposited spray droplets because an aqueous droplet can leave a dark blue stain on WSP with a yellow surface (Salyani et al., 2013). Droplet spot analysis such as spray coverage and number of spots per unit area can subsequently be determined by image analysis techniques (Zhu et al., 2008). Fox et al. (2003) compared three methods of evaluating spot distributions on WSP. They found that the imaging system could provide consistent measurements of droplet size and spray coverage. Further relationship between stain diameter and coverage on WSP was addressed by Cerruto et al. (2019). With a high degree of coverage, the spread factor needs to be adjusted without considering overlapped stains.

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

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

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

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

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

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

2.2 Material and Methods

2.2.1 Design and application parameters for Expt 1 and Expt 2

Expt 1 and Expt 2 were conducted as randomised single factor designs with seven treatments in Expt 1 and ten treatments in Expt 2 on 4th December 2018 and 17th January 2019, respectively. There were three replicates for each. Water-sensitive papers (WSP, 26x76 mm, Teejet, USA) were used as artificial spray targets to assess spray coverage. WSP was positioned horizontally at a specific height below the nozzles (70 cm in Expt 1, 50 cm in Expt 2). Film antitranspirant Vapor Gard (a.i. di-1-*p* menthene 96%, Miller Chemicals and Fertilizer, Hanover, USA) and water as control were sprayed with the amounts of water-insoluble titanium dioxide (TiO_2, CI 77891, ReAgent, Cheshire, UK) as a spray marker shown in Table 2.2.1. The proportion of AT and TiO_2 was 1:1 across all the AT-related treatments. A custom-built automatic pot sprayer with a pair of nozzles (Hypro Flat Fan 110–03, Retrofitparts, UK) in the glasshouse at Harper Adams University (HAU) was used at 0.2 MPa pressure and nominal 1 m s^{-1} forward speed (Figure 2.2.1). The volume of application in both experiments was nominal 200 L ha^{-1} according to manufacturer settings. To minimise technical errors from the sprayer, the actual application volume was estimated at 70 cm and 50 cm height below nozzles with ten replicates using filter paper in a Petri dish. There was no significant difference between the two heights (p = 0.342, data not shown), so the actual application volume was averaged over the two heights and was approximately 250 L ha^{-1}. WSP was allowed to dry for several minutes after spraying, followed by separate storage in sealable plastic bags for the image analysis. Both experiments were conducted in an enclosed chamber to reduce air and droplet movement variations.

Figure 2.2.1 The customized built-in pot sprayer inside an enclosed chamber with a pair of nozzles.

Table 2.2.1 Overview of treatment composition including the nominal and actual dose rates of film antitranspirant, and the corresponding amount of TiO_2 in four experiments. The volume of the sprayer tank used was 100 mL.

Expts	Treatments	Dose rates of AT ($L\ ha^{-1}$)		Mixture in the tank		
		Nominal	Actual	TiO_2 (g)	AT (mL)	water (mL)
Expt 1	Water	0.0	0.0	0.0	0.0	100.0
	Water +1 g TiO_2	0.0	0.0	1.0	0.0	100.0
	Water + 2 g TiO_2	0.0	0.0	2.0	0.0	100.0
	1AT	1.0	1.3	0.0	0.5	99.5
	2AT	2.0	2.5	0.0	1.0	99.0
	1AT + 1 g TiO_2	1.0	1.3	1.0	0.5	99.5
	2AT + 2 g TiO_2	2.0	2.5	2.0	1.0	99.0
Expt 2	Water	0.0	0.0	0.0	0.0	100.0
	Water +1 g TiO_2	0.0	0.0	1.0	0.0	100.0
	Water + 2 g TiO_2	0.0	0.0	2.0	0.0	100.0
	Water + 3 g TiO_2	0.0	0.0	3.0	0.0	100.0

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

2.2.2 Spray coverage analysis in Expt 1 and Expt 2

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

2.2.3 Droplet size analysis in Expt 1

In Expt 1, the Dropcounter (Appendix. Figure 1) (Billericay Farm Services Ltd, Essex, UK) was placed 50 cm below the nozzles. The device uses infrared light to measure the number and droplet size within an area of 0.7 cm^2 (Kateley et al., 2016). The volumetric droplet size spectra parameters for analysis were $D_{V0.1}$, $D_{V0.5}$, $D_{V0.9}$, relative span (RS = ($D_{V0.9}$ - $D_{V0.1}$)/ $D_{V0.5}$) and uniformity of the spray distribution was determined by the coefficient of variation (CV) of $D_{V0.5}$. $D_{V0.5}$ (also known as volume median diameter, VMD) is the diameter at which half of the volume of spray contained in droplets are smaller or bigger than this median value. $Dv_{0.1}$ and $D_{V0.9}$ is the diameter at 10% and 90% of the volume of spray contained in droplets are at or smaller than these values, respectively (Ferguson et al., 2015). Additionally, droplet class size distributions were analysed in 12 class sizes with a range from 30 μm to > 200 μm, with a relative increment of 10 μm between class sizes (Cunha et al., 2012).

2.2.4 Leaf coverage analysis in Expt 3 and Expt 4

In Expt 3 and Expt 4, seeds of spring rapeseed (cv. Mirakel; NPZ-Lembke, Germany) were sown into seedling-planter trays filled with John Innes No. 2 compost (loam, peat, coarse sand and base fertilizer, John Innes Manufacturers Association, Reading, UK) on 26th April and 25th July 2019, respectively. Seedlings at the fourth true leaf stage were transplanted into 1 L pots (one plant per pot). Each pot contained ~500 g John Innes No. 2 compost at 22±1% volumetric water content measured with a soil moisture probe (ML2X theta probe; Delta-T-device, Cambridge, UK). Pots were well arranged in the glasshouse at HAU with sodium vapour lamps supplemented (16 h-8 h light-dark photoperiod) and daily temperature on average was approximately 17 °C in Expt 3 and 21 °C in Expt 4 before treatments started. All pots were well irrigated every two days until drainage from the bottom occurred. Nitrogen was applied at a rate of 100 kg/ha before flowering stage, i.e., 0.3g Ammonium nitrate (34.5% N) per pot.

Both experiments were conducted using a complete randomised block design and treatments are shown in Table 2.2.1. Each treatment was replicated three times in each experiment. AT solutions was applied on the canopy without leaves overlapped between pots at the flowering stage (GS 6.0) (Lancashire et al., 1991) on 12th July in Expt 3 and 14th November 2019 in Expt 4 The adaxial surface of leaves was sprayed with AT solutions uniformly using the same custom-built automatic pot sprayer (Flat Fan 110-03, 0.2 MPa, 1 m s^{-1} forward speed) at nominal 200 L ha^{-1} while the actual volume rate was about 250 L ha^{-1} as described above. The distance between nozzles and plant canopy was kept at ~50 cm. After spraying, the first fully expanded leaf from the top and two leaves below were collected for leaf coverage analysis. In both experiments, we estimate that the distance from nozzles to the first fully expanded leaf was approximately 70-90 cm and the interval between two leaves was ~5cm.

In Exp 3 and Exp 4, leaves collected were scanned by the TASKalfa 3252ci Printer (Kyocera, UK) with high resolution (600 × 600 dpi). Files were saved as the colour JPEG. Three representative parts from each leaf were selected ranging from 0.4 to 1.0 cm^2 to avoid the main and lateral veins where possible. Then, selected areas were saved as new colour images for leaf coverage analysis in MATLAB (R2018a). The following procedures about image segmentation and thresholding were the same as spray coverage analysis in 2.2.2. Therefore, leaf coverage was calculated as the percentage of pixels of the white area of interest to the total number of pixels of the whole image (examples from Expt 3 also shown in Figure 2.3.4). Data from three leaves and means were used for the statistical analysis.

2.2.5 Statistical analysis

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

2.3 Results and discussion

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

According to the ISO draft standard (ISO 25358, 2018), six spray quality boundaries are defined based on the combination of different nozzles and specific pressures to classify droplet size spectra. Despite minor changes in $Dv_{0.1}$, $Dv_{0.5}$ and $Dv_{0.9}$ across treatments as shown in Table 2.3.1, spray quality of droplets was all classified as fine. We found that TiO_2 had no significant effects on the droplet size spectra of AT solutions or water control in Expt

1. In terms of spray distribution uniformity, CV values ranged from 3.43% to 16.22% while all treatments had similar values in relative span.

Table 2.3.1 Droplet size spectra with three replicates (n = 3) measured by the Dropcounter, relative span and ISO 25358 spray quality classification based on Dv0.1, Dv0.5 and Dv0.9, and the output of one-way ANOVA in Expt 1.

Treatments	$D_{V0.1}$	$Dv_{0.9}$	$Dv_{0.5}$		Relative	ISO
	µm	µm	µm	CV (%)	span	classification
Water	93.00	345.67	193.46	9.71	1.30	Fine
Water+ 1 g TiO_2	93.00	406.67	206.46	16.22	1.49	Fine
Water+ 2 g TiO_2	87.00	315.67	180.97	3.68	1.26	Fine
AT 1 L ha^{-1}	85.00	357.33	187.74	11.14	1.44	Fine
AT1 L ha^{-1} + 1 g TiO_2	105.00	441.67	212.65	8.98	1.57	Fine
AT 2 L ha^{-1}	93.00	355.00	193.62	3.43	1.35	Fine
AT 2 L ha^{-1} + 2 g TiO_2	111.67	357.33	203.07	11.65	1.20	Fine
ANOVA						
SEM	†	39.4	11.8	-	0.11	-
P-values	0.15	0.39	0.53	-	0.25	-

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

To evaluate the droplet class size distribution, droplets were grouped into 12 classes according to the diameter, ranging from 30 µm to > 200 µm. Figure 2.3.1 shows the profile of the droplet class size distribution of AT and AT+TiO_2. The diameter of most droplets was within the range of 30 µm - 40 µm, accounting for 59%-64% of the total number of droplets measured, followed by the group of 40-50 µm droplets with the percentage of 9% - 11%. According to the Chi-square test, it showed that there was no significant difference between these two groups of treatments (p = 0.332). Different chemical compounds with similar physical properties can produce similar spray characteristics including droplet size and droplet number (Butler Ellis et al., 1997). The advantage of TiO_2 would be that it can be visible in ordinary light, compared to fluorescein dyes that require special light to be visualised.

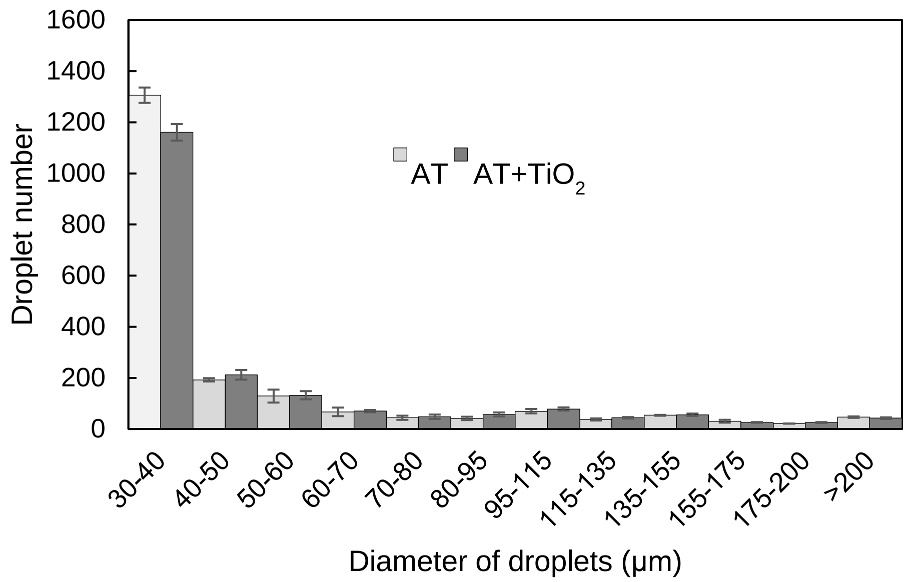


Figure 2.3.1 Number of droplets for each class size diameter measured by the Dropcounter in Expt 1 (n = 3). Data are means of film antitranspirant (AT) treatments with/without TiO_2 and error bars represent standard error of means.

It is expected that differences in nozzle types and operation settings of measuring system may result in considerable variations in spray droplet characteristics (Nuyttens et al., 2009). As Kateley et al. (2016) verified, the Dropcounter can work effectively to discriminate droplet size from different nozzles in a similar way to laser techniques. Regarding the evidence above, we failed to reject the null hypothesis that there were no effects of TiO_2 on droplet size and spray distribution of AT solutions.

2.3.2 Effects of TiO_2 on WSP spray coverage

We found that spray coverage on WSP was 46.8% on average in Expt 1 (Figure 2.3.2). The overall *ANOVA* showed that there was a borderline significant effect of treatments on spray coverage ($p = 0.048$). Except that treatment of water + 2 g TiO_2 was significantly lower than AT only at 1 L ha^{-1}, there were no significant differences among the remaining treatments on spray coverage. In Expt 2, the mean of spray coverage was 57.3% across all treatments (Figure 2.3.3). No significances were observed between treatments, except treatments of water + 1 g TiO_2 and water + 2 g TiO_2. These results indicate the null hypothesis cannot be rejected that TiO_2 had no significant effects on WSP spray coverage at different dose rates of AT.

Compared to Expt 1, spray coverage increased by an average of ~22% due to the shorter distance between nozzles to WSP (Expt 1: 70 cm; Expt 2: 50 cm) using the same type of sprayer and application volume rate. It suggests that volume of spray deposited per unit area changed with the boom height, which is consistent with Ferguson et al. (2016), that the higher coverage on WSP was observed from the top card than the ground card when volume rate was constant. Hanna et al. (2009) also found that fungicide deposition coverage on WSP reduced by degrees from the top to the middle and bottom.

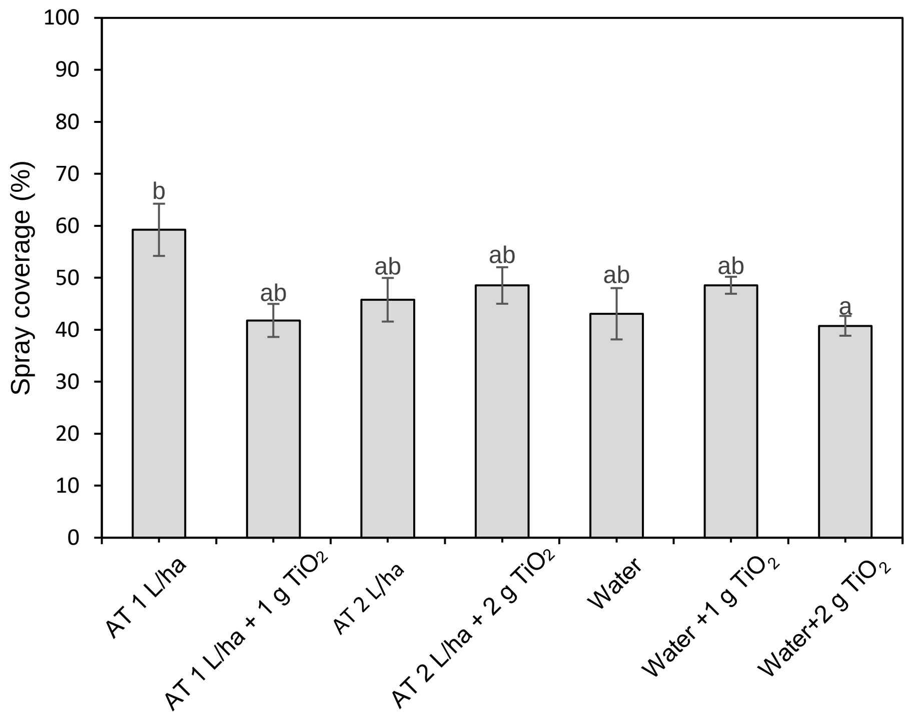


Figure 2.3.2 Spray coverage using water sensitive papers with film antitranspirant (AT) application with/without TiO_2 in Expt 1. Data are means of replicates (n = 3) and error bars represent standard error of means. Treatments with the same letters are not significantly different according to Tukey's test at p = 0.05.

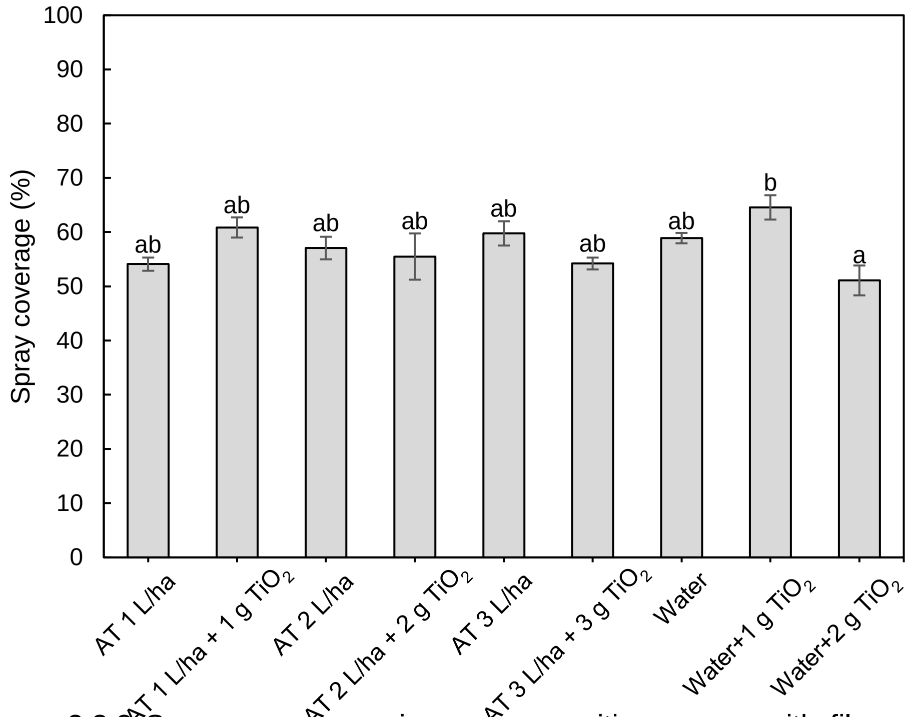


Figure 2.3.3 Spray coverage using water sensitive papers with film antitranspirant (AT) application with/without TiO_2 in Expt 2. Data are means of replicates (n = 3) and error bars represent standard error of means. Treatments with the same letters are not significantly different according to Tukey's test at p = 0.05.

Considering the droplet size between AT at 1 L ha^{-1} and water with 2g TiO_2 (Table 2.3.1), a great difference between them makes it difficult to explain from the present data. It might be attributed to the variation from measurements between replicates (Berger-Neto et al., 2017). The result from water with 1 g TiO_2 and with 2 g TiO_2 observed significantly different in Expt 2 was not found in Expt 1, suggesting a chance occurrence. One possible reason is that TiO_2 was not adequately mixed up in the tank with water before spraying for the 2 g treatment. On the other hand, fluctuations observed from different treatments can result from slight changes in spread factor, influencing the spot size on WSP (Fox et al., 2001). However, some variation from image processing software (i.e., Matlab in this study) using pixel recognition may affect results directly. Accuracy decreases along with the decreased spot size on WSP, and mistakes would be made when deposits on WSP are too dense, leading to plenty of overlapped deposits which cannot be discriminated by the program (Zhu et al., 2011). In Expt 1 and 2, spray coverage was nearly ~50%, at which plenty of overlapping deposits were observed by eyes or the imaging software system. The contrast between stains and background will be lost when the coverage is heavy (Panneton, 2002).

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

2.3.3 Relationship between film antitranspirants and leaf coverage

To explore the dose-response between leaf coverage and dose rates of AT, regression analysis in groups showed that both experiments could be displayed in parallel lines (Figure 2.3.5). Leaf coverage was 14% and 9% on average in Expt 3 and 4 respectively. Despite the two experiments being conducted at different times, the data showed a significantly positive relationship between leaf coverage and dose rates of AT ($p < 0.001$, $R^2 = 0.99$). In Expt 3, the highest and lowest leaf coverages were observed with 2.0 L ha^{-1} AT (18.62%) and 0.5 L ha^{-1} AT (6.61%) respectively. In Expt 4, the highest and lowest value were 14.64% from 2.0 L ha^{-1} and only 2.12% from 0.5 L ha^{-1}.

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

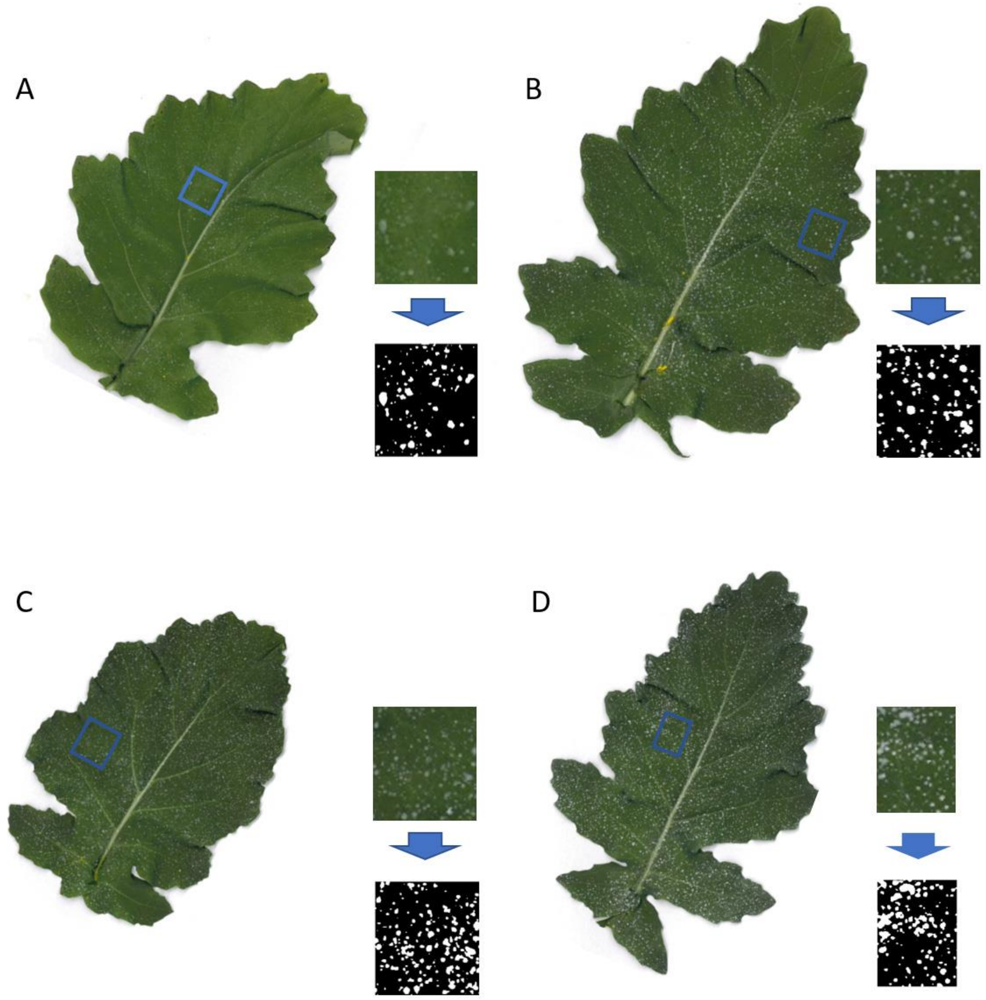


Figure 2.3.4 Images of the scanned 1st leaf and corresponding representative selections after thresholding at 3x magnification when rapeseed was sprayed at nominal dose rates of 0.5 L ha^{-1} (A), 1.0 L ha^{-1} (B), 1.5 L ha^{-1} (C) and 2.0 L ha^{-1} (D) in Expt 3.

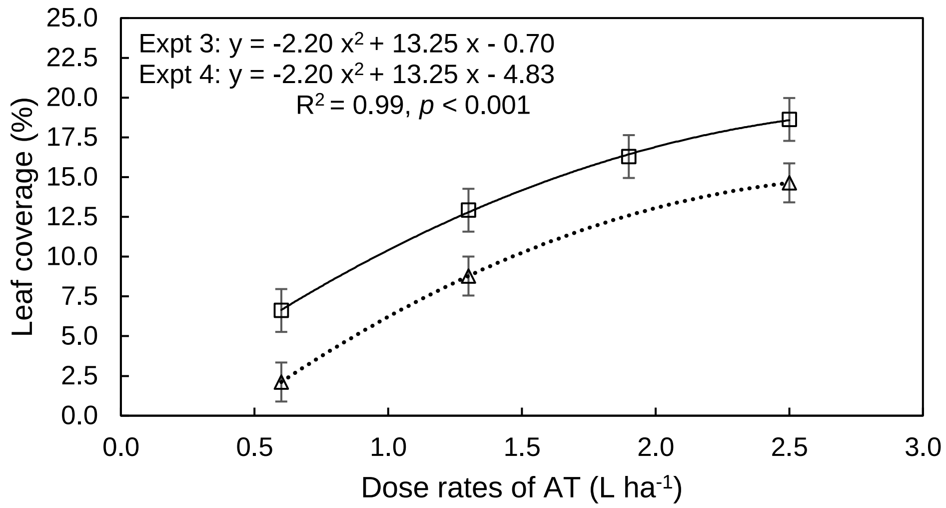


Figure 2.3.5 Relationship between leaf coverage of film antitranspirant (AT) estimated from TiO_2 and actual dose rates of AT in Expt 3 (open squares) and Expt 4 (open triangles). Lines were fitted using stepwise regression analysis in groups (solid and dashed for Expt 3 and 4, respectively). Data points are means (n = 9) and error bars represent standard deviations (SD).

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

As shown in Table 2.3.2, only AT had consistently significant effects on the leaf coverage in both Expts ($p < 0.001$ in Expt 3 and $p = 0.009$ in Expt 4), while no significant effects were observed from leaf position alone or interaction between AT and leaf position. It implies that differences in the application volume for the different layers cannot account for the variability in coverage between leaves. The observation can also confirm this during the research. Despite that, assessed leaves of interest were partially obstructed by the inflorescence and leaves above them, which can change the general route of flow liquid and thus affect the retention on the surface.

Table 2.3.2 Leaf coverage from three leaves of each treatment in Expt 3 and 4, and probability values from repeated measure *ANOVA* with one leaf position equivalent to one measurement affected by different dose rates of film antitranspirant (AT).

Experiments	Concentrations of AT	Dose rates of AT ($L\ ha^{-1}$)		Leaf coverage at three leaf positions (%)			
	(%)	Nominal	Actual	1st	2nd	3rd	Mean
Expt 3	0.25	0.5	0.6	6.64	6.77	6.43	6.61
	0.50	1.0	1.3	13.61	15.05	11.37	13.13
	0.75	1.5	1.9	17.09	14.79	16.99	16.29
	1.00	2.0	2.5	18.28	20.09	17.50	18.62
Expt 4	0.25	0.5	0.6	1.96	2.04	1.87	2.20
	0.50	1.0	1.3	7.44	10.41	6.76	9.45
	1.00	2.0	2.5	10.68	12.03	13.07	13.19
ANOVA							
P values							
Expt 3	AT		0.001				
	Linear		< 0.001				
	Quadratic		0.151				
	Deviations		0.679				
	Leaf position (LP)		0.619				
	AT*LP		0.621				

Expt 4	AT	0.009
	Linear	0.003
	Deviations	0.420
	Leaf position (LP)	0.088
	AT*LP	0.442

Leaf coverage increased with an increased dose rate of AT at constant volume rate, indicating that deposition efficiency (i.e., leaf coverage) was highly related to the concentration of AT involved under the same spray operating conditions. This is in line with Herrington et al. (1981) that there was a positive relationship between the volume retained on the various component zones of apple tree like trunk, branches and shoots, and the volume of copper fungicide sprayed corresponding to the same level of application rates. van Zyl et al. (2013) also observed an increase in the percentage covered by fluorescent pigments, i.e., deposition quantity, with increased concentrations of copper oxychloride on detached mandarin leaves. Apart from sprayer, nozzle types and operation parameters, changes in the coverage may be in part dependent on the mode-of-action of chemicals, affecting the performance on the target plant (Wise et al., 2010). AT (a.i., di-1-*p*-menthene) at higher concentrations would allow more active surfactants to improve spreading properties by reducing surface tension in spray water droplets more effectively (accessed from the PPDB).

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

2.4 Conclusions

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

Chapter 3 Evaluation of the concentration-response relationship between film antitranspirant and yield of rapeseed (*Brassica napus* L.) under drought

(Published in *Agricultural Water Management*)

3.1 Introduction to Chapter 3

With climate projections indicating more frequency and intensity of extreme regional events (such as droughts), negative impacts from drought on the yield of crops are more likely to take place in the future (Pachauri et al., 2014). Drought is usually defined as the period when the water uptake by the plants exceeds the water attained by precipitation during the growing season from an agricultural point of view (Tuberosa, 2012). Great impacts of drought on the crops are leading to a large loss of crop production in arid and semi-arid regions around the world (Lesk et al., 2016).

Rapeseed (*Brassica napus* L.) (also known as canola or oilseed rape) has become the third most essential crop globally for edible oil, fodder, and biofuel production after soybean and palm oil (FAOSTAT). Previous studies have reported that rapeseed can be highly affected by water stress during the flowering stage in terms of seed yield and yield components such as pod number and seed number per pod (Johnston et al., 2002; Istanbulluoglu et al., 2010; Hess et al., 2015). Ahmadi and Bahrani (2009) compared the effect of water stress imposed at three different growth stages (flowering, pod development and seed filling stage) on rapeseed. It showed that during the flowering stage, plants under drought experienced the largest reduction of seed yield and oil yield by 29.5% and 31.7%, respectively. Likewise, Tesfamariam et al. (2010) observed that seed yield was reduced by 30% and 51% over two growing seasons, respectively, when water stress occurred at flowering stage, compared to well-watered control treatments. Therefore, improving the resistance of rapeseed to drought especially at flowering stage is crucial in sustaining the seed yield when plants are subjected to water stress.

It is important to optimise water use efficiency by reducing water loss while sustaining the yield under drought conditions (AbdAllah et al., 2018). Film antitranspirants (AT) are emulsions sprayed on the surface of leaves that create a waterproof layer to block stomata, thereby decreasing the diffusion of water vapour (Gale and Poljakoff-Mayber, 1967; Kettlewell, 2014; Abdullah et al., 2015). The reduction of water loss from AT application is usually accompanied by the limitation on photosynthesis due to the low permeability of films to CO_2 entering the leaf, accordingly, film AT is mainly applied to ornamental species, in which photosynthesis is less important than the reduction of transpiration (Das and Raghavendra, 1979).

There is increasing evidence that the application of AT regulates plant water status and gas exchange to sustain the growth and yield of crops exposed to drought, such as tomato (AbdAllah et al., 2018), pea (Aldasoro et al., 2019) and sweet pepper (del Amor et al., 2010). There are only a few previous published papers investigating the mechanism of AT applied around the sensitive growth stage, which can mitigate the damage of drought on the yield when compared to unsprayed droughted plants (Kettlewell et al., 2010). For example, AT application relative to an unsprayed droughted control gave a rise in the grain yield of winter rapeseed by 39% (Faralli et al., 2016) and wheat by 11%-16% (Weerasinghe et al., 2016; Mphande et al., 2021). Based on previous studies, however, the mechanism of how film AT mitigates the drought damage to crops during the critical growth stage still merits further investigation. In rapeseed, the correlations between gas exchange, leaf water status and yield components have not been widely explored under drought conditions. Furthermore, to our knowledge, effects of film AT applied at different concentrations on the yield and yield components of rapeseed under drought have not been reported in the literature.

Therefore, three pot experiments were carried out in our study with the aim of understanding the physiological mechanism of film AT applied on rapeseed plants at flowering stage and its effects on the final seed yield. The null hypothesis we tested is that there is no concentration-response of rapeseed to film AT on gas exchange, seed yield and yield components when plants are subjected to drought during the flowering stage.

3.2 Materials and methods

3.2.1 Planting material and research environment

Spring rapeseed seeds (*Brassica napus* L. var. Mirakel; NPZ-Lembke, Germany) were sown into 1-L pots containing ~0.5 kg John Innes No. 2 compost (loam, peat, coarse sand and base fertiliser, John Innes Manufacturers Association, Reading, UK) on 19th January 2019 in the glasshouse at Harper Adams University (HAU), at a rate of three seeds per pot with a spacing of 4 cm in Expt 1. Seedlings were thinned out at the fourth-leaf stage and one plant was left in each pot. In Expts 2 and 3, seeds were sown in seedling trays at a rate of 25 seeds per tray on 7th May and 12th September 2019, respectively. One seedling was transplanted into each 7.5-L pot at the fourth leaf stage, containing ~4.5 kg John Innes No. 2 compost and seedlings were watered immediately. The inter-pot spacing was ~25 cm and ~45 cm in Expt 1 and the other two experiments (Expts 2 and 3), respectively (Appendix. Figure 2). Plants from three experiments were grown in the glasshouse at the light-dark photoperiod of 16-8 h, supplemented with high-pressure sodium vapour lamps (Feilo Sylvania Europe Ltd, East Sussex, UK). Daily air temperature and relative humidity in the specific compartment for each experiment during the growing season were monitored by the AS3 Aspirated Screen Sensor and T200 logger installed in the glasshouse (TomTech (UK) Ltd, Spalding, UK). Daily solar radiation (MJ m^{-2}) was obtained from the weather station on campus. To estimate the effects of air temperature on rapeseed plants, thermal time in growing degree days (GDD) was estimated using Equation 1:

$$GDD = (T_m - T_b) \triangle t \quad 1)$$

Where Tm is the means of daily air temperature, Tb is the base temperature and $\triangle t$ is the number of days. In the present study, we used 5°C as the base temperature (Aiken et al., 2015).

In Expt 1, plants were fertilised with ammonium nitrate (34.5% N, Yara Prilled N Fertiliser, Wynnstay, UK) at 0.15 g pot^{-1} and 0.3 g pot^{-1} before and after spraying AT, respectively. Nutrigrow triple-16 (Yara Universal 16 Fertiliser, Wynnstay, UK) was applied at 0.02 g pot^{-1} after spraying AT. In Expt 2, plants were fertilised with Nutrigrow triple-16 (0.7 g pot^{-1}) before spraying AT. Ammonium nitrate (1 g pot^{-1}) and Nutrigrow triple-16 (0.7 g pot^{-1}) were applied before spraying in Expt 3.

3.2.2 Experimental design

All the pots in three experiments were arranged in a randomised complete block design (RCBD) with two factors including two levels of irrigation (IR), well-watered (WW) and water stressed (WS); and film AT at different concentrations. There were five levels of AT in Expts 1 and 2 including 0 (water), 0.25%, 0.5%, 0.75% and 1%; and three levels in Expt 3 including 0 (water), 0.5% and 1%. There was a total of five blocks and 60 pots in Expts 1 and 2 while seven blocks and 42 pots in total in Expt 3 to increase the power of analysis of variance. Details of treatments in each experiment are shown in Table 3.2.1 and a schematic diagram of RCBD is in Appendix. Figure 2.

Table 3.2.1 Treatments in Expt 1-3 (WW, well-watered; WS, water-stressed).

Expts	No.	Treatments	Concentration of AT (%)	Dose rate of AT (L ha^{-1})	Volume of AT (mL)	Volume of water (mL)
Expts 1&2	1	WW 0AT	0.00	0.00	0.00	100.00

	2	WS 0AT	0.00	0.00	0.00	100.00
	3	WW 0.25AT	0.25	0.60	0.25	99.75
	4	WS 0.25AT	0.25	0.60	0.25	99.75
	5	WW 0.5AT	0.50	1.30	0.50	99.50
	6	WS 0.5AT	0.50	1.30	0.50	99.50
	7	WW 0.75AT	0.75	1.90	0.75	99.25
	8	WS 0.75AT	0.75	1.90	0.75	99.25
	9	WW 1AT	1.00	2.50	1.00	99.00
	10	WS 1AT	1.00	2.50	1.00	99.00
Expt 3	1	WW 0AT	0.00	0.00	0.00	100.00
	2	WS 0AT	0.00	0.00	0.00	100.00
	3	WW 0.5AT	0.50	1.30	0.50	99.50
	4	WS 0.5AT	0.50	1.30	0.50	99.50
	5	WW 1AT	1.00	2.50	1.00	99.00
	6	WS 1AT	1.00	2.50	1.00	99.00

3.2.3 Irrigation supply

According to the soil water-retention curve (SWRC) of John Innes No. 2 compost (Saeed, 2008; unpublished data), the volumetric water content (VWC) at field capacity (FC) and permanent wilting point (PWP) of the compost was 45% and 7.5%, respectively. Available water content (AWC) of the compost was then calculated as the difference between FC and PWP, i.e., 37.5%. Across three experiments, WW pots were well-irrigated to ensure optimal growth throughout the growing season. In WS, drought was imposed over the flowering stage (BBCH 60-69, Lancashire et al., 1991) such that 70% of plant available water was allowed to deplete before irrigation was applied (i.e., 30% AWC) with reference to Faralli et al., (2017a). The inverted saucers of suitable size were placed beneath each pot in all three experiments to allow for free drainage from the bottom of the pots and to prevent any water uptake from the bench (Appendix. Figure 2). The application of irrigation in three experiments is summarised in Table 3.2.2 and more details are as follows:

In Expts 1 and 2, VWC of WW and WS pots at a depth of ~8 cm was measured by ML2X theta probe (Delta T-devices, Cambridge, UK) every morning (9.00-10.00 am). In the afternoon (4.00-5.00 pm), WW pots were watered to ~FC using the hose pipe in Expt 1 while in Expt 2, WW pots were rewatered to 40% VWC (i.e., 87%) to quantify soil water status. The amount of water required for WS pots in Expts 1 and 2, and WW pots in Expt 2 during the period of drought was calculated according to Equations 2 and 3, respectively.

$$WR_{ws} = (\theta_{AWC30} - \theta_m) \times \text{compost volume} \qquad 2)$$

Where WR is water requirement in units of ml; θ_{AWC30} is VWC at the 30% AWC level between FC and PWP (θ = 18.8%); θ_m is measured VWC expressed as percentage; Compost volume for a 1-L pot is 675 and for a 7.5-L pot is 6100 in units of ml.

$$WR_{ww} = (\theta_{AWC87} - \theta_m) \times \text{compost volume} \qquad 3)$$

Where θAWC87 is VWC at the 87% AWC between FC and PWP (θ = 40%), Compost volume for a 7.5-L pot is 6100 in units of ml.

In Expt 3, the timing and irrigation management for both WW and WS pots were the same as in Expt 2. In addition, extra water (30~50 mL) was added to each WS pot daily to avoid large fluctuations in the dynamic change of soil moisture depending on the temperature through the weather forecast to estimate water loss through evapotranspiration. In the afternoon (4.30-5.00 pm), VWC was measured again to check if it was at the target VWC and re-watered if necessary.

3.2.4 Antitranspirant application

In Expts 1 and 2, film AT (Vapor Gard, a.i. di-1-*p*-menthene 96%, VG. Miller Chemicals and Fertilizer, Hanover, USA) was applied when the target AWC 30% was achieved. In Expt 3, AT was applied on the same day irrigation stopped in WS pots (Table 3.2.2). The adaxial surface of leaves was uniformly sprayed with water (0 AT) or a solution containing AT at an application rate of 250 L ha^{-1} using a custom-built pot sprayer (Flat Fan 110/002, 0.2 MPa) in an enclosed chamber located in a separate part of the glasshouse to simulate crop spraying in the field. Concentrations (%) and corresponding dose rates (L ha^{-1}) of AT in each experiment are shown in Table 3.2.1. We cleaned the tank and sprayed water only without plants in the chamber prior to sprays. Water was always sprayed first for the 0AT treatment, followed by AT solutions from the lowest to highest concentrations. To avoid cross-contamination between sprays of AT solutions, we washed the tank thoroughly with the brush until residues were removed. Given the high solubility of the AT product, there was no need to use detergent.

Table 3.2.2 Summary of irrigation management and film antitranspirant (AT) application in three experiments.

Events	Expt 1				Expt 2				Expt 3			
	GS	Date	DAP	GDD	GS	Date	DAP	GDD	GS	Date	DAP	GDD
Sowing		2019/01/19				2019/05/07				2019/09/12		
Irrigation stopped	GS61	2019/04/08	79	741	GS61	2019/06/24	48	549	GS63	2019/12/04	83	897
Target stress (30% AWC) achieved	GS61	2019/04/09	80	752	GS61	2019/06/26	50	576	GS63	2019/12/08	87	905
AT application	GS61	2019/04/10	81	762	GS61	2019/06/28	52	618	GS63	2019/12/04	83	897
Optimal irrigation re-started	GS69/71	2019/04/28	99	981	GS69/71	2019/07/16	70	889	GS69/71	2020/01/02	112	1147
Harvest	GS89	2019/06/26	158	1713	GS89	2019/09/09	126	1781	GS89	2020/03/09	179	1742

† AWC, available water content; GS, growth stage of plants (BBCH, Lancashire et al., 1991); DAP, days after planting; GDD, growing degree days (℃ - d, base 5 ℃, Aiken et al., 2015).

3.2.5 Gas exchange measurements

Stomatal conductance (g_s, mol m^{-2} s^{-1}) and photosynthesis rate (A, µmol m^{-2} s^{-1}) were measured on the youngest fully expanded leaf of the top canopy (n = 3) using the LC*pro*-SD advanced portable photosynthesis system (ADC BioScientific Limited, UK) with a broad leaf chamber (6.25 cm^2) between 10.00- 13.00 at three days after spraying (DAS) in three experiments. The temperature of leaf chamber was maintained at ~25 ℃, and photosynthetically active photon flux density was 1044 µmol m^{-2} s^{-1} provided by an attached mixed Red/Blue LED array at a flow rate of 300 µmol s^{-1} through the leaf chamber. The CO_2 concentration of inlet air through the leaf chamber was ~380 ppm. All the data were recorded when steady-state photosynthesis was achieved after 3~5 mins. The flow check was calibrated before every use in three experiments to check that the cycle times were long enough for the gas in the analysis cell to become stable before the absorption was measured. Intrinsic water-use efficiency (WUEi, µmol (CO_2) mol (H_2O)$^{-1}$) was calculated from A divided by g_s.

3.2.6 Yield and yield component measurements

At maturity, all plants in Expts 2 and 3 were harvested while pod number per plant was counted. All the pods were then threshed by hand to determine seed yield based on dry matter. Seeds were dried in the oven at 60 ℃ to constant weight for 72 h, recorded as seed dry weight per plant using a 0.0001-g precision resolution balance. Seed number per plant was counted using "Analyse particles" programme in Image J software by taking photos of seeds spread out on white paper. Seed number per pod was then determined by dividing the seed number per plant by pod number and thousand-seed weight (TSW) was determined by the ratio of seed dry weight to seed number per plant and multiplied by 1000.

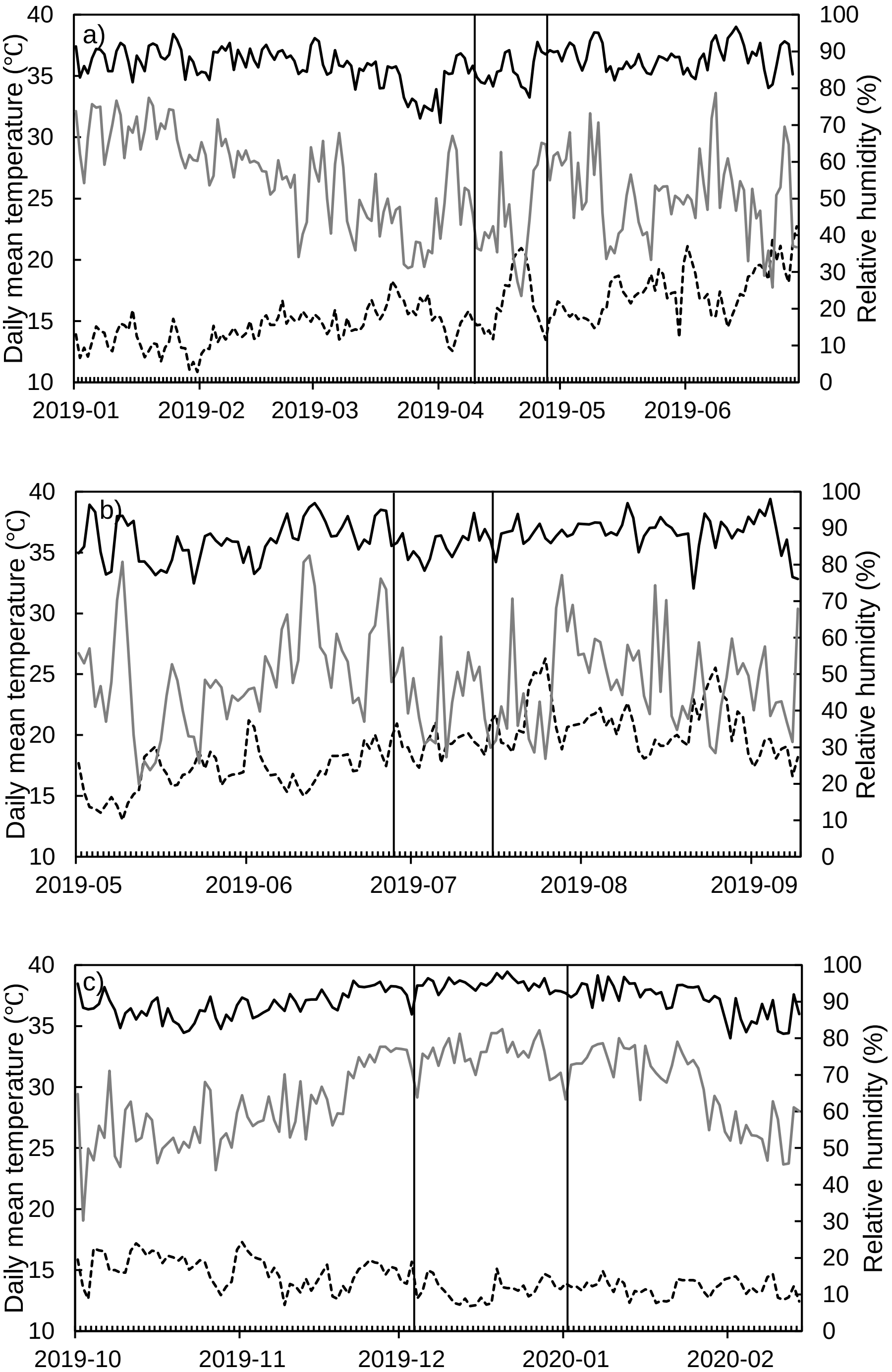
3.2.7 Statistical analysis

Data were analysed using GenStat 18th edition (VSN International, Hemel Hempstead, UK). Shapiro-Wilk and Levene tests were used for the estimation of normality and homogeneity of variance. Two-way analysis of variance (*ANOVA*) was employed to explore the AT concentration responses of plants to gas exchange and yield-related parameters with irrigation and AT as two factors with quadratic contrasts in Expt 1 (yield data excluded) and Expt 2, while linear contrasts in Expt 3. Multiple comparisons were performed to compare the significant difference between treatments according to Tukey's test (p = 0.05), where there was a significant interaction between irrigation and AT. Linear regression with groups analysis was used to test the relationships in g_s and A against AT concentrations, and between seed yield and yield components.

3.3 Results

3.3.1 Environmental conditions

The environmental conditions in the compartment of glasshouse varied among three experiments. The daily mean air temperature for the growing season was 16 ℃, 19 ℃ and 14 ℃ on average in Expts 1, 2 and 3, respectively (Figure 3.3.1-a, b, c). It showed similar patterns in terms of maximum RH with an average of 87% for Expts 1 and 2, and 91% for Expt 3, whereas the mean of minimum RH differed slightly, which was 52%, 48% and 65% in Expts 1, 2 and 3, respectively (Figure 3.3.1-a, b, c). Plants received the same supplementation from artificial light. However, daily solar radiation (SR) differed substantially between experiments. The average of SR was 11 MJ m^{-2} in Expt 1 and 16 MJ m^{-2} in Expt 2 while only 4.2 MJ m^{-2} in Expt 3 (Figure 3.3.1-d, e, f). The flowering started early in Expt 2 with the accumulation of 549 GDD (base 5 °C) before onset, compared to Expts 1 and 3 (741 and 897 GDD, respectively). Concurrently, the duration of the flowering stage varied, which was ~20 days in Expts 1 and 2, and more than a month in Expt 3 (Table 3.2.2). Despite these differences, the duration of whole growing season was similar between experiments with the range of 1713 to 1781 GDD.


a)
b)
c)
Daily mean temperature (℃)
Relative humidity (%)
40
35
30
25
20
15
10
100
90
80
70
60
50
40
30
20
10
0
2019-01
2019-02
2019-03
2019-04
2019-05
2019-06
2019-07
2019-08
2019-09
2019-10
2019-11
2019-12
2020-01
2020-02

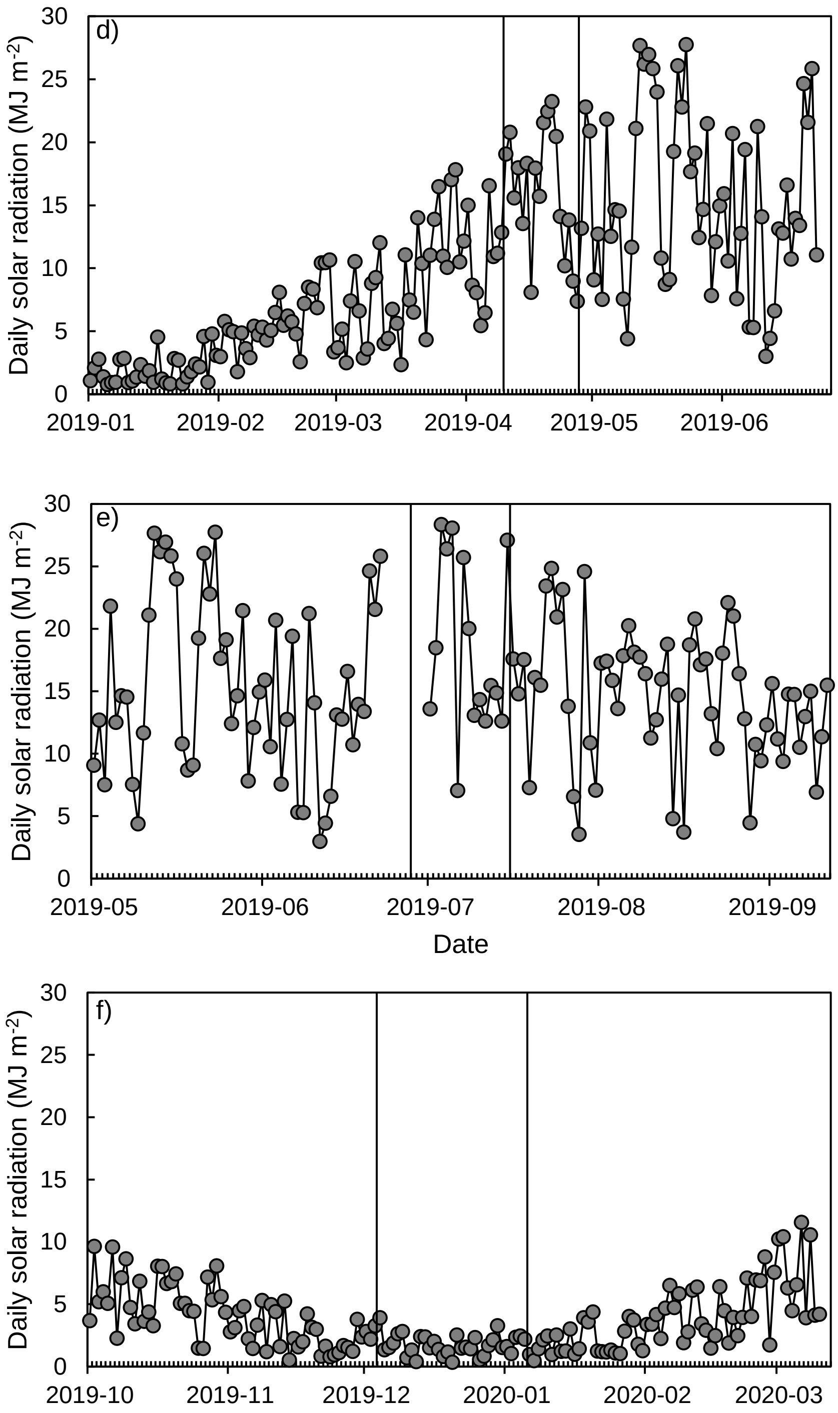
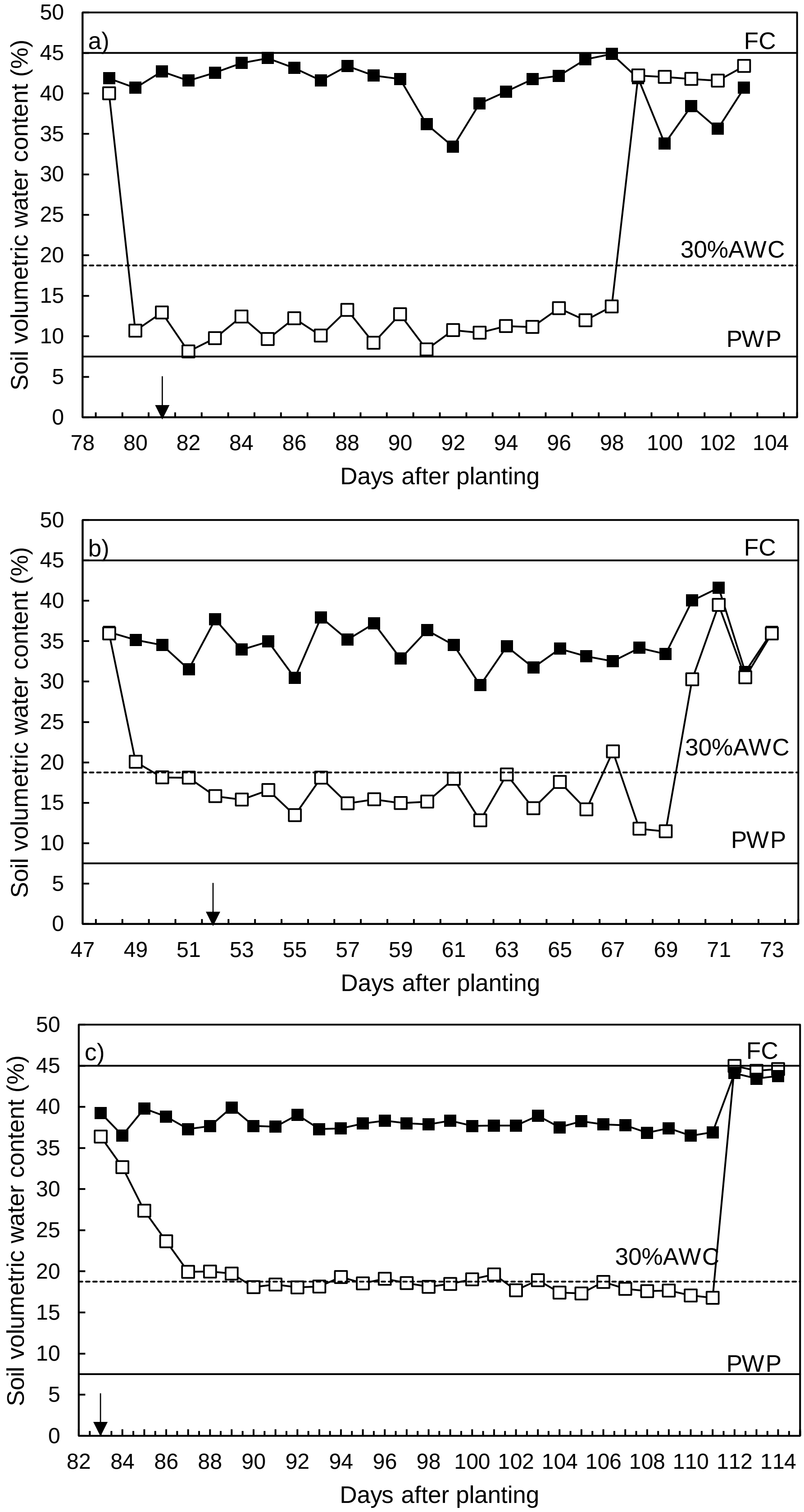


Figure 3.3.1 Daily mean temperature (°C, ----) and maximum (——) and minimum (——) relative humidity (%) and daily solar radiation (MJ m^{-2}, —●—) during the growing season of rapeseed plants in Expt 1(a, d), Expt 2 (b, e) and Expt 3 (c, f), respectively. Two vertical lines represent the day of spraying film antitranspirant and the day when full irrigation was restarted in WS pots after drought, respectively.

3.3.2 Soil water status

Soil VWC and soil matric potential (SMP) as converted according to SWRC were shown in Figure 3.3.2. In Expt 1, soil VWC (SMP) in the WW pots was maintained at the level of ~42% (SMP, -0.02 MPa) over the period of drought, whereas the average of soil VWC in WS was ~11% (-1 MPa) with substantial fluctuations around 8%-14%, i.e., a SMP of between -1.48 ~ -0.70 MPa. It returned to a similar level as WW immediately after rewatering (Figure 3.3.2-a, d). WS plants experienced less severe drought in Expts 2 and 3, compared to Expt 1. Means of soil VWC (SMP) in WW were ~34% (-0.05 MPa) and ~38% (-0.03 MPa) in Expts 2 and 3, respectively. VWC in WS was ~16% (-0.58 MPa) and ~20% (-0.34 MPa) before rewatering, and after rewatering, soil VWC increased to ~35% (-0.05 MPa) and ~44% (-0.01 MPa) in Expts 2 (Figure 3.3.2-b, d) and 3 (Figure 3.3.2-c, f), respectively.


a)
b)
c)
FC
30%AWC
PWP
Soil volumetric water content (%)
Days after planting

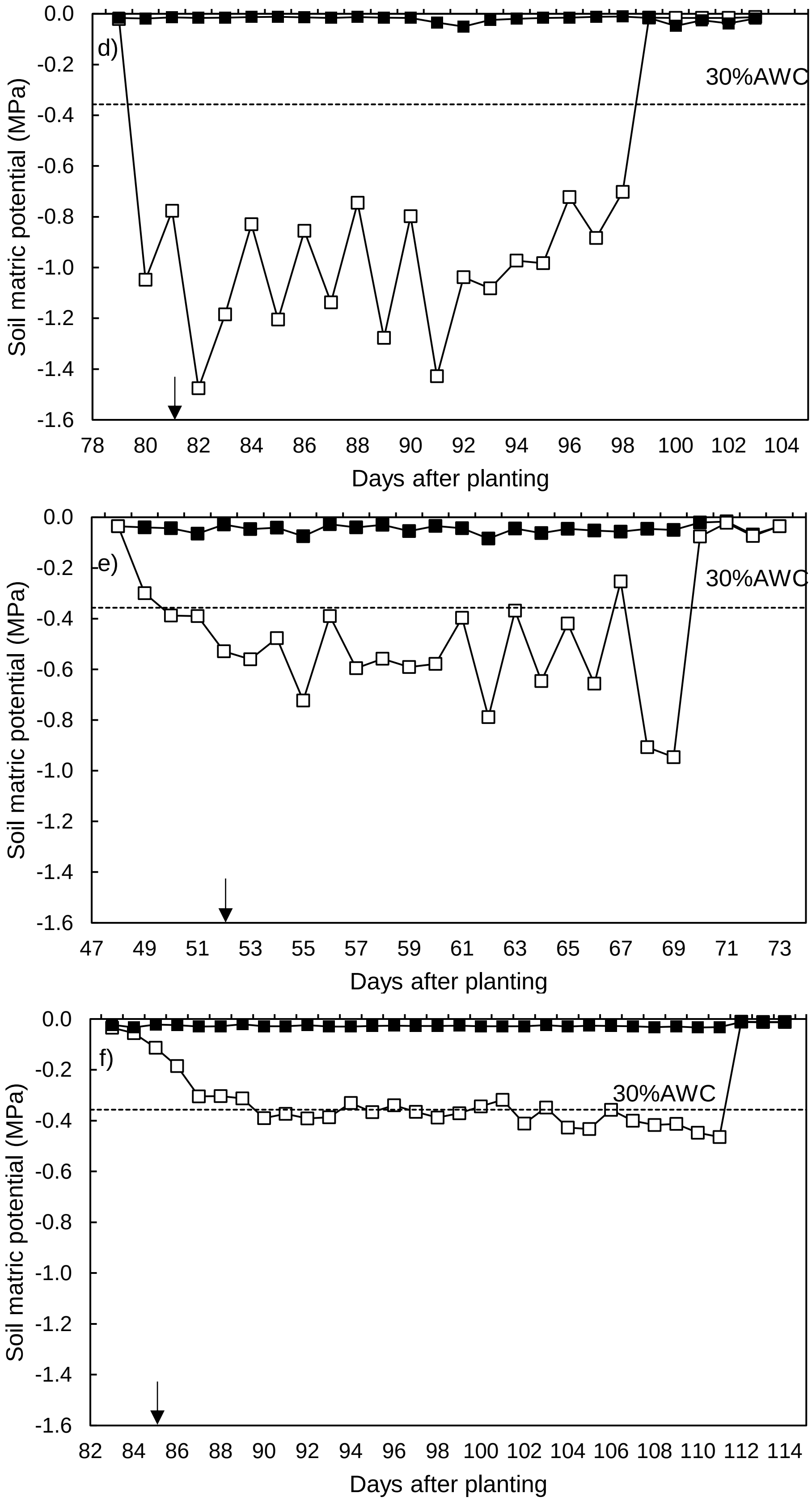

d)
e)
f)
30%AWC
Soil matric potential (MPa)
Days after planting
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
78 80 82 84 86 88 90 92 94 96 98 100 102 104
47 49 51 53 55 57 59 61 63 65 67 69 71 73
82 84 86 88 90 92 94 96 98 100 102 104 106 108 110 112 114

Figure 3.3.2 Soil volumetric water content (VWC) (Expt 1- a; Expt 2- b; Expt 3- c) and soil matric potential (SMP) (Expt 1- d; Expt 2- e; Expt 3- f) of pots under well-watered (WW- —■—) and water stressed (WS- —□—) conditions before and after spraying film antitranspirant. Two horizontal closed lines represent field capacity (FC) and permanent wilting point (PWP) and dotted lines represent the target VWC/SMP of WS treatments, i.e., 30% available water content (30%AWC). Arrows represent the day of spraying film antitranspirant. Data are means of replicates (n= 5 in Expts 1 and 2; n= 7 in Expt 3).

3.3.3 Gas exchange

Across all experiments, IR at the flowering stage had significant effects on g_s, A and WUEi. The significant interaction IR×AT was observed in g_s in Expt 1, WUEi in Expt 2 and A in Expt 3 (Table 3.3.1). However, the difference between WW and WS was substantially larger than that between AT concentrations, and the interactions did not affect the very large mean effect of WS compared with WW. Thus, means of gas exchange between WW and WS groups were logically comparable. In Expts 1-3, g_s in WW was 0.33, 0.34 and 0.36 mol m^{-2} s^{-1}, respectively. WS plants exhibited a reduction in g_s by 89%, 63% and 62% as compared to WW plants (Figure 3.3.3-a, b, c). The A in WW was 14.7, 15.1 and 16.3 µmol m^{-2} s^{-1} in Expts 1-3, respectively. When compared to WW plants, A in WS decreased by 72%, 23% and 21% in Expts 1-3, respectively (Figure 3.3.3-d, e, f). WUEi was 45.9, 47.6 and 46.0 µmol (CO_2) mol (H_2O)$^{-1}$ under WW conditions in Expts 1-3. Water deficit increased WUEi of WS plants by 145%, 90% and 107%, when compared to WW control (Figure 3.3.3-g, h, i) for these respective experiments.

Averaging WW and WS groups, the average g_s of unsprayed plants was ~0.3 mol m^{-2} s^{-1} in Expts 2 and 3. There was a linear relationship between g_s and AT concentrations, and g_s decreased by 12%-19% and 9%-24% relative to the control when AT was applied at the concentrations of 0.25%-1% in Expt 2 and 0.5%-1% in Expt 3, respectively. With a 1% increase in AT, g_s and A were predicted to decrease by 16% and 13% and by 25% and 17% in Expt 2 (Figure 3.3.3-b, e) and Expt 3 (Figure 3.3.3-c, f), respectively, as compared to corresponding mean of WW and WS control (0AT). For Expt 3, there was a significant linear interaction between IR and AT in g_s and A where g_s of WW declined greatly with increasing concentrations of AT, whereas WS only decreased slightly as AT concentrations increased (Figure 3.3.3 - c, f; Table 3.3.1). A similar trend was concurrently observed in A. In Expt 2, the interaction IRxAT was significant for WUEi, primarily accounted for the large deviation in 0.25AT and 1AT (Figure 3.3.3-h; Table 3.3.1). Except that, there appeared to be no significant impacts from AT or IR×AT in the other two experiments (Figure 3.3.3-g, i).

Table 3.3.1 Probability values from *ANOVA* for stomatal conductance (g_s), photosynthesis rate (A) and intrinsic water use efficiency (WUEi) as affected by irrigation (IR) and film antitranspirant (AT) in three experiments. Bold numbers indicate significant differences at $p < 0.05$.

Experiments	Factors	d.f.	*p values*		
			g_s	A	WUEi
Expt 1	Irrigation (IR)	1	**< .001**	**< .001**	**< .001**
	Antitranspirant (AT)	4	0.095	0.382	0.702
	Linear	1	0.687	0.141	0.993
	Quadratic	1	0.467	0.729	0.766
	Deviations	2	**0.028**	0.392	0.363
	IR×AT	4	**0.040**	0.352	0.927
	Linear	1	0.234	0.187	0.505
	Quadratic	1	0.192	0.414	0.997
	Deviations	2	**0.027**	0.363	0.815
Expt 2	Irrigation (IR)	1	**< .001**	**< .001**	**< .001**
	Antitranspirant (AT)	4	0.115	0.383	**0.008**
	Linear	1	**0.043**	**0.048**	0.578
	Quadratic	1	0.326	0.952	0.458
	Deviations	2	0.284	0.944	**0.002**
	IR×AT	4	0.071	0.888	**0.026**
	Linear	1	0.264	0.462	0.675
	Quadratic	1	0.863	0.869	0.789
	Deviations	2	**0.025**	0.763	**0.005**

Expt 3	Irrigations (IR)	2	**< .001**	**< .001**	**< .001**
	Antitranspirant (AT)	2	**0.042**	**0.009**	0.752
	Linear	1	**0.014**	**0.004**	0.524
	Deviations	1	0.633	0.210	0.697
	IR×AT	4	0.095	**0.019**	0.686
	Linear	1	**0.036**	**0.011**	0.442
	Deviations	1	0.656	0.176	0.702

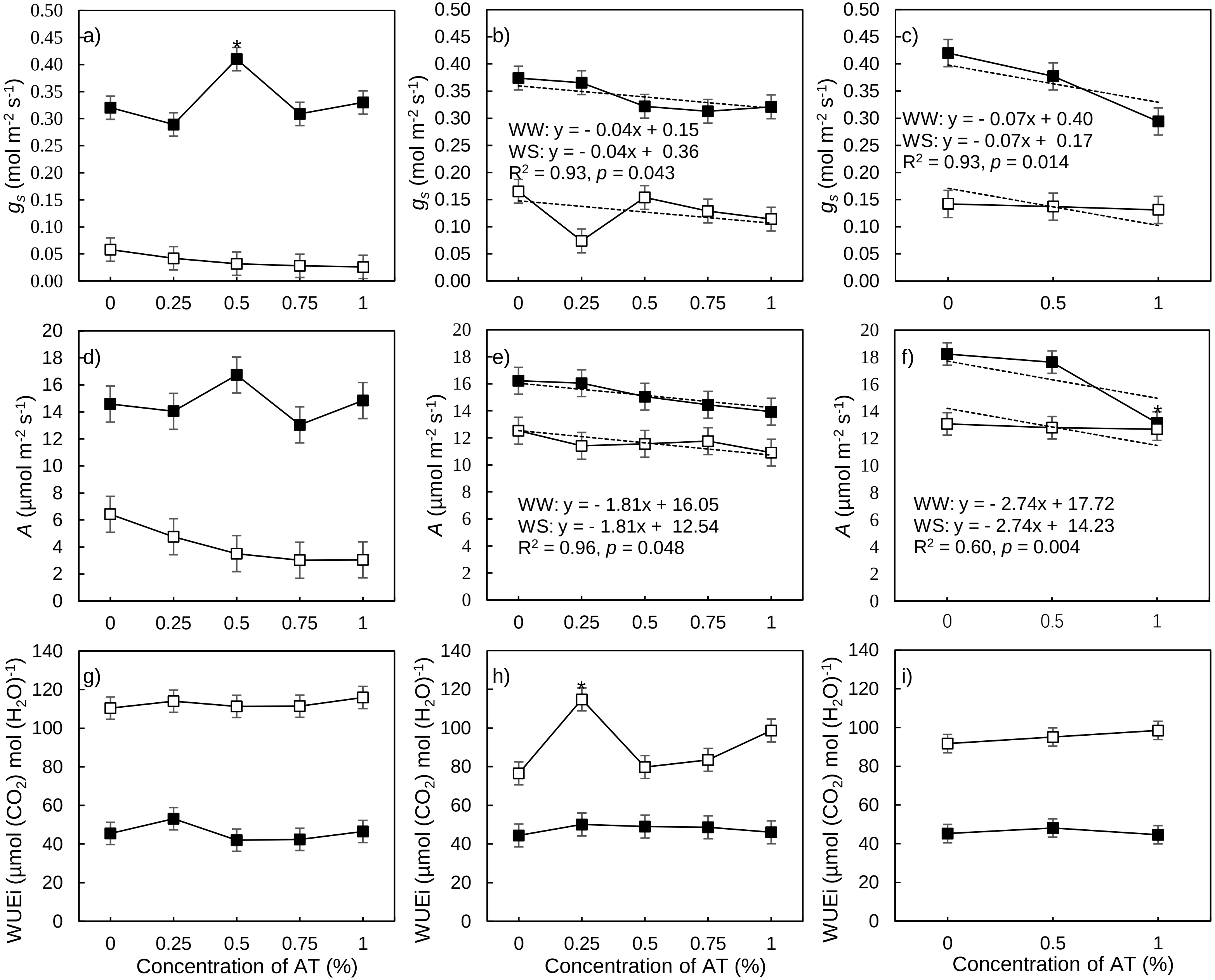

a)
b)
c)
d)
e)
f)
g)
h)
i)
*
g_s (mol m^{-2} s^{-1})
A (µmol m^{-2} s^{-1})
WUEi (µmol (CO_2) mol (H_2O)$^{-1}$)
WW: y = - 0.04x + 0.15
WS: y = - 0.04x + 0.36
R^2 = 0.93, p = 0.043
WW: y = - 0.07x + 0.40
WS: y = - 0.07x + 0.17
R^2 = 0.93, p = 0.014
WW: y = - 1.81x + 16.05
WS: y = - 1.81x + 12.54
R^2 = 0.96, p = 0.048
WW: y = - 2.74x + 17.72
WS: y = - 2.74x + 14.23
R^2 = 0.60, p = 0.004
Concentration of AT (%)

Figure 3.3.3 Stomatal conductance (gs), photosynthesis rate (A) and intrinsic water use efficiency (WUEi) of rapeseed plants at three days after spraying film antitranspirant (AT) at different concentrations under well-watered (WW, —■—) and water stressed (WS, —□—) conditions in Expt 1 (a, d, g), Expt 2 (b, e, h) and Expt 3 (c, f, i). The linear regression model with two groups (WW and WS) was fitted to gs and A against concentrations of AT in Exps 2 (b, e) and 3 (c, f) where gs and A had a significant linear relationship with AT concentrations without significant interaction ($p < 0.05$, Table 3.3.1). Asterisks represent significant differences compared to corresponding unsprayed control according to Tukey's test ($p = 0.05$). Data are means of replicates (n= 5 in Expts 1 and 2; n= 7 in Expt 3) ± standard error of the mean (SEM).

3.3.4 Yield and yield components

At maturity, no reliable seed yield data were available from Expt 1 due to the low quantity of pods. Across Expts 2 and 3, water deficit showed significantly negative impacts on seed yield and yield components, except for TSW in Expt 3 (Table 3.3.2). In Expt 2, plants grown under WW conditions had a production of ~19 g seeds and ~274 pods per plant with ~22 seeds per pod and ~3.3 g in TSW (Table 3.3.3). When compared to WW control, water deficit resulted in a reduction of 59% and 37% in seed dry weight and pod number, respectively, and to a lesser extent, 23% and 17% in seed number per pod and TSW, respectively (Figure 3.3.4-a, c, e, g). Compared to Expt 2, seed yield and yield components were numerally lower in Expt 3. Plants under WW conditions produced ~16 g of seeds and ~405 pods per plant, as well as ~8 seeds per pod and ~5 g regarding TSW (Table 3.3.3). There was a consistent but greater reduction caused by water deficit in seed dry weight, pod number and seed number per pod by 81%, 77% and 38%, respectively, while the change in TSW was negligible (Figure 3.3.4-b, d, f, h).

Averaging all concentrations of AT, consistent results from both experiments showed no significant AT or IRxAT effects in seed dry weight (Table 3.3.2). However, responses in yield components were observed, albeit in varying degrees. In Expt 2, AT application decreased pod number significantly while increased TSW with borderline significance by 4% and 5%, respectively (Table 3.3.3). Further, there was a significant linear interaction between IRxAT in pod number per plant and TSW (Table 3.3.2). With an increase in AT concentrations from 0% to 1%, pod number of WW plants increased greatly but decreased slightly for WS (Figure 3.3.4-c). On the contrary, TSW in WW decreased marginally from 0% to 1% concentration of AT and the opposite was true in WS (Figure 3.3.4-g). In Expt 3, borderline significances of AT and IRxAT interaction were observed in seed number per pod (Table 3.3.2).

Taking WW and WS as groups in regression, seed yield was highly associated with both pod number per plant and seed number per pod in Expts 2 and 3 (Figure 3.3.5). There was a positive linear relationship of seed yield with pod number, explaining 64% of the variation with irrigation as a significant factor in Expt 2 (Figure 3.3.5-a) while 87% in Expt 3 across WW and WS groups (Figure 3.3.5-b). Similarly, seed yield was less positive but still significantly correlated to the number of seeds per pod in Expt 2 ($R^2 = 0.82$) and Expt 3 ($R^2 = 0.65$), taking differences between WW and WS plants into account (Figure 3.3.5-c, d).

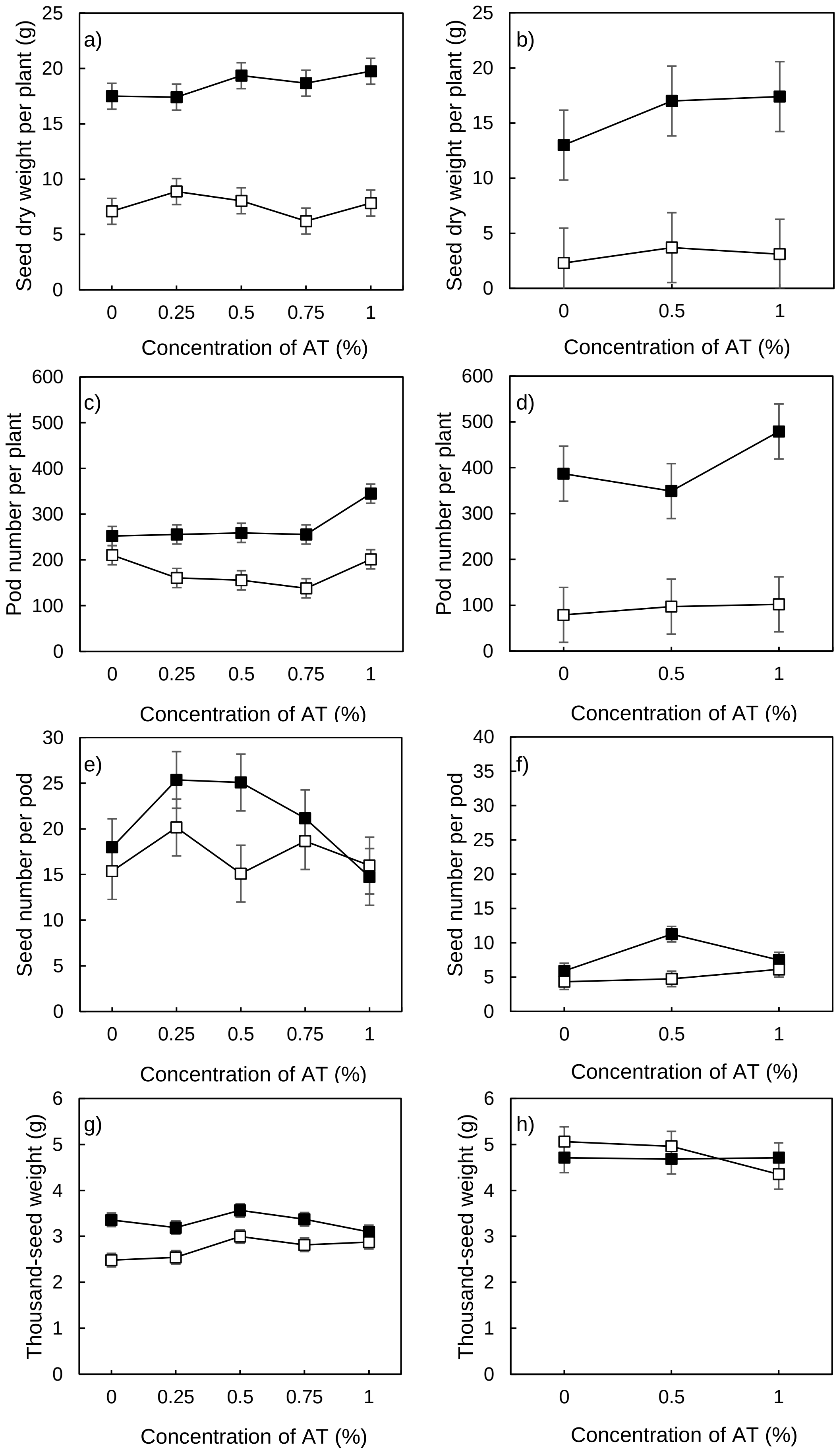
Table 3.3.2 Probability values from *ANOVA* for seed dry weight (SDW), pod number per plant (PP), seed number per pod (SP) and thousand-seed weight (TSW) as affected by irrigation and film antitranspirant in Expts 2 and 3. Bold numbers indicate significant differences at $p < 0.05$.

Expts	Factors	d.f.	*P* values			
			SDW	PP	SP	TSW
Expt 2	Irrigation (IR)	1	**< .001**	**< .001**	**0.027**	**< .001**
	Antitranspirant (AT)	4	0.601	**0.008**	**0.040**	0.061
	IR×AT	4	0.498	0.202	0.363	0.305
	Linear	1	0.195	**0.023**	0.315	**0.042**
	Quadratic	1	0.869	0.656	0.119	0.857
	Deviations	2	0.446	0.820	0.651	0.763
Expt 3	Irrigation (IR)	1	**< .001**	**< .001**	**0.002**	0.737
	Antitranspirant (AT)	2	0.642	0.486	0.053	0.519
	IR×AT	2	0.847	0.586	0.051	0.498
	Linear	1	0.583	0.570	0.918	0.288
	Deviations	1	0.875	0.391	**0.016**	0.618

Table 3.3.3 The average seed dry weight (SDW), pod number per plant (PP), seed number per pod (SP) and thousand-seed weight (TSW) as affected by the two main factors of irrigation including well-watered (WW) and water stressed (WS); and film antitranspirant (AT) including unsprayed AT (-AT) and sprayed AT (+AT) in Expts 2 and 3. The difference between WS and +AT in comparison to the corresponding control WW and -AT, respectively, were present as percentages, with positive values indicating increases and vice versa. Data are means with standard error of means (SEM), and asterisks represent the statistical significance according to Tukey's test (p = 0.05).

Expts	Factors	Treatments	SDW	Difference	PP	Difference	SP	Difference	TSW	Difference
Expt 2	Irrigation	WW	18.5		273.7		22.0		3.3	
		WS	7.6	-59%*	173.3	-37%*	17.1	-23%*	2.7	-17%*
		SEM	0.5		9.3		1.4		0.1	
	Antitranspirant	-AT	12.3		231.5		16.7		2.9	
		+AT	13.3	8%	221.5	-4%*	20.3	21%*	3.1	5%
		SEM	0.8		14.8		2.2		0.1	
Expt 3	Irrigation	WW	15.8		405.0		8.2		4.7	
		WS	3.0	-81%*	92.7	-77%*	5.1	-38%*	4.8	2%
		SEM	1.8		34.6		0.7		0.2	
	Antitranspirant	-AT	7.7		233.0		5.1		4.9	
		+AT	10.3	35%	256.8	10%	7.4	45%	4.7	-4%

SEM	2.2	42.4	0.8	0.2



a)
b)
c)
d)
e)
f)
g)
h)
Seed dry weight per plant (g)
Pod number per plant
Seed number per pod
Thousand-seed weight (g)
Concentration of AT (%)

Figure 3.3.4 Seed dry weight per plant and yield components of rapeseed plants under well-watered (WW, —■—) and water stressed (WS, —□—) conditions with application of film antitranspirants (AT) at different concentrations in Expt 2 (a, c, e, g) and Expt 3 (b, d, f, h). Data are means of replicates (n = 5 in Expt 2; n = 7 in Expt 3) ± standard error of the mean (SEM).

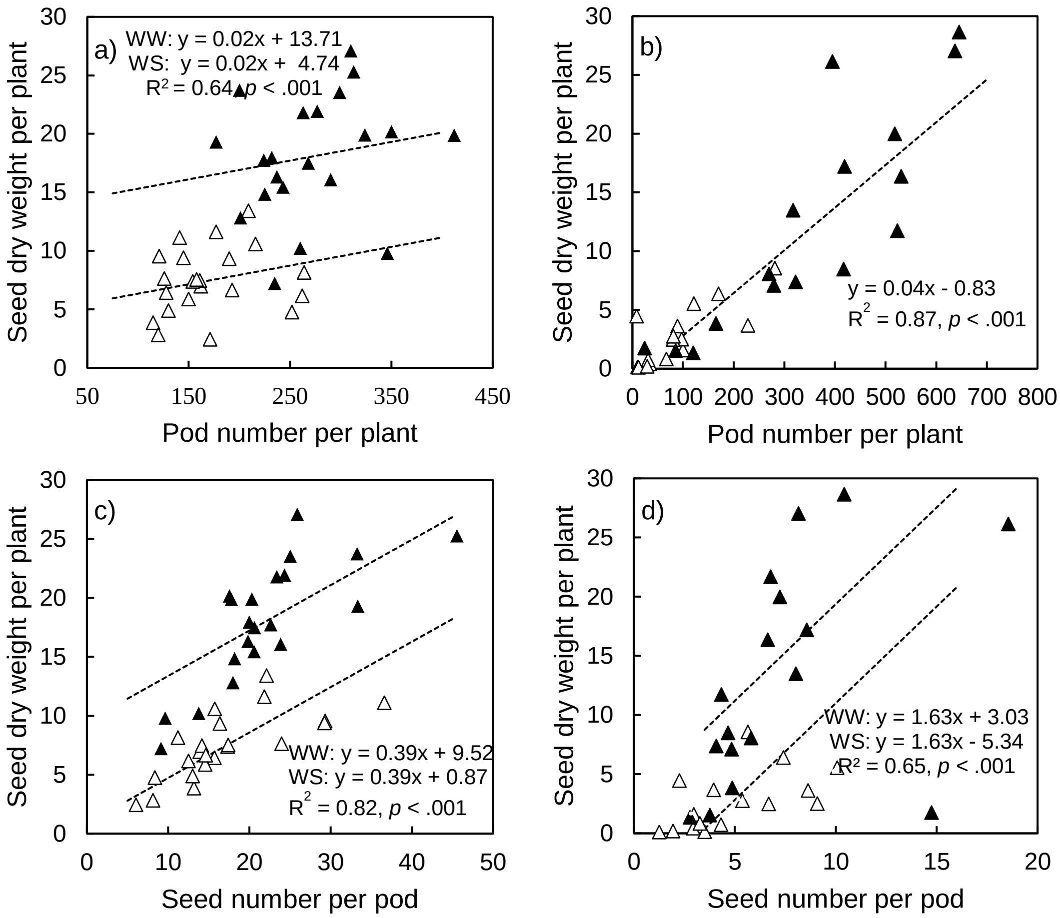


Figure 3.3.5 Relationships between seed dry weight and yield components in Expt 2 (a, c) and Expt 3 (b, d). Parallel/common dotted lines are fitted with the linear regression model with/without irrigation as groups including well-watered (WW- ▲) and water-stressed (WS- △). Data are replicates in each treatment from Expt 2 (n = 5) and Expt 3 (n = 7).

3.4 Discussion

3.4.1 Water stress depressed stomatal conductance with concomitant decrease in photosynthesis rate

Large variations between three experiments in gas exchange and final seed yield production were accounted for by the size of pots and various environmental conditions.

Compared to 7.5-L pots used in Expts 2 and 3, 1-L pots exhibited large constraints on the root access to water, resulting in drastic fluctuations in soil water content (SMP) during the period of drought (Figure 3.3.2). Despite daily air temperature and solar radiation varied slightly between Expts 1 and 2, plants in Expt 3 experienced a substantial reduction in both temperature and radiation during the growing season (Figure 3.3.1). Given incident radiation and temperature having large effects on assimilation rate and the duration of flowering stage (Weymann et al., 2015b), the difference of both factors in Expt 3 resulted in an elongation of the flowering stage, i.e., duration of drought imposed being longer than the former two experiments (Figure 3.3.1; Table 3.3.2). In addition, water stress in Expt 3 appeared to be less severe, compared to Expts 1 and 2 (Figure 3.3.2-c). Previous studies have shown that water stress can have detrimental effects on rapeseed plants and it is most pronounced during the flowering stage, followed by vegetative and seed filling stages (Tesfamariam et al., 2010). This is also supported by our studies involved with spring rapeseed. Gas exchange was consistently affected by water stress of varying degrees across three experiments. When compared to WW, water stress reduced g_s and A by 89% and 72%, respectively, in Expt 1 (~11% VWC); and to a lesser extent, by 62.5% and 22%, respectively, for Expts 2 and 3 on average (~18% VWC). The larger reduction of g_s and A that occurred in Expt 1 compared to the other two experiments was mainly attributed to the greater severity of water stress (Figure 3.3.3). With the same type of compost (John Innes No. 2), Faralli et al. (2017a) found that g_s and A of winter rapeseed reduced by 77.2% and 64.4% under severe water stress (~10% VWC), while under less severe water deficit (~20% VWC), there was a reduction of 48.5% and 41.0% in g_s and A, respectively. The difference in the reduction *of* A compared to our study, particularly under 20% VWC (41% vs 22%), might be explained by different species and/or the size of pots with restrictions on the root volume (Poorter et al., 2012). Across three experiments, WUEi from WS plants was 2-2.5 times higher than from WW plants, regardless of AT treatments (Figure 3.3.3-g, h, i). This indicates that the assimilation rate, to certain degree, can be sustained with reduced water loss through stomata as a result of the higher proportional limitation of g_s compared to A as widely reported in the literature (Palliotti et al., 2013).

3.4.2 The concentration-response of rapeseed to AT application under water stress in gas exchange

Film AT inhibits the diffusion of gas by forming a film on the leaf to block the stomata physically, thereby decreasing water loss from stomata as well as reducing photosynthetic efficiency (Gale and Hagan, 1966). In the present study, AT-treated plants showed a greater reduction in g_s than A (Figure 3.3.3), which is in agreement with the findings of Faralli et al. (2016) on winter rapeseed and Abdullah et al. (2015) on wheat. Besides, g_s and A across

WW and WS were linearly and negatively associated with the concentration of AT in two of three experiments in our study (Table 3.3.1). The g_s was projected to decrease 1.4 times faster than *A* with increasing concentrations of AT (Figure 3.3.3-b, c, e, f). Those observations indicate that the suppression on gas exchange induced by AT was highly associated with the concentration of AT, although the significant effects from AT on WUEi were found only in Expt 2 (Figure 3.3.3-h).

Gale and Poljakoff-Mayber (1967) reported that films formed by polyethylene, were distributed with unevenly varying thickness, and within some gaps as well as micropores. There is no doubt that the area covered by AT, i.e., leaf coverage, has direct impacts on the performance of AT. That is, the increase in leaf coverage induced by an increasing concentration of AT may result in further inhibition of gas exchange (Xiang et al., 2021). Faralli et al. (2017b) compared three dose rates of AT, 1, 2 and 4 L ha^{-1} (i.e., 1%, 2% and 4%) on winter rapeseed in the field conditions. There was no additional reduction at higher dose rates (2 and 4 L ha^{-1}) compared to 1 L ha^{-1}. However, whether AT beyond 1% would inhibit gas exchange further still needs further investigation in specific crop varieties and under specific environmental conditions.

The g_s and *A* increased at some concentrations after AT application in WW groups of Expt 1 (Figure 3.3.3-a, d), which is contrary to the general view about film AT that partial stomata blocked by AT physically would increase the resistance to diffusion of water vapour from stomata and consequently reduce *A* as discussed previously. The increased g_s could be caused in part by wider stomatal apertures induced by increasing leaf water potential as a result of AT application (Davenport et al., 1972). Further work is still needed to explore the stomatal size after the application of AT with specific concentrations and the interaction between stomatal blockage by AT and the gas exchange processes involved.

3.4.3 Effects of water stress and AT application on seed yield and yield components

Compared to the corresponding WW control, a great yield loss occurred in WS plants (59% and 81% in Expts 2 and 3, respectively) when water deficit was imposed during the flowering stage (Table 3.3.3). The decrease in seed dry weight was associated with a reduction in seed number per plant (data not shown) related to both pod number and seed number per pod. This is also supported by strong correlations between seed dry weight and pod number per plant (Figure 3.3.5-a, b) and seed number per pod (Figure 3.3.5-c, d). Rapeseed is most susceptible to water stress over the flowering stage, during which pollen development can be restricted by water stress, and its effects lead to pod abortion by preventing flowers from developing into pods and pod abscission (Ahmadi and Bahrani, 2009). Concurrently, the decline of the assimilate availability caused by drought shown in a great reduction in *A*

(Figure 3.3.3-e) could have had detrimental effects on the development of pods and, in turn, seeds during the seed filling stage (Istanbulluoglu et al., 2010). However, very few pods formed from rapeseed plants grown in 1-L pots may be primarily accounted for by the small size of pots, as discussed earlier. Limited rooting volume could accelerate the severity of soil water deficit developed by withholding irrigation for WS plants as seen that soil VWC on two occasions approached PWP (-1.5 MPa) (Figure 3.3.2-a, d), which could have resulted in a large quantity of pod abortion (Poorter et al., 2012).

Water stress at the critical stage, particularly at late flowering, has multiple impacts on the assimilate supply of pods, and concurrently it also restricts the capacity of surviving pods and seeds for compensatory growth (Kirkegaard et al., 2018). In the current work, yield components showed different responses to AT across two experiments, making it difficult to interpret because of multiple variabilities from the individual plant, environmental conditions etc. However, we failed to identify a statistically significant relationship between irrigation and AT in seed yield, although improvements (not significant) were observed from AT-treated WS plants. Overall, the opposite trend was overserved for the number of seeds per plant (data not shown) and thousand seed weight, implying that the compensation of yield components occurred following AT application during the late flowering stage and/or seed filling stage by altering number of seeds and individual seed weight. Labra et al. (2017) stressed that rapeseed has the plasticity to adjust its potential seed number and size according to the assimilates produced at different stages. One possible hypothesis to explain the lack of effect of AT application is that the concentration of AT may not be high enough to detect the yield benefits significantly on rapeseed due to the limitation of leaf coverage, which is highly related to the concentration of AT. The leaf coverage from AT application at 1% was estimated to be up to 19%, depending on the growth of plants (Xiang et al., 2021).

The duration, intensity and timing of water stress would affect crop yield (Müller et al., 2012). Those factors are also highly associated with the response of crops to AT application. Extremely severe or mild water stress may prevent the efficacy of AT application on rapeseed (Faralli et al., 2017a). The water stress in our experiments was imposed over a short timescale, and rapeseed plants would exhibit isohydric behaviours at gas-exchange level with tight control of leaf water potential when subjected to this type of water stress, although irreversible damage still can be caused by prolonged and/or intense water deficit (Bodner et al., 2015). Moreover, rapeseed can show different adaptations if water stress is imposed progressively (Ilami and Contour-Ansel, 1997). Future work should therefore compare different timescales and severities of drought imposition. In addition, as abscisic acid plays a role in mitigating drought damage to crops following AT application (Mphande et al., 2021b), it is worth assessing the efficacy of AT on crops in terms of the synergistic effects of hydraulic and hormone signals.

3.5 Conclusions

We showed that the range of water stress during the flowering stage, as indicated by soil matric potential (-1 ~ -0.34 MPa) across three experiments, depressed the gas exchange of rapeseed plants. Consequently, water stress resulted in a substantial reduction in seed dry weight of rapeseed with the largest effects on pod number, compared to the other two yield components (seed number per pod and thousand-seed weight). Application of film AT inhibited gas exchange of both well-watered and water-stressed plants in two out of three experiments. Furthermore, the magnitude of inhibition was linearly related to AT concentration that stomatal conductance decreased ~1.4 times faster than photosynthesis rate with a 1% increase in AT concentrations irrespective of irrigation. The increases induced by AT application in some yield components were observed at some concentrations, however, the compensating trade-off between pod number and seed number per pod resulted in the lack of significant improvement in seed yield from AT in water-stressed or well-watered plants. Therefore, the response of rapeseed treated with higher concentrations (> 1%) of AT under both glasshouse and field conditions with different stress scenarios requires further investigation to identify an optimum dose rate and to understand the situations when AT has the highest efficacy to mitigate drought damage.

Chapter 4 A preliminary study about leaf coverage and stomatal density of rapeseed under simulated field conditions in the glasshouse

4.1 Introduction to Chapter 4

The mode of action of film AT is to block stomata physically by forming a film, thereby reducing water loss through transpiration. Leaf coverage is determined by multiple factors, among which plant canopy plays an important role, particularly leaf orientation. From Chapter 2, we have demonstrated that TiO_2 can be used as a spray marker to estimate leaf coverage. In the glasshouse with well-spaced plants, leaf coverage showed a significant relationship with AT concentrations. Leaf coverage increased from an average of 4% to 17% when AT was applied from 0.25% to 1% v/v. Then, in Chapter 3, we applied the same concentrations of AT on droughted rapeseed at the early flowering stage in the glasshouse, showing non-significant improvements in seed yield but only yield components. The lack of yield responses can be explained by the yield compensation during the late flowering stage and/or seed filling stage by altering the number of seeds and individual seed weight after rewatering. Alternatively, AT concentrations may not achieve good leaf coverage to improve leaf water status. Accordingly, we propose our hypothesis for Chapter 5 that increasing AT concentrations to above 1% can improve seed yield and yield components of rapeseed subjected to terminal drought, which occurs most commonly in agricultural drought events. It is important to estimate the leaf coverage when plants are treated with higher concentrations of AT applied under field conditions. Considering the feasibility and reliability of the work, therefore, a preliminary study was conducted to estimate stomatal density and leaf coverage by simulating field-growing conditions in terms of plant arrangement. The null hypothesis tested is that there is no significant difference in leaf coverage of film AT from 1% to 3% when applied at the flowering stage of rapeseed.

4.2 Materials and Methods

4.2.1 Agronomic management and experimental design

Seeds of spring rapeseed (cv. Mirakel; NPZ-Lembke, Germany) were sown into seedling-planter trays filled with John Innes No. 2 compost on August 9^{th}, 2021. About two weeks after sowing, seedlings were transplanted into pots at fourth true leaf stage, with one plant per pot. The size of pots used here was 1 L and each pot contained ~500 g John Innes No. 2 compost at 22 ± 1% volumetric water content measured with a soil moisture probe (ML2X theta probe; Delta-T-device, Cambridge, UK). Pots were arranged in the glasshouse at HAU with sodium vapour lamps supplemented (16 h–8 h light-dark photoperiod) and irrigated every two days until free drainage from the bottom occurred. Nitrogen was applied at 100 kg/ha before flowering stage, i.e., 0.3g Ammonium nitrate (34.5% N) per pot. The average daily air temperature was ~17 °C before treatments started. This experiment was conducted as a randomised single factor design with five levels: 0.5%, 1%, 1.5%, 2% and 3%. Details of each treatment are shown in Table 4.2.1

Table 4.2.1 Overview of treatment compositions, including concentrations of film antitranspirant (AT), nominal and actual dose rates of AT, and the corresponding amount of AT, TiO_2 and water in the sprayer tank (100 mL).

Treatments	AT (%)	Dose rates of AT (L ha^{-1})		Mixture in the tank		
		Nominal	Actual	TiO_2 (g)	AT (mL)	Water (mL)
1 AT + 1 g TiO_2	0.5	1.0	1.25	1.0	0.5	99.5
2 AT + 2 g TiO_2	1.0	2.0	2.50	2.0	1.0	99.0
3 AT + 3 g TiO_2	1.5	3.0	3.75	3.0	1.5	98.5
4 AT + 4 g TiO_2	2.0	4.0	5.00	4.0	2.0	98.0
6 AT + 6 g TiO_2	3.0	6.0	7.50	6.0	3.0	97.0

4.2.2 Stomatal density analysis

At flowering stage just one day before AT application, an epidermal impression from each of three leaves including the first fully expanded leaf and two leaves below (also for leaf coverage analysis) was collected by coating the adaxial and abaxial leaf surfaces with the nail varnish in the mid-area between the central vein and the leaf edge. Then, peeled off the dried film of nail varnish using clear tape and mounted it onto a glass slide (Figure 4.2.1), assuming that the inevitable removal of wax from nail vanish had no effects on following AT treatments because the size per impression was negligible as compared to the whole leaf. Stomata per unit area were then viewed and counted under the compound light microscope (CX31, Olympus UK & Ireland, UK) with a camera (Moticam 1080, Motic, PeplerTech Ltd, UK) at ×10 magnification. Ten random microscopic fields (~0.14 mm^2) were captured using an attached camera (examples shown in Figure 4.2.2). Stomatal density per unit area (mm^2) was then determined by dividing the number of stomata by 0.14. Data were not independent from three leaves or two sides of the leaf, so stomatal density was not included in statistical analysis.

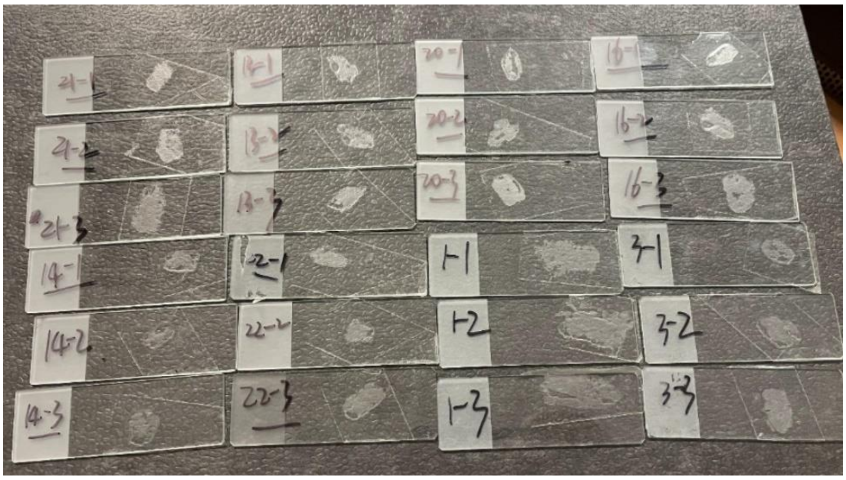


Figure 4.2.1 Examples of epidermal stomata impressions from the adaxial surface of three leaves for each rapeseed plant.

Figure 4.2.3 The arrangement of plants when film antitranspirant (AT) was sprayed in the chamber with a customised built-in sprayer (left: AT 1.5%; right: 2%).

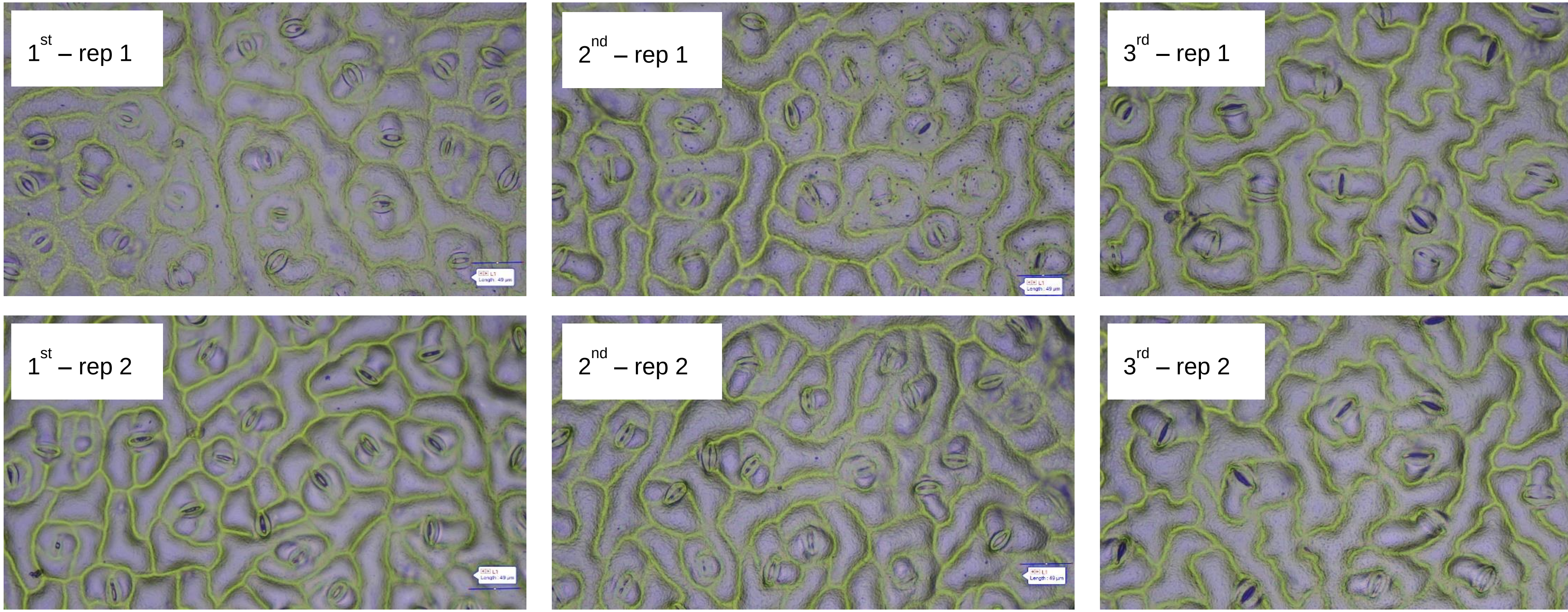


Figure 4.2.2 Examples of adaxial stomata images under the microscope from three leaf positions of one rapeseed plant. Two replicates from each position had the same stomatal density. The number of stomata per mm^{-2} from 1st to 3rd was 183, 162 and 113, respectively.

4.2.3 Spray application and leaf coverage analysis

At the early flowering stage on October 1st, 2021, film AT with TiO_2 was applied on the adaxial surface of leaves uniformly using the same custom-built automatic pot sprayer with reference to Section 3.2.1. The distance between nozzles and plant canopy was kept at ~50 cm. Four pots were placed 15 cm between plants in a row (along with spraying direction) and 25 cm apart between rows to simulate field growing conditions (Figure 4.2.3). After spraying, the first fully expanded leaf and two leaves below were collected for leaf coverage analysis. The method of leaf coverage analysis was referred to as Section 3.3.4.

4.2.4 Statistical analysis

Data were checked for normality by examining residual plots and presented as means ± standard error (SD). As the position of leaves was not randomly allocated, repeated measures *ANOVA* was conducted on the leaf coverage from three leaves, considering one leaf position equivalent to the data from one measurement time. A stepwise regression model was fitted using mean leaf coverage of three replicates from three positions against concentrations of AT. All data analysis was performed by GenStat 18th edition (VSN International, Hemel Hempstead, UK).

4.3 Results and discussion

4.3.1 Stomatal density

The mean stomatal density across three positions was 157 and 228 from the adaxial and abaxial surfaces, respectively. The stomatal density, especially from the adaxial surface, decreased from high- to low-ranked leaves (Table 4.3.1). The higher stomatal density from the abaxial surface than from the adaxial surface is also reported by Liu et al. (2021), although values were significantly different between eight rapeseed genotypes. It is well known that higher abaxial stomatal density over the adaxial surface plays an important role for crops because the underside of the leaf is cooler and less prone to water loss, especially under drought conditions. As stomatal density is highly dependent on species and environmental conditions, differences in stomatal density can be reasonably observed from the literature. Usually, smaller stomata with higher stomatal density are beneficiary for crops to survive under drought conditions as they can result in a fast stomatal movement, leading to stomatal closure and reducing water loss (Bertolino et al., 2019). Zhu et al. (2021) compared the stomatal density of two rapeseed genotypes under water-stressed and well-watered conditions at the fourth-leaf stage. Results showed that the tolerant genotype had higher stomatal density than the drought-sensitive rapeseed genotype. Under drought conditions by withholding irrigation for five days, stomatal density and stomatal closure rate (percentage of closed stomata per unit area) of both genotypes increased significantly, indicating that the interaction between stomatal development and water stress may have the potential to improve drought tolerance of rapeseed at early vegetative stage. Dellero et al. (2021) reported that abaxial stomatal density significantly decreased from young-to-mature-to-senescent leaves while stomatal aperture showed an opposite trend to support photosynthesis with respect to senescence-driven changes of leaf anatomy and Rubisco amounts by boosting CO_2 diffusion toward chloroplasts. The difference in phonological stages may explain the lower stomatal density from lower-ranked leaves (Dellero et al., 2021).

Table 4.3.1 Adaxial and abaxial stomatal density (stomata mm^{-2}) of rapeseed leaves from three positions. Data are means (n = 20) ± standard deviations (SD).

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

4.3.2 Leaf coverage

AT significantly influenced leaf coverage without effects on the leaf position or the interaction between AT and leaf position (Table 4.3.2). Leaf coverage was linearly correlated with AT concentrations, although deviations were also significant. The linear regression showed that as AT concentrations increased by 1%, leaf coverage was predicted to increase by 9.6% (Figure 4.3.1). A positive relationship was also observed in well-spaced potted rapeseed treated with AT from 0.25% to 1% in Chapter 2. However, this linear relationship was accompanied by significant deviations, indicating that leaf coverage was not always linearly correlated to AT concentrations tested in the present study (1% - 3%).

As discussed in Chapter 2, the deposition of sprays on leaves is highly associated with canopy characteristics, which may result in large variabilities between experiments regarding leaf coverage. Compared to Chapter 2, results from the current study showed a substantial reduction in 0.5% AT that was mainly attributed to overlapped leaves because of the close distance between plants. Unexpectedly, 1% AT had higher leaf coverage (20%)

compared to the glasshouse study (13% and 19% in Expts 3 and 4, respectively), which could have caused a lower coverage as 0.5% AT. This might be from random effects such as improper mixing of AT solutions with TiO_2, resulting in less uniform spray coverage. For discussions about the limitation of using TiO_2 as a spray marker refer to Chapter 2. Nonetheless, the greater leaf coverage from higher concentrations is important to enhance film AT efficiency as a greater number of stomata on the leaf are blocked.

Table 4.3.2 Leaf coverage from three leaves of each treatment, and probability values from repeated measure *ANOVA* with one leaf position (LP) equivalent to one measurement affected by different concentrations of film antitranspirant (AT).

Concentrations	Dose rates of AT (L ha^{-1})		Leaf coverage at three positions (%)			
of AT (%)	Nominal	Actual	1st	2nd	3rd	Mean
0.5	1.0	1.3	3.32	2.08	3.77	3.06
1.0	2.0	2.5	16.83	22.86	19.57	19.75
1.5	3.0	3.8	15.07	17.16	21.42	17.88
2.0	4.0	5.0	28.40	21.34	20.74	23.49
3.0	6.0	7.5	30.18	33.58	29.35	31.04
ANOVA - *p* values						
AT		< 0.001				
Linear		< 0.001				
Quadratic		0.238				
Deviations		0.011				
LP		0.847				
AT * LP		0.186				

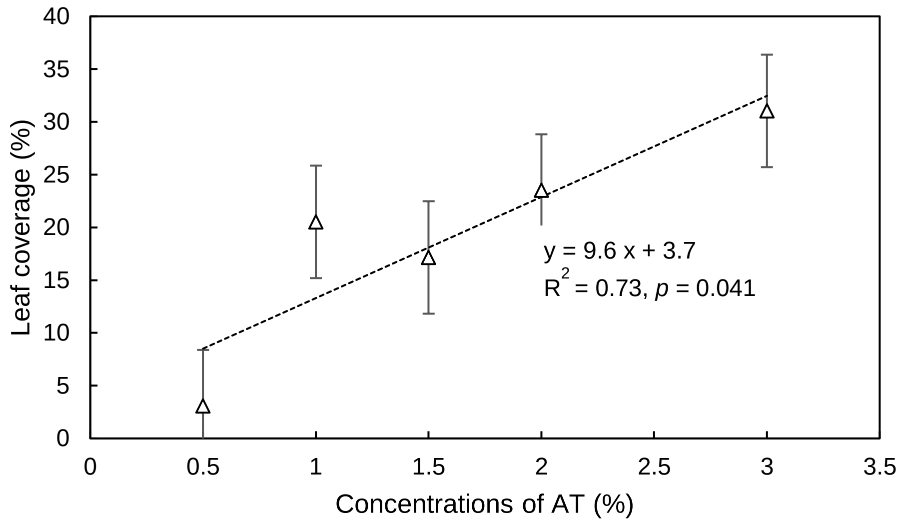


Figure 4.3.1 Relationship between leaf coverage of film antitranspirant (AT) estimated from TiO_2 and concentrations of AT (0.5%, 1%, 1.5%, 2% and 3% with corresponding dose rates of 1, 2, 3, 4 and 6 L ha^{-1}, respectively). A straight line was fitted using stepwise regression analysis. Data points are means (n=12), and error bars represent standard deviations (SD).

4.4 Conclusions

In conclusion, rapeseed plants had unequal stomatal distribution, with 59% on the abaxial surface and 41% on the adaxial surface. Young leaves appeared to have higher adaxial stomata density but had similar abaxial stomatal density to low-ranked leaves. Using TiO_2 as a spray marker, leaf coverage under field-simulated conditions was linearly and positively correlated with AT concentrations. Further, adaxial leaf coverage increased from 3% to 31% when AT was sprayed from 0.5% to 3%. Therefore, further research will focus on the application of AT on field crops with a series of concentrations tested in the present study and explore the physiological response of rapeseed to terminal drought under field conditions and yield and its components.

Chapter 5: Increasing the concentration of film antitranspirant enhances yield benefits on rapeseed (*Brassica napus* L.) under terminal drought

5.1 Introduction to Chapter 5

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

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

However, there are limited publications about rapeseed. Patil and De (1978) sprayed film AT (Mobileaf, 10%) once at the early flowering stage of *Brassica campestris* under dryland conditions in addition to the other two types of ATs. All ATs resulted in yield benefits, among which film AT improved the seed yield by 26%, accompanied by greater leaf relative water content, compared to 0AT. More recently, Faralli et al. (2016) applied film AT 1% (di-p-menthene) on winter rapeseed (*Brassica napus*) at the flowering stage under controlled drought, and they reported that AT improved seed dry weight by 17%, primarily by increased pod number. Later, the same authors found similar improvement in winter rapeseed from the field conditions, and AT efficacy for yield benefits was highly related to the magnitude of water stress (Faralli et al., 2017a, 2017b). We initially investigated the concentration-response between AT and the yield of spring rapeseed in the glasshouse to explore the optimal cost-effective concentration of AT (Xiang et al., 2022). Plants showed significant physiological responses to AT concentrations in gas exchange. However, the lack of consistent responses in yield and yield components highly indicates the need for further research with higher concentrations in the field conditions.

Therefore, two field experiments were conducted to assess the effect of different concentrations of AT on yield and its components of rapeseed under terminal drought, which occurs most commonly during spring and early summer. Some physiological parameters are also determined to estimate plant water status, gas exchange and endogenous ABA content in plant tissues. The null hypothesis is tested that there is no significant difference in yield and its components of rapeseed treated with film antitranspirant at increasing concentrations from 1% to 3%.

5.2 Materials and Methods

5.2.1 Experimental sites and agronomic management

Two field experiments were conducted at Bird's Nest (BN) and Flat Nook (FN), Harper Adams University (HAU) (52°46′N, 2°25′W) in 2021. The previous crop of both was wheat. Ten soil samples at both sites were collected randomly in the top 30 cm using a rotary corer drill prior to the start of trials, and they were mixed up in a sealable plastic bag to form one representative sample for each. Two soil samples were then stored at 4 °C and sent for physicochemical analysis in the private laboratory (NRM Coopers Bridge, Bracknell, UK). Results are shown in Table 5.2.1. The soil at BN and FN was ploughed and power-harrowed before sowing. To simulate terminal drought by keeping the rain out, the polytunnel at BN was fixed, covering all plots, while four polythene shelters were installed at FN on fixed metal frames for each block after the soil was cultivated. Two TinyTag loggers (Gemini Data Loggers, UK) monitored air temperature and relative humidity (RH) at BN and two selected polytunnels at FN. Daily solar radiation (SR) data were obtained from the meteorological station based at HAU, located within a one-kilometre distance from both research sites. The size of one plot was 1.5 m long (L) × 1 m wide (W) at both sites except for the benchmark plot (L:1 m × W: 1m) at FN (see more details in Appendix. Figure 3).

Spring rapeseed (*Brassica napus* L. var. Mirakel; NPZ-Lembke, Germany) was sown by hand in four rows with ~15 cm between rows and ~8 cm between plants within rows at1–2 cm depth. Seedlings at the early leaf development stage were thinned to ~50 plants m^{-2}. The sowing dates of the two experiments are listed in Table 5.2.2, alongside AT application and harvesting dates. All treatments received light irrigations to ensure uniform seed germination and plant emergence. Following the establishment of seedlings, irrigation in experimental plots was withheld at ~1 month after planting (i.e., GS12/13), whereas benchmark plots were irrigated with three 1 m-dripper tapes (dripper spacing- 20 cm & output- 250 L h^{-1}, TSX 506 T-Tape, Access Irrigation Ltd, Northampton, UK) for 1 h (approximately 17 mm h^{-1}) on Mondays, Wednesdays, and Fridays at 9:00–10:00 am until harvest.

At BN, nitrogen fertiliser (ammonium nitrate, 34.5% N) at 100 kg N ha^{-1} was incorporated into the soil using a tractor-drawn plough on 2nd March; fungicide (Propulse, a.i. carboxamide and triazolinthione, Bayer Crop Science Ltd, UK) at 1 L ha^{-1} applied on 20th May. At FN, nitrogen fertiliser was applied at 100 kg ha^{-1} on 21st May and foliar multi-nutrient fertiliser (3X Solution, OMEX Agriculture Ltd, UK) at 5 L ha^{-1} on 01st July. Due to an unexpected delay in receiving soil analysis results from the laboratory, the same amount of nitrogen fertiliser was applied to both sites to reduce soil variance and to ensure plant optimum growth. A mixture of insecticide (Hallmark Zeon, a.i. lambda-cyhalothrin, Syngenta UK Ltd, UK) at 75 ml ha^{-1} and fungicide — Propulse at 1 L ha^{-1} was applied on 02nd July. Weeding was manually done at both sites.

Table 5.2.1 Soil physical and chemical characteristics (0-30 cm in depth) at BN and FN.

Sites	Texture	pH	OM[a] (%)	FC[b] (%)	PWP[c] (%)	Available nutrients			
						N (kg ha^{-1})	P (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})
BN	Sandy loam	7.3	3.8	26	13	300	86.6	353	120
FN	Sandy loam	7.3	2.3	21	8	40	61.4	156	68

[a] – organic matter; [b] – field capacity; [c] – permanent wilting point.

Table 5.2.2 Dates of planting, film antitranspirant (AT) application and harvest at BN and FN.

Sites	Planting	AT application				Harvest			
		GS[a]	Date	DAP[b]	GDD[c]	GS	Date	DAP	GDD

BN	19th March	63	02nd June	75	644	89	02nd August	136	1669
FN	19th April	62	15th June	57	557	89	17th August	120	1478

[a] – growth stage; [b] – days after planting; [c] – growing degree days (base temperature, 5 °C) (Wintermantel et al., 2020).

5.2.2 Experimental design

The study was conducted in a randomised complete block design with a single factor, including eight blocks at BN and four blocks at FN. Details of treatments in each block are summarised in Table 5.2.3. At BN, each block consisted of four drought plots; four well-watered benchmark plots were located on one side of the polytunnel to estimate the magnitude of drought. At FN, each rain shelter was treated as one block, and each block comprised six drought plots and two benchmark plots located at one end of each rain shelter. Note that benchmark plots of two experiments were not part of randomisation such that they were not included in statistical analyses. Appendix Supp. Figure 1 shows the layout of drought and benchmark plots from both sites.

5.2.3 Spray application

Film antitranspirant (AT, Vapor Gard, a.i. di-1-*p* menthane, Miller Chemical and Fertilizer Corp., Hanover, PA) was applied to the canopy at a 0.5 m distance from the nozzle at the early flowering stage. Di-1-*p*-menthane is a terpene polymer known as Pinolene, produced by distillation from conifer resins. A range of concentrations of AT from 1% to 3% was applied, and unsprayed droughted plots (i.e., 0AT) were treated with water only, using a hand-held boom sprayer (flat-fan 110/03, 2 bar, 200 L ha^{-1}, Lunch Box Sprayer, Trials Equipment (UK) Ltd, Essex, UK). Details of treatments are listed in Table 5.2.3. To prevent cross-contamination between treatments, solutions/water were prepared in individual tanks and nozzles were brushed thoroughly using corresponding solutions/water prior to spraying on plants.

Table 5.2.3 Details of treatments within one block at BN and FN.

Sites	Treatments	Concentration of AT (%)	Dose rate of AT (L ha^{-1})	Volume of AT (mL)	Volume of water (mL)
BN	0AT (control)	-	-	-	5000

	1AT	1	2	50	4950
	3AT	3	6	150	4850
FN	0AT (control)	-	-	-	5000
	1AT	1	2	50	4950
	1.5AT	1.5	3	75	4925
	2AT	2	4	100	4900
	3AT	3	6	150	4850

5.2.4 Soil moisture dynamics

Access tubes were installed in the central part of representative plots within each block at two sites to monitor the dynamic soil moisture in both drought and benchmark plots as shown in Appendix Figure 3. Soil volumetric water content (VWC) in the depths of 0-20 cm, 20-40 cm and 40-60 cm were measured by a time domain reflectometry (TDR, TRIME-TDR, IMKO Micromodultechnik GmbH, Ettlingen, Germany) ~twice a week during the growing season. To compare the magnitude of drought between the two sites, soil VWC was converted to soil matric potential (SMP) according to the corresponding soil water retention curve (SWRC).

SWRC from two sites was determined on undisturbed soil using the pressure membrane apparatus (0700CG23F1 Manifold, Soil Moisture Equipment Corp., USA) (Bittelli and Flury, 2009). Low-pressure steps, including 0.01, 0.1, 0.5, and 1 bar, were applied with a 1-bar pressure plate, and 3, 5, 10, and 15 bars were used for high-pressure steps with a 5-bar pressure plate. Soil samples were collected at a depth of 0-30 and 30-60 cm using the steel corer (diameter: 5 cm, height: 5 cm), and then samples were shaped using the knife to fit the size of the ring (diameter: 5 cm, height: 1 cm) for the pressure application. A minimum of two days without any outflow were required for the equilibration to be attained. Once the equilibrium was established, samples were removed from the pressure plates to measure gravimetric water content (GWC). There were three replicates for each depth and each pressure step. The porous plates were cleaned thoroughly with deionised water to prevent cross effects between pressures. Bulk density (BD) was measured on sub-soil samples for SWRC at depths of 0-30 and 30-60 cm, respectively. Soil VWC was then calculated by multiplying GWC by the mean of two depths of BN, followed by the linear model fitted with VWC against log transformed SMP to obtain the equation of SWRC (Appendix. Figure 4).

5.2.5 Thermal image collection and analysis

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

5.2.6 Gas exchange analysis

Stomatal conductance (g_s, mol m^{-2} s^{-1}) and net photosynthesis rate (A, µmol m^{-2} s^{-1}) were determined by using an infrared gas analyser (IRGA) - LC pro-SD (ADC BioScientific Ltd, UK) on the youngest fully expanded leaves (the 5th/6th leaf counting down from the top of canopy) during 10:00-13:00 at 3 DAS and 8 DAS. The temperature of the leaf chamber (6.25 cm^2) was maintained at ~25 °C, and the photosynthetically active photon flux density was 1044 µmol m^{-2} s^{-1} provided by an attached mixed red/blue LED array with a flow rate of 300 µmol s^{-1}. The CO_2 concentration of inlet air through the leaf chamber was ~380 ppm. All the data were recorded when steady-state photosynthesis was achieved after 3~5 mins. The flow check was calibrated before the first sampling time for each of the two experiments to check if the cycle times were long enough for the gas through the analysis cell to become stable before taking readings. Intrinsic water-use efficiency (WUEi, µmol (CO_2) mol (H_2O)$^{-1}$) was calculated from A divided by g_s. The dates on which readings were taken are listed in Table 5.2.4.

5.2.7 Endogenous ABA concentration and leaf relative water content

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

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

5.2.8 Yield and yield components analysis

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

Table 5.2.4 Summary dates of thermal images, gas exchange, and leaf/pod sampling for endogenous ABA concentration (Endo-ABA) at BN and FN.

Sites	Thermal images			Gas exchange			Sampling for Endo-ABA		
	Date	DAP[a]	DAS[b]	Date	DAP	DAS	Date	DAP	DAS
BN	04th June	77	2	05th June	78	3	09th June_leaf	82	7
	09th June	82	7	10th June	83	8	16th June_leaf	89	14
							30th June_pod	10	28
FN	17th June	59	2	18th June	60	3	22nd June_leaf	64	7
	22nd June	64	7	23rd June	65	8	29th June_leaf	71	14
							13th July_pod	85	28

[a] – days after planting; [b] – days after spraying film antitranspirant.

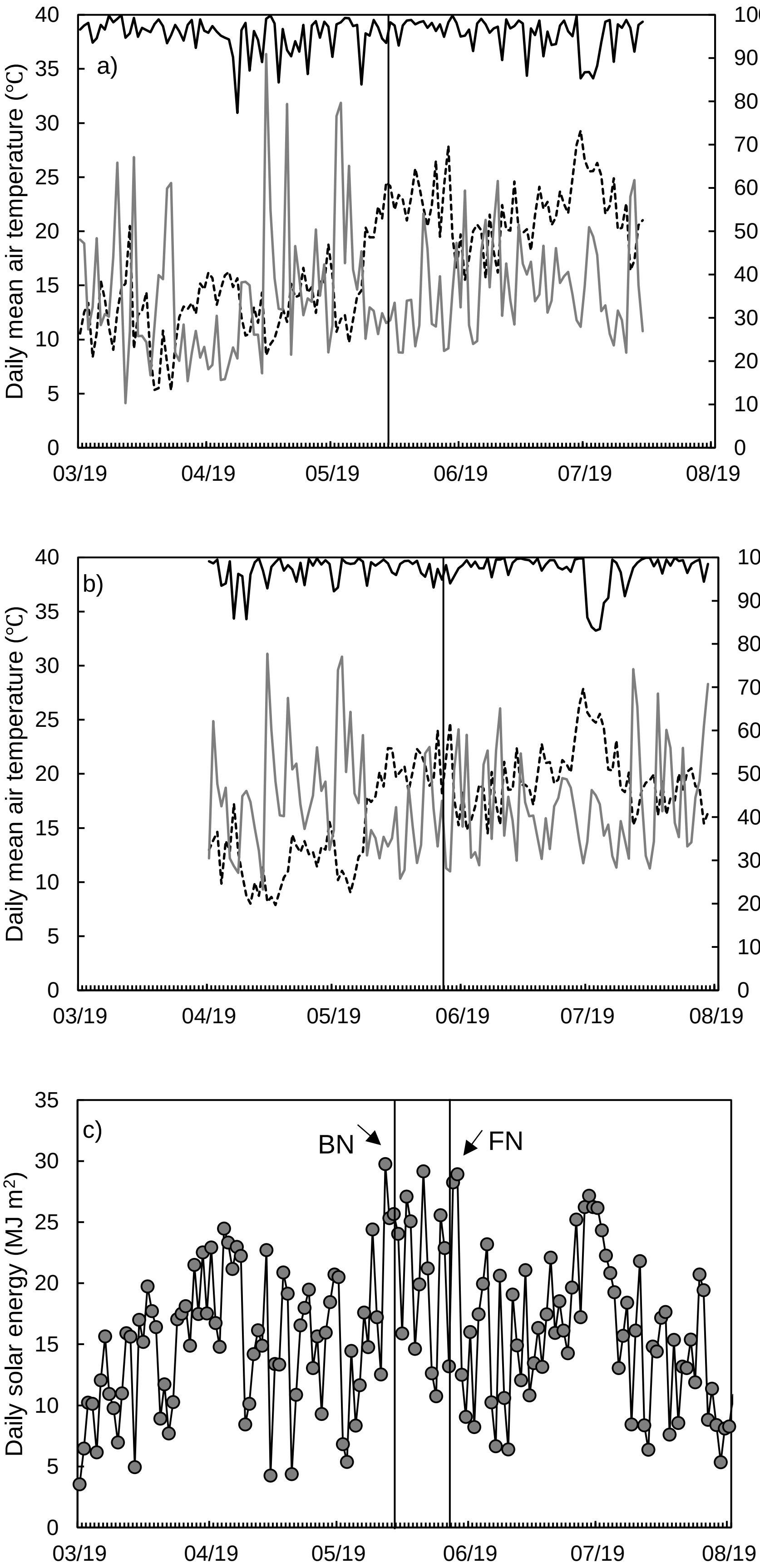
5.2.9 Statistical analysis

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

5.3 Results

5.3.1 Environmental conditions

The mean daily air temperature at both sites was ~ 17 °C through the growing seasons, and the minimum and maximum RH were 36.1%, 95.5 % and 43.4%, 97.0% at BN and FN, respectively (Figure 5.3.1 – a, b). After spray application until harvest, the daily air temperature on average was 21.8 °C at BN, slightly higher than FN (19.6 °C). However, min- and max-RH were lower at BN over FN, i.e., 36.8%, 95.6% and 43.0%, 97.1%, respectively. During the whole season, the average SR received were 16.3 and 16.7 MJ m^{-2} day^{-1} at BN and FN, respectively; it was 17.7 and 16.1 MJ m^{-2} day^{-1} after spraying (Figure 5.3.1 - c). Compared to BN with early planting, rapeseed plants at FN started flowering earlier and had a shorter growing season by 11% in terms of thermal time (Table 5.2.2).

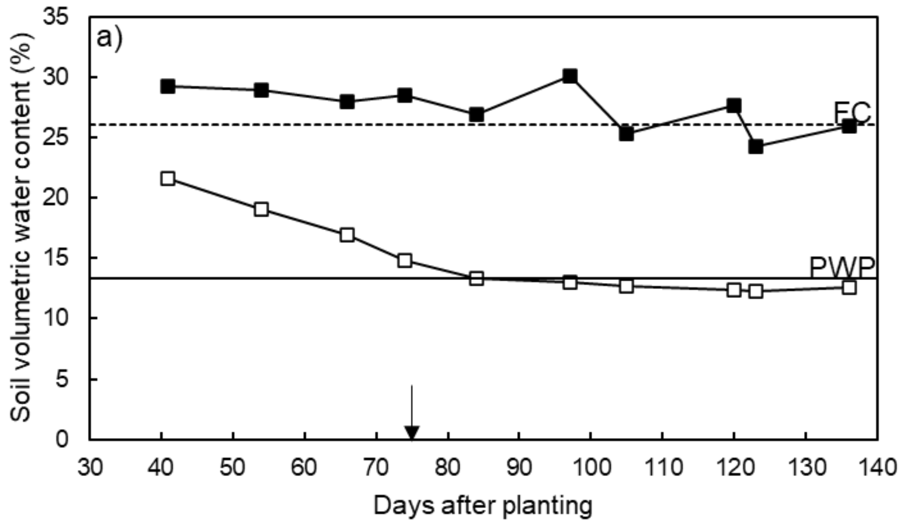


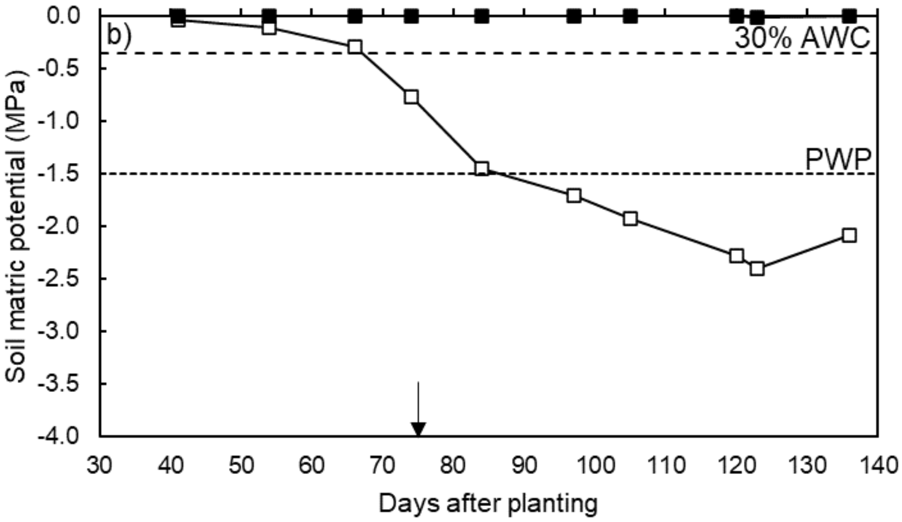
a)
Daily mean air temperature (℃)
Relative humidity (%)
03/19
04/19
05/19
06/19
07/19
08/19
b)
Daily mean air temperature (℃)
Relative humidity (%)
c)
BN
FN
Daily solar energy (MJ m2)

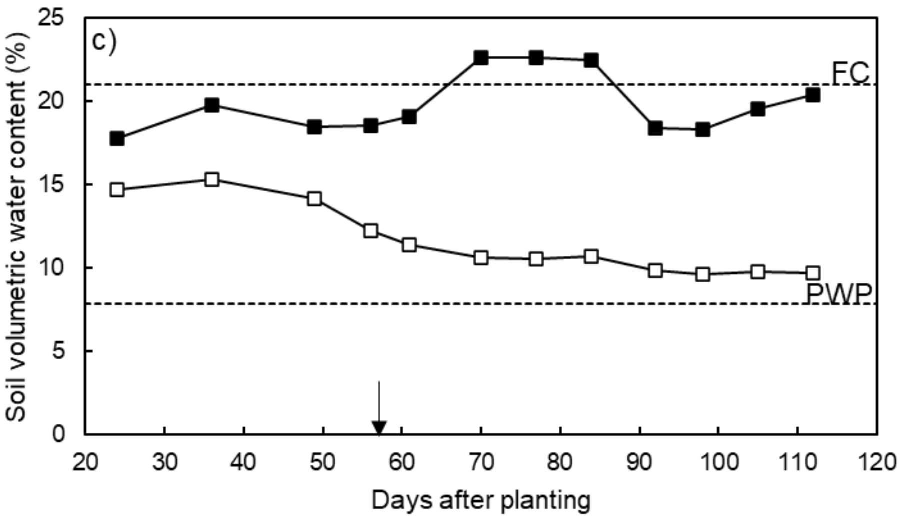
Figure 5.3.1 Daily mean air temperature (°C, ----), minimum (——) and maximum (——) relative humidity (%) during the growing season of rapeseed plants at BN (a) and FN (b); and daily solar radiation (MJ m^{-2}, —●—) from both sites (c) with vertical lines indicating the day of spraying film antitranspirant.

5.3.2 Soil water status

Average soil VWC (SMP) in benchmark plots was maintained at 27.5% (<0.01 MPa) and 19.8% (-0.01 MPa) throughout the growing season at BN and FN, respectively. In contrast, soil moisture (VWC/SMP) in the top 60 cm significantly decreased with increasing DAP at BN and FN, showing no differences between AT-treated plots relative to 0AT from regression analysis in groups (data not shown). Overall, soil water deficits were greater at BN than at FN. At harvest, soil moisture (VWC/SMP) decreased to below PWP at BN, but it was not the case at FN, particularly in SMP (Figure 5.3.2). At BN, soil VWC decreased from an average of 21.6% at 41 DAP to 14.8% just above PWP at 74 DAP (one day before spraying), and it further gradually declined to 12.6% at harvest (i.e., 136 DAP) (Figure 5.3.2 – a). The corresponding readings of SMP were from -0.04 MPa to -0.77 MPa, and ultimately to -2.08 MPa (Figure 5.3.2 – b). At FN, soil VWC decreased from the average of 15.32% at 36 DAP to 12.2% at 56 DAP (one day before spraying), and then it continually reduced to 9.69% at 112 DAP near harvest, with corresponding SMP values of -0.10, -0.22 and -0.67 MPa, respectively (Figure 5.3.2 – c, d).




b)
30% AWC
PWP
Soil matric potential (MPa)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
30
40
50
60
70
80
90
100
110
120
130
140
Days after planting


c)
FC
PWP
Soil volumetric water content (%)
25
20
15
10
5
0
20
30
40
50
60
70
80
90
100
110
120
Days after planting

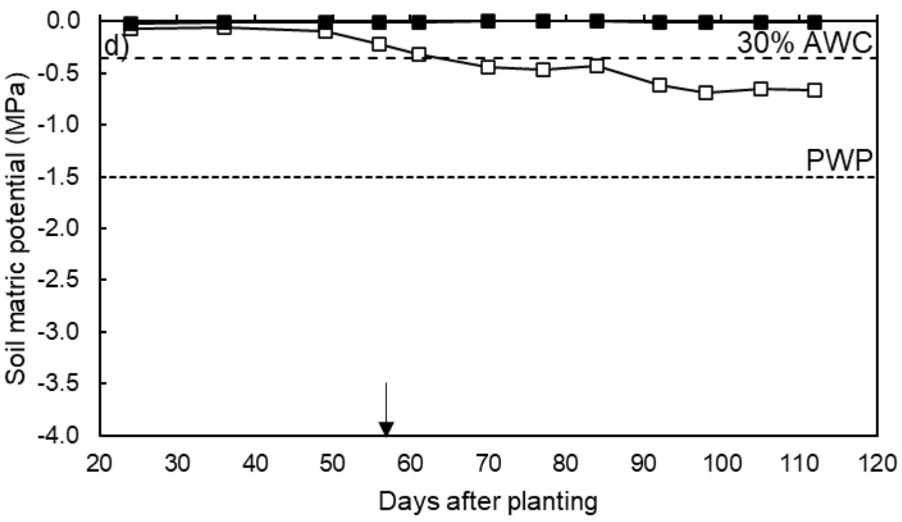

d)
30% AWC
PWP
Soil matric potential (MPa)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
20
30
40
50
60
70
80
90
100
110
120
Days after planting

Figure 5.3.2 Soil volumetric water content and matric potential in the top 60 cm from the well-watered benchmark (—■—) and drought plots (—□—) at BN (a, b) and FN (c, d). Dashed lines represent field capacity (FC) and permanent wilting point (PWP) as indicated on the graph, and densely dashed lines represent the level of water stress, i.e., 30% available water content (AWC) imposed in the glasshouse study (Chapter 3). Arrows represent the day of spraying film antitranspirant. Data are means of replicates (WS: n = 8 at BN and n = 4 at FN; WW: n = 2 at BN and n = 4 at FN).

5.3.3 Leaf temperature

Leaf temperature highly fluctuated depending on the weather conditions of sampling across experiments (Figure 5.3.3). Relative to the well-watered benchmark, spray treatments increased leaf temperature significantly to varying extents at both sites, except for BN at 2 DAS (Table 5.3.1). L_T at 2 DAS in 0AT was 4.3 °C and 1.3 °C at BN and FN, respectively, while at 7 DAS, LT was 1.5 °C and 1.4 °C (Figure 5.3.3). AT had significant effects on L_T at three sampling dates of two sites, and a linear relationship was observed at FN, although deviations from FN at 7DAS were significant. When compared to 0AT, AT increased L_T by 67% and 35% at BN and FN, respectively, averaging concentrations and two sampling times.

5.3.4 Gas exchange

Drought inhibited g_s and A, and improved WUEi substantially relative to the benchmark at both sites (without statistical comparisons due to the non-randomisation of benchmark plots (Figure 5.3.4). With the development of terminal drought, responses in gas exchange to AT application were more prominent at 8 DAS over 3 DAS across two trials (Table 5.3.1). At 8DAS in BN, there was a significant and linear relationship between g_s, WUEi and AT concentrations. Readings of g_s, A and WUE_i at 8DAS were 0.3 mol m^{-2} s^{-1}, 16.5 µmol m^{-2} s^{-1} and 57.6 µmol (CO_2) mol (H_2O)$^{-1}$ in 0AT, respectively. When AT increased from 0% to 3% at 8 DAS, g_s decreased by an average of 16%, while WUE_i increased by 12% (Figure 5.3.4 - b, j). At FN, g_s, A and WUE_i in 0AT were 0.7 mol m^{-2} s^{-1}, 19.8 µmol m^{-2} s^{-1} and 32.2 µmol (CO_2) mol (H_2O)$^{-1}$. Only A had a significant and linear relationship with AT concentrations (Table 5.3.1). AT reduced A by an average of 18% across all concentrations at FN (Figure 5.3.4 - h).

Table 5.3.1 Probability values from *ANOVA* for L_T (leaf temperature – benchmark), stomatal conductance (g_s), photosynthesis rate (*A*) and intrinsic water use efficiency (WUEi) as affected by the application of film antitranspirant (AT) at BN and FN. Polynomial contrasts were conducted between concentrations of AT, including droughted control treated with water (0AT). Bold numbers indicate significant differences at $p < 0.05$.

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

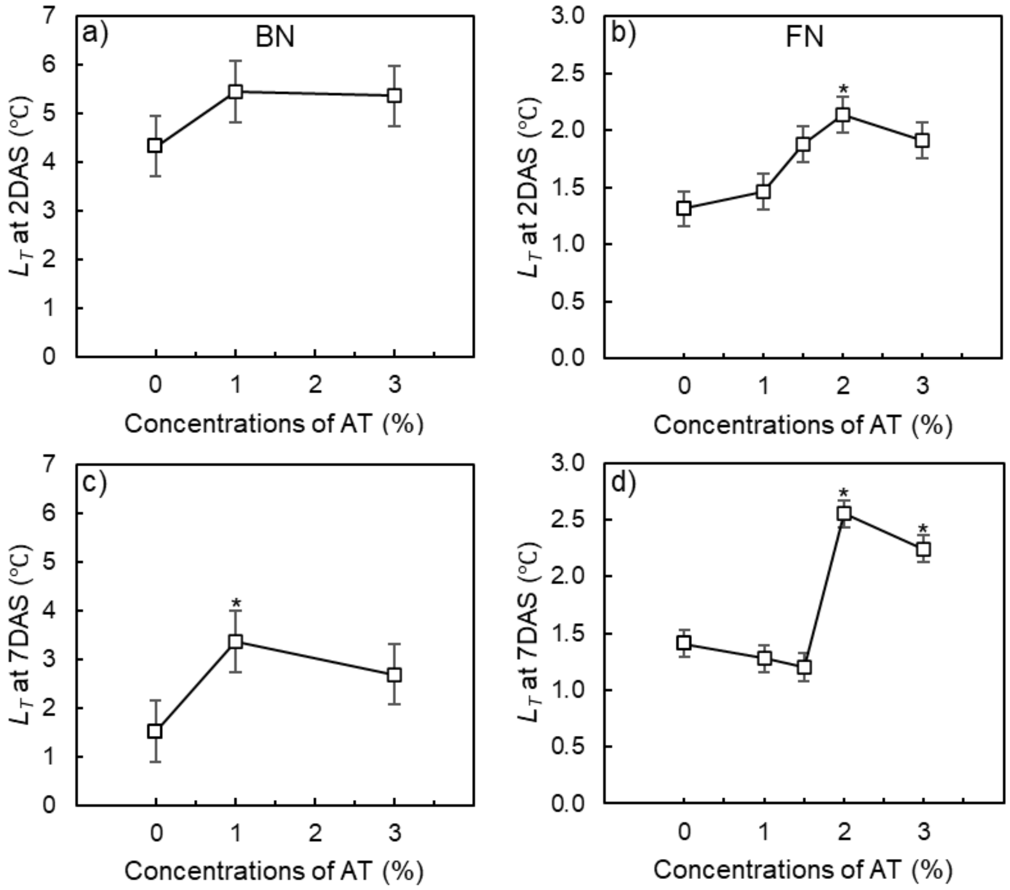
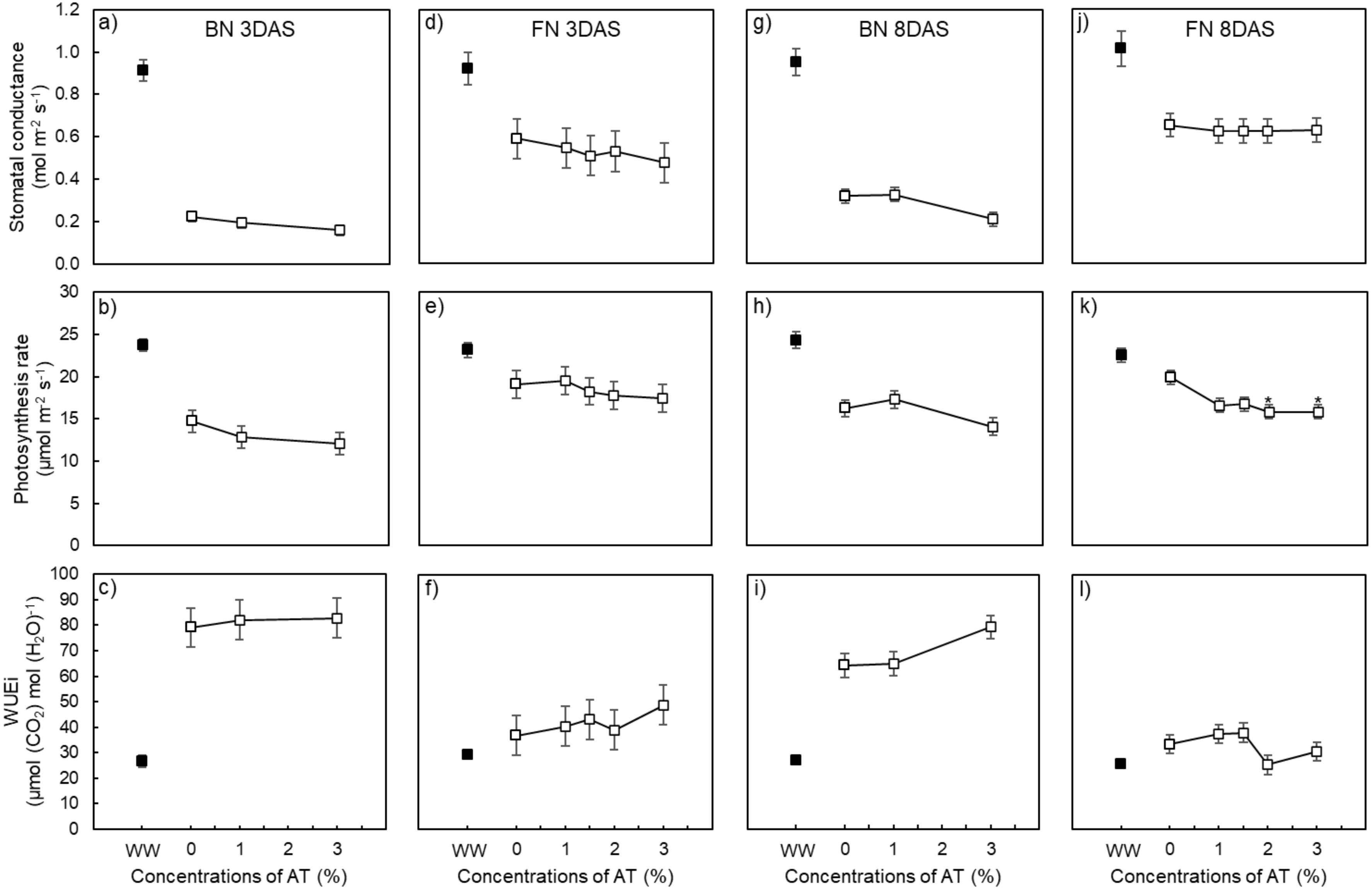


Figure 5.3.3 Difference in leaf temperature (terminal drought – benchmark plots [L_T]) at 2 and 7 days after spraying (DAS) film antitranspirant (AT) at BN (a, b) and FN (c, d). Data are means (n = 8 at BN and n = 4 at FN) ± standard error of the mean (SEM). Asterisks (*) represent the significance compared to drought control (0AT) according to Tukey's test at p = 0.05.



a)
BN 3DAS
d)
FN 3DAS
g)
BN 8DAS
j)
FN 8DAS
b)
e)
h)
k)
c)
f)
i)
l)
Stomatal conductance (mol m-2 s-1)
Photosynthesis rate (µmol m-2 s-1)
WUEi (µmol (CO2) mol (H2O)-1)
WW
Concentrations of AT (%)

Figure 5.3.4 Stomatal conductance, photosynthesis rate and intrinsic water use efficiency (WUEi) of rapeseed plants at 3 and 8 days after spraying (DAS) film antitranspirant (AT) from the well-watered benchmark (WW—■—) and drought plots (—□—) at BN (a-c, g-i) and FN (d-f, j-l). Data are means of replicates (n = 8 at BN; n = 4 at FN) ± standard error of the mean (SEM). Asterisks (*) represent the significance compared to drought control (0AT) according to Tukey's test at $p = 0.05$.

5.3.5 Yield and yield components

At both sites, the application of AT significantly influenced AGB, seed yield and pod number ha^{-1}, although with borderline significances in AGB and seed yield at BN (Table 5.3.2). Compared to the benchmark, drought decreased AGB and seed yield by an average of 43% and 37% at BN, respectively (Figure 5.3.5 – a, b), and more markedly by 49% and 56% at FN (Figure 5.3.6 – a, b), although non-randomised benchmark plots were not included in statistical analysis. AGB and seed yield in 0AT were 13.5, 5.6 t ha^{-1} and 5.6, 1.5 t ha^{-1} at BN and FN, respectively. AT at 1%-3% increased AGB and seed yield by an average of 12% and 14% at BN, respectively, and to a greater extent, by 31% and 34% at FN relative to 0AT (Table 5.3.2). Further, 3% AT increased AGB and seed yield significantly by 48% and 52% at FN only. Similarly, compared to the benchmark, terminal drought decreased pod number by an average of 38% and 34% at BN (Figure 5.3.5 – d) and FN (Figure 5.3.6 – d), respectively. AT across concentrations increased pod number ha^{-1} by 7% and 25% at BN and FN, respectively, compared to 0AT (i.e., 57 and 15.1 million ha^{-1} at BN and FN, respectively). 3% AT was significantly higher than 0AT, increased by ~42% from both sites (Table 5.3.2). The oil content from the benchmark was ~42% and ~47% at BN and FN, respectively. Compared to the benchmark, oil content from drought plots was slightly lower, decreased by 6% and 2% at BN and FN, respectively (Figure 5.3.5 – g, Figure 5.3.6 – g). Compared to 0AT, 3% AT increased oil yield by 31% and 45% at BN and FN, respectively, with the latter showing significance (Table 5.3.2). Averaging concentrations, AT application increased oil yield by 15% and 28% at BN and FN, respectively.

Seed yield at BN and FN was linearly associated with AT concentrations, and so were AGB, pod number and oil yield (Table 5.3.2). With a 1% increase in the concentration of AT, the projected increase in AGB, seed yield and oil yield were, respectively: 1.35, 0.61, 0.25 t ha^{-1} at BN (Figure 5.3.5 – a, b, h) and 0.85, 0.23, 0.10 t ha^{-1} at FN (Figure 5.3.6 – a, b, h). Pod number at BN showed very high variabilities due to significant deviations, so it was not included in the linear regression model (Table 5.3.2). At FN, every 1% increase in AT concentrations was predicted to increase pod number per ha by 1.92×10^6 (Figure 5.3.6 – d).

From the regression of yield with its components, we found that seed yield was highly associated with pod number, followed by seed per pod at BN (Figure 5.3.7 – a, c). This is also consistent with FN in seed yield, showing the highest positive correlation with pod number (Figure 5.3.7 – b) but a negative correlation with seed per pod with large variabilities (Figure 5.3.7 – d). Although leaf RWC was not significantly affected by AT application at either sampling of two sites (Appendix. Figure 6), pod number was highly associated with leaf RWC from the regression analysis on combined data with BN and FN showing different linear trends (Figure 5.3.8 – b). Pod number was predicted to increase at BN nearly six times more than it would be at FN, with a 1% improvement in leaf RWC.

Table 5.3.2 Probability values from *ANOVA* for aboveground biomass (AGB), seed yield (SY), harvest index (HI), pod number per ha (Pod), seed number per pod (SP), thousand-seed weight (TSW), oil content and oil yield (OY) as affected by application of film antitranspirant (AT) at BN and FN. Polynomial contrasts were conducted between concentrations of AT, including droughted control treated with water (0AT). Bold numbers indicate significant differences at $p < 0.05$.

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

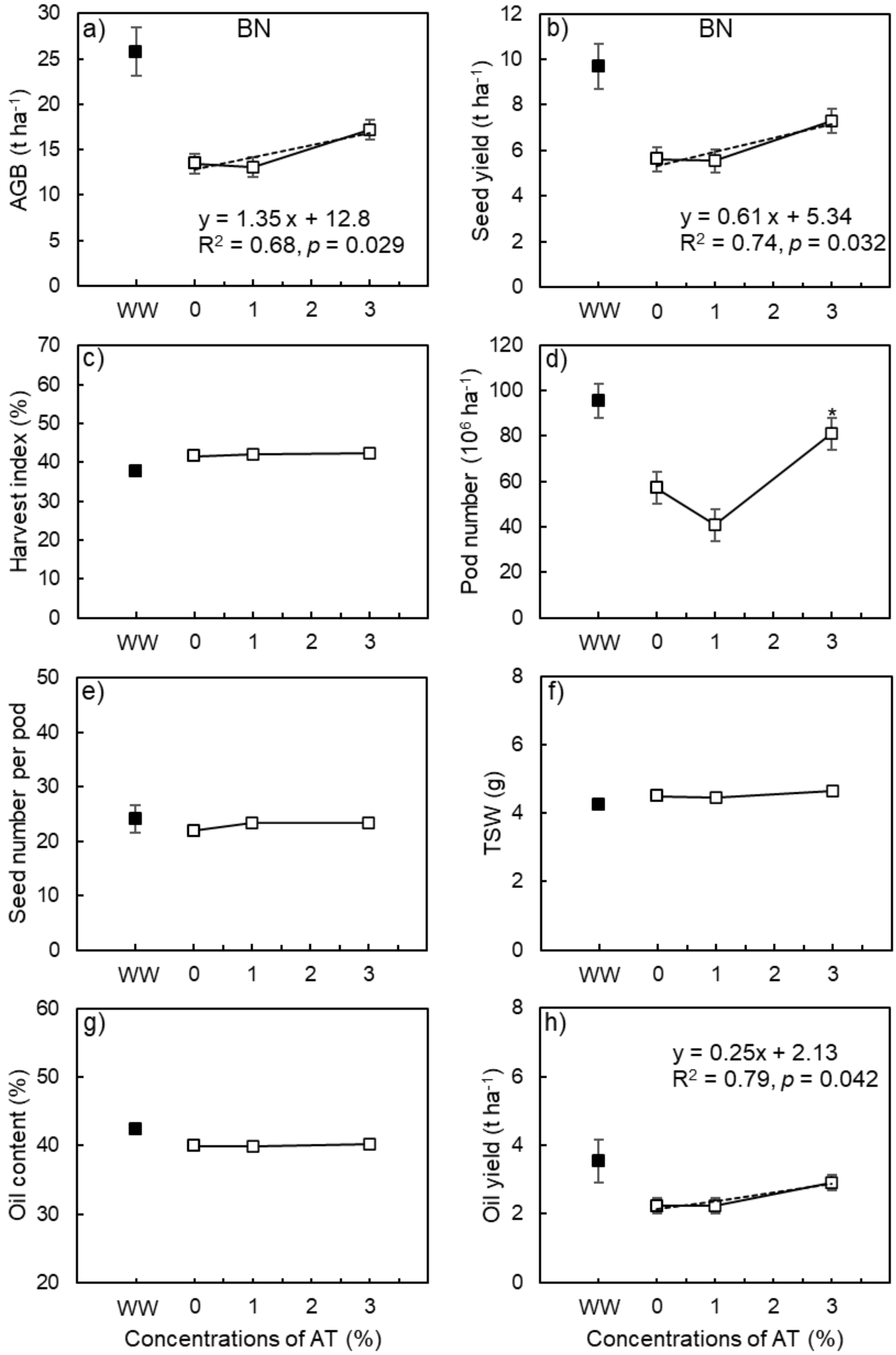


Figure 5.3.5 Aboveground biomass (AGB, a), seed yield (b), harvest index (c), yield components (d, e, f), oil content (g) and oil yield (h) of rapeseed plants from the well-watered benchmark (WW, —■—) and terminal drought plots (—□—) following the application of film antitranspirant (AT) at BN. The linear regression model was fitted with AGB, seed yield and

oil yield against concentrations of AT, where parameters had significant and linear relationships with AT concentrations (Table 5.3.2). Note that pod number at BN had significant deviations in addition to the linear contrast between concentrations, so a linear fitted line with low R-squared is not present. Data are means of replicates (n = 8) ± standard error of the mean (SEM). Asterisks (*) represent the significance compared to drought control (0AT) according to Tukey's test at $p = 0.05$.

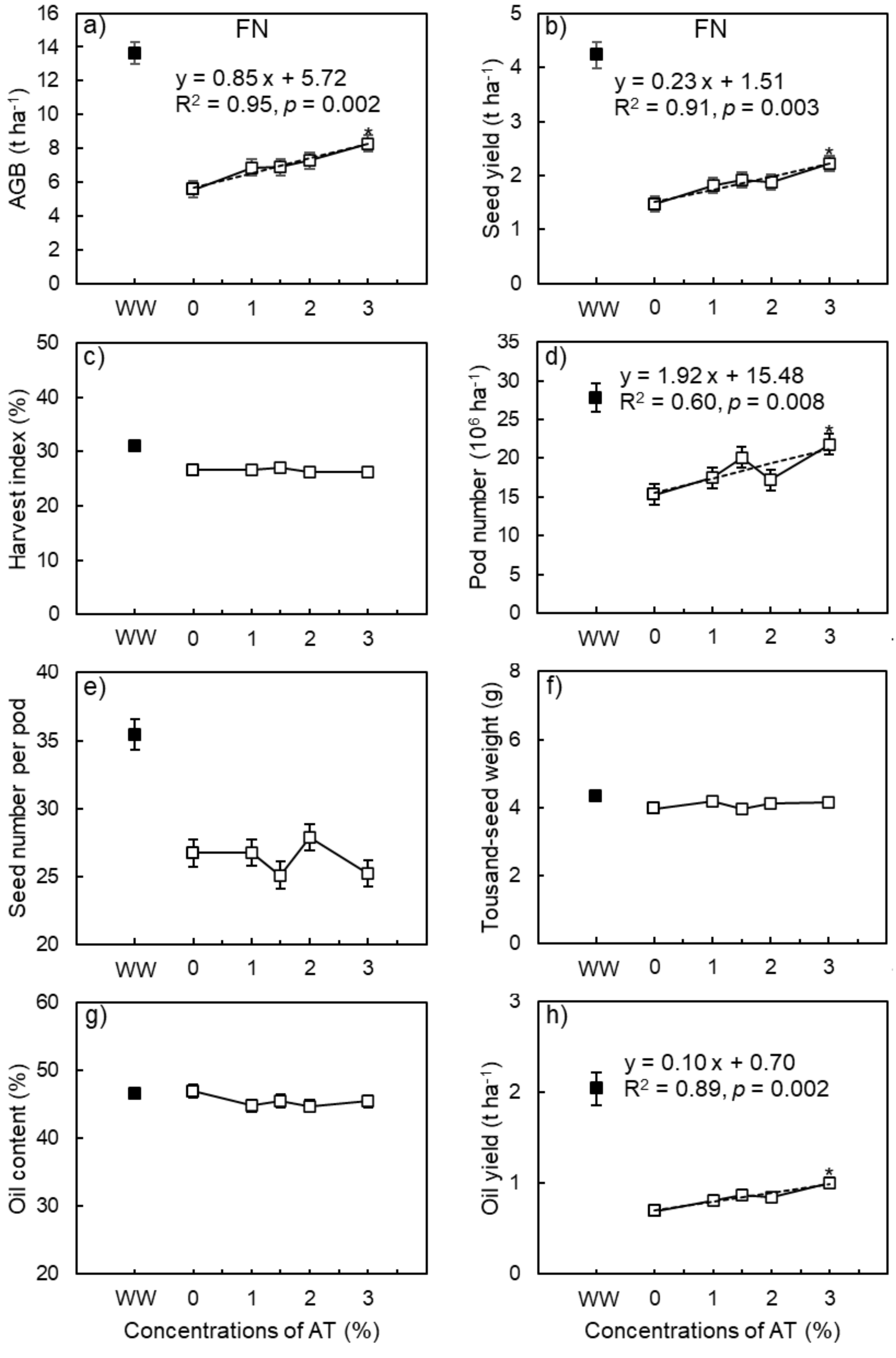


Figure 5.3.6 Aboveground biomass (AGB, a), seed yield (b), harvest index (c), yield components (d, e, f), oil content (g) and oil yield (h) of rapeseed plants from the well-watered benchmark (WW, —■—) and drought plots (—□—) following the application of film antitranspirants (AT) at different concentrations at FN. The linear regression model was

fitted with AGB, seed yield, pod number and oil yield against concentrations of AT, where parameters had significant and linear relationships with AT concentrations (Table 5.3.2). Data are means of replicates (n = 4) ± standard error of the mean (SEM). Asterisks (*) represent the significance compared to drought control (0AT) according to Tukey's test at $p = 0.05$.

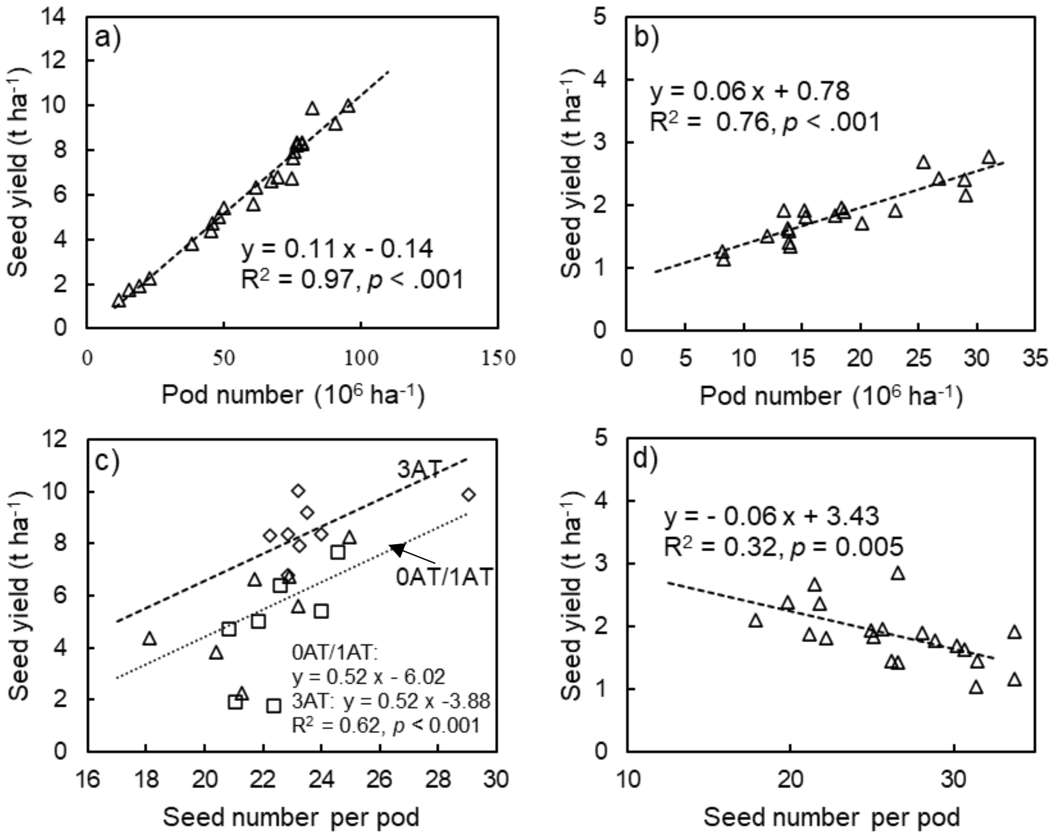


Figure 5.3.7 Relationships between seed yield and yield components at BN (a, c) and FN (b, d). Note that symbols of triangle (△), square (□) and tilted square (◇) represent the concentrations of film antitranspirant (AT) at 0% (0AT), 2% (2AT) and 3% (3AT), respectively in panel C. Parallel/common dotted lines are fitted with the linear regression model with/without treatments as groups. Data are replicates from BN (n = 9) and FN (n = 4).

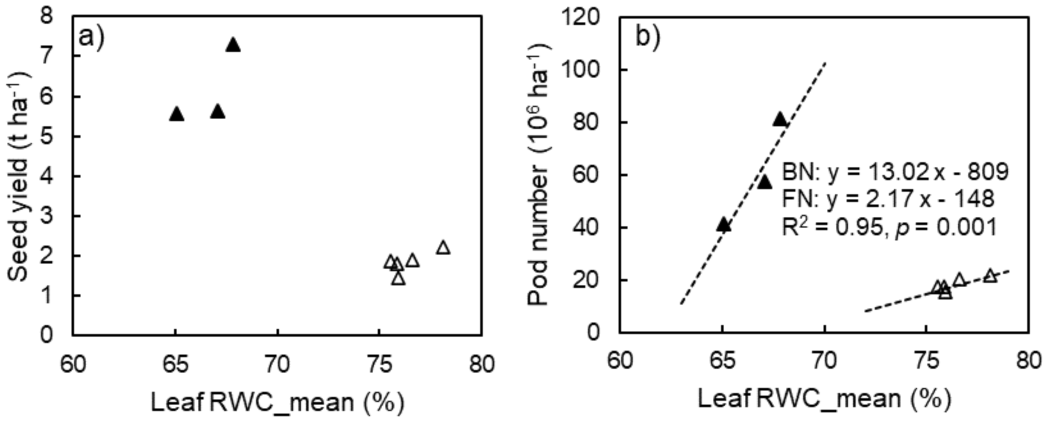


Figure 5.3.8 Relationships in seed yield (a) and pod number (b) against the mean of leaf relative water content (RWC) at 2 and 14 days after spraying film antitranspirant from BN (▲) and FN (△) combined data. Simple linear regression analysis with experiments as groups are conducted. Based on results from regression analysis at the level of 5%, separate lines are fitted with pod number against leaf RWC in Panel b, along with equations, adjusted R^2 and probability values of fitted linear regression model. Data points are means of replicates (n = 8 at BN; n = 4 at FN).

5.4 Discussion

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

Stomatal closure is the earliest response to drought for the maintenance of leaf water potential, which restricts the diffusion of water and CO_2 into the leaf (Flexas and Medrano, 2002). We showed that drought reduced g_s and A by an average of 73%, 37% and 39%, 22% across two sampling times at BN and FN, respectively; and improved WUEi by 176% and 31% as compared to the well-watered benchmark (Figure 5.3.4). The depression of drought on crops has been widely reported from previous studies on rapeseed (Hess et al., 2015; Yan et al., 2016), the degree of which is positively aossociated with the magnitude of water stress (Faralli et al., 2016). An increase in WUEi of droughted rapeseed has also been reported by Elferjani and Soolanayakanahally (2018) and Biswas et al. (2019). Compared to FN, the further suppression of g_s at BN could be explained by the greater and longer-lasting soil water deficit, and drought at BN developed about three times faster than at FN in terms of soil SMP (Figure 5.3.2).

From the previous glasshouse study under controlled drought conditions, we found *that* g_s and *A* of AT-treated rapeseed plants had a significant and negative relationship with AT concentrations (0.25% - 1%). The greater detrimental effects of increasing concentrations from 1% to 3% were also observed from some sampling times of two sites in the present study under terminal drought, but with large variabilities between them due to variable environmental conditions in the field (Table 5.3.1). As concentrations increased, more stomata were blocked with higher leaf coverage, which augmented the suppression of g_s (Xiang et al., 2021). To confirm this, a preliminary pot experiment was conducted to estimate leaf coverage of different AT concentrations by arranging four replicates at an interspace the same as plants in the field experiment. Results showed that leaf coverage and AT concentrations were positively correlated, and leaf coverage increased from 3% to 31% when AT was applied from 1% to 3% (Figure 4.3.1 in Chapter 4). Apart from concentrations, drought intensity would also impact gas exchange responses to AT application (del Amor et al., 2010).

Under drought conditions, rapeseed plants adopting a “conventional” strategy via stomatal closing would result in less leaf cooling (Elferjani and Soolanayakanahally, 2018), which could also be confirmed by increased leaf temperature from droughted plots (Figure 5.3.3). After AT application, reduced g_s is expected to reduce leaf transpiration, thus increasing leaf temperature (Gale and Hagan, 1966). This is also confirmed in the present study that When compared to 0AT, the application of AT increased leaf temperature by 1.1–1.8 °C averaging all concentrations at both sites (Figure 5.3.3). This is consistent with previous studies by Faralli et al. (2016) on winter rapeseed and Gatti et al. (2016) on grapevines. Faralli et al. (2016) found that VG at 1% increased leaf temperature significantly compared to well-watered control, and leaf temperature increased by <1 °C compared to unsprayed droughted plants. Similarly, Gatti et al. (2016) applied a higher concentration of VG at 2% and found that sprayed leaves were moderately warmer by 1-2 °C than unsprayed ones. The heating effect can be explained by greater resistance to the passage of water vapour from AT application, thereby slowing evaporative cooling effects (Davenport et al., 1972). In addition, L_T increased linearly as AT concentrations increased from 1% to 3%, but with highly significant deviations at both sites (Table 5.3.1). This suggests that the relationship between L_T and AT concentrations may not always be linear, and a more complex relationship may involve leaf metabolic processes following blocked stomata, or canopy structure could impact the relationship.

ABA accumulation in guard cells triggers stomatal closure, thus preventing plants from losing excessive water under drought conditions (Kollist et al., 2014). Previous studies conducted by Faralli et al. (2017a) showed that AT application at the flowering stage of winter rapeseed reduced Endo-ABA concentration in the leaf and pod at 7 and 16 DAS. Similar findings have also been reported on wheat (Mphande et al., 2021b). In our study, however, differences between treatments in Endo-ABA of leaves at 7, 14 DAS or of pods at 28 DAS were not significant (Appendix. Figure 5). This might be explained by the dynamic responses of Endo-ABA to different environmental conditions. Leaf synthesised ABA might be loaded to the phloem and transported to the roots (Wilkinson and Davies, 2002). However, if ABA concentration in the phloem increases still needs further analysis. Alternatively, our colleagues compared VG and fluridone (ABA inhibitor) on wheat and found no significant improvement from fluridone application, but VG-sprayed only due to the conservation of plant water by suppressing transpiration (Mphande W., 2021, unpublished data). Nevertheless, the role of ABA in the activity of film AT remains elusive and requires further research.

During flowering and pod set, the relationship between source and sink regulates the availability of assimilates necessary for seed filling (Diepenbrock, 2000). Numerous studies have reported that terminal drought can cause massive yield losses in rapeseed (Faralli et al., 2017b; Elferjani and Soolanayakanahally, 2018), which is also supported by our study, showing that terminal drought resulted in ~ 46.6% seed yield losses across two sites, compared to the benchmark (Figure 5.3.5 – b, Figure 5.3.6 – b). The large decrease was highly associated with reductions in AGB and pod number by 46.6% and 36.4%, respectively (Figure 5.3.7 – a–d), which can be explained by a substantial decline of available assimilates during the flowering stage, which negatively affected the formation of pods and seed size (Johnston et al., 2002; Wang et al., 2011), leading to abortion and/or abscission of pods particularly (Tesfamariam et al., 2010).

However, yield losses caused by drought were lower from BN than from FN, which was 36.5 % and 56.7% relative to the benchmark, respectively (without statistical comparisons because of non-randomisation in benchmark plots). One possible explanation would be the huge difference in initial nutrients available for plants in the soil, as both sites showed similar levels of air temperature and RH. Initial available N from BN was almost eight times higher than from FN (300 vs 70 kg ha^{-1}, respectively). Greater N supply can improve individual plant growth through a higher leaf area index and a prolonged period of photosynthetic activity, thereby producing high yields (Wang et al., 2014). Alternatively, plants at BN had a

13% longer growth period than at FN in terms of thermal time due to early planting, which resulted in greater dry matter accumulation (Sieling et al., 2017). Taken together, these two important factors boosted plant growth at BN and produced a much higher yield at BN than at FN and other related parameters (Figures 5.3.5 and 5.3.6). Although fungicide and insecticide were applied at both sites, plants at FN were still affected by pollen beetles and fungus disease, particularly during the reproductive stage, which may have partially accounted for yield losses.

In the current study, we first demonstrated that seed yield had a linear and positive relationship with concentrations of AT from 1% to 3%. Similar relationships were also observed in AGB, pod number/ha and oil yield with AT concentrations (Table 5.3.2). An increase in AGB by ~22% across AT concentrations and two sites relative to 0AT is consistent with Faralli et al. (2016), who studied winter rapeseed and found two types of film AT at 1% increased aboveground dry matter by an average of film AT about 17% under terminal drought. This improvement in shoot biomass has also been demonstrated in other crops such as sweet corn ((Shekour et al., 1987), fava bean (Davenport et al., 1972), wheat (Mphande et al., 2021a) and olive (Cirillo et al., 2021). From the glasshouse study, however, we only observed improvements in some yield components (Xiang et al., 2022). The individual plants in a controlled environment can experience different light and water conditions compared to those in the field, resulting in different responses to AT application. There may be two reasons for the difference in yield response in our glasshouse and field studies. First, soil hydraulics varied between these two studies that affect the expansive growth of reproductive organs (pod and seed) in varying degrees, leading to pod and seed abortion (Turc and Tardieu, 2018). Soil moisture decreased constantly and gradually throughout the whole season in the field experiments (Figure 5.3.2) while potted plants in the glasshouse experienced rapid onset of water deficit and moisture was maintained at a similar level (30% VWC equals to ~20% VWC) only during the flowering stage, albeit with fluctuations. A non-limiting shoot (leaf and pod) water status could have been maintained for longer with AT application under slower soil drying, thereby preventing some of the pod abortion seen in drought-stricken plants without AT. Second, ways of imposing drought can impact the capability of yield compensation during seed filling stage. As terminal drought developed with increasing DAP in the field, drought may have imposed more restrictions on the capacity of surviving pods and seeds for compensatory growth at the later seed filling stage (Kirkegaard et al., 2018).

Improving leaf water status, particularly during the flowering stage, is vital in determining final crop production as it is closely associated with gas exchange (Raza et al., 2017). The application of AT physically blocks leaf stomata and reduces water loss through transpiration, improving leaf water status (Davenport et al., 1972). This is also reported by

Faralli et al. (2017a) on winter rapeseed, showing that plants treated with AT 1% exhibited significantly higher RWC relative to unsprayed droughted control. However, in the present study, we failed to see a significant increase in leaf RWC at 2 or 14 DAS from two sites compared to 0AT (Appendix. Figure 6). The lack of statistical significance in leaf RWC might be attributed to variable environmental conditions at the time of sampling; different types of drought and varieties may also result in the discrepancy compared with Faralli et al. (2017a).

Nevertheless, pod number and leaf RWC were significantly and positively correlated, although separate lines from two experiments (Figure 5.3.8 – b), indicating the greater pod number from increasing AT concentration appeared to be mediated by leaf RWC. Since seed yield was strongly related to pod number (Figure 5.3.7 – a, b), it can be deduced that greater seed yield from increasing AT concentration may also have been mediated by RWC (Figure 5.3.8 – a).

Our study showed a greater yield from increasing AT concentrations, which confirms findings from a previous study conducted at FN on winter rapeseed (Faralli et al., 2017b). Researchers sprayed three concentrations of film AT (VG) on winter rapeseed subject to terminal drought, showing that AT at 1 (0.5%), 2 (1%) and 4 L ha^{-1} (2%) increased seed yield by 14%, 14% and 21%, respectively when AT applied at the flowering stage. This strong relationship with AT concentrations are also reported by Fahey and Rogiers (2019) on grape bunch loss dipped in 0%, 1%, 2% and 3% VG solutions, showing a linear and positive relationship between the bunch mass loss and AT concentrations. For droughted rapeseed, therefore, we can improve seed yield by increasing concentrations of film AT. Based on the current price of rapeseed, the average cost of spraying and AT product (Vapor Gard), the improvement from 1% (2 L ha^{-1}) and 3% (2 L ha^{-1}) at flowering of droughted rapeseed would approximately result in an economic benefit of £29.36/ha and £555.88/ha (more details available in Table 5.4.1). However, higher concentrations would increase the cost of products and reduce economic profits, which is less cost-effective and practical. Since rapeseed stomata are unequally distributed between the surfaces, with ~44% of total stomata number on the adaxial and 56% on the abaxial surface (Table 4.3.1 in Chapter 4), an alternative way is to optimise the spraying method to achieve higher leaf coverage, i.e., stomatal blockage of both adaxial and abaxial surface. According to results of leaf coverage from the preliminary study in Chapter 4, leaf coverage was approximately 20% and 30% at the concentration of 1% and 3% AT, respectively (Figure 4.3.1 in Chapter 4). Therefore, in future work, it would also be worth developing spray technology to increase abaxial stomatal blockage in addition to studying the mechanism of film AT.

Table 5.4.1 Economic benefits from AT at 1% and 3% on rapeseed yield in the UK.

AT concentrations	AT cost (VG)[1]	Spraying cost[2]	Yield benefit[3]	Rapeseed price[4]	Economic benefit
1% (2 L/ha)	£15.25/L	£16.30/ha	0.14 t/ha	£544/ha	£29.36/ha
3% (6 L/ha)	£15.25/L	£16.30/ha	1.22 t/ha	£544/ha	£555.88/ha

[1] Seed Ranch, 2022 URL (https://www.seedranch.com/Millers-Vapor-Gard-Concentrate-1-Gallon-p/vapor-gard-gallon.htm) (accessed 18/10/2022).

[2] NAAC CONTRACTING PRICES SURVEY 2022 (https://stmaaprodfwsite.blob.core.windows.net/assets/sites/1/2022/05/NAAC-contracting-prices2.pdf?_ga=2.120630152.1661377273.1655111315-735006727.1653313056) (accessed 18/10/2022).

[3]mean of two field experiments (BN and FN).

[4] AHDB UK delivered oilseed prices (https://ahdb.org.uk/cereals-oilseeds/uk-delivered-prices) (accessed 18/10/2022).

5.5 Conclusions

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

Chapter 6 General discussion and conclusions

6.1 General discussion

In the present study, six glasshouse experiments and two field experiments were conducted to test the central hypothesis that "film antitranspirant has the potential to mitigate the drought damage to rapeseed at flowering stage and increase the yield of rapeseed". Using TiO_2 as a spray marker, we demonstrated that leaf coverage was positively associated with the concentration of AT under glasshouse and field-simulated conditions in Chapters 2 and 4. From glasshouse experiments in Chapter 3, concentrations of AT from 0.25% to 1% at the flowering stage showed linear relationships with the reduction in stomatal conductance and photosynthesis rate when rapeseed is subjected to controlled drought. However, the compensatory trade-off between pod number and seed number per pod prevented significant yield benefits from being shown. Subsequently, increased concentrations of AT were applied from 1% to 3% in the field where rapeseed plants were under terminal drought, showing the greater yield benefits from increased AT concentrations because of the improvement in leaf water status leading to an increase in pod number. Overall, we accepted the central hypothesis.

Under controlled drought in the glasshouse, results showed that g_s and A of AT-treated rapeseed at 3 DAS had linear and negative relationships with AT concentrations from two of three glasshouse experiments. Similarly, this linear relationship was only significant at 8 DAS in g_s and A against AT concentrations from 1% to 3% at BN and FN under terminal drought, respectively, but not at 3 DAS. The discrepancy here could be possibly attributed to the different types of drought and environmental conditions, making it difficult to directly compare glasshouse with field experiments. For example, soil moisture was depleted to controlled level of 30% AWC in one (Figure 3.3.2) while it was allowed to deplete steadily through the post flowering period in another (Figure 5.3.2). Nevertheless, the overall inhibition on g_s more than A leading to increased WUEi was consistent in both studies, although by varying degrees. As stomata play a pivotal role in leaf gas exchange processes, especially for isohydric plants (Tsialtas et al., 2017), the present study further confirms that film AT could effectively improve intrinsic water use efficiency by regulating g_s of droughted rapeseed.

In the previous study, Faralli et al. (2016, 2017a) found yield improvements from AT application (1%) on winter rapeseed under controlled drought only during the flowering stage. However, these yield benefits were not observed on spring rapeseed under the same type of drought. One factor that contributes to the discrepancy might be the way of imposing drought. In the present study, a soil moisture sensor was used for monitoring soil volumetric water content in the glasshouse study (Chapter 3) while a gravimetric method was conducted in Faralli et al. (2016, 2017a). The sensor uses capacitance to measure dilectric permittivity of the surrounding medium (manual in https://delta-t.co.uk/wp-content/uploads/2016/11/ML2-Thetaprobe-UM.pdf), which can provide accurate results in a simple and effective way especially when the samples size is large. However, soil sampling volume by the sensor is limited. Readings may not be representative for water status of the whole pot when the volume of soil used in the pot is much larger than the sampling volume detected. Under such circumstances, using gravimetric method can potentially fix this issue, but the growth of roots and soil microorganisms along the time may result in underestimating the magnitude of soil water stress. Therefore, it is worthwhile to conduct a comparative study with two different methods to explore the concentration response of rapeseed and to investigate the interaction of magnitudes of water stress and AT in a quantitative way.

Alternatively, Faralli et al. (2016, 2017a) used a smaller size of pots (5 L) and winter variety, while we used a bigger one (7.5 L) and spring variety. Pot size could restrict root volume and affect plant growth (Poorter et al., 2012). Dambreville et al. (2017) found a significant interactive effect of pot size and watering regime on rapeseed grown in two sizes of pots (0.22 L and 6 L), showing that the ratio of root: shoot dry weight increased in large pots but not in small pots.

Further, water deficit decreased epidermal leaf cell area in large pots but not in small ones. Although those measurements were only from rapeseed seedlings, they still have implications that the interaction between pot size and drought might affect yield responses to AT application. That is, the pot size may impact rapeseed responses to water stress, and the degree of restrictions from pots depends on the crop growth (Poorter et al., 2012). Given the difference discussed above, confounding factors from a controlled environment also increase the complexity of explaining the discrepancy relative to Faralli et al. (2016, 2017), which appears elusive. Therefore, much work is still required to clarify the interactive relationship between drought, AT, and varieties.

It was concluded from the glasshouse study that the impression on gas exchange from AT at lower concentrations might not be enough to detect significant differences in final yield and yield production. In addition, the yield compensation between components may have occurred during the seed filling stage since regular irrigation was resumed (refers to the discussion in Chapter 3). Yet, findings from controlled environments in the glasshouse may not be extrapolated to field conditions because of considerable variabilities. Apart from restrictions on the root volume mentioned above, multiple factors acting together, and their interactive effects regulate plant physiological processes, thus influencing plant responses to drought and AT application (Qaderi et al., 2006). Therefore, two field experiments were conducted in 2021 to explore the concentration-response relationship between AT and yield responses of rapeseed under terminal drought. As was hypothesized, results showed that AT improved seed yield significantly from concentrations of 1% - 3%. Compared to unsprayed droughted control, AT across concentrations increased seed yield by an average of 14% and 34% at BN and FN, respectively (Chapter 5).

Given that drought was more severe at BN than at FN, the interaction between drought and AT could have contributed to the degree of yield improvements, apart from increased AT concentrations. Different responses of rapeseed treated with AT to the magnitude of water stress under controlled environment have been reported by Faralli et al. (2017a), showing that AT worked more effectively on sustaining seed production under severe water-stressed conditions (10-20% AWC). As discussed in Chapter 5, under terminal drought in the field, progressive drought still was developing until harvest, which could substantially reduce the availability of carbohydrates and impair the capacity of surviving pods and seeds for compensatory growth at the later seed filling stage (Kirkegaard et al., 2018).

Besides the overall yield improvement from AT application, seed yield improved as AT concentrations increased from 1% to 3% at both sites. This greater yield from higher concentrations of AT has also been reported in a previous study conducted at FN on winter rapeseed (Faralli et al., 2017b). For more discussions between studies in AT concentrations, refer to Chapter 5. In contrast, Kettlewell and Holloway (2010) found no yield benefits in wheat from higher rates of film AT at 0, 0.1, 1.0 and 2.5 L ha^{-1}, which were equal to 0%, 0.1%, 1% and 2.5%, respectively (confirmed from personal communication). This conclusion, however, has limitations since the results were only from a single study in one year. Therefore, the patterns of concentration-response may vary depending on species and subsequently affect the efficiency of film AT for sustaining the yield of droughted crops. Future research requires more work related to the optimum concentration for specific crops under certain environmental conditions.

One of the concerns about film AT is that curtailed transpiration by blocking stomata could reduce cooling effects, leading to increased leaf temperature that potentially impairs metabolic processes. In field experiments (Chapter 5), leaf temperature increased with increasing AT concentrations as more percentage of stomata were blocked; however, the effect from AT application might be marginal without a threat to final yield production.

High temperature and water stress could frequently occur simultaneously; hence, drought's detrimental effects on crop growth and yield production may be exacerbated by high temperatures (Biswas et al., 2019). Qaderi et al. (2006) explored the interactive effects of CO_2, temperature, and drought on rapeseed plants during the vegetive stage, showing that the lowest biomass from combined water stress and high temperature (28/22 °C vs control 22/18 °C - day/dark). When combining water stress (30% field capacity) with heat stress (29/18 °C vs control 23/18 °C - day/dark) during the reproductive stage, photosynthesis was reduced to a larger extent, leading to substantial yield losses more than water stress alone (Elferjani and Soolanayakanahally, 2018). Faraji et al. (2009) found that seed yield was highly correlated with high-temperature stress during the latter phases of reproductive development when rapeseed was grown under Mediterranean conditions (i.e., terminal drought). That evidence implies that rapeseed plants might be sensitive to drought and heat stress. In the field study (Chapter 5), there were many days when the maximum air temperature exceeded 28 °C, especially during the reproductive stage at both sites (Appendix. Figure 7), although this partially resulted from the inadequate ventilation inside the polytunnel that was compromised to prevent rain out. A short period of very high temperature at a sensitive stage can be as critical to rapeseed as mild temperature stress over a long period (Aksouh-Harradj et al., 2006). It would be worthwhile investigating the interactive effects of drought and heat stress on rapeseed and relate to AT efficiency under such conditions.

6.2 General conclusions

This study proposed a novel approach to estimating leaf coverage of film AT on rapeseed and initially explored the concentration response of film AT (a.i., di-1-*p*-menthene) on rapeseed to different concentrations of AT under controlled and terminal drought in terms of physiological performances and yield and yield components. There were eight glasshouse experiments and two field experiments conducted to test the central hypothesis that film AT has the potential to mitigate the drought damage to rapeseed at the flowering stage and increase the yield of rapeseed". The general conclusions are presented below:

1. TiO_2 can be considered a valid marker to visualise AT on artificial targets (WSP) and natural leaves to estimate coverage. Under glasshouse (well-spaced) and field-simulated (15 cm apart) conditions, leaf coverage and AT concentrations were positively correlated, although with large variabilities between experiments depending on several factors like a mixture of solutions and crop growth (Chapters 2 and 4).

2. Film AT exhibited higher impressions on stomatal conductance than photosynthesis of both well-watered and droughted rapeseed plants, leading to increased intrinsic water use efficiency. Further, this impression increased with increasing AT

concentrations (Chapters 3 and 5). These findings suggest that film AT (di-1-*p*-menthene) can effectively improve leaf water status by reducing stomatal conductance to an extent closely associated with concentrations, although at the expense of photosynthesis.

3. Rapeseed yield is prominently determined by pod number under controlled-environment or field conditions. Pod number is also most influenced by water stress, while thousand-seed weight is the least (Chapters 3 and 5).
4. Film AT can increase seed yield of droughted rapeseed by pod number per unit area, which was highly associated with leaf relative water content. These observations imply that the yield benefit from AT application appeared to be mediated by improving leaf water status (Chapter 5).

6.3 Contributions, limitations, and future research

This study initially provides evidence that TiO_2 can be used as a spray marker for Vapor Gard (a.i., di-1-*p*-menthene), the most common film AT from the commercial market, to estimate leaf coverage after spraying AT. For its strength and drawbacks, refer to Chapter 2. We also first explored the concentration response of film AT on droughted rapeseed in terms of leaf coverage, gas exchange, endogenous abscisic acid, aboveground biomass, seed and oil yield, and yield components. Results from this study can be an important part of film AT research on rapeseed and help understand the physiological mechanism of how film AT ameliorates drought damage to crops. Further, this study can also be considered a reference for farmers to choose the most cost-effective concentration of film AT product on rapeseed, specifically Vapor Gard (a.i., di-1-*p*-menthene).

From the glasshouse experiments (Chapter 3), we used a moisture sensor to monitor soil moisture for drought imposition in the glasshouse, assuming soil water was distributed uniformly in the pot. Soil water tends to be accumulated in the bottom of pots due to evapotranspiration and gravity. Subsequently, readings from the sensor may not represent the whole pot, which is an inherent drawback of imposing drought in this way. So, developing methods of imposing drought at different levels would help understand the interaction between drought and AT application in future studies.

From field experiments (Chapter 5), the greater yield benefits from increasing concentrations of AT were mainly from the improvement in leaf water status in an indirect way, as there was a significant relationship between pod number and AT concentration. Apart from leaf relative water content, osmotic adjust might be involved by accumulating osmolytes like soluble sugars and amino acids (del Amor et al., 2010). Therefore, more direct evidence related to the effect of AT application on leaf water status, such as leaf relative water content and water potential, may be required to clarify our conclusion for future work.

There are mainly three types of antitranspirants: film-formed, metabolic, and reflective. Reflective AT (such as Kaolin) can effectively control heat stress by increasing canopy reflectance of infrared and ultraviolet radiations, thereby obtaining a lower temperature of both the leaf and the fruit tissues. Combining film AT with reflective AT would potentially reduce the detrimental effects of increasing temperature while reducing transpiration to improve leaf water status (Rodriguez et al., 2019). Reflective AT is a less expensive compound than film AT, which can also reduce the cost of products, particularly if high concentrations are required in practice. Therefore, future work can focus on using different types of antitranspirants on various crops under different environmental conditions.

In conclusion, the data have shown that increasing AT concentrations can enhance yield benefits on rapeseed under terminal drought if applied at the flowering stage. However, higher AT concentrations may bring up issues like increasing leaf temperature and raising the cost, as mentioned above. Accordingly, improving the leaf coverage by developing spraying and sprayer types in the field merit further investigations. One of the suggestions is to increase the frequency of spraying without changing the amount of film AT product to achieve good leaf coverage, but it may increase the spraying cost. Although spray droplets from the "conventional" spraying would mostly deposit on the adaxial surface, spray deposits might occur on abaxial surfaces facing upwards due to leaf rolling or bending. Given that more than half of stomata are usually distributed on the abaxial surface of rapeseed leaves, increasing abaxial leaf coverage may be a promising target considered for developing specific sprayers and nozzles for rapeseed crops (e.g., DroplegUL, Lechler GmbH, Germany).

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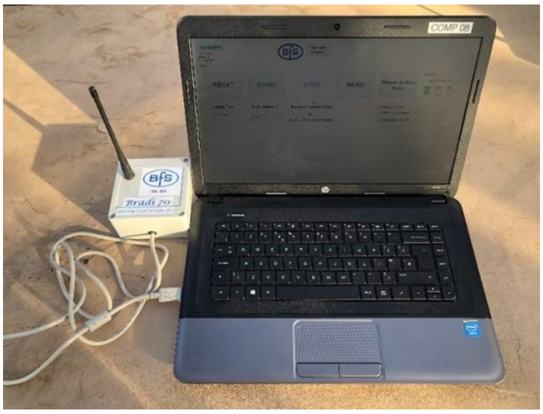
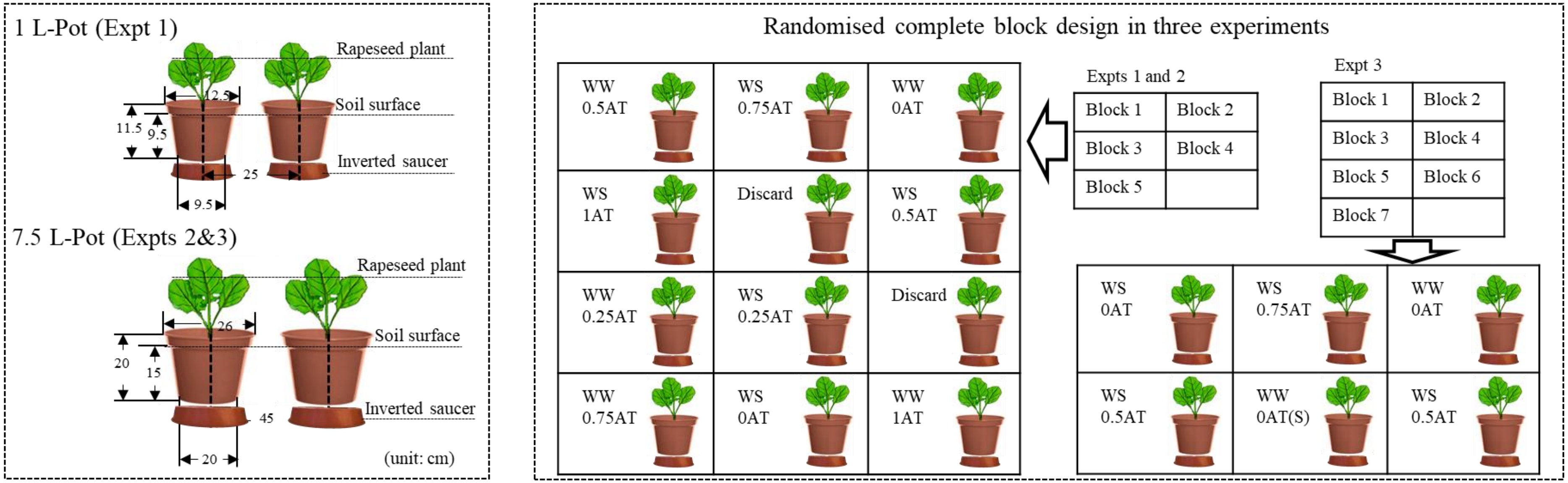
Appendix Tables and Figures

Appendix. Table 1 Probability values from *ANOVA* in leaf relative water content (LRWC) and endogenous ABA content (Endo-ABA) as affected by the application of film antitranspirant (AT) at BN and FN. Polynomial contrasts were conducted between concentrations of AT, including droughted control treated with water (0AT).

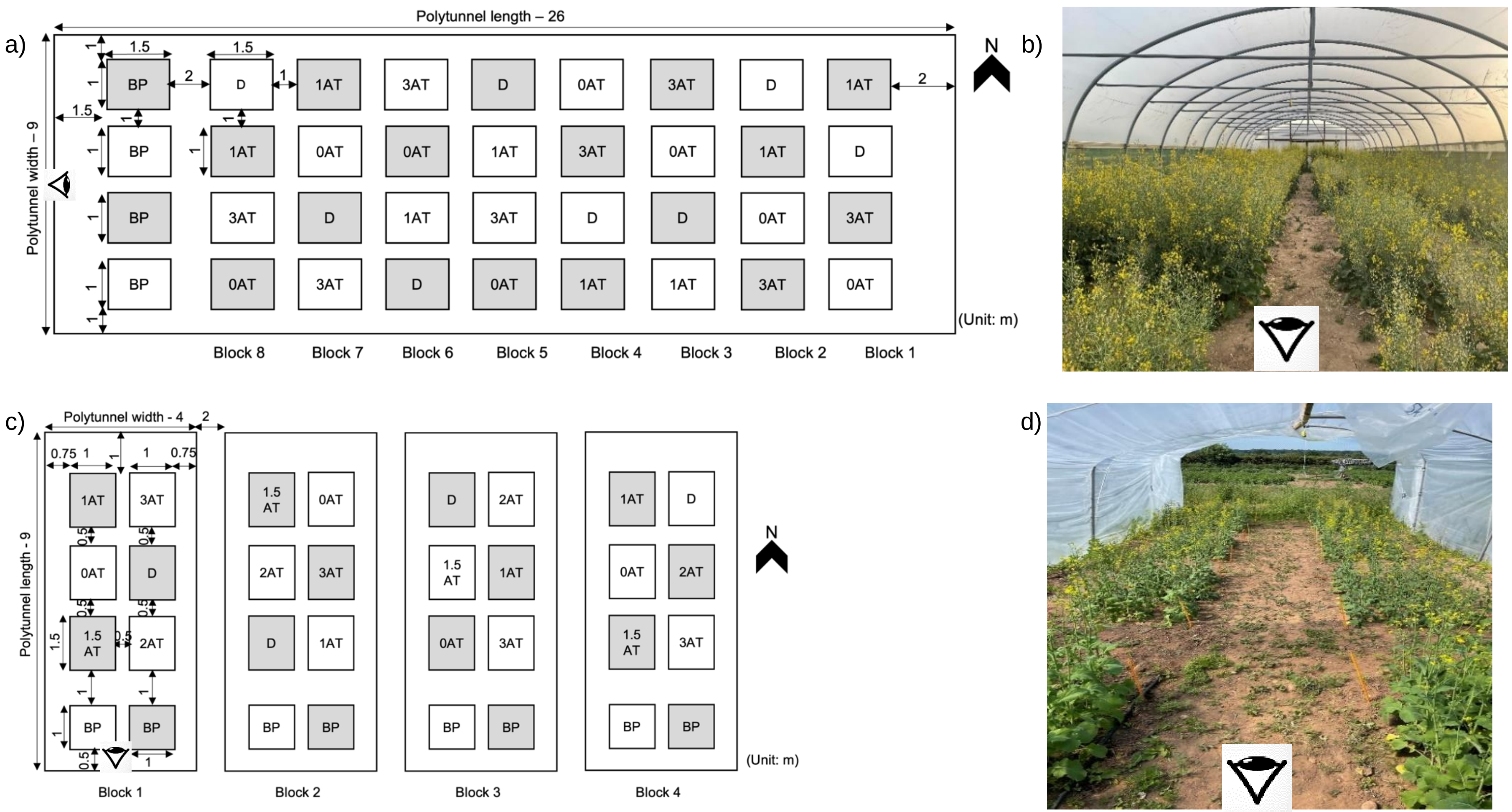
			LRWC		Endo-ABA		
Sites	Factors	d.f.	2 DAS	14 DAS	7 DAS	14 DAS	28 DAS
					Leaf	Leaf	Pod
BN	Treatments	3	0.950	0.163	0.061	0.394	0.488
	Linear	1	0.838	0.425	0.100	0.207	0.340
	Deviations	1	0.812	0.084	0.072	0.634	0.475
FN	Treatments	4	0.869	0.785	0.304	0.386	0.448
	Linear	1	0.604	0.621	0.357	0.969	0.244
	Quadratic	1	0.826	0.504	0.963	0.061	0.454
	Deviations	2	0.651	0.622	0.147	0.871	0.419



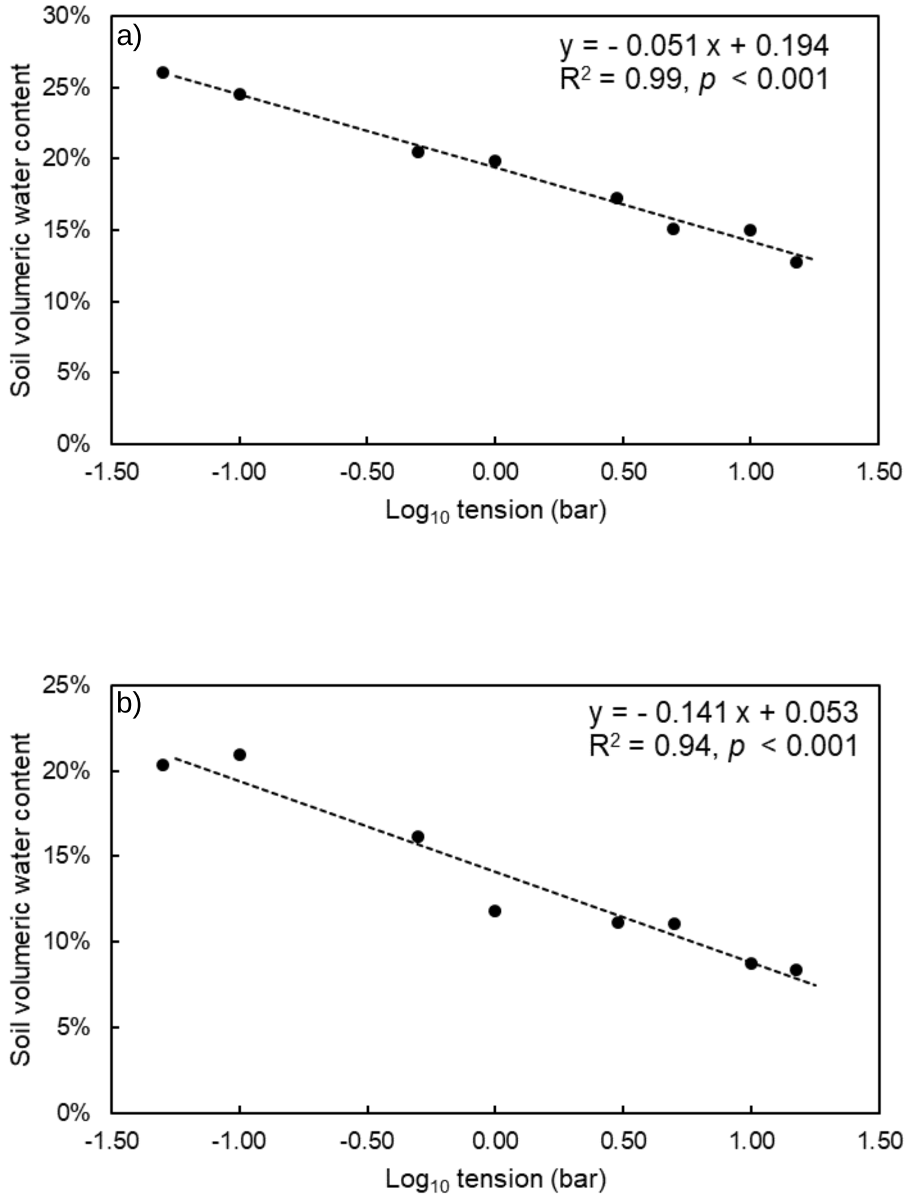
Appendix. Figure 1 Measuring unit of the Dropcounter and the imaging system.



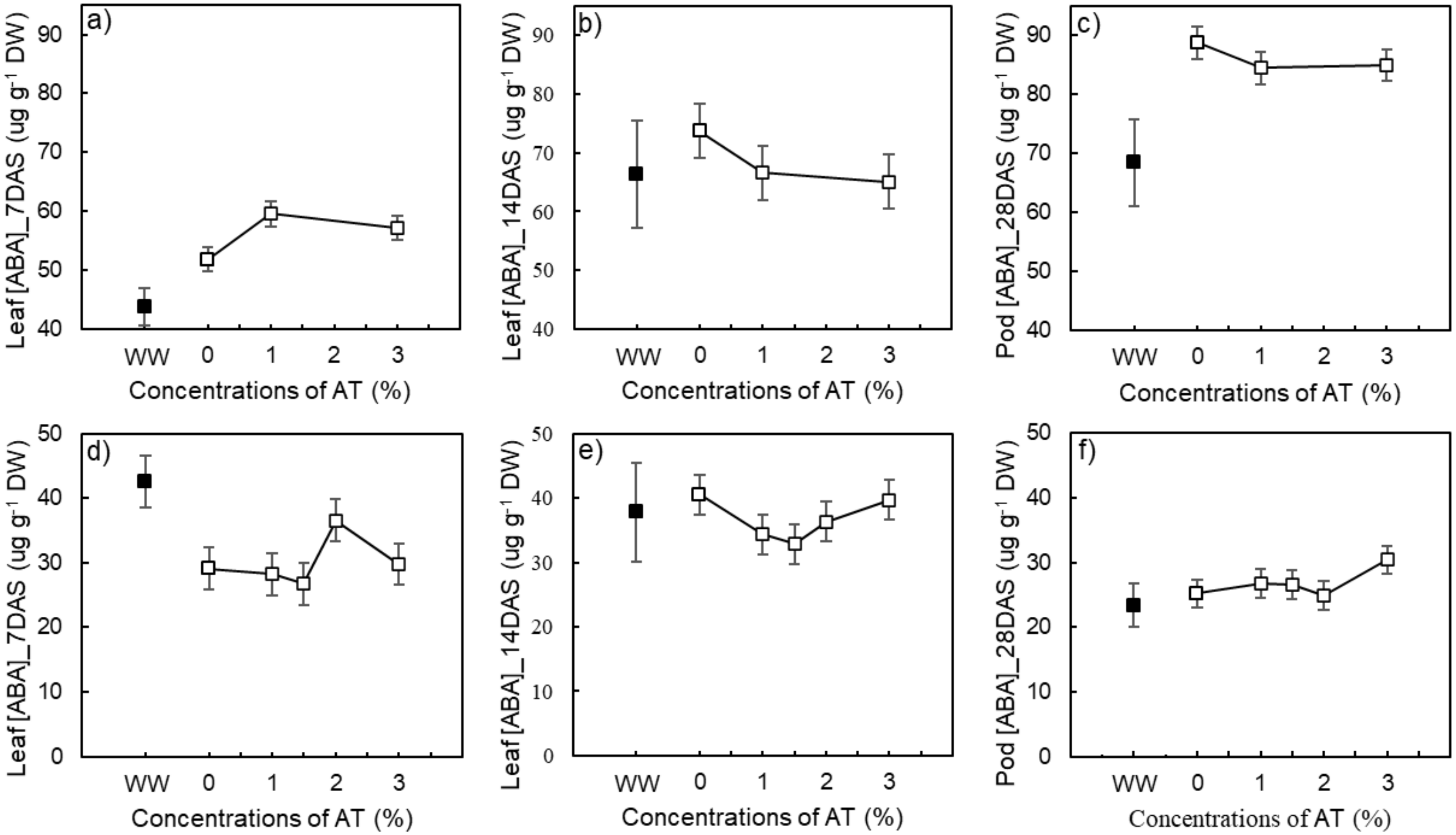
Appendix. Figure 2 The pot dimensions and inter-pot spacing (a), and the randomised complete block design with two factors: irrigation, well-watered (WW) and water-stressed); and concentrations of antitranspirants (AT) at 0 (water), 0.25% (0.25AT), 0.5% (0.5AT), 0.75% (0.75AT) and 1% (1AT) in three experiments and examples of treatments in a block from Expts 1&2 and Expt 3 (b). Note that two plants from each block were discarded in Expts 1 and 2.



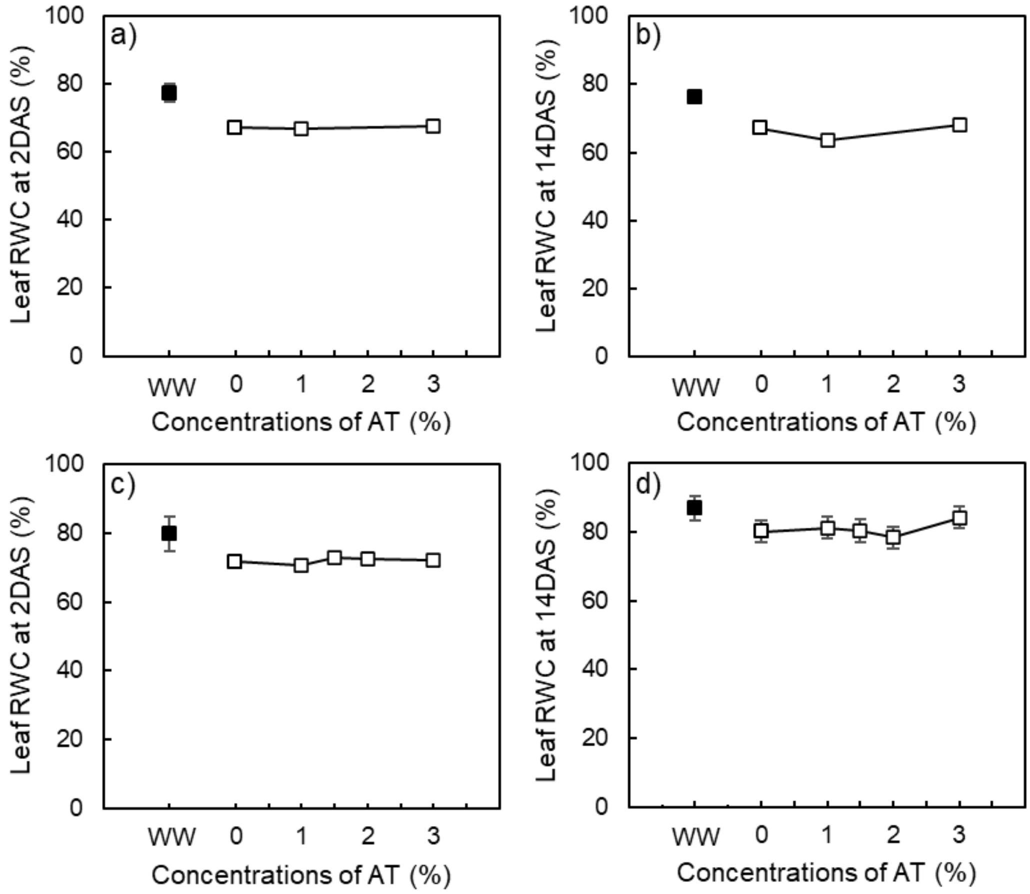
Appendix. Figure 3 The experimental layout of well-watered benchmark plots (BP) and experimental plots treated with film antitranspirant (AT) at different concentrations at BN (a) and FN (c); photos of the whole experiment at BN (b) and block 4 at FN (d) when rapeseed plants were at flowering stage. Plots in grey represent the locations where access tubes were installed. Note that plots labelled with the capital letter D were not used for any experiment.



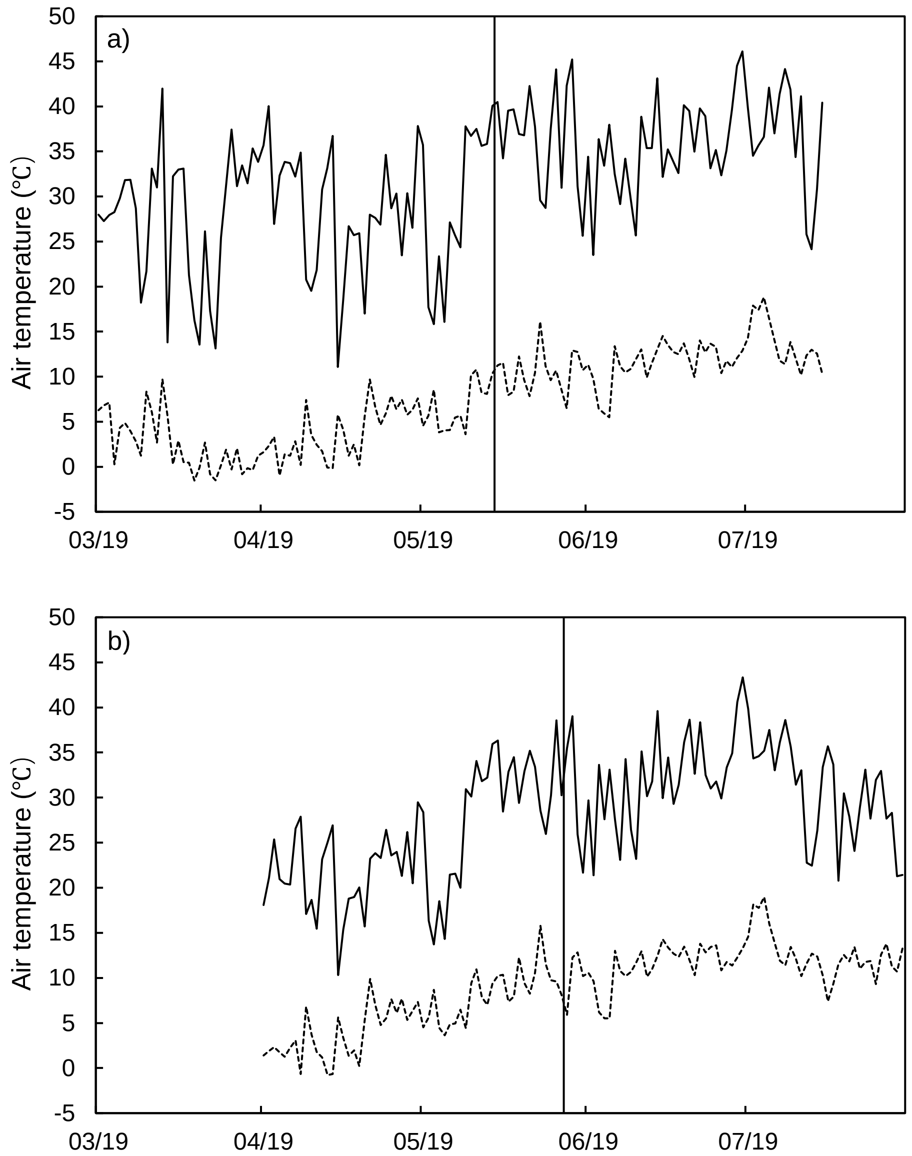
Appendix. Figure 4 Soil water retention curve at BN (a) and FN (b). A linear regression model was fitted with soil volumetric water content against the common logarithm of tension applied using the pressure membrane. Data points are means of 0-30 cm and 30-60 cm depths (n=3).



Appendix. Figure 5 Endogenous ABA concentration ([ABA]) in leaf and pod from well-watered benchmark (WW, —■—) and drought plots (—□—) at BN (a-c) and FN (d-f). Leaf samples were collected at 7 and 14 days after spraying film antitranspirant (7 DAS and 14 DAS, respectively); pod samples were collected at 28DAS. Data are means of replicates (n = 8 at BN; n = 4 at FN) ± standard error of the mean (SEM).



Appendix. Figure 6 Leaf relative water content (RWC) from the well-watered benchmark (WW, —■—) and drought plots (—□—) at BN (a, b) and FN (c, d). Samples were collected at 2 and 14 days after spraying film antiitransprant (AT) (2DAS and 14DAS, respectively). Data are means of replicates (n = 8 at BN; n = 4 at FN) ± standard error of the mean (SEM).



Appendix. Figure 7 Daily minimum (____) and maximum (--------) air temperature during the growing season of rapeseed plants at BN (a) and FN (b) with vertical lines indicating the day of spraying film antitranspirant.