Harper Adams University

Agronomic and physiological responses of sorghum drought tolerance to film antitranspirant

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Abstract

Drought is one of the most limiting environmental constraints to plant survival and productivity globally. Thus, studying means of improving drought resistance of plants continues to assume importance. Hence the need to explore other means of reducing plant water stress apart from breeding drought tolerant plant varieties. Application of transpiration suppressants also called antitranspirants can alleviate the effect of water stress on plants by decreasing transpiration and thereby increasing plant water status and prolonging plant survival under water stress. Transpiration suppressants are of different types one of which is the film antitranspirant. Although a drought tolerant crop, significant yield losses have been recorded in sorghum due to water stress. The main objective of this study was to evaluate the response of sorghum drought tolerance to film antitranspirant with a view to optimizing sorghum yield under water stress through antitranspirant application. The study was carried out between February 2015 and February 2017 and involved eight different experiments, six in the glasshouse and two in the field under rain out shelters. Transpiration, growth and yield of sorghum were investigated using three sorghum cultivars under drought and antitranspirant at different plant growth stages. Drought significantly decreased transpiration and grain number but growth and grain yield and other yield components were not significantly reduced. The antitranspirant significantly decreased transpiration and significantly increased green area index, but yield and other yield components were not significantly affected. The reason for a lack of significant effects of the antitranspirant on grain yield and other yield components is attributable to yield compensation in sorghum under water stress which obscured any improvements in yield by the antitranspirant. In conclusion, antitranspirant reduced transpiration and increased growth in droughted sorghum but did not increase yield and yield components. However, grain yield and yield components were not lower in the droughted sprayed compared with the unsprayed plants either. Thus, application of antitranspirant has potentials to improve drought tolerance in sorghum.

Author Declaration

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

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CHAPTER 1

General introduction

1.1. Introduction

Sorghum (*Sorghum bicolor* L. Moench), is ranked the world's fifth most widely cultivated cereal crop after wheat, maize, rice and barley (Belton and Taylor, 2004). It is cultivated on about 40 million hectares in 105 countries with 70 million tonnes of grains harvested worldwide in 2013 (Sultan, *et al*., 2013). Sorghum is a dietary staple for more than 500 million poor and most food-insecure people in more than 30 countries, mostly in the marginal agricultural regions of Africa, Asia, Oceania and the Americas (Kumar *et a*l., 2011). Over 70 % of the global sorghum area is in Africa and Asia; where about 40 % of world sorghum output is utilized for human consumption and crop production is mostly rain-fed and drought prevalent (Kumar *et al*., 2011). Sorghum is highly suited for hot and dry agro-ecologies where other food grains fail to yield substantially or find it entirely difficult to grow (Hariprasana and Rakshit, 2016). In addition, sorghum is a C_4 crop species with high photosynthetic and nitrogen and water use efficiencies (Reddy, *et al*., 2011), however compared with maize another C_4 plant, it can thrive better on marginal soils (Kholova, *et al*., 2013; Mutava, *et al*., 2015). The sorghum based world economy comprises of two distinct sectors: a traditional smallholder farming category, mainly found in Asia and Africa as subsistence farming and a modern high-input large scale farming sector, largely in the developed countries and in Latin America (FAO, 1996).

Although sorghum is known as a drought tolerant crop, drought stress at any of the growth stages can cause yield loss. Water stress during the reproductive phase is the most damaging to grain yield (Assefa, *et al*., 2010). Water stress from growing point differentiation through anthesis suppresses grain number (Assefa, *et al*., 2010; van Oosterom and Hammer, 2008; Tolk, *et al*., 2013; Prasad, *et al*., 2008; Blum, 2005; Craufurd and Peacock, 1983, Peacock, 1982; Eck and Musick, 1979) and during booting minimizes the rate of head exertion which limits flowering and reduces chances of pollination success (Gerik, *et al*., 2003). While during anthesis water stress may cause floral abortion and suppress grain number, but from anthesis through the dough stage weight per grain is decreased (Ockerby *et al*., 2001).

Several methods are being adopted with varying levels of success to address sorghum yield loss caused by drought. Some of these approaches include agronomic practices, traditional plant breeding (Reddy, *et al*., 2009) and genetic engineering (Reddy, *et al*., 2005). Even though these drought mitigating strategies have shown some benefits, they also have significant shortcomings. Agronomic practices being adopted including planting suitable early maturing and improved plant varieties, so they mature before the onset of drought and selecting varieties with deep roots do have their disadvantages. Planting early maturing varieties shortens the growth duration of the plant leading to the grains ripening under humid conditions. These usually come under heavy attack by insect pests and various types of moulds (*Fusarium* spp) (Andrews, 1972) which destroy some of the grains, render some unhealthy for human consumption and ultimately lead to lower grain quality and yield (Curtis, 1968; Flower, 1996; Andrews, 1972). Selecting plants with deep roots and smaller leaf areas may divert carbon into non-harvestable sinks, whereas the processes of breeding and genetic engineering are expensive. Nevertheless, classical breeding for instance has resulted in the successful development of high yielding, highly adapted sorghum cultivars (Iqbal, *et al*., 2010). But because the majority of sorghum is cultivated without irrigation in areas characterised by irregular and sometimes extremes of high and low rainfall, leading to soil water alternating between drought and saturation which adversely effects plant growth (Wung and Zhang, 2018), there is need to complement plant breeding with other agronomic practices. Complementing plant breeding with agronomic practices will contribute to improving sorghum drought tolerance and yield under drought thereby harnessing its full potential as a food security crop. For instance, in Northern Nigeria sorghum crops are usually harvested long after the rainy season thus the crop completes its final growth cycles on residual moisture (Andrews. 1972), therefore the grains are produced under a condition of very low soil moisture thereby exposing the crop to terminal drought stress. Under low soil moisture condition, the developing sorghum may exhaust the stored water in the soil, before grain filling is completed, leading to little weight per grain (Flower, 1996). Underscoring the place of agronomic practices as complementary measures to drought resistant varieties to help farmers cope with less and more erratic rainfall characteristic of the agro-ecology where sorghum is mostly grown, Lobell, *et al*., (2009) argued that agronomic strategies offers greater potential benefits than improved crop varieties for managing moisture stress particularly in rain-fed systems because they offer a rapid solution to crop yield losses. Furthermore, Turner (1996) stressed that although new technologies of genetic engineering hold considerable potential for improving drought resistance of our food and fibre crops, they will need to be coupled to agronomy if they are to be fully utilized. Accordingly there is a need for the continuous exploration, evaluation and adoption of agronomic practices including chemical treatments to improve plant yield under drought generally and sorghum in particular. Chemical treatments that have been promoted and used commercially to reduce the effect of transpirational water loss on plants include application of antitranspirants. Antitranspirants are chemical compounds, when applied onto the plant leaf, coat the leaf surface making it less permeable to water loss from the plant through transpiration. The application of antitranspirants is a promising soil and plant water management strategy for improving soil and plant moisture content thereby optimizing soil and plant water use. This strategy has been advocated as a tool for reducing plant water deficit in field crops such as sorghum whose yield is sensitive to reproductive-stage water stress (Kettlewell, 2014). The literature showed three main types of antitranspirant as follows: those that act by forming a film layer on the leaf surface: these are polymers sprayed as emulsions and they comprise hydrocarbons and terpenoids (Kettlewell, *et al*., 2010); stomatal closing types, that act by reducing stomatal aperture and the reflecting type that reflects a part of the incident solar radiation on the leaves. The stomatal closing and the reflecting types and their effect on plants have been extensively studied, whereas the film forming types have received scant attention in the study of antitranspirant usage and technology. Antitranspirants are commercially available and are used extensively in plant species such as ornamentals where photosynthesis is less important and transpiration is deleterious. With regard to their application on cereal and food crops, the fact that they reduced photosynthesis which is essential for yield formation did not encourage further investigations into their application to these other agricultural crops. Thus in the 20th century, it was concluded that although antitranspirants have the desirable effect of reducing transpiration thereby enhancing plant water status, they also restrict photosynthesis (Davenport, *et al*.,1974). However, Kettlewell, *et al*., (2010) argued against the above conclusion observing that it appeared to consider photosynthesis and transpiration as two disconnected events in the course of plant growth and development, and as an alternative advocated for a position that views plant growth and yield (at any time) to be determined by the integration of physiological and metabolic processes with the stage of plant development. In other words, plant growth and /or yield is not a product of transpiration and photosynthesis occurring as distinct processes, but is/are the outcome of the interplay of transpiration and photosynthesis among others with plant developmental stage. As such, the impact that any change in the physiological processes such as transpiration and photosynthesis might have on plant growth and yield is dependent upon the plant development stage. If environmental conditions exist that impairs or enhances either or both transpiration and photosynthesis as the case may be, the resultant effect on the plant in terms of yield will be determined not only by the rates of photosynthesis and/or transpiration alone, but also by the growth stage at which the environmental conditions appeared. This understanding has now opened up a new vista in the study and application of antitranspirant to cereal and other food crops from 2010 to date leading to remarkable results showing significant improvements in yield and yield components of some field crops sprayed with antitranspirants at particular stages of plant development. Kettlewell and Holloway (2010) showed as much as a 42 % increase in grain yield of droughted wheat sprayed with film antitranspirant over the droughted unsprayed and Abdullah, *et al*., (2015) recorded only a 10 % reduction from drought in grain yield of wheat sprayed with film antitranspirant compared with a 40 % reduction from drought in grain yield of unsprayed plants, thereby gaining up to 10 % yield over the unsprayed, whereas Faralli (2017) showed a 29 % yield increase in droughted oil seed rape compared with the well-watered control. With these recent successes recorded in the use of film antitranspirants to significantly sustain yield under drought there is a renewed attention to the role that film antitranspirant application could play in ameliorating water stress in droughted field crops.

As regards sorghum, little has been reported pertaining to its yield responses to film antitranspirant application under drought, perhaps due the fact that it is already known as as a drought tolerant crop. This is despite the fact that most of the crop is grown under conditions of limited moisture in Africa and Asia where it is highly valued as a staple crop. Previous research on the effect of film antitranspirant on yield of sorghum under limited soil moisture in the USA in the early 1970's is the only major work reported in this area (Fuehring, 1973) and there is no evidence in the literature to date of any attempt to further the investigation within the USA or outside of it. Hence, the response of sorghum drought tolerance to film antitranspirant remained largely uninvestigated since Fuehring's publication in 1973. Meanwhile experimental evidence by Fuehring (1973) has shown the potential of film antitranspirant application prior to booting growth stage to improve drought tolerance and significantly increase grain yield of droughted sorghum. Therefore, further investigation into the role of film antitranspirants in ameliorating drought stress and increasing grain yield in droughted sorghum is worthwhile. The research reported in this thesis is the first work on the response of sorghum drought tolerance to the application of modern terpene film-forming antitranspirant.

The current study aims to fill the gap created by the paucity or complete absence of knowledge about the response of sorghum drought tolerance to film antitranspirant compared with other world major crops like wheat, maize and potatos, since the work of Fuerhing in the USA in 1973. By exploring the effects of the film antitranspirant on transpiration, growth and yield of sorghum under water stress, this project will contribute towards defining the responses of sorghum drought tolerance to film antitranspirant under glasshouse and field conditions which is critical to further research that could form the basis for the adoption of the application of antitranspirant as a drought management strategy to optimize sorghum yield under drought especially in Africa and Asia.

1.2. Objectives of the study

The following are the objectives of the study:

1. To evaluate the effect of film antitranspirant on transpiration of droughted sorghum.
2. To determine the effect of film antitranspirant on the growth of droughted sorghum.
3. To assess the effect of film antitranspirant on yield and yield components of droughted sorghum.

CHAPTER 2

Literature Review

2.1. Plant general response to water stress

Plant strategies for survival, growing and producing grain, with part of its life cycle under water stress is known as drought resistance (Fageria, 1992). Drought resistance is classified into escape, avoidance and tolerance strategies. Drought escape strategies entails the successful completion of reproduction by means of a faster growth rate, a short life cycle, or efficient storage and use of reserves for seed production before the onset of water stress(Barnabas, *et al*., 2008). Drought avoidance results from deep roots, early stomatal closure to reduce water vapor loss which results into a higher plant water status despite exposure to external water stress (Fageria, 1992; Levit, 1980). Drought tolerance is the ability of the plant to grow and produce yield at reduced soil and plant water potentials (Fageria, 1992). Other plant responses to water stress involves anatomical adaptations and includes production and deposition of a thicker cuticle that reduces water loss through the cuticle and maximized water uptake by increasing assimilate investment into the roots leading in some cases to the development of a robust root system for the enhancement of rooting depth (Fischer and Turner, 1978; Blum and Arkin, 1984). To free up water, some plants shed older leaves and reallocate nutrients to the stem or younger leaves to aid in their survival in a water deficit environment (Larcher, 2003). In others, soil drying causes desiccation of the root tissues, which triggers the expression of drought-induced genes that synthesize various hormones, in particular abscisic acid (ABA). ABA is translocated from roots to leaf tissues, and there it binds to the plasma membrane of the stomatal guard cells (Taiz and Zeiger, 2002). This results in a flux of ions across the cell membrane, leading to rapid osmotic adjustments (increase in organic solute content because of stress), shrinkage and closure of the stomatal guard cells. The production of ABA in the roots and its subsequent transport to the leaves is a mechanism for transmitting chemical signal to the plant on the water status of the soil (Schachtman and Goodger, 2008). The ABA-induced stomatal closure is an important physiological mechanism employed to limit water loss and to increase water-use efficiency particularly in isohydric plant species (Bansal, 2015). Isohydric plants operate a strict water conservation strategy; when water is abundant or under drought conditions, stomatal conductance is reduced to limit transpiration. Anisohydric plants maintain open stomata even in the presence of decreasing water potential (Sade, *et al*., 2012). Leaf elongation is one of the most sensitive growth processes to drought in plants. Under mild water stress, there is reduction in rate of leaf expansion and final size. Under severe drought, rate of elongation decreases and leaf growth can cease and total leaf area decreases due to reduction in initiation of new leaves, which limits water loss through stomatal or cuticular transpiration. Prolonged drought stress can accelerate leaf senescence and the death of leaf tissue, resulting in leaf drop particularly of the old and mature leaves. Drought influences various important plant physiological activities, such as photosynthesis, stomatal conductance and chlorophyll fluorescence. Drought can influence photosynthesis either indirectly through inducing stomatal closure and decreasing CO_2 flow into mesophyll tissues (Chaves *et al*.,1991) or by directly impairing metabolic activities marked by declines in regeneration of ribulose bisphosphate (RuBP) and ribulose 1, 5-biphosphate carboxylase/oxygenase (Rubisco) and decreased rubisco activity among others (Bota *et al*., 2004). Generally, metabolic impairment is not an immediate response to drought in plants, but occurs depending on the severity of water stress. At the onset of drought stress, decline in photosynthesis is primarily due to decreased stomatal conductance and with increasing severity; drought stress can cause tissue dehydration, which leads to metabolic impairment (Prasad, *et al*., 2008).

2.2. Sorghum

2.2.1. General description

Figure 2.1. Shows a diagram of sorghum giving the major botanical parts.

Sorghum inflorescence is a branched terminal panicle that varies from compact to open or loose, on which branches are arranged on the rachis in whorls. Spikelets occur in pairs, one pedicelled and the other sessile. Flowers are attached to the branches rather than the main axis. A single panicle carries between 800 and 3000 kernels. Grain size is very variable even within same variety classified as large (> 35 g/1000 grains), medium (25 - 35 g/1000 grains) and small (< 25 g/1000 grains) (Doggett, 1988, Curtis, 1967). Sorghum is a self-pollinating crop, although some cross-pollination does infrequently occur (Fagaria, *et al*., 2011). Anthesis begins near the tip of the panicle, about 0 – 3 days after the boot stage, and flowering proceeds basipetally for 4 – 7 days.

The stem is erect and solid, dry or juicy, with diameter from 1 cm to 5 cm and range in height from 0.8 m to 6.0 m (Doggett, 1988). The leaves possess a distinct mid-rib and the leaf blades are 8 – 13 cm wide and 30 – 135 cm long. The leaves on the main stem vary in number from 7 – 24 according to variety; usually they are alternate in two ranks and not opposite sides of the stem with the flag leaf out of line with the leaf arrangement on the stem. The stomata occur in single or double files on both surfaces of the leaf amphistomatous (Jones, 2014) with more stomata on the abaxial (lower) than on the adaxial (upper) surface (Liang *et al*., 1975), and a row of motor cells located near the midrib which are responsible for longitudinal rolling of the leaf inwards under drought conditions to reduce transpiration. It is common to find the leaf epidermis coated with a layer of white wax exudate known as bloom. This waxy surface layer and extensive cutinisation further limits water loss (Doggett, 1988).

Sorghum has two distinct types of root system as it is with maize: the seminal roots which develop from the embryo below the ground and the adventitious roots which arise from the lower stem nodes near the soil surface. The bulk of the root system in mature sorghum plants is adventitious (Fagaria, *et al*., 2011; Doggett, 1988). Maximum root weight occurs at about anthesis when the roots can extend to a depth of more than 1.5 m. Compared to corn, sorghum roots have a greater mass percentage in the upper soil profile and explores deeper sections of the soil profile (Assefa *et al*., 2014). About 84 % of the roots are found in the top 30 cm of soil with maximum root activity restricted to the top 25 cm (Mayaki, *et al*., 1976). Characteristics such as root length, density, mass, volume and thickness are highly correlated with drought tolerance (Beyene *et al*., 2015). Thus, a narrower root angle permitting deeper root penetration and a faster elongation rate are associated with drought tolerance (Singh *et al*., 2010).

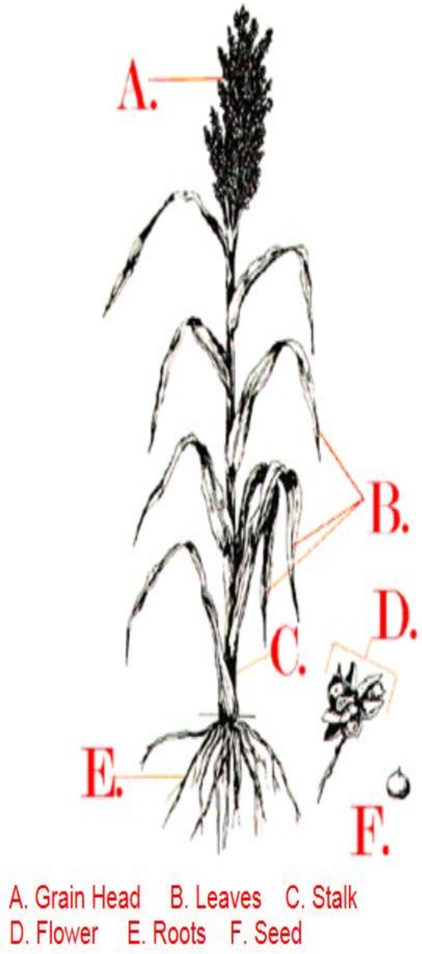

Figure 2.1. A diagram of sorghum showing major botanical parts (http://archive.gramene.org/species/sorghum/sorghum_anatomy.html).

2.2.2. Sorghum growth stages

Cell growth and division are the most elementary processes of growth resulting into final plant organ, whole plant size and shape. Agronomists generally define growth as increase in dry matter, and this encompasses the process of differentiation which contributes to dry matter accumulation. Plant development on the other hand is the result of growth by cell division, enlargement and differentiation.

The need to understand and communicate the physiological and morphological state of agricultural plants has led to the study and identification of various growth stages in agricultural plants and this has led to the development of growth stage scales (Smith, 1995).This is a scale used to identify the phenological development stages of a plant. An understanding of plant growth stages aids in the design and operation of best management, cultural and agronomic practices to optimize yield.

Studies on sorghum development stages by Vanderlip and Reeves (1972) have shown that development stages in the sorghum plant are divided into ten: zero (emergence) through nine (physiological maturity). The time taken to reach a particular stage depends on the hybrid and the environment in which sorghum is growing. A description of developmental stages, approximate days after emergence to reach a particular stage and some observable features for a grain sorghum crop are given in Table 2.1 and Figure 2.2. Furthermore, Eastin (1972) categorised sorghum growth and development into three major stages as follows: GS1, vegetative stage from emergence to bud initiation taking an average of 38 days; GS2, floral stage characterized by commencement of flowering lasting an average of 31 days and GS3, grain filling stage, physiological maturity taking an average of 26 days. The divisions of sorghum developmental stages presented Table 2.1 and Figure 2.2 were made solely on the basis of anatomical or morphological observations therefore these divisions are rather arbitrary, since events occurring in GS1 influence events in GS2 which in turn influence final grain production during GS3.

Table 2.1. Sorghum developmental stages, approximate time intervals between growth stages and characteristics.

Dev. stage	Growth stage	Days after emergence	Observed features
0	GS1	0	Emergence, coleoptile visible at soil surface
1	GS1 3-leaf stage	5	Appearance of the collar of the 3rd leaf.
2	GS1 5-leaf stage	10-15	Appearance of the collar of the 5th leaf.
3	GS1 Growing point differentiation stage	25 - 30	Appearance of 7 – 10 fully developed leaves; may have lost 1 – 3 leaves from the bottom of the plant. Growing point visible above the soil surface, there is rapid elongation of culm and stalk.
4	GS2 Flag leaf stage	35 -50	Emergence of the final leaf (flag leaf). Full expansion of all leaves except the last three. At least 80% of the total leaf area present. Lower 2 -5 leaves may be lost.
5	GS2 Booting stage	40 - 55	Booting characterised by head enclosed in the flag leaf sheath. Peduncle elongation starts pushing head out of sheath.
6	GS2 Half-bloom stage	55 - 65	50 % of plants in the field are in some stage of flowering
7	GS2 Soft dough stage	65 - 80	Only 8 -12 functional leave remains; grain can easily be squeezed between the fingers.
8	GS3 Hard dough stage	80 - 90	Cannot squeeze grain between fingers; grain contents have solidified.
9	GS3 Physiological maturity stage	90 - 110	Dark spot at the tip of the kernel.
10	Harvest	>150	Grains assume a distinct colour depending on variety

(Based on Vanderlip and Reeves, 1972).

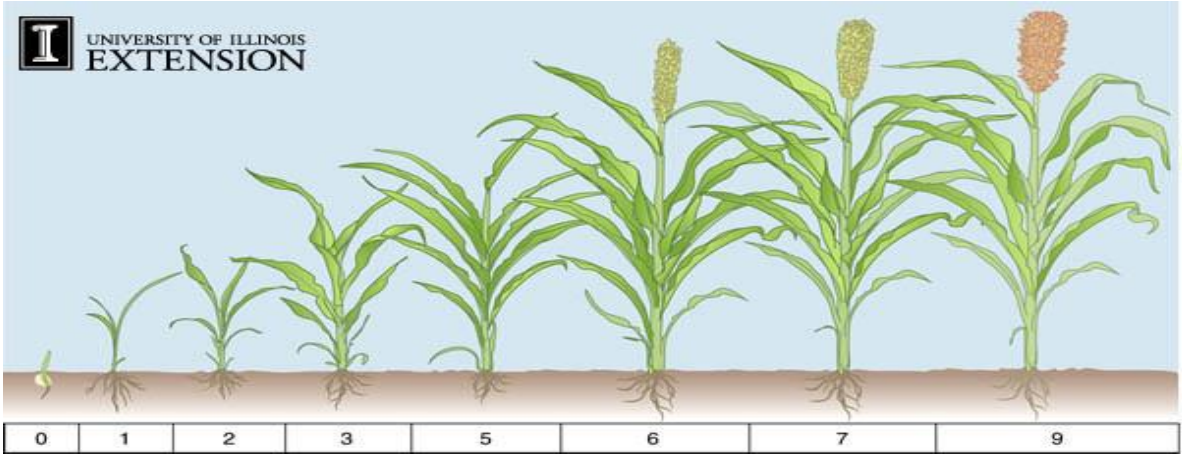

Figure 2.2. Sorghum growth stages from emergence to maturity.

Source (http://weedsoft.unl.edu/documents/GrowthStagesModule/Sorghum/Sorg.htm).

2.2.3. Sorghum response to drought stress

A great proportion of the world’s sorghum is grown under unirrigated conditions with several factors limiting its production. Amongst these are: water stress, nutrient deficiencies, weeds, insects, diseases, heat, cold and bird attacks. Although the aforementioned factors significantly limit sorghum production, drought stress is recognized as the major constraint (Anami, *et al*., 2016), causing significant damage to growth and yield. An analysis of 52 years of research on sorghum production in Kansas, USA, identified drought stress as the major yield-limiting factor in about 30 of the 52 years. Drought stress was not only the most frequently mentioned factor, but also the most described in terms of duration and intensity (Assefa, *et al*., 2010).

2.2.3.1. Stomatal conductance and transpiration

Stomatal conductance is an estimate of the rate of gas exchange, CO_2 uptake and transpirational water loss through the leaf stomata. In operation, this gas exchange takes place between plants and the atmosphere and is regulated by the stomata that opens and closes to optimize CO_2 fixation and minimize transpirational water loss (Schroeder *et al*., 2001).Therefore, stomatal conductance is determined by the degree of opening of the stomatal aperture, and the physical resistances to the movement of gases between the air and the interior of the leaf. A widely open stomata permits greater conductance which translates into potentially higher photosynthesis and transpiration, than one that is not as widely open. Reduction in stomatal conductance in response to soil moisture deficit has been reported in various sorghum cultivars despite its reputation for drought tolerance. Shimshi and Shaphat (1975) showed positive correlation between reduced stomatal conductance and yield in wheat under water stress and this may apply also to sorghum. Thus sorghum drought tolerance which aids the crop’s survival under soil moisture stress can be understood to be at the expense of yield reduction (Assefa, *et al*., 2010; Peacock, 1982). In other words since stomatal conductance is reduced in sorghum under water stress, then it suggests that sorghum yield could also decrease under drought stress. In an assessment of sorghum response to water stress, Munamava and Riddoch (2001) applied water stress at the vegetative (5-8 fully expanded leaves), booting (panicle developed to nearly full size) and flowering growth stages (50 % of head has flowered) by withholding water for 10 days at the vegetative and for an average of 5 days at the booting and flowering stages. Stomatal conductance decreased in the stressed plants at all growth stages, especially at the later growth stages. Reduction in stomatal conductance in response to water stress in sorghum was found to be due to a decrease in leaf water potentials. Reports by Al-hamdani *et al*., (1991) in which four sorghum genotypes BOX 11, TX622, BOK 111 and IN-15 were subjected to water stress at 50 % and 25 % of saturation with 100 % saturation as the control until leaf rolling and wilting were observed in most of them, showed decreased stomatal conductance associated with reduced leaf water potential with the magnitude of the reduction being dependent upon the rate of change in leaf water potentials. For instance under increasing soil water stress, stomatal conductance in the TX622 genotype declined by 0.11 mol m^{-2} s^{-1} for a 0.86 MPa decline in water potential whereas under the same water stress IN-15 recorded only 0.040 mol m^{-2} s^{-1} decrease in stomatal conductance in response to a smaller reduction of 0.036 MPa in water potential. Tsuji *et al*., (2003) quantified the physiological basis for drought tolerance using stomatal conductance in three sorghum cultivars, Gadambalia, Arous and Tabat by measuring stomatal conductance and transpiration rate under a drought stress regime of 118 days and showed that stomatal conductance and transpiration were lower in Tabat than in Gadambalia and the leaf water potentials and relative water contents were lower in Tabat than in the Gadambalia, thus lower leaf water potentials resulted in lower conductance. Thus, reduction in leaf water potential in response to water stress causes decrease in stomatal conductance, which also reduces transpiration because decreased leaf water potential, increases resistance to vapor transport, thereby decreasing transpiration (Sumayao, *et al*., 1977). With regard to plant developmental stage, Yadav, *et al*., (2005) observed that soil water stress led to significant reduction in stomatal conductance irrespective of the sorghum growth stage, as pot-grown sorghum droughted at anthesis and grain filling stages for 10 days by withholding irrigation indicated a 95.6 % and 87.4 % reduction in stomatal conductance as well as in relative water content and leaf water potential compared with the well-watered control plants. A field experiment carried out by Sumayao, *et al*., (1977) to determine effect of soil moisture content on net carbon dioxide exchange and transpiration in sorghum under water stressed and well-watered conditions suggests that transpiration and net carbon dioxide exchange rates were significantly reduced at available soil moisture content of ≥ 35 %. Their results indicated that above the 35 % available soil moisture content, transpiration was more dependent on temperature (heat energy) than soil moisture content.

2.2.3.2. Germination and seedling emergence

Soil moisture is an essential requirement for germination of seeds (Cardwell, 1984). Although the embryo in the seed is highly tolerant to desiccation due to its dormancy, after they start to germinate and emerge, seeds can become susceptible to moisture stress (Blum, 1996). Under PEG induced water stress the rate and percentage of germination and the early growth of sorghum seedlings were significantly reduced (Bayu, *et al*., 2006). A greater significant reduction in germination percentage, root length and shoot length was observed at a stress level of -9 bar than at 0 and -6 bars (Amiri and Mojaddam, 2014). At the seedling stage, traits vary in their responses to water stress as the shoot related traits namely, shoot length, shoot fresh weight and shoot dry weight were more susceptible to water stress than root growth consequently, a significantly higher reduction in final biomass occurred due to water stress at the early stage, when mainly only leaf was growing, than at a later stage when mainly only stem was growing (Mastrorilli, *et al*., 1999). However, the effect of water stress on germination will depend certain factors including the intensity of drought (Bayu, *et al*., 2006; Amiri and Mojaddam, 2014) as well as the duration of water stress, the depth of sowing and the soil type. An assessement of the germination of sorghum seeds conducted in vertisol soil type in the Central Rift Valley of Ethiopia (Marga, *et al*., 2014) showed that planting depths accounted for large variations in plant population with several interactions between the planting depths and water regimes. However, the 5 cm planting depth with no water applied until 15 days after planting gave the greatest seedling establishment of 80 %, while seeds planted at 7 cm depth and droughted until 15 and 30 days after planting had a 12 % seedling establishment.

2.2.3.3. Vegetative growth

Sufficient leaf area and functional root system are identified as essential requirements to attain maximum grain yield in sorghum. Water stress at the vegetative growth stage of sorghum leads to inadequate leaf area index, significant reduction in plant height and leaf area and reduction in rate of leaf appearance (Mastrorilli, *et al*., 1995; Craufurd, *et al.,* 1993; Stout *et al*., 1978). On the other hand, root-to-shoot ratio in water stressed sorghum increases with increasing drought stress (Salih, *et al*., 1999). Munamava and Riddoch, (2001) reported a 42 % increase in the root-to-shoot ratio in sorghum droughted at the vegetative growth stage as early exposure to water stress, facilitates preferential allocation of carbon towards the development of more below ground biomass at the expense of shoot growth, to sustain plant growth under water deficit.

2.2.3.4. Reproductive growth

Studies have shown that drought stress delays panicle development and flowering in sorghum. Craufurd *et al*., (1993) showed water stress delayed panicle initiation, and flowering by 2 – 25 days and 1 – 59 days respectively. Drought stress increased the period between panicle initiation and flowering by retarding the rate of panicle development. Severe water stress at any stage between panicle initiation and flowering induces complete cessation of panicle development (Craufurd *et al*., 1993). The booting to flowering stage is the most sensitive to water stress in sorghum (Doorknobs and Kassam, 1979; Lewis, *et al*., 1974). Therefore, avoiding water stress at this stage is very important to gaining optimum yield from the crop (Doorknobs and Kassam, 1979). Water stress imposed during booting to flowering (Lewis, *et al*., 1974) and early booting to heading (Eck and Musick, 1979) significantly lowered grain yield by 34 % and 50 % respectively, retarded the rate of panicle development and in severe cases induced complete cessation of panicle development (Inuyama, *et al*., 1976, Craufurd *et al*., 1993), while significantly reducing grains per head and seed size (Inuyama, *et al*., 1976). Within the booting to flowering stage, the precise stage of sensitivity of sorghum to water stress, and the injury caused has not been determined. This may be probably identical or similar to maize, where pollen sterility has been reported (Saini and Westgate, 1999).

2.2.3.5. Grain yield and yield components

Sorghum grain yield is a function of a number of factors including panicles harvested, seeds per panicle, individual seed weight and water supply. Although sorghum tolerates short-term water deficit, long term and severe water stress significantly reduces its growth and final grain yield (Assefa, *et al.,* 2010). Eck and Musick (1979) experimented with a range of water stress durations on sorghum under irrigation at early booting, heading, and early grain filling stages. Water stress of between 13 to 15 days at early booting, heading, and early grain filling growth stages did not decrease grain yield, while water stress of between 27 to 28 days at early booting, heading and early grain filling stages reduced yield by 27 %, 27 % and 12 % respectively. Reduced seed size and seed number were responsible for yield decline at the boot stage, while at heading yield decline was attributed to reduction only in seed size. There is evidence that sorghum growth stages responded differently to the same degree of water stress with a substantial reduction in grain yield during the booting to bloom stage under moderate water deficit. A decrease to –12.9 bar in soil water potential from late vegetative to bloom stage resulted into a 17 % decrease in sorghum grain yield. However, the same water potential imposed from boot to bloom and milk to soft dough stages led to 34 % and 10 % reductions in yield respectively (Lewis, *et al*., 1974).

2.3. The use of antitranspirants to relieve water stress in some world's major food crops.

2.3.1. Maize

Water stress in maize (*Zea Mays* L.) results in disruption of silking interval and causes yield reduction hence the application of antitranspirants to reduce yield loss from drought. Fuehring and Finker (1983) investigated the response of droughted maize to antitranspirant Folicate (a hydrocarbon film material) over nine field experiments at New Mexico in the USA, and concluded that antitranspirant application is a feasible method of increasing maize yield under drought. Plants were sprayed with varying doses of the antitranspirant at different water volumes after complete leaf emergence. Application on both leaf surfaces just prior to tasselling at 2 L/ha resulted in significantly higher yields of up to 11 to 17 % in the sprayed plants. Shekour *et al*. (1987) found that antitranspirant Vapor Gard led to a mean increases of 5.7 % in plant height and leaf area as well as a 42 % in fresh weight and total dry matter in dry season sweet corn under water stress compared with plants under irrigation. Increases in fresh weight and dry matter are desirable to sustain the plant through the dry season thereby prolonging the time during which it remained productive.

2.3.2. Soybean

Soybean (*Glycine max* L.) is an important leguminous crop providing a source of essential protein to humans, feed for livestock and bio-fuel for industry (Wilcox, 2004). Water stress during the pod filling period in soybean reduced yield by 5 - 38 % and seed size by 11 – 35 %, but the grain number remained unaffected, while leaf senescence was accelerated and photosynthesis was reduced as a consequence (Egli and Bruening, 2004). Application of an antitranspirant FZ to soybean for three times during the pod filling and pod bearing stages under lower and upper irrigation limits of 40 – 70 % field capacity increased seed yield and water use efficiency by 24 % and 21 % respectively, but did not affect final biomass (Ji, *et al*., 2017). According to Egli and Bruening (2004) one of the reasons for decreased seed yield in soybean under water stress is the reduction in the grain filling period due to leaf senescence. Probably the antitranspirant increased yield of soybean under water stress by reducing the rate of leaf senescence thereby improving photosynthesis and yield under drought.

2.3.3. Wheat

As indicated in Chapter 1, antitranspirant has been used with remarkable success to decrease yield loss of droughted wheat (*Triticum aestivum*) (Kettlewell *et al*., 2010; Weerasinghe *et al*., 2010). Weerasinghe *et al*. (2010) reported a non-significant difference in grains per ear between well-watered and droughted wheat, after application of 2.5 L/ha of Vapor Gard to droughted wheat at BBCH GS 33, GS 39, GS 41 and GS 59 growth stages. Furthermore, the mechanism by which the antitranspirant protects against significant yield loss in droughted wheat has been elucidated (Weerasighe, *et al*., 2016; Abdallah, *et al*., 2016). The main physiological factors responsible for decreased yield damage from drought following antitranspirant treatment are higher leaf water potential and pollen viability under water stress (Weerasighe, *et al*., 2016) and sustained photosynthetic rates with greater grain production in sprayed than unsprayed droughted plants (Abdallah, *et al*., 2016).

2.3.4. Sorghum

Although there is limited information in the literature on the application of film antitranspirant to irrigated and droughted sorghum, available information on the response of water-stressed sorghum to film-forming antitranspirants suggests that moisture loss can be reduced during boot to early heading stages leading to higher grain yields. Results of field experiments in the USA by Fuehring (1973) aimed at investigating the effect of film antitranspirants on grain yield of sorghum demonstrate the effectiveness of film antitranspirants in significantly increasing grain yield in water–stressed sorghum. The experiments involved two irrigation regimes: a) two irrigations - at planting and booting and b) four irrigations - at planting (mid-may), June 25, at booting (July ending) and on August 25, with two levels of the antitranspirant Folicote, sprayed at concentrations of 2.1 L/ha and 3.2 L/ha, while the control plants were left unsprayed. The irrigated treatments were the main plots, while the time of application of antitranspirants were the subplots with six replications of the main plots used. Results showed a 9.3 % and 10 % increase in average grain yield kg/ha of the sprayed treatments over the control (irrigated unsprayed) under two and four irrigations respectively using the lower 2.1 L/ha rate of antitranspirant application. However, at 3.2 L/ha rate of antitranspirant application, 2.5 % and 4.8 % increases in average grain yield kg/ha under two and four irrigation regimes respectively over the control (irrigated unsprayed) were recorded. In addition, there were no statistically significant differences in the average grain yield kg/ha of sorghum treated with Folicote at 2.1 L/ha and 3.1 L/ha at two and four irrigations.

2.4. Conclusion

Numerous studies have explored water stress and water stress effects on plants including sorghum using a variety of approaches which shows the complexity of the problem of water stress in plants. Some of these studies considered water stress from an intensity (level-of-stress) point of view, while others looked at it in terms of duration (how long) of stress. Other studies assessed water stress as a combination of drought intensity and duration under the overarching influence of high temperature (heat stress) generated as a consequence. Reducing the effect of drought stress using techniques like antitranspirant application has been carried out under specified drought intensity and duration at particular growth stages in food crops including sorghum. However, the literature has not reported on the use modern film forming terpene based antitranspirant to reduce drought stress in sorghum; this is what the current study is designed to explore. A summary of the experiments and key factors used in the study is presented on Table 2.2.

Table 2.2. A summary of experiments reported in the thesis.

Experiments	**Experimental design**	**Duration of drought (days)**	**Growth stage of drought imposition**	**Growth stage of spraying with antitranspirant**	**Concentration of antitranspirant (L/ha)**
Glasshouse Expt.1.	2 x 2 factorial, RCB in 6 replicates	31	GS 5.5	GS 5.5	1.0
Glasshouse Expt. 2.	2 x 2 factorial, RCB in 6 replicates	24	GS 5.1	GS 5.1	1.0
Glasshouse Expt.3.	2 x 4 RCB in 6 replicates	18	GS 5.1	GS 5.1	0.0, 1.0, 2.0, 3.0
Glasshouse Expt.4.	1 x 5 RCB In 12 replicates	Terminal	3-leaf, 5-leaf, 8-leaf and panicle emergence	No antitranspirant applied	na
Glasshouse Expt.5.	1 x 3 RCB in 16 replicates	Terminal	3-leaf	GS 5.1	1.0
Glasshouse Expt.6.	1 x 4 RCB in 15 replicates	Terminal	3-leaf	GS 5.1	1.0
Polytunnel Expt.1.	2 x 2 factorial, split-plot in 8 replicates	94	GS 2.0	GS 5.1	1.0
Polytunnel Expt.2.	1 x 3 factorial in 4 replicates	Terminal	3-leaf	GS 5.1	1.0

CHAPTER 3

Evaluation of the effect of drought stress and film antitranspirant on transpiration, growth and yield of sorghum using two cultivars

3.1. Introduction

Glasshouse experiments 1 and 2 reported in this chapter were undertaken to determine the effect of drought stress and antitranspirant on transpiration, growth and yield of sorghum.

The following null hypothesis was used:

- There is no significant effect of drought stress and film antitranspirant and their interaction on volumetric soil moisture content, transpiration, growth and yield in sorghum.

The following objectives were set in order to test this null hypothesis:

To evaluate the effect of drought stress and antitranspirant and their interactions on:

- Volumetric soil moisture content of pots.
- Transpiration, relative water content and leaf temperature of sorghum.
- Plant height of sorghum.
- Yield and yield components of sorghum.

Glasshouse experiments were conducted at Harper Adams University Edgmond (52°46′ N, 2°25′ W) using two distinct sorghum cultivars namely, Pen110 and SAMSORG-40. Pen110 is adapted to the UK weather where it is being grown as a game cover whereas SAMSORG-40 was bred for tropical environments in Nigeria although it has been grown in the growth chamber in the UK. The short maturity attributes of Pen 110 and the small grains may be a limitation in terms of responding to the treatments. In the same vein, the SAMSORG cultivar in the current project was grown in the glasshouse. Light was programmed to be switched off in the bay after 12 hours illumination. However, more light durations would have been recorded in the bay due to light from the other bays in the glasshouse and longer days would have been experienced since the plants were being grown in the summer. These altered the growth pattern of the plant as sorghum is a short day plant. At 50 days after emergence, it was observed that out of the 24 Pen110 variety, 12.5 % had flag leaves fully emerged, 50.0 % were at booting, while 37.5 % had come into flowering, whereas 100 % of the SAMSORG-40 variety were at the fifth leaf stage, and none was booting or flowering. Because of this difference in growth stage, it was decided that the two varieties be treated separately. Thus, experiments with Pen110 and SAMSORG-40 were designated as glasshouse experiments 1 and 2 respectively.

Glasshouse experiment 1 Using Pen110 cultivar

3.2. Materials and methods

3.2.1 Plant growth environment

3.2.1.1. Glasshouse environmental conditions

The glasshouse conditions comprise night temperatures of 25 ± 2 °C and day temperatures of 31 ± 2 °C, with an average day length of 12 hours and relative humidity of between 30 ± 2% and 40 ± 3 %; these were set and monitored by an automatic control system (Tomtech Ltd. Salisbury, UK). In addition to natural light received in the glasshouse, the bay was fitted with 12 400 watts SON-T lamps (Thermoforce, Cumbria, UK) that were switched on from 06:00 to 18:00 hours daily, to provide additional illumination.

3.2.1.2. Plant growth medium

The plant growth medium consists of 8.0 kg sandy loam soil collected from Crab Tree Leasow field, Harper Adams University, UK - sieved and mixed with 10 % John Innes no. 2 compost (Keith Singleton, Egremont, Cumbria, UK) with a bulk density of 1 g/cm^3 and placed plastic pots, OPTIPOT 26F (Congleton Plastic Company, Congleton, Cheshire, UK) of diameter of 13.0 cm and depth of 23.0 cm with a volume of about 3.05 litres. As at the time that the soils of Harper Adams University (52° 46' N, 2° 25 'W) were being analysed as in Beard (1988) Crab Tree Leasow was not included. However, the soils at Buttery Hill which adjoins Crab Tree Leasow were analysed. Therefore, the properties of Buttery Hill soils will be adopted for the soils used in the glasshouse experiments in this project. According to Beard (1988), the soils belongs to the Arrow Series characterised by deep permeable light loams with slightly sandy loam top soils capable of growing a wide range of crops.The organic matter is normally small leading to a weak top soil structure prone to compaction and slaking. Available water in the soil for growth during the summer is moderate to large and sufficient to sustain arable crops.

3.2.2. Antitranspirant used

The antitranspirant used in the study was di-1-*p*-menthene, also known as Vapor Gard. Vapor Gard is a non-ionic water emulsifiable concentrate consisting of 96 % di-1-*p*-menthene as active ingredient and 4 % inert materials. The product used in this project was obtained from Miller Chemicals, USA. Upon application onto the plant leaf surface, Vapor Gard dries to form a clear glossy film layer that reduces moisture loss and normal plant respiration and growth.

3.2.3. Plant material

Pen 110 (Grainseed Ltd, Suffolk UK) variety, a dwarf sorghum cultivar of average height between 100 - 150 cm with broad leaves and a substantial seed-head. It possesses excellent standing power throughout the season and is mainly used for game cover.

3.2.4. Experimental design and treatments

The experimental design was a 2 x 2 factorial with 6 replicates giving a total of 24 plants in a randomised complete block design. Drought and antitranspirant sprays were the two factors each at two levels. The two levels of drought were ‘drought’ and ‘irrigated’ and the two levels of antitranspirant sprays were ‘sprayed’ and ‘unsprayed’. Pot arrangement in the glasshouse is shown on Figure 3.2.1.

3.2.5. Sowing and thinning

Grain sorghum (*Sorghum bicolor*, L.) seeds were manually sown at the rate of 3 - 4 seeds per pot at depths of 3.0 – 5.0 cm. At three weeks after emergence the number of plants were thinned manually to one plant per pot.

3.2.6. Drought application

Drought was imposed on the plants by restricting irrigation to the ‘droughted’ pots, while irrigation was applied by unrestricted watering of the ‘irrigated’ pots. To the droughted pots, 1L of water was added manually once every other day since pot weights were not being taken. Whereas the ‘irrigated’ pots remained on drippers set to automatically deliver water at the rate of 1L per minute for 1 minute thrice daily at 07:30 hours, 12:30 hours, and 19:30 hours. Drought was initiated at 70 days after emergence, GS 5.1 – 5.9 head in flag leaf to head full size in flag leaf, and lasted for 30 days at GS 6.1 – 6.5 which were at 10 % bloom from tip of head downwards to 50 % bloom based on the growth stage numbering system in accordance to the extended BBCH (Agvita BBCH, 2008). Thereafter manual addition of water was stopped and irrigation resumed for the ‘droughted’ pots in the same manner as in the ‘irrigated’ pots. The soil analysis NRM detail is presented on Table 3.3.1., and no additional nutrients were supplied in the course of the experiment.

3.2.7. Antitranspirant application

Spraying with the antitranspirant was done at 70 days after emergence, GS 5.1 – 6.1 head in flag leaf to bloom (Agvita BBCH, 2008). Four plants at a time of the six replicates were sprayed in a custom-made precision pot sprayer with antitranspirant solution at a concentration of 0.5 v/v % using Flat Fan nozzle (FF110 – 03), at a 2-bar pressure at the rate equivalent to 200 L/ha. The nozzle of the sprayer was placed at approximately 90 cm above the flag leaf or plant canopy where the flag leaf was yet to emerge. The antitranspirant solution was applied to the whole plant, while ensuring that the flag leaves or most recently emerged leaves were adequately sprayed with the solution. Thereafter, the pots were returned to the bay and drippers for irrigation were fixed into the 'irrigated sprayed' pots. Details of main experimental operations carried out and some events during the experiment are on Table 3.2.1.

Table 3.2.1. Some significant dates and events during Expt.1.

Year	Date	Events
2015	August 12	Sowing
	August 17	Emergence
	October 27	Drought application and antitranspirant spraying
	November 27	Stopping drought and resumption of full irrigation
	December 31	Harvest

3.2.8. Statistical analysis

Data were analysed using GENSTAT, 16th edition (VSN International Ltd, Hemel Hempstead, UK) with three-way (time x drought x antitranspirant) repeated measures analysis of variance (ANOVA) for volumetric soil moisture content, stomatal conductance and leaf temperature. A two-way (drought x antitranspirant) ANOVA was conducted on data from each day of assessment where interaction with time was recorded and for yield and yield components, to provide information on effects of the treatments on each day of measurements. Data were checked for normality and variance homogeneity by examining the residual plot. Skeleton ANOVA of measurements are shown on tables ranging from Table 3.2.2 to 3.2.9.

Table 3.2.2. Skeleton ANOVA of volumetric soil moisturecontent measurements (Expt.1)

Source of variation	Degrees of freedom
Blocks stratum	5
Blocks x subject stratum	
Antitranspirant	1
Drought	1
Antitranspirant x drought	1
Residual	15
Blocks x subject x time stratum	
Time	7
Time x antitranspirant	7
Time x drought	7
Time x antitranspirant x drought	7
Residual	138
Total	189

Table 3.2.3.Skeleton ANOVA of the adaxial stomatal conductancemeasurements (Expt. 1)

Source of variation	Degrees of freedom
Blocks stratum	5
Blocks x subject stratum	
Antitranspirant	1
Drought	1
Antitranspirant x drought	1
Residual	15
Blocks x subject x time stratum	
Time	6
Time x antitranspirant	6
Time x drought	6
Time x antitranspirant x drought	6
Residual	108(12)
Total	155(12)

(Numbers in parenthesis are missing values)

Table 3.2.4.Skeleton ANOVA of the abaxial stomatal conductance measurements (Expt.1)

Source of variation	Degrees of freedom
Blocks stratum	5
Blocks x subject stratum	
Antitranspirant	1
Drought	1
Antitranspirant x drought	1
Residual	15
Blocks x subject x time stratum	
Time	6
Time x antitranspirant	6
Time x drought	6
Time x antitranspirant x drought	6
Residual	106(14)
Total	153(14)

(Numbers in parenthesis are missing values)

Table 3.2.5. Skeleton ANOVA of the total stomatal conductancemeasurements (Expt.1)

Source of variation	Degrees of freedom
Blocks stratum	5
Blocks x subject stratum	
Antitranspirant	1
Drought	1
Antitranspirant x drought	1
Residual	15
Blocks x subject x time stratum	
Time	6
Time x antitranspirant	6
Time x drought	6
Time x antitranspirant x drought	6
Residual	115(5)
Total	162(5)

(Numbers in parenthesis are missing values)

Table 3.2.6. Skeleton ANOVA of leaf temperature measurements (Expt.1)

Source of variation	Degrees of freedom
Blocks stratum	5
Blocks x subject stratum	
Antitranspirant	1
Drought	1
Antitranspirant x drought	1
Residual	15
Blocks x subject x time stratum	
Time	5
Time x antitranspirant	5
Time x drought	5
Time x antitranspirant x drought	5
Residual	91(9)
Total	134(9)

(Numbers in parenthesis are missing values)

Table 3.2.7. Skeleton ANOVA of relative water contentmeasurements (Expt.1)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x units stratum	
Antitranspirant	1
Drought	1
Antitranspirant x drought	1
Residual	9
Total	15

Table 3.2.8. Skeleton ANOVA of plant height measurements (Expt.1)

Source of variation	Degrees of freedom
Blocks stratum	5
Blocks x units stratum	
Drought	1
Antitranspirant	1
Drought x antitranspirant	1
Residual	15
Total	23

Table 3.2.9. Skeleton ANOVA yield and yield components measurements (Expt.1)

Source of variation	Degrees of freedom
Blocks stratum	5
Blocks x unit stratum	
Antitranspirant	1
Drought	1
Antitranspirant x drought	1
Residual	15
Total	23

3.2.9. Measurements

3.2.9.1. Volumetric soil moisture content

Soil moisture content was taken by inserting the TDR (Fieldscout TDR 100/200 Soil Moisture Meter, Spectrum Technologies, Inc., USA) probe into the soil in each pot and recording the readings as percent moisture content, that is volumetric soil moisture content (%).

3.2.9.2. Field capacity and permanent wilting point

To determine the field capacity of soils for the experiment, ten 10-inch pots were filled with a mixture of field soil and 10 % John Innes No.2 compost (Keith Singletons Horticultural products, Cumbria, UK). Each pot was ‘saturated’ with water and placed on a saucer to enable free drainage and the top covered with a saucer to prevent water loss via evaporation. Thereafter, volumetric soil moisture contents (%) and weights (g) of the individual pots were taken at 0, 2, 4, 24, 48 and 96 hours after initial ‘saturation’ using soil moisture probe and weighing balance. After taking these measurements, moisture contents in the ten pots averaged 22 % to 25 % by volume, this was used as a benchmark volumetric soil moisture content at which the well-watered pots will be kept, which is the ‘field capacity’ and from which the ‘permanent wilting point’ was determined. The ‘permanent wilting point’ was determined to be a moisture content of around 50 % of the well-watered pots. This was between 11.0 % and 12.2 % which would fairly stress the soil and was set as the moisture content for the droughted pots. Therefore, the available moisture content was calculated to be between 11.0 % and 12.8 %. Thus water stress is expected to be initiated at a volumetric soil moisture content of between 50 to 70 % of the available water which equals 5.5 % to 7.7 % and 6.4 % to 8.9 %. No reference known to the author has been used to determine permanent wilting point this way.

3.2.9.3. Stomatal conductance

Stomatal conductance (mmol m^{-2} s^{-1}) was measured using a porometer (AP4 Delta –T Devices, UK). Prior to each measurement session, the porometer was calibrated using the calibration plate provided. Data were collected between 09:30 am and 12:30 am on all plants in the experiment. Measurements were taken from the adaxial and abaxial surfaces of the flag leaf or the most recently emerged leaf from the top of the plant, where the flag leaf was yet to emerge. Four measurements were taken per plant; two from the adaxial and two from the abaxial surfaces at two different points around the middle of the leaf avoiding the mid-rib. Adaxial stomatal conductance per plant was calculated as the average of the two measurements on the adaxial leaf surface and abaxial stomatal conductance per plant as the average of the two values of stomatal conductance from the abaxial surface of the leaf. However, the total stomatal conductance per plant is the average of the adaxial and abaxial stomatal conductance.

3.2.9.4. Leaf temperature

This was measured using a handheld infrared thermometer (Omega Engineering, Inc., Stamford Connecticut, USA). Measurements were taken between 10.00 am – 1.00 pm on all plants from the adaxial surface of the flag leaf or the most recently emerged leaf from the top of the plant where the flag leaf was yet to emerge. Two different spots were randomly chosen around the middle of the leaf for data collection. The infrared thermometer was manually pointed at right angles to the leaf blade, but not on the mid-rib, at a distance of 15 cm and the resulting measurement on the thermometer recorded. Leaf temperature (˚C) per plant was calculated as the mean of the two measurements.

3.2.9.5. Relative water content

Relative water conten was measured at 31 days after spraying. Fresh leaf samples obtained from the fifth leaf from the bottom of the sorghum plant were excised from 16 plants selected randomly, representing four plants from each treatment using a 10 cm diameter disc. Each leaf disc was weighed on an electric balance (Precisa XT 1220M, Precisa Instruments Ltd., Switzerland) to get the fresh leaf weight (FW), and immediately soaked in distilled water in petri dishes and left for for 12 hours in the refridgerator and then removed and gently wiped with a blotting paper. Thereafter, the leaf discs were weighed again and the turgid leaf weight (TW) was recorded. The leaf discs were then oven dried at 80 ^{o}C for 24 hours after which they were weighed to obtain the oven dried leaf weights (DW). In this way the dry weight, the turgid weight and dry weight of the samples were obtained and relative water content was computed as follows in accordance with Weatherly (1950).

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} \times 100$$

3.2.9.6. Plant height

Plant height (cm) was taken from all plants in the experiment using a measuring tape. The tape was placed at the base of the plant above the ground surface and pulled upwards to the tip of the flag leaf; the flag leaf was extended upwards by hand.

3.2.9.7. Yield and yield components

At harvest each panicle was cut from the main stem at the base leaving the stalk in place and thereafter the remaining stalks were each cut at the base near the ground surface. The stalks were shredded in a mechanical shredder (Viking GE 150 Shredder VIKING GmbH, Austria) and the resulting biomass were oven dried in a convection oven at 70˚C to a nearly constant weight. Similarly, the panicles were threshed in an electric thresher (Wintersteiger, Austria) and the grains separated from the spikes. The grains were oven dried at 70˚C in a convection oven until nearly constant weight and then counted by a grain counter (CountAmatic Console, Farm-Tec Whitby, UK). The yield and yield components per plant comprising grain yield, seed number, weight per seed, stalk weight, biomass and harvest index were computed as described by Sylvester-Bradley, *et al*. (1985).

Figure 3.2.1.Sorghum growing in pots in the glasshouse (Expt. 1)

3.3. Results

3.3.1. Soil moisture content and properties

3.3.1.1. Field capacity and permanent wilting point*:* The soil had a volumetric soil moisture content at field capacity of 22.0 % and at permanent wilting point of 11.0 %.

3.3.1.2. Soil analysis*:* The result of soil analysis of the soil is presented on Table 3.3.1.

Table 3.3.1. Result of soil analysis (standard) on 1.00 kg of the soil taken from a depth of 0 – 50 cm at Crab Tree Leasow, Harper Adams University (52°46' N, 2°25' W) for Expts. 1 and 2.

Chemical property	Values obtained	Date of analysis
Soil pH	6.8	16thSeptember, 2015
Available P_2O_5	126 kg/ha	
Available K_2O	384 kg/ha	
Available Mg_2O_2	86.6 kg/ha	

3.3.2. Volumetric soil moisture content

The results from the analysis of data on response of volumetric soil moisture content to drought imposition and antitranspirant application and their interactions is presented in Table 3.3.2 and Figure 3.3.1. There was a significant difference in volumetric soil moisture content of pots between the droughted and irrigated treatments. Drought imposed significantly ($P < 0.001$) reduced mean volumetric soil moisture content from 33.7 % in the irrigated to 17.8 % in the droughted pots. Measurement of air filled porosity was not carried out, but soil water content in the droughted pots was about 2 % of the available water capacity. There was no significant difference in the volumetric soil moisture content of pots between the unsprayed and sprayed treatments. There was no significant drought x antitranspirant interaction. However, there was a significant effect of time ($P < 0.001$) because the overall mean volumetric water content was lower at the end of the measurement period than at the beginning. The time x drought ($P < 0.001$) interaction was significant as the drought effect was less at the last date of measurement. The two-way analysis of variance for each day after spraying (DAS) (Table 3.3.3) showed that the significant time x antitranspirant ($P = 0.039$) interaction occurred because there were significant differences (reduction) in volumetric soil moisture content between the unsprayed and sprayed treatments on only two occasions at the beginning of the measurement period: at 3 DAS ($P = 0.054$) and 6 DAS ($P = 0.013$). Drought x antitranspirant x time interaction effects was not significant.

Table 3.3.2. Average volumetric soil moisture content (%) of pots under drought and antitranspirant applied at 71 days after emergence of sorghum during booting to flowering from October to November 2015 (n = 24) ± Standard error of the differences of the mean (SED) (Expt.1).

	Days after spraying								
Treatments	3	6	9	12	15	18	21	28	Mean
Irrigated	35.34	34.93	34.23	35.94	32.28	34.99	34.72	27.32	33.72
Droughted	22.15	18.91	16.98	16.78	16.58	16.84	16.57	17.53	17.79
Unsprayed	29.78	28.68	26.16	26.79	24.95	25.6	26.08	21.17	26.15
Sprayed	27.71	25.15	25.06	25.93	23.91	26.23	25.20	23.68	25.36
Irrigated unsprayed	35.98	36.25	34.58	36.92	33.28	34.48	35.13	25.30	33.99
Irrigated sprayed	34.70	33.60	33.88	34.97	31.27	35.5	34.31	29.33	33.44
Droughted unsprayed	23.58	21.12	17.73	16.67	16.62	16.72	17.03	17.03	18.31
Droughted sprayed	20.72	16.70	16.23	16.90	16.55	16.97	16.10	18.02	17.27
Mean	28.75	26.92	25.61	26.36	24.43	25.92	25.64	22.42	25.76
df									138
SED									2.684
CV (%)									10.4
P values									
Drought									< 0.001
Antitranspirant									0.095
Drought x antitranspirant									0.589
Time									< 0.001
Time x drought									< 0.001
Time x antitranspirant									0.039
Time x drought x antitranspirant									0.611

Table 3.3.3 Two-way analysis of variance on the effect of irrigation, drought and antitranspirants on volumetric soil moisture content of pots during booting to flowering from October to November, 2015 (n = 24) (Expt.1).

	Days after spraying							
	3	6	9	12	15	18	21	28
P values								
Drought	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	< 0.001	< 0.001
Antitranspirant	0.054	0.013	0.388	0.522	0.151	0.54	0.418	0.124
Drought x antitranspirant	0.437	0.495	0.751	0.417	0.177	0.71	0.865	0.337
SED								
Drought	0.991	1.262	1.239	1.309	0.688	1.011	0.925	1.538
Antitranspirant	0.991	1.262	1.239	1.309	0.688	1.011	0.925	1.538
Drought x antitranspirant	1.401	1.785	1.752	1.852	0.974	1.430	1.308	2.176
df	15	15	15	15	15	15	13	15

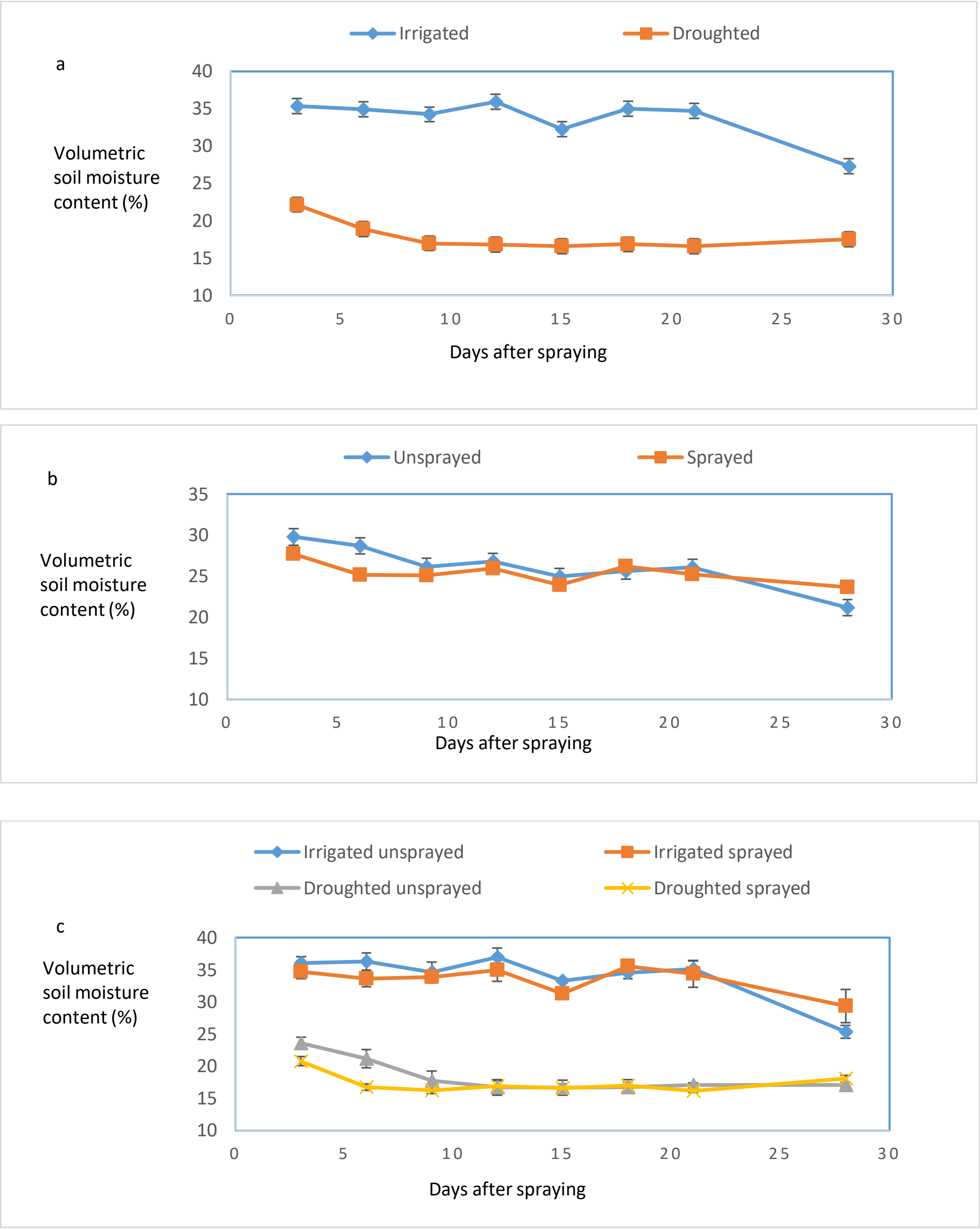

Figure 3.3.1. Response of volumetric soil moisture content (%) to (a) drought (b) antitranspirant and (c) drought x antitranspirant interaction during booting to flowering of sorghum grown in the glasshouse from October to November 2015. (Drought, $P < 0.001$; antitranspirant, $P = 0.095$; drought x antitranspirant, $P = 0.498$) Error bars are SEM ($n = 24$) (Expt.1).

3.3.3. Adaxial Stomatal conductance

Results of data analysis on response of adaxial stomatal conductance to drought and antitranspirant application and their interactions are shown on Tables 3.3.4 and Figure 3.3.2.

Effect of drought and antitranspirant factors on adaxial stomatal conductance over time showed drought had significant ($P = 0.005$) effect on adaxial stomatal conductance leading to a reduction of 15.6 % in the droughted compared with the irrigated treatments; while the antitranspirant effect was not significant. The drought x antitranspirant ($P = 0.005$) interaction effects on adaxial stomatal conductance was however significant as there was a 13.6 % increase in the irrigated sprayed over the unsprayed and a 20.4 % reduction in the droughted sprayed compared with the unsprayed treatments. There was a significant effect of time ($P < 0.001$) on the measurement as the adaxial stomatal conductance decreased with increase in days after spraying (DAS). Two-way analysis of variance on individual DAS (Table 3.3.5) showed that the significant time x drought ($P = 0.031$) interaction effects, was recorded due to significant differences (reduction) in adaxial stomatal conductance between droughted and irrigated treatments at 9 DAS ($P = 0.001$). Although the interaction effects of time x antitranspirant was not significant from the repeated measures analysis of variance, the two-way analysis of variance indicated that the antitranspirant application did induce significant differences in adaxial stomatal conductance between the unsprayed and sprayed treatments on two occasions at the end of measurement period as adaxial stomatal conductance was significantly reduced at 24 DAS ($P = 0.030$) and 26 DAS ($P = 0.026$). The time x drought x antitranspirant interaction effects were not significant.

Table 3.3.4. Average adaxial stomatal conductance (mmols $m^{-2} s^{-1}$) recorded on the flag leaf under drought and antitranspirant applied at 71 days after emergence of sorghum grown in the glasshouse from October to November 2015 measured at 4, 9, 13, 16, 22, 24 and 26 days after spraying (n = 24) ± Standard error of the differences of the mean (SED)(Expt.1).

	Days after spraying							
Treatments	4	9	13	16	22	24	26	Mean
Irrigated	48.72	54.20	29.79	25.05	23.05	22.27	15.10	31.17
Droughted	54.31	31.18	24.70	22.52	22.48	17.19	11.87	26.32
Unsprayed	47.31	44.31	25.70	23.47	25.84	22.42	15.66	29.24
Sprayed	55.72	41.07	28.79	22.06	21.72	17.04	11.31	28.24
Irrigated unsprayed	38.86	48.33	25.75	26.77	23.05	24.84	16.69	29.18
Irrigated sprayed	58.58	60.08	33.83	23.33	23.04	19.7	13.51	33.15
Droughted unsprayed	55.75	40.30	25.66	24.92	23.88	20.00	14.63	29.31
Droughted sprayed	52.86	22.07	23.74	20.12	21.07	14.38	9.11	23.34
Means	51.51	42.69	27.25	23.53	23.02	19.73	13.49	28.74
df								108
SED								11.954
CV %								41.6
								Pvalues
Drought								0.005
Antitranspirant								0.513
Drought x antitranspirant								0.005
Time								< 0.001
Time x antitranspirant								0.374
Time x drought								0.031
Time x drought x antitranspirant								0.238

Table 3.3.5. Two-way analysis of variance on the effect of drought and antitranspirants applied at 71days after emergence on adaxial stomatal conductance recorded on the flag leaf measured at 4, 9, 13, 16, 22, 24 and 26 days after spraying during booting to flowering of sorghum grown in the glasshouse from October to November, 2015 (n = 24) (Expt.1).

	Days after spraying						
	4	9	13	16	22	24	26
P values							
Drought	0.093	0.001	0.579	0.803	0.330	0.140	0.148
Antitranspirant	0.295	0.771	0.317	0.837	0.280	0.030	0.026
Drought x antitranspirant	0.099	0.026	0.129	0.660	0.401	0.985	0.693
SED							
Drought	2.84	5.37	5.97	4.26	2.57	1.294	1.541
Antitranspirant	2.84	5.37	5.97	4.26	2.57	1.294	1.641
Drought x antitranspirant	4.02	7.60	8.44	6.02	3.63	1.83	2.321
df	15	14	12	13	12	13	14

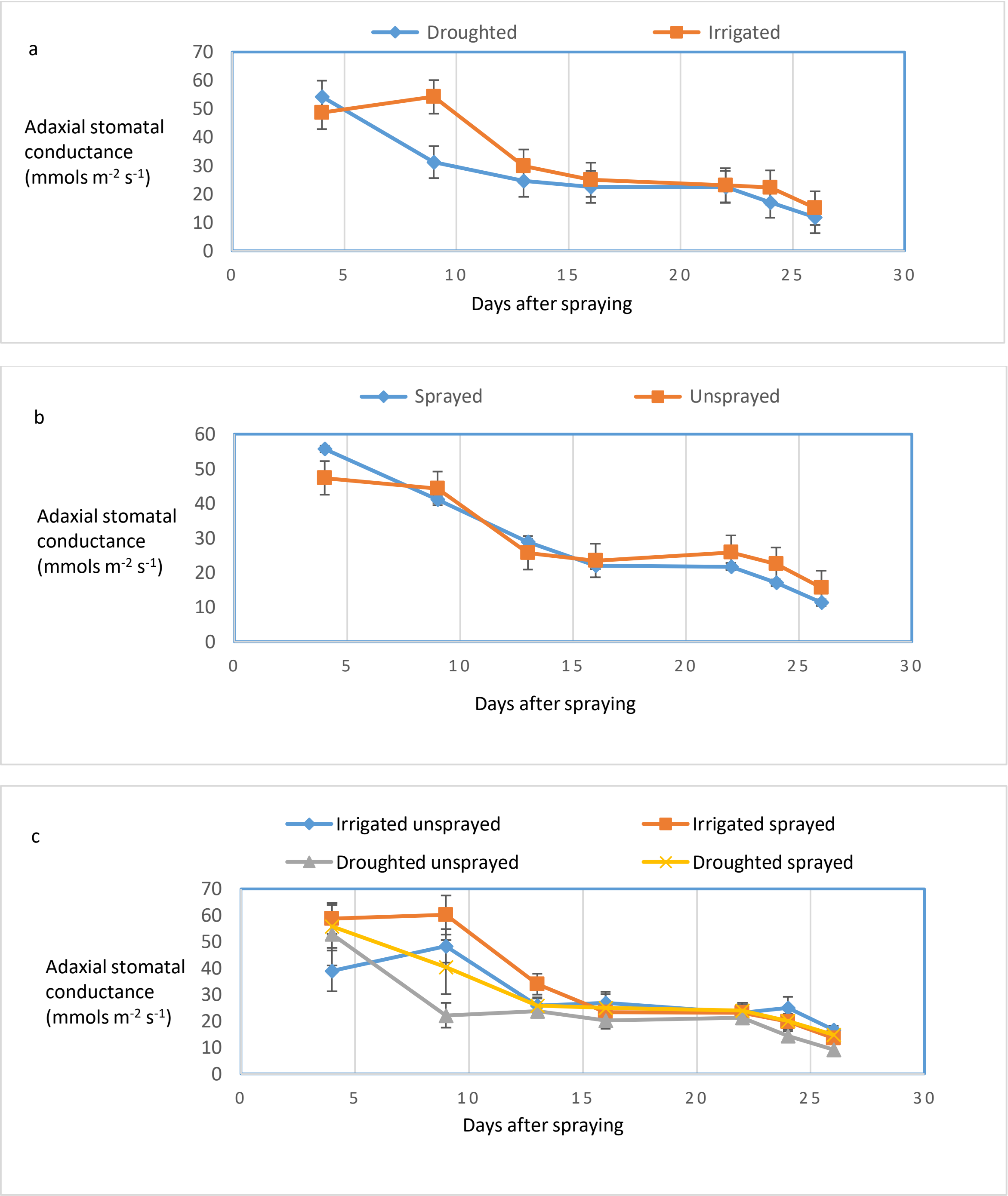

Figure 3.3.2. Response of adaxial stomatal conductance (mmol m^{-2} s^{-1}) of sorghum to (a) drought (b) antitranspirant and (c) drought x antitranspirant interaction recoreded on the flag leaf at 71 days after emergence grown in the glasshouse during booting to flowering from October to November 2015 (n = 24). (Drought, P = 0.005; antitranspirant, P = 0.513; drought x antitranspirant, P = 0.005). Error bars are SEM (Expt.1).

3.3.4. Abaxial Stomatal conductance

Results from analysis of data on response of abaxial stomatal conductance to drought and antitranspirant application and their interactions are shown on Table 3.3.6 and Figure 3.3.3. There was a significant difference in abaxial stomatal conductance between the droughted and irrigated plants. The drought significantly ($P < 0.001$) decreased abaxial stomatal conductance by 26.5 % in the droughted compared with the irrigated treatments on average over the time measurements were taken while the antitranspirant treatments caused no significant differences in abaxial stomatal conductance. However, the drought x antitranspirant interaction effects on abaxial stomatal conductance was significant ($P = 0.027$). In the irrigated treatments, antitranspirant increased abaxial stomatal conductance by 12.3 % in the sprayed over the unsprayed treatments, while in the droughted treatments abaxial stomatal conductance was reduced by 22.3 % in the sprayed compared with the unsprayed treatment. A significant time ($P < 0.001$) effect on abaxial stomatal conductance led to differences in the measurements between the days showing a greater conductance at the beginning than at the end of the measurement periods. Results from a Two-way analysis of variance on different days after spraying (DAS) (Table 3.3.7) showed that the significant time x drought ($P < 0.010$) interaction effects occurred because drought induced significant differences in abaxial stomatal conductance between the irrigated and droughted treatments at some points in the measurements as conductance was reduced at 9 DAS ($P < 0.001$), 13 DAS ($P < 0.001$), 16 DAS ($P < 0.001$), 24 DAS ($P < 0.024$) and 26 DAS ($P < 0.001$) in the droughted compared with the irrigated treatments. Also, a significant time x antitranspirant ($P < 0.032$) interaction effect resulted as the antitranspirant application gave rise to significant differences in abaxial stomatal conductance between the unsprayed and sprayed treatments with significant reductions at 13 DAS ($P < 0.039$) and 16 DAS ($P < 0.039$) in the sprayed compared with the unsprayed treatments. However, no significant time x drought x antitranspirant interaction effect on abaxial stomatal conductance was shown.

Table 3.3.6. Average abaxial stomatal conductance (mmols $m^{-2}s^{-1}$) recorded on the flag leaf under drought and antitranspirant applied at 71 days after emergence of sorghum grown in the glasshouse from October to November 2015 measured at 4, 9, 13, 16, 22, 24 and 26 days after spraying (n = 24) ± Standard error of the differences of the mean (SED) (Expt.1).

	Days after spraying							
Treatments	4	9	13	16	22	24	26	Means
Irrigated	61.61	48.36	51.47	61.61	38.97	39.45	38.92	48.63
Droughted	34.22	42.80	55.29	34.22	23.38	28.29	32.15	35.76
Unsprayed	54.31	40.94	48.06	54.31	34.30	35.94	33.38	43.03
Sprayed	41.51	50.22	58.70	41.51	28.05	31.80	37.69	41.35
Irrigated unsprayed	60.51	40.81	39.68	60.51	40.07	45.03	34.11	45.82
Irrigated sprayed	62.71	37.13	57.03	62.71	38.83	57.92	43.73	51.44
Droughted unsprayed	48.12	27.79	42.2	48.12	31.82	51.08	32.66	40.26
Droughted sprayed	20.32	18.96	43.4	20.32	24.77	59.49	31.64	31.27
Means	47.91	38.38	49.48	47.91	32.52	43.63	35.54	42.20
df								106
SED								12.749
CV %								30.2
								P values
Antitranspirant								0.581
Drought								< 0.001
Drought x antitranspirant								0.027
Time								< 0.001
Time x antitranspirant								0.032
Time x drought								0.010
Time x antitranspirant x drought								0.325

Table 3.3.7. Two-way analysis of variance on the effect of drought and antitranspirants applied at 71 days after emergence on abaxial stomatal conductance recorded on the flag leaf measured at 4, 9, 13, 16, 22, 24 and 26 days after spraying during booting to flowering of sorghum grown in the glasshose from October to November, 2015 (n = 24) (Expt.1).

	Days after spraying						
	4	9	13	16	22	24	26
P values							
Drought	0.408	< 0.001	< 0.001	< 0.001	0.825	0.046	< 0.001
Antitranspirant	0.191	0.140	0.039	0.039	0.213	0.360	0.100
Drought x antitranspirant	0.307	0.590	0.018	0.018	0.748	0.096	0.348
SED							
Drought	7.12	3.62	5	5	6.27	3.51	1.562
Antitranspirant	7.12	3.62	5	5	6.27	3.51	1.562
Drought x antitranspirant	10.08	5.13	7.07	7.07	8.86	4.96	2.209
df	14	14	12	12	121	13	14

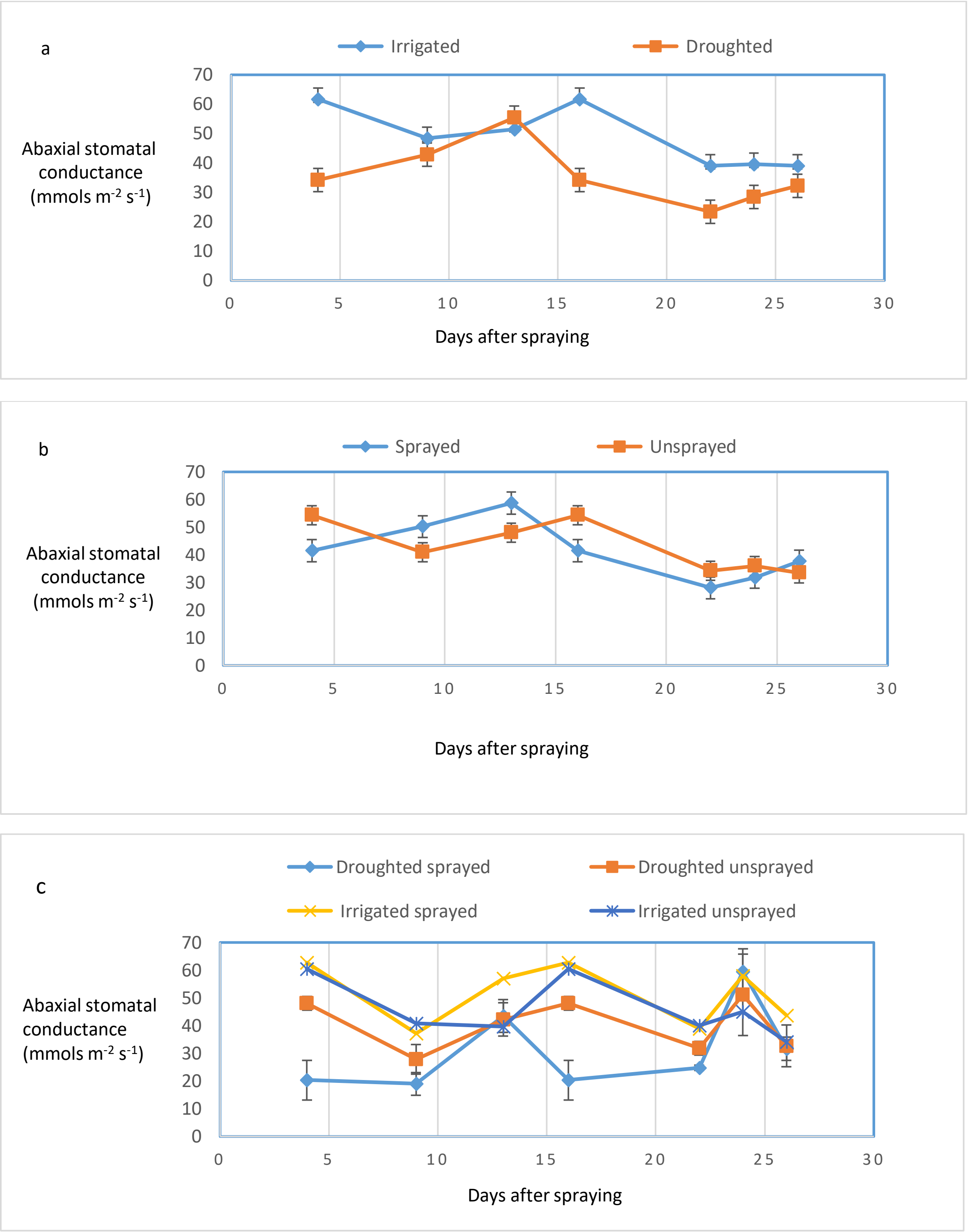

Figure 3.3.3. Response of abaxial stomatal conductance (mmol m^{-2} s^{-1}) of sorghum to (a) drought (b) antitranspirant and (c) drought x antitranspirant interaction recorded on the flag leaf at 71 days after emergence during booting to flowering grown in the glasshouse, from October to November 2015. (Drought, $P < 0.005$; antitranspirant, $P=0.581$; drought x antitranspirant $P = 0.027$) Error bars are SEM ($n = 24$) (Expt.1).

3.3.5. Total stomatal conductance

Data on response of total stomatal conductance to drought and antitranspirant application and their interactions is shown on Table 3.3.8 and Figure 3.3.4.

There were significant differences in total stomatal conductance between droughted and irrigated treatments, whereas there were no significant differences in total stomatal conductance between the unsprayed and sprayed plants with antitranspirant application. Drought ($P < 0.001$) significantly decreased total stomatal conductance by 21.8 % in the droughted compared with the irrigated treatments. However, drought x antitranspirant interaction effects on total stomatal conductance was significant ($P = 0.002$) as the antitranspirant increased total stomatal conductance by 16.7 % in the irrigated sprayed over the unsprayed and decreased it with 13.3 % in the sprayed compared with the unsprayed droughted treatments. There was a significant effect of time ($P < 0.001$) on the total stomatal conductance being progressively reduced with time from the first to the last day of measurement. Two-way analysis of variance (Table 3.3.9) showed interaction effects of time x drought (0.044) was significant because drought imposition resulted into significant differences in total stomatal conductance between the irrigated and droughted treatments with significant decreases in total stomatal conductance on 9 DAS ($P < 0.001$), 12 DAS ($P < 0.001$), 17 DAS ($P < 0.014$), 23 DAS ($P < 0.030$) and 27 DAS ($P < 0.001$) in the droughted compared with the droughted treatments. The time x antitranspirant interaction effect on total stomatal conductance was not significant.

Table 3.3.8. Average total stomatal conductance (mmol m^{-2} s^{-1}) recoreded on the flag leaf under drought and antitranspirant applied at 71 days after emergence of sorghum grown in the glasshouse from October to November 2015 measured at 4, 9, 13, 16, 22, 24 and 26 days after spraying during booting to flowering (n = 24) ± Standard error of the differences of the mean (SED) (Expt.1).

	Days after spraying							
Treatments	4	9	13	16	22	24	26	Means
Irrigated	57.16	46.59	39.07	44.22	32.25	36.01	27.01	40.33
Droughted	45.89	27.97	32.51	28.47	26.10	37.48	22.51	31.56
Unsprayed	52.81	39.31	32.08	38.89	30.89	34.37	24.52	36.12
Sprayed	50.25	35.25	39.50	33.80	27.45	39.11	25.00	35.77
Irrigated unsprayed	53.68	44.57	32.72	41.78	31.08	33.2	25.40	37.82
Irrigated sprayed	60.65	48.61	45.43	46.67	33.42	38.81	28.62	42.84
Droughted unsprayed	51.93	34.05	31.45	36.00	28.37	35.54	23.65	34.43
Droughted sprayed	39.85	21.89	33.57	20.94	23.82	39.41	21.37	28.69
Means	51.53	37.28	35.79	36.35	29.17	36.74	24.76	35.95
df								115
SED								9.734
CV %								27.1
P values								
Antitranspirant								0.811
Drought								< 0.001
Drought x antitranspirant								0.002
Time								< 0.001
Time x antitranspirant								0.241
Time x drought								0.044
Time x antitranspirant x drought								0.409

Table 3.3.9. Two-way analysis of variance on the effect of drought and antitranspirants applied at 71 days after emergence on total stomatal conductance recorded on the flag leaf measured at 4, 9, 13, 16, 22, 24 and 26 days after spraying during booting to flowering of sorghum grown in the glasshouse from October to November, 2015 (n = 24) (Expt.1).

	Days after spraying						
	4	9	13	16	22	24	26
P values							
Drought	0.169	< 0.001	0.014	< 0.001	0.665	0.030	< 0.001
Antitranspirant	0.123	0.307	0.537	0.139	0.260	0.967	0.008
Drought x antitranspirant	0.61	0.042	0.033	0.008	0.936	0.141	0.346
SED							
Drought	4.54	3.42	4.05	3.25	4.46	2.00	1.11
Antitranspirant	4.54	3.42	4.05	3.52	4.46	2.00	1.12
Drought x antitranspirant	6.42	4.84	5.73	4.60	6.31	2.83	1.58
df	15	14	15	15	14	13	14

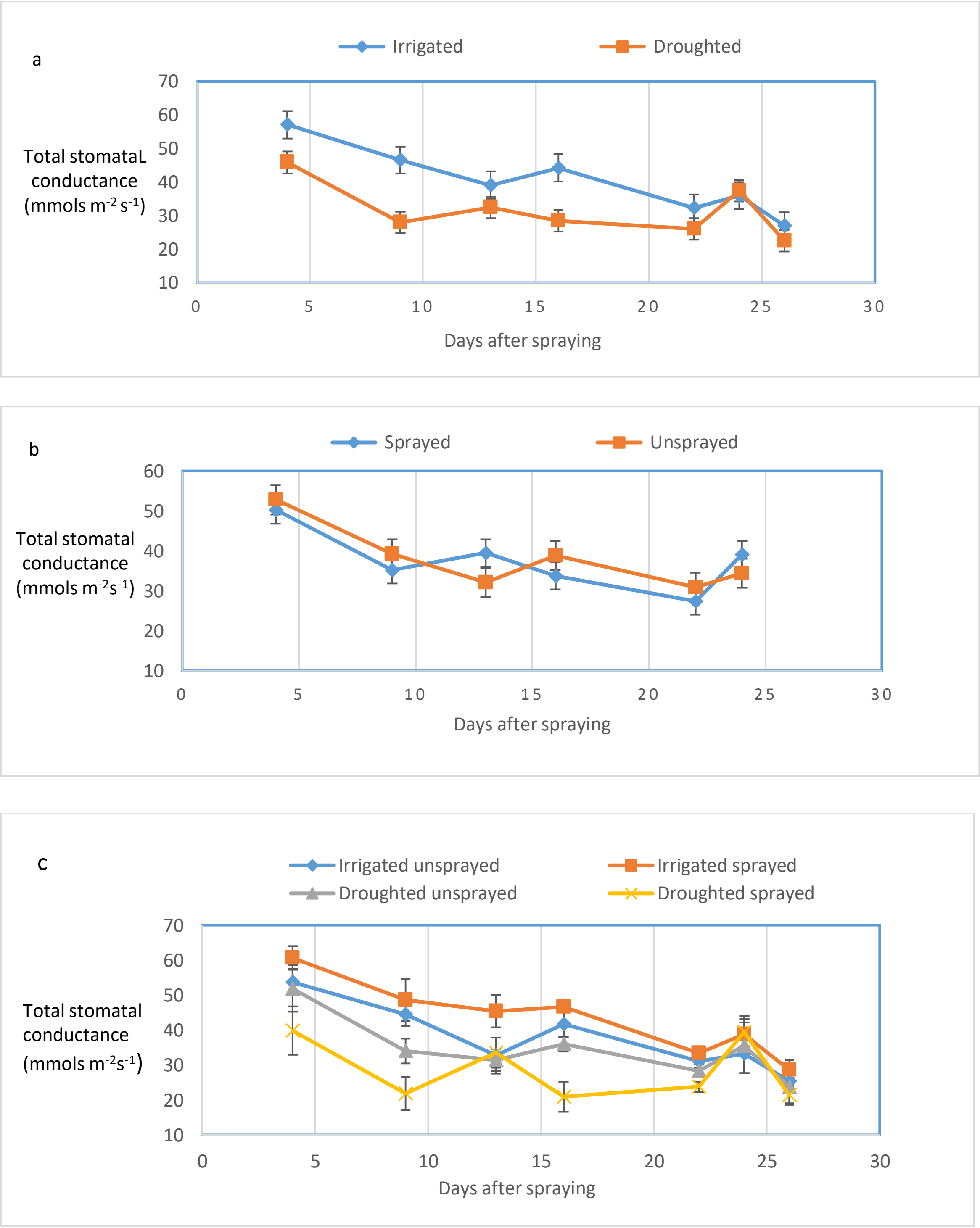

Figure 3.3.4. Response of total stomatal conductance (mmol m^{-2} s^{-1}) of sorghum to (a) drought (b) antitranspirant and (c) drought x antitranspirant interaction recorded on the flag leaf at 71 days after emergence during booting to flowering grown in the glasshouse from October to November 2015. (Drought, $P < 0.001$; antitranspirant, $P = 0.811$; drought x antitranspirant, $P = 0.002$) Error bars are SEM ($n = 24$) (Expt.1).

3.3.6. Leaf temperature

Tables 3.3.10, 3.3.11 and figure 3.3.5 shows results of analysis of response of leaf temperature to drought and antitranspirant treatments and the interaction effects. There were no significant differences in leaf temperature between the droughted and the irrigated and between the unsprayed and sprayed treatments. Similarly, the drought x antitranspirant interaction effect on leaf temperature was not significant. However, there was a significant effect of time on leaf temperature (P = 0.023) as the leaf temperature increased with increase in DAS and was lower at the beginning than at the end of the measurement period. The time x drought, as well as the time x antitranspirant and the time x drought x antitranspirant interaction effects on leaf temperature were not significant.

Table 3.3.10. Average leaf temperature (˚C) recorded on the flag leaf under drought and antitranspirant applied at 71 days after emergence of sorghum grown in the glasshouse from October to November 2015 measured at 4, 9, 13, 16, 22 and 24 days after spraying during booting to flowering (n = 24) ± Standard error of the differences of the mean (SED)(Expt.1).

	Days after spraying						
Treatments	4	9	13	16	22	24	Mean
Irrigated	26.78	26.65	26.04	25.11	23.14	26.42	25.69
Droughted	28.19	26.59	26.59	25.51	25.93	25.83	26.44
Unsprayed	27.56	26.25	25.92	24.88	24.55	25.53	25.78
Sprayed	27.41	26.99	26.72	25.73	24.52	26.73	26.35
Irrigated sprayed	26.07	26.60	26.23	25.32	23.11	27.47	25.80
Irrigated unsprayed	27.48	26.70	25.85	24.89	23.17	25.37	25.58
Droughted sprayed	28.75	27.38	27.20	26.15	25.93	25.98	26.90
Droughted unsprayed	27.63	25.80	25.98	24.88	25.93	25.68	25.99
Means	24.78	26.62	26.32	25.31	24.53	26.13	26.06
df							91
SED							2.41
CV %							9.2
							P values
Drought							0.165
Antitranspirant							0.288
Drought x antitranspirant							0.513
Time							0.023
Time x drought							0.240
Time x antitranspirant							0.727
Time x antitranspirant x drought							0.557

Table 3.3.11. Two-way analysis of variance on the effect of drought and antitranspirants applied at 71 days after emergence on leaf temperature (˚C) recorded on the flag leaf measured at 4, 9, 13, 16, 22 and 24 days after spraying during booting to flowering of sorghum grown in the glasshouse from October to November, 2015. (n = 24) (Expt.1).

	Days after spraying					
Treatments	4	9	13	16	22	24
P values						
Drought	0.116	0.956	0.483	0.764	0.010	0.475
Antitranspirant	0.862	0.500	0.312	0.282	0.884	0.227
Drought x antitranspirant	0.156	0.594	0.419	0.833	0.331	0.442
SED						
Drought	0.848	0.765	1.313	0.878	1.165	1.066
Antitranspirant	0.848	0.765	1.313	0.878	1.165	1.066
Drought x antitranspirant	1.199	1.082	1.856	1.241	1.648	1.508
df	15	15	8	14	14	15

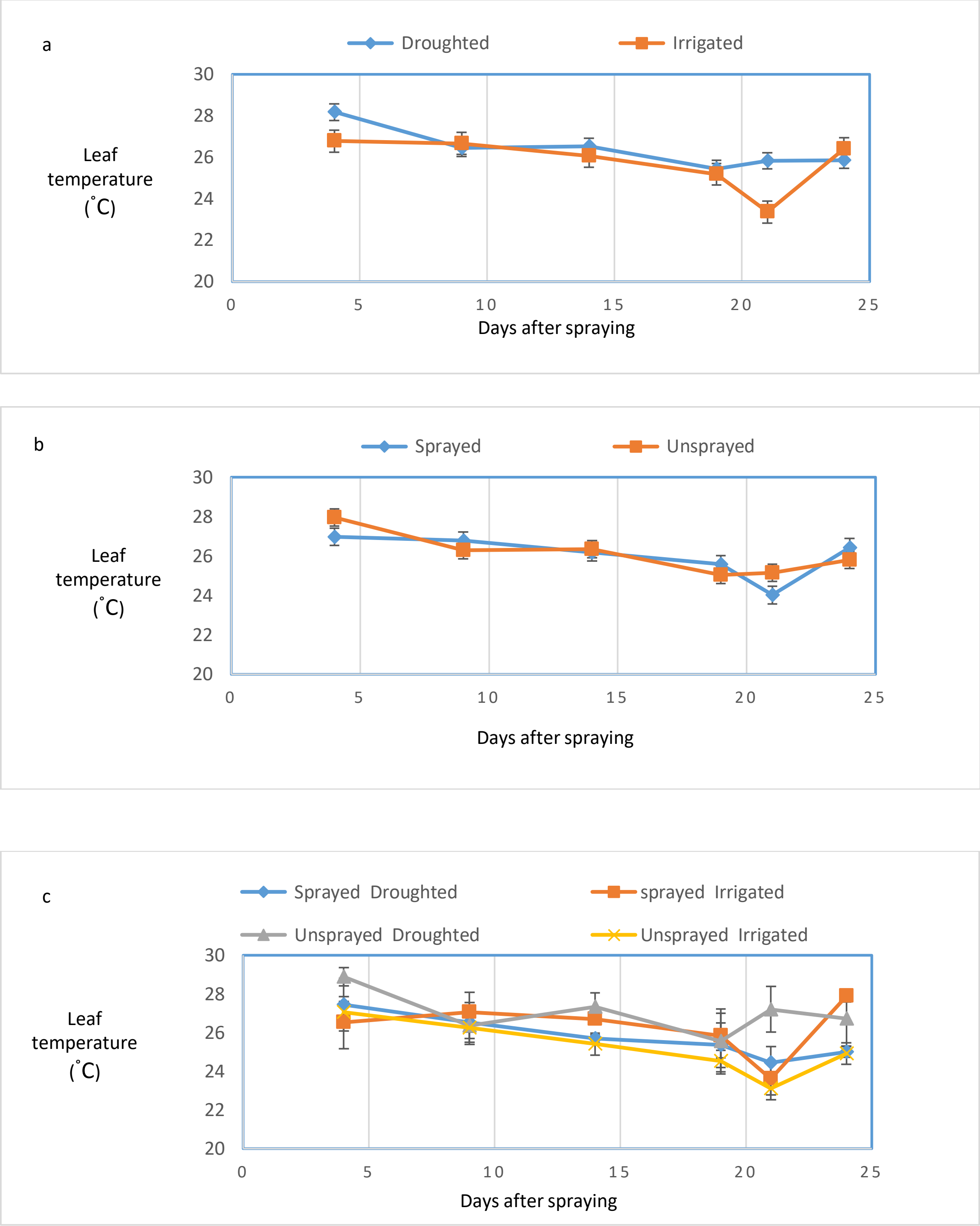

Figure 3.3.5. Response of leaf temperature (˚C) to (a) drought (b) antitranspirant and (c) drought x antitranspirant interaction recorded on the flag leaf at 71 days after emergence during booting to flowering of sorghum grown in the glasshouse from October to November 2015 (Drought, P < 0.285; antitranspirant, P = 0.426; drought x antitranspirant, P = 0.267). Error bars are SEM (n = 24) (Expt.1).

3.3.7. Relative water content and plant height

Results of analysis with regard to relative water content and plant height upon drought imposition and antitranspirant application is shown on Table 3.3.12.

Differences in relative water content between the droughted and irrigated treatments were observed with the effect of drought being close to significance (P = 0.061) and leading to a 9.3 % decrease in relative water content in the droughted compared with the irrigated treatments. There was no significant interaction effect of drought x antitranspirant on relative water content. With regards to plant height, there were significant differences between droughted and irrigated treatments. Drought (P = 0.01) significantly decreased plant height by 11.7 % in the droughted compared with the irrigated treatments, while the antitranspirant induced no significant differences in plant height between the unsprayed and sprayed treatments. There was no significant drought x antitranspirant interaction effects on plant height.

Table 3.3.12. Average relative water content (%) recoreded on the fifth leaf from the bottom of sorghum taken at 31 and plant height (cm) taken at 24 days after spraying during booting to flowering in the glasshouse from October to November 2015 (Relative water content, n = 6; Plant height, n = 24) ± Standard error of the differences of the mean (SED) (Expt.1).

Treatments	Relative water content (%)	Plant height (cm)
Irrigated	58.00	83.2
Droughted	52.60	73.5
Unsprayed	56.60	78.2
Sprayed	54.00	78.5
Droughted sprayed	53.40	75.0
Droughted unsprayed	51.80	72.0
Irrigated sprayed	54.60	82.0
Irrigated unsprayed	61.30	84.3
Mean	55.28	78.3
Df	9	15
SED	4.97	8.03
CV %	9.0	10.3
	P values	P values
Drought	0.061	0.010
Antitranspirant	0.336	0.920
Drought x antitranspirant	0.133	0.429

3.3.8. Yield and yield components

The results of the yield and yield components of sorghum resulting from drought imposition and antitranspirant application and their interactions are shown on Table 3.3.13.

Drought application caused no significant differences in grain yield and grain number per plant, weight per grain, and stalk weight per plant, biomass and harvest index per plant between droughted and irrigated treatments. Similarly, antitranspirant treatments did not produce any significant differences between the unsprayed and sprayed treatments; and the drought x antitranspirant interaction effects were also not significant for yield and all yield components. As per grain number per plant, there was no significant difference caused by drought between the droughted and irrigated treatments. Whereas the antitranspirant did not cause any significant differences between droughted and irrigated treatments and there was no significant drought x antitranspirant interaction effects on grain number per plant. The results also showed no significant differences in weight per grain between the droughted and irrigated treatments and between the unsprayed and sprayed treatments and there was no significant drought x antitranspirant interaction effect on weight per grain. In addition, stalk weight per plant was not significantly different between the droughted and irrigated treatments; and was also not significantly different between the unsprayed and sprayed treatments. Interactions effects of drought x antitranspirant factors was not significant for this yield component. Drought imposition did not show any significant differences between droughted and irrigated treatments in biomass per plant and there were no significant differences in biomass per plant between unsprayed and sprayed treatments with antitranspirant application either. However, the biomass per plant tended (P = 0.077) to marginally increase in the droughted plants by 2.1 % with drought application. The effect of drought x antitranspirant interaction on biomass per plant was not significant. The response of harvest index to drought did not show any significant differences between droughted and irrigated treatments and the harvest index was not significantly different between the unsprayed and sprayed treatments with antitranspirant application.

Table 3.3.13. Grain yield and yield components under drought and antitranspirant applied at 71 days after emergence during booting to flowering of sorghum grown in the glasshouse from October to November 2015 (n = 24) ± Standard error of the differences of the mean (SED) (Expt.1).

Treatments	Grain yield per plant (g)	Grain number per plant	Weight per seed (mg)	Stalk weight per plant (g)	Biomass (g)	Harvest Index (%)
Irrigated	11.74	570	21.96	22.17	33.91	34.7
Droughted	10.61	499	20.99	20.52	34.60	34.5
Unsprayed	11.59	562	21.25	22.07	33.66	34.5
Sprayed	10.76	507	21.70	20.62	31.38	34.7
Irrigated Sprayed	12.10	522	21.16	21.84	33.22	35.0
Irrigated unsprayed	11.38	618	19.82	22.50	34.60	34.4
Droughted sprayed	10.13	492	21.24	19.39	29.53	35.0
Droughted unsprayed	11.08	505	22.68	22.50	32.72	34.4
Mean	11.17	534	21.48	21.34	32.52	34.6
df	15	15	15	15	15	15
SED	1.439	111.40	4.247	3.73	3.595	5.11
CV %	12.9	20.8	19.0	15.8	11.1	14.8
P values						
Drought	0.073	0.136	0.583	0.249	0.077	0.929
Antitranspirant	0.176	0.249	0.800	0.309	0.140	0.931
Drought x antitranspirant	0.845	0.380	0.294	0.573	0.544	0.701

Glasshouse experiment 2 Using SAMSORG-40 Cultivar

3.4. Materials and methods

The materials and methods used are identical to the ones used in glasshouse Expt. 1 above except for the following:

3.4.1. Plant material

The sorghum variety used in this experiment, SAMSORG-40 (Institute of Agricultural Research, Ahmadu Bello University, Nigeria) sorghum cultivar is semi-dwarf and semi-compacted sorghum with average height 140 -150 cm. It was bred for and adapted to the Sudan and Sahel savanna zones. It is high yielding, drought tolerant, and early maturing, between 75 – 80 days. It possesses hard grains with good local food and malting quality.

3.4.2. Drought application

Drought was imposed on the plants by restricting irrigation to the ‘droughted’ pots, while irrigation was applied by unrestricted watering of the ‘irrigated’ pots. 1L of water was added manually once every other day to the droughted pots since pot weights were not being taken. Whereas the ‘irrigated’ pots remained on drippers set to automatically deliver water at the rate of 1L per minute thrice daily at 07:30 hours, 12:30 hours, and 19:30 hours. Drought was initiated at 100 days after emergence, GS 5.1 – 5.5 head in flag leaf to mid booting to GS 6.1, early bloom (AgVita BBCH 2008) and lasted for 24 days.

3.4.3. Antitranspirant application

Spraying with the antitranspirant was done at 100 days after emergence at GS 5.1 – 5.5 head in flag leaf to mid booting (AgVita BBCH 2008) with the antitranspirant solution mixed at a concentration of 0.5 v/v with a hand-held lunch box boom sprayer (Trials Equipment Ltd, Essex UK) using a Flat Fan nozzle (FF110 -03), at 2 bar pressure, 200 L/ha and forward speed of 6 km/ha. Because the plants were too high to be fitted into custom made precision pot sprayer, they were brought outside the glasshouse and sprayed manually. Thereafter, all sprayed plants were returned to the bay in the glasshouse and the drippers fixed into the irrigated pots. Dates of some events and operations during the experiment are presented on Table 3.4.1. Skeleton ANOVA of measurements are shown on tables ranging from Table 3.4.2 to 3.4.6.

Table 3.4.1. Some significant dates and events during Expt.2.

Year	Date	Events
2015	August 12	Sowing
	August 17	Emergence
	December 7	Drought application and antitranspirant spraying
	December 31	Stopping drought and resumption of full irrigation
2016	March 8	Harvest

Table 3.4.2 Skeleton ANOVA of volumetric soil moisture content measurements(Expt. 2)

Source of variation	Degrees of freedom
Blocks stratum	5
Blocks x subject stratum	
Antitranspirant	1
Drought	1
Antitranspirant x drought	1
Residual	15
Blocks x subject x time stratum	
Time	5
Time x antitranspirant	5
Time x drought	5
Time x antitranspirant x drought	5
Residual	98(2)
Total	141(2)

(Numbers in parenthesis are missing values)

Table 3.4.3 Skeleton ANOVA of adaxial, abaxial and total stomatal conductance measurements (Expt. 2)

Source of variation	Degrees of freedom
Blocks stratum	5
Blocks x subject stratum	
Antitranspirant	1
Drought	1
Antitranspirant x drought	1
Residual	15
Blocks x Subject x Time stratum	
Time x antitranspirant	4
Time x drought	4
Time x antitranspirant x drought	4
Residual	80
Total	119

Table 3.4.4 Skeleton ANOVA of leaf temperature measurements (Expt. 2)

Source of variation	Degrees of freedom
Blocks stratum	5
Blocks x subject stratum	
Drought	1
Antitranspirant	1
Drought x antitranspirant	1
Residual	15
Blocks x subject x time stratum	
Time	3
Time x drought	3
Time x antitranspirant	3
Time x drought x antitranspirant	3
Residual	60
Total	95

Table 3.4.5 Skeleton ANOVA of plant height and RWC measurements (Expt. 2)

Sources of variation	Degrees of freedom
Blocks stratum	5
Blocks x unit stratum	
Antitranspirant	1
Drought	1
Drought x antitranspirant	1
Residual	15
Total	23

*RWC = relative water content

Table 3.4.6. Skeleton ANOVA of yield and yield components measurements (Expt. 2)

Source of variation	Degrees of freedom
Blocks stratum	5
Blocks x units stratum	
Antitranspirant	1
Drought	1
Antitranspirant x drought	1
Residual	14(1)
Total	22(1)

(Numbers in parenthesis are missing values)

3.5. Results

3.5.1. Volumetric soil moisture content

Table 3.5.1 and Figure 3.3.6 shows results of analysis of response of volumetric soil water content of pots to drought and antitranspirant treatments and the interaction effects. There were significant differences in volumetric soil moisture content of pots between the droughted and irrigated pots. No significant differences in the volumetric soil moisture content between unsprayed and sprayed treatments was recorded. Drought significantly ($P < 0.001$) reduced the volumetric soil water content of pots from 36.5 % in the irrigated to 28.9 % in the droughted. The drought x antitranspirant interaction effect on volumetric soil moisture content was not significant. A significant time (P < 0.001) effect indicated by differences between the volumetric soil moisture content of pots at various days after spraying (DAS) was observed. Two-way analysis of variance on each of the days after spraying (DAS) (Table 3.5.2) showed that the significant time x drought (P < 0.001) effect on volumetric soil moisture content was as result of significant differences shown by reduction in volumetric soil moisture content between the droughted and irrigated pots at 10 DAS (P < 0.001), 15 DAS (P < 0.001), 20 DAS (P < 0.001), 25 DAS (P < 0.001) and 31 DAS (P < 0.001). Whereas there were no significant time x antitranspirant, and time x drought x antitranspirant interaction effects on soil volumetric moisture content.

Table 3.5.1 Average volumetric soil moisture content (%) of pots under drought and antitranspirant applied at 112 days after emergence of sorghum grown in the glasshouse in December 2015 measured during booting to flowering at 3, 10, 15, 20, 25 and 31 days after spraying (n = 24) ± Standard error of the differences of the mean (SED) (Expt. 2).

	Days after spraying						
Treatments	3	10	15	20	25	31	Means
Irrigated	37.88	36.98	36.99	35.25	35.93	35.89	36.49
Droughted	36.65	28.47	26.97	26.94	26.07	28.41	28.92
Unsprayed	37.38	33.23	31.86	30.84	31.53	32.34	32.86
Sprayed	37.15	32.22	32.1	31.35	30.47	31.96	32.54
Irrigated unsprayed	37.33	36.8	36.57	35.45	35.55	33.95	35.94
Irrigated sprayed	38.42	37.17	37.4	35.05	36.3	37.83	37.03
Droughted unsprayed	36.97	27.63	27.62	27.25	25.38	29.97	29.14
Droughted sprayed	36.33	29.3	26.32	26.63	26.75	26.85	28.70
Means	32.76	32.73	31.98	31.10	31.00	32.15	32.70
df							98
SED							3.071
CV %							9.4
P values							
Drought							< 0.001
Antitranspirant							0.413
Drought x antitranspirant							0.066
Time							<0.001
Time x drought							< 0.001
Time x antitranspirant							0.867
Time x drought x antitranspirant							0.243

Table 3.5.2 Two-way analysis of variance on the effect of drought and antitranspirant on volumetric soil moisture content (%) of pots during booting to flowering of sorghum from December 2015. (n = 24) (Expt.2).

	Days after spraying					
	3	10	15	20	25	31
P values						
Drought	0.418	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Antitranspirant	0.881	0.516	0.773	0.704	0.200	0.665
Drought x antitranspirant	0.568	0.677	0.403	0.935	0.702	0.001
SED						
Drought	1.172	1.528	1.121	1.311	0.790	0.867
Antitranspirant	1.472	1.528	1.121	1.311	0.790	0.867
Drought x antitranspirant	2.082	2.161	1.585	1.845	1.117	1.226
df	15	15	13	15	15	15

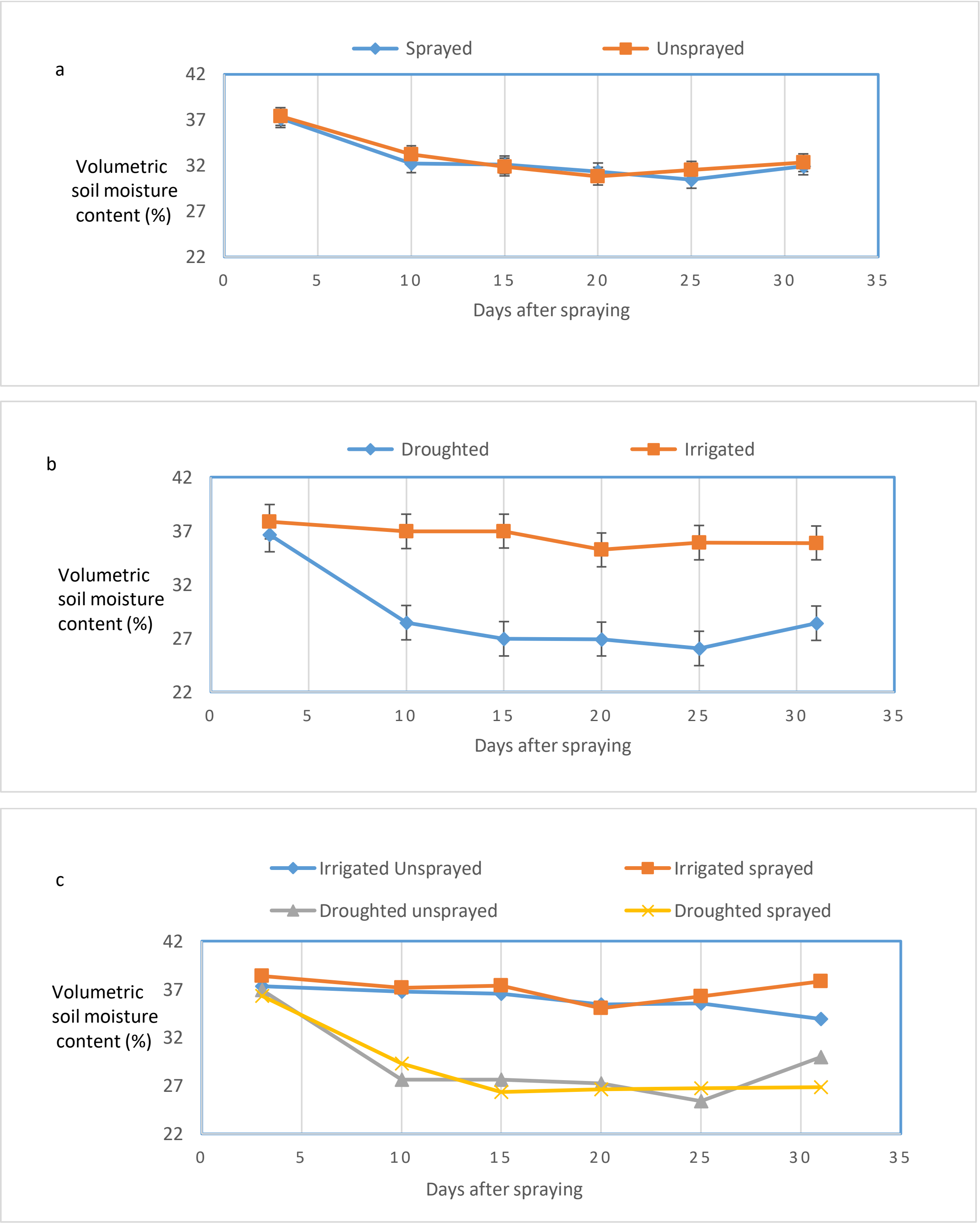

Figure 3.3.6. Response of volumetric water content (%) to (a) antitranspirant (b) drought and (c) drought x antitranspirant interaction during booting to flowering of sorghum grown in the glasshouse in December 2015 (n = 24). (Drought, $P < 0.001$, antitranspirant, $P = 0.413$, drought x antitranspirant = 0.066). Error bars are SEM. (Expt.2).

3.5.2. Adaxial stomatal conductance

Results of analysis on response of adaxial stomatal conductance (mmol $m^{-2}s^{-1}$) to drought and antitranspirant application and their interactions is shown on Table 3.5.3 and Figure 3.3.7.

Adaxial stomatal conductance was significantly different in droughted and irrigated treatments as well as in unsprayed and sprayed treatments. Drought significantly ($P < 0.001$) decreased adaxial stomatal conductance by 21.2 % in the droughted compared with the irrigated treatments. Antitranspirant application also significantly ($P = 0.002$) reduced adaxial stomatal conductance by 12.5 % in the unsprayed compared with the sprayed treatments. The drought x antitranspirant interaction effect on adaxial stomatal conductance was not significant and there was no significant effect of the treatments with time. Also, the time x drought, time x antitranspirant and time x drought x antitranspirant interaction effects were not significant.

Table 3.5.3. Average adaxial stomatal conductance (mmols m^{-2} s^{-1}) recorded on the flag leaf under drought and antitranspirant applied at 112 days after emergence of sorghum grown in the glasshouse in December 2015 measured during booting to flowering at 3, 7, 11, 14 and 17 days after spraying (n = 24) ± Standard error of the differences of the mean (SED) (Expt. 2).

	Days after spraying					
Treatments	3	7	11	14	17	Means
Irrigated	25.00	24.55	26.25	25.26	23.32	24.88
Droughted	22.27	19.95	18.48	18.62	18.70	19.60
Unsprayed	26.32	23.62	23.82	22.52	22.32	23.72
Sprayed	20.95	20.88	20.91	21.35	19.70	20.76
Irrigated unsprayed	29.42	27.31	28.83	25.63	24.4	27.12
Irrigated sprayed	20.58	21.80	23.67	24.88	22.25	22.64
Droughted unsprayed	23.22	19.93	18.80	19.41	20.24	20.32
Droughted sprayed	21.31	19.97	18.15	17.82	17.16	18.88
Means	23.63	22.25	22.36	21.94	21.01	22.24
df						80
SED						3.088
CV %						13.9
						P values
Drought						< 0.001
Antitranspirant						0.002
Drought x antitranspirant						0.066
Time						0.105
Time x drought						0.086
Time x antitranspirant						0.243
Time x drought x antitranspirant						0.117

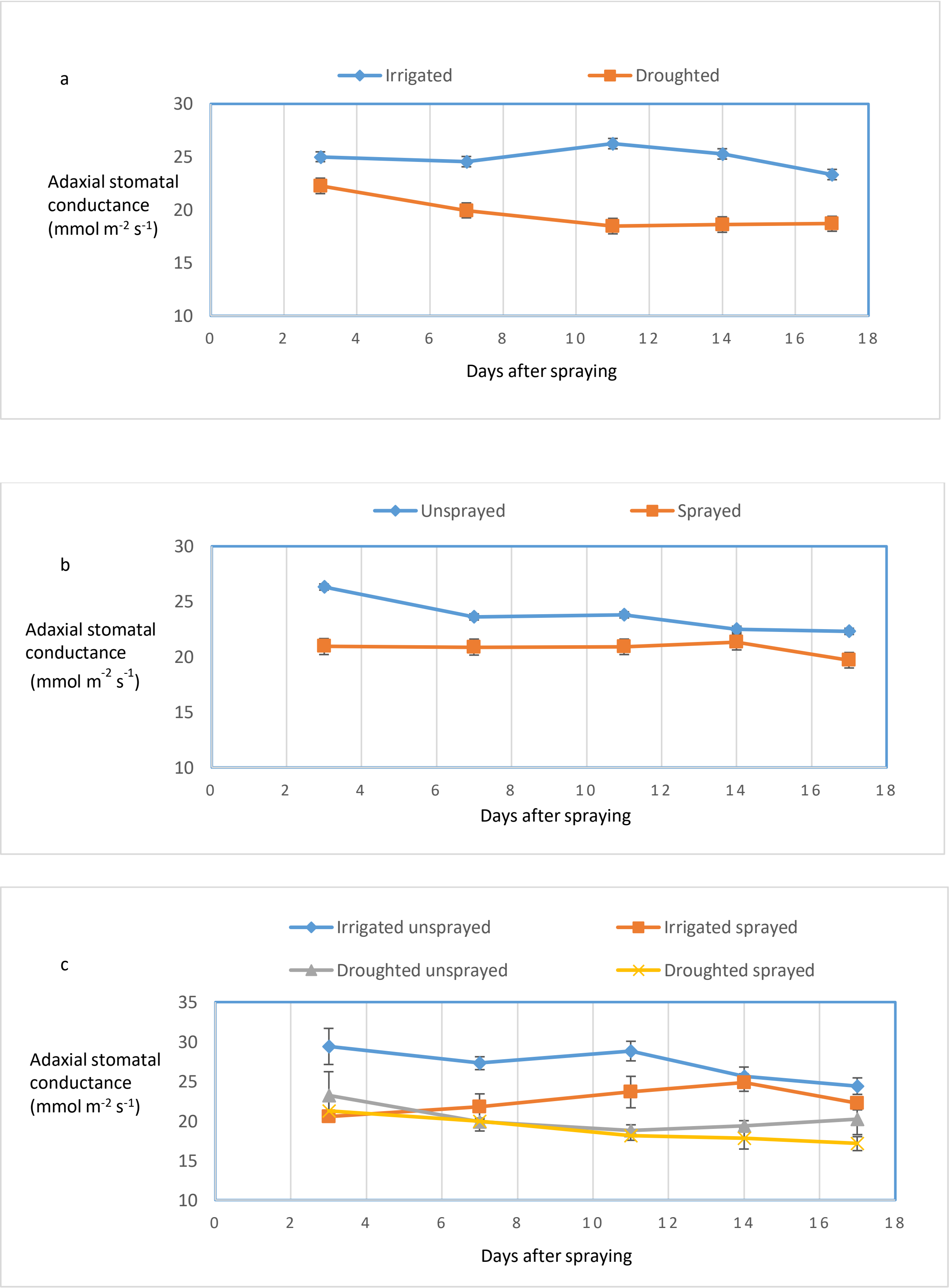

Figure 3.3.7. Response of adaxial stomatal conductance (mmol m^{-2}s^{-1}) to (a) drought (b) antitranspirant and (c) drought x antitranspirant interaction at 112 days after emergence recorded on the flag leaf measured at 3, 7, 11, 14 and 17 days after spraying during booting to flowering of sorghum grown in the glasshouse in December 2015 (n = 24). (Drought, $P < 0.001$; antitranspirant, $P = 0.002$; drought x antitranspirant, $P = 0.066$). Error bars are SEM. (Expt. 2).

3.5.3. Abaxial stomatal conductance

Results of analysis of data on response of abaxial stomatal conductance to drought and antitranspirant application and their interactions is shown on Table 3.5.4 and Figure 3.3.8. There were significant differences in abaxial stomatal conductance between droughted and irrigated treatments with drought imposition and with antitranspirant application. Drought significantly ($P < 0.001$) decreased abaxial stomatal conductance by 24.1 % in droughted compared with irrigated treatments and with antitranspirant application, abaxial stomatal conductance was significantly ($P = 0.002$) decreased by 13.4 % in the sprayed compared with the unsprayed treatments. The drought x antitranspirant interaction effect was significant ($P = 0.032$) because antritranspirant reduced abaxial stomatal conductance differently in irrigated and droughted treatments. In the irrigated treatments, abaxial stomatal conductance was decreased by 18.5 % in the sprayed compared with the unsprayed plants while in the droughted treatments it was reduced by only 6.0 % in the sprayed compared with the unsprayed plants. There were also significant differences in abaxial stomatal conductance over time ($P < 0.001$) with the abaxial stomatal conductance decreasing as days after spraying (DAS) progresses. A two-way analysis of variance on each of the DAS (Table 3.5.5) showed that the significant drought x time ($P = 0.049$) interaction effect was because of significant differences in abaxial stomatal conductance between irrigated and droughted treatments on some occasions. Drought significantly decreased abaxial stomatal conductance at 3 DAS ($P < 0.001$), 7 DAS ($P = 0.005$) and 17 DAS (0.002) in the droughted compared with irrigated treatments. Similarly, the Two-way analysis of variance also showed the antitranspirant x time ($P = 0.012$) interaction effects were significant on abaxial stomatal conductance, due to significant differences (reduction) in abaxial stomatal conductance between unsprayed and sprayed treatments at 17 DAS ($P = 0.010$). However, the time x drought x antitranspirant interactions were not significant on abaxial stomatal conductance.

Table 3.5.4. Average abaxial stomatal conductance (mmols m^{-2} s^{-1}) recorded on the flag under drought and antitranspirant applied at 112 days after emergence of sorghum grown in the glasshouse in December 2015 measured during booting to flowering at 3, 7, 11, 14 and 17 days after spraying (n = 24) ± Standard error of the differences of the mean (SED) (Expt. 2).

	Days after spraying					
Treatments	3	7	11	14	17	Mean
Irrigated	36.65	32.8	30.74	29.92	28.37	31.71
Droughted	29.10	23.35	25.32	19.62	22.94	24.07
Unsprayed	34.73	29.83	28.79	26.1	29.98	29.89
Sprayed	31.02	26.41	27.28	23.44	21.33	25.90
Irrigated unsprayed	39.44	35.7	31.96	32.82	34.83	34.95
Irrigated sprayed	33.87	30.07	29.53	27.02	21.9	28.48
Droughted unsprayed	30.03	23.95	25.62	25.62	25.12	24.82
Droughted sprayed	28.17	22.75	25.03	19.86	20.77	23.32
Mean	32.88	28.12	28.03	25.55	25.66	27.89
Df						80
SED						3.272
CV %						11.7
P values						
Drought						< 0.001
Antitranspirant						0.002
Drought x antitranspirant						0.032
Time						< 0.001
Time x antitranspirant						0.012
Time x drought						0.049
Time x antitranspirant x drought						0.428

Table 3.5.5. Two-way analysis of variance on the effect of drought and antitranspirant applied at 112 days after emergence on abaxial stomatal conductance (mmol m^{-2} s^{-1})recorded on the flag leaf measured at 3, 7, 11, 14 and 17 days after spraying during booting to flowering of sorghum grown in the glasshouse in December 2015 (n = 24) (Expt.2).

	Days after spraying				
	3	7	11	14	17
P values					
Drought	< 0.001	0.005	0.193	0.082	0.002
Antitranspirant	0.131	0.346	0.177	0.916	0.010
Drought x antitranspirant	0.753	0.856	0.726	0.846	0.258
SED					
Drought	1.646	1.525	2.310	1.836	2.090
Antitranspirant	1.646	1.525	3.270	2.597	2.960
Drought x antitranspirant	2.327	2.157	3.270	2.597	2.960
df	15	15	15	15	15

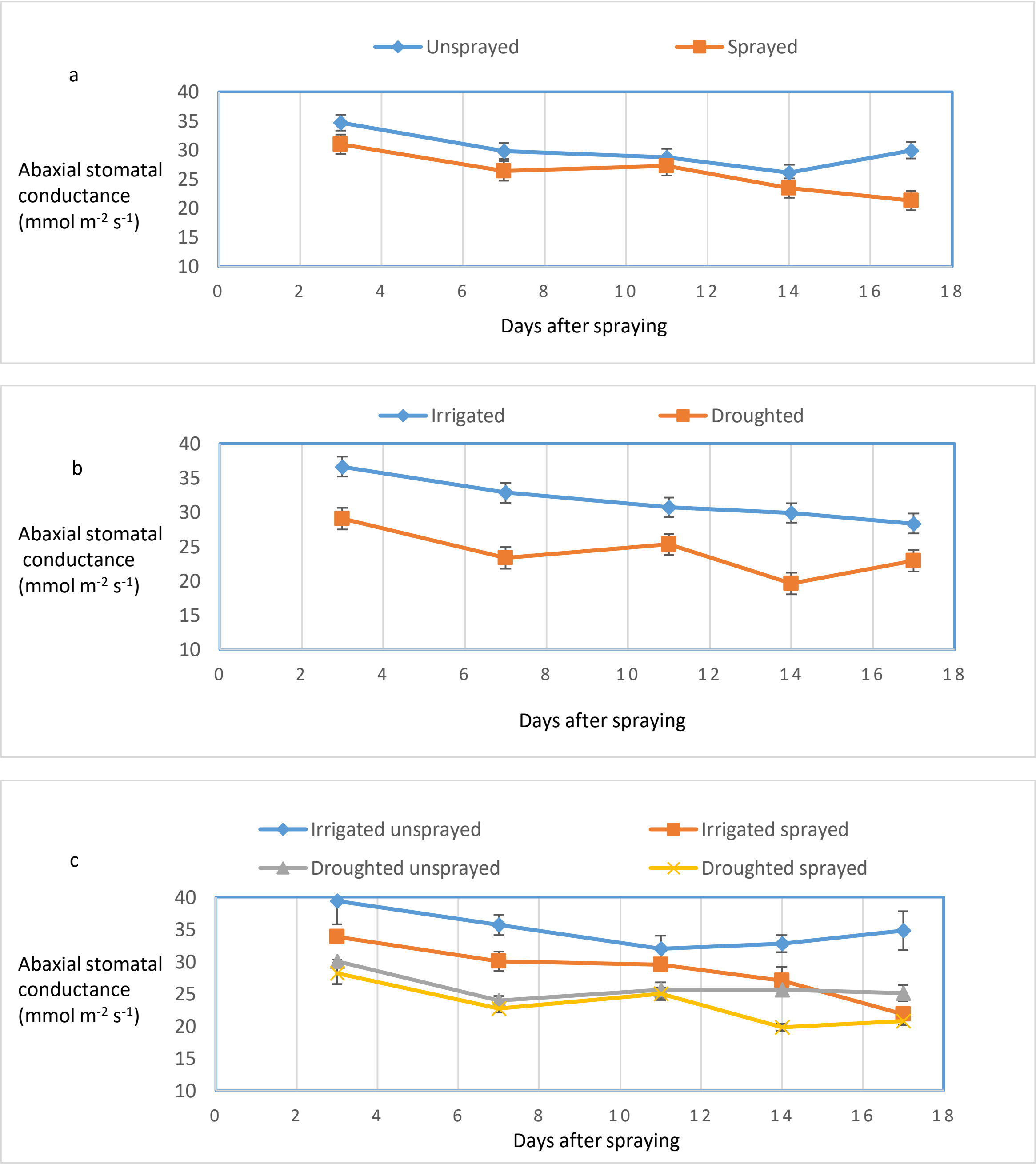

Figure 3.3.8. Response of abaxial stomatal conductance (mmol $m^{-2}s^{-1}$) to (a) antitranspirant (b) drought (c) drought x antitranspirant interaction applied at 112 days after emergence recorded on the flag leaf during booting to flowering of sorghum grown in the glasshouse in December 2015 (n = 24). (Drought, $P < 0.001$; antitranspirant, $P = 0.002$; drought x antitranspirant, $P = 0.032$). Error bars are SEM. (Expt. 2).

3.5.4 Total stomatal conductance

Results from data analysis on response of total stomatal conductance to drought and antitranspirant application and their interaction is shown on Tables 3.5.6 and Figure 3.3.9. Drought led to significant differences in total stomatal conductance between the irrigated and droughted treatments. Similarly, with the antitranspirant significant differences were recorded in total stomatal conductance between the unsprayed and sprayed treatments. The drought produced a significant (P < 0.001) reduction of 23.54 % in total stomatal conductance in the droughted compared with the irrigated plants whereas the antitranspirant significantly (P < 0.001) decreased total stomatal conductance by 13.55 % in the sprayed compared with unsprayed treatments. There was a significant (P < 0.001) effect of time on total stomatal conductance, as the magnitude increased with increase in days after spraying (DAS). The drought x antitranspirant interaction effects on total stomatal conductance was also significant (P=0.016). The total stomatal conductance was decreased by 8.15 % in the irrigated sprayed compared with the unsprayed but by only 2.04 % in the droughted sprayed compared with the unsprayed plants. The time x drought interaction effects(P = 0.001) showed highly significant effects on total stomatal conductance as the conductance increased under irrigation between 3 DAS and 7 DAS and later declined at 11 DAS, 14 DAS and 17 DAS. Whereas under drought there was an increase in conductance between 3 DAS and 11 DAS and a decline at 14 DAS and later increased at 17 DAS. There was also a significant time x antitranspirant effect on total stomatal conductance, because there was an increase in total stomatal conductance between 3 DAS and 11 DAS followed by a decline from 14 DAS to 17 DAS in the sprayed treatments, while there was an increase at 3 DAS to 11 DAS and a decline at 14 DAS followed by an increase at 17 DAS. In addition, results of Two-way analysis of variance on individual days after spraying (DAS) (Table 3.5.7) showed significant drought x antitranspirant interaction effects on total stomatal conductance on four occasions. First, at 7 DAS (P = 0.003) total stomatal conductance was reduced in the irrigated sprayed by 20.94 % compared with the unsprayed, and by 7.10 % in the droughted sprayed compared with the unsprayed treatments. Secondly, at 7 DAS (P = 0.036) a reduction of 16.98 % in total stomatal conductance was recorded in the irrigated sprayed compared with the unsprayed, while only a 3.64 % reduction was found in the droughted sprayed compared with the unsprayed treatments. At 14 DAS (P = 0.035) there was a 13.50 % reduction in total stomatal conductance in the irrigated sprayed in comparison with the unsprayed, and a 1.1 % decrease in the droughted unsprayed compared with the sprayed treatments and thirdly, at 17 DAS (P = 0.016) total stomatal conductance declined by 29.80 % in the irrigated sprayed in comparison with unsprayed and by 16.70 % in the droughted sprayed compared with the unsprayed treatments.

Table 3.5.6. Average total stomatal conductance (mmols $m^{-2}s^{-1}$) recorded on the flag under drought and antitranspirant applied at 112 days after emergence of sorghum grown in the glasshouse in December 2015 measured during booting to flowering at 3, 7, 11, 14 and 17 days after spraying (n = 24) ± Standard error of the differences of the mean (SED) (Expt. 2).

	Days after spraying					
Treatments	3	7	11	14	17	Mean
Irrigated	30.83	45.16	43.87	42.54	40.03	40.49
Droughted	25.68	33.33	34.56	28.93	32.29	30.96
Unsprayed	30.53	41.64	40.70	37.36	41.14	38.27
Sprayed	25.98	36.85	37.73	34.11	31.19	33.17
Irrigated unsprayed	34.43	49.35	46.37	45.63	47.03	44.56
Irrigated sprayed	27.22	40.97	41.36	39.46	33.02	36.41
Droughted unsprayed	26.63	33.92	35.02	29.09	35.24	31.98
Droughted sprayed	24.74	32.73	34.11	28.77	29.35	29.94
Means	28.25	39.24	39.21	35.74	36.16	35.72
df						80
SED						3.325
CV %						9.1
						P values
Antitranspirant						< 0.001
Drought						< 0.001
Drought x antitranspirant						0.016
Time						< 0.001
Time x antitranspirant						0.008
Time x drought						0.001
Time x antitranspirant x drought						0.768

Table 3.5.7. Two-way analysis of variance on the effect of drought and antitranspirant on total stomatal conductance (mmol $m^{-2}s^{-1}$) applied at 112 days after emergence recorded on the flag leaf measured at 3, 7, 11, 14 and 17 days after spraying during booting to flowering in December 2015 (n = 24) (Expt.2).

	Days after spraying				
	3	7	11	14	17
P values					
Drought	0.003	< 0.001	< 0.001	< 0.001	< 0.001
Antitranspirant	0.007	0.008	0.122	0.022	< 0.001
Drought x antitranspirant	0.086	0.036	0.274	0.035	0.016
SED					
Drought	1.446	1.567	1.807	1.266	1.499
Antitranspirant	1.446	1.567	1.807	1.266	1.499
Drought x antitranspirant	2.045	2.216	2.556	1.790	2.119
df	15	15	15	15	15

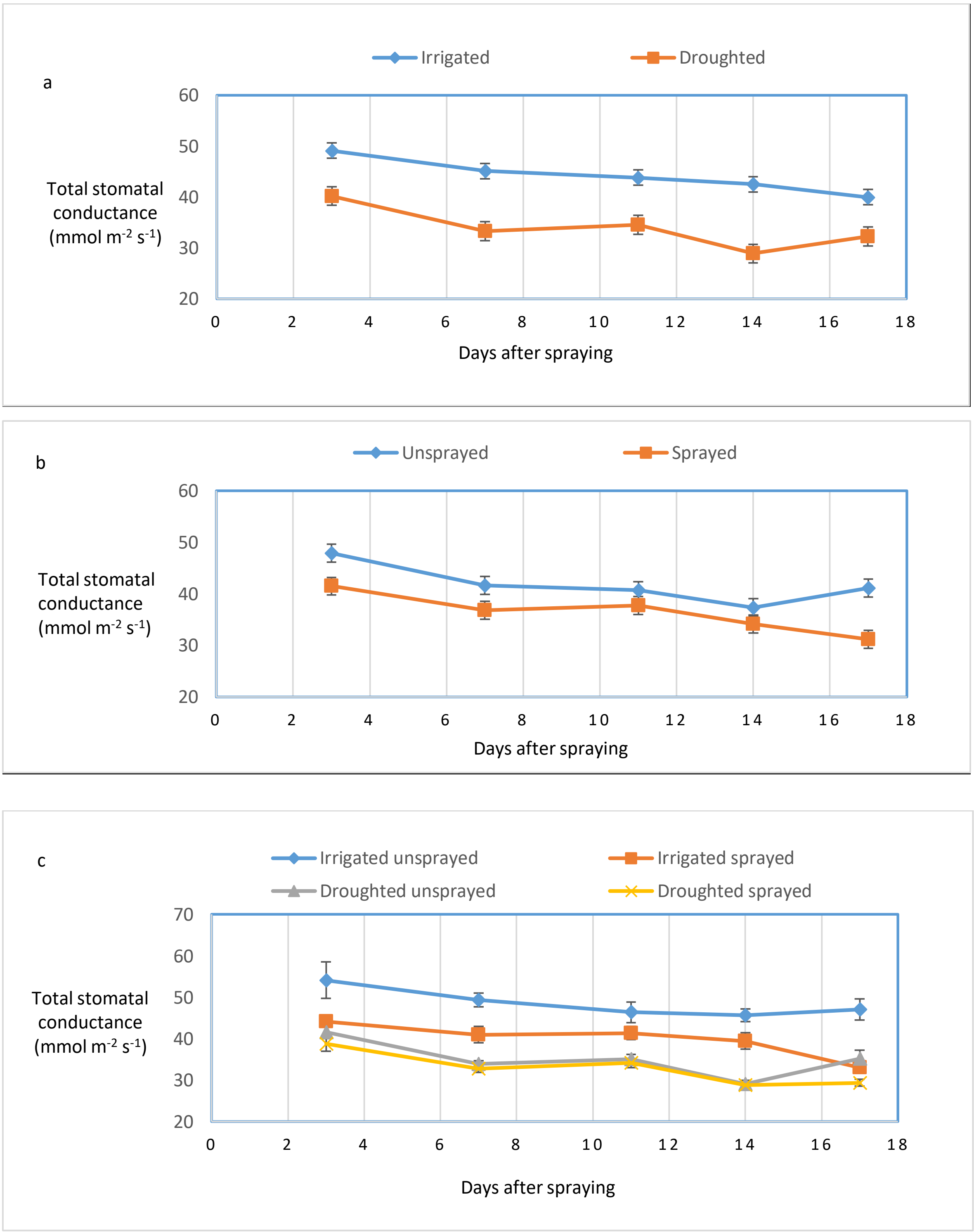

Figure 3.3.9. Response of total stomatal conductance (mmol $m^{-2}s^{-1}$) of sorghum to (a) drought (b) antitranspirant and (c) drought x antitranspirant interaction applied at 112 days after emergence recoreded from the flag leaf of sorghum during booting and flowering grown in the glasshouse in December 2015 (n = 24). (Drought, $P < 0.001$, antitranspirant, $P < 0.001$, drought x antitranspirant, $P = 0.020$) Error bars are SEM (Expt. 2).

3.5.5. Leaf temperature

Table 3.5.8 and Figure 3.3.10; shows results of analysis of response of leaf temperature to drought and antitranspirant treatments and the interaction effects. There was no significant effect of drought and antitranspirant treatments on leaf temperature. The drought x antitranspirant interaction on leaf temperature was not significant. Similarly, the time x drought and time x antitranspirant interaction effects on leaf temperature were not significant. However, the interaction effects of time x drought x antitranspirant was significant on leaf temperature. Two-way analysis of variance performed for each of the days after spraying (DAS) (Table 3.5.9) showed a significant (P = 0.001) drought x antitranspirant interaction effects at 17 DAS. The interaction effect on leaf temperature was due to 22.4 % increase in the irrigated sprayed compared with the unsprayed and a 16.3 % decrease in the droughted sprayed compared with the unsprayed treatments. However, this interaction was observed on only one day and so may be a random occurrence for unclear reasons.

Table 3.5.8. Average leaf temperature (˚C) recoreded on the flag leaf under drought and antitranspirant applied at 112 days after emergence of sorghum grown in the glasshouse in December 2015 measured during booting to flowering at 14, 17, 20 and 28 days after spraying (n = 6) ± Standard error of the differences of the mean (SED) (Expt. 2).

	Days after spraying				
Treatments	14	17	20	28	Mean
Irrigated	24.53	25.29	26.54	27.03	25.85
Droughted	26.31	24.28	25.84	27.29	25.93
Unsprayed	25.72	24.59	26.63	27.10	26.01
Sprayed	25.11	24.98	25.75	27.23	25.77
Irrigated unsprayed	24.96	22.74	27.87	26.93	25.63
Irrigated sprayed	24.10	27.83	25.22	27.13	26.07
Droughted unsprayed	26.48	26.44	25.40	27.27	26.40
Droughted sprayed	26.13	22.13	26.28	27.13	25.46
Mean	25.42	24.78	26.19	27.16	25.89
df					60
SED					2.617
CV %					10.1
					P values
Drought					0.870
Antitranspirant					0.625
Drought x antitranspirant					0.180
Time					0.033
Time x drought					0.266
Time x antitranspirant					0.736
Time x drought x antitranspirant					0.003

Table 3.5.9. Two-way analysis of variance on the effect of drought and antitranspirant appied at 112 days after emergence on leaf temperature (˚C) recorded on the flag laef measured at 14, 17, 20 and 28 days after spraying during booting to flowering of sorghum in December 2015 (n = 24) (Expt.2).

	Days after spraying			
	14	17	20	28
P values				
Drought	0.052	0.416	0.577	0.687
Antitranspirant	0.479	0.749	0.482	0.845
Drought x antitranspirant	0.767	0.001	0.170	0.906
SED				
Drought	0.840	1.201	1.227	0.629
Antitranspirant	0.840	1.201	1.227	0.629
Drought x antitranspirant	1.188	1.699	1.735	0.890
df	15	15	15	15

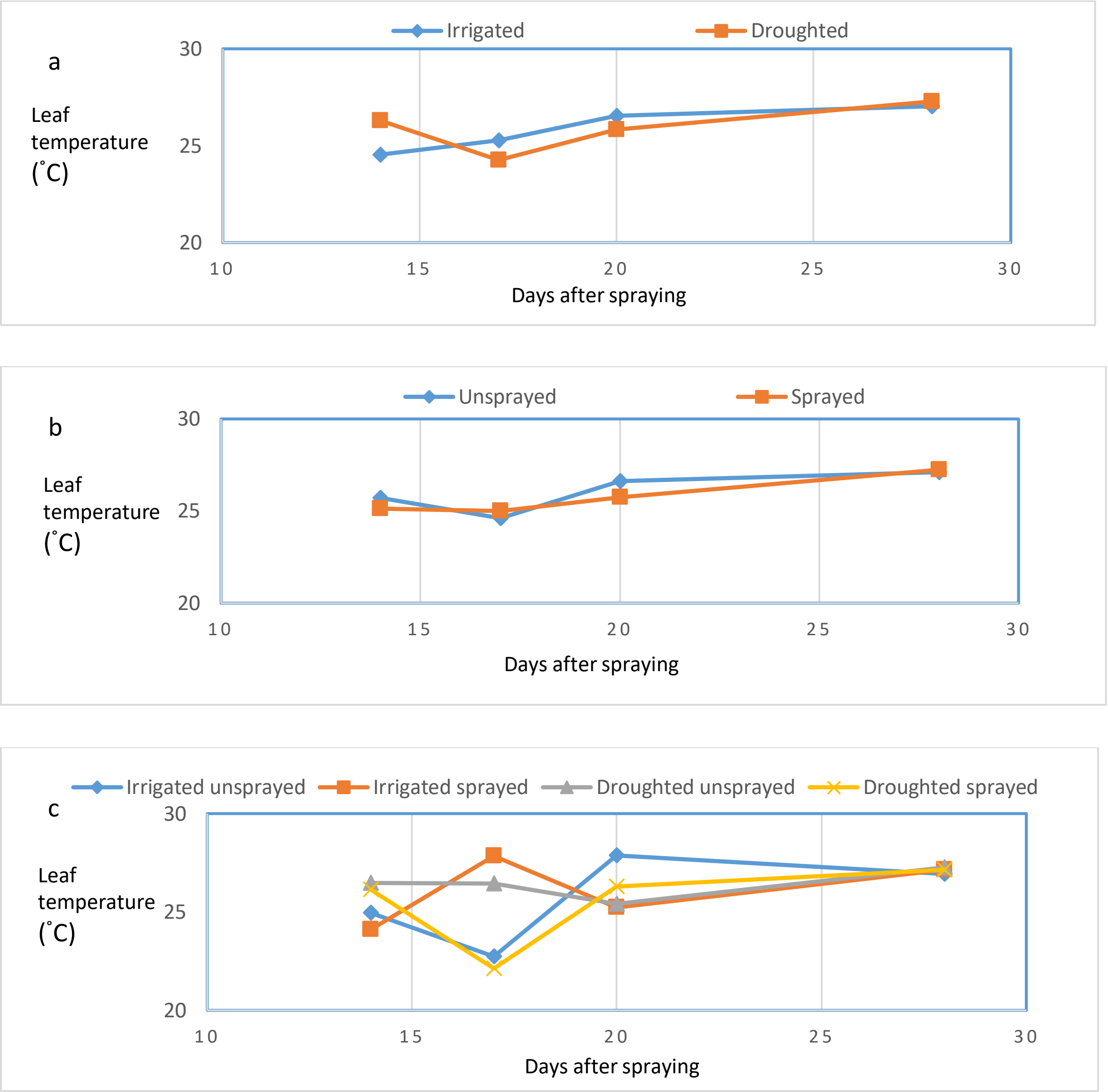

Figure 3.3.10. Response of leaf temperature (˚C) to (a) drought (b) antitranspirant and (c) drought x antitranspirant interaction at 112 days after emergence recorded on the flag leaf during booting and flowering of sorghum grown in the glasshouse in December 2015 (n = 24). (Drought $P = 0.870$, antitranspirant $P < 0.625$, drought x antitranspirant $P = 0.180$) (Expt. 2).

3.5.6. Relative water content and plant height

Table 3.5.10 shows results of analysis of data on response of relative water content (%) and plant height (cm) to drought and antitranspirant application. There was no significant difference in relative water content between droughted and irrigated treatments with drought imposition and between the unsprayed and sprayed treatments with antitranspirant application. Thus, no significant effects of drought and antitranspirant on relative water content were observed. And no significant drought x antitranspirant interaction effects on relative water content was observed. With respect to plant height, there were significant differences in plant height between droughted and irrigated treatments as well as between unsprayed and sprayed treatments. Drought imposition nearly significantly (P < 0.006) decreased plant height by 12.74 % in the droughted compared with the irrigated treatments, whereas the antitranspirant effect was not significant. In addition, there was a significant (P < 0.015) drought x antitranspirant interaction effect on plant height, as in the irrigated treatments antitranspirant increased plant height by 8.58 % in the sprayed compared with the unsprayed plants and in the droughted treatments plant height was reduced by 14.24 % in the sprayed compared with unsprayed plants.

Table 3.5.10. Average relative water content (%) at (DAS) and plant height (cm) at (DAS) under drought and antitranspirant during booting to flowering of sorghum grown in the glasshouse in December 2015 (n = 6) ± Standard error of the differences of the mean (SED) (Expt.2).

Treatments	Relative water content (%)	Plant height (cm)
Irrigated	79.59	204.1
Droughted	80.51	178.1
Unsprayed	80.16	193.7
Sprayed	79.94	188.5
Irrigated unsprayed	80.82	195.7
Irrigated sprayed	78.35	212.5
Droughted unsprayed	79.49	191.7
Droughted sprayed	81.52	164.5
Mean	80.50	191.1
df	15	15
SED	4.423	10.74
CV %	5.5	5.6
	P values	P values
Drought	0.618	0.006
Antitranspirant	0.904	0.530
Drought x antitranspirant	0.233	0.015

3.5.7. Yield and yield components

Table 3.5.11 shows results of analysis of data on yield and yield components of sorghum resulting from drought and antitranspirant application and their interactions.

There were no significant differences in grain yield between droughted and irrigated, as well as between unsprayed and sprayed treatments. The differences in grain number per plant between droughted and irrigated treatments as well as that between unsprayed and sprayed treatments were not significant. Thus no significant effects of both drought and antitranspirant application on grain number per plant were found. Also, the drought x antitranspirant interaction effects on grain number per plant was not significant. Weight per grain was not significantly different in droughted compared with the irrigated treatments. No significant difference was recorded between unsprayed and sprayed treatments with antitranspirant application as well. In addition, the interaction effects of drought x antitranspirant on weight per grain was not significant. Drought and antitranspirant treatments did not produce any significant differences in stalk weight between droughted and irrigated as well as between unsprayed and sprayed treatments. Therefore, the effect of drought and the effect of antitranspirant on stalk weight were not significant. In addition, interaction effects of drought x antitranspirant was not significant. Similarly, no significant difference was obtained in biomass between the droughted and irrigated treatments, as well as between the unsprayed and sprayed treatments. Thus, no significant effect of drought and antitranspirant application was recorded in biomass. However, the drought x antitranspirant interaction effect was significant ($P = 0.043$) on the biomass per plant. The antitranspirant increased biomass per plant in both irrigated and droughted treatments; the increase was greater in the droughted 29.62 %, than in the irrigated 0.29 % in favour of the sprayed treatments. Harvest index (HI) was not significantly different between droughted and irrigated treatments and between unsprayed and sprayed treatments. No significant effect of drought imposition and antitranspirant application on harvest index was recorded. Similarly, the drought x antitranspirant effects on harvest index was not significant.

Table 3.5.11. Average grain yield and yield components under drought and antitranspirant applied at 112 days after emergence of sorghum grown in the glasshouse in December 2015 (n = 6) ± Standard error of the differences of the mean (SED) (Expt. 2).

Treatments	Grain yield per plant (g)	Grain number per plant	Weight per grain (mg)	Stalk weight per plant (g)	Biomass (g)	Harvest index (%)
Irrigated	16.37	443	36.20	74.80	88.30	15.52
Droughted	15.82	440	37.30	82.10	98.00	16.35
Unsprayed	15.12	419	36.50	74.00	83.00	16.57
Sprayed	17.07	464	37.10	82.90	98.00	15.31
Irrigated unsprayed	16.17	449	36.40	75.70	90.20	14.62
Irrigated sprayed	16.56	437	38.30	73.90	90.46	16.43
Droughted unsprayed	14.06	389	36.50	72.40	86.46	16.00
Droughted sprayed	17.58	492	35.90	91.90	109.48	16.71
Mean	16.09	442	36.80	78.5	93.1	15.94
df	14	15	15	15	15	15
SED	3.251	86	4.94	14.59	14.87	3.627
CV%	20.2	19.5	13.5	16.3	16.0	22.8
P values						
Drought	0.687	0.646	0.591	0.942	0.134	0.584
Antitranspirant	0.162	0.734	0.747	0.714	0.134	0.409
Antitranspirant x drought	0.258	0.489	0.531	0.472	0.043	0.716

Table 3.6. Summary table of means of measurements over time for the effect of drought and antitranspirant and drought x antitranspirant interaction for Expts. 1 and 2.

Measurements	Glasshouse Expt. 1			Glasshouse Expt. 2		
	Drought	Antitranspirant	Drought x antitranspirant	Drought	Antitranspirant	Drought x antitranspirant
VWC	*	ns	ns	*	ns	ns
Adaxial stomatal conductance	*	ns	*	*	*	ns
Abaxial stomatal conductance	*	ns	*	*	*	*
Total stomatal conductance	*	ns	*	*	*	*
Leaf temp.	ns	ns	ns	ns	ns	ns
Relative water content	*	ns	ns	ns	ns	ns
Plant height	*	ns	ns	*	ns	*
Grain yield	ns	ns	ns	ns	ns	ns
Grain number	*	ns	ns	ns	ns	*
Weight per grain	*	ns	ns	ns	ns	ns
Stalk weight	ns	ns	ns	ns	ns	ns
Biomass	ns	ns	ns	ns	ns	ns
Harvest index	ns	ns	ns	ns	ns	ns

*** =** *Significant effect. ns = not significant effect.* VWC = *volumetric soil moisture content*

3.6. Discussion

3.6.1 The effect of drought stress

A summary of drought and antitranspirant effects and their interactions on the measurements is presented on Table 3.6. Drought imposition significantly ($P < 0.001$) decreased volumetric soil moisture content of pots in Experiments 1 and 2. Therefore, the null hypothesis was rejected regarding the drought treatment effects. In Expt.1, the volumetric soil moisture content of pots was decreased from 33.7 % in the irrigated to 17.8 % in the droughted pots, and in Expt. 2 from 36.5 % in the irrigated to 29.0 % in the droughted pots. In both experiments volumetric soil moisture content in the irrigated pots were clearly above the 22.0 % volumetric soil moisture content at field capacity and for the droughted pots above the 11.0 % volumetric soil moisture content at the permanent wilting point. In Expt. 1, the volumetric soil moisture content of the droughted pots was 61.8 % higher than at permanent wilting point and 19.1 % lower than at field capacity, and in Expt. 2 it was more than two times greater than at permanent wilting point and 31.8 % lower than at field capacity. Hence, although the droughted pots were below field capacity, they were above permanent wilting point, hence the drought was mild.

Drought stress also significantly decreased stomatal conductance in both experiments, thus the null hypothesis is rejected in terms of stomatal conductance. In agreement with the above results, significant reduction in stomatal conductance in sorghum under drought stress have been reported in the literature. Al-Hamdani, *et al*., (1991) used reduction in the magnitude of stomatal conductance to evaluate different sorghum genotypes for resistance to soil water stress and found that stomatal conductance decreased with increasing soil water stress in all genotypes. Similarly, in other reports stomatal conductance in sorghum was drastically reduced under water stress imposed at different growth stages and in different cultivars (Yadav, *et al*., 2005; Munamava and Riddoch, 2001). Significant reduction in stomatal conductance and invariably transpiration could have resulted from closure of stomata due to reduction in leaf moisture content or poor uptake of soil water by the roots system due to soil water stress. Stomatal closure characterised by reduction in stomatal conductance is a very common way in which plants including sorghum respond to soil water stress. Furthermore, results of stomatal conductance from Expts. 1 and 2 appeared to correspond with reduction in the volumetric soil moisture content, all of which showed a progressive decline from the first to the last day of the measurements. Thus, it can be argued that the soil water stress led to a reduction in leaf moisture content which must have induced stomatal closure and the reduction in stomatal conductance. It should be noted that transpiration was reduced in the treatments despite the volumetric soil moisture content being above the field capacity and permanent wilting point, except in Expt.1. Shimsi (1963) reported a similar situation in which a reduction in transpiration was recorded in maize growing in soils with moisture above the wilting range and attributed this to the effect of decrease in capillary conductivity typical in potted plants. In that experiment, transpiration decreased from 8.14 cm^{-2} hr^{-1} at field capacity of 18 % soil moisture to 5.82 cm^{-2} hr^{-1} at a soil moisture of 13 %, whereas the permanent wilting point was at 10 % soil moisture. In the current study volumetric soil moisture content were 28.92 % and 17.79 % in the droughted treatments in Expts. 1 and 2 respectively above the 11.0 % volumetric soil moisture content at permanent wilting point, yet total stomatal conductance decreased from 44.15 mmol m^{-2} s^{-1} to 33.87 mmol m^{-2} s^{-1} in Expt. 1 and from 40.33 mmol m^{-2} s^{-1} to 31.56 mmol m^{-2} s^{-1} in Expt. 2. Shimshi (1963) explained that in a small pot the soil is so densely permeated by the roots that it dries uniformly, and no untapped soil remains, thus before the soil dries down to the wilting point, the decrease in capillary conductivity may restrict substantial water supply to the plant.

Results showed there was no significant effect of drought stress on leaf temperature thus the null hypothesis is accepted in terms of the leaf temperature. The lack of significant effects of drought on leaf temperature does not however correspond with the significant effects on stomatal conductance, as it is expected that with reduction in conductance the cooling effect of transpiration on the leaves would have been decreased and there would have been an elevation in leaf temperature. Stuart *et al*., (1985) observed in Johnson grass (*Sorghum halepense*) under both well-watered and water-stressed conditions that decreased conductance resulted into increasing leaf temperatures, however the sensitivity of leaf temperature was dependent on soil water content and not conductance. Thus, the lack of significant effect of drought on leaf temperature may be due to water-stress not being severe enough and not directly related to the stomatal conductance. However, in Expt. 1 drought created a significant decrease in plant height and in Expt. 2 there was a significant effect of drought leading to a reduction in plant height. Therefore, with regards to plant height, the null hypothesis is rejected. In conformity with this result Eck and Musick (1979) recorded significant reduction in sorghum plant height droughted at booting and attributed this to decreasing peduncle extension, which according to (Inuyama, *et al*., 1976) is vigorous during the booting stage, whereas Somadiredja and Sutoro (1989) reported a significant reduction in plant height of sorghum under water stress compared with corn. Reduction in plant height in the current study may be associated with decrease in relative water content (Expt. 1) which must have decreased moisture available for cell expansion and growth. In Expt. 2 although the relative water content was not significantly reduced, there is possibility that because of the duration of growth, 204 days compared with 136 days in Expt. 1 and the height attained, 191.1 cm compared to 78.3 cm in Expt. 1, the pots would have been so densely packed by the plant roots and the plant may have exhausted nutrients available in the pot and these may have caused reduction in growth.

Relative water content in both experiments was not significantly affected by the drought treatment. This could be due to inadequacy of the water stress regime imposed to put the plant under severe stress to cause a significant reduction in relative water content. In this study, the plant leaves were at a relative water content of greater than 50 % in both experiments so drought intensity is a more probable reason for non-significant effect than duration. Drought duration was about 30 days in both experiments which was sufficient to induce stress in sorghum as Mastrorilli *et al*., (1999) reported that after stopping irrigation the first significant difference in leaf water potential between stressed and unstressed sorghum occurred in about 15 days. With regard to intensity, volumetric soil moisture content of pots was above field capacity and permanent wilting point, in both droughted and irrigated pots hence drought intensity could not have been severe enough and a drastic reduction in relative water content could not have occured.

From the results, there was no significant effect of drought on grain yield in both experiments. Therefore, the null hypothesis is accepted with regards to grain yield, grain number, weight per grain, and stalk weight, biomass and harvest index in both experiments.

In contrast to some of the results of the present study, grain yield reduction under water stress is commonly recorded in sorghum (Garrity, *et al*., 1982; Lewis *et al*., 1974; Yadav, *et al*., 2005; Inuyama, *et al*., 1976; Blum, 1973). Thus, the finding of the current study is certainly not general but may be the result of experimental conditions. According to Blum (1973) drought stress decreases sorghum grain yield through its effects on various yield components which are being affected depending on the timing and magnitude of water stress. Furthermore, Inuyama *et al*., (1976) observed that grain yield is indirectly affected by the effects of water stress on growth before heading. Thus, since significant effects of the drought have not been recorded in the yield contributing parameters, grain yield will remain unaffected by the treatment. Although drought imposition was done at the booting stage after Lewis *et al*., (1974) who reported significant reduction in grain yield of sorghum under a drought stress of – 12 bars the severity of water stress in the current study may be less than this reference point. It should be noted that droughted pots retained volumetric soil moisture content above the permanent wilting point and the irrigated pots had volumetric soil moisture content above the field capacity. Therefore, the plants may have had sufficient water supply before and during stress imposition to sustain assimilate availability for yield production without any significant effects of water stress on yield. In other words, the plants were not sufficiently droughted to induce significant yield reductions.

With regard to other yield components, similar results showing water deficit at booting in sorghum having a major effect on reducing grains per head, and little effect on increasing weight per grain in contradiction to the crrent results has been reported (Inuyama *et al*., 1976; Yadav, *et al*., 2005). The non-significant effect of drought on stalk weight, biomass and harvest index could be due to the intensity and duration of water stress imposed. Inuyama *et al*., (1976) showed that under different levels of water deficits, plant elongation was stunted and dry matter accumulation was sustained for some time after stoppage of plant elongation. However at severe water deficits, both plant elongation and dry matter accumulation completely ceased. This can be confirmed from the result of this study as plant height was significantly decreased under drought, but growth parameters related to dry matter accumulation such as stalk weight, biomass and harvest index remained unaffected. This may be probably due to the drought being mild.

3.6.2. The effect of antitranspirant

With respect to antitranspirant treatment effects on volumetric soil moisture content of pots the null hypothesis is accepted as no significant effect of antitranspirant on the volumetric soil moisture content of pots was recorded in both experiments. On the other hand, there was no significant mean antitranspirant effect on stomatal conductance in Expt.1, while the antitranspirant application significantly decreased stomatal conductance in Expt.2. Therefore, the null hypothesis on stomatal conductance is accepted as it regards Expt. 1 and rejected in the case of Expt. 2.

Reduction in stomatal conductance of droughted sorghum owing to antitranspirant application as found in this study, has not been reported anywhere in the literature. However, using another film antitranspirant phenylmercuric acetate (PMA), Shimshi (1963) found that PMA induced stomatal closure and significantly reduced transpiration in young maize and sunflower in response to various water stress regimes under field conditions. Differences in the effects of antitranspirant on stomatal conductance between Expt. 1 and 2 may be attributed to the differences in cultivars used. Since genotypic variation has been observed for gas exchange (Kidambi, *et al*., 1990) and stomatal sensitivity (Henzell *et al*., 1976) in sorghum under water stress, differences in the response of the two cultivars in terms of stomatal conductance upon application of antitranspirant might be related to the sorghum cultivar. But, this observation cannot be supported from the literature as the effect of antitranspirant on stomatal conductance in droughted sorghum has not been reported.

Results showed there was no significant effect of antitranspirant on leaf temperature, plant height and on relative water content, thus the null hypothesis is accepted in terms of the leaf temperature, plant height and relative water content. While the same argument as to the reason that drought imposition did not significantly elevate leaf temperature of droughted plants can be posited for the lack of significant antitranspirant effects, the same cannot be said to be the reason behind the lack of significant effect of antitranspirant on relative water content and plant height. The effect of antitranspirant on relative water content and plant height will become more pronounced if they were highly significantly decreased under drought, but from the current results the differences between irrigated and droughted treatments do not give enough room to statistically show improvements in these parameters.

As it concerns response of grain yield and yield components to antitranspirant, the results of this study are contrary to Fuerhing (1973) in which significant differences in yield and yield components were recorded in sorghum between irrigated and droughted and the unsprayed and sprayed at booting. Similarly, under limited irrigation Boobathi and Singh (1984) found significant increases in yield of sorghum upon application of antitranspirant; however their result showed that the more frequent the irrigation the greater the yield upon antitranspirant application. Differences between the aforementioned results and that of the current study may be ascribed to sorghum cultivar, the intensity and duration of drought, the type and concentration of antitranspirant used, and the environmental conditions. For instance, Fuerhing (1973) used a petroleum based antitranspirant, and the spray was done twice, and the plants were raised in the field as opposed to spraying once in the glasshouse in the current experiments. A common trend found in both the Boobathi and Singh (1984) and Fuerhing (1973) reports is that the efficacy of the antitranspirant to increase yield in droughted plants appears to be greater with limited irrigation , when severe plant moisture stress is not likely to be a problem, than under drought conditions. Thus, given that in the current experiments, the drought was not severe, a similar effect is anticipated but that was not the case.

3.6.3. Interactions

There were no significant drought x antitranspirant interaction effects on volumetric soil moisture contents of pots in both Expts. 1 and 2, thus the null hypothesis is upheld. However, drought x antitranspirant interaction effects on total stomatal conductance were significant in both Expts. 1 and 2. So, the null hypothesis on stomatal conductance is rejected. The interaction effects consistently show a decline in stomatal conductance in the sprayed compared with the unsprayed treatments on most days in the irrigated treatments, but in the droughted treatments, results showed little or no differences in stomatal conductance between the unsprayed and sprayed on most of the days. This could imply that the effect of antitranspirant on stomatal conductance may be dependent upon the volumetric soil moisture content, plant water content and probably the leaf area covered by the antitranspirant.

With regard to seed number per plant in Expt. 2, the null hypothesis is rejected as significant drought x antitranspirant interactions were recorded. The antitranspirant increased grain number in the droughted but reduced it in the irrigated treatments. Perhaps because grain number was compensated by higher weight per grain in the irrigated and not in the droughted treatment. Drought x antitranspirant interaction effects on other yield parameters, namely grain weight per plant, weight per grain, stalk weight per plant, biomass per plant and harvest index in both Expts. 1 and 2 were not significant, therefore the null is accepted in these cases. However, a yield compensation process is apparent with regard to grain number and weight per grain in both experiments showing that in the irrigated treatments in Expt. 1, low weight per grain is compensated for by a high grain number and in the droughted treatments, low grain number is compensated for by high weight per grain. And in Expt. 2, low grain number is compensated by high weight per grain, while in the droughted treatments low weight per grain is compensated for by high grain number. Interaction of antitranspirant and drought with time indicates that at certain times after spraying, drought and antitranspirant can have significant effects on stomatal conductance.

3.7. Conclusions

The overall aim of this study was to evaluate the response of transpiration, growth, and yield of sorghum under water stress to film antitranspirant application using two different cultivars. In conclusion, although drought imposed reduced soil volumetric moisture content and stomatal conductance, the magnitude and duration of the water stress was not sufficient to cause deleterious effects on yield. On the other hand, although antitranspirant application could reduce stomatal conductance, it could not remedy the drought effects on volumetric soil moisture content, growth and yield. Further studies in the subsequent chapters would involve changing to another cultivar due to the limitations of small seeds in Pen 110 and growth alteration in SAMSORG - 40 cultivars. Adjusting the drought duration and intensity to achieve reduction in grain yield under glasshouse conditions and varying the concentrations of antitranspirant to determine optimum dose rates will be carried out.

CHAPTER 4

Response of sorghum to varying concentrations of antitranspirant and terminal drought initiated at different growth stages

4.1. Introduction to chapter 4

The results of preceding Expts. 1 and 2 have shown that the drought reduced transpiration, but did not reduce yield and yield components, also the antitranspirant reduced transpiration, but did not increase grain yield and yield components of sorghum. This result could be due to the magnitude of the drought not being severe enough to decrease grain yield or not being imposed at the appropriate growth stage at which it can cause significant reduction in grain yield or both. In this project, it is essential to demonstrate significant yield losses from drought to clearly validate yield increases from antitranspirants. As per the effect of drought stress, Lewis *et al*., (1974) noted that the yield reduction resulting from a certain magnitude of water stress applied at a certain plant growth stage is useful for soil – water management purposes, and so the growth stage at which this occurs needs to be determined. Some reports have underscored this position having shown that the effects of drought stress on sorghum grain yields were influenced by the magnitude of the water deficit as well as the stage of plant development (See Chapter 2). The results from the experiments reviewed in Chapter 2 confirms that the growth stage at which drought occurs, the duration of drought and the severity of the drought situation are critical to yield and yield component reduction in droughted sorghum.

Regarding the antitranspirant effect, there is evidence in the literature that antitranspirant if applied at certain rates reduces the degree and duration of moisture stress by restricting moisture loss from individual leaves thus increasing plant water availability and crop yield. See reports by Fuehring (1973) on sorghum, Holloway and Kettlewell (2010) on wheat and Faralli *et al*., (2017) on oil seed rape in Chapter 2. It is obvious from these published results that the rate of application of antitranspirant is critical hence the need for further studies on optimum levels of application in this project.

Another dimension to antitranspirant usage is the restriction in the usefulness of established commercial grade antitranspirant in agriculture because of cost and toxic side effects (Willmer and Finker, 1996; Meidner and Mansfield, 1968). Appraising the potentials of oils derived from some locally grown and available plants for use as antitranspirant has been suggested in Kettlewell (2014). In this regard, cheaper and environmentally friendly antitranspirants had been formulated from oils derived from some plants, for example castor bean (Javan, *et al*., 2012) and soybean (Ji, et *al*., 2017) and used with some successes (See Chapter 2). In this project Neem Oil derived from the seed and fruit of the neem tree (*Azadirachta indica*) is used as an antitranspirant to ameliorate plant water stress for the first time. Neem Oil was used because it contains terpenoids whose derivatives exhibited antitranspirant property on barley (Johannes and Grossmann 1985).

Therefore, the subsequent experiments in this chapter were designed to assess the effect of various concentrations of antitranspirant, drought at various growth stages and types of antitranspirant on transpiration, growth and yield of sorghum. Expt.3 was designed to evaluate the effects of increasing concentrations of antitranspirant above the 1.0 L/ha used in the previous Expts. 1 and 2, to 2.0 L/ha and 3.0 L/ha and drought at booting on transpiration, growth and yield of sorghum. Expt. 4 considered drought imposition at four different growth stages namely: 3-leaf, 5-leaf, 8-leaf to booting and panicle emergence and antitranspirant at the rate of 1.0 L/ha sprayed at booting to flowering on the grain yield of sorghum, while Expt.5 assessed the effects of drought at the 3-leaf stage and antitranspirant at 1.0 L/ha sprayed at booting on the grain yield of sorghum. Expt.6 was a comparison of the effect of Vapor Gard with Neem Oil on sorghum droughted at the 3-leaf growth stage and sprayed with Vapor Gard or Neem Oil at 1.0 L/ha on the grain yield of sorghum under drought.

Glasshouse experiment 3

Effect of varying concentrations of antitranspirant and drought imposed at booting to flowering on transpiration, growth and yield of sorghum

The null hypothesis for this study was:

- There is no significant effect of drought and varying concentrations of antitranspirant on drought tolerance in sorghum.

The objectives of the study were to determine the effects of varying concentrations of antitranspirant and drought at booting to flowering on:

- Volumetric soil moisture content,
- Transpiration,
- Growth and yield and yield components of sorghum.

4.2. Materials and methods

Materials and methods were identical to Expts. 1 and 2 except for the following:

4.2.1. Experimental design and treatments 80

The experimental design was a 2 x 4 factorial in 6 replicates with a total of 48 plants in a randomised complete block design. Drought and antitranspirant are the two factors. The two levels of drought were ‘drought’ and ‘no drought’ with four levels of the antitranspirants sprays as described in section 4.2.2. Skeleton ANOVA of measurements are shown on tables ranging from Table 4.2.1 to 4.2.3.

4.2.2. Antitranspirant application

Various concentrations of antitranspirant at the following rates were used: 0.0 L/ha, 1.0 L/ha, 2.0 L/ha, and 3.0 L/ha. These correspond with concentrations of 0.0 v/v %, 0.5 v/v %, 1.0 v/v % and 1.5 v/v % of the antitranspirant in water and also unsprayed, spray 1,spray 2 and spray 3 in conjunction with ‘irrigated’ or ‘droughted’ according to watering regime. Table 4.2.3 shows some events occurring and operations carried out during the experiment.

4.2.3. Plant material

The sorghum variety used in this experiment was BirdGO Grain Sorghum (Bright Seeds, UK).

Table 4.2.1. Some significant dates and events during Expt.3.

Year	Date	Operation
2016	February 26	Sowing
	March 7	Emergence
	May 13	Drought application and antitranspirant spraying
	May 31	Stopping drought and resumption of full irrigation
	July 4	Harvest

Table 4.2.2. Skeleton ANOVA of the volumetric soil moisture content measurements (Expt. 3)

Source of variation	Degrees of freedom
Blocks stratum	5
Blocks x subject stratum	
Drought	1
Antitranspirant	3
Drought x antitranspirant	3
Residual	35
Blocks x subject x time stratum	
Time	4
Time x drought x antitranspirant	4
Time x antitranspirant	12
Time x drought x antitranspirant	12
Residual	160
Total	239

Table 4.2.3. Skeleton ANOVA of adaxial and abaxial stomatal conductance measurements (Expt. 3)

Source of variation	Degrees of freedom
Blocks stratum	5
Blocks x subject stratum	
Drought	1
Antitranspirant	3
Drought x antitranspirant	3
Residual	35
Blocks x subject x time stratum	
Time	4
Time x drought	4
Time x antitranspirant	12
Time x drought x antitranspirant	12
Residual	160
Total	239

Table 4.2.4. Skeleton ANOVA of the yield and yield components measurements (Expt. 3)

Source of variation	Degrees of freedom
Blocks stratum	5
Blocks x units x stratum	
Drought	1
Antitranspirant	3
Drought x antitranspirant	3
Residual	28 (7)
Total	40 (7)

4.2.5. Statistical analysis

Data were analysed using GENSTAT, 16th edition (VSN International Ltd, Hemel Hempstead, UK). Repeated measures analysis of variance (ANOVA) was used for volumetric soil moisture content and stomatal conductance whereas a two-way ANOVA was used if interactions with time were significant as well as for yield and yield components. Data were checked for normality and variance homogeneity by examining the residual plots.

4.3. Results

4.3.1. Volumetric soil moisture content

Results of analysis of data on effect of drought and varying concentrations of antitranspirant on volumetric soil moisture content of pots are presented in Table 4.3.1 and Figure 4.3.1a and 4.3.1b. Drought significantly ($P < 0.001$) reduced the mean volumetric soil moisture content of pots over time from 32.3 % in the irrigated to 15.1 % in the droughted pots. The effect of the antitranspirant on volumetric soil moisture content was however not significant.

But there was a border-line significant (P = 0.062) drought x antitranspirant interaction effect on volumetric soil moisture content, because volumetric soil moisture content decreased with increasing antitranspirant concentration in the droughted pots but increased with increasing antitranspirant concentrations in the irrigated pots. There was a significant effect of time (P < 0.001) as volumetric soil moisture content of pots consistently declined from the first to the last day of measurements. Time x drought (P = 0.005) interaction effect on volumetric soil moisture content was significant because the reduction due to drought became greater with time. Results from the two-way analysis of variance performed for each day of measurement showed significant (P = 0.001) differences (reduction) in the soil volumetric moisture content between irrigated and droughted pots on all days after spraying (DAS) with the differences (reduction) becoming greater with time. The time x antitranspirant interaction and the time x drought x antitranspirants interaction on volumetric soil moisture content were not significant. Even though significant and borderline effects of drought x antitranspirant were found in the two way analysis at 5 DAS (P = 0.010) and 14 DAS (P = 0.062) respectively, no consistent effects were observed from the repeated measures analysis. Two-way analysis of variance on DAS (Table 4.3.2) indicated a significant (P = 0.001) response of the volumetric soil moisture content to drought at all DAS, whereas various concentrations of the antitranspirant treatment induced significant differences in volumetric soil moisture content at 3 DAS (P = 0.015).

Table 4.3.1. Average volumetric soil water content (%) of pots under drought and varying concentrations of antitranspirant applied at 67 days after emergence of sorghum grown in the glasshouse in May 2016 measured at 3, 5, 11, 14 and 27 days after spraying during booting to flowering (n = 48) ± Standard error of the differences of the mean (SED) (Expt.3).

	Days after spraying					
Treatments	3	5	11	14	27	Mean
Irrigated	34.14	32.17	31.82	31.51	32.29	32.39
Droughted	20.07	15.06	15.38	12.41	12.35	15.05
Unsprayed	25.98	23.75	23.99	24.55	21.14	23.88
Sprayed 1 L/ha	24.64	22.86	22.67	21.26	22.53	22.79
Sprayed 2 L/ha	28.36	23.82	23.53	21.07	22.83	23.92
Sprayed 3 L/ha	29.45	24.06	23.58	22.5	21.85	24.29
Irrigated unsprayed	33.47	31.86	32.66	32.13	28.56	31.73
Irrigated sprayed 1 L/ha	29.66	29.28	30.84	30.04	32.92	30.54
Irrigated sprayed 2 L/ha	36.76	31.86	32.13	32.66	28.56	33.21
Irrigated sprayed 3 L/ha	36.68	29.28	30.04	30.84	32.92	34.06
Droughted unsprayed	18.48	15.64	15.85	16.45	13.71	16.03
Droughted sprayed 1 L/ha	19.61	16.43	15.31	11.68	12.14	15.03
Droughted sprayed 2 L/ha	19.95	14.75	12.21	14.98	11.26	14.63
Droughted sprayed 3 L/ha	22.21	13.43	11.34	15.37	10.23	14.52
Mean	27.10	23.62	23.44	22.35	22.08	23.72
df						160
SED						3.69
CV %						15.6
						P values
Drought						< 0.001
Antitranspirant						0.369
Drought x antitranspirant						0.062
Time						< 0.001
Time x drought						0.005
Time x antitranspirant						0.208
Time x drought x antitranspirant						0.291

Table 4.3.2. Two-way ANOVA on effect of varying concentrations of antitranspirant and drought on volumetric soil moisture contents (%) of pots during booting to flowering sorghum grown in the glasshouse in May, 2016. (n = 48)(Expt. 3).

	Days after spraying				
	3	5	11	14	27
Drought	0.001	0.001	0.001	0.001	0.001
Antitranspirant	0.015	0.768	0.618	0.094	0.958
Drought x antitranspirant	0.184	0.011	0.687	0.062	0.149
df	35	35	35	35	35
SED	3.807	2.955	2.465	5.913	3.662

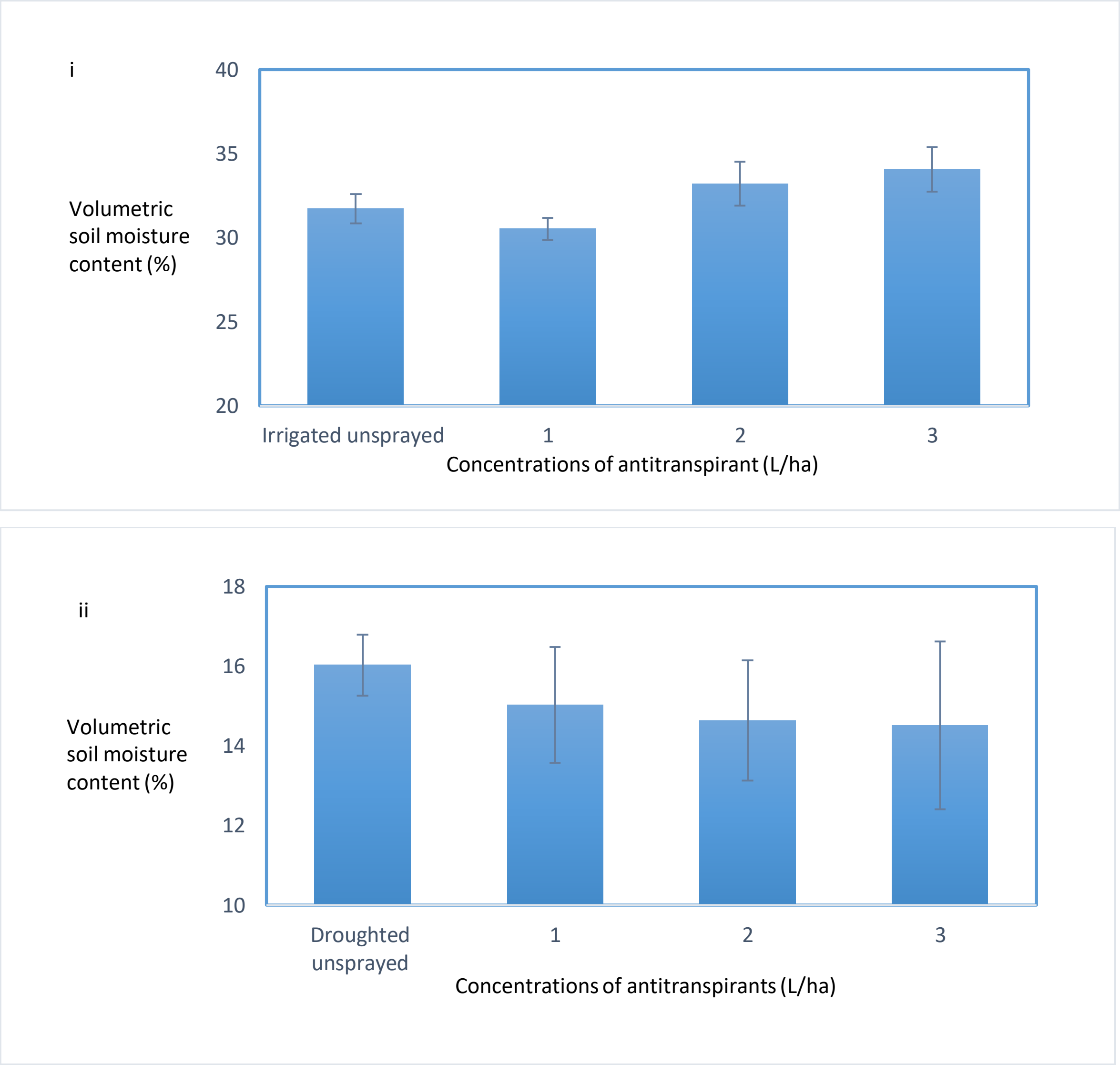

Figure 4.3.1a. Interactions between drought x antitranspirant on volumetric soil moisture content (%) in (i) irrigated and (ii) droughted pots during booting to flowering of sorghum grown in the glasshouse in May 2016. (n = 48). (Drought P < 0.001, antitranspirant P = < 0.369; drought x antitranspirant P = 0.062) Error bars are SEM (Expt.3).

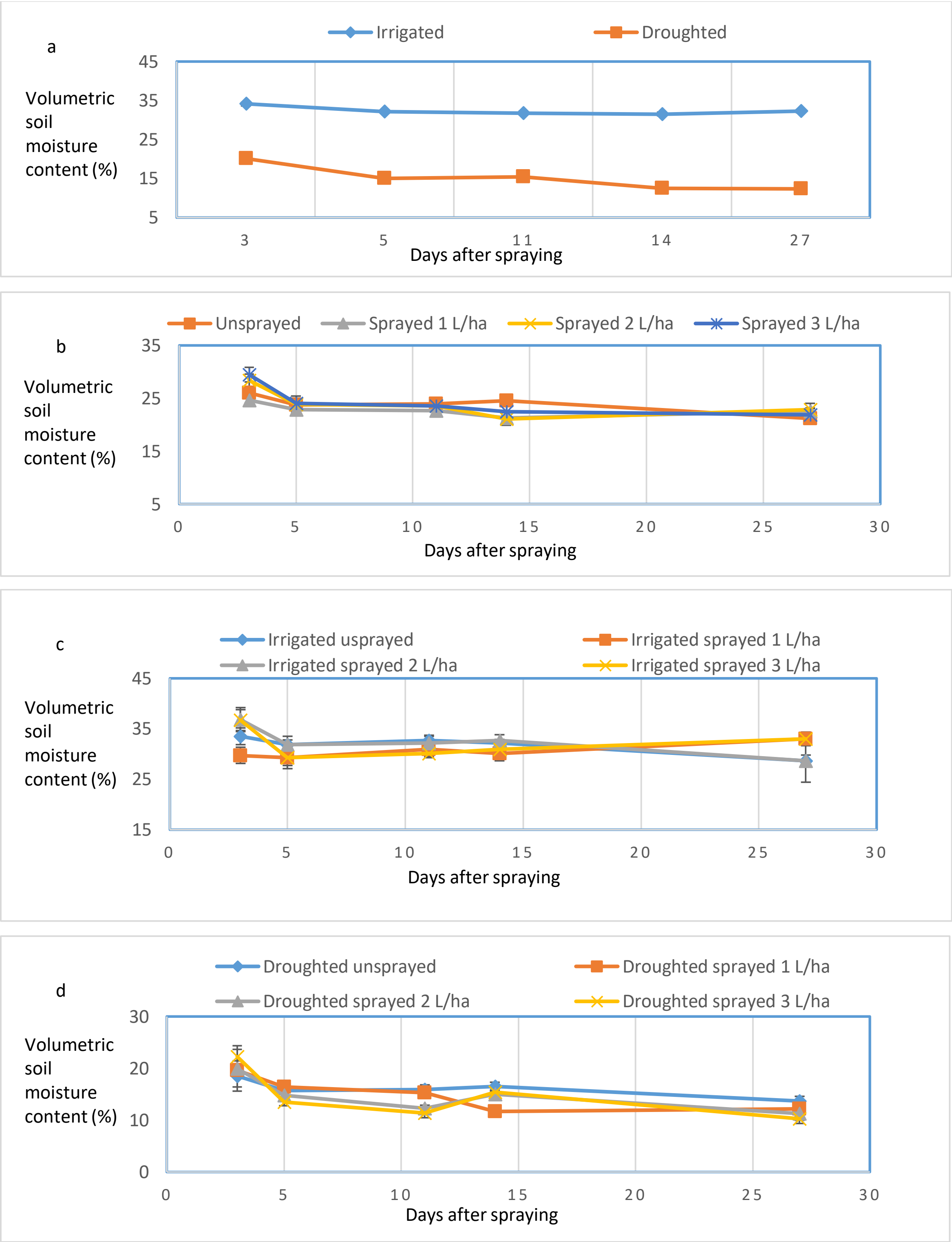

Figure 4.3.1b. Response of volumetric soil moisture content (%) to (a) drought (b) antitranspirants (c) drought x antitranspirant interaction effects (irrigated) and (d) drought x antitranspirant interaction effects (droughted) during booting to flowering of sorghum grown in the glasshouse in May 2016. (n = 48) (Drought $P < 0.001$, antitranspirants $P = 0.369$; drought x antitranspirants $P = 0.062$). Error bars are SEM (Expt.3).

4.3.2. Adaxial stomatal conductance

The results of data analysis on response of adaxial stomatal conductance to drought and varying concentrations of antitranspirants are presented in Table 4.3.3 and Figure 4.3.2. There were significant differences in adaxial stomatal conductance between irrigated and droughted as well as between unsprayed and sprayed treatments. Drought treatment significantly ($P < 0.001$) decreased mean adaxial stomatal conductance by 19.2 % in the droughted compared with the irrigated, and the antitranspirant treatment significantly ($P < 0.001$) reduced mean adaxial stomatal conductance in the sprayed plants by 13.5 % in 1.0 L/ha, 14.9 % in 2.0 L/ha and 12.0 % in 3.0 L/ha compared with the unsprayed plants. There was a significant ($P = 0.005$) drought x antitranspirant interaction effect as decrease in adaxial stomatal conductance due to antitranspirant was greater in the irrigated than in the droughted treatments. The effect of time was significant ($P < 0.001$) showing a consistent decline in adaxial stomatal conductance as the days after spraying (DAS) progresses. There was a highly significant ($P < 0.001$) time x drought interaction on adaxial stomatal conductance as the differences between the irrigated and droughted treatments decreased as DAS increased. According to two-way analysis of variance significant differences (reduction) in adaxial stomatal conductance between the droughted and the irrigated treatments occurred at 3 DAS ($P < 0.001$), 7 DAS ($P < 0.001$) and 12 DAS ($P = 0.043$) but not at 17 DAS and 22 DAS. The antitranspirant treatment gave a highly significant ($P < 0.001$) time x antitranspirant interaction effect as the spray decreased adaxial stomatal conductance in the sprayed compared with unsprayed treatments at 7 DAS, 12 DAS, 17 DAS and 22 DAS, whereas at 3 DAS an a greater adaxial stomatal conductance in the sprayed over the unsprayed treatments was observed. A two-way analysis of variance showed highly significant differences in adaxial stomatal conductance between unsprayed and sprayed treatments at 7 DAS ($P < 0.001$), 12 DAS ($P < 0.001$) and 17 DAS ($P < 0.001$) and significant differences at 22 DAS ($P = 0.001$) and no significant difference at 3 DAS. Furthermore, a significant ($P = 0.002$) time x drought x antitranspirant interaction effect was observed on adaxial stomatal conductance. The data showed that this interaction occurred because differences in adaxial stomatal conductance between the unsprayed and the sprayed treatments were smaller in the droughted than the irrigated treatments at 3 DAS, 7 DAS and 12 DAS but the differences were greater in the droughted than the irrigated at 17 DAS and at 22 DAS although at 22 DAS differences between unsprayed and sprayed was greater in the irrigated than the droughted in the 1.0 L/ha dosage. Results of two-way analysis of variance (Table 4.3.4) showed that drought x antitranspirant interaction was significant at 3 DAS ($P = 0.010$), 7 DAS ($P < 0.001$), 12 DAS ($P = 0.054$) and 17 DAS ($P = 0.037$).

Table 4.3.3. Average adaxial stomatal conductance (mmol $m^{-2}s^{-1}$) recorded on the flag leaf under drought and varying concentrations of antitranspirant applied at 67 days after emergence of sorghum grown in the glasshouse in May 2016 measured during booting to flowering at 3, 7, 12, 17 and 22 days after spraying (n = 48) ± Standard error of the differences of the mean (SED) (Expt.3).

	Days after spraying					
Treatments	3	7	12	17	22	Mean
Irrigated	31.31	31.34	27.02	23.03	22.22	29.98
Droughted	25.54	26.60	24.56	22.23	22.21	24.23
Unsprayed	26.25	32.77	32.59	26.14	24.63	28.48
Sprayed 1 L/ha	27.04	29.03	27.28	20.53	19.38	24.65
Sprayed 2 L/ha	28.69	25.89	22.15	22.04	22.40	24.23
Sprayed 3 L/ha	31.72	28.18	21.15	21.79	22.45	25.06
Irrigated unsprayed	32.61	39.18	36.60	24.78	24.27	31.49
Irrigated sprayed 1 L/ha	28.74	30.07	26.77	21.73	18.80	25.22
Irrigated sprayed 2 L/ha	29.66	25.88	22.55	24.10	23.10	25.06
Irrigated sprayed 3 L/ha	34.25	30.22	22.17	21.48	22.72	26.17
Droughted unsprayed	19.89	26.35	28.58	27.50	25.00	25.47
Droughted sprayed 1 L/ha	25.34	28.00	27.78	19.33	19.97	24.08
Droughted sprayed 2 L/ha	27.73	25.90	21.75	19.98	21.69	23.41
Droughted sprayed 3 L/ha	29.18	26.15	20.13	22.10	22.18	23.95
Mean	28.43	28.97	25.79	22.63	22.22	25.61
df						160
SED						3.85
CV %						15
						P values
Drought						< 0.001
Antitranspirant						< 0.001
Drought x antitranspirant						0.005
Time						< 0.001
Time x drought						< 0.001
Time x antitranspirant						< 0.001
Time x drought x antitranspirant						0.002

Table 4.3.4. Two-way ANOVA on the effect of drought and varying concentrations of antitranspirant applied at 67 days after emergence on adaxial stomatal conductance recorded on the flag leaf measured at 3,7,12,17 and 22 days after spraying during booting to flowering of sorghum grown in the glasshouse in May, 2016. (n = 48)(Expt. 3).

	Days after spraying				
	3	7	12	17	22
Drought	<0.001	<0.001	0.043	0.361	0.989
Antitranspirant	0.009	<0.001	<0.001	<0.001	0.001
Drought x antitranspirant	0.100	<0.001	0.054	0.037	0.694
df	35	35	35	35	35
SED	3.953	3.953	4.047	2.98	2.927

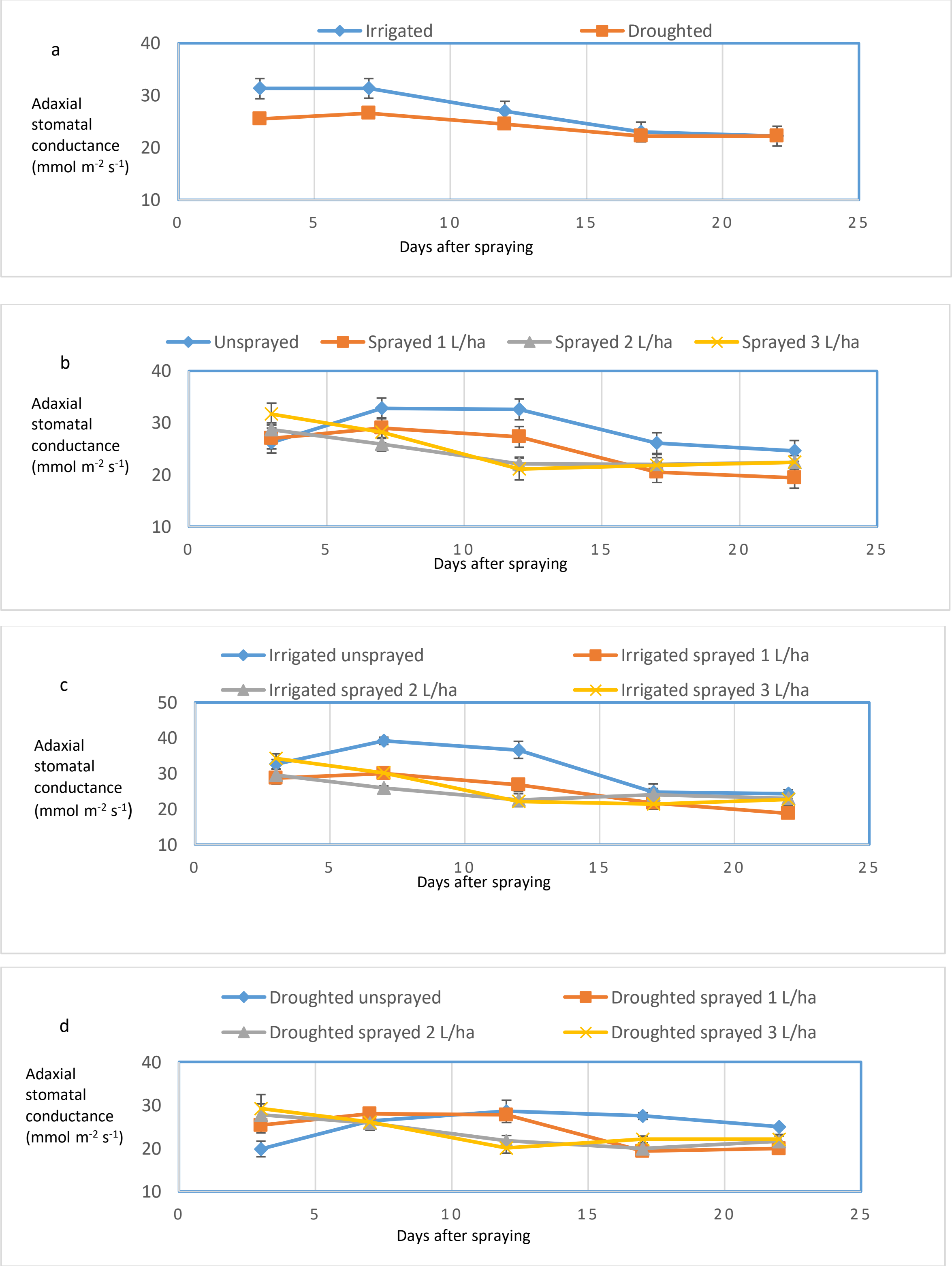

Figure 4.3.2. Response of adaxial stomatal conductance (mmol $m^{-2}s^{-1}$) to (a) drought (b) antitranspirant (c) drought x antitranspirant interaction effects (irrigated) and (d) drought x antitranspirant interaction effects (droughted) applied at 67 days after emergence recorded on the flag leaf during booting to flowering of sorghum grown in the glasshouse in May 2016. (n = 48). (Drought $P < 0.001$; antitranspirant P = < 0.001; drought x antitranspirant P = 0.005). Error bars are SEM (Expt.3).

4.3.3. Abaxial stomatal conductance

The response of abaxial stomatal conductance to drought imposition and application of varying concentrations of antitranspirant are presented on Table 4.3.5 and Figure 4.3.3. Significant differences in abaxial stomatal conductance were recorded between irrigated and droughted and between unsprayed and sprayed plants. Drought treatment significantly ($P < 0.001$) reduced mean abaxial stomatal conductance by 13.4 % in the droughted compared with the irrigated plants. The antitranspirant significantly ($P = 0.001$) decreased abaxial stomatal conductance by 10.6 % in 1.0 L/ha and 0.2 % in 2.0 L/ha, whereas in 3.0 L/ha there was an increase of 0.3 % in abaxial stomatal conductance. Interaction effects of drought x antitranspirant were not significant. The effect of time on abaxial stomatal conductance was highly significant ($P < 0.001$) showing that mean abaxial stomatal conductance decreased as days after spraying (DAS) increased. No significant interaction of time x drought was recorded, but the time x antitranspirant interaction effect was highly significant ($P < 0.001$). The time x antitranspirant interaction occurred for a variety of reasons: in the unsprayed and the 3.0 L/ha treatments, abaxial stomatal conductance decreased with increasing DAS up to 7 DAS and thereafter increased with increasing DAS to a peak at 12 DAS and later decreased with increasing DAS. Whereas in the 1.0 L/ha it increased with increasing DAS to a peak at 12 DAS and subsequently decreased with increasing DAS. However, in the 2.0 L/ha treatment, abaxial stomatal conductance consistently decreased with increasing DAS. Two-way analyses of variance (Table 4.3.6) for each DAS showed significant differences (decreases) between irrigated and droughted treatments at 1 DAS ($P < 0.001$), 3 DAS ($P < 0.001$) and 12 DAS ($P < 0.001$) and significant differences (reductions) between unsprayed and sprayed plants at 1 DAS ($P < 0.001$), 3 DAS ($P = 0.031$), 8 DAS ($P = 0.016$), 12 DAS ($P = 0.002$) and 16 DAS ($P = 0.002$). The time x drought x antitranspirant interaction effect was significant ($P = 0.001$) at 3 DAS and 12 DAS occurring for no obvious reason.

Table 4.3.5. Average abaxial stomatal conductance (mmol $m^{-2}s^{-1}$) recorded on the flag leaf under drought and varying concentrations of antitranspirant applied at 67 days after emergence of sorghum grown in the glasshouse in May 2016 measured during booting to flowering at 3, 7, 12, 17 and 22 days after spraying (n = 48) ± Standard error of the differences of the mean (SED) (Expt.3).

	Days after spraying					
Treatments	3	7	12	17	22	Mean
Irrigated	43.86	40.23	40.31	40.17	32.88	39.49
Droughted	36.99	34.49	37.33	33.59	29.48	34.38
Unsprayed	39.75	39.27	40.54	42.01	30.47	38.41
Sprayed 1 L/ha	34.69	35.93	36.6	35.34	25.68	33.65
Sprayed 2 L/ha	45.05	39.37	35.35	33.92	32.61	37.26
Sprayed 3 L/ha	42.22	34.89	42.81	36.25	35.95	38.42
Irrigated unsprayed	42.49	43.95	39.83	41.43	34.63	40.47
Irrigated sprayed 1 L/ha	38.44	39.58	39.93	38.75	24.25	36.19
Irrigated sprayed 2 L/ha	46.89	42.65	37.98	41.25	33.13	40.38
Irrigated sprayed 3 L/ha	47.61	34.75	43.5	39.25	39.5	40.92
Droughted unsprayed	37	34.58	41.25	42.58	26.32	36.35
Droughted sprayed 1 L/ha	30.94	32.27	33.26	31.93	27.12	31.1
Droughted sprayed 2 L/ha	43.21	36.08	32.72	26.58	32.1	34.14
Droughted sprayed 3 L/ha	36.83	35.03	42.11	33.25	32.4	35.92
Mean	40.43	37.36	38.82	36.88	31.18	36.93
df						160
SED						5.036
CV %						13.6
						P values
Drought						< 0.001
Antitranspirant						0.001
Drought x antitranspirant						0.861
Time						< 0.001
Time x drought						0.197
Time x antitranspirant						< 0.001
Time x drought x antitranspirant						0.001

Table 4.3.6. Two-way ANOVA on effect of drought and varying concentrations of antitranspirant and drought applied at 67 days after emergence on abaxial stomatal conductance recorded on the flag leaf measured at 3, 7, 12, 17 and 22 days after spraying during booting to flowering of sorghum grown in the glasshouse in May, 2016 (n = 48) (Expt. 3).

	Days after spraying				
	3	7	12	17	22
Drought	< 0.001	< 0.001	0.097	< 0.001	0.064
Antitranspirant	< 0.001	0.031	0.016	0.002	0.002
Drought x antitranspirant	0.259	0.056	0.357	0.005	0.107
df	35	35	35	35	35
SED	4.455	4.361	6.054	5.004	6.152

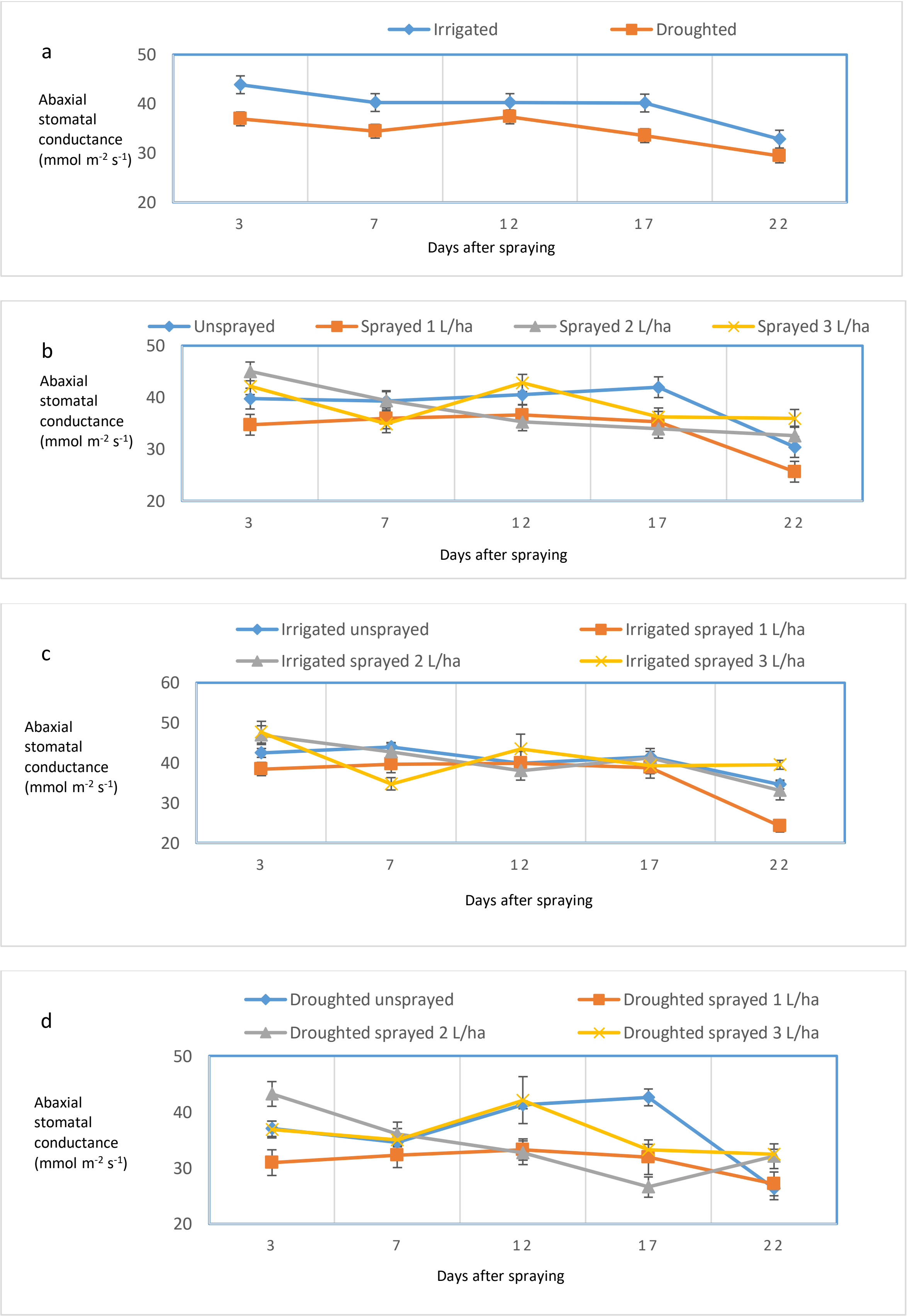

Figure 4.3.3. Response of abaxial stomatal conductance (mmol $m^{-2}s^{-1}$) to (a) drought (b) antitranspirant (c) drought x antitranspirant interaction effects (irrigated) and (d) drought x antitranspirants interaction effects on (droughted) applied at 67 days after emergence recorded on the flag leaf during booting to flowering of sorghum grown in the glasshouse in May 2016. (n = 48). (Drought $P < 0.001$; antitranspirants $P= 0.001$; drought x antitranspirants $P = 0.861$). Error bars are SEM. (Expt.3).

4.3.4. Total stomatal conductance

The results of data analysis on the effect of drought imposition and application of various concentrations of antitranspirant on total stomatal conductance are presented on Table 4.3.7 and Figure 4.3.4. There was a significant difference in total stomatal conductance between the droughted and irrigated as well as unsprayed and sprayed plants. Drought imposition significantly reduced mean total stomatal conductance by 11.9 % in the droughted compared with the irrigated plants. Antitranspirant application decreased total stomatal conductance in by 12.4 % in 1.0 L/ha; 8.0 % in 2.0 L/ha and 5.1 % in the 3.0 L/ha sprayed compared with the unsprayed plants. No significant drought x antitranspirant interaction effects on total stomatal conductance occurred. Time ($P < 0.001$) was significant with the magnitude of mean total stomatal conductance being reduced from the first to the last day of measurement. Time x drought interaction effect was not significant. But the time x antitranspirant interaction effect was highly significant ($P < 0.001$) due to certain trends in the data: In the unsprayed treatment total stomatal conductance increased with increasing DAS and reached a peak at 12 DAS and later declined whereas in the 1.0 L/ha and 2.0 L/ha treatments it consistently decreased with increasing DAS. However in the 3.0 L/ha treatment, total stomatal conductance decreased with increasing DAS and reached the lowest point at 17 DAS and later increased with increasing DAS. Significant interaction effects of time x drought x antitranspirant occurred because in both the irrigated and droughted treatments, total stomatal conductance increased with increasing DAS and later decreased with increasing DAS in the unsprayed, 1.0 L/ha and 2.0 L/ha irrigated and droughted treatments, but in the 3.0 L/ha in both irrigated and droughted treatments, total stomatal conductance decreased somewhat consistently and later increased with increasing DAS. Two-way analysis of variance on each day after spraying (DAS) (Table 4.3.8) indicated that there were significant differences (reduction) in total stomatal conductance between irrigated and droughted treatments at 3 DAS ($P < 0.001$), 7 DAS ($P < 0.001$), 12 DAS ($P < 0.013$) and 17 DAS ($P < 0.001$). Significant drought x antitranspirant interactions occurred at 3 DAS ($P = 0.041$), 7 DAS ($P < 0.001$) and 17 DAS ($P = 0.002$), but not at other dates.

Table 4.3.7. Average total stomatal conductance (mmol $m^{-2}s^{-1}$) recorded on the flag leaf under drought and varying concentrations of antitranspirant applied at 67 days after emergence of sorghum grown in the glasshouse in May 2016 measured during booting to flowering at 3, 7, 12, 17 and 22 days after spraying (n = 48) ± Standard error of the differences of the mean (SED) (Expt.3).

	Days after spraying					
Treatments	3	7	12	17	22	Mean
Irrigated	37.59	35.79	33.67	31.60	27.55	33.24
Droughted	31.26	30.55	30.95	27.91	25.85	29.30
Unsprayed	33.00	36.02	36.57	34.08	27.55	33.44
Sprayed 1 L/ha	30.86	32.48	31.94	27.94	22.53	29.15
Sprayed 2 L/ha	36.87	32.63	28.75	27.98	27.50	30.75
Sprayed 3 L/ha	36.97	31.54	31.98	29.02	29.20	31.74
Irrigated unsprayed	37.55	41.57	38.22	33.11	29.45	35.98
Irrigated sprayed 1 L/ha	33.59	34.83	33.35	30.24	21.53	30.71
Irrigated sprayed 2 L/ha	38.27	34.27	30.27	32.68	28.11	32.72
Irrigated sprayed 3 L/ha	40.93	32.48	32.83	30.37	31.11	33.54
Droughted unsprayed	28.45	30.47	34.92	35.04	25.66	30.91
Droughted sprayed 1 L/ha	28.14	30.13	30.52	25.63	23.54	27.59
Droughted sprayed 2 L/ha	35.47	30.99	27.23	23.28	26.90	28.77
Droughted sprayed 3 L/ha	33.00	30.59	31.12	27.68	27.29	29.94
Mean	34.43	33.17	32.31	29.75	26.70	31.27
d.f.						160
SED						3.292
CV %						10.5
						P values
Drought						< 0.001
Antitranspirant						< 0.001
Drought x antitranspirant						0.615
Time						< 0.001
Time x drought						0.009
Time x antitranspirant						< 0.001
Time x drought x antitranspirant						< 0.001

Table 4.3.8. Two-way ANOVA on effect of drought and varying concentrations of antitranspirants applied at 67 days after emergence on total stomatal conductance (mmols $m^{-2}s^{-1}$) recorded on the flag leaf measured at 3, 7, 12, 17 and 22 days after spraying during booting to flowering grown in the glasshouse in May, 2016. (n = 48)(Expt. 3).

	3	7	12	17	22
Drought	< 0.001	< 0.001	0.013	< 0.001	0.133
Antitranspirant	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Drought x antitranspirant	0.041	< 0.001	0.952	0.002	0.217
df	35	35	35	35	35
SED	2.770	2.562	3.610	3.299	3.883

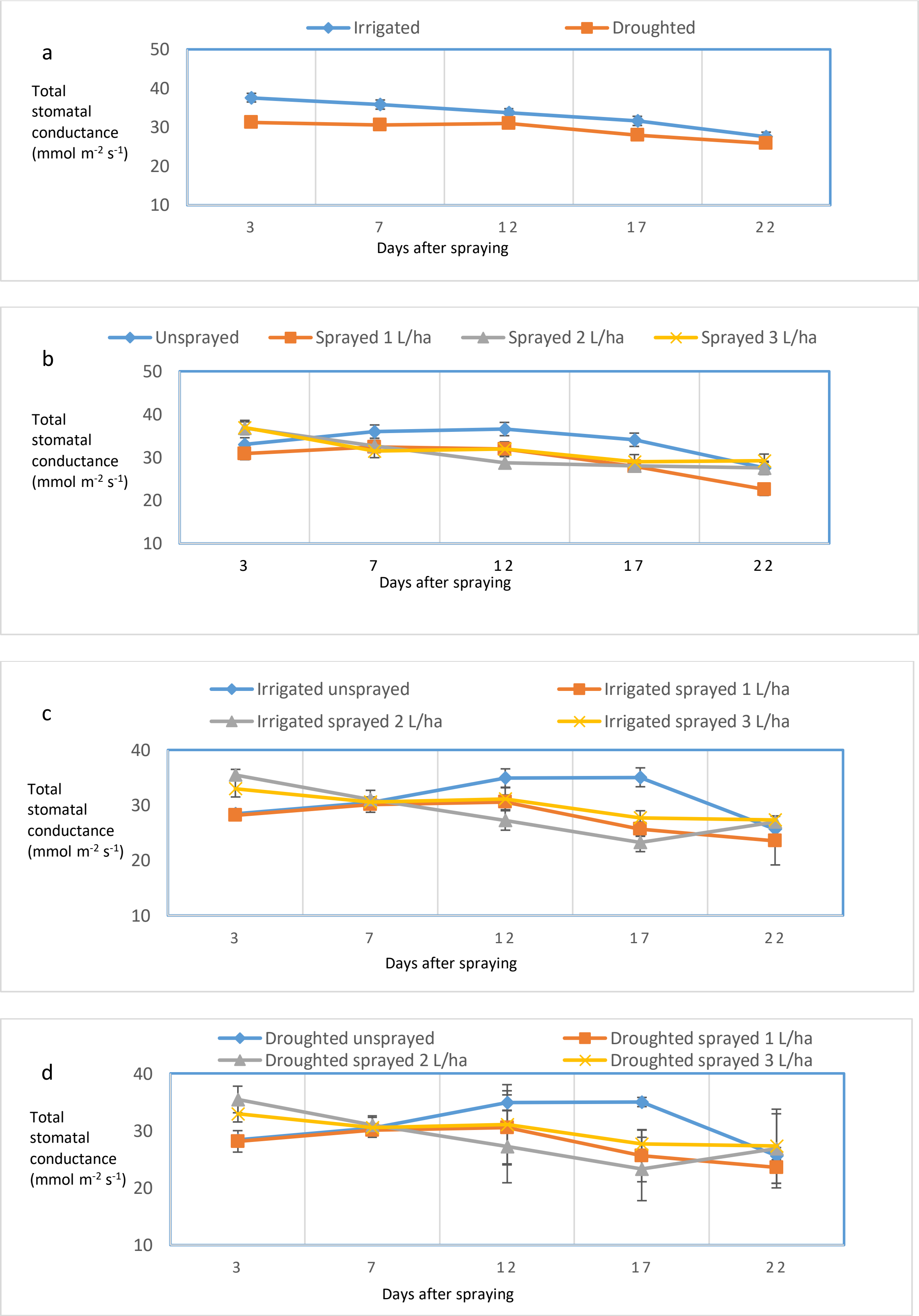

Figure 4.3.4. Response of total stomatal conductance (mmol $m^{-2}s^{-1}$) to (a) drought (b) antitranspirants (c) drought x antitranspirant interaction effects (irrigated) and (d) drought x antitranspirant interaction effects (droughted) applied at 67 days after emergence recorded on the flag leaf during booting to flowering of sorghum grown in the glasshouse in May 2016. (Drought P < 0.001; antitranspirants P< 0.001; drought x antitranspirants P = 0.615). Error bars are SEM. (Expt.3).

4.3.5. Yield and yield components

Results of analysis of the response of sorghum yield and yield components per plant to varying concentrations of antitranspirant and drought application are presented on Table 4.3.9. There were no significant differences in grain yield found between droughted and irrigated plants with drought imposition, similarly the various concentrations of antitranspirant applied did not induce any significant differences in grain yield between the unsprayed and sprayed treatments. Thus, drought and antitranspirant treatments did have any significant effect on grain yield. In addition, no significant drought x antitranspirant interaction effect on grain yield was found. There was a highly significant ($P < 0.001$) difference in grain number between the droughted and irrigated plants with the grain number in the droughted being 24.35 % lower than in the irrigated treatments. However, the antitranspirant caused no significant effect on the grain number per plant, and no drought x antitranspirant interaction effect on grain number per plant was recorded. A higly significant difference ($P < 0.001$) in weight per grain was recorded between irrigated and droughted treatments in favour of the droughted treatments, whereas no significant differences in weight per grain were found between unsprayed and sprayed treatments. Thus, drought significantly increased weight per grain by 22.14 % in the droughted compared with irrigated plants, while with antitranspirant treatments no significant effect was recorded. Interaction effect of drought x antitranspirant on weight per grain was not significant. The differences in stalk weight between the irrigated and droughted treatments were significant ($P = 0.004$), as drought decreased stalk weight by 14.09 % in the droughted compared with the irrigated plants. However, no significant antitranspirant and drought x antitranspirant effects on stalk weight were shown. On the other hand, there was a significant differences in biomass between irrigated and droughted plants. Drought significantly ($P = 0.014$) decreased biomass by 5.9 % in the droughted compared with the irrigated plants. With the antitranspirant, no significant differences in biomass between the unsprayed and sprayed plants was observed. Also, drought x antitranspirant interaction effects on biomass were not significant. There were no significant difference in harvest index between the unsprayed and sprayed plants. Hence, both drought and antitranspirant concentrations have no significant effect on harvest index. In addition, no drought x antitranspirant effect on harvest index was found.

Table 4.3.9. Average grain yield and yield components under drought and varying concentrations of antitranspirant applied at 67 days after emergence of sorghum grown in the glasshouse in May 2016 (n = 48) ± Standard error of the differences of the mean (SED) (Expt.3).

Treatments	Grain yield per plant (g)	Grain number per plant	Weight per grain (mg)	Stalk weight (g)	Biomass (g)	Harvest index (%)
Irrigated	14.44	542	27.60	19.09	33.5	42.9
Droughted	13.46	410	33.71	16.40	26.3	40
Unsprayed	14.84	521	29.86	19	33.8	44.4
Sprayed 1 L/ha	13.65	462	30.27	17.45	31.1	43.7
Sprayed 2 L/ha	12.61	439	29.88	17.1	25.3	35.5
Sprayed 3 L/ha	14.72	483	32.62	17.44	29.3	42.1
Irrigated usprayed	14.97	552	28.09	16.58	36.4	41
Irrigated sprayed 1 L/ha	13.75	508	27.39	16.96	31.7	43.1
Irrigated sprayed 2 L/ha	13.76	504	28.96	15.97	32	42.8
Irrigated sprayed 3 L/ha	15.3	603	25.98	16.08	34.1	44.5
Droughted unsprayed	14.72	490	31.63	16.58	31.3	47.9
Droughted sprayed 1 L/ha	13.54	415	33.16	16.96	30.5	44.2
Droughted sprayed 2 L/ha	11.46	374	30.8	15.97	18.7	28.2
Droughted sprayed 3 L/ha	14.14	362	39.26	16.08	24.6	39.7
Mean	13.95	476	30.66	17.75	29.9	41.4
Df	35	35	35	35	35	35
SED	2.637	123.9	5.461	2.992	7.38	10.4
CV %	18.9	26	17.8	16.9	24.7	25.1
P values						
Drought	0.206	< 0.001	< 0.001	0.004	0.002	0.353
Antitranspirant	0.150	0.427	0.557	0.415	0.053	0.159
Drought x antitranspirant	0.741	0.326	0.071	0.466	0.223	0.091

4.4. Discussion

4.4.1. The effect of drought stress

The drought applied had significant effects on volumetric soil moisture content of pots, stomatal conductance, weight per seed, stalk weight and biomass therefore the null hypothesis is rejected with regard to these parameters. On the other hand, drought imposed had no significant effects on grain yield, grain number, and harvest index, hence the null hypothesis is accepted.

The overall means of volumetric soil moisture content of irrigated was above the field capacity of 22.0 % and permanent wilting point of 11.0 %, and droughted pots were below the field capacity and above the permanent wilting point. The volumetric soil moisture content was not only significantly reduced in the droughted compared with the irrigated pots, but there was a consistent decline in the volumetric soil moisture content in the irrigated and droughted pots as the drought period increases. Minimal but not statistically significant reduction in volumetric soil moisture content in the irrigated pots were recorded due perhaps to the evaporation from the soil surface due to heat developed within the glasshouse and also due to plant growth. Declining soil water content upon drought imposition at the booting stage is consistent with the findings from other pot experiments with sorghum, where soil moisture content decreased as the duration of the drought period increased (Younis, *et al*., 2000). Despite these moisture regimes, significant reduction in stomatal conductance were recorded. According to Al-Hamdani *et al*., (1991) stomatal conductance in sorghum is very sensitive to small changes in leaf water potential, and the decrease in leaf water potential increased with increasing water stress. That implies that in this experiment the drought magnitude might have been sufficient to cause significant reduction in leaf water potential leading to a decrease in stomatal conductance. However, response of leaf water potential to soil water stress was not measured to validate the role it must have played in reducing the stomatal conductance. Nevertheless, decrease in stomatal conductance and hence transpiration in response to drought stress as shown in this study is widely reported mainly as a drought response characteristic in plants (Chaves, *et al*., 2003) including sorghum (Mutava, 2001; Kidambi, *et al*., 1989; Mastrorilli, 1999).

It is expected that under water stress a significant reduction in grain yield should occur in sorghum as many studies have shown (Assefa, *et al*., 2010). Water stress is expected to reduce the production and translocation of assimilates to the grains leading to fewer and smaller grains however, that is not shown in this experiment. Probably, the drought imposed although sufficient to reduce stomatal conductance was not of such severity as to reduce yield, thus the droughted plants could have been subjected to only a mild instead of severe drought stress and so enjoyed adequate moisture. In addition, the non-significant lower grain number appeared to have been compensated for by the significantly higher weight per grain in the droughted compared with the irrigated. Literature reporting substantial yield decrease in sorghum under drought is characterised by imposition of high soil water potential resulting into severe water stress often lasting through the reproductive stage e.g. Lewis *et al*., (1974) and Inuyama *et al*., (1976). However, in this experiment although the duration of drought imposed reached the reproductive stage as in the reports cited, soil moisture content was not assessed in terms of water potential. On the other hand, reports by Munamava and Riddoch (2001) and Craufurd and Peacock (1993) agree with this study as they showed non-significant differences in grain yield between irrigated and droughted sorghum as well as a numerically lower grain yield in the droughted compared with the irrigated plants with some cultivar differences playing a role. Grain number per plant was highly significantly different in the irrigated than droughted plants in agreement with findings by Craufurd and Peacock (1993). Grain number is responsible for 70 % of the final grain yield (Gerik, *et*. *al*., 2003) in sorghum and along with panicle size are determined shortly after growing point differentiation. The growing point differentiation is the stage at which the sorghum plant transits from the vegetative to the reproductive, and the total number of leaves, potential head size and grain number per plant are determined (Vanderlip and Reeves, 1972). Consequently, any type of stress at this point which can impede panicle development, will reduce the number of grains to be formed and lower final grain yield. The emergence of the flag leaf indicates the end of further leaf formation and when the flag leaf collar appears, the plant enters the booting stage at which panicle development is complete (Gerik, *et*. *al*., 2003). At this stage the plant is set for flowering, has attained maximum leaf area and accumulated approximately 60 % of its total biomass. Thus the effect of drought on grain number in this experiment (although not significant) was because water stress imposed at the booting to flowering stage reduced potential grain number which was already being determined therefore the final grain number was severely inhibited. In other words the timing of the drought must have coincided with determination of potential grain number in the plant. Weight per grain was significantly different in irrigated and droughted treatments, with the drought influencing greater weight per grain in the droughted over the irrigated. This came about as a result of compensation for lower grain number in the droughted compared with the irrigated plants, which is in accordance with other findings (Saeed, *et al*., 1986; Yang, *et al*., 2010). Because plant development sequences are correlated, component interactions may compensate by increases in some components for reduction in others under stress (Blum, 1973). Whereas grain number is often regarded as the main component of yield (Saeed, *et al*., 1986; Craufurd and Peacock, 1993; van Oosterom and Hammer, 2008), the weight per grain component is a yield stabilizing factor whose role increases in significance as yield levels decreased with production limitations (Saeed, *et al*., 1986). Thus, the effect of drought in increasing weight per grain in the droughted over the irrigated was to stabilize yield by compensating for decline in grain number caused by production limitation imposed by drought stress. Stalk weight was significantly different in irrigated and droughted plants, with the irrigated giving higher stalk weight than the droughted treatments. A similar reduction was observed by Tsuji, *et al*., (2003) and Borrel *et al*., (2000) in some grain sorghum genotypes. This agrees with the significant reduction in volumetric soil moisture content in the droughted compared with the irrigated pots. However, lower transpiration in the droughted than the irrigated did not cause higher stalk weight in the droughted than the irrigated plants. Similarly, biomass was significantly reduced in the droughted compared with the irrigated, this follows from significant reduction in volumetric soil moisture content and stalk weight. Biomass is the result of grain yield and stalk weight, but grain yield was not significantly different in the droughted than the irrigated, therefore the significant reduction in the stalk weight and volumetric soil moisture content was responsible for the significant effect on biomass. Harvest index was not significantly different in the irrigated and droughted treatments because harvest index is a ratio, and both parts of the ratio namely grain yield and stalk weight were reduced due to drought.

4.4.2. The effect of varying concentrations of antitranspirant

Varying antitranspirant doses had no significant effects on volumetric soil moisture content of pots, grain yield, weight per grain, stalk weight, biomass and harvest index, but caused significant effects on stomatal conductance and borderline significant effect on grain number. Therefore the null hypothesis is rejected with regard to stomatal conductance and grain number, but accepted in terms of volumetric soil content, weight per grain, stalk weight, biomass and harvest index.

The effect of the antitranspirant was significant in reducing transpiration as adaxial, abaxial and total stomatal conductance were significantly reduced by the antitranspirant treatment. Reduction in transpiration was greatest in the 2.0 L/ha, 1.0 L/ha and 2.0 L/ha treated plants in the adaxial, abaxial and total stomatal conductance measurements respectively, which implies that the antitranspirant at 1.0 L/ha and 2.0 L/ha doses significantly reduces transpiration in sorghum. But, the higher 3.0 L/ha rate did not give a lower conductance than the 1.0 L/ha and 2.0 L/ha. Reduction in transpiration by antitranspirant at 1.0 L/ha is consistent with results from other studies on oil seed rape (Faralli *et al*., 2017) and preceding experiments in the current study. However significant decrease in transpiration from 2.0 L/ha Vapor Gard antitranspirant rate of application has not been reported with regard to sorghum anywhere in the literature, rather significant reduction in stomatal conductance were recorded from 2.5 L/ha Vapor Gard application in wheat under glasshouse (Abdallah, *et al*., 2015) and field conditions (Weerasinghe, *et al*., 2016). Nevertheless, there was a drought x antitranspirant interaction effect on adaxial stomatal conductance, indicating the role of drought in the development of significant antitranspirant effect on adaxial stomatal conductance. But, since the reduction in adaxial stomatal conductance was greater in the irrigated than in the droughted treatments, it can be inferred that the antitranspirant was largely responsible for the significant reduction in transpiration.

There was no significant effect of antitranspirant on grain yield, hence differences between the unsprayed and sprayed treatments were not significant. Reduction in transpiration and greater soil and plant moisture content must have impeded creation of significant drought stress effects on volumetric soil moisture content leading to no significant effects of the antitranspirant on grain yield. Hence, the non-significant effect of the antitranspirant does not have to do with the lack of effectiveness of the antitranspirant, but lack of sufficient drought development. Grain number was affected by the antitranspirant doses which led to decreases from 613 in the 1.0 L/ha to 609 in the 2.0 L/ha and and an increase to 656 in the 3.0 L/ha sprayed treatments. Whereas the unsprayed with a grain number of 623 was greater than in 1.0 L/ha and 2.0 L/ha but less than 3.0 L/ha treatments. This represents a decrease in grain number in the sprayed compared with the unsprayed treatments in contrast to some published results in wheat (Abdallah, *et al*., 2015; Weerasinghe, *et al*., 2016). The current results could be attributed to the effect of the antitranspirant spray on photosynthesis which is normally reduced upon antitranspirant application. In this instance photosynthesis must have been reduced leading to reduced production and supply of assimilates for reproductive development and the consequent lower grain number in the sprayed than unsprayed treatments. In addition reduction in grain number following antitranspirant application points to the already known fact that antitranspirant have the potential to cause yield damage when applied late in the plant reproductive stage (Kettlewell, 2010) whereby the damage caused by reduced photosynthesis could outweigh the benefits obtained from reduced transpiration. However, measurements of carbon dioxide assimilation needs to be carried out to support this position. But, in these experiments (Expts. 1 and 2) particularly and perhaps in sorghum generally late application of antitranspirant may not lead to the deleterious effect of yield reduction, as the inherent compensatory mechanism often leads to the formation of greater weight per grain to compensate for lower grain number.

With regard to the rate of antitranspirant application, the trend in the results from the current study showing greater grain number resulting from higher rates of application is not consistent with other findings which showed that no further yield benefits arose from applying antitranspirant at higher rates beyond 2.5 L/ha in wheat (Kettlewell and Holloway, 2010; Abdallah, *et al*., 2015). The differences in the published results and the current study could be due to the plant type used.

Weight per grain was not significantly different in the sprayed and unsprayed treatments. The non-significant effect of the antitranspirant on weight per grain follows from the lack of significant effect on grain yield and the time of drought imposition. The effect of the antitranspirant on weight per grain is difficult to interpret in this experiment, because drought was imposed prior to flowering stage at which time any effect on weight per seed may not be observed clearly. Because, in sorghum it is post- anthesis rather than pre anthesis drought stress that reduces weight per grain (Olufayo, *et al*., 1997). Thus, the effect of antitranspirant on weight per grain could be better evaluated under post-anthesis rather than pre-anthesis water stress. However, numerical increases in the weight per grain of the sprayed over the unsprayed as well as a concurrent increase with increased antitranspirant concentrations, shows a potential for the antitranspirant to increase weight per grain.

Glasshouse experiment 4

Effect of terminal drought initiated at varying growth stages on transpiration, growth and yield of sorghum

The null hypothesis for this study was:

- There is no significant effect of terminal drought initiated at varying growth stages on drought tolerance of sorghum.

The objective of the study was:

- To evaluate the response of volumetric soil moisture content, transpiration, growth, yield and yield components of sorghum to terminal drought initiation at 3-leaf, 5-leaf, 8-leaf to booting and panicle emergence growth stages.

4.5. Materials and methods

Materials and methods were identical to Glasshouse Expts. 1, 2 in Chapter 3 and Glasshouse Expt. 3 in the current Chapter except for the following:

4.5.1. The stage of drought application

As stated in the introduction in section 4.1, significant reduction in grain yield of sorghum due to water deficit-imposed stress has not been demonstrated thus far in the current study. This contrasts with the conclusions drawn from the literature. This result could be attributed to the drought imposed not being severe enough to cause a significant decrease in grain yield or the drought was imposed at a growth stage during which the expected significant yield reduction may not occur or both. In the preceding Expts. 1, 2 and 3 the sorghum growth stage at which drought was imposed was at the booting to flowering and severity of water stress comprised a duration of between 19 – 31 days before re-watering commencing at the end of flowering and lasting until maturity when the grains feel hard. Watering regime was set to be achieved by keeping the volumetric soil moisture content at 22.0 %, which is at field capacity for the irrigated and 11.0 %, which is at permanent wilting point for the droughted plants. Also, the highest volumetric soil moisture content achieved were greater than the field capacity at 35.94 %, 37.38 % and 34.14 % for Expts. 1, 2 and 3 respectively. Similarly, the lowest volumetric soil moisture content reached were greater that the permanent wilting point at 16.57 %, 26.07 % and 12.35 % for Expts. 1, 2 and 3 respectively. To achieve one of this project's objectives of getting significant reduction in sorghum grain yield under water stress the sorghum growth stage of at which drought was imposed in previous Expts.1, 2 and 3 as well as the drought severity were altered such that in the succeeding Expt.4 the drought was imposed at more than one sorghum growth stage including the booting to flowering stage with each considered as a treatment and the drought severity was increased by increasing the intensity and duration water stress.

Specifically, the following modifications were carried out:

i) The sorghum growth stage at which drought was imposed was modified to include the 3-leaf stage, 5- leaf stage, 8-leaf stage and panicle emergence for separate treatment groups of plants, which corresponds with very early, early, mid and panicle emergence treatments.

ii) Volumetric soil moisture content was reduced from 22.0 % to 17.0 % for irrigated and from 11.0 % to 10.0 % for the droughted plants.

iii) Drought continued from the time/ growth stage of drought imposition to harvest.

4.5.2. Experimental design and treatments

The experiment was a one-way randomized complete block design with 5 treatments consisting of one fully irrigated and four droughted at different times as follows: fully irrigated, very early drought, early drought, mid drought and panicle emergence. The four drought treatments above represent different times of drought imposition on the soil and plant. The irrigated as well as the drought treatments were each assigned 12 pots representing replicates of these treatments giving 60 pots in total. These 60 pots were arranged into 12 blocks of 5 pots per block. Details of the treatments and stages of irrigation and drought imposition as well as some events and operations during the experiment are shown on Tables 4.5.1 and 4.5.2. Figure 4.5.1 shows the pot arrangement with sorghum growing in the glasshouse. Skeleton ANOVA of measurements are shown on tables ranging from Table 4.5.1 to 4.5.10.

4.5.3 Plant material: The sorghum variety used in this experiment was BirdGO Grain Sorghum (Bright Seeds, UK).

Figure 4.5.1. Sorghum growing in pots in the glasshouse (Expt. 4).

4.5.3. Measurements

4.5.3.1 Determination of field capacity and permanent wilting point

Field capacity and permanent wilting point were determined using the pressure plate and the gravimetric methods.

4.5.3.1.1 Using pressure plate

Field capacity and permanent wilting point of soil were determined using pressure plate membrane apparatus (Soil Moisture Equipment Corp. Santa Barbara, USA) was done as follows: Ceramic plate and sandy loam soil samples of bulk density 1.2 were placed into eight of the rings supplied with the equipment were soaked overnight and thereafter placed inside the pressure chamber. The outflow pipe for water was connected, and the lid of the chamber sealed and the appropriate pressure for permanent wilting point and field capacity applied. When the water ceases to be released from the outflow pipe for each appropriate pressure applied, the apparatus was turned off, and the soil samples removed and immediately weighed; the result of which was recorded as the wet weight (WW). These samples are then dried to a nearly constant weight in oven at 105˚C, to give the dry weight (DW). The above procedure was followed with eight samples of soil each for the determination of field capacity at 0.05 bar and permanent wilting point at 15 bar (Hall, *et al*., 1977).

Statistical analysis

Data were analysed using GENSTAT, 16th edition (VSN International Ltd, Hemel Hempstead, UK). Repeated measures analysis of variance (ANOVA) was used for volumetric soil moisture content and stomatal conductance whereas a two-way ANOVA was used if interactions with time were significant as well as for yield and yield components. The two-way ANOVA was eployed to show the responses to the treatments on individual days of measurements. Data were checked for normality and variance homogeneity by examining the residual plots. Skeleton ANOVA of measurements to show residuals of the treatments are shown on tables ranging from Table 4.5.3 to 4.5.10.

The target volumetric soil moisture content for irrigated and droughted plants were set at 17.0 % and 10 % respectively.

Table 4.5.1. Irrigation and drought levels and growth stages of sorghum with the degree and duration of irrigation and stages and duration of drought application (Expt. 4).

	Growth stages			
Treatments	**I** **3-leaf** **28 days after emergence**	**II** **5-leaf** **39 days after emergence**	**III** **8-leaf to booting** **49 days after emergence**	**IV** **Panicle Emergence** **63 days after emergence**
Fully irrigated	fully irrigated	fully irrigated	fully irrigated	fully irrigated
Very early drought	**stopped watering**	water stressed	water stressed	water stressed
Early drought	fully irrigated	**stopped watering**	water stressed	water stressed
Mid drought	fully irrigated	fully irrigated	**stopped watering**	water stressed
Panicle emergence	fully irrigated	fully irrigated	fully irrigated	**stopped watering**

Table 4.5.2. Dates and activities regarding drought application and duration during the experiment (Expt. 4).

Dates	Treatments	Time of drought application (days after emergence)	Drought duration (days)
March 24	Emergence	0	0
April 21	Very early drought treatment starts	28	77
May 2	Early drought treatment starts	39	66
May 12	Mid-drought treatment starts	49	56
May 30	Panicle emergence treatment starts	63	32
July 2	Harvest	105	na

Table 4.5.3 Skeleton ANOVA of volumetric soil moisture content measurements (Expt. 4)

Source of variation	Degrees of freedom
Blocks stratum	11
Block x subject stratum	
Drought	4
Residual	44
Block x subject x time stratum	
Time	14
Drought x time	56
Residual	758 (12)
Total	887 (12)

(Numbers in parenthesis are missing values)

Table 4.5.4 Skeleton ANOVA of the adaxial stomatal conductance measurements (Expt. 4)

Source of variation	Degrees of freedom
Blocks stratum	11
Blocks x subject stratum	
Drought	4
Residual	44
Block x subject x time stratum	
Time	4
Time x drought	16
Residual	201(21)
Total	280 (21)

(Numbers in parenthesis are missing values)

Table 4.5.5 Skeleton ANOVA of the abaxial stomatal conductance measurements (Expt. 4)

Source of variation	Degrees of freedom
Blocks stratum	11
Blocks x subject stratum	
Drought	4
Residual	44
Block x subject x time stratum	
Time	4
Time x Drought	16
Residual	199 (19)
Total	278 (19)

(Numbers in parenthesis are missing values)

Table 4.5.6. Skeleton ANOVA of the total stomatal conductance measurements (Expt. 4)

Source of variation	Degrees of freedom
Blocks stratum	11
Blocks x subject stratum	
Drought	4
Residual	44
Block x subject x time stratum	
Time	4
Time x drought	16
Residual	201 (19)
Total	280 (19)

(Numbers in parenthesis are missing values)

Table 4.5.7. Skeleton ANOVA of the plant height measurements (Expt. 4)

Source of variation	Degrees of freedom
Blocks stratum	11
Block x subject stratum	
Drought	4
Residual	44
Block x subject x time stratum	
Time	1
Time x drought	4
Residual	55
Total	119

(Numbers in parenthesis are missing values)

Table 4.5.8. Skeleton ANOVA of the leaf area measurements (Expt. 4)

Source of variation	Degrees of freedom
Blocks stratum	11
Block x unit x stratum	
Drought	4
Residual	42(2)
Total	57(2)

(Numbers in parenthesis are missing values)

Table 4.5.9. Skeleton ANOVA of the grain yield, grain number, weight per grain and harvest index measurements (Expt. 4)

Source of variation	Degrees of freedom
Blocks stratum	11
Block x unit x stratum	
Drought	4
Residual	17 (27)
Total	32 (27)

(Numbers in parenthesis are missing values)

Table 4.5.10. Skeleton ANOVA of the stalk weight and biomass measurements (Expt. 4)

Source of variation	Degree of freedom
Blocks stratum	11
Block x unit x stratum	
Drought	4
Residual	42 (2)
Total	57 (2)

(Numbers in parenthesis are missing values)

4.6. Results

4.6.1. Volumetric soil moisture content

Volumetric soil moisture content at field capacity was 17.1 % while the permanent wilting point was 9.6 %. The pot weight at field capacity was 9000 grams and at permanent wilting point was 8300 grams. Results of analysis of data on effect of drought and different stages of application and their interactions on volumetric soil moisture content of pots are presented in Table 4.6.1 and Figure 4.6.1. Drought imposition at the different growth stages caused significant differences ($P < 0.001$) in the mean volumetric soil moisture contents between irrigated and droughted pots. Therefore, the null hypothesis is rejected with regards to the volumetric soil moisture content. Tukey's test on the mean volumetric soil moisture contents of treatments showed the mean volumetric soil moisture content of the irrigated, very early, early, mid and panicle emergence treatments were all significantly different from each other. The mean volumetric soil moisture content of the irrigated was significantly different from those of all the drought treatments, and the mean of the droughted treatments were significantly different from each other. Drought significantly reduced mean volumetric soil moisture content in the droughted pots by 46.55% in the very early, by 39.23 % in the early, and 25.70 % in the mid and by 14.64 % in the late compared with the irrigated treatments. In the very early drought treatments, volumetric soil moisture content reached the lowest point at 97 DAE and in the early drought treatments at 69 DAE, whereas in the mid drought it was at 84 DAE and in the panicle emergence at 97 DAE. All of these lowest points in the various treatments were lower than the volumetric soil moisture content at permanent wilting point. There was a significant ($P < 0.001$) effect of time on the volumetric soil moisture content because the mean volumetric soil moisture of pots on individual days was being progressively reduced as the days after emergence (time) progresses. The drought x time interaction was also significant because, from 21 – 42 DAE, volumetric soil moisture content was maintained at close to field capacity as the DAE increases, whereas from 56 – 97 DAE, it decreased with increasing DAE.

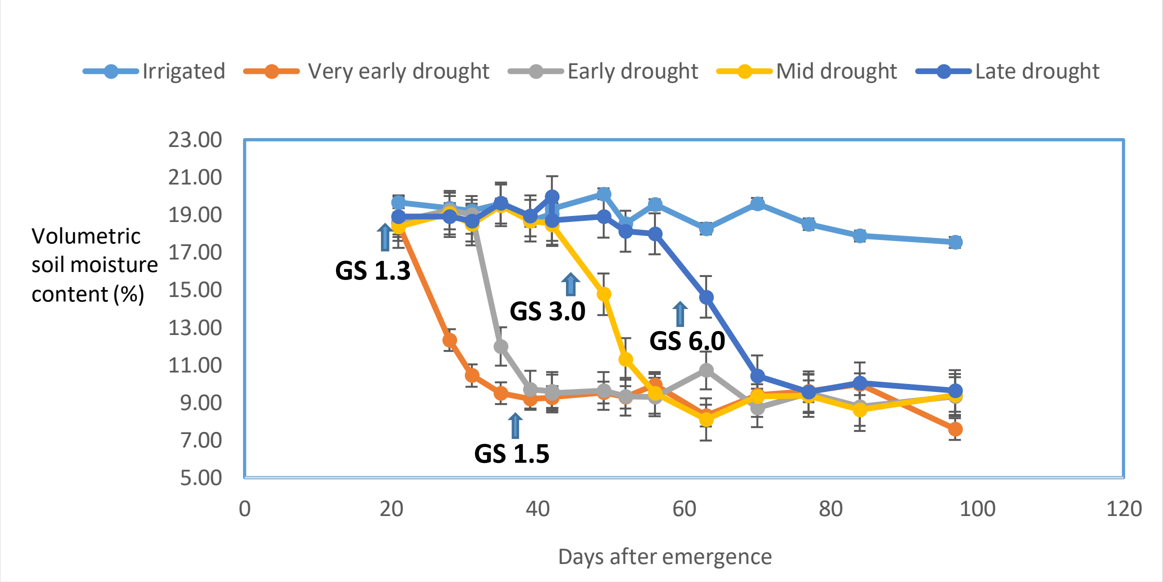

Figure 4.6.1. Response of volumetric soil moisture content (%) to drought imposition at 28, 39, 49 and 63 days after emergence corresponding to GS 1.3, GS 1.5, GS 3.0 and GS 6.0 growth stages of sorghum grown in the glasshouse in April 2017 (n =12) (drought P < 0.001; time < 0.001; drought x time P < 0.001). Error bars are SEM. (Expt.4).

A one-way analysis of variance performed on each day after emergence (DAE) (Table 4.6.2) showed there were significant (P < 0.001) differences in volumetric soil moisture content between the irrigated and the droughted treatments on all DAE with the volumetric soil moisture content being significantly reduced in the droughted compared with the irrigated pots on all occasions.

Table 4.6.1. Average volumetric soil moisture content (%) of pots under irrigation and drought at the very early, early, mid and panicle emergence corresponding to 28, 39, 49 and 63 days after emergence respectively of sorghum grown in the glasshouse in April 2017 measured at 21, 28, 31, 35, 39, 41, 44, 48, 51, 55, 65, 69, 77, 84 and 97 days after emergence. Means within a column followed by same letter are not significantly different according to Tukey's test at $P \le 0.05$. (n = 60) ± Standard error of the differences of the mean (SED) (Expt.4).

	Days after emergence															
Treatments	21	28	31	35	39	41	44	48	51	55	65	69	77	84	97	Means
Irrigated	19.67	19.35	19.21	19.58	18.69	19.05	19.32	20.12	18.53	19.55	18.27	19.62	18.51	17.88	17.54	18.99
Very early drought	18.54	12.33	10.44	9.51	9.21	9.27	9.29	9.53	9.28	9.95	8.30	9.39	9.59	9.98	7.60	10.15
Early drought	18.64	19.26	19.02	11.99	9.70	9.61	9.49	9.63	9.32	9.30	10.73	8.72	9.52	8.79	9.35	11.54
Mid drought	18.38	19.07	18.49	19.51	18.69	18.53	18.48	14.78	11.33	9.52	8.10	9.35	9.37	8.61	9.41	14.11
Panicle emergence	18.93	18.91	18.69	19.64	18.95	19.96	18.72	18.90	18.13	18.00	14.63	10.42	9.56	10.03	9.63	16.21
Mean	18.83	17.78	17.17	16.05	15.05	15.28	15.06	14.59	13.32	13.26	12.01	11.50	11.31	11.06	10.71	14.20
d.f.																758
SED																1.4414
CV %																10.2
P Values																
Time																< 0.001
Drought																< 0.001
Time x drought																< 0.001
Tukey's test																
Irrigated																18.99 e
Very early drought																10.15 a
Early drought																11.54 b
Mid drought																14.11 c
Panicle emergence																16.21 d

Table 4.6.2. One-way analysis of variance on the effect of irrigation and drought at the very early, early, mid and panicle emergence growth stages on volumetric soil moisture content of pots. Data are P values and stratum standard errors (Expt.4).

Days after emergence	21	28	31	35	39	41	44	48	51	55	65	69	77	84	97
Drought															
P values	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
SED	0.667	1.265	1.006	1.204	0.889	2.099	0.700	2.221	1.421	2.355	2.441	1.222	0.670	1.316	1.055
df	44	44	44	44	43	44	44	43	42	44	43	43	42	42	42

4.6.4. Adaxial stomatal conductance

The response of adaxial stomatal conductance to drought and different times of drought imposition and their interactions is shown on Table 4.6.3 and Figure 4.6.2.

The adaxial stomatal conductance in the irrigated and droughted treatments were significantly different at $P < 0.001$; thus, different times of drought imposition significantly affected adaxial stomatal conductance in the various treatments compared with the irrigated. Tukey's test results showed there was a significant difference in mean adaxial stomatal conductance between the treatments. The mean of the irrigated was significantly different from the means of the very early, early and mid-drought treatments but was not significantly different from the mean of the panicle emergence treatment. However, the mean of the very early, early, mid and panicle emergence treatments were not significantly different from each other. The mean of the treatments were reduced by 24.01 % in the very early, 25.49 % in the early, 19.18 % in the mid and 12.74 % in the panicle emergence compared with the irrigated treatments. There was a significant effect of time ($P < 0.001$) on adaxial stomatal conductance as the magnitude declined with time with the one-way analysis of variance (Table 4.6.4) showing significant differences ($P < 0.001$) between the means occurring at 21 days after emergence (DAE) and 25 DAE. The drought x time interaction effect on adaxial stomatal conductance was not significant.

Table 4.6.3. Average adaxial stomatal conductance (mmols $m^{-2}s^{-1}$) recorded on the flag leaf under irrigation and drought imposed at very early, early, mid and panicle emergence growth stages corresponding to 28, 39, 49 and 63 days after emergence respectively of sorghum grown in the glasshouse in April 2017 measured at 21, 25, 46, 55 and 65 days after emergence. Means within a column followed by same letter are not significantly different according to Tukey's test at P ≤ 0.05. (n = 59) ± Standard error of the differences of the mean (SED) (Expt.4).

	Days after emergence					
Treatments	21	25	46	55	65	Means
Irrigated	56.07	56.07	41.41	43.93	49.07	49.31
Very early drought	32.73	34.55	40.33	28.8	50.93	37.47
Early drought	35.65	37.32	34.86	28.82	47.06	36.74
Mid-drought	42.83	50.33	31.8	27.28	47.00	39.85
Panicle emergence	45.14	49.31	37.11	35.62	47.94	43.03
Means	42.48	45.51	37.1	32.89	48.4	41.28
df						199
SED						13.991
CV %						33.9
P values						
Drought						< 0.001
Time						< 0.001
Drought x time						0.126
Tukey's test						
Irrigated						49.31 b
Very early drought						37.47 a
Early drought						36.74 a
Mid-drought						39.85 a
Panicle emergence						43.03 ab

Table 4.6.4. One-way analysis of variance on the effect of irrigation and drought at the very early, early, mid and panicle emergence growth stages applied at 28, 39, 49 and 63 days after emergence on adaxial stomatal conductance recorded on the flag leaf at 21, 25, 46, 55 and 65 days after emergence of sorghum grown in the glasshouse in April 2017. Data are P values and stratum standard errors (Expt.4).

	Days after emergence				
	21	25	46	55	65
Drought					
P Values	0.006	0.020	0.386	0.427	0.883
SED	15.27	17.61	9.9	8.54	7.78
df	43	43	37	37	37

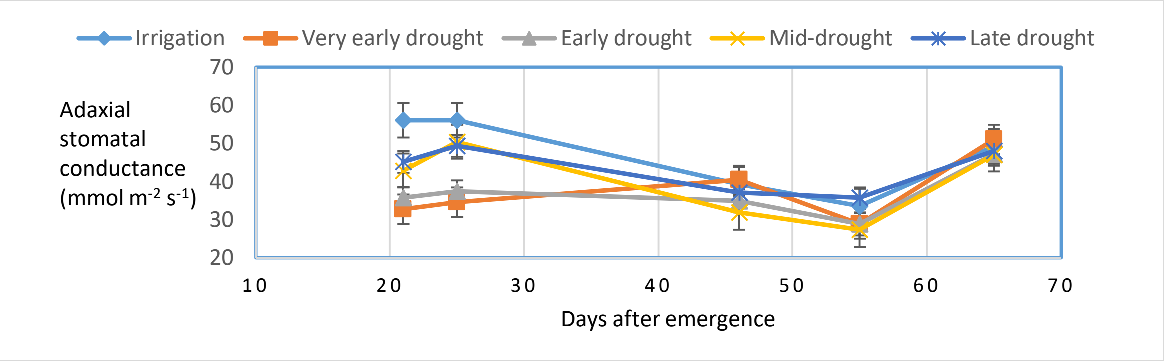

Figure 4.6.2. Response of adaxial stomatal conductance to irrigation and drought at very early, early, mid and panicle emergence growth stages imposed at 28, 39, 49 and 70 days after emergence recorded on the flag leaf at 21, 25, 46, 55 and 65 days after emergence of sorghum grown in the glasshouse in April 2017 (n = 59) (drought, P < 0.006; time, P = < 0.001; drought x time P = 0.126). Error bars are SEM. (Expt.4).

4.6.5 Abaxial stomatal conductance

Results from data analysed on effect of drought and that of different times of drought imposition and interactions on abaxial stomatal conductance are presented on Table 4.6.5 and Figure 4.6.3.

There were differences in abaxial stomatal conductance between the droughted and irrigated treatments as the drought imposition significantly (P < 0.001) decreased adaxial stomatal conductance in the droughted compared with the irrigated treatments. According to Tukeys test the mean of the irrigated was significantly different from the means of the very early, early, mid and panicle emergence treatments. Whereas the means of the very early, early, mid and panicle emergence treatments were not significantly different from each other. Mean abaxial stomatal conductance were decreased by 26.7 % in the very early, 25.2 % in the early, 17.4 % in the mid and 14.6 % in the panicle emergence compared with the irrigated treatment. There was a significant effect of time (P < 0.001) on the measurements as stomatal conductance on each successive day from the first to the last day of measurement were different, but no trend was clear. However, there was no significant time x drought interaction effects on abaxial stomatal conductance, but the one-way analysis of variance (Table 4.6.6) showed there were significant effects of drought on abaxial stomatal conductance in the irrigated and droughted treatments at 21 (P = 0.006) and 25 (P = 0.020) DAE.

Table 4.6.5. Average abaxial stomatal conductance (mmols $m^{-2}s^{-1}$) recorded on the flag leaf under irrigation and drought imposed at very early, early, mid and panicle emergence growth stages corresponding to 28, 39, 49 and 63 days after emergence respectively of sorghum grown in the glasshouse in April 2017 measured at 21, 25, 46, 55 and 65 days after emergence. Means within a column followed by same letter are not significantly different according to Tukeys test at P ≤ 0.05. (n = 59) ± Standard error of the differences of the mean (SED) (Expt.4).

Treatments	Days after emergence					
	21	25	46	55	65	Means
Irrigated	50.65	42.52	49.09	49.89	42.05	46.84
Very early drought	32.23	26.59	43.26	31.29	38.33	34.34
Early drought	33.00	29.73	36.05	35.8	40.64	35.05
Mid-drought	48.00	32.07	36.73	37.08	39.55	38.68
Panicle emergence	40.48	35.08	46.23	38.93	39.28	40
Means	40.87	33.2	42.27	38.6	39.97	38.98
df						201
SED						10.702
CV %						27.50
P values						
Drought						< 0.001
Time						< 0.001
Drought x Time						0.056
Tukeys test						
Irrigated						46.84 b
Very early drought						34.34 a
Early drought						35.05 a
Mid-drought						38.68 a
Panicle emergence						40.00 a

Table 4.6.6. One-way analysis of variance on the effect of irrigation and drought at the very early, early, mid and panicle emergence growth stages on abaxial stomatal conductance recorded on the flag leaf in sorghum at 21, 25, 46, 55 and 65 days after emergence grown in the glasshouse in April 2017. Data are P values and stratum standard errors (Expt.4).

	Days after emergence				
	21	25	46	55	65
Drought					
P values	< 0.001	< 0.001	0.328	0.609	0.883
df	43	44	38	37	39
SED	11.45	6.89	15	10.94	7.78

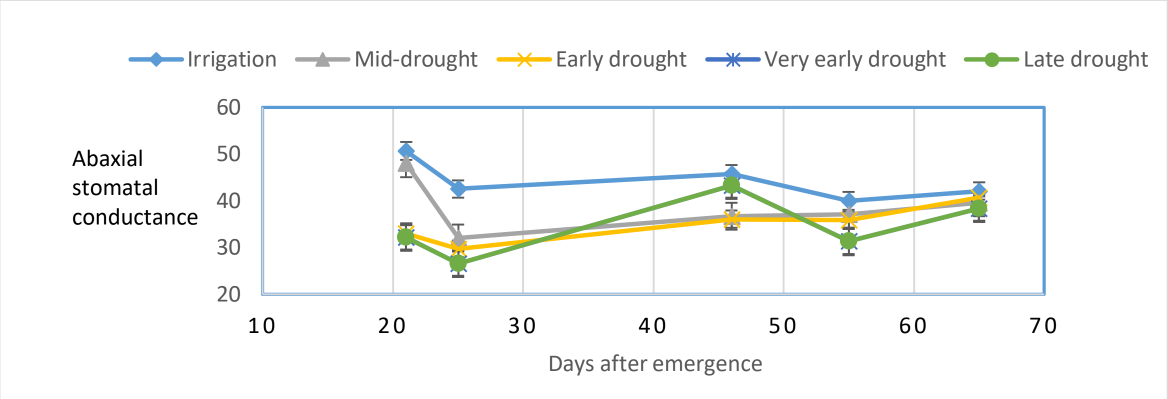

Figure 4.6.3. Response of abaxial stomatal conductance to irrigation and drought imposed at very early, early, mid and panicle emergence growth stages of sorghum recorded on the flag leaf at 21, 25, 46, 55 and 65 days after emergence grown in the glasshouse in April 2017 (n = 59) (drought, $P < 0.001$; time, P = < 0.001; drought x time $P = 0.056$). Error bars are SEM. (Expt.4).

4.6.6. Total stomatal conductance

The results of data analysed on the response of total stomatal conductance to drought imposed at different times are presented on Table 4.6.7 and Figure 4.6.4.

The results showed there were significant differences in total stomatal conductance between the irrigated and droughted treatments. Drought imposition significantly ($P < 0.001$) reduced total stomatal conductance in the droughted compared with the irrigated plants. From results of the Tukey's test the mean of the irrigated treatment was significantly different from the means of very early, early, mid and panicle emergence treatments, and the means of the very early, early, mid and panicle emergence treatments were not significantly different from each other. A significant time ($P < 0.001$) effect on total stomatal conductance was recorded showing different mean values on each day of measurement, and there was a trend towards a progressive decline from the beginning to the end of measurements at 65 DAE. The drought x time interaction effect on total stomatal conductance was significant ($P < 0.017$) showing no clear trend and results from the one-way analysis of variance on each day after emergence (DAE) (Table 4.6.8) showed significant ($P < 0.001$) differences (reduction) in total stomatal conductance between the irrigated and droughted treatments at 21 DAE and at 25 DAE.

Table 4.6.7. Average total stomatal conductance (mmols $m^{-2}s^{-1}$) recorded on the flag leaf under irrigation and drought imposed at very early, early, mid and panicle emergence growth stages corresponding to 28, 39, 49 and 63 days after emergence respectively of sorghum grown in the glasshouse in April 2017 measured at 21, 25, 46, 55 and 65 days after emergence. Means within a column followed by same letter are not significantly different according to Tukey's test at P ≤ 0.05. (n = 59) ± Standard error of the differences of the mean (SED) (Expt.4).

Treatments	Days after emergence					
	21	25	46	55	65	Means
Irrigated	53.36	49.29	45.25	46.91	45.56	48.07
Very early drought	32.55	30.82	41.51	29.94	44.52	35.87
Early drought	34.33	33.52	35.46	32.31	43.86	35.90
Mid-drought	45.41	41.20	34.26	32.18	43.27	39.27
Panicle emergence	42.81	42.20	41.67	37.28	43.61	41.52
Means	41.69	39.41	39.63	35.72	44.17	40.12
df						201
SED						9.632
CV %						24
						P values
Drought						< 0.001
Time						< 0.001
Drought x Time						0.015
Tukey's test						
Irrigated						48.07 b
Very early drought						35.87 a
Early drought						35.9 a
Mid-drought						39.27 a
Panicle emergence						41.52 a

Table 4.6.8. One-way analysis of variance on the effect of irrigation and drought at the very early, early, mid and late growth stages on total stomatal conductance in sorghum recorded on the flag leaf at 21, 25, 46, 55 and 65 days after emergence grown in the glasshouse in April 2017. Data are P values and stratum standard errors (Expt.4).

	Days after emergence				
	21	25	46	55	65
Drought P values	< 0.001	< 0.001	0.413	0.520	0.859
df	43	38	37	37	39
SED	11.79	9.93	11.67	8.25	6.435

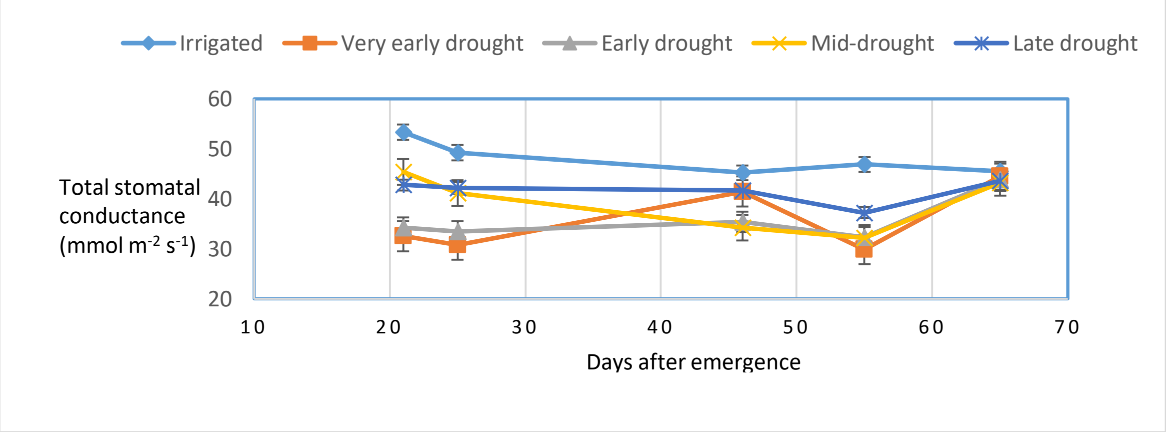

Figure 4.6.4. Response of total stomatal conductance to irrigation and drought imposed at very early, early, mid and panicle emergence growth stages of sorghum recorded on the flag leaf at 21, 25, 46, 55 and 65 days after emergence grown in the glasshouse in April 2017 (n = 59) (drought, $P < 0.001$; time, $P = < 0.001$; drought x time $P = 0.015$). Error bars are SEM. (Expt.4).

4.6.7. Plant height and leaf area

Table 4.6.9 shows results of data analysed on the responses of plant height and leaf area to drought imposed at different times and growth stages of sorghum.

There was a significant difference in plant height and in leaf area between the irrigated and droughted treatments, hence the null hypothesis is rejected regarding growth. Drought had significant ($P < 0.001$) effects leading to a reduction of 13.4 %, 21 %, 8.7 % and 0.8 % in plant height in the very early, early, mid and panicle emergence treatments respectively compared with the irrigated treatments. The magnitude of reduction in plant height in the droughted compared with the irrigated were greatest in the mid and least in the panicle emergence treatments. Tukey's test revealed that the means of the irrigated, very early, mid and panicle emergence treatments were not significantly different from each other, but were significantly different from the mean of the early drought. The effect of time was significant on plant height as the mean plant height differed and increased over time. The drought x time interaction effect was not significant. With respect to the leaf area, drought effect was significant at $P = 0.006$ with considerable reductions in some of the droughted compared with the irrigated treatments. The very early drought treatment was reduced by 22.5 %, the early by 5.0 %, the mid by 12.9 % and the panicle emergence by 19.0 % compared with the irrigated. However, Tukey's test showed that the mean of the irrigated treatment was not significantly different from any of the drought treatments. The means of the mid and panicle emergence treatments, were significantly higher than the mean of the very early drought treatment.

Table 4.6.9. Average plant height (cm) and leaf area (m^2) measured on the third and fifth leaves under irrigation and drought at the very early, early, mid and late growth stages corresponding to 28, 39, 49 and 63 days after emergence respectively of sorghum grown in the glasshouse in May 2017.Plant height was measured at 28 and, 49, leaf area at 63 days after emergence. Means within a column followed by same letter are not significantly different according to Tukey's test at P ≤ 0.05. (n = 59) ± Standard error of the differences of the mean (SED) (Expt.4).

	Days after emergence			Leaf area(cm^2)
Treatments	28	49	Means	63
Irrigated	15.03	23.46	19.25	68.5
Very early drought	14.83	18.50	16.67	53.10
Early drought	14.10	16.32	15.21	65.10
Mid drought	14.37	20.79	17.58	77.30
Panicle emergence	14.27	23.92	19.09	81.50
Mean	14.52	20.6	17.56	69.10
df			55	42
SED			5.393	18.85
CV %			30.70	27.30
P values				
Drought			< 0.001	0.006
Time			< 0.001	
Drought x time			0.105	
Tukey's test				
Irrigated			19.25 b	68.50 ab
Very early drought			16.67 ab	53.10 a
Early drought			15.21 a	65.10ab
Mid drought			17.58 ab	77.30 b
Panicle emergence			19.09 b	81.50 b

4.6.8. Yield and yield components

Table 4.6.10 shows results of response of grain yield and yield components of sorghum to drought imposition at different times and growth stages.

Drought imposition led to significant differences in grain yield, grain number, and weight per grain, stalk weight and biomass between irrigated and droughted treatments, however no significant difference between irrigated and droughted treatments was observed in harvest index. Therefore, the null hypothesis is rejected in terms grain yield, grain number, weight per grain, stalk weight and biomass, but accepted as per the harvest index. Drought imposition had significant ($P = 0.026$) effect and reduced grain yield in the droughted compared with the irrigated treatments. Grain yield decreased by 25.49 %, 19.65 %, 19.52 % and 12.07 % in the very early, early, mid and panicle emergence treatments respectively compared with the irrigated treatments. The reduction was greatest in the very early and least in the panicle emergence treatments. The Tukey's test showed that the mean of irrigated treatment was not significantly different from the means of the early, mid and panicle emergence treatments, but was significantly different from the means of the very early drought treatments. The test also showed the mean of the very early drought treatment was not significantly different from the mean of the early, mid and panicle emergence, but was significantly different from the means of the irrigated treatments. Whereas the mean of the very early, early mid and panicle emergence treatments was not significantly different from the means of the irrigated, treatments, but was significantly different from the mean of the very early drought treatment. Drought had highly significant ($P < 0.016$) effect on grain number. There was a 37.88 %, 27.90 %, 33.60 % and 1.43 % reduction in grain number in the very early, early, mid and panicle emergence treatments respectively in comparison with the irrigated treatment, with the greatest reduction in the very early and least in the panicle emergence treatments. Results of the Tukey's test revealed no significant differences between the means of the irrigated and the very early, early, mid and panicle emergence treatments. On the other hand weight per grain was significantly ($P = 0.017$) affected by drought and led to significant increase in the droughted compared with the irrigated treatments. The drought imposed increased weight per grain by 19.95 %, 11.43 %, 21.21 % in the very early, early and, mid drought treatments, while decrease in weight per grain of 10.80 % was recorded in the panicle emergence treatment. The increase was greatest in the mid drought and least in the early drought treatments. According to Tukey's test, the mean of the irrigated was significantly different from the means of the very early, and early drought treatments. However, the means of the very early, early, and mid panicle emergence treatments were not significantly different. In addition the means of the very early, early and panicle emergence treatments were not significantly different, so also were the means of the very early, early and panicle emergence treatments. The effect of drought treatment on stalk weight was significant (P = 0.050) with a 25.0 %, 15.0 %, 15.17 %, 12.05 %and 3.05 % decrease in very early, early, mid and panicle emergence treatments respectively in comparison with the irrigated treatments. The reduction in stalk weight was greatest in the very early and least in the panicle emergence treatments. Results from the Tukey's test showed the mean of the irrigated, early, mid and panicle emergence treatments were not significantly different, while the means of the very early, early, mid and panicle emergence treatments were also not significantly different. But the means of the irrigated was significantly different from the very early drought treatment.

Drought application which significantly (P < 0.001) decreased biomass in the very early, early, mid and panicle emergence treatments by 25.25 %, 42.01 %, 15.84 % and 7.63 % respectively in comparison with the irrigated treatments. Reduction in biomass was greatest in the early and least in the panicle emergence treatments. Tukey's test revealed that the mean of the irrigated treatment was significantly different from the means of the very early, early, mid and panicle emergence treatments and there were no significant differences between the means of the very early, early, mid and panicle emergence treatments. With regard to the harvest index, no significant differences were recorded between the droughted and irrigated treatments, thus the effect of drought on harvest index was not significant.

Table 4.6.10. Average grain yield and yield components under drought imposed at the very early, early, mid and panicle emergence growth stages corresponding to 28, 39, 49 and 63 days after emergence respectively of sorghum grown in the glasshouse in April 2017. Means within a column followed by same letter are not significantly different according to Tukey's test at P ≤ 0.05. (n = 59) ± Standard error of the differences of the mean (SED) (Expt.4).

Treatments	Grain yield per plant (g)	Grain number per plant	Weight per grain (mg)	Stalk weight (g)	Biomass (g)	Harvest Index (%)
Irrigated	14.91	491	30.37	14.44	29.35	51
Very early drought	11.11	305	36.43	10.83	21.94	51
Early drought	11.98	354	33.84	12.27	17.02	49
Mid drought	12.00	326	36.81	12.70	24.70	49
Panicle emergence	13.11	484	27.09	14.00	27.11	48
Mean	12.64	392	32.91	12.85	24.02	49
df	17	17	17	42	42	17
SED	2.714	151	8.67	3.096	6.987	7.255
CV %	21.5	38.5	24	24.1	31	14.5
P values						
Drought	0.026	0.016	0.017	0.050	< 0.001	0.746
Tukey's test						
Irrigated	14.91 b	491a	30.37ab	14.44 b	29.35 b	51a
Very early drought	11.11 a	305a	36.43 ab	10.83 a	21.94 a	51 a
Early drought	11.98 ab	354a	33.84ab	12.27 ab	17.02 a	49a
Mid drought	12.00 ab	326a	36.81b	12.7ab	24.70 a	49a
Panicle emergence	13.11 ab	484a	27.09 a	14.00ab	27.11 a	48 a

4.7. Discussion

4.7.1. Effect of terminal drought stress on volumetric soil moisture content, transpiration, growth and yield of sorghum

The effect of drought stress was significant on volumetric soil moisture content, stomatal conductance, plant height, grain yield, seed number, weight per grain, stalk weight, biomass and leaf area, therefore, the null hypothesis was rejected. However, no- significant effects were observed on harvest index, thus the null hypothesis was accepted.

Volumetric soil moisture content of the irrigated pots was at 16.9 % on average, whereas in the droughted pots an average of 13.1 % was obtained. Therefore, in the irrigated pots the volumetric soil moisture content was a little less than the field capacity of 17.1 %, but in the droughted pots it was a little above the permanent wilting point of 9.6 %. The very early drought treatment reached a minimum volumetric soil moisture content of 9.0 % whereas the early drought treatment reached a minimum of 8.8 % and the mid and panicle emergence treatments were at of 8.6 % and 9.6 % minimum volumetric soil moisture content respectively. Therefore, sufficient water stress was developed in the soil, leading to a significant reduction in volumetric soil moisture content in the droughted compared with the irrigated pots.

Drought imposition decreased stomatal conductance in all treatments with the irrigated showing greater conductance than the droughted plants. Also, stomatal conductance was significantly reduced in very early, early, mid and panicle emergence treatments compared with the irrigated treatments but the means were not significantly different. Therefore, drought imposed reduced the volumetric soil moisture content as well as transpiration. The decrease in volumetric soil moisture content would have reduced water availability and transport to the leaves thus decreasing the leaf water status thereby reducing stomatal aperture, conductance and transpiration in the droughted treatments, whereas in the irrigated treatments, plant water absorption due to growth, and evaporation may be the cause. However, the effects of the drought on stomatal conductance did not follow a consistent pattern and so difficult to interpret. The non-consistent response of transpiration to water stress here could be due to the effects of light intensity, temperature and humidity as stomatal conductance is generally sensitive to ambient light, temperature and humidity (Aphalo and Jarvis, 1991; Jones, 1992) or it could be due to error in measurements on stomatal conductance. Cloud cover and the white painting on the outer sides of the glasshouse in the course of the experiment may have introduced variations to the natural light intensity and distribution inside the glasshouse as stomatal response to changing light is variable(Jones, 1992). Therefore data collected on the cloudy days may show differences from those on less cloudy or non-cloudy days and those from plants placed near the painted sides may differ from those that were further away. Relative humidity in the glasshouse may also be a contributory factor to this seemingly irregular stomatal response as stomatal conductance is known to fluctuate in relation to humidity (Jones, 1992). An increase in the relative humidity may reduce transpiration rates and vice versa therefore it is possible that the data were collected under various conditions of relative humidity which on some days might be high and on others low, hence the inconsistencies recorded.

Plant height was reduced in the droughted compared with the irrigated and mean plant height decreased by about 20.0 % in the droughted compared with the irrigated treatments. Also, plant height was significantly reduced in the very early, early, mid and panicle emergence compared with the irrigated treatments. Under the droughted treatments, the early and late terminal drought treatments gave the lowest and the highest plant heights respectively, while the mid and very early terminal drought treatments appeared to be of similar plant heights. In addition, plant height measurements in the very early and early terminal drought treatments were lower than those in the mid and late terminal drought treatments. According to Kramer (1963) plant growth and this includes plant height, is directly controlled by plant water stress and indirectly by soil water stress. Therefore, in this experiment plant height was indirectly influenced by the soil water regime as reduction in volumetric soil moisture content must have decreased the volume of moisture supplied to the plant from the soil and led to lower plant water content which subsequently generated plant water stress resulting in decreased plant height. In addition, greater reduction in plant height at the early terminal drought treatment compared with the very early, mid and late terminal drought treatments suggest that the effect of terminal drought imposition could be greatest on plant height in sorghum at this growth stage. In the early terminal drought treatment, drought was initiated at the 5-leaf growth stage at which sorghum enters its ‘grand period of growth’ when the root system develops rapidly, dry matter accumulates at a nearly constant rate if conditions are favourable, and the developmental potential of the plant is determined (Vanderlip, 1993). Accordingly, the significant reduction in volumetric soil moisture content may have decreased root growth and soil moisture absorption which reduced plant water content and dry matter accumulation leading to a reduction in the plant height. Measurements of plant water content and root growth were not made to verify this position.

Leaf area was decreased under very early drought compared with mid and panicle emergences, and this relates to the effect of drought in reducing the volumetric soil moisture content. Due to significant reduction in the volumetric soil moisture content, the soil moisture available cannot satisfy all the plant water requirements, thus at the leaf level, cell turgor and cell growth decreases and this reduces especially the cellular elongation and consequently the leaf area (Meier, *et al*., 1992).

Grain yield was significantly reduced in the droughted compared to the fully irrigated treatments with the mean of grain yield in the droughted being 19.18 % lower than the fully irrigated treatments. Amongst the droughted treatments, the very early drought gave the lowest grain yield, followed by the early, mid and panicle emergence. Accordingly, the very early terminal drought was more effective in reducing grain yield than the early, mid and late terminal drought treatments because it was under water deficit for a longer duration and hence suffered greater water stress than the early, mid and late terminal drought treatments. Significant reduction in volumetric soil moisture content and leaf area must have caused the significant reduction in grain yield in the droughted compared with the irrigated treatments. A reduction in leaf area had led to a concomitant decrease in intercepted radiation as the proportion of intercepted light depends on the leaf area and this would have decreased biomass production (Tardieu, 2013) which in turn contributes to reduction in grain yield. Similar results were obtained by Legg *et al*., (1979) in which a yield reduction of between 10 – 40 % in water stressed barley was attributed to decrease in intercepted radiation.

Grain number was decreased by an average of 25.20 % in the droughted compared with the fully irrigated plants.. Within the droughted treatments, the very early gave the lowest grain number, followed by the mid, then the early, and late terminal drought treatments. Thus the most adverse effect of drought in decreasing grain number occurred at the very early terminal drought treatment. The decreases in grain number followed a similar pattern as in grain yield therefore, the decrease in grain yield was largely caused by reduction in grain numbers. In addition, reduction in the grain number also mirrors the volumetric soil moisture content data as it did in the grain yield. From the current results, both grain yield and number were significantly reduced under water stress, and reduction in grain number appears to be the main driver of grain yield reduction, which supports the observation that grain yield and grain number in sorghum are strongly correlated (Craufurd and Peacock 1993). Of all the drought treatments, the highest grain number was recorded from the late terminal drought treatment.

On the other hand, weight per grain increased by 10.44 % in the droughted over the irrigated treatments; and also in the very early, early, and mid terminal drought over the irrigated treatment. Of all the treatments whose magnitude increased, the irrigated gave the lowest weight per grain and among the droughted treatments, the late terminal drought gave the lowest, followed by the early, the mid and the very early terminal drought treatments. The terminal drought treatments where the most deleterious effect of drought on weight per grain occurred were the very early and mid-drought treatments. . The results indicate that weight per grain responded differently and in the opposite direction to grain number except in the panicle emergence treatments as the effect of water stress in reducing grain number was compensated for by increasing weight per grain. Hence, whereas weight per grain increased, grain number decreased in these droughted treatments. This compensatory phenomena conforms to the observation by Berenguer and Faci (2001) who showed that water stress reduced grain number per panicle that is partly compensated by an increase in weight per grain in sorghum.

Drought stress reduced stalk weight significantly in the droughted compared with the irrigated treatments. Droughted plants yielded 13.78 % lower mean stalk weight than the irrigated. Within the terminal drought treatments, the very early terminal drought treatment produced the lowest stalk weight, followed by the early, mid and late terminal drought treatments hence, the most adverse effect on stalk weight was recorded under the very early terminal drought treatment. Whereas the early and mid-drought treatments yielded lower and similar results compared to the irrigated and late terminal drought treatment, the stalk weight from the late terminal drought treatment was close to the irrigated control. In the very early terminal drought treatment the plants were subjected to a longer duration of drought which must have reduced assimilates available for stalk growth compared with the other treatments. While, in the early, mid and late terminal drought treatments a shorter drought duration leading to less severe drought effects on growth, more advanced growth stages which gives the advantage of greater resistance to drought and a longer duration of assimilate production through photosynthesis enhanced greater stalk weights than in the very early drought treatments. Reduction in stalk weight in this experiment must also have been as a consequence of significant decreases volumetric soil moisture content, leaf area and plant height in the droughted compared with the irrigated plants.

Biomass was also significantly reduced under drought stress by 22.69 % when the mean of the droughted was compared with the irrigated treatments. This is expected because grain yield and stalk weight were both significantly reduced under water stress compared with the irrigated treatment. The early terminal drought treatment yielded the lowest biomass indicating that water stress reduced biomass the most compared with the other treatments. There were lower biomass produced by the very early and early compared with the mid and late terminal drought treatments. Reduction in biomass must have resulted from a decrease in grain yield, leaf area and stalk weight which constitute the biomass. Harvest index was not significantly reduced in the droughted compared with the irrigated treatments. Therefore the very early, early, mid and panicle emergence treatments did not cause any significant difference between the irrigated and droughted treatments. And amongst all the treatments, there appeared to be no much numerical differences between the treatments, although the panicle emergence treatment gave the lowest harvest index.

4.8. Conclusions

This experiment was carried out to evaluate the effect of drought and its initiation at different growth stages on transpiration, growth and yield of sorghum with the objective of determining the drought initiation growth stage at which the greatest reduction in grain yield would occur. It was hypothesised that terminal drought initiated at different growth stages do not cause significant differences in transpiration, growth and yield between irrigated and droughted sorghum. This null hypothesis was rejected and the following conclusions drawn:

1. A soil moisture content regime of between 20.0 % and 10.0 % field capacity and permanent wilting point respectively, maintained until harvest decreased volumetric soil moisture content in the droughted compared with the irrigated treatments.

2.The very early terminal drought treatment at which drought was initiated at the 3-leaf stage was the most effective in attaining significant yield reduction in sorghum because it decreased transpiration, growth, grain yield and yield components to a larger extent than the other treatments in the droughted compared with irrigated sorghum.

3. The result suggests that subjecting sorghum to terminal water stress initiated at the 3-leaf stage unto harvest may be another way of applying drought to sorghum and cause significant reduction in grain yield.

Glasshouse experiment 5

Effects of terminal drought initiated at 3-leaf growth stage and antitranspirant on drought tolerance of sorghum

4.9. Introduction

The effect of varying concentrations of the antitranspirant and initiating terminal drought at various growth stages were investigated in Expts. 3 and 4 with the aim of defining the appropriate antitranspirant dosage and growth stage for antitranspirant and drought application to achieve significant yield improvements from the antitranspirant and reduction from the drought in sorghum. The result showed that increasing antitranspirant application above 1.0 L/ha did not significantly improve yield and drought application at the 3-leaf growth stage significantly reduced yield compared with other concentrations of antitranspirant and growth stages investigated. These two variables were tested in the preceding Expt. 5 to further validate the effect of antitranspirant in improving drought tolerance in sorghum.

The null hypothesis for this study was:

- Terminal water stress initiated at the 3-leaf growth stage and antitranspirant applied at booting to flowering do not improve drought tolerance of sorghum.

The objectives of the study were:

- To determine the effect of terminal drought initiated at the 3-leaf growth stage on volumetric soil moisture content, transpiration, growth, yield and yield components of sorghum.
- To assess the effect of film antitranspirant on volumetric soil moisture content, transpiration, yield and yield components of sorghum droughted at the 3-leaf growth stage.

4.10. Materials and methods

Materials and methods were identical to Expts. 1, 2, 3 and 4 except for the following:

4.10.1. Experimental design and treatments

The experiment was a one-way randomized complete block design with three treatments in 16 blocks consisting of one fully irrigated and one droughted unsprayed and one droughted sprayed. Pot arrangement in the glasshouse is shown in Figure 4.10.1 and skeleton ANOVA of measurements are shown on Tables ranging from 4.10.2 to 4.10.8.

4.10.2 Plant material: The sorghum variety used in this experiment was BirdGO Grain Sorghum (Bright Seeds, UK).

4.10.3. Management

Some events and management operations during the experiment are presented on Table 4.10.1.

Table 4.10.1. Some significant dates and events during Expt. 5.

Dates	Activity	Days after emergence
May 30	Emergence	0
June 23	Transplanting	24
July 1	Drought starts	32
July 21	Antitranspirant spray	52
September 14	Harvest	107

Table 4.10.2 Skeleton ANOVA of the volumetric soil moisture content measurements (Expt. 5)

Source of variation	Degrees of freedom
Block stratum	15
Block x subject stratum	
Antitranspirant	2
Residual	30
Blocks x subject x time stratum	
Time	6
Time x treatments	12
Residual	270
Total	335

(Numbers in parenthesis are missing values)

Table 4.10.3. Skeleton ANOVA of the adaxial stomatal conductance measurements (Expt. 5)

Source of variation	Degrees of freedom
Blocks stratum	15
Blocks x subject stratum	
Antitranspirant	2
Residual	30
Blocks x subject x time stratum	
Time	4
Time x Antitranspirant-	8
Residual	177(3)
Total	236 (3)

(Numbers in parenthesis are missing values)

Table 4.10.4 Skeleton ANOVA of the abaxial stomatal Conductance measurements (Expt. 5).

Source of variation	Degrees of freedom
Blocks stratum	15
Blocks x subject stratum	
Antitranspirants	2
Residual	30
Blocks x subject x time stratum	
Time	4
Time x antitranspirants	8
Residual	178 (2)
Total	237 (2)

(Numbers in parenthesis are missing values)

Table 4.10.5 Skeleton ANOVA of the total stomatal conductance measurements (Expt. 5)

Source of variation	Degrees of freedom
Blocks stratum	15
Blocks x subject stratum	
Antitranspirants	2
Residual	30
Blocks x subject x time stratum	
Time	4
Time x antitranspirant	8
Residual	179 (1)
Total	238(1)

(Numbers in parenthesis are missing values)

Table 4.10.6 Skeleton ANOVA of the relative water content measurements (Expt. 5)

Source of variation	Degrees of freedom
Blocks stratum	4
Blocks x units x stratum	
Treatments	2
Residual	8
Total	14

(Numbers in parenthesis are missing values)

Table 4.10.7 Skeleton ANOVA of the leaf area measurements (Expt.5)

Source of variation	Degrees of freedom
Blocks stratum	15
Blocks x units x stratum	
Treatments	2
Residual	30
Total	47

(Numbers in parenthesis are missing values)

Table 4.10.8 Skeleton ANOVA of the stalk weight measurements (Expt. 5)

Source of variation	Degrees of freedom
Blocks stratum	15
Blocks x units x stratum	
Treatments	2
Residual	30
Total	47

(Numbers in parenthesis are missing values)

Figure 4.10.1. Sorghum growing in pots in the glasshouse after flowering.

4.11. Results

4.11.1. Volumetric soil moisture content

Results of analysis of data on effect of treatments (drought and antitranspirants) on volumetric soil moisture content of pots are presented in Table 4.11.1 and Figure 4.10.2. The treatments induced significant differences ($P < 0.001$) in the mean volumetric soil moisture contents between irrigated and droughted pots. Therefore, the null hypothesis was rejected with regards to the volumetric soil moisture content. Drought significantly reduced volumetric soil moisture content from 19.9 % in the irrigated to 13.3 % and to 13.8 % in the unsprayed and sprayed whereas the antitranspirant had no effect on the volumetric soil moisture content. Time had significant ($P < 0.001$) effects as the mean volumetric soil moisture content of pots decreased as the days after emergence progresses and there was a significant ($P < 0.001$) treatment x time interaction because the volumetric soil moisture content decreased between 32 to 59 days after emergence (DAE) and later increased at 67 and 80 DAE in the irrigated, but increased at 67 DAE and decreased at 80 DAE in the unsprayed and sprayed treatments respectively. Tukey's test showed significant differences in the mean volumetric soil moisture content between the irrigated and droughted treatments as the mean of the irrigated was significantly different from the droughted treatments and the mean of the unsprayed was not significantly different from the sprayed. One way analysis of variance (Table 4.11.2) showed significant reduction in volumetric soil moisture content at 37 DAE ($P = 0.021$), 46 DAE ($P = 0.021$), 52 DAE ($P = 0.021$), 59 DAE ($P = 0.021$), 67 DAE ($P = 0.021$), and 80 DAE ($P = 0.021$)

Table 4.11.1. Average volumetric soil moisture content (%) of pots under irrigation, drought and antitranspirant 52, 59, 65, 67 and 73 days after emergence grown in the glasshouse in May 2017. Drought and antitranspirant were applied at 32 and 52 days after emergence respectively. Means within a column followed by same letter are not significantly different according to Tukey's test at P ≤ 0.05. (n = 48) ± Standard error of the differences of the mean (SED) (Expt.5).

	Days after emergence							
Treatments	32	37	46	52	59	67	80	Mean
Irrigated	20.51	18.49	18.51	20.93	18.99	20.8	21.03	19.89
Droughted unsprayed	20.02	14.9	14.32	10.78	10.64	12.26	10.44	13.34
Droughted sprayed	20.23	15.86	15.75	11.22	11.95	10.74	10.57	13.76
Means	20.25	16.41	16.19	14.31	13.86	14.6	14.01	15.66
Df								15
SED								0.587
CV %								3.7
P values								
Treatments								< 0.001
Time								< 0.001
Treatments x time								< 0.001
Tukey's test								
Irrigated								19.89 b
Droughted unsprayed								13.34 a
Droughted sprayed								13.76 a

Table 4.11.2. One-way analysis of variance on the effect of irrigation, drought and antitranspirant on volumetric soil moisture content (%) of pots at 52, 59, 65, 67 and 73 days after emergence grown in the glasshouse in May 2017 (n = 48) (Expt.5).

	Days after emergence						
	32	37	46	52	59	67	80
Treatments							
P values	0.378	0.021	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
SED	0.995	2.595	4.046	1.136	3.307	2.61	0.938
df	30	30	30	30	30	30	30

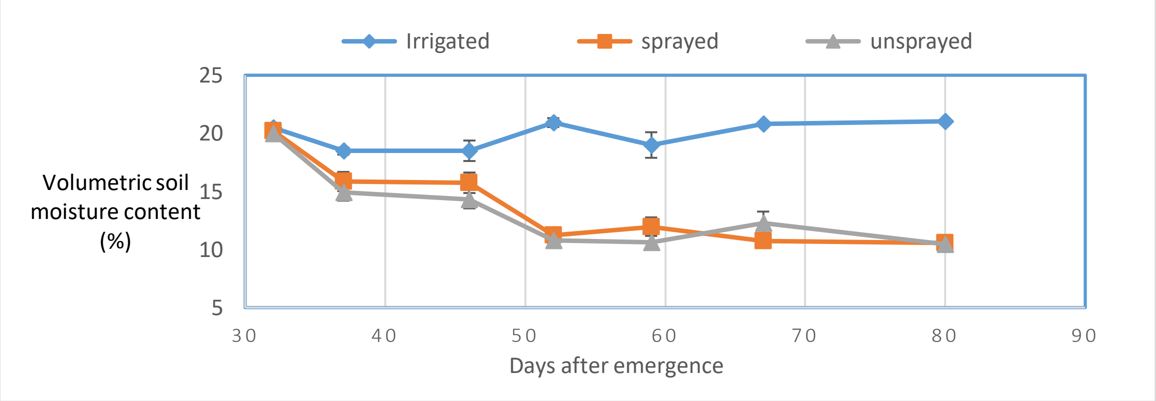

Figure 4.10.2. Response of volumetric soil moisture content (%) of pots to irrigation, drought and antitranspirants at 52, 59, 65, 67 and 73 days after emergence grown in the glasshouse in May 2017 (n = 48) (treatment, P < 0.001; time, P < 0.001; treatment x time P < 0.001). Error bars are SEM. (Expt.5).

4.11.2. Adaxial stomatal conductance

The results of analysis of data on the effect of treatments on adaxial stomatal conductance is presented on Table 4.11.3 and Figure 4.10.3.

There was a significant (P < 0.004) effect of the treatments on adaxial stomatal conductance, thus the null hypothesis was rejected with regards to the adaxial stomatal conductance. In comparison to the irrigated, there was a 2.8 % and 19.7 % reduction in adaxial stomatal conductance in the unsprayed and sprayed treatments respectively whereas there was a 17.4 % reduction in the adaxial stomatal conductance in the sprayed compared with the unsprayed treatments. The adaxial stomatal conductance was not significantly affected by the time and treatment x time interaction effects. Tukey's test showed that the means of the irrigated and unsprayed treatments were not significantly different, but was significantly different from the mean of the sprayed treatments. Significant effects of the treatments on the adaxial stomatal conductance was recorded according to the one-way ANOVA (Table 4.11.4) at 52 DAE (P = 0.032), however at 59 DAE (P = 0.647), 65 DAE (P = 0.111), 67 DAE (P = 0.942) and 73 DAE (0.8111), treatments did not significantly affect adaxial stomatal conductance.

Table 4.11.3. Average adaxial stomatal conductance (mmols $m^{-2}s^{-1}$) recorded on the flag leaf under irrigation, drought and antitranspirant taken at 52, 59, 65, 67 and 73 days after emergence of sorghum grown in the glasshouse in May 2017. Drought and antitranspirant were applied at 32 and 52 days after emergence respectively. Means within a column followed by same letter are not significantly different according to Tukey's test at $P \leq 0.05$ (n = 48) ± Standard error of the differences of the mean (SED) (Expt.5).

	Days after emergence					
Treatments	52	59	65	67	73	Mean
Irrigated	104.9	87.8	75.5	75.6	82.7	85.3
Droughted unsprayed	102.1	78.8	84.7	77.9	71.0	82.9
Droughted sprayed	68.2	78.3	60.6	74.7	60.9	68.5
Mean	91.7	81.7	73.6	76.1	71.6	78.9
df						177
SED						39.27
CV %						49.8
						P values
Treatments						0.004
Time						0.099
Treatments x time						0.601
Tukey's test						
Irrigated						85.3 b
Droughted unsprayed						82.9 b
Droughted sprayed						68.5 a

Table 4.11.4. One-way analysis of variance on the effect of irrigation, drought and antitranspirant on adaxial stomatal conductance (mmols $m^{-2}s^{-1}$) recorded on the flag leaf in sorghum at 52, 59, 65, 67 and 73 days after emergence grown in the glasshouse in May 2017 (n = 48) (Expt.5).

	Days after emergence				
	52	59	65	67	73
Drought P values	0.032	0.647	0.111	0.942	0.181
df	30	30	29	29	29
SED	41.46	32.21	34.38	35.57	29.53

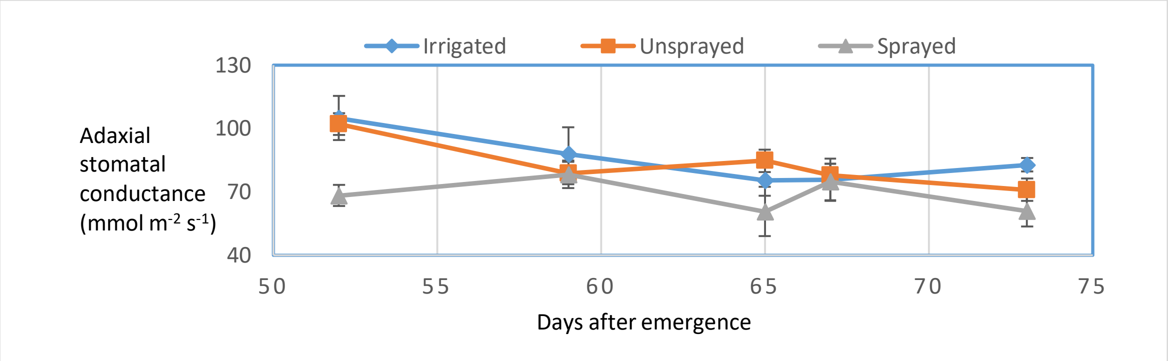

Figure 4.10.3. Response of adaxial stomatal conductance (mmol $m^{-2}s^{-1}$) to irrigation, drought and antitranspirant at 52, 59, 65, 67 and 73 days after emergence recorded on the flag leaf of sorghum grown in the glasshouse in May 2017 (n = 3) (treatment, P < 0.004; time, P = 0.099; treatment x time P = 0.601). Error bars are SEM. (Expt.5).

4.11.3. Abaxial stomatal conductance

The response of abaxial stomatal conductance to treatments (drought and antitranspirants) is presented on Table 4.11.5 and Figure 4.10.4.

The treatments caused significant differences in adaxial stomatal conductance (P < 0.039) between the irrigated and droughted plants. Thus, the null hypothesis is rejected in the case of adaxial stomatal conductance. Drought application reduced adaxial stomatal conductance by 7.9 % in the unsprayed and 20.0 % in the sprayed compared with the irrigated, while the antitranspirants reduced mean adaxial stomatal conductance by 13.1 % in the sprayed compared to the unsprayed treatments. The effect of time was significant (P < 0.014) as the magnitude of adaxial stomatal conductance differed over the days after emergence (DAE) and the treatments x time effects were significant because as the DAE increases, the adaxial stomatal conductance decreased in the irrigated, but increased in the droughted unsprayed and sprayed treatments. From the result of the Tukey's test, the mean of the irrigated was not significantly different from the mean of the unsprayed, but differed significantly from the mean of the sprayed droughted treatment. However, the mean of the unsprayed was not significantly different from the mean of sprayed treatment. One-way analysis of variance (Table 4.11.6) showed significant differences in the means of treatments at initial stages of drought application at 52 DAE (P < 0.001) and 59 DAE (P < 0.001), but at 65 DAE, 67 DAE and 73 DAE no significant differences were recorded however, the effect of the treatments diminished with time.

Table 4.11.5. Average abaxial stomatal conductance (mmols $m^{-2}s^{-1}$) recorded on the flag leaf under irrigation, drought and antitranspirant taken at 52, 59, 65, 67 and 73 days after emergence of sorghum grown in the glasshouse in May 2017. Drought and antitranspirant were applied at 32 and 52 days after emergence respectively. Means within a column followed by same letter are not significantly different according to Tukey's test at $P \leq 0.05$ (n = 48) ± Standard error of the differences of the mean (SED) (Expt.5).

	Days after emergence					
Treatments	52	59	65	67	73	Mean
Irrigated	125.1	77.0	91.2	75.3	74.1	88.5
Droughted unsprayed	67.6	64.1	116.5	76.7	82.9	81.5
Droughted sprayed	67.2	52.5	90.3	81.4	62.5	70.8
Mean	86.6	64.5	99.3	77.8	73.2	80.3
df						178
SED						44.03
CV %						54.8
						P values
Treatments						0.039
Time						0.014
Treatments x time						0.047
Tukey's test						
Irrigated						88.5 b
Droughted unsprayed						81.5 ab
Droughted sprayed						70.8 a

Table 4.11.6. One-way analysis of variance on the effect of irrigation, drought and antitranspirant on abaxial stomatal conductance (mmol $m^{-2}s^{-1}$) recorded on the fag leaf in sorghum at 52, 59, 65, 67 and 73 days after emergence grown in the glasshouse in May 2017 (n = 48) (Expt.5).

	Days after emergence				
	52	59	65	67	73
Drought P values	< 0.001	< 0.001	0.615	0.885	0.196
SED	38.8	16.37	73.4	35.4	31.16
df	30	30	29	29	30

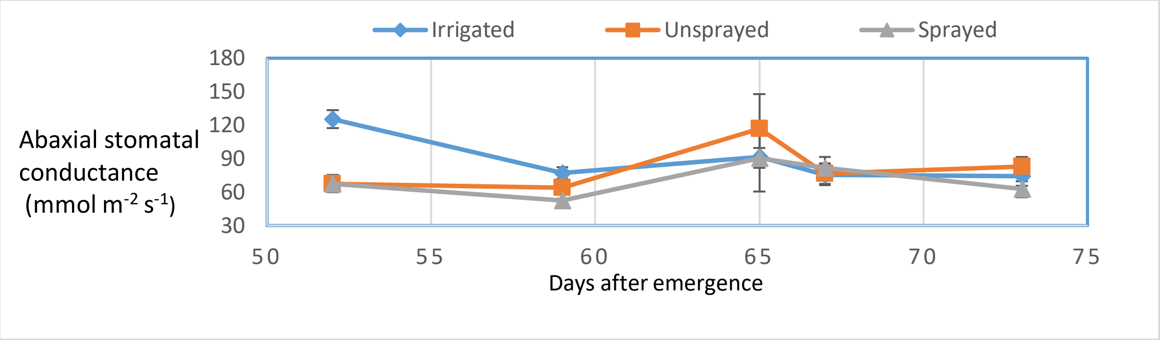

Figure 4.10.4. Response of abaxial stomatal conductance (mmol $m^{-2}s^{-1}$) to irrigation, drought and antitranspirants at 52, 59, 65, 67 and 73 days after emergence recorded on the flag leaf of sorghum grown in the glasshouse in May 2017 (n = 48) (treatment, $P < 0.039$; time, $P = 0.014$; treatment x time $P = 0.047$). Error bars are SEM. (Expt.5).

4.11.4. Total stomatal conductance

The effect of treatments on total stomatal conductance is presented on Table 4.11.7 and Figure 4.10.5.

Total stomatal conductance was significantly affected by treatments, so the null hypothesis was rejected as per the total stomatal conductance. Drought application significantly ($P < 0.001$) decreased in the total stomatal conductance by 5.2 % and 20.0 % in the unsprayed and sprayed treatments respectively. The antitranspirant on the other hand decreased the total stomatal conductance by 15.6 % in the sprayed compared with the unsprayed treatment. Time was significant ($P < 0.056$) showing reduction in total stomatal conductance as days after emergence (DAE) increased and there was no significant time x treatment interaction. The Tukey's test showed that the means of the irrigated and unsprayed were not significantly different, but were significantly different from that of the sprayed treatment. One-way analysis of variance (Table 4.11.8) showed there were significant effect of treatments on the total stomatal conductance at 52 ($P = 0.002$) and 59 (0.045) DAE, whereas and at 65 ($P = 0.263$), 67 ($P = 0.988$) and 73 DAE ($P = 0.109$), no significant response to treatments was recorded.

Table 4.11.7. Average total stomatal conductance (mmols $m^{-2}s^{-1}$) recorded on the flag leaf under irrigation, drought and antitranspirant taken at 52, 59, 65, 67 and 73 days after emergence of sorghum grown in the glasshouse in May 2017. Drought and antitranspirant were applied at 32 and 52 days after emergence respectively. Means within a column followed by same letter are not significantly different according to Tukey's test at $P \leq 0.05$ (n = 48) ± Standard error of the differences of the mean (SED) (Expt.5).

	Days after emergence					
Treatments	52	59	65	67	73	Mean
Irrigated	115.0	82.4	83.3	75.7	77.0	86.7
Droughted unsprayed	84.8	71.4	100.6	77.3	77.0	82.2
Droughted sprayed	67.7	65.4	75.4	76.8	61.7	69.4
Mean	89.2	73.1	86.4	76.6	71.9	79.4
df						179
SED						33.8
CV %						42.5
						P values
Treatments						< 0.001
Time						0.056
Treatments x time						0.127
Tukey's test						
Irrigated						86.7 b
Droughted unsprayed						82.2 b
Droughted sprayed						69.4 a

Table 4.11.8. One-way analysis of variance on the effect of irrigation, drought and antitranspirant on total stomatal conductance (mmols $m^{-2}s^{-1}$) recorded on the flag leaf in sorghum at 52, 59, 65, 67 and 73 days after emergence grown in the glasshouse in May 2017 (n = 48) (Expt.5).

	Days after emergence				
	52	59	65	67	73
Drought					
P values	0.002	0.045	0.263	0.988	0.109
df	30	30	29	30	30
SED	34.20	18.56	42.48	28.78	22.80

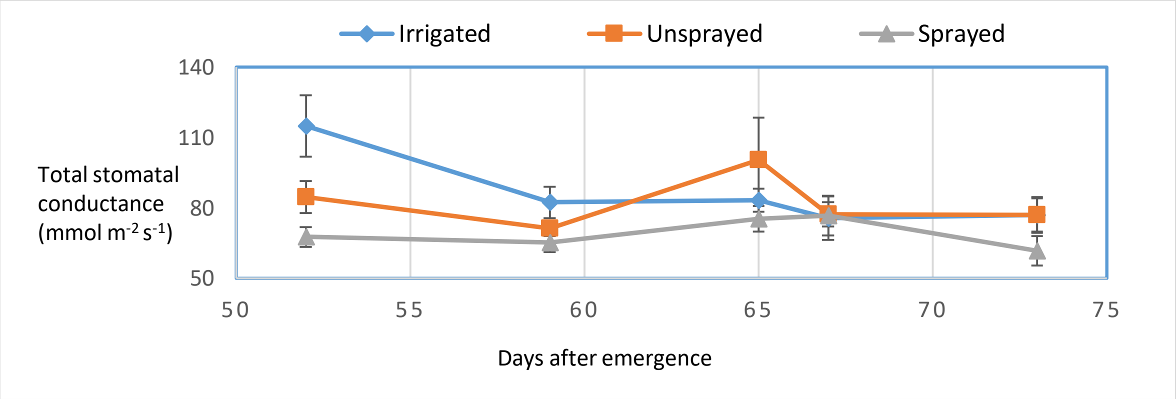

Figure 4.10.5. Response of total stomatal conductance (mmol $m^{-2}s^{-1}$) to irrigation, drought and antitranspirant at 52, 59, 65, 67 and 73 days after emergence recorded on the flag leaf of sorghum grown in the glasshouse in May 2017 (n = 3) (treatment, $P < 0.001$; time, $P = 0.056$; treatment x time $P = 0.127$). Error bars are SEM. (Expt.5).

Table 4.11.9. Average daily temperatures in the glasshouse from 87 - 95 days after emergence of sorghum (Expt.5).

Dates		Days after emergence	Average daily temperatures (˚C)
August 2017	25	87	31.2
	26	88	31.8
	27	89	31.8
	28	90	31.7
	29	91	31.2
	30	92	31.2
	31	93	31.3
September 2017	1	94	31.2
	2	95	31.1

4.11.5. Stalk weight, leaf area relative water content

The effect of treatments (drought and antitranspirant) on stalk weight, leaf area and relative water content are presented on Table 4.11.10.

The treatments had significant effects ($P < 0.001$) thus the null hypothesis is rejected with regards to the stalk weight. Drought significantly reduced stalk weight by 23.23 % and 21.50 % in the unsprayed and sprayed compared with the irrigated treatments and the antitranspirant increased the stalk weight by 2.37 % in the sprayed compared with the unsprayed treatment. The Tukey's test revealed that there was a significant difference between the means of the irrigated and the droughted treatments, but there was no significant difference in the means of the unsprayed and sprayed treatments. Treatments had significant ($P < 0.001$) effects on the leaf area. The drought led to a reduction of 31.96 % and 33.51 % in the unsprayed and sprayed treatments respectively compared with the irrigated, while the antitranspirant led to a decrease of 2.18 % in the sprayed compared with the unsprayed. Tukey's test showed that, while there was a significant difference between the means of the irrigated and droughted treatments, the mean of the unsprayed and sprayed droughted treatments were not significantly different. There was a significant ($P = 0.003$) effect of treatments on relative water content. The drought treatment reduced relative water content by 17.75 % and 18.89 % in the unsprayed and sprayed droughted respectively compared with the irrigated treatments and the antitranspirant increased the relative water content in the sprayed compared with the unsprayed treatment. According to Tukey's test, the mean of the irrigated treatment was significantly different from the droughted unsprayed and sprayed treatments whereas the means of the unsprayed and sprayed treatments were not significantly different.

Table 4.11.10. Average stalk weight (g), leaf area (cm^2) and relative water content (RWC) (%) of sorghum under full irrigation, drought and antitranspirant grown in the glasshouse in May 2017. Leaf area was measured at on the flag leaf and youngest leaf and RWC (%) was measured on the fifth leaf from the bottom. Means within a column followed by same letter are not significantly different according to Tukey's test at $P \leq 0.05$. (n = 16 ± Standard error of the differences of the mean (SED) (Expt.5).

Treatments	Stalk weight (g)	Leaf area (cm^2)	RWC (%)
Irrigated	22.00	148.30	57.85
Droughted unsprayed	16.89	100.90	47.58
Droughted sprayed	17.27	98.60	46.92
Mean	18.72	115.9	50.79
df	15	15	4
SED	1.862	20.25	2.124
CV %	9.9	17.5	4.2
P values			
Treatments	< 0.001	< 0.001	0.003
Tukey's test			
Irrigated	22.00 b	148.3 b	57.85 b
Droughted unsprayed	16.89 a	100.9 a	47.58 a
Droughted sprayed	17.27 a	98.6a	46.92a

4.12. Discussion

4.12.1. Effect of drought imposition at the 3-leaf growth stage on volumetric soil moisture content, transpiration, growth, yield and yield components of sorghum

The imposition of drought was successful as observed by consistent decrease in volumetric soil moisture content, and there was an indication that the antitranspirant may have limited the severity of the decrease in volumetric soil moisture content. Transpiration in the irrigated was consistently greater than in the droughted, while the sprayed maintained a consistently lower transpiration than the unsprayed droughted treatments. These results are in keeping with those of previous experiments in the current study, where significant reduction in transpiration occurred as drought imposed significantly decreased volumetric soil moisture content. Stalk weight and leaf area were significantly different in the irrigated and droughted, showing that sufficient moisture was not available to support cell elongation and growth processes, and this led to reduction in growth under drought than under irrigated conditions. The sprayed plants also had lower relative water content than the unsprayed, as has previously been reported in sorghum adapted to mild temperate climate (Barbanti *et al*., 2015).

4.12.2. Effect of antitranspirant on volumetric soil moisture content, transpiration, and growth of sorghum

Antiranspirant reduced water loss leading to more moisture being held in the soil. This result is in contrast to previous results in this study, because of differences in the drought regimes which allowed for significant reduction in transpiration. Transpiration was significantly lower in the sprayed than in the unsprayed and irrigated treatments as the adaxial, abaxial and total stomatal conductance measurements were significantly lower on some occasions in the sprayed than in the irrigated and unsprayed treatments.

4.12.3. Effect of drought and antitranspirant on grain yield, grain number, weight per grain, biomass and harvest index of sorghum

Grain yield data was unavailable because at the time of harvest the plants did not produce seed. On August 24, 2017 which corresponds to 86 days after emergence, the plants were in full bloom and appeared normal, with no discoloration or disease symptoms, however on September 1, 2017 corresponding to 96 days after emergence, by which time they should have seeded it was observed that all the pollen had fallen off from both irrigated and droughted plants (Figure 4.10.1). This was an anomaly which could be explained by the effects of high temperatures on sorghum at the reproductive stage. The result of data on temperature in the glasshouse showed that between 88 – 90 days after emergence average daily temperatures rose up to 31.8 (˚C) (Table 4.11.9), this was around the optimum of 32 (˚C) daytime and far above the 22 (˚C) night time temperatures under which sorghum thrives best. It has been shown that the developmental stage most sensitive to high temperature in sorghum is during a period 10 – 15 days around anthesis when grain number is determined (Prasad, *et al*., 2008; van Oosterom and Hammer, 2008). Prasad, *et al*., (2006) exposed sorghum to a season-long array of high temperatures consisting daytime maximum/night time minimum of 32/22 (˚C), 36/26 (˚C), 40/30 (˚C) and 44/34 (˚C). The results showed that while treatments at 40/30 (˚C) and 44/34 (˚C) inhibited panicle emergence, those at 36/26 ˚C decreased pollen production, pollen viability, seed set, seed yield, and harvest index in comparison to 32/22 (˚C). Similarly, Downes (1972) reported depression in sorghum grain yield under controlled high temperatures (day/night 33/28 (˚C)), as plants exposed to high temperatures late in panicle development stages suffered floret abortion and even those under moderate temperatures after anthesis were associated with embryo abortion. These adverse effects of high temperatures were attributed to high night temperatures in particular which is responsible for a possible reduction in assimilate availability for dark respiration. Therefore, the reason for the lack of grain setting was not severity of water stress per se, but high temperatures. However, in contrast to the reports of Prasad, *et al*., (2006) and Downes (1972), in the present study there was a complete failure by the plants to produce seeds. Although this can still be attributed to high temperature stress caused by high temperatures which exceeded the optimum by 10 (˚C), the effect was more adverse than mere reduction in grain yield. The exceedingly high temperature must have inflicted significant damage leading to pollens being detached from the panicles and blown away. It is necessary to carry out further investigations on the actual mechanism of the damages caused by excessive heat after flowering in order to explain the reason for lack of grains being produced. Furthermore, although significant differences were not shown between unsprayed and sprayed treatments in relative water content, leaf area was slightly reduced in the sprayed compred with the unsprayed. However, drought application significantly decreased leaf area and relative water content. Nevertheless, the values of relative water content in glasshouse Expt. 5 are very low at 50.79 % whereas the relative water content of 78.30 % in Expt. 1 and 80.50 % in Expt. 2 agrees with some published values (Jones and Turner, 1977). The reason for the very low relative water content of treatments in Expt. 5 could be because there could have been an increase in the size of the turgid leaves since these were placed in the open on the laboratory tables during the incubation period.

4.13. Conclusion

Drought imposition at the 3-leaf growth stage of sorghum has been shown to decrease volumetric soil moisture content, transpiration and growth. The antitranspirant increased volumetric soil moisture content, and decreased transpiration but did not show any significant benefits in increasing stalk weight, leaf area and relative water content in droughted sorghum.

Glasshouse experiment 6

Effect of film antitranspirant Vapor Gard and Neem Oil and terminal drought initiated at the (3-leaf) growth stage on transpiration, growth and yield of sorghum.

4.14. Introduction

Despite arguments against the use of antitranspirants to ameliorate plant water stress which includes restriction of photosynthesis, toxicity and high cost of material (Fuerhing and Finker, 1983; Kettlewell, 2014) significant successes has been achieved in the application of antitranspirant as an agronomic means of improving the yield of crops under drought to warrant continuous investigations (Abdallah, *et al*., 2015; Weerasinghe, *et al*., 2016; Faralli, *et al*., 2017; Abdallah, *et al*., 2017; Ji, *et al*., 2017). With regard to reducing the high cost of material, further research and development of antitranspirant particularly involving mineral oils, low-cost polymers and some plant based oils which could act as antitranspirants has been suggested. (Kettlewell 2014). Kettlewell (2014) suggested evaluating the antitranspirant potentials of oils from locally grown plants for example groundnut oil produced by smallholder farmers in Africa and Asia where drought stress adversely reduces crop yield. In this regard, the suitability of other oils from plant sources to act as antitranspirants have been tested with some remarkable success (Deswarte, et al., 2007, Shanan, et al., 2017).These plant based oils are cheaper to obtain and bio gradable, and therefore environmentally friendly. Deswarte, *et al*., (2007) showed a potential for plant waxes derived from wheat straw, a low-value and high-volume plant by-product to produce a polymer, which may be used as antitranspirants. Also, glycerol used as antitranspirant on *Monstera deliciosa* (Shanan, *et al*., 2011) caused decreased leaf water loss and pigment degradation leading to prolonged vase life compared with other antitranspirants, $MgCO_3$ and Na_2CO_3. However, there is no information in the literature regarding the practical use of oils derived from plants found in some areas where water stress causes significant yield losses to arable crops like sorghum, for example India and Nigeria. This experiment was designed to evaluate the effect of Neem Oil in comparison to Vapor Gard applied as antitranspirants on the yield of droughted sorghum.

The null hypothesis for this study was: There is no significant effect of Vapor Gard and Neem Oil in improving drought tolerance in sorghum.

The objective of the study was:

- To investigate the effects of antitranspirant Vapor Gard and Neem Oil on volumetric soil moisture content, transpiration, yield and yield components of sorghum droughted at the very early (3-leaf) growth stage.

4.14.1. Materials and methods

Materials and methods were identical to Expts. 1, 2, 3 and 4 except for the following:

4.14.1.2. Experimental design and treatments

The experiment was a one-way randomized complete block design with 4 treatments consisting of one fully irrigated, one droughted unsprayed, one droughted sprayed with Vapor Gard (Droughted sprayed VG) and one droughted sprayed with Neem Oil (Droughted sprayed NO) arranged in 15 blocks. Dates of some events and operations during the experiment are presented on Table 4.14.1. Skeleton ANOVA tables are given on Tables 4.14.2 to 4.14.7.

4.14.2. Plant material: The sorghum variety used in this experiment was BirdGO Grain Sorghum (Bright Seeds, UK).

Table 4.14.1. Some significant dates and events during Expt. 6.

Dates	Activity
July 10	Emergence
August 15	Transplanting
August 21	Drought imposition
October 27	Antitranspirant spray
Nov. 10	Harvest

Table 4.14.2. The skeleton ANOVA of the volumetric soil water content measurements (Expt.6).

Source of variation	Degrees of freedom
Blocks stratum	14
Blocks x subject stratum	
Treatments	3
Residual	42
Blocks x subject x time stratum	
Time	3
Time x treatments	9
Residual	168
Total	239

Table 4.14.3. The skeleton ANOVA for the adaxial and abaxial stomatal conductance measurements (Expt.6).

Source of variation	Degrees of freedom
Blocks stratum	13(1)
Blocks x subject stratum	
Treatments	3
Residual	35(7)
Blocks x subject x time stratum	
Time	3
Time x treatments	9
Residual	144 (24)
Total	207(32)

(Numbers in parenthesis are missing values)

Table 4.14.4. The skeleton ANOVA for the total stomatal conductance measurements (Expt.6).

Source of variation	Degrees of freedom
Blocks stratum	13(1)
Blocks x subject stratum	
Treatments	3
Residual	35(7)
Time	3
Time x treatments	9
Residual	144(24)
Total	207(32)

(Numbers in parenthesis are missing values)

Table 4.14.5. The skeleton ANOVA for the grain yield, weight per grain and grain number measurements (Expt.6).

Source of variation	Degrees of freedom
Blocks stratum	13(1)
Blocks x units x stratum	
Treatments	3
Residual	22(20)
Total	38(21)

(Numbers in parenthesis are missing values)

Table 4.14.6. The skeleton ANOVA for the stalk weight and biomass measurements (Expt.6).

Source of variation	Degrees of freedom
Blocks stratum	14
Blocks x units x stratum	
Treatments	3
Residual	42
Total	59

(Numbers in parenthesis are missing values)

Table 4.14.7. The skeleton ANOVA for the harvest index measurements (Expt.6).

Source of variation	Degrees of freedom
Blocks stratum	14
Blocks x units x stratum	
Treatments	3
Residual	25(17)
Total	42(17)

(Numbers in parenthesis are missing values)

4.14.2. Results

4.14.2.1. Volumetric soil moisture content

Results of data analysis on response of volumetric soil moisture content of pots to drought and film antitranspirant Vapor Gard and Neem Oil are shown on Table 4.14.8, 4.14.9 and Figure 4.14.1. The effect of treatments on volumetric soil moisture content was significant (P < 0.001). Compared with the irrigated treatments, drought reduced mean volumetric soil moisture content by 41.5 %, 42.6 % and 35.5 % in the unsprayed, sprayed with neem oil and Vapor Gard respectively. According to contrast analysis ANOVA, volumetric soil moisture content in the irrigated and droughted unsprayed, and the unsprayed and sprayed pots were highly significantly different (P < 0.001). Volumetric soil moisture content in the irrigated was 70.9 % greater than in the droughted unsprayed pots. Also, differences in volumetric soil moisture content between the droughted sprayed with Neem Oil and Vapor Gard were highly significant. Nevertheless, the volumetric soil moisture content in the pots with plants sprayed with Neem Oil were numerically greater than in the pots sprayed with Vapor Gard. Time (P < 0.001) significantly reduced the volumetric soil moisture content which was 16.3 % at the first and 11.2 % at the last day of measurement. The treatment x time interaction effect was significant (P < 0.001) due to the fact that while the differences between the irrigated and droughted treatments increased with time, the differences between the unsprayed and sprayed treatments became smaller with time as it was greater at 3 DAS than at any other day. One-way analysis of variance showed significant (P < 0.001) effects of treatments on volumetric soil moisture content at 3, 9, 14 and 19 days after spraying (DAS).

Table 4.14.8. Average volumetric soil moisture content (%) of pots under irrigation, drought and Vapor Gard (VG) and Neem Oil (NO) at 3, 6, 14 and 19 days after spraying in sorghum grown in the glasshouse in November 2017. Drought was imposed at 19 and Vapor Gard (VG) and Neem Oil (NO) were applied at 109 days after emergence respectively (n = 60) ± Standard error of the differences of the mean (SED) (Expt.6).

	Days after spraying				
Treatments	3	9	14	19	Mean
Irrigated	20.17	18.79	19.21	19.55	19.43
Droughted unsprayed	14.39	10.93	10.94	9.20	11.37
Droughted sprayed NO	14.46	10.65	10.84	8.70	11.16
Droughted sprayed VG	16.00	12.09	11.60	10.41	12.53
Mean	16.25	13.12	13.15	11.19	13.62
df					14
SED					0.824
CV %					6.0
					P values
Treatments					< 0.001
Time					< 0.001
Treatments x time					< 0.001
Contrast analysis					Mean
Irrigated					19.43
Droughted unsprayed					11.37
Droughted sprayed NO					11.16
Droughted sprayed VG					12.53
Treatments					< 0.001
Irrigated vs Droughted					< 0.001
Unsprayed vs Sprayed					< 0.001
NO vs VG					0.008

Table 4.14.9. One-way analysis of variance on the effect of irrigation, drought and Vapor Gard (VG) and Neem Oil (NO) at 3, 6, 14 and 19 days after spraying on volumetric soil moisture content of pots (%) in sorghum grown in the glasshouse in November, 2017. Drought was imposed at 19 and Vapor Gard (VG) and Neem Oil (NO) were applied at 109 days after emergence respectively. (n = 60) ± Standard error of the differences of the mean (SED) (Expt.6).

	Days after spraying			
	3	9	14	19
P values				
Treatments	< 0.001	< 0.001	< 0.001	< 0.001
SED	2.19	2.12	1.36	1.98
df	42	42	42	42

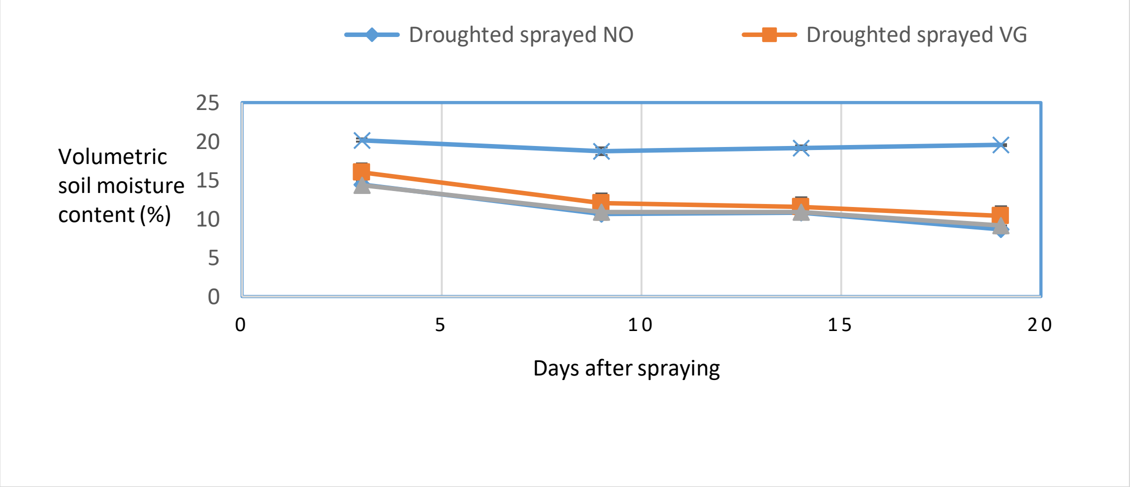

Figure 4.14.1. Response of volumetric soil water content (%) of pots to drought, Vapor Gard (VG) and Neem Oil (NO) treatments at 3, 6, 14 and 19 days after spraying in sorghum grown in glasshouse in November, 2017 (Treatments, P = < 0.001; time < 0.001; treatments x time < 0.001) ± standard error of mean (SEM (Expt. 6).

4.14.2.2. Adaxial stomatal conductance

The response of adaxial stomatal conductance to drought, Vapor Gard (VG) and Neem Oil (NO) applications are presented on Table 4.14.10, 4.14.11 and Figure 4.14.2.

Abaxial stomatal conductance was significantly reduced by 27.20 %, 33.00 % and 41.90 % in the droughted unsprayed, sprayed with Neem Oil and Vapor Gard respectively compared with the irrigated. From the contrast ANOVA result, the treatments induced significant (P < 0.001) differences in mean conductance between the irrigated and droughted (P < 0.001), the unsprayed and sprayed (P = 0.028), but no significant differences were observed between the means of plants treated with Neem Oil and Vapor Gard. However, mean adaxial stomatal conductance was numerically lower in plants treated with Vapor Gard than those with Neem Oil. Time significantly affected the treatments, and the mean on individual days did not show any consistent pattern of response except that the mean on the first and last days of measurements were similar. According to one-way analysis of variance there were significant differences in the treatments at 3 DAS (P < 0.003), 6 DAS (P = 0.002), 9 DAS (P = 0.014) and 14 DAS (P < 0.001).

Table 4.14.10. Adaxial stomatal conductance (mmol $m^{-2}s^{-1}$) of sorghum under irrigation, drought, Vapor Gard and Neem Oil (NO) recorded on the flag leaf at 3, 6, 14 and 19 days after spraying in sorghum grown in the glasshouse in November, 2017. Drought was imposed at 19 and Vapor Gard (VG) and Neem Oil (NO) were applied at 109 days after emergence respectively. (n = 60) ± Standard error of the differences of the mean (SED) (Expt.6).

	Days after spraying				
Treatments	3	6	14	19	Mean
Irrigated	33.78	32.71	37.37	39.04	35.73
Droughted unsprayed	25.68	27.36	25.84	25.14	26.01
Droughted sprayed NO	24.25	21.8	27.28	22.43	23.94
Droughted sprayed VG	22.27	18.87	23.68	18.22	20.76
Mean	26.49	25.19	28.54	26.21	26.61
df					13
SED					3.8
CV %					14.1
	P values				
Treatments					< 0.001
Time					0.048
Time x treatments					0.094

Contrast analysis	Mean
Irrigated	35.73
Droughted unsprayed	26.01
Droughted sprayed NO	23.94
Droughted sprayed VG	20.76
	P values
Treatments	< 0.001
Irrigated vs Droughted	< 0.001
Unsprayed vs Sprayed	0.028
NO vs VG	0.084

Table 4.14.11. One-way analysis of variance on the effect of irrigation, drought, Vapor Gard and Neem Oil (NO) recorded on the flag leaf at 3, 6, 14 and 19 days after spraying on adaxial stomatal conductance (mmol $m^{-2}s^{-1}$) in sorghum grown in the glasshouse in November, 2017. Drought was imposed at 19 and Vapor Gard (VG) and Neem Oil (NO) were applied at 109 days after emergence respectively. (n = 60) ± Standard error of the differences of the mean (SED) (Expt.6).

	Days after spraying			
	3	6	14	19
P values				
Treatments	0.003	0.002	0.014	< 0.001
SED	8.11	9.66	11.78	10.13
df	35	35	35	35

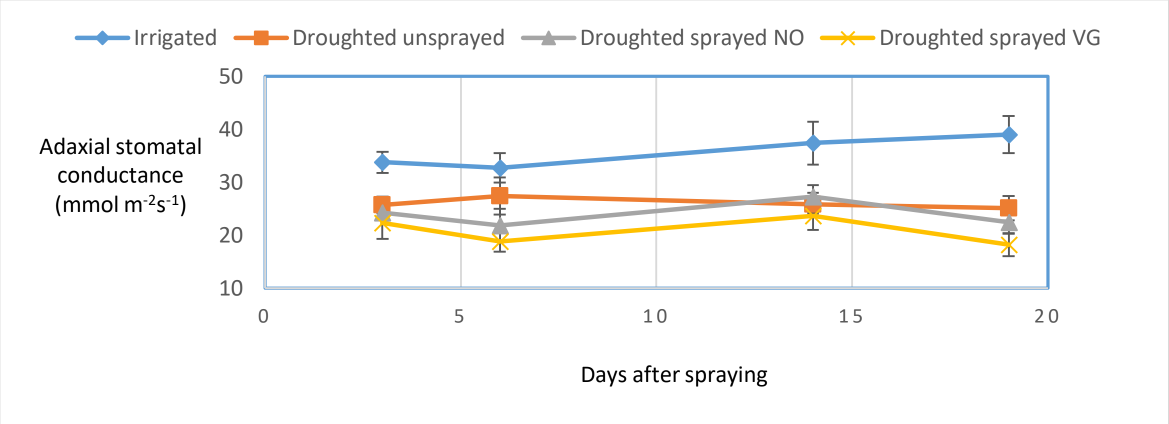

Figure 4.14.2. Response of adaxial stomatal conductance to drought, Vapor Gard (VG) and Neem Oil (NO) treatments recorded on the flag leaf at 3, 6, 14 and 19 days after spraying in sorghum grown in glasshouse in November, 2017 (Treatments, P = < 0.001; time = 0.048; treatments x time = 0.094) ± standard error of mean (SEM (Expt. 6).

4.14.2.3. Abaxial stomatal conductance

Results of data on the effect of drought, Vapor Gard (VG) and Neem Oil (NO) treatments on abaxial stomatal conductance is presented on Table 4.14.12, 4.14.13 and Figure 4.14.3. The treatments had a significant (P < 0.001) effect on abaxial stomatal conductance and reduced it by 19.20 % in the droughted unsprayed and by 27.49 % and 26.02 % in the sprayed with Neem Oil and Vapor Gard respectively compared with the irrigated. Contrast ANOVA revealed significant (P < 0.001) differences in abaxial stomatal conductance between the irrigated and droughted as well as the unsprayed and sprayed plants, but no differences were found in abaxial stomatal conductance between the Neem Oil and Vapor Gard sprayed treatments. Reduction in stomatal conductance were greater in plants sprayed with Neem Oil and Vapor Gard, than in those unsprayed compared with the irrigated. Time and treatments x time interaction effects were not significant. However, one-way analysis of variance showed significant effects and differences in the treatments at 3 DAS (P < 0.001), 6 DAS (P = 0.005), 9 DAS (P = 0.005) and 14 DAS (P = 0.037)

Table 4.14.12. Abaxial stomatal conductance (mmol $m^{-2}s^{-1}$) of sorghum under irrigation, drought and Vapor Gard (VG) and Neem Oil (NO) recorded on the flag leaf at 3, 6, 14 and 19 days after spraying in sorghum grown in the glasshouse in November, 2017. Drought was imposed at 19 and Vapor Gard (VG) and Neem Oil (NO) were applied at 109 days after emergence respectively. (n = 60) ± Standard error of the differences of the mean (SED) (Expt.6).

	Days after spraying				
Treatments	3	6	14	19	Mean
Irrigated	43.21	38.15	42.81	38.34	40.63
Droughted unsprayed	31.69	33.42	34.3	31.89	32.83
Droughted sprayed NO	31.2	28.91	30.23	27.49	29.46
Droughted sprayed VG	29.93	26.8	32.16	31.36	30.06
Mean	30.41	31.82	34.87	32.27	33.24
Df					13
SED					8.8
CV %					26.3
					P values
Treatments					< 0.001
Time					0.126
Treatments x time					0.566
Contrast analysis					Mean
Irrigated					40.63
Droughted unsprayed					32.83
Droughted sprayed NO					29.46
Droughted sprayed VG					30.06
					P values
Treatments					< 0.001
Irrigated vs Droughted					< 0.001
Unsprayed vs Sprayed					0.033
NO vs VG					0.688

Table 4.14.13. One-way analysis of variance on the effect of irrigation, drought, Vapor Gard (VG) and Neem Oil (NO) at 3, 6, 14 and 19 days after spraying on abaxial stomatal conductance (mmols $m^{-2}s^{-1}$) recorded on the flag leaf in sorghum grown in the glasshouse in November, 2017. Drought was imposed at 19 and Vapor Gard (VG) and Neem Oil (NO) were applied at 109 days after emergence respectively. (n = 60) ± Standard error of the differences of the mean (SED) (Expt.6).

	Days after spraying			
	3	6	14	19
P values				
Treatments	< 0.001	0.005	0.005	0.037
SED	8.06	8.89	9.24	9.52
df	35	35	35	35

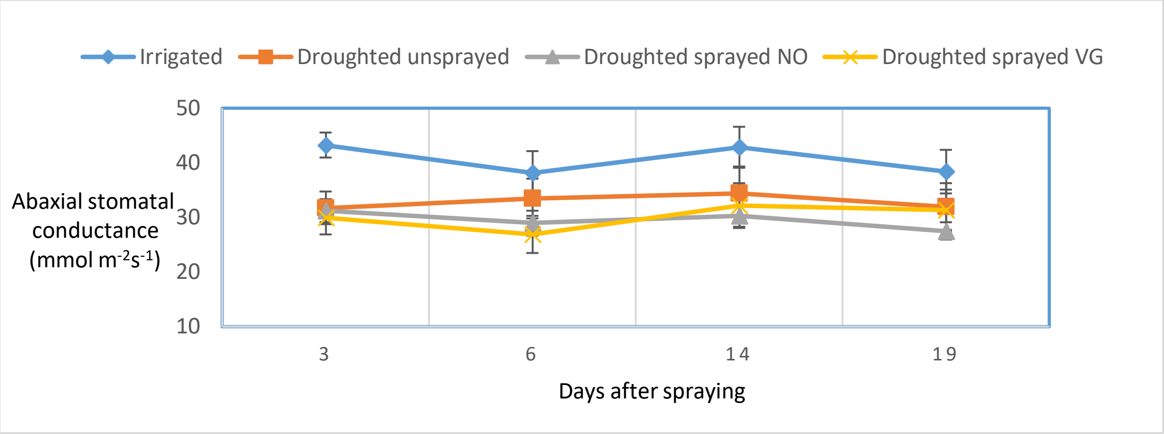

Figure 4.14.3. Response of abaxial stomatal conductance to drought, Vapor Gard (VG) and Neem Oil (NO) treatments recorded on the flag leaf at 3, 6, 14 and 19 days after spraying in sorghum grown in glasshouse in November, 2017 (Treatments, P = < 0.001; time = 0.126; treatments x time = 0.566) ± standard error of mean (SEM (Expt. 6).

4.14.2.4. Total stomatal conductance

The response of total stomatal conductance to drought, Vapor Gard (VG) and NO (Neem Oil) treatments are presented on Table 4.14.14, 4.14.15 and Figure 4.14.4.

The treatments had significant ($P < 0.001$) effect on the total stomatal conductance. In the droughted unsprayed, droughted sprayed with Neem Oil and sprayed with Vapor Gard, total stomatal conductance decreased by 22.92 %, 30.78 % and 33.43 % respectively compared with the irrigated. From the contrast ANOVA result, the treatments led to significant ($P < 0.001$) differences between the irrigated and droughted ($P = 0.001$), the unsprayed and sprayed ($P = 0.010$), but no significant differences were observed between the treatments sprayed Neem Oil and those with Vapor Gard. According to one-way analysis of variance there were significant differences in the treatments at 3 DAS ($P < 0.001$), 6 DAS ($P < 0.001$), 19 DAS ($P < 0.001$) and 14 DAS ($P < 0.001$).

Table 4.14.14. Average total stomatal conductance (mmol $m^{-2}s^{-1}$) of sorghum under irrigation, drought, Vapor Gard (VG) and Neem Oil (NO) recoreded on the flag leaf at 3, 6, 14 and 19 days after spraying in sorghum grown in the glasshouse in November, 2017. Drought was imposed at 19 and Vapor Gard (VG) and Neem Oil (NO) were applied at 109 days after emergence respectively. (n = 60) ± Standard error of the differences of the mean (SED) (Expt.6).

	Days after spraying				
Treatments	3	6	14	19	Mean
Irrigated	38.49	35.43	40.09	38.68	38.17
Droughted unsprayed	28.69	30.39	30.07	28.52	29.42
Droughted sprayed NO	27.73	25.36	28.75	24.96	26.7
Droughted sprayed VG	26.1	22.84	27.92	24.79	25.41
Mean	30.25	28.5	31.71	29.4	29.92
df					13
SED					5.7
CV %					19.2
					P values
Treatments					< 0.001
Time					0.012
Time x treatments					0.492
Contrast analysis					
					Mean
Irrigated					38.17
Droughted unsprayed					29.42
Droughted sprayed NO					26.7
Droughted sprayed VG					25.41
					P values
Treatments					< 0.001
Irrigated vs Droughted					0.001
Unsprayed vs Sprayed					0.01
NO vs VG					0.327

Table 4.14.15. One-way analysis of variance on the effect of irrigation, drought, Vapor Gard (VG) and Neem Oil (NO) at 3, 6, 14 and 19 days after spraying on total stomatal conductance (mmol $m^{-2}s^{-1}$) recorded on the flag leaf in sorghum grown in the glasshouse in November, 2017. Drought was imposed at 19 and Vapor Gard (VG) and Neem Oil (NO) were applied at 109 days after emergence respectively. (n = 60) ± Standard error of the differences of the mean (SED) (Expt.6).

	Days after spraying			
	3	6	14	19
P values				
Treatments	< 0.001	< 0.001	< 0.001	< 0.001
SED	5.4	6.52	8.12	6.5
df	35	35	35	35

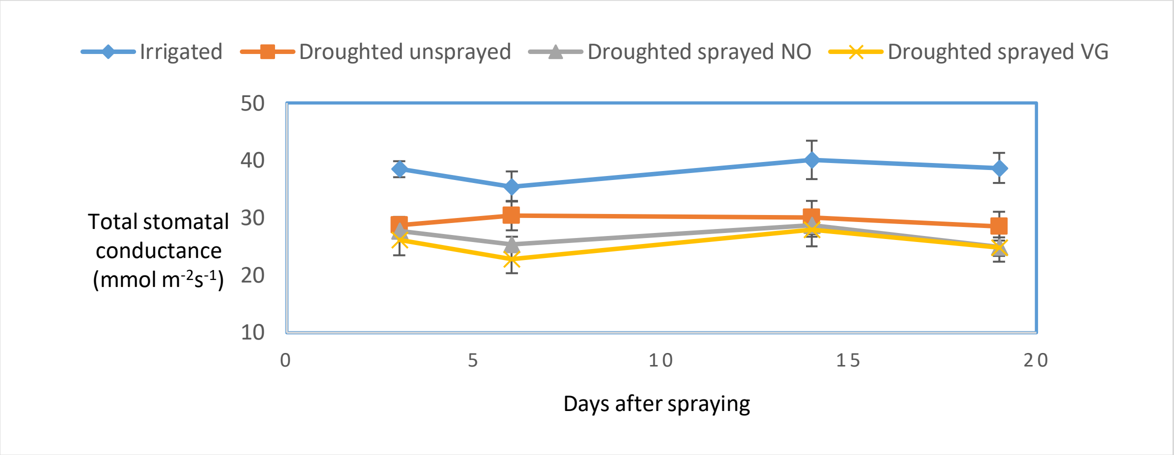

Figure 4.14.4. Response of total stomatal conductance to drought, Vapor Gard (VG) and Neem Oil (NO) treatments recorded on the flag leaf at 3, 6, 14 and 19 days after spraying in sorghum grown in glasshouse in November, 2017 (Treatments, P = < 0.001; time = 0.012; treatments x time = 0.492) ± standard error of mean (SEM (Expt. 6).

4.14.2.5. Yield and yield components

Results from data analysed on responses of grain yield and yield components to application of drought, Vapor Gard (VG) and Neem Oil (NO) are presented on Table 4.14.16.

Treatments had highly significant (P < 0.001) effects on grain yield as the drought decreased grain yield by 53.03 %, 38.36 % and 22.64 % in the droughted unsprayed, sprayed with Neem Oil and Vapor Gard respectively compared with the irrigated. Contrast ANOVA revealed that the mean grain yield of the irrigated and droughted unsprayed were highly significantly (P < 0.001) different, so also the means of the plants sprayed with antitranspirants and those unsprayed were highly significantly (P < 0.001) different. The mean grain yield of plants sprayed with Neem Oil and those sprayed with Vapor Gard were significantly (P = 0.027) different. It can be observed that reduction in grain yield in the unsprayed treatment was lower than in the treatments sprayed with Vapor Gard and Neem oil and that the grain yield in the plants treated with Vapor Gard was greater than the unsprayed and sprayed with Neem Oil.

There was a highly significant (P < 0.001) effect of the treatments on the grain number which was reduced under drought by 42.70 %, 26.13 % and 11.52 % in the droughted unsprayed, sprayed with Neem Oil and Vapor Gard respectively compared with the irrigated. According to contrast ANOVA, there was a highly significant (P < 0.001) difference in grain number between the irrigated and droughted, the unsprayed and sprayed but only a significant (P = 0.025) difference between the plants sprayed with Neem Oil and those sprayed with Vapor Gard. With regard to the droughted treatments, reduction in grain number was greatest in the unsprayed plants than in the Neem Oil and Vapor Gard sprayed treatments.

Weight per grain was also significantly (P = 0.083) affected by the treatments leading to a decrease of 25.98 % in the treatments that were unsprayed, while the treatments sprayed with Neem Oil and Vapor Gard decreased by 14.50 % and 8.46 % respectively below the irrigated treatments. Contrast ANOVA showed significant differences (P = 0.05) in weight per grain between the irrigated and the droughted treatments, but no sigficant differences occurred between treatments sprayed with Neem Oil and those with Vapor Gard.

However, the treatments had no significant effects on stalk weight, but biomass was highly significantly (P < 0.001) affected by the treatments and was reduced by 43.52 % in the droughted unsprayed treatment, 44.91 % and 52.55 % in the treatments sprayed with Neem Oil and those sprayed with Vapor Gard respectively. Whereas the contrast ANOVA showed there were significant differences between the irrigated and droughted treatments, no significant differences were observed between the means of the unsprayed and sprayed and those of treatments sprayed with Neem Oil and those with Vapor Gard. There was a significant (P < 0.002) effect of the treatments on harvest index leading to a reduction of 23.19 % in the droughted unsprayed and a marginal increase of 5.12 % in the Neem Oil but a slight decrease of 9.96 % in the Vapor Gard treated plants. The treatment caused significant differences (P = 0.002) between the unsprayed and sprayed plants; also significant differences (P = 0.039) were recorded between the Neem Oil and Vapor Gard sprayed plants.

Table 4.14.16. Average grain yield and yield components of sorghum under irrigation, drought, Vapor Gard (VG) and Neem Oil (NO) in sorghum grown in the glasshouse in November, 2017. Drought was imposed at 19 and Vapor Gard (VG) and Neem Oil (NO) were applied at 109 days after emergence respectively. (n = 60) ± Standard error of the differences of the mean (SED) (Expt.6).

Treatments	Grain yield (g)	Grain number per plant	Weight per grain (mg)	Stalk weight (g)	Biomass (g)	Harvest Index (%)
Irrigated	31.49	972	33.1	13.23	43.2	70.3
Droughted unsprayed	14.79	557	24.5	11.13	24.4	54
Droughted sprayed (NO)	19.41	718	28.3	11.99	23.8	73.9
Droughted sprayed (VG)	24.36	860	30.3	11.33	20.5	63.3
Mean	22.50	777	29.1	11.92	28.0	65.4
P values	< 0.001	< 0.001	0.083	0.192	< 0.001	0.002
df	22	22	22	42	38	22
CV %	5.694	160.3	8.77	2.858	7.9	13.25
SED	25.3	20.6	30.2	24	28.2	20.3
Contrast Analysis						
Irrigated	31.49	972	33.1	13.23	43.2	70.3
Droughted unsprayed	14.79	557	24.5	11.13	24.4	54
Droughted sprayed (NO)	19.41	718	28.3	11.99	23.8	73.9
Droughted sprayed VG	24.36	860	30.3	11.33	20.5	63.3
Treatments	< 0.001	< 0.001	0.083	0.192	< 0.001	0.002
Irrigated vs Droughted	< 0.001	< 0.001	0.050	0.046	< 0.001	0.107
Unsprayed vs Sprayed	< 0.001	< 0.001	0.099	0.563	0.386	0.002
NO vs VG	0.027	0.025	0.549	0.535	0.255	0.039

4.15. Discussion

Treatments significantly affected volumetric soil moisture contents of pots, transpiration, grain yield, grain number, weight per grain, biomass and harvest index, thus the null hypothesis is rejected with reference to these measurements. Reduction in volumetric soil moisture content is in agreement with previous results in Expts. 4 and 5 in the current chapter and points to the adequacy of the soil water stress applied. Significant differences between irrigated and droughted plants and between unsprayed and sprayed plants confirms previous findings in the current study (Expts. 4 and 5 in the current chapter). Similarly, significant reduction in adaxial, abaxial and total stomatal conductance concurs with the significant reduction in volumetric soil moisture content recorded in this study. The reason for the lack of significant differences in adaxial, abaxial and total stomatal conductance between the Vapor Gard and Neem Oil treated plants could be attributed to both Vapor Gard and Neem Oil treatments having similar or the same effects on stomatal conductance. However, this observation can only be sustained if the experiment was repeated, and that could not be done due to time constraints.

The treatments had significant effects on grain yield, grain number, weight per grain, biomass and harvest index, therefore the null hypothesis was rejected. Grain yield was significantly decreased in the droughted compared with the irrigated consistent with the findings from Expt. 5. Antitranspirant significantly increased grain yield in the sprayed compared with the unsprayed signifying certain advantageous effects of antitranspirant to grain yield, but in favour of Vapor Gard and not Neem Oil as the former gave a 25.50 % significantly higher grain yield than the Neem Oil treated plants. There was a highly significant and drastic reduction in the grain number in the droughted compared with the irrigated as a result of drought, thus drought effect must have been so severe on the plant to trigger conservatism in setting grain number in order to forestall further yield damage. Grain setting must have been adversely interrupted by the severe water stress, and this could not be ameliorated by the antitranspirant activity of reducing transpiration.

Weight per grain was not significantly affected in the droughted compared with the irrigated and the antitranspirant numerically increased weight per grain in the sprayed over the unsprayed treatments. Further reductions in weight per grain the sprayed treatments could have been caused by the blockage of the stomata by the antitranspirant leading to a probabale reduction in CO_2 uptake and subsequent interruption of the grain filling processes.

Drought and antitranspirant treatments led to significant differences in biomass in the irrigated and droughted plants and this could be attributed to significant reductions in grain yield and number. However, Vapor Gard and Neem Oil treatments did not cause any significant differences in biomass because no significant differences in stalk weight which is a component of biomass occurred from the antitranspirants. Harvest index was significantly affected by the treatments. The drought did show significantly different results and the antitranspirant increased harvest index mainly due to increases in grain yield. Neither the Vapor Gard nor the Neem Oil gave significantly different harvest index because marginal increases were recorded with Neem Oil application whereas decreases were sustained with Vapor Gard application. The stalk weight remained largely unaffected, and the greater grain yield and weight per grain in these treatments must have concealed whatever differences there would have been between the treatments.

4.16. Conclusions

Both Vapor Gard and Neem Oil were effective as antiranspirant as they decreased transpiration and numerically improved grain yield and yield components of droughted sorghum over the unsprayed. Although, there were no significant differences in their performances toward improving grain yield; numerically greater grain yield, was recorded by the Vapor Gard over the Neem Oil treated plants. Thus, Vapor Gard may be better than the Neem Oil in terms of their antitranspirant actions of improving grain yield in droughted sorghum. Nevertheless, considerations for practical application should involve the economics around the cost of the products and notjust their efficacies. However, there would be need to repeat the experiment under controlled and field conditions to draw plausible conclusions.

4.17. Summary and conclusions on chapter 4.

The experiments done in this chapter aimed at demonstrating optimum concentration of antitranspirants that would produce the greatest yield in droughted sorghum and the drought severity under which significant reduction in sorghum grain yield would occur. From the results of the experiments the following can be concluded:

1. Since theantitranspirant dosage at 1.0 L/ha which had been used in previous Expts. 1 and 2 gave no significantly lower yield than the higher concentrations it can be used in formulating antitranspirant solution to spray sorghum as the 1.0 L/ha dosage had given significantly higher yield in wheat.
2. Drought imposition at the three and five-leaf growth stages had greater adverse effects on sorghum grain yield than the booting to flowering used in Expts. 2 and 3 and the 8-leaf to booting and panicle emergence growth stages used in Expt.5, hence in addition to the booting to flowering reported in the literature, the three and five-leaf growth stages can be considered as most susceptible to drought in sorghum.

3. There was no significant yield benefit gained from applying Vapor Gard over Neem Oil when applied as antitranspirants on droughted sorghum at the same rate under same environmental conditions, therefore Neem Oil can serve as alternative to Vapor Gard and indeed has the potential to be used as an antitranspirant.

CHAPTER 5

Effect of drought and antitranspirant on transpiration, growth and yield of sorghum under field conditions

5.0. Introduction

The effect of drought and antitranspirant on the transpiration, growth and yield of sorghum was evaluated using glasshouse experiments reported in preceding chapters 3 and 4. The results across the experiments showed that drought significantly reduced transpiration, growth and yield while the antitranspirant significantly decreased transpiration and increased growth and gave a numerical but not significant increase in grain yield and yield components in droughted sorghum. Thus, the antitranspirant has shown potential for improving plant water relations, growth and yield in droughted sorghum. Therefore, there is a need to further investigate the effect of the antitranspirant on the yield of droughted sorghum in field plots. The following field experiments (Polytunnel Expts. 1 and 2) were designed to determine the effectiveness of the antitranspirant in improving the yield and yield components of droughted sorghum under field conditions.

The null hypothesis tested in the Polytunnel Expts. 1 and 2 was:

- There is no significant effect of drought stress and antitranspirant on transpiration, growth and yield of sorghum.

The objective was to evaluate the effect of drought stress and antitranspirant on the following:

- Volumetric soil moisture content of plots.
- Transpiration and leaf temperature of sorghum.
- Leaf area and green area index of sorghum.
- Yield and yield components of sorghum.

Polytunnel Expt. 1 (Expt. 7)

5.1. Materials and methods

5.1.1. Field location and conditions

The polytunnel experiments were carried out at flat Nook field located at Harper Adams University, Shropshire, UK (52° 46 'N, 2° 25 'W). Previous crop in the field in 2015 – 16 was spring barley. Soil analysis carried out on a sample of the soil taken at 0 – 30 cm depth in 2016 showed that the soil had a pH = 6.4, available nitrogen = 30.2 kg/ha, phosphorus = 55.8 mg/l and potassium = 114 mg/l (Table 5.11). The field was ploughed and harrowed, and rain-out shelters erected for two weeks over the plough-harrowed field prior to sowing.

The soil texture in Flat Nook is sandy loam according to Toogod (1958). At a depth of 20 cm, it contains 75.8 % sand, 20.8 % silt and 3.4 % clay with a bulk density of 1.74 g cm^{-3}. At the 40 cm depth the percentage of sand was 78.9 %, and 71.2 % and 72.1 % for the 60 and 80 cm depths respectively, while the silt percentages was ~20 %. Clay concentrations were 6.4 % and 5.4 % at the 60 cm and 80 cm depths respectively. Whereas bulk density were 1.76, 1.78 and 1.84 g/cm^{-3} at 20 cm, 40 cm and 60cm depths respectively. According to Weerasinghe, *et al*. (2016), the field capacity of Flat Nook at a depth of 80 cm was determined to be 160 mm using neutron probe (Institute of Hydrology Neutron Probe System, Wallingford, UK) and the permanent wilting point at 80 cm was 62 mm (Hall, 1977), thus the available soil water capacity was 98 mm.

5.1.2. Experimental design and layout

The experiment was carried out under rain-out shelters and the experimental design was a split-plot design. The reason for using the rain-out shelters is to standardize sorghum to the condition where it is dominantly cultivated in order to simulate the occurrence and effect of drought. Each rain-out shelter was made of a number of metal hoops 2.3 m high whose ends were inserted into the ground and covered by polythene on all sides with a door fitted to one side enclosing an area of dimension 9 m x 5 m. (Figures 5.1.1 to 5.1.6). There were eight rain-out shelters in total allocated to four blocks giving two per block. For the drought treatment, in each block a whole rain-out shelter constitutes the main plot, one of which is droughted and another irrigated. With regard to the antitranspirant spray, within each of the droughted or irrigated rain-out shelters as the case may be, there were four subplots: two of which were sprayed and the other two unsprayed randomly, giving four unsprayed and four sprayed subplots per block. Therefore, 'drought' is the main plot, while the 'antitranspirant' is the subplot. Planting distance within each shelter was 45 cm apart between rows and 30 cm apart within rows, giving 6 rows in each rain-out shelter with 23 stands per row making a total of 138 stands per rain-out shelter. 7 to 10 seeds of grain sorghum (*Sorghum bicolor*, L.) BirdGO Grain Sorghum (Bright Seeds, UK) variety were sown manually at each stand at depths of ~5cm. Plant stands were separated by a distance of 1m from the ends of the rain-out shelter and 50 cm from the sides while a 1 m space was left in the middle of each rain-out shelter. The subplots were marked out as 300 cm x 350 cm (Figure 5.1.7).

5.1.2.1. Fertilizer application

On 27th July, corresponding to 30 days after emergence, ammonium nitrate and murate of potash fertilizers were applied using a spreader to the entire plants in each polytunnel. 130 g of ammonium nitrate and 1.5 kg of murate of potash per polytunnel were applied at the same time. The murate of potash was applied at the rate 10 g per
Plant.

5.1.3. Treatments

Drought and antitranspirant sprays were the 2 factors each at 2 levels. The 2 levels of drought were ‘drought’ and ‘irrigated’ while the 2 levels of antitranspirant sprays were ‘sprayed’ and ‘unsprayed’.

5.1.4. Irrigation and drought imposition

All plants were fully irrigated by trickle tape up to the five leaf stage 15th July, 2016 at GS 1.5. Thus irrigation was suspended on the droughted plots at GS 1.5. However, irrigation continued on the irrigated plots only; at the rate of 1.4 L/min/polytunnel at least once every two days until 17th July 2016, GS 5.9, when it was resumed for all treatments (Irrigated and droughted) and stopped finally on 17th October 2016, at GS 6.9. All growth stages were determined according to the extended BBCH modified for sorghum (AgVita, 2008).

5.1.5. Antitranspirant application

A commercial terpene based film-forming antitranspirant Vapor Gard® (Miller Chemicals, Pennsylvannia USA containing 96 % di-1-*p*-methene active ingredient and 4 % inert materials) at a concentration of 0.5 ml in 100 ml of water was sprayed on the ‘droughted sprayed’ and ‘irrigated sprayed’ treatments manually at booting with a hand-held lunch box boom sprayer (Trials equipment, Essex, UK) using a Flat Fan nozzle (FF110 -03), at 2 bar pressure, a rate of 200 L/ha and forward speed of 6 km/hour.

5.1.6. Plant material

The sorghum variety used in this experiment was BirdGO Grain Sorghum (Bright Seeds, UK).

Table 5.1. Some significant dates and events during Polytunnel Expt.1 (Expt.7) from June to October, 2016.

Dates	Activities
June 20	Sowing
June 20	Pre-emergence herbicide applied
June 27	Emergence (about 70 %)
July 11	Complete emergence
July 13	Thinning
July 15	Drought imposition
July 27	Fertilizer application
August 17	Antitranspirant application
October 27	Harvest

5.1.7. Measurements

All measurements were carried out as in the glasshouse experiments except for the following:

5.1.7.1. Volumetric soil moisture content

Volumetric soil moisture content (%) was taken by inserting the TDR (Fieldscout TDR 100/200 Soil Moisture Meter, Spectrum Technologies, Inc., USA) probe into the soil at a depth of 30 cm at two different places on each treatment subplot/plot and recording the readings.

5.1.7.2. Leaf area

The leaf area was determined using a leaf area scanner (LI-3000C Portable Area Meter, LI-COR Biosciences). Measurements were taken on the flag leaf of two plants per subplot randomly selected and the average reported as leaf area. Leaf area measurements were taken three times during the experiment.

5.1.7.3. Green area index

Green area index measurement was carried out using a Ceptometer (AccuPAR Model, LP – 80. Decagon Devices Inc., Washington, USA). The Ceptometer is battery-operated menu driven device consisting of a microprocessor-driven data logger and a probe with 80 sensors. It is used to measure light interception by plant canopies to compute the leaf area index. At each measurement session, the probe was placed at different points above and beneath the plant canopy on a subplot representing a treatment and the resulting values recorded at each point as the green area index of the treatment.

5.1.7.4. Yield component measurements

The panicles of plants in middle rows of all subplots comprising ten plants were harvested at maturity manually using secateurs and main shoots were left in the ground. The panicles were put in paper bags labelled according to treatments and subplots and placed in a glasshouse bay set at 31°C and 28°C day and night temperatures respectively to dry. The panicles were later threshed in an electric thresher (Wintersteiger Austria) and the grains collected in separate bags according to treatments and placed in a convection oven set at 80°C, and continuously weighed until nearly constant weight to the nearest gram. The main shoots were later harvested and placed in a soil cupboard at 30°C to dry to a nearly constant weight to the nearest gram. Grain yield per plot was taken by weighing the entire grains from the ten plants harvested on a balance and recording the weight. Grain number per plot was obtained by counting the seeds in each bag using a counting machine (CountAmatic Cosole, Farm-Tec Whitby, UK). To obtain the stalk weight per plot, the entire main shoot from each plot was weighed directly on a Floor Scale (AE Adam, CPW plus – 75) and the weight recorded. The biomass and harvest index were computed on plot basis according to Sylvester-Bradley *et al*., (1985).

Therefore, the weight of subsample is the weight of grains harvested from a plot and the number of grains in a subsample is the grain number from a plot.

5.1.7.5. Soil properties

A sample of the soil between depths of 0 - 30 cm was taken for chemical analysis on nitrogen, phosphorous, potassium and magnesium.

5.1.7.6. Meteorological data

Temperature, was recorded by data loggers (Tinytag Plus, Gemini data loggers, Chichester, UK) placed in three of the rain-out shelters throughout the experimental period.

5.1.7.7. Statistical analysis

Data were analysed using GENSTAT, 17th edition (VSN International Ltd, Hemel Hempstead, UK). All data were subjected to split-plot analysis of variance (ANOVA). Data were tested for normality and variance homogeneity by checking the residual plots during analysis. Where data were taken over a range of days after spraying, repeated measures ANOVA was used in the analysis. Skeleton ANOVA for the measurements are presented on the range of tables from 5.2 to 5.11.

Figure 5.1.1. Side view of a polytunnel covered with plastic material to raise temperature and create drought conditions for sorghum growth.

Figure 5.1.2. Front view of polytunnels showing doors and pipes for irrigation.

Figure 5.1.3. Sorghum growing under drought inside polytunnels at 52 days after emergence. Showing stage of antitranspirant application (Polytunnel Expt.1) (Expt.7).

Figure 5.1.4. Sorghum growing under irrigation inside polytunnels at 52 days after emergence. Showing stage of antitranspirant application (Polytunnel Expt.1) (Expt.7).

Figure 5.1.5. Sorghum growing under irrigation inside polytunnels at 85 days after emergence. (Polytunnel Expt.1) (Expt.7).

Figure 5.1.6. Sorghum growing under drought inside polytunnels at 85 days after emergence. (Polytunnel Expt.1) (Expt.7).

Table 5.2. Skeleton ANOVA of the experimental design (Polytunnel Expt.1) (Expt.7)

Source of variation	Degrees of freedom
Block stratum	3
Main plots stratum	
Drought	1
Residual	4
Subplots stratum	
Antitranspirant	1
Drought x antitranspirant	1
Residual	21
Total	31

Table 5.3. Skeleton ANOVA of the volumetric soil moisture content measurements (Polytunnel Expt.1) (Expt.7)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x subject stratum	
Drought	1
Antitranspirants	1
Irrigation x antitranspirants	1
Residual	25
Blocks x subject x time stratum	
Time	5
Timex drought	5
Time x antitranspirants	5
Time x drought x antitranspirants	5
Residual	140
Total	191

Table 5.4. Skeleton ANOVA of the adaxial stomatal conductance measurements (Polytunnel Expt.1) (Expt.7)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x subject stratum	
Antitranspirant	1
Droughted	1
Antitranspirant x drought	1
Residual	25
Blocks x drought x subject	
Antitranspirant	1
Drought x antitranspirant	1
Residual	22
Blocks x subject x time stratum	
Time	5
Time x antitranspirants	5
Time x drought	5
Time x antitranspirant x drought	5
Residual	139(1)
Total	190(1)

(Numbers in parenthesis are missing values)

Table 5.5. Skeleton ANOVA of the abaxial stomatal conductance measurements (Polytunnel Expt.1) (Expt.7)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x drought stratum	
Drought	1
Residual	3
Blocks x drought x subject stratum	
Antitranspirants	1
Drought x antitranspirants	1
Residual	22
Blocks x drought x subject x time stratum	
Time	5
Drought x time	5
Time x antitranspirants	5
Drought x time x antitranspirants	5
Residual	140
Total	191

Table 5.6. Skeleton ANOVA of the leaf temperature measurements (Polytunnel Expt.1) (Expt.7)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x drought stratum	
Drought	1
Residual	3
Blocks x drought x subject stratum	
Antitranspirants	1
Drought x antitranspirants	1
Residual	22
Blocks x drought x subject x time stratum	
Time	2
Drought x time	2
Time x antitranspirants	2
Irrigation x time x antitranspirants	2
Residual	56
Total	95

Table 5.7. Skeleton ANOVA of the leaf area measurements (Polytunnel Expt.1) (Expt.7)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x drought stratum	
Irrigation	1
Residual	3
Blocks x drought x units x stratum	
Antitranspirants	1
Drought x antitranspirants	1
Residual	22
Total	31

Table 5.8.Skeleton ANOVA of the green area index measurements (Polytunnel Expt.1) (Expt.7)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x subject stratum	
Antitranspirants	1
Drought	1
Antitranspirants x drought	1
Residual	25
Blocks x subject x time stratum	
Time	2
Time x antitranspirants	2
Time x drought	2
Time x antitranspirants x drought	2
Residual	56
Total	95

Table 5.9. Skeleton ANOVA of the yield and yield component measurements (Polytunnel Expt.1) (Expt.7)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x drought stratum	
Drought x antitranspirants	1
Residual	3
Blocks x drought x units x stratum	
Antitranspirants	1
Drought x antitranspirants	1
Residual	20(3)
Total	29(3)

(Numbers in parenthesis are missing values)

Table 5.10. Skeleton ANOVA of the total stomatal conductance measurements (Polytunnel Expt.1) (Expt.7)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x drought stratum	
Drought	1
Residual	3
Blocks x drought x subject stratum	
Antitranspirants	1
Drought x antitranspirants	1
Residual	22
Blocks x drought x subject x time stratum	
Time	5
Drought x time	5
Time x antitranspirants	5
Drought x time x antitranspirants	5
Residual	138(2)
Total	189(2)

(Numbers in parenthesis are missing values)

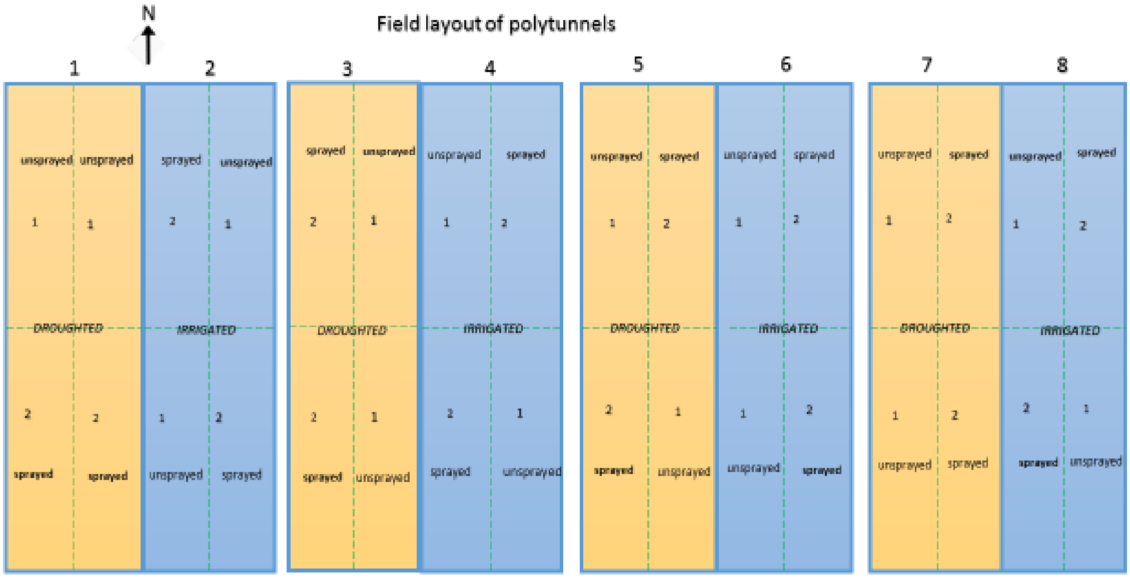

Figure 5.1.7. Field layout of irrigated, droughted, sprayed and unsprayed polytunnels 1 to 8, showing arrangement of two polytunnels per block and treatments inserted. (Polytunnel Expt.1) (Expt.7).

5.2. Results

Table 5.11. Result of soil analysis (standard) on 1.00 kg of the soil taken from a depth of 0 – 30 cm at Flat Nook, Harper Adams University (52° 46 'N, 2° 25 'W) for Polytunnel Expt.1.

Chemical property	Values obtained	Date of analysis
Soil pH	6.4	July 26, 2016
Available Phosphorus	55.8 mg/l	
Available Potassium	114 mg/l	
Available Magnesium	61.7 mg/l	
Nitrate Nitrogen (Fresh)	7.29 mg/kg	
Ammonium nitrogen (Fresh	0.76 mg/kg	
Dry Matter (Fresh)	87.5 %	

5.2.1. Volumetric soil moisture content

The response of volumetric soil moisture content to drought and antitranspirants is presented on Table 5.12 and Figure 5.1.8. Drought significantly affected mean volumetric soil moisture content at 0 – 30 cm soil depth, while the antitranspirant effect was not significant. Volumetric soil moisture content was reduced from 30.15 % in the irrigated to 15.84 % in the droughted plots, while the antitranspirant did not cause any significant differences in the volumetric soil moisture contents of plots. However, there was a significant ($P < 0.001$) effect of time on all the treatments with the overall mean on each day being different and greater at the beginning than at the end of the experiment. There was no significant drought x antitranspirant interaction effect on volumetric soil moisture content, but the time x drought interaction effect was significant because the differences in volumetric soil moisture content between the irrigated and droughted treatments decreased with increasing DAS from 1 DAS to 10 DAS, but increased with increasing DAS from 10 DAS to 24 DAS and later declined at 28 DAS and increased with increasing DAS from then on. The time x antitranspirant interaction effect was not significant as antitranspirant application did not significantly change volumetric soil moisture content with time and time x drought x antitranspirant interaction effects were not significant. Two-way analysis of variance (Table 5.13) indicated that the drought treatment caused highly significant ($P < 0.001$) reductions in volumetric soil moisture content at all DAS, whereas the antitranspirant induced no significant effects on any of the DAS.

Table 5.12. Average volumetric soil moisture content (%) of plots at 0 - 30 cm depth under drought imposed at 18 and antitranspirant applied at 51 days after emergenceof sorghum grown in polytunnels in July – August, 2016 measured at 1, 10, 15, 22, 28 and 38 days after spraying (n = 32) ± Standard error of the differences of the mean (SED) (Polytunnel Expt.1) (Expt.7).

	Days after spraying						
Treatments	1	10	15	22	28	38	Mean
Irrigated	29.23	25.82	29.82	32.73	28.72	34.59	30.15
Droughted	19.47	15.34	15.81	16.25	13.48	14.71	15.84
Unsprayed	24.75	20.66	22.31	24.68	21.22	25.13	23.13
Sprayed	23.95	20.49	23.32	24.29	20.98	24.16	22.86
Irrigated unsprayed	29.69	26.39	29.55	32.44	28.94	35.66	30.44
Irrigated sprayed	28.76	25.24	30.09	33.01	28.50	33.52	29.85
Droughted unsprayed	19.81	14.94	15.07	16.93	13.50	14.61	15.81
Droughted sprayed	19.13	15.74	16.55	15.57	13.46	14.81	15.87
Mean	24.35	20.58	22.81	24.49	21.1	24.65	23.0
df							140
SED							2.671
CV %							11.6
							P values
Drought							< 0.001
Antitranspirant							0.495
Drought x antitranspirant							0.396
Time							< 0.001
Time x drought							< 0.001
Time x antitranspirant							0.668
Time x drought x antitranspirant							0.603

Table 5.13. Two-way analysis of variance on the volumetric soil moisture content (%) at 0 – 30 cm depth of plots in sorghum under drought imposed at 19 and antitranspirant applied at 51 days after emergence in sorghum grown in the polytunnels in July - August 2016 taken at 1, 10, 15, 22, 28 and 38 days after spraying. Data are P values and stratum standard errors (n = 32) ± Standard error of the differences of the mean (SED) (Polytunnel Expt. 1) (Expt.7).

	Days after spraying					
	1	10	15	22	28	38
P values						
Drought	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Antitranspirant	0.297	0.885	0.167	0.163	0.797	0.393
Drought x antitranspirant	0.868	0.416	0.514	0.22	0.834	0.307
SED	2.13	3.32	2	2.171	2.611	3.164
df	25	25	25	25	25	25

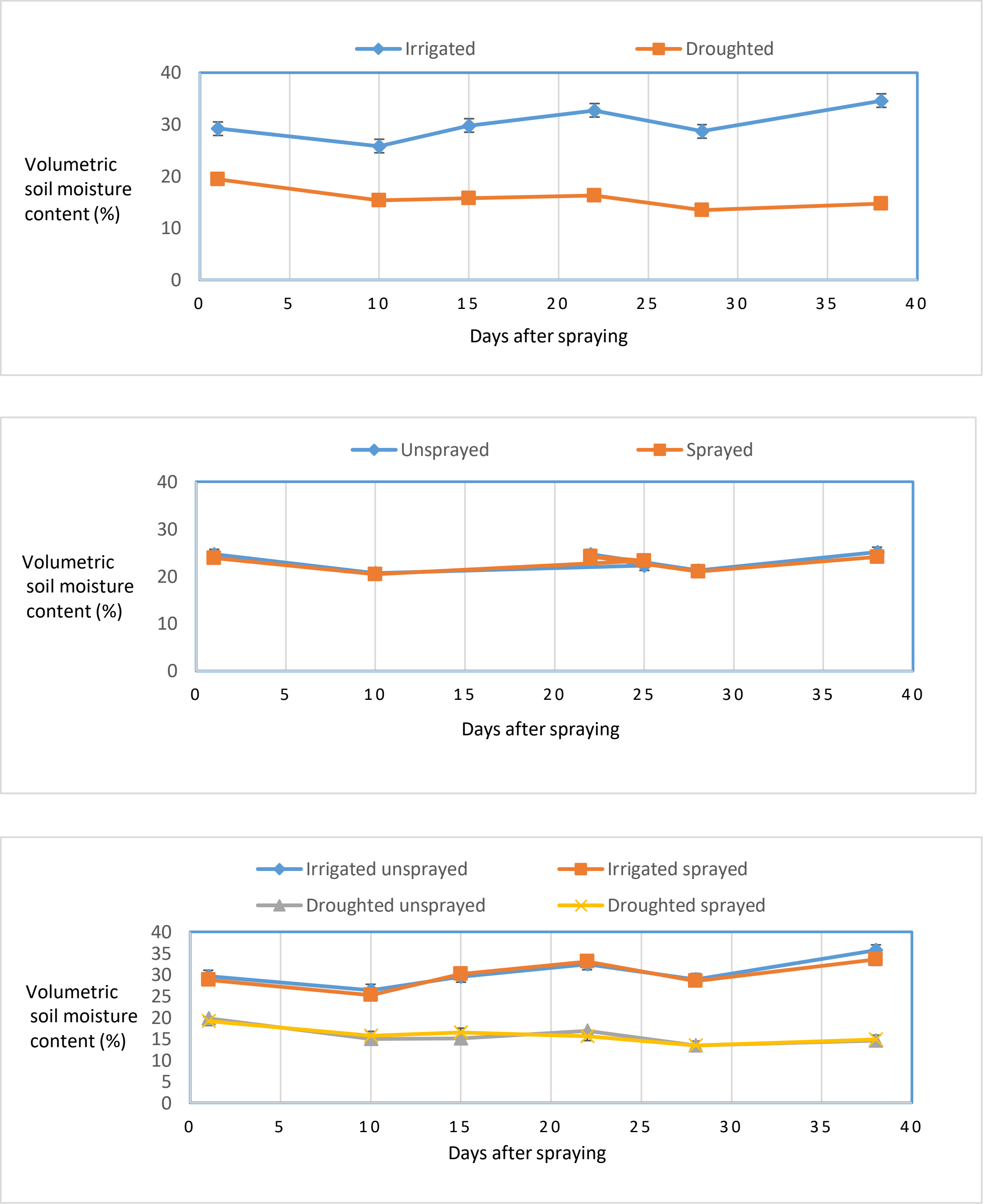

Figure 5.1.8. Response of volumetric soil moisture content (%) of plots at 0 – 30 cm depth to irrigation, drought and antitranspirants at 1, 10, 15, 22, 28 and 38 days after emergence grown in the glasshouse in July – August, 2016 (n = 32) Drought was imposed at 18 and antitranspirant applied at 51 days after emergence. (Drought, $P < 0.001$; antitranspirant, $P = 0.495$; drought x antitranspirant, $P = 0.396$). Error bars are SEM. (Polytunnel Expt.1) (Expt.7).

5.2.3. Adaxial stomatal conductance

Results of analysis of data on response of adaxial stomatal conductance to drought and antitranspirant application is presented on Table 5.14 and Figure 5.1.9.

There was no significant difference in adaxial stomatal conductance between droughted and irrigated treatments, but the antitranspirant caused significant differences in adaxial stomatal conductance between the unsprayed and sprayed plants. Drought application did not significantly affect adaxial stomatal conductance, however the antitranspirant significantly ($P = 0.001$) decreased mean adaxial stomatal conductance by 11.96 % in the sprayed compared with the unsprayed treatments. There was no significant drought x antitranspirant effect on adaxial stomatal conductance. But the effect of time was highly significant ($P < 0.001$) causing adaxial stomatal conductance to differ across the DAS and to be greater at the beginning than at the end of the experiment. The drought treatment did not have any significant interaction with time, so no significant interactions between irrigated and droughted plants occurred with time. However, the time x antitranspirant interaction effect was significant ($P = 0.010$) as the differences between unsprayed and sprayed treatments decreased with increasing DAS from 1 day after spraying to 31 DAS. The time x drought x antitranspirant interaction effects were not significant. Two way analysis of variance (Table 5.15) showed drought effect was significant on two occasions at 31 DAS (0.030) and 36 DAS ($P = 0.009$) whereas the antitranspirant showed significant effects earlier at 1 ($P < 0.001$) and 4 DAS ($P = 0.030$) and apparently sustained nearly significant effects at 12 ($P =0.063$) and 20 DAS ($P = 0.067$).

Table 5.14. Average adaxial stomatal conductance (mmols $m^{-2}s^{-1}$) of sorghum recorded on the flag leaf and one other leaf under drought imposed at 18 and antitranspirant applied at 51 days after emergence grown in the polytunnel in July – August, 2016 taken at 1, 4, 12, 20, 31 and 46 days after spraying (n = 32) ± standard error of the differences of the mean (SED) (Polytunnel Expt.1) (Expt.7).

	Days after spraying						
Treatments	1	4	12	20	31	46	Mean
Irrigated	293.6	258.8	173.3	131.0	68.5	92.3	169.6
Droughted	306.2	265.2	136.4	123.8	50.0	62.8	157.4
Unsprayed	321.6	282.5	166.6	134.2	57.6	80.9	173.9
Sprayed	278.2	241.5	143.1	120.6	60.8	74.2	153.1
Irrigated unsprayed	310.8	285.9	174.3	139.4	69.9	100.8	180.2
Irrigated sprayed	276.4	231.7	172.4	122.7	67.0	83.8	159.0
Droughted unsprayed	332.4	279.0	158.9	129.0	45.3	61.1	167.6
Droughted sprayed	280.0	251.3	113.9	118.6	54.6	64.6	147.2
Mean	299.9	262.0	154.9	127.4	59.2	77.6	163.5
df							139
SED							34.77
CV %							21.6
P values							
Drought							0.180
Antitranspirant							0.001
Drought x antitranspirant							0.950
Time							<0.001
Time x drought							0.163
Time x antitranspirant							0.010
Time x drought x antitranspirant							0.097

Table 5.15. Two-way analysis of variance on the adaxial stomatal conductance (mmols $m^{-2}s^{-1}$) in sorghum recorded on the flag leaf and one other leaf under drought imposed at 18 and antitranspirant applied at 51 days after emergence grown in the glasshouse in July - August 2016 taken at 1, 4, 12, 20, 31 and 46 days after spraying. (n = 32) Data are P values and stratum standard errors (Polytunnel Expt.1) (Expt.7).

	Days after spraying					
	1	4	12	20	31	46
P values						
Drought	0.662	0.724	0.060	0.197	0.030	0.009
Antitranspirant	< 0.001	0.030	0.063	0.067	0.450	0.632
Drought x antitranspirant	0.023	0.462	0.084	0.728	0.253	0.359
SED	34.73	5.32	36.86	30.21	24.83	18.49
df	24	25	25	25	25	25

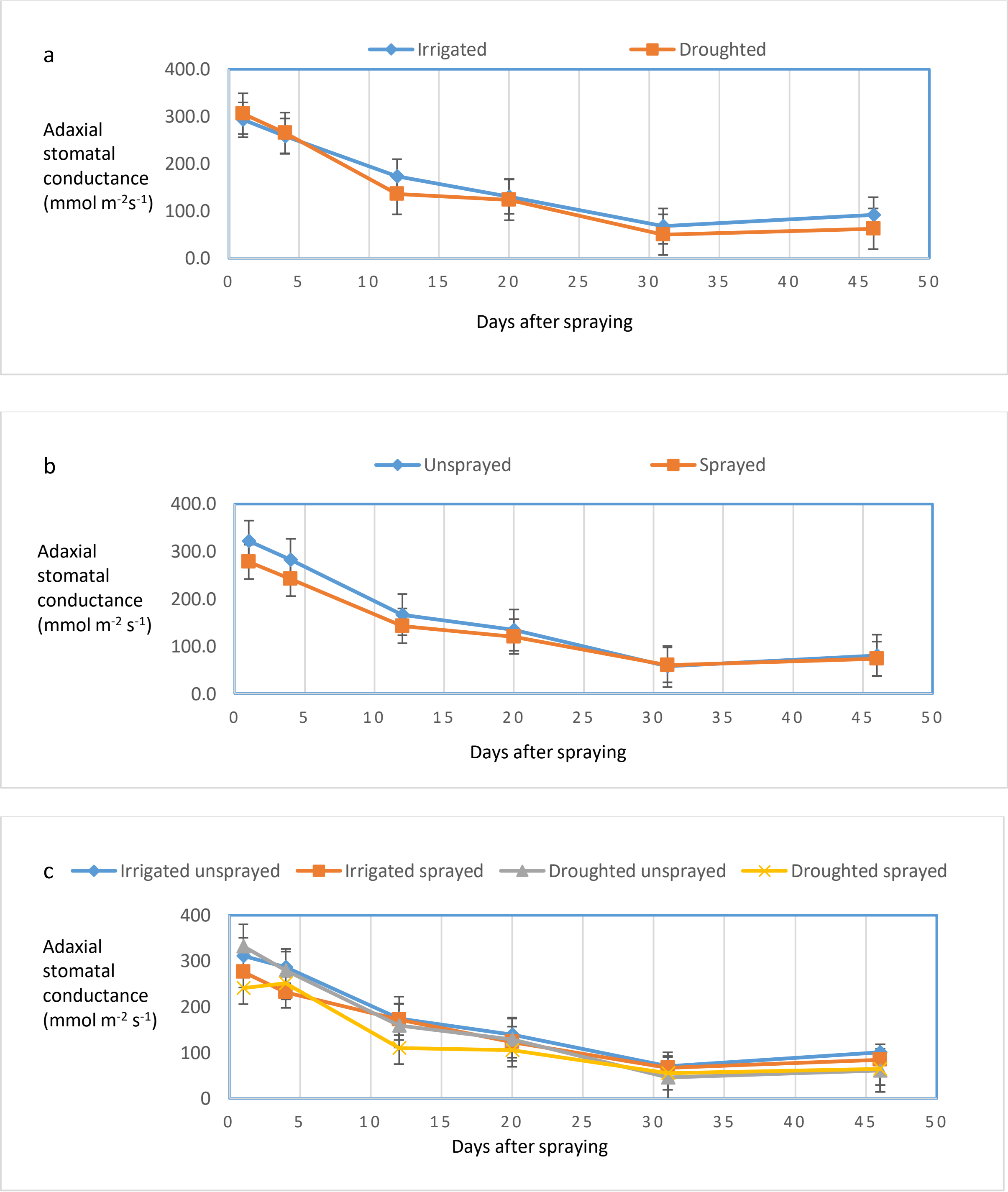

Figure 5.1.9. Response of adaxial stomatal conductance (mmols $m^{-2}s^{-1}$) to (a) drought (b) antitranspirant and (c) drought x antitranspirant interactions recorded on the flag leaf and one other leaf at 1, 4, 12, 20, 31 and 46 days after spraying in the polytunnel in July – August, 2016 (n = 32).Drought was imposed at 18 and antitranspirant applied at 51 days after emergence. (Drought, P = 0.180; antitranspirant, P = 0.001; drought x antitranspirant, P = 0.95). Error bars are SEM. (Polytunnel Expt.1) (Expt.7).

5.2.4. Abaxial stomatal conductance

Results of analysis of data on the effect of drought and antitranspirant application on abaxial stomatal conductance is presented on Table 5.16 and Figure 5.1.10.

There was no significant difference in abaxial stomatal conductance between droughted and irrigated treatments, as well as between the unsprayed and sprayed plants. Therefore, neither drought nor antitranspirant application significantly affect affected abaxial stomatal conductance. Similarly, there was no significant drought x antitranspirant effect on abaxial stomatal conductance. But the effect of time was highly significant ($P < 0.001$) leading to decreases in abaxial stomatal conductance across the DAS that were greater at the beginning than at the end of the measurement. However, the drought treatment showed highly significant ($P < 0.001$) interaction with time, for the reason that abaxial stomatal conductance increased with increasing DAS from 1 day after spraying to 4 DAS and later decreased with increasing DAS from 4 DAS to 46 DAS in the irrigated treatments, however it increased with increasing DAS from 1 day after spraying to 4 DAS and declined with increasing DAS from 4 DAS to 46 DAS in the droughted treatments. Thus, it was greater in the irrigated than in the droughted at 1 day after spraying, 12 DAS, 20 DAS and 46 DAS, and lower in the irrigated than the droughted 4 DAS and 31 DAS. Abaxial stomatal conductance was not significantly affected by the time x antitranspirant and the time x drought x antitranspirant interaction effects. Two-way analysis of variance (Table 5.17) showed significant reductions in abaxial stomatal conductance at 1 DAS ($P = 0.022$), 12 DAS ($P < 0.001$) and 20 DAS (0.027), but significant and highly significant increases at 4 DAS ($P = 0.037$) and 31 DAS ($P < 0.001$) respectively.

Table 5.16. Average abaxial stomatal conductance (mmols $m^{-2}s^{-1}$) of sorghum recorded on the flag leaf and one other leaf under drought imposed at 18 and antitranspirant applied at 51 days after emergence grown in the polytunnel in July – August, 2016 taken at 1, 4, 12, 20, 31 and 46 days after spraying (n = 32) ± standard error of the differences of the mean (SED) (Polytunnel Expt.1) (Expt.7).

	Days after spraying						
Treatments	1	4	12	20	31	46	Mean
Irrigated	404.7	373.5	261.4	213.8	141.4	174.7	261.6
Droughted	375.5	422.2	223.4	165.1	214.4	162.8	260.6
Unsprayed	400.8	405.8	246.9	195	180.9	175	267.4
Sprayed	379.4	389.9	237.9	183.9	174.9	162.5	254.7
Irrigated unsprayed	408.7	359.2	265.1	227.4	140.5	185.7	264.4
Irrigated sprayed	400.6	387.9	257.6	200.2	142.4	163.6	258.7
Droughted unsprayed	392.8	452.4	228.6	162.6	221.4	164.2	270.4
Droughted sprayed	358.2	391.9	218.2	167.5	207.4	161.3	250.8
Mean	390.1	397.8	242.4	189.5	177.9	168.7	261.1
df							3
SED							17.75
CV %							6.8
							P values
Drought							0.820
Antitranspirant							0.110
Drought x antitranspirant							0.370
Time							< 0.001
Time x drought							< 0.001
Time x antitranspirant							0.970
Time x drought x antitranspirant							0.195

Table 5.17. Two-way analysis of variance on the abaxial stomatal conductance (mmols $m^{-2}s^{-1}$) in sorghum recorded on the flag leaf and one other leaf under drought imposed at 18 and antitranspirant applied at 51 days after emergence grown in the glasshouse in July - August 2016 taken at 1, 4, 12, 20, 31 and 46 days after spraying. (n = 32) Data are P values and stratum standard errors (Polytunnel Expt.1) (Expt.7).

	Days after spraying					
	1	4	12	20	31	46
P values						
Drought	0.022	0.037	< 0.001	0.027	< 0.001	0.167
Antitranspirant	0.057	0.185	0.548	0.595	0.548	0.147
Drought x antitranspirant	0.137	0.007	0.432	0.448	0.432	0.262
SED	50.27	27.18	27.98	58.8	27.98	23.58
df	25	25	25	25	25	25

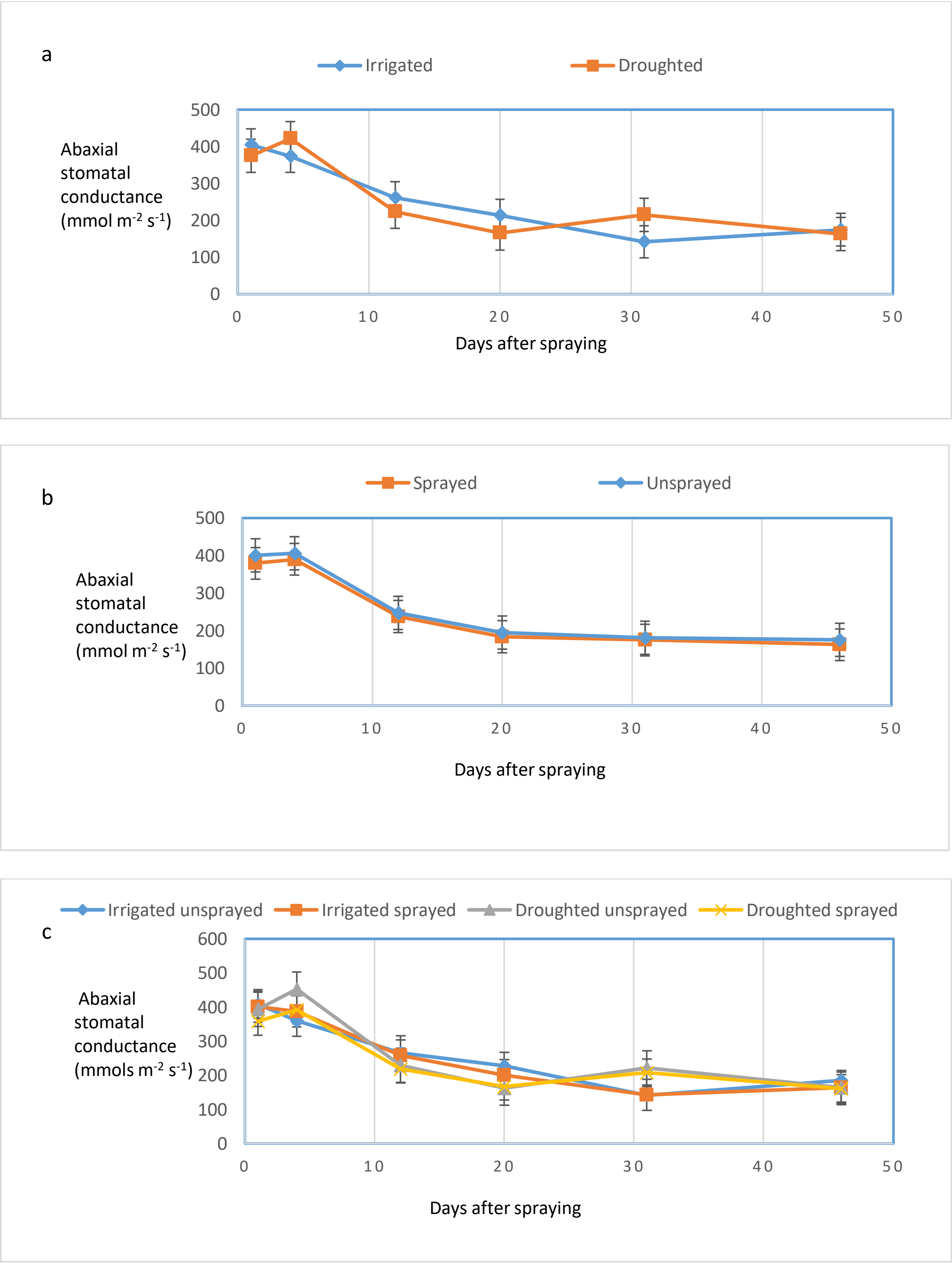

Figure 5.1.10. Response of abaxial stomatal conductance (mmols $m^{-2}s^{-1}$) to (a) drought (b) antitranspirant and (c) drought x antitranspirant interaction recorded on the flag leaf and one other leaf measured at 1, 4, 12, 20, 31 and 46 days after spraying grown in the polytunnel in July - August 2016 (n = 32). Drought was imposed at 18 and antitranspirant applied at 51 days after emergence. (Drought, P = 0.820; antitranspirant, P = 0.110; drought x antitranspirant, P = 0.370). Error bars are SEM. (Polytunnel Expt.1) (Expt.7).

5.2.5. Total stomatal conductance

The results of analysis of data on the effect of drought and antitranspirant application on total stomatal conductance is presented on Table 5.18 and Figure 5.1.11.

There was no significant difference in total stomatal conductance between droughted and irrigated treatments, but significant differences occurred between the unsprayed and sprayed plants. Therefore, the drought treatment did not have significant effect whereas the antitranspirant application had significant ($P = 0.001$) effect on total stomatal conductance. The drought x antitranspirant interaction effect on total stomatal conductance was not significant, but the effect of time was highly significant ($P < 0.001$) as observed in declining total stomatal conductance across the DAS that were greater at the beginning than at the end of the experiment. However, the drought treatment showed highly significant ($P < 0.001$) interaction with time; the total stomatal conductance was greater in the irrigated than the droughted at 1 day after spraying, 12 DAS and 20 DAS, while it was lower in the irrigated than the droughted at 4 DAS and 31 DAS. The time x antitranspirant and the time x drought x antitranspirant interaction effect on total stomatal conductance were not significant. From Table 5.19, the two-way analysis of variance showed significant decreases owing to drought treatment at 12 DAS ($P < 0.001$), and 46 DAS ($P = 0.010$) and significant increases at 4 DAS ($P = 0.05$) 20 DAS ($P = 0.023$) and 31 DAS ($P = 0.010$), whereas the antitranspirant effect was significant on three consecutive occasions at the beginning of the measurements leading to significant reductions in total stomatal conductance at 1DAS ($P = 0.009$), 4 DAS ($P = 0.007$) and 12 DAS ($P = 0.045$).

Table 5.18. Average total stomatal conductance (mmols $m^{-2}s^{-1}$) of sorghum recorded on the flag leaf and one other leaf under drought imposed at 18 and antitranspirant applied at 51 days after emergence grown in the polytunnel in July – August, 2016 taken at 1, 4, 12, 20, 31 and 46 days after spraying (n = 32) ± standard error of the differences of the mean (SED) (Polytunnel Expt.1) (Expt.7).

	Days after spraying						
Treatments	1	4	12	20	31	46	Mean
Irrigated	350.9	316.2	217.4	172.4	105.0	133.5	215.9
Droughted	339.0	343.7	179.9	144.4	132.2	112.8	208.7
Unsprayed	362.9	344.1	206.7	164.6	119.3	128.0	220.9
Sprayed	326.9	315.7	190.5	152.3	117.9	118.3	203.6
Irrigated unsprayed	363.2	322.6	219.7	183.4	105.2	143.3	222.9
Irrigated sprayed	338.5	309.8	215.0	161.5	104.7	123.7	208.9
Droughted unsprayed	362.6	365.7	193.8	145.8	133.4	112.7	219
Droughted sprayed	315.4	321.6	166.8	143.1	131.0	113.0	198.3
Mean	344.9	329.9	198.6	158.4	118.6	123.1	212.3
df							3
SED							10.990
CV %							5.2
							P values
Drought							0.300
Antitranspirant							0.001
Drought x antitranspirant							0.490
Time							< 0.001
Time x drought							< 0.001
Time x antitranspirant							0.319
Time x drought x antitranspirant							0.443

Table 5.19. Two-way analysis of variance on the total stomatal conductance (mmols $m^{-2}s^{-1}$) in sorghum recorded on the flag leaf and one other leaf under drought imposed at 18 and antitranspirant applied at 51 days after emergence grown in the glasshouse in July - August 2016 taken at 1, 4, 12, 20, 31 and 46 days after spraying. (n = 32) Data are P values and stratum standard errors (Polytunnel Expt.1) (Expt.7).

	Days after spraying					
	1	4	12	20	31	46
P values						
Drought	0.214	0.050	< 0.001	0.023	< 0.001	0.010
Antitranspirant	0.009	0.007	0.045	0.234	0.821	0.210
Drought x antitranspirant	0.327	0.087	0.138	0.640	0.887	0.196
SED	43.31	31.83	23.03	36.38	17.73	21.1
df	24	25	25	25	25	25

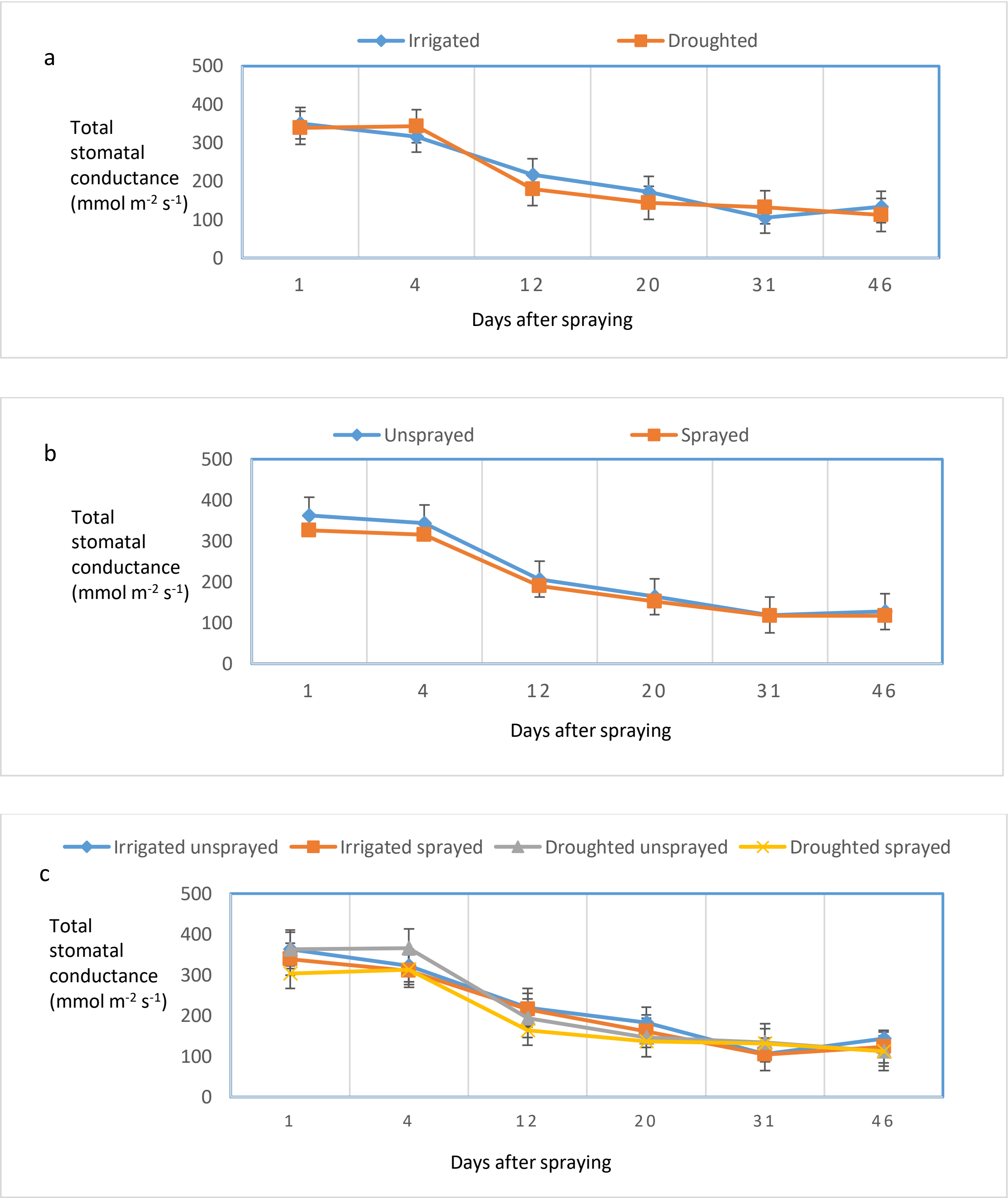

Figure 5.1.11. Response of total stomatal conductance (mmols $m^{-2}s^{-1}$) recorded on the flag leaf and one other leaf to (a) drought (b) antitranspirant and (c) drought x antitranspirant measured at 1, 4, 12, 20, 31 and 46 days after spraying grown in polytunnel in July – August, 2016 (n = 32). Drought was imposed at 18 and antitranspirant applied at 51 days after emergence. (Drought, P = 0.300; antitranspirant, P = 0.001; drought x antitranspirant, P = 0.490). Error bars are SEM. (Polytunnel Expt.1) (Expt.7).

5.2.6. Leaf temperature

The results of analysis of data on leaf temperature measurements after drought and antitranspirant applications are shown in Table 5.20 and Figure 5.1.12.

There was no significant difference in leaf temperature between the irrigated and droughted and the unsprayed and sprayed treatments, therefore drought and antitranspirant applications had no significant effect on leaf temperature. However, there was a significant effect of time (P < 0.001) as the mean leaf temperature over DAS were different, and was lower at the beginning than at the end of the experiment. The drought x antitranspirant as well as the time x drought, time antitranspirant and time x drought x antitranspirant interaction effects were not significant.

Table 5.20. Average leaf temperature (°C) of sorghum recorded on the flag leaf under drought imposed at 18 and antitranspirant applied at 51 days after emergence grown in the polytunnel in July – August, 2016 taken at 8, 12 and 20 days after spraying (n = 8) ± standard error of the differences of the mean (SED) (Polytunnel Expt.1) (Expt.7).

	Days after spraying			
Treatments	8	12	20	Mean
Irrigated	22.22	21.62	28.07	23.97
Droughted	22.56	22.01	28.39	24.32
Unsprayed	22.62	21.55	28.54	24.24
Sprayed	22.16	22.07	27.91	24.05
Irrigated unsprayed	22.76	21.16	28.07	24.00
Irrigated sprayed	21.69	22.07	28.06	23.94
Droughted unsprayed	22.47	21.94	29.01	24.47
Droughted sprayed	22.64	22.07	27.76	24.16
Mean	22.39	21.81	28.23	24.14
df				19
SED				0.452
CV %				1.9
				P values
Drought				0.490
Antitranspirant				0.340
Antitranspirants x drought				0.510
Time				< 0.001
Time x drought				0.998
Time x antitranspirant				0.651
Time x antitranspirant x drought				0.620

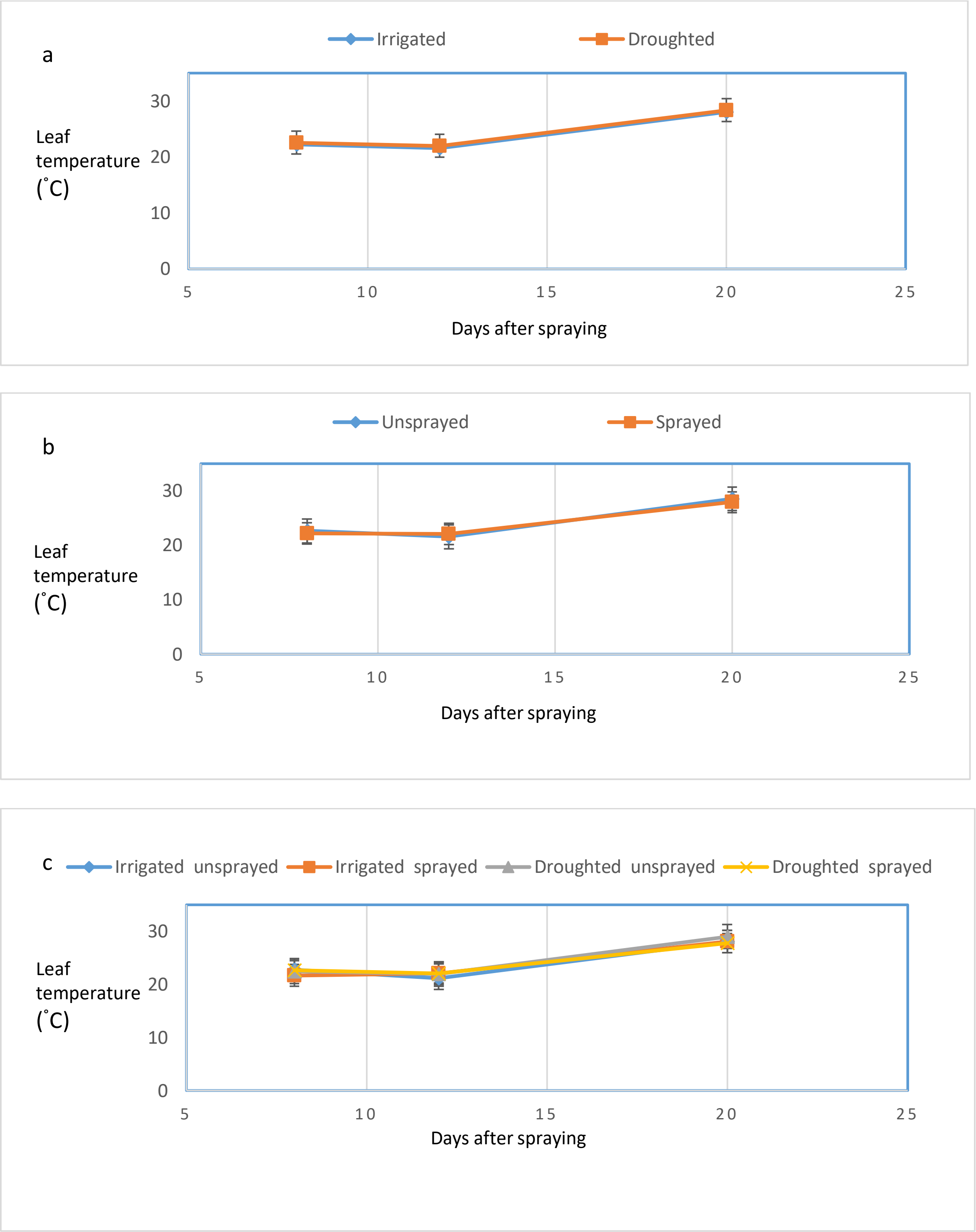

Figure 5.1.12. Response of leaf temperature (˚C) recorded on the flag leaf to (a) drought (b) antitranspirant and (c) drought x antitranspirant interaction applied at 51 days after emergence measured at 8, 12 and 20 days after emergence grown in the polytunnel in July – August, 2016 (n = 16) (Drought, P = 0.490; antitranspirant, P = 0.340; drought x antitranspirant, P = 0.510). Error bars are SEM. (Polytunnel Expt.1) (Expt.7).

5.2.7. Leaf area

The results of analysis of data on response of leaf area to drought and antitranspirant applications are shown in Table 5.21 and Figure 5.1.13.

There was no significant difference in leaf area between the irrigated and droughted and between the unsprayed and sprayed treatments. Drought and antitranspirant did not have any significant effects on leaf area and drought x antitranspirant interaction effect on leaf area was not significant. Similarly, leaf area remained not significantly affected by time, and the time x drought, time x antitranspirant as well as the time x drought x antitranspirant interaction effects showed no significant effects on leaf area.

Table 5.21. Average leaf area (cm^2) of sorghum recorded on the flag leaf under drought imposed at 18 and antitranspirant applied at 51 days after emergence grown in the polytunnel in July – August, 2016 taken at 2, 12 and 18 days after spraying (n = 8) ± standard error of the differences of the mean (SED) (Polytunnel Expt.1) (Expt.7).

Treatments	Days after spraying			
	2	12	18	Mean
Irrigated	302.3	327.8	300.4	310.1
Droughted	275.3	277.1	293.3	281.9
Unsprayed	282.7	298.0	298.3	293.0
Sprayed	294.8	306.9	295.4	299.0
Irrigated unsprayed	294.5	314.8	322.2	310.5
Irrigated sprayed	310.0	340.9	278.6	309.8
Droughted unsprayed	270.9	281.3	274.3	275.5
Droughted sprayed	279.6	273.0	312.2	288.3
Mean	288.8	302.5	296.8	296.0
df				56
SED				65.51
CV %				22.1
				P values
Drought				0.082
Antitranspirant				0.561
Drought x antitranspirant				0.520
Time				0.645
Time x drought				0.393
Time x antitranspirant				0.835
Time x drought x antitranspirant				0.199

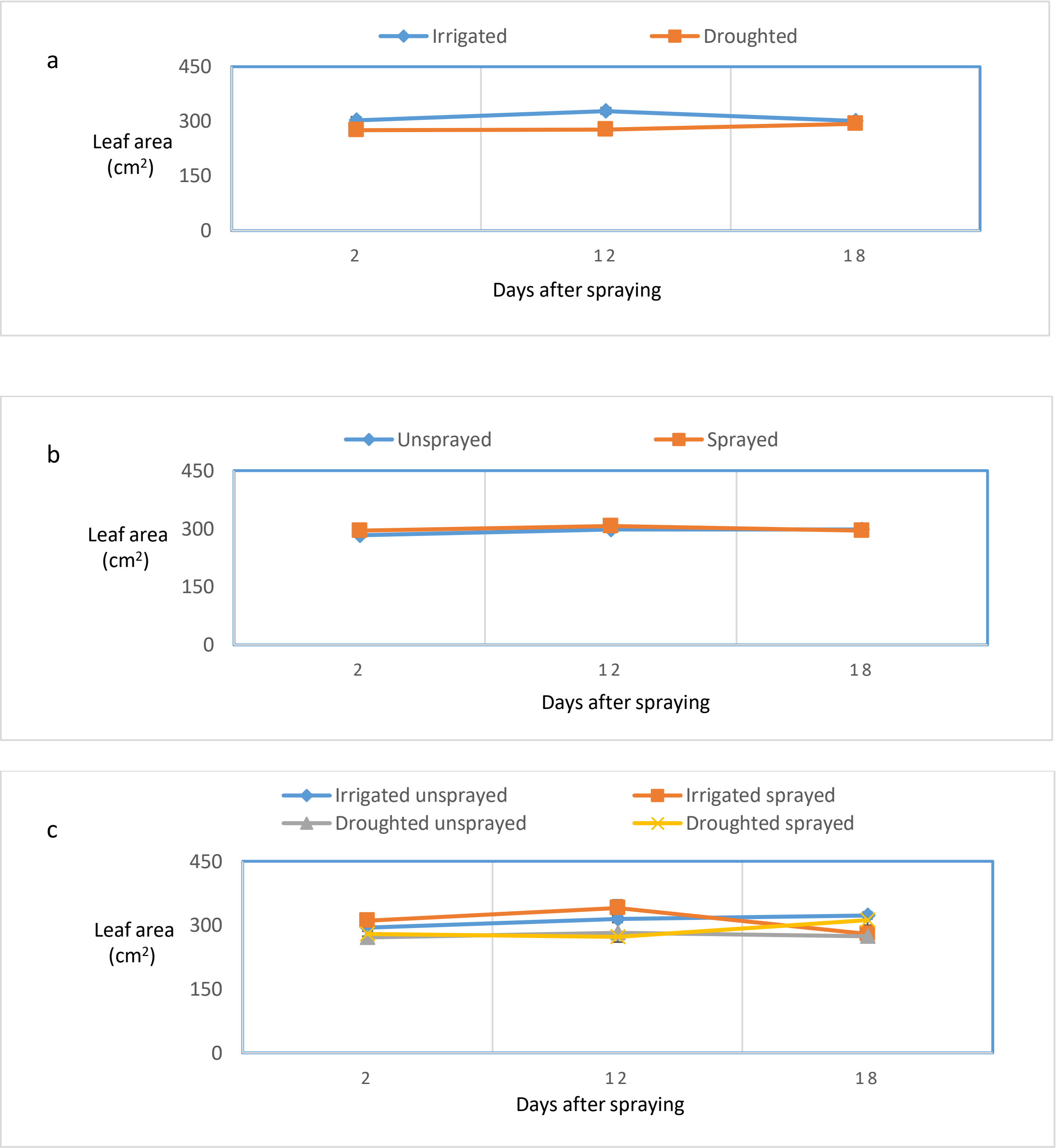

Figure 5.1.13. Response of leaf area (cm^2) recorded on the flag leaf to (a) drought (b) antitranspirant and (c) drought x antitranspirant at 2, 12 and 18 days after emergence grown in the polytunnel in July – August, 2016 (n = 10) (drought, $P < 0.001$; antitranspirant, $P = 0.495$; drought x antitranspirant, $P = 0.396$). Error bars are SEM. (Polytunnel Expt.1) (Expt.7).

5.2.8. Green area index

The response of green area index to drought and antitranspirant applications and their interaction is presented on Table 5.22 and Figure 5.1.14.

There were significant differences in green area index between the irrigated and droughted and between the unsprayed and sprayed treatments.

The effect of the drought treatment on green area index was highly significant ($P < 0.001$) leading to a reduction of 52.00 % in the droughted compared with the irrigated treatment.

Also the antitranspirant application also induced significant (P = 0.022) effects on green area index causing an 11.39 % increase in the sprayed over the unsprayed treatments. The drought x antitranspirant interaction effect was not significant. There was a highly significant effect of time (P < 0.001) as the mean green area index increased over DAS and was lower at the beginning than at the end of the measurements. The time x drought interaction effect on green area index was highly significant (P < 0.001) as there were significant reductions in the droughted compared with the irrigated at 15 DAS (P < 0.001), 20 DAS (P < 0.001) and 31 DAS (P < 0.001). And because with increasing DAS the differences in green area index between the irrigated and droughted treatments increased. In addition, the green area index increased in the irrigated but decreased in the droughted treatments with increasing DAS. Similarly, the time x antitranspirant interaction effect was significant (P = 0.012) showing increasing differences between the irrigated and droughted treatments as DAS increases and becoming significant at 31 DAS (P = 0.031). And while the green area index in the unsprayed was greater than in the antitranspirant treated plants at 20 DAS, the antitranspirant treated plants gave greater green area index than the unsprayed at 31 DAS. The time x drought x antitranspirant interaction effect was significant (P = 0.054) as green area index was highly significantly influenced due to drought x antitranspirant effect at 15 DAS (P < 0.001) where the antitranspirant spray decreased green area index in the irrigated but increased it in the droughted treatments. In addition at 20 DAS the antitranspirant decreased green area index in the irrigated but increased it in the droughted and at 31 DAS the antitranspirant increased green area index in both the irrigated and droughted treatments. Two-way analysis of variance on DAS (Table 5.23) indicated significant (P < 0.001) effects of drought in reducing green area index at 15 DAS, 20 DAS and 31 DAS by 26.19 %, 23.35 % and 75.17 % respectively, while the antitranspirant significantly (P = 0.013) increased green area index at 31 DAS by 31.18 %.

Table 5.22. Average green area index of sorghum recorded on the flag leaf under drought imposed at 18 and antitranspirant applied at 51 days after emergence grown in the polytunnel in July – August, 2016 taken at 15, 20 and 31 days after spraying (n = 8) ± standard error of the differences of the mean (SED) (Polytunnel Expt.1) (Expt.7).

	Days after spraying			
Treatments	15	20	31	Mean
Irrigated	0.504	0.501	1.176	0.727
Droughted	0.372	0.384	0.292	0.349
Unsprayed	0.438	0.454	0.635	0.509
Sprayed	0.438	0.430	0.833	0.567
Irrigated unsprayed	0.535	0.516	1.018	0.690
Irrigated sprayed	0.472	0.485	1.335	0.764
Droughted unsprayed	0.341	0.392	0.252	0.329
Droughted sprayed	0.404	0.375	0.331	0.370
Mean	0.438	0.442	0.734	0.538
df				56
SED				0.134
CV %				24.9
				P values
Drought				< 0.001
Antitranspirant				0.022
Drought x antitranspirant				0.490
Time				< 0.001
Time x drought				< 0.001
Time x antitranspirant				0.012
Time x drought x antitranspirant				0.054

Table 5.23. Two-way analysis of variance on the green area index of sorghum recorded on the flag leaf under drought imposed at 18 and antitranspirant applied at 51 days after emergence grown in the glasshouse in July - August 2016 taken at 15, 20 and 31 days after spraying. (n = 8) Data are P values and stratum standard errors (Polytunnel Expt.1) (Expt.7).

	Days after spraying		
	15	20	31
P values			
Drought	< 0.001	< 0.001	< 0.001
Antitranspirant	1.000	0.284	0.013
Drought x antitranspirant	< 0.001	0.760	0.121
SED			
Drought	0.014	0.022	0.074
Antitranspirant	0.014	0.022	0.074
Drought x antitranspirant	0.020	0.032	0.105

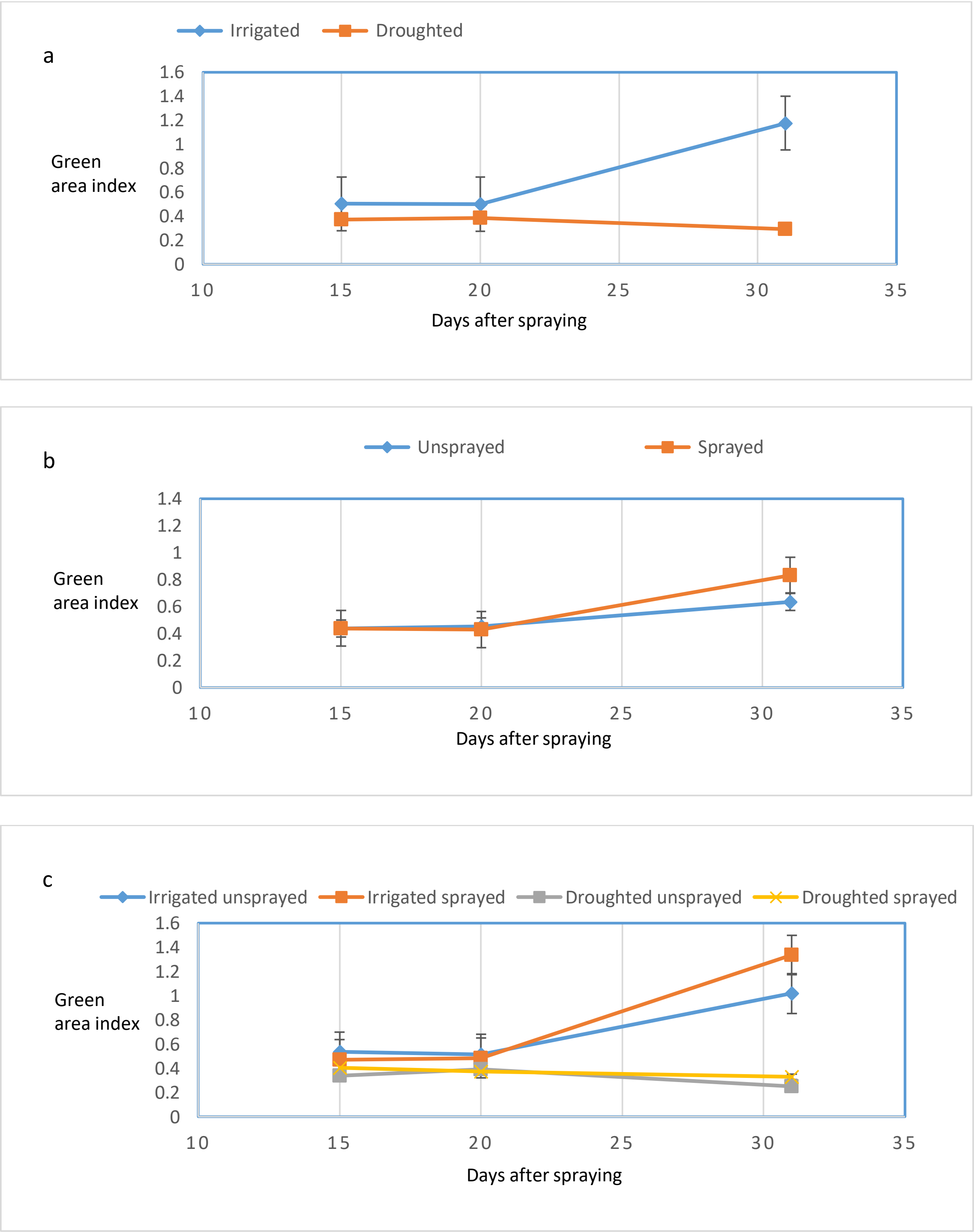

Figure 5.1.14. Response of green area index to (a) drought (b) antitranspirant and (c) drought x antitranspirant at 15, 20 and 31 days after spraying grown in the polytunnel in 2016 (n = 32) (drought, $P < 0.001$; antitranspirant, $P = 0.022$; drought x antitranspirant, $P = 0.51$). Error bars are SEM. (Polytunnel Expt.1) (Expt.7).

5.2.9. Yield and yield components

Table 5.24 shows results of data analysis on the effect of drought and antitranspirant and their interactions on the grain yield and yield components of sorghum grown in polytunnels. There were no significant differences between treatments in yield and yield components in this experiment. However, there was a nearly significant drought x antitranspirant interaction effect on weight per grain as the antitranspirant decreased weight per grain in the irrigated plants by 9.44 % in the sprayed compared with the unsprayed, but increased it in the droughted plants by 2.90 % in the sprayed compared with the unsprayed.

Stalk weight was not significantly different in irrigated and droughted and also in the unsprayed and sprayed treatments. So there were no significant effects of drought and antitranspirant on the stalk weight. The drought x antitranspirant interaction effect on stalk weight was also not significant. There was no significant difference in biomass between the irrigated and droughted treatments and between the unsprayed and sprayed treatments, thus drought and antitranspirant had no significant effect on biomass. The drought x antitranspirant interaction effect on biomass was not significant. Similarly, drought induced no significant differences in the harvest index between the irrigated and droughted treatments and the antitranspirant caused no significant differences in harvest index between the unsprayed and sprayed treatments. Consequently, there was no significant effect of drought and antitranspirant on the harvest index. The drought x antitranspirant interaction effect on harvest index was not significant.

Table 5.24. Average grain yield and yield components under irrigation, drought and antitranspirant of sorghum grown in the polytunnel from July – August, 2016. Data are means (n = 32) (Polytunnel Expt.1) (Expt.7).

Treatments	Grain yield (g)	Grain number per plant	Weight per grain (mg)	Stalk weight (g)	Biomass (gm^{-2})	Harvest index (%)
Irrigated	515	14949	34.83	891	1417	38.2
Droughted	500	15073	33.60	761	1262	40.7
Unsprayed	497	14446	34.84	779	1286	40.2
Sprayed	519	15575	33.59	874	1393	38.7
Irrigated unsprayed	507	13980	36.56	878	1405	38.6
Irrigated sprayed	524	15918	33.11	905	1429	37.9
Droughted unsprayed	487	14913	33.12	680	1167	41.8
Droughted sprayed	514	15233	34.08	842	1356	39.5
Mean	508	15011	34.22	826	1339	39.5
df	20	15	15	19	19	19
SED	89.6	2749.4	2.544	238.8	251.1	7.27
CV %	17.6	18.3	7.4	28.9	18.8	18.4
P values						
Drought	0.623	0.902	0.222	0.507	0.407	0.633
Antitranspirant	0.496	0.509	0.228	0.275	0.245	0.571
Drought x antitranspirant	0.887	0.633	0.060	0.433	0.365	0.774

Polytunnel Expt. 2 (Expt.8).

5.3. Materials and methods

5.3.1. Field location and conditions

The polytunnel experiment (Polytunnel Expt. 2) was carried out at flat Nook field located at Harper Adams University, Shropshire, UK (52° 46 'N, 2° 25 'W). Previous crop in the field in 2015 – 16 was spring barley. Soil analysis carried out on a sample of the soil taken at 0 – 30 cm depth in 2016 showed that the soil had a pH = 6.4, available nitrogen = 30.2 kg/ha, phosphorus = 55.8 mg/l and potassium = 114 mg/l. The field was ploughed and harrowed, and rain-out shelters erected for four weeks over the plough-harrowed field prior to sowing. The soil texture in Flat Nook is sandy loam according to Toogod (1958). At a depth of 20 cm, it contains 75.8 % sand, 20.8 % silt and 3.4 % clay with a bulk density of 1.74 g cm^{-3}. At the 40 cm depth the percentage of sand was 78.9 %, and 71.2 % and 72.1 % for the 60 and 80 cm depths respectively, while the silt percentages was ~20 %. Clay concentrations were 6.4 % and 5.4 % at the 60 cm and 80 cm depths respectively. Whereas bulk density were 1.76, 1.78 and 1.84 g/cm^{-3} at 20 cm, 40 cm and 60cm depths respectively. According to Weerasinghe, *et al*. (2016), the field capacity of Flat Nook at a depth of 80 cm was determined to be 160 mm using neutron probe (Institute of Hydrology Neutron Probe System, Wallingford, UK) and the permanent wilting point at 80 cm was 62 mm (Hall, 1977), thus the available soil water capacity was 98 mm.

However the following modifications were carried out in the current experiment in comparison with Ploytunnel Expt. 1.

1. In this experiment, seeds were not sown directly, but sorghum seedlings grown in the glasshouse in 7 cm pots filled with 50 g of compost and watered continuously until 14 DAE and were transplanted into field soil in the polytunnel.

2. The polytunnels were modified to facilitate better air circulation and decrease condensation compared with Polytunnel Expt.1 (Figures 5.3.1 to 5.3.4).

5.3.2. Experimental design and layout

The experimental design and layout consists of four rain-out shelters in total allocated to four blocks giving one per block. Within each polytunnel/block there were three plots assigned as: irrigated, droughted sprayed and droughted unsprayed. The irrigated plots were separated from the droughted plots by a distance of 1 m. The plots were separated by a distance of 50 cm from each end and the sides of the polytunnels. For ease of delivering water through trickle tapes the irrigated plots were located near the entrance of the polytunnels, the unsprayed and sprayed plots were randomized within each block. Thus, there were a total of four blocks and 12 plots (Figure. 5.3.5).

5.3.3. Treatments

Drought and antitranspirant sprays were the two factors each at two levels. The two levels of drought were ‘drought’ and ‘irrigated’ while the two levels of antitranspirant sprays were ‘sprayed’ and ‘unsprayed’. The ‘watered’ plots were fully irrigated from the day of transplanting which corresponds with GS 3.0 BBCH (AgVita, 2008) whereas the droughted plots were not watered throughout the period of the experiment. Drought imposition was considered to have been effectively commenced from the time of transplanting as at GS 3.0. BBCH (AgVita, 2008). At 64 days after emergence GrowHOW number seven® fertilizer NPK, N =17 %, P_2O_5 = 17 %, K_2O = 17 % was applied at rate of ~10 g per plant to all plants in the experiment, and immediately after application, the droughted plants were watered with 500 ml of water per plant to dissolve the fertilizer. The irrigated plants were watered at the rate of 1.4 L/min using the trickle tapes.

Some significant dates and activities carried out during the experiment are shown on Table 5.27. Skeleton ANOVA for each of the measurements are presented on the range of tables from Table 5.28 to 5.36.

5.3.4. Antitranspirant application

Antitranspirant Vapor Gard at a concentration of 0.5 ml dissolved in 100 ml of water was applied at GS 5.1. BBCH (AgVita, 2008), corresponding to 61 days after emergence using a lunch box sprayer at the rate of 200 l/ha at a speed of 600 km/h. Only four plots designated as ‘sprayed’ were treated with the antitranspirant.

5.3.5. Plant material

The sorghum variety used in this experiment was BirdGO Grain Sorghum (Bright Seeds, UK).

Table 5.25. Significant dates and activities and during Polytunnel Experiment 2 (Expt.8) from May to October, 2017

Dates	Activity
May 30	Sowing
June 13	Emergence
June 29	Transplanting to the polytunnels from glasshouse
July 7	Moisture sensors installed
August 1	Antitranspirant spray
August 3	Fertilizer application
October 23	Harvest

5.3.6. Measurements

5.3.6.1. Volumetric soil moisture content

The volumetric soil moisture content was measured using TRIME-PICO IPH/T3 (IMKO, GmbH Ettlingen, Germany) moisture sensor, a measurement device for continuous non-destructive determination of volumetric soil moisture content with a probe of 22 cm length. Access tubes for soil moisture data collection were inserted into the soil at a depth of 60 cm on eight plots, two per polytunnel out of which four were placed on the irrigated plots, two on the droughted sprayed and two on the droughted unsprayed. Volumetric soil moisture content readings were taken at depths of 20, 40 and 60 cm and recorded.

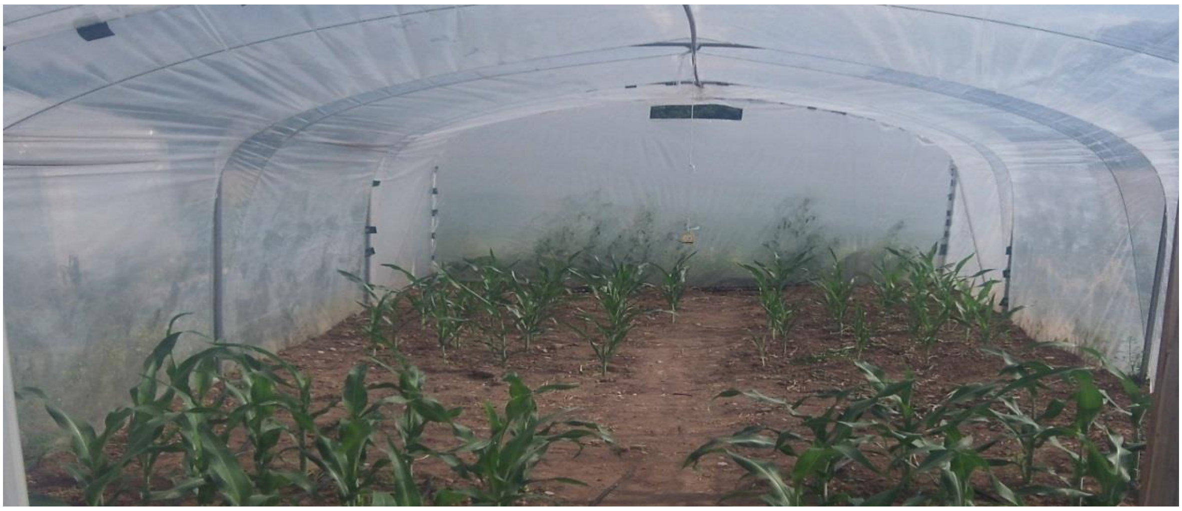

Figure 5.3.1. Sorghum growing inside a polytunnel which has been modified by creating a vent at rear end. Showing the vent at the rear. Polytunnel Expt. 2 (Expt.8).

Figure 5.3.2. Sorghum growing on four plots within the same polytunnel at 41 days after emergence. Showing four different plots. Polytunnel Expt. 2 (Expt.8).

Figure 5.3.3. Sorghum growing on irrigated and droughted plots within the same polytunnel at 49 days after emergence. Showing the stage at which spraying with antitranspirant was carried out. Polytunnel Expt. 2 (Expt.8).

Figure 5.3.4. Sorghum growing on irrigated and droughted plots within the same polytunnel at 79 days after emergence. Showing differences in growth between irrigated and droughted plots. Polytunnel Expt. 2 (Expt.8).

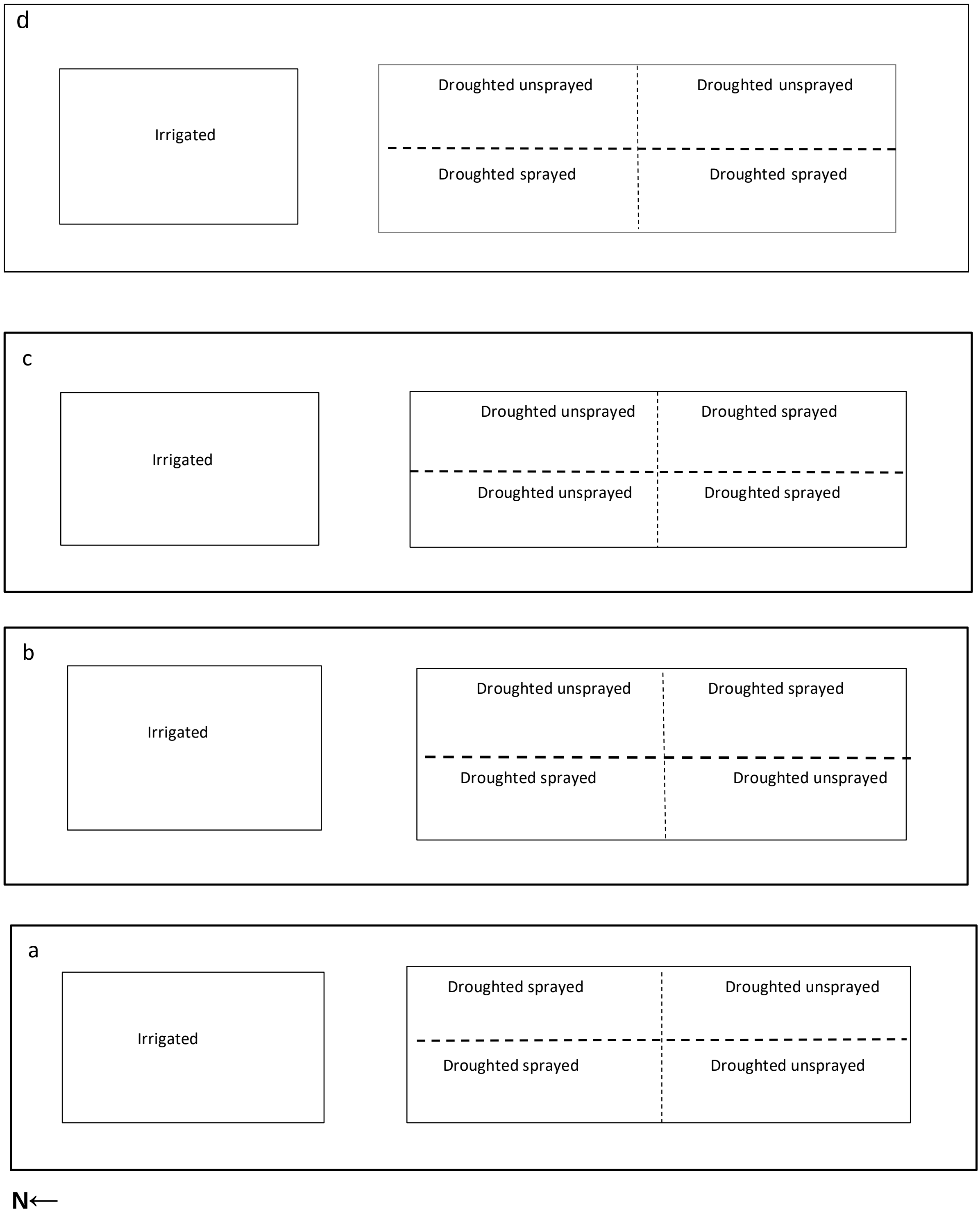

Figure 5.3.5. Layout of polytunnels a, b, c and d serving as blocks showing locations/plots allocated to irrigated, droughted unsprayed and droughted sprayed treatments. Polytunnel Expt. 2 (Expt.8).

Table 5.26. Skeleton ANOVA VWC of the measurements (Polytunnel Expt.2) (Expt.8)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x subject stratum	
Treatments	1
Depths	2
Treatments x depths	2
Residual	15
Blocks x subject x time stratum	
Time	9
Time x treatments	9
Time x depths	18
Time x treatments x depths	18
Residual	162
Total	239

*VWC = Volumetric soil moisture content

Table 5.27. Skeleton ANOVA of the stomatal conductance measurements (Polytunnel Expt.2) (Expt.8)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x subject stratum	
Treatments	2
Residual	6
Blocks x subject x time stratum	
Time	3
Time x treatments	6
Residual	27
Total	47

Table 5.28. Skeleton ANOVA of the leaf temperature measurements (Polytunnel Expt.2) (Expt.8)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x subject stratum	
Treatments	2
Residual	6
Blocks x subject x time stratum	
Time	3
Time x treatments	6
Residual	27
Total	47

Table 5.29. Skeleton ANOVA of the leaf area measurements (Polytunnel Expt.2) (Expt.8)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x subject stratum	
Treatments	2
Residual	6
Blocks x subject x time stratum	
Time	2
Time x treatments	4
Residual	18
Total	35

Table 5.30. Skeleton ANOVA of the green area index measurements (Polytunnel Expt.2) (Expt.8)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x subject stratum	
Treatments	2
Residual	6
Blocks x subject x time stratum	
Time	2
Time x treatments	4
Residual	18
Total	35

Table 5.31. Skeleton ANOVA of the grain yield and yield components measurements (Polytunnel Expt.2) (Expt.8)

Source of variation	Degrees of freedom
Blocks stratum	3
Blocks x units x stratum	
Treatments	2
Residual	6
Total	11

5.4. Results

5.4.1. Volumetric soil moisture content

Results from data analysed on response of volumetric soil moisture content to drought and antitranspirant are presented on Table 5.34 and Figure 5.4.1.

Volumetric soil moisture content was significantly reduced in the droughted by 38.38 % compared with the irrigated treatments, whereas volumetric soil moisture content was not significantly affected by the drought at different depths, although there was a progressive increase in volumetric soil moisture content as the depth increases. But time was significant (P < 0001) as the volumetric soil moisture content differed as the days of measurements progressed showing a progressive increase from the 7 to 28 July and decreasing from 7 August to 7 September. The treatment x depth interaction effects on volumetric soil moisture content was not significant. However, the time x treatments interaction effects significantly (P < 0.001) affected the volumetric soil moisture content because the reduction in mean volumetric soil moisture content with time was greater in the droughted than in the irrigated treatments at the end of the measurements. The time x depths and time x treatments x depths interaction effects on volumetric soil moisture content were not significant.

Table 5.32. Volumetric soil moisture content (%) at 20, 40 and 60 cm depths taken on plots under irrigation, drought and antitranspirant in the polytunnels at varios times between July – September, 2017. Drought was imposed at 16 while antitranpirant was applied at 49 days after emergence. (n = 24) ± Standard error of the differences of the mean (SED) (Polytunnel Expt.2) (Expt.8).

		Times of measurements										
		7 Jul.	14 Jul.	21 Jul.	28-Jul	7 Aug.	10 Aug.	18 Aug.	25 Aug.	1 Sept.	7 Sept.	
	Overall means	31.31	36.13	35.2	36.64	21.92	18.09	19.3	20.15	19.72	20.95	25.94
	Depths											Mean
Irrigation	0 - 20	30.40	37.21	40.68	43.15	28.22	22.17	26.02	26.28	26.28	31.47	31.19
	20 - 40	34.88	41.99	40.24	42.32	25.40	23.66	26.75	27.04	26.02	26.41	31.47
	40 - 60	34.56	47.96	42.44	46.83	27.09	24.01	26.09	29.05	28.56	29.71	33.63
	means	33.28	23.28	42.39	26.29	26.95	41.12	27.46	44.1	26.9	29.20	32.10
Drought	0 - 20	25.79	25.94	24.64	24.11	13.35	9.84	8.70	10.95	9.38	9.87	16.26
	20 - 40	30.79	31.50	30.60	31.06	18.16	14.12	13.88	13.78	13.45	13.73	21.11
	40 - 60	31.47	32.18	32.57	32.38	19.33	14.74	14.34	13.78	14.61	14.50	21.99
	Means	29.35	12.90	29.87	12.31	12.48	29.27	12.83	29.18	16.95	12.70	19.78
df												3
SED												0.992
CV %												3.8
												Pvalues
Treatments												< 0.001
Depths												0.191
Treatments x depths												0.560
Time												< 0.001
Time x treatments												< 0.001
Time x depths												0.279
Time x treatments x depths												0.442

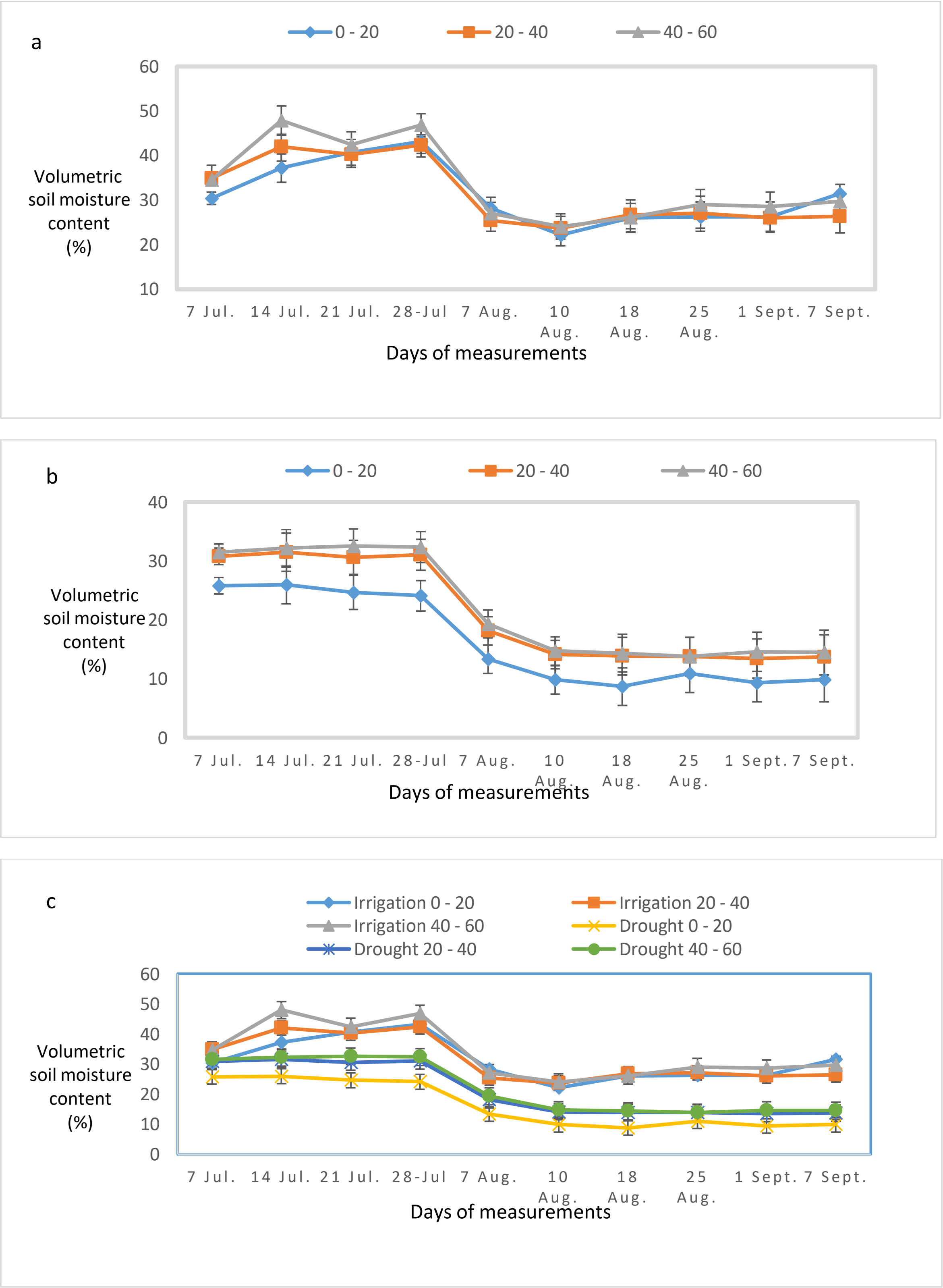

Figure 5.4.1. Volumetric water content (%) of (a) irrigated (b) droughted and (c) irrigated and droughted plots at 0 – 20 cm, 20 – 40 cm and 40 – 60 cm in polytunnels from July - September 2017 (treatments, P = < 0.001; depths = 0.191; treatments x depths = 0.560). Error bars are SEM.

5.4.3. Adaxial stomatal conductance

Results of analysis of data on the effect of drought and antitranspirant treatments on adaxial stomatal conductance is presented on Table 5.35 and Figure 5.4.2.

The effect of the treatments on adaxial stomatal conductance was highly significant ($P < 0.001$) leading to a reduction of 28.89 % on average in the droughted unsprayed and sprayed compared with the irrigated treatment. The effect of time was significant ($P = 0.020$) causing the adaxial stomatal conductance to increase as the days after spraying increases, however the differences in magnitude at 25 DAS and 27 DAS was only marginal compared to that between the first and last days of data collection at 12 DAS and 34 DAS respectively. But, the treatments x time interaction effect on adaxial stomatal conductance was not significant. Contrast analysis revealed that drought imposed caused a highly ($P < 0.001$) significant difference in adaxial stomatal conductance with a 21.28 % and 38.23 % decrease in the unsprayed and sprayed droughted respectively compared with the irrigated treatment. Whereas the antitranspirant spray induced a significant ($P = 0.002$) difference between the unsprayed and sprayed plants with a reduction of 21.53 % in the sprayed compared with the unsprayed plants.

Table 5.33. Average adaxial stomatal conductance (mmol $m^{-2}s^{-1}$) of sorghum recorded on the flag leaf under drought and antitranspirant applied at 16 and 49 days after emergence respectively grown in the polytunnel from June – October, 2017 taken at 12, 25, 27 and 34 days after spraying (n = 12) ± standard error of the differences of the mean (SED) (Polytunnel Expt.2) (Expt.8).

	Days after spraying				
Treatments	12	25	27	34	Mean
Irrigated	335.0	312.2	348.0	371.2	341.6
Unsprayed	236.7	292.0	254.2	292.5	268.9
Sprayed	169.5	187.0	203.2	285.0	211.0
Mean	247.1	263.8	268.5	316.2	273.9
df					3
SED					20.61
CV %					7.5
					P values
Treatments					< 0.001
Time					0.020
Treatments x time					0.422
Contrast analysis					P values
Treatments					< 0.001
Irrigated vs droughted					< 0.001
Droughted unsprayed vs droughted sprayed					0.002

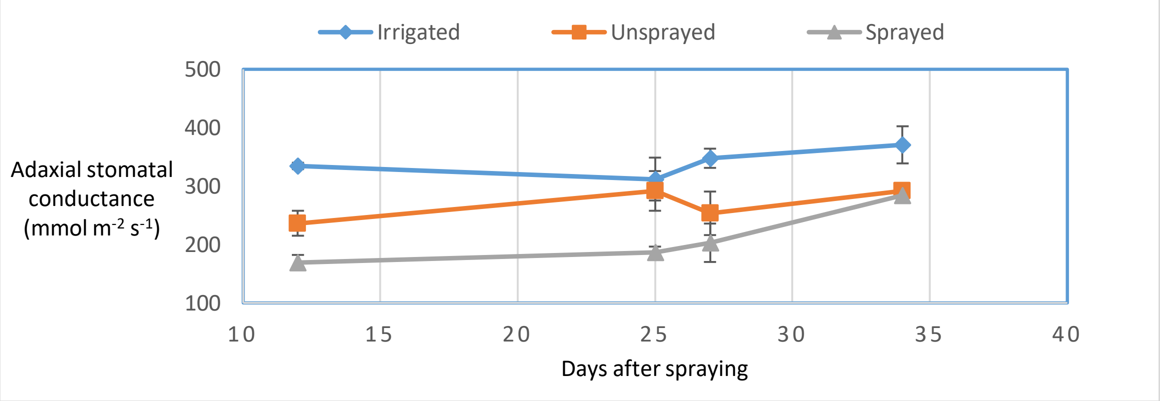

Figure 5.4.2. Response of adaxial stomatal conductance (mmol $m^{-2}s^{-1}$) to drought and antitranspirant applied at 16 and 49 days after emergence respectively measured at 12, 25, 27 and 34 days after spraying grown in the polytunnel June to October, 2017 (n = 3) (treatments, $P < 0.001$; time, $P = 0.020$; treatments x time, $P = 0.422$). Error bars are SEM. (Polytunnel Expt.2) (Expt.8).

5.4.4. Abaxial stomatal conductance

Results of analysis of data on the effect of drought and antitranspirant treatments on abaxial stomatal conductance is presented on Table 5.36 and Figure 5.4.3.

There was a significant ($P = 0.053$) effect of the treatments on abaxial stomatal conductance which caused a reduction of 16.59 % in the droughted unsprayed and 13.76 % in the sprayed compared with the irrigated treatments. The time effect on abaxial stomatal conductance was highly significant ($P < 0.001$) because there was a progressive decline in magnitude as time progressed up to 27 DAS. However, the value increased at 34 DAS to almost twice that at the beginning of the measurements, but the treatment x time interaction effect on abaxial stomatal conductance was not significant. The contrast analysis showed that the drought imposed led to significant ($P = 0.020$) reduction in abaxial stomatal conductance between the irrigated and droughted treatments, whereas the antitranspirant applied did not cause any significant differences between the droughted unsprayed and sprayed treatments.

Table 5.34. Average abaxial stomatal conductance (mmol $m^{-2}s^{-1}$) of sorghum recorded on the flag leaf under drought and antitranspirant applied at 16 and 49 days after emergence respectively grown in the polytunnel from June – October, 2017 taken at 12, 25, 27 and 34 days after spraying (n = 12) ± standard error of the differences of the mean (SED) (Polytunnel Expt.2) (Expt.8).

	Days after spraying				
Treatments	12	25	27	34	Mean
Irrigated	468	440	320	606	458
Unsprayed	318	447	204	558	382
Sprayed	266	353	310	649	395
Means	350	413	278	604	411
df					3
SE					70.9
CV %					17.2
					P values
Treatments					0.053
Time					< 0.001
Treatments x time					0.442
Contrast analysis					Mean
Treatments					0.053
Irrigated vs droughted					0.021
Droughted unsprayed vs droughted sprayed					0.636

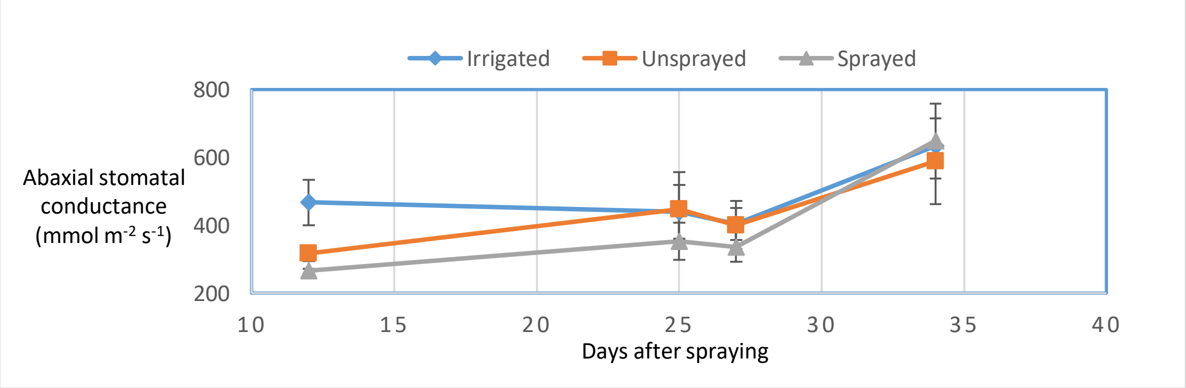

Figure 5.4.3. Response of abaxial stomatal conductance (mmol $m^{-2}s^{-1}$) to drought and antitranspirant applied at 16 and 49 days after emergence respectively recorded on the flag leaf measured at 12, 25, 27 and 34 days after spraying grown in the polytunnel June to October, 2017 (n = 3) (treatments, P = 0.053; time, P < 0.001; treatments x time, P = 0.442). Error bars are SEM. (Polytunnel Expt.2) (Expt.8).

5.4.5. Total stomatal conductance

The result of data analysed on the effect of drought and antitranspirant treatments on total stomatal conductance is presented on Table 5.37 and Figure 5.4.4.

Total stomatal conductance was significantly (P = 0.056) affected by the treatments and reduced by 17.07 % in the droughted unsprayed and sprayed on average compared with the irrigated. And there was a significant (P = 0.013) effect of time as the total stomatal conductance varied across the days after spraying with a greater value at the end than at the beginning of the measurements. The treatment x time effect was not significant on the total stomatal conductance. Contrast analysis showed that there was a significant difference between the irrigated and droughted treatments as the drought induced a reduction of 15.73 % and 18.40 % in the unsprayed and sprayed droughted compared with the irrigated treatments, but the antitranspirant gave no significant differences between the unsprayed and sprayed plants.

Table 5.35. Average total stomatal conductance (mmol $m^{-2}s^{-1}$) of sorghum recorded on the flag leaf under drought and antitranspirant applied at 16 and 49 days after emergence respectively grown in the polytunnel from June – October, 2017 taken at 12, 25, 27 and 34 days after spraying (n = 12) ± standard error of the differences of the mean (SED) (Polytunnel Expt.2) (Expt.8).

	Days after spraying				
Treatments	12	25	27	34	Mean
Irrigated	401	376	334	387	375
Unsprayed	277	370	229	387	316
Sprayed	218	270	257	481	306
Mean	299	339	273	418	332
df					3
SED					23.3
CV %					7
					P values
Treatments					0.063
Time					0.013
Treatments x time					0.127
Contrast analysis					
					P values
Treatments					0.056
Irrigated vs droughted					0.020
Droughted unsprayed vs droughted sprayed					0.730

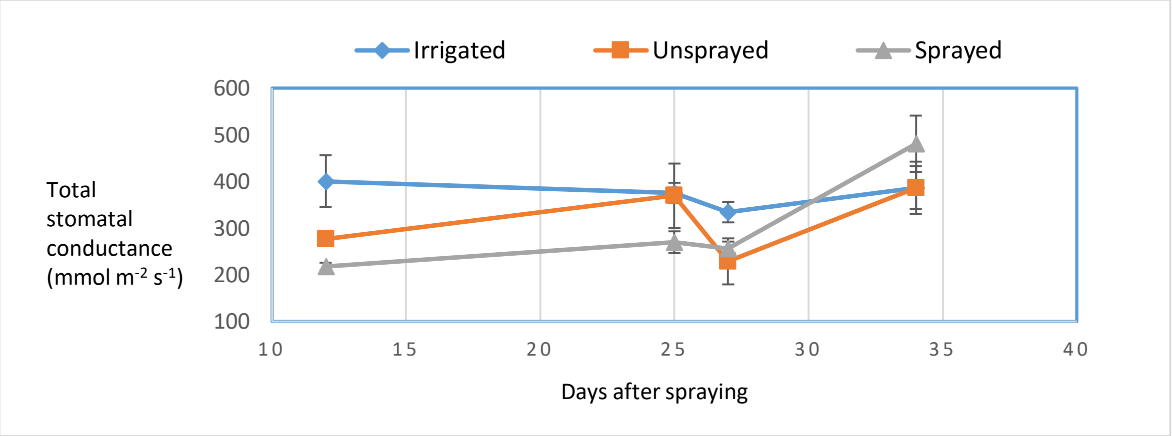

Figure 5.4.4. Response of total stomatal conductance (mmol $m^{-2}s^{-1}$) to drought and antitranspirant applied at 16 and 49 days after emergence respectively measured at 12, 25, 27 and 34 days after spraying grown in the polytunnel from June to October, 2017 (n = 3) (treatments, P = 0.063; time, P = 0.013; treatments x time, P = 0.127). Error bars are SEM. (Polytunnel Expt.2) (Expt.8).

5.4.6. Leaf temperature

The result of data analysed on the effect of drought and antitranspirant treatments on leaf temperature is presented on Table 5.38 and Figure 5.4.5**.**

There was a significant (P = 0.046) effect of the treatments on leaf temperature which increased by 6.8 % on average in the droughted compared with the irrigated plants. The effect of time was highly significant (P < 0.001) as the leaf temperature fluctuated over the DAS, with an increase between 22 DAS and 26 DAS, and a slight decrease from 26 DAS to 28 DAS, but increased substantially at 30 DAS over the other days during which it was fairly constant. There was no significant treatment x time interaction effect on the leaf temperature. Although the treatments significantly affected the leaf temperature, contrast analysis showed drought caused significant differences between the irrigated and the droughted treatment, however no significant differences were recorded between the droughted unsprayed and sprayed treatments owing to antitranspirant application.

Table 5.36. Average leaf temperature (˚C) of sorghum under drought and antitranspirant applied at 16 and 49 days after emergence respectively grown in the polytunnel from June – October, 2017 taken at 22, 26, 28 and 30 days after spraying (n = 12) ± standard error of the differences of the mean (SED) (Polytunnel Expt.2) (Expt.8).

	Days after spraying				
Treatments	22	26	28	30	Mean
Irrigated	16.99	17.75	17.65	28.70	20.27
Droughted unsprayed	18.75	19.38	19.25	28.58	21.49
Droughted sprayed	19.83	19.40	19.30	28.73	21.81
Mean	18.52	18.84	18.73	28.67	21.19
df					3
SED					1.379
CV %					6.5
P values					
Treatments					0.046
Time					< 0.001
Treatments x time					0.647
Contrast analysis					Pvalues
Treatments					0.046
Irrigated vs droughted					0.018
Droughted unsprayed vs droughted sprayed					0.536

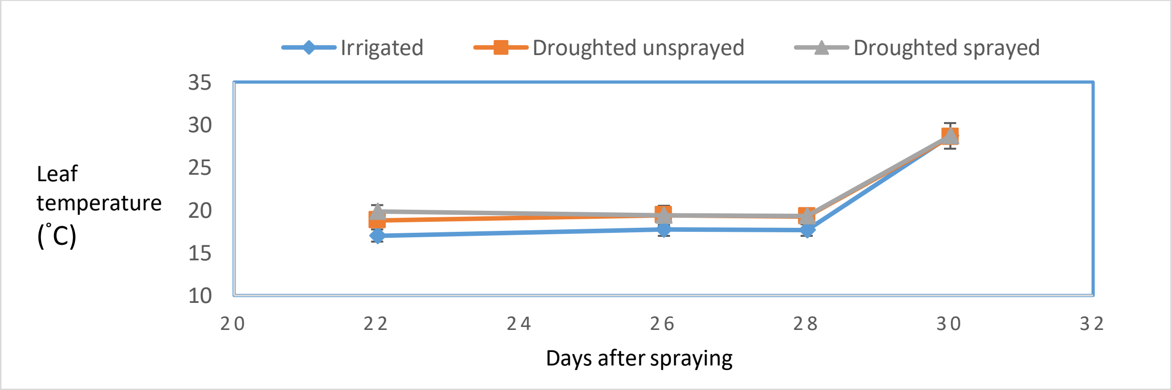

Figure 5.4.5. Response of leaf temperature (˚C) to drought and antitranspirant applied at 16 and 49 days after emergence respectively measured at 22, 26, 28 and 30 days after spraying grown in the Polytunnel from June – October 2017 (n = 3) (treatments, P = 0.046; time, P < 0.001; treatments x time, P = 0.647). Error bars are SEM. (Polytunnel Expt.2) (Expt.8).

5.4.7. Leaf area

The result of data analysed on the effect of drought and antitranspirant treatments on leaf area is presented on Table 5.39 and Figure 5.4.6**.**

Leaf area was significantly (P = 0.042) affected by the treatments with a reduction of 21.66 % on average in the drought compared with the irrigated treatments. There were no significant differences in the leaf area with time in the irrigated and droughted treatments and the treatment x time interaction effect on leaf area was not also significant. However, contrast analysis revealed drought imposition caused a significant difference in leaf area between the irrigated and droughted treatments as there was a 43.32 % and 29.55 % reduction in the droughted unsprayed and sprayed respectively compared with the irrigated treatments. But the antitranspirant created no significant differences between the unsprayed and sprayed droughted treatments.

Table 5.37. Avergae leaf area (cm^2) of sorghum recorded on the flag leaf under drought and antitranspirant applied at 16 and 49 days after emergence respectively grown in the polytunnel from June – October, 2017 taken at 16, 30 and 32 days after spraying (n = 12) ± standard error of the differences of the mean (SED) (Polytunnel Expt.2) (Expt.8).

	Days after spraying			
Treatments	16	30	32	Mean
Irrigated	262	233	246	247
Droughted unsprayed	165	119	135	140
Droughted sprayed	154	203	167	174
df				18
SED				63.8
CV %				34.1
Mean	194	185	183	187
				P values
Treatments				0.042
Time				0.833
Treatments x Time				0.622
Contrast analysis				P values
Treatments				0.042
Irrigated vs Droughted				0.019
Droughted unsprayed vs Droughted sprayed				0.327

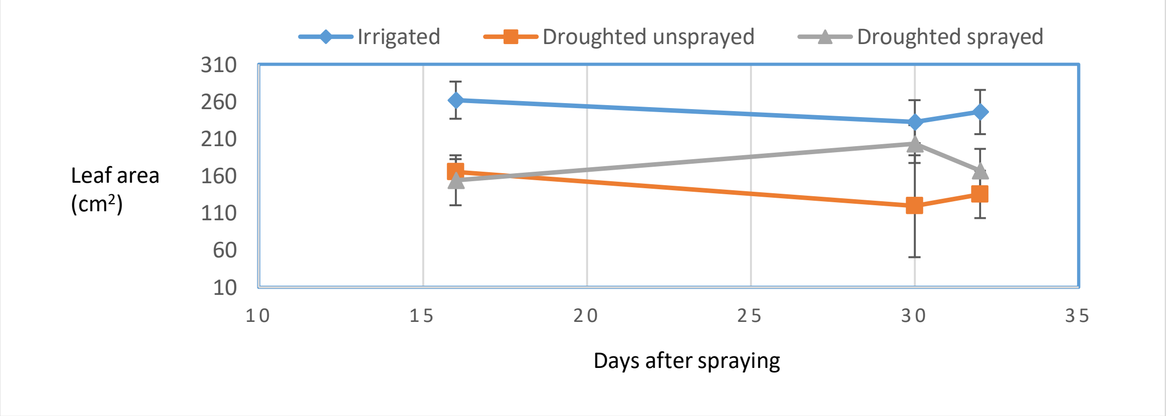

Figure 5.4.6. Response of leaf area (cm^2) recorded on the flag leaf to drought and antitranspirant applied at 16 and 49 days after emergence respectively measured at 16, 30 and 32 days after spraying in sorghum grown in the polytunnel from June – October 2017 (n = 3) (treatments, P = 0.042; time, P = 0.833; treatments x time, P = 0.622). Error bars are SEM. (Polytunnel Expt.2) (Expt.8).

5.4.8. Green area index

The result of data analysed on the effect of drought and antitranspirant treatments on green area index is presented on Table 5.40 and Figure 5.4.7.

Treatments caused significant (P = 0.044) differences in green area index between the irrigated and droughted plants with a reduction on average of 24.49 % in the droughted compared with the irrigated treatments. Time did not cause any significant difference in green area index in the treatments. Also, the treatment x time interaction effects did not significantly affect green area index. However, contrast analysis showed that the drought applied caused significant (P = 0.058) difference in green area index between the irrigated and droughted plants cuasing a 38.78 % and 10.20 % reduction in the droughted unsprayed and sprayed treatments respectively. The antitranspirant created a significant (P = 0.057) difference between the unsprayed and sprayed droughted treatments and increased green area index by 46.67 % in the droughted sprayed compared with the droughted unsprayed treatments.

Table 5.38. Average green area index of sorghum under drought and antitranspirant applied at 16 and 49 days after emergence respectively grown in the polytunnel from June – October, 2017 taken at 18, 22 and 32 days after spraying (n = 12) ± standard error of the differences of the mean (SED) (Polytunnel Expt.2) (Expt.8).

	Days after spraying			
Treatments	18	22	32	Mean
Irrigated	0.094	0.094	0.105	0.098
Droughted unsprayed	0.066	0.074	0.041	0.060
Droughted sprayed	0.087	0.089	0.087	0.088
df				18
SED				0.022
CV %				26.9
				P values
Treatments				0.044
Time				0.608
Treatments x time				0.348
Contrast analysis				P values
Treatments				0.044
Irrigated vs droughted				0.058
Droughted unsprayed vs droughted sprayed				0.057

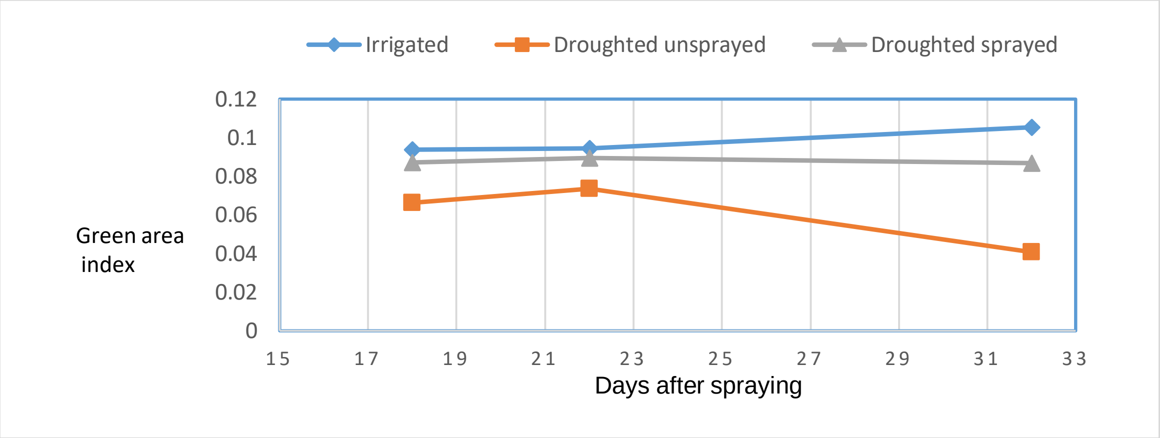

Figure 5.4.7. Response of green area index to drought and antitranspirant applied at 16 and 49 days after emergence respectively measured at 18, 22 and 32 days after spraying grown in the polytunnel from June – October, 2017 (n = 3) (treatments, P = 0.044 time, P = 0.608; treatments x time, P = 0.348). Error bars are SEM. (Polytunnel Expt.2) (Expt.8).

5.4.9. Yield and yield components

The result of data analysed on the effect of drought and antitranspirant treatments on grain yield and yield components is presented on Table 5.41.

Treatments did not produce any significant effect on grain yield, grain number, biomass and harvest index, but the stalk weight was significantly affected by the treatments.

According to contrast analysis, drought application did not lead to any significant difference in grain yield and yield components between the irrigated and the droughted treatments and the antitranspirant caused no significant differences between the droughted unsprayed and sprayed treatments. Nevertheless there was a numerical but not statistically significant greater grain number in the droughted sprayed than the droughted unsprayed treatments.

But, the weight per grain remained not significantly affected by the drought and antitranspirant treatments as drought caused no significant differences between the irrigated and droughted plants, and the antitranspirant induced no significant differences between the unsprayed and sprayed droughted plants.

The effect of drought on stalk weight was highly significant ($P < 0.001$) leading to significant differences between the irrigated and droughted treatments and showing a 24.72 % decrease on average in the droughted compared with the irrigated treatment. Although the antitranspirant applied led to no significant differences in stalk weight between the unsprayed and sprayed droughted plants, a numerical but not statistically significant increase in stalk weight occurred in the sprayed over the unsprayed droughted plants.

Drought led to significant ($P = 0.049$) differences in biomass between the irrigated and droughted treatments with the irrigated producing 18.54 % higher biomass than the droughted treatment, whereas the antitranspirant caused no significant differences in biomass between the unsprayed and sprayed droughted treatments. But there was a greater biomass in the unsprayed than the sprayed droughted treatments, although not statistically significant.

Upon drought application, the harvest index was not significantly different in the irrigated and droughted treatments and the antitranspirant did not create any significant differences in harvest index between the unsprayed and sprayed droughted plants.

Table 5.39. Grain yield and yield components under drought and antitranspirant applied at 16 and 49 days after emergence respectively of sorghum grown in the polytunnel from June to October 2017. Data are means (Polytunnel Expt.2) (Expt.8).

Treatments	Grain yield (g)	Grain number per plant	Weight per grain (mg)	Stalk weight (g)	Biomass (g m $^{-2}$)	Harvest index (%)
Irrigated	175	5737	30.90	145.2	321	53.7
Droughted unsprayed	141	4261	33.90	104.5	245	57.2
Droughted sprayed	164	4746	34.30	114.1	278	57.9
df	6	6	6	6	6	6
SED	44.1	551	3.47	10.79	43	8.21
CV %	27.6	11.3	10.5	8.9	15.3	14.6
			P values			
Treatments	0.563	0.267	0.378	0.004	0.121	0.754
Contrast analysis						
			P values			
Treatments	0.562	0.267	0.378	<0.001	0.090	0.751
Irrigated vs Droughted	0.425	0.394	0.254	<0.001	0.049	0.468
Droughted unsprayed vs Droughted sprayed	0.484	0.579	0.856	0.216	0.302	0.901

5.5. Discussion

5.5.1. Effect of drought

In Polytunnel Expt. 1, drought had significant effects on volumetric soil moisture content of plots and green area index therefore the null hypothesis is rejected with regard to these measurements. On the other hand, drought applied had no significant effects on stomata conductance, leaf temperature, leaf area, grain yield, grain number, weight per grain, stalk weight, biomass and harvest index, hence the null hypothesis is accepted. In Polytunnel Expt. 2, under drought imposition, volumetric soil moisture content, transpiration, leaf temperature, leaf area and green area index, stalk weight and biomass were all significantly different in the irrigated and droughted treatments, thus the null hypothesis is rejected for these parameters. However, the grain yield, grain number, weight per grain, and harvest index were not significantly different in the irrigated and droughted treatments, therefore the null hypothesis is rejected in terms of these measurements.

In Polytunnel Expt. 1, volumetric soil moisture content in the irrigated was significantly different from the droughted plots. In the irrigated plots volumetric soil moisture content fluctuated between 35.0 % and 26.0 %, while in the droughted plots it was between 20.0 % and 14.0 % at all days of measurements. However at the end of the measurements the irrigated had a 47.5 % greater volumetric soil moisture content than the droughted plots. And on average volumetric soil moisture content of the irrigated plots was 30.2 % while the droughted was 15.8 %. These results are comparable to those obtained previously by Faralli (2017) in a similar experiment with oil seed rape on plots located nearby, from the same soil depth using a different instrument which showed that the volumetric soil moisture content ranged between 40.0 – 43.0 % in the irrigated and 17.0 – 12.0 % in the droughted plots and decreased in the droughted plots by 40.0 – 30.0 % compared with the irrigated. Whereas Faralli (2017) used a neutron probe inserted into the soil and sampled at a depth of 20 – 80 cm (data from 40 – 80 cm not considered here), in the current study a TDR soil moisture meter with a probe of 20.0 cm long was used. Although, measurements of field capacity and permanent wilting point could not be taken in the present study, the upper limit of 40.0 % in the irrigated plots and the lower limit of 12.0 % in the droughted plots as shown in Faralli (2017) could be used. Therefore, volumetric soil moisture content in the irrigated and droughted plots were slightly below the field capacity and above the permanent wilting point in the current study.

In Polytunnel Expt. 2, on average volumetric soil moisture content in the irrigated treatments decreased from 33.28 % to 32.10 % and in the droughted treatments it reduced from 29.35 % to 19.78 %, indicating a greater reduction in magnitude in the droughted than in the irrigated treatments. At the end of the measurements volumetric soil moisture content decreased from 32.10 % in the irrigated to 19.78 % in the droughted, showing that the irrigated held a 38.38 % greater volumetric soil moisture content than the droughted plots. The volumetric soil moisture content in the irrigated was lower than the 40.0 % field capacity and greater than the 12.0 % permanent wilting point earlier used by Faralli (2017) on a nearby plot. And compared with Polytunnel Expt.1., where volumetric soil moisture content of the irrigated and droughted plots were 30.15 % and 15.84 % respectively, in the current experiment it was greater in both irrigated and droughted plots at 32.10 % and 19.78 % respectively. The differences between the current results and those of Faralli (2017) and Polytunnel Expt. 1 may be ascribed to the different equipment used and the conditions under which measurements were carried out. However, despite this soil moisture regime, the drought treatment was severe to create soil water reduction that induced significant differences between the irrigated and droughted plots and a statistically significant soil water deficit in the droughted compared with the irrigated plots. It should be noted that while the volumetric soil moisture content was not significantly different with depths, the differences between the values obtained at the top 0 – 20 cm layer of the soil and the subsequent 20 – 40 cm and 40 – 60 cm depths was greater in the droughted than the irrigated, this shows greater evaporation and plant water uptake from the top 0 – 20 cm depth than from the 20 – 40 cm and 40 – 60 cm depths.

There were no significant differences in adaxial, abaxial and total stomatal conductance between irrigated and droughted plants, thus transpiration was not significantly affected in the course of the Polytunnel Expt. 1. This is contrary to expectations and the general norm, as significant reduction in volumetric soil moisture content should have produced significant reduction in stomatal conductance and hence transpiration. However, Jones *et al*., (1981) noted that for drought to affect a plant community, soil water deficit must lead to plant water deficit, and that the degree to which a certain soil water deficit influences the plant is determined by a number of factors including the vapour pressure deficit, transpiration rate, and the plant's specific response to water stress. Thus, there is the possibility that significant reduction in volumetric soil moisture content could not lead to significant plant water deficit and reduction in stomatal conductance and transpiration in the Polytunnel Expt. 1, due to a number of reasons including the low vapour pressure deficit and slow transpiration rate created by the polytunnel environment and the nature of the sorghum plant. It was observed during the experiment that there were heavy condensations of water vapour on the inner side of the top of the polytunnels housing both irrigated and droughted treatments. This micro-climate could have led to high humidity, low leaf-to-air vapor pressure deficit and slowed transpiration rates thereby impeding the development of significant plant water stress. However, the low vapor pressure deficit may have played a greater role than humidity in the response of stomatal conductance under the polytunnels because Assman and Grantz (1990) reported that stomatal conductance is better correlated with vapor pressure deficit than humidity in sorghum and sugar cane. Furthermore, this experiment was carried out in the field, so the root system of the sorghum plants which is highly branched, deep and spreading and can get to a soil depth of 180 cm, could have aided in the capture of water from greater soil depths, thereby countering or reducing the effect of soil water deficit on stomatal conductance by maintaining high leaf water potential. It has been recognised that the drying of the top soil around the plant's crown inhibits the emergence of new crown roots and that the existing roots compensate in growth and penetrate into deeper layers (Blum and Ritchie, 1984; Blum and Arkin, 1984; Jordan *et al*., 1979). Comparing soil water absorption of maize and sorghum under drought, Singh and Singh (1995) noted that maize absorbed more water from the top soil (0 – 45 cm) than sorghum, while sorghum extracted greater water than maize from the sub-soil (45 – 135 cm). Thus, sorghum's ability to draw moisture from the subsoil under drought could have reduced the effect of drought on the plant water content, hence the lack of significant effect of drought on the stomatal conductance. However, measurements of root depth was not carried out to show how this must have happened. Yet, the numerical values showed a reduction in the stomatal conductance in the droughted compared with the irrigated plots. Whereas in Polytunnel Expt. 2, significant effect of drought on transpiration occurred, despite a greater water regime than in the Polytunnel Expt. 1. Since the water regime in Polytunnel Expt. 1, was comparable to that in the current experiment in terms of the maximum and minimum moisture levels attained in the irrigated and droughted plots, the differences in the result could be accounted for by the differences in the design and environmental conditions of the two experiments. Polytunnel Expt. 1 was a split-plot design with four treatments and thirty two plots, whereas the present experiment was a one-way design with three treatments and twelve plots. With regards to the environmental conditions, each of the polytunnels in which the current experiment was carried out had a rectangular opening at the top end of the sides opposite the door. This modification allowed for air circulation between the polytunnel enclosure and the outside through the door and the rectangular opening which decreased the build-up of high humidity as was observed from the absence of condensations on the inner side of the polytunnel and probably increased vapor pressure deficit, consequently reduction in transpiration was more clearly observed and better determined. The effect of the likelihood for increased vapor pressure deficit on the overall transpiration process can be observed from greater magnitudes of adaxial, abaxial and total stomatal conductance in the current experiment compared with in Polytunnel Expt.1., thus as humidity declines vapour pressure deficit and transpiration increased. This trend was observed in maize grown under rain shelters by Yang *et al*., (2012).

Leaf temperature was not significantly different in the droughted and irrigated treatments upon drought application in Polytunnel Expt. 1, in correspondence with the non-significant effect of drought on stomatal conductance. However, this is in contrast to Stuart, *et al*., (1985) where drought decreased stomatal conductance and increased leaf temperature in sorghum. The differences in water stress levels, sorghum variety and growth conditions may account for the differences in the response of leaf temperature to drought in the present study and Staurt, *et al*., (1985). Since drought imposed was not severe enough to cause significant reduction in transpiration, elevation in leaf temperature as a consequence of stomatal closure in response to water stress in the droughted plants compared with the irrigated could not have occurred. However, there was a significant increase in leaf temperature in the droughted compared with the irrigated treatments in Polytunnel Expt. 2. This could be attributed to the drought effect that caused significant reduction in volumetric soil water content which led to significant decrease in transpiration and the consequent elevation in leaf temperature. As can be shown by the current results, Pallas *et al*., 1967 noted that leaf temperature is usually negatively correlated with soil water and transpiration while reports on millet (Singh and Kanemasu, 1983) showed that average leaf temperature were higher in droughted than irrigated plants. However, the results from the Polytunnel Expt. 2, contrasts with findings from both glasshouse and Polytunnel Expt.1 in this project, perhaps due to differences in growing conditions and drought severity.

Leaf area in Polytunnel Expt. 1, was not significantly different between the irrigated and droughted plants, so no significant effect of drought on leaf area was recorded despite significant effect of drought on volumetric soil moisture content. The lack of significant effect of drought on leaf area could have occurred due to the lack of development of significant plant water deficit so the rates of cell division and expansion were not substantially inhibited in the droughted treatments. In other words, the significant reduction in volumetric soil moisture content, could not lead to significant reduction in the sorghum plant water content which in turn did not lead to significant decrease in leaf area. The high humidity and low vapour pressure deficit under the polytunnels and decreased transpiration from the plants did not allow for the development of significant plant water stress despite significant reduction in soil moisture content. The current results is in contrast with Stout, *et al*., (1978b) who found that leaf length, an aspect of the leaf area was significantly different in irrigated and droughted NK300 sorghum variety grown under rain out shelters. In contrast with the result of Polytunnel Expt. 1, drought imposed caused a significantly lower leaf area in the droughted compared with the irrigated plants in Polytunnel Expt. 2. This happened perhaps due to differences in the polytunnel design which ensured a greater vapour pressure and plant water deficit leading to reduced leaf area in polytunnel Expt. 2 than in Polytunnel Expt.1. Reduction in leaf area as observed in Polytunnel Expt. 2, aimed to limit plant transpiration surface thereby decreasing soil and plant water loss. Hence, Blum and Arkin (1984) found in their study that when soil moisture was decreased to below 20 % of available water, transpiration in sorghum was mainly reduced owing to a reduction in leaf area through leaf senescence. Tsuji, *et al*., (2003) also reported a 28 % - 63 % decrease in leaf area in droughted compared with irrigated treatments in three cultivars of field grown sorghum. The mechanism for reduction in leaf area under water stress in sorghum involves both reduction in rate of cell division and in cell expansion (McCree and Davies, 1974) as both cell division and expansion were inhibited by water stress (Hsiao, 1973). Thus reduction in leaf area due to drought in the current study could be due to decrease in the rate of cell division and expansion. However, measurements of cell division and expansion were not carried out in this study to support this position, but it is generally recognised that plant growth and leaf area are reduced under water stress (Hsiao, 1973).

In Polytunnel Expt. 1, green area index was significantly different in the droughted and irrigated treatments as the effect of the drought treatment caused a reduction of 52.00 % in the droughted compared with the irrigated treatment. In agreement with these results Singh and Singh (1995) and Garofalo, *et al*., (2011) reported a 62.00 % and a 36.59 % reduction in leaf area index respectively in severely droughted compared with the well-irrigated sorghum. The significant reduction in green area index in the droughted compared with the irrigated treatment in the current study could be due to the significant reduction in volumetric soil moisture content, as leaf area per se was not significantly reduced by the drought, so could not have contributed to reduction in green area index. The lack of correspondence in the results of leaf area and green area index could be attributed to the differences in the way they were measured. While the green area index takes the entire plant canopy into consideration, the average area of two individual leaves was recorded as the leaf area per plant. Nevertheless, the significant reduction in volumetric soil moisture content by the drought imposition must have significantly reduced plant canopy in the droughted compared with the irrigated treatments probably through a reduction in leaf number. Craufurd and Peacock (1993) working on sorghum remarked that the imposition of water stress immediately resulted into a reduction in leaf appearance rate, and as the water stress increased, leaf appearance eventually ceased. Thus in the Polytunnel Expt.1, drought imposition must have retarded the leaf appearance rate, thereby decreasing leaf number and hence the plant canopy in the droughted compared with the irrigated treatment. Measurements of leaf number were taken only once and did not show any significant differences between the droughted and irrigated treatments (data not shown). However there is a possibility that drought caused lower leaf number in the droughted compared with the irrigated, as the droughted plants would have avoided the production of new leaves, and/or shed the more older leaves and preferentially allocated more resources to maintaining the main shoot and leaf area in fewer rather than initiating new leaves. In Polytunnel Expt. 2, green area index was reduced significantly in the droughted compared with the irrigated plants in conformity with the results in Polytunnel Expt.1. This is related to the significant reduction in volumetric soil moisture content and leaf area. As explained under Polytunnel Expt. 1, leaf area is a part of the whole canopy being measured as green area index, thus decrease in leaf area is responsible for the decrease in green area index under drought application in Polytunnel Expt. 2. Borrell, *et al*., 2000 found that drought imposition reduced green leaf area/leaf area index by 67 % in the droughted compared with the fully irrigated sorghum and attributed this to a 12 % decrease in leaf area primarily caused by a significant reduction in the size of the leaves. This suggests that water stress in Polytunnel Expt.2 was sufficiently severe to limit cell division and expansion which are some of the known main sources of leaf size reduction in plants including sorghum under water stress.

Drought did not cause any significant difference in grain yield, grain number as well as harvest index between the irrigated and droughted treatments in both Polytunnel Expt. 1 and 2. However, stalk weight and biomass were significantly different in irrigated and droughted treatments after contrast analysis in Polytunnel Expt. 2. The responses in terms of grain yield and some yield components not being significantly reduced by drought is in marked contrast to the effect of water stress on sorghum under field conditions where significant decrease in grain yield and yield components were recorded (Craufurd and Peacock, 1993; Inuyama, *et al*., 1976; Lewis, *et al*., 1974; Fuerhing, 1973; Blum, 1973; Tolk, *et al*., 2013). In Polytunnel Expts. 1 and 2, the volumetric soil moisture content in the droughted plots were predominantly greater than the 12.0 % lower limit of volumetric soil moisture content required to effect plant water stress in the droughted plots as obtained in Faralli (2017). Therefore, perhaps the drought imposed despite being severe enough to cause a significant reduction in the volumetric soil moisture content was not sufficiently severe to create adequate plant water stress that would have led to a reduction in yield and yield components. In addition in Polytunnel Expt. 1, stomatal conductance and leaf area were not significantly decreased by the drought in congruence with results from grain yield and yield components. Therefore, transpiration and light interception were not significantly reduced as to cause a reduction in assimilate production and availability for grain yield, grain number, thousand grain weight, stalk weight and biomass development. In polytunnel Expt.2, there was a significant effect of drought in reducing transpiration, leaf area and green area index, but these did not translate into lower grain yield, grain number, thousand grain weight and harvest index. This is contrary to what would be expected as sorghum is reported to be source than sink limited (Fischer and Wilson, 1975). Perhaps reductions due to drought were not of such magnitude as to restrict assimilate supply for grain production hence the grain yield, grain number and thousand grain weight were not concomitantly reduced. The occurrence of significant reduction in stalk weight and biomass in Polytunnel Expt. 2 as a consequence drought imposition could be ascribed to the reduction in leaf area and green area index, which are a component of the biomass. Also, there is the probability that due to the severity of water stress, the plant preferentially allocated more resources to the grains than the stalk. Nevertheless, in Polytunnel Expt. 1, the significant effect of drought on green area index was reflected in the numerical, but not significant decrease in grain yield, grain number, weight per gran, stalk weight and biomass. Furthermore, given that the profile available water was 98 mm, the easily available water which is 60 % of the available water is equivalent to 40.80 mm. And using the average biomass of 1339.0 g/m^2 in Polytunnel Expt. 1, and an assumed water use efficiency of 40.72 for sorghum (Kuganathan and Palaniappan, 1980), the water requirement to give this biomass can be computed as 32.88 mm which is the volume of water assumed to be consumed by the plant in addition to water loss via evaporation from the soil surface. These water losses are sufficient to deplete a significant amount of the available water and create sufficient drought intensity to cause yield reduction, however this could not occur. Nevrtheless in Polytunnel Expt. 2, with an average biomass of 281.3 g/m^2, and an assumed water use efficiency of 40.72 (Kuganathan and Palaniappan, 1980) as in Polytunnel Expt. 1, the water requirement to produce this amount of biomass is equivalent to 2.91 mm, which is only a fraction of the easily available water. Thus, drought and reduction in yield under drought could not happen because so much water was left in the soil to counteract any drought formation. .

5.5.2. Effect of antitranspirant

In Polytunnel Expt. 1, the antitranspirant did not have any significant effect on volumetric soil moisture content and volumetric soil moisture was also not significantly reduced. Moreover, in Polytunnel Expts. 1 and 2, leaf area, leaf temperature, grain yield and yield components were also not significantly affected by the antitranspirant, therefore the null hypothesis is accepted with regard to these measurements. On the other hand the antitranspirant application had significant effect on transpiration and green area index in polytunnel Expt.1 and 2, thus the null hypothesis is accepted in these cases.

In Polytunnel Expt. 1, volumetric soil moisture content in the sprayed was not significantly different than in the unsprayed plots. The expected increase in volumetric soil moisture content in the sprayed over the unsprayed due to the antitranspirant application was not also recorded as the numerical values of volumetric soil moisture content showed that the unsprayed plots held more soil moisture than the sprayed plots on most of the days of measurements. This indicate that the coverage of the stomata by the antitranspirant could not cause a reduction in transpiration of such magnitude as to lead to soil moisture conservation in the sprayed compared with the unsprayed plots. In addition, the possible degradation of the antitranspirant on the plant leaves would have made the effect of the antitranspirant to be of no consequence in conserving significant amount of soil moisture over time. On the other hand, the antitranspirant could have caused soil moisture conservation but the micro-climate in the polytunnels characterized by high temperature developed by the enclosure could have led to high evaporation of moisture from the soil and of the antitranspirant from the leaf surfaces thereby depleting any additional moisture conserved in the soil and plant due to the antitranspirant. In Polytunnel Expt. 2, measurements of volumetric soil water content were not carried out on the basis of unsprayed and sprayed treatments, because the access tubes were inserted according to the drought and not the antitranspirant factor, so the replicates were not adequate for statistical analysis.

However, in Polytunnel Expt. 1, there was a significant effect of the antitranspirant in reducing transpiration as the adaxial stomatal conductance decreased by 11.96 % and the total stomatal conductance by 7.83 % in the sprayed compared with the unsprayed treatments. And in Polytunnel Expt. 2, adaxial stomatal conductance was reduced by 21.53 % in the sprayed compared with the unsprayed treatments. This gives an average reduction of ~16.75 % in the adaxial stomatal conductance across the two experiments. These results correspond with the reduction in transpiration in sorghum by the antitranspirant in glasshouse experiments in the current project and also in wheat under similar polytunnel/field conditions in previous reports by Weerasinghe (2013). This indicates the effectiveness of the antitranspirant in effecting stomatal coverage thus decreasing stomatal conductance and transpiration in the sprayed compared with the unsprayed treatments. It is evident in the sprayed treatments that stomatal closure have been greater and conductance lower at the adaxial leaf surface where the antitranspirant have been sprayed than at the abaxial surface which was not sprayed. Hence, the significant reduction in adaxial stomatal conductance by the antitranspirant must have contributed to the overall significant reduction in total stomatal conductance and transpiration. This is the first report showing significant decrease in transpiration in sorghum grown in rain out shelters using a film antitranspirant in general and Vapor Gard in particular.

Leaf temperature was not significantly different in the unsprayed and sprayed treatments in both Polytunnel Expts. 1 and 2, thus the antitranspirant had no significant effect on leaf temperature. It is expected that since transpiration has been decreased by the antitranspirant, the cooling effect of transpiration will also decrease and leaf temperature is expected to increase (Han, 1990). As can be observed from the data, the effect of the antitranspirant in increasing leaf temperature was not clearly evident in Polytunnel Expt. 1 as the unsprayed gave numerically higher values than the sprayed treatments on most occasions, but in Polytunnel Expt. 2 the antitranspirant appeared to have an effect as the sprayed gave numerically greater leaf temperature values than the unsprayed. These trends apparently validate a positive antitranspirant effect in reducing transpiration as in antitranspirant usage the aim is to restrict transpiration and not to prevent it entirely, therefore only a part of the cooling effect will be reduced by their use. It is important to point out that the lack of significant effect of the antitranspirant on leaf temperature could be attributed to the ambient temperature in the polytunnel not being so high which made the leaves already cool; thus any elevation in temperature caused by reduction in transpiration by the antitranspirant may not be revealed. According to Meidner and Mansfield (1968) the cooling effect of transpiration on leaves varies according to insolation, wind speed and the environment (hot or temperate) and whereas it is between 2 ^{o}C and 4 ^{o}C in the temperate areas, it could be down by up to 8 ^{o}C in the hotter places. Therefore, it is likely that the decrease in leaf temperature by the antitranspirant was obscured by the cooling effect of transpiration on already cool leaves. In hotter countries like Nigeria and India, where a reduction of 8 ^{o}C due to the cooling effect of transpiration is expected the effect of the antitranspirant may be readily observed, as part of the expected 8 ^{o}C reduction could be removed by the antitranspirant effect. On the other hand, green area index were significantly different in the unsprayed and the sprayed treatments in both Polytunnel Expts. 1 and 2. The antitranspirant increased green area index by 11.39 % in Polytunnel Expt. 1, and by 46.67 % in Polytunnel Expt.2, however the leaf area which would have contributed to greater green area index was not significantly increased in the sprayed compared with the unsprayed treatments. A probable reason for the increase in green area index in the sprayed compared with the unsprayed treatments could be an increase in leaf water content due to a reduction in transpiration in the antitranspirant sprayed over the unsprayed plants. Measurements carried out in this experiment showed no significant benefit of the antitranspirant to relative water content (data not shown) perhaps because it was done only once, but other workers (Abdallah, *et al*., 2015) found that wheat sprayed with antitranspirant/Vapor Gard maintained a significantly greater leaf turgor than those of the unsprayed plants, consistent with the recognized position that film antitranspirant reduces plant water loss and prolongs turgor maintenance under drought (del Amor, *et al*., 2010). Thus, it is possible that an increase in the leaf water content, though not indicated by the relative water content data, could have increased individual leaf and foliage weight that covered greater space in the sprayed compared with the unsprayed plots. In other words, with more viable green leaves the plant canopy architecture occupied a larger portion and led to higher green area index in the sprayed than in the unsprayed plots mainly due to higher leaf number than greater individual leaf areas as explained in section 5.7.1. In addition, the green area index in the Polytunnel experiments were very low, this occurred because the plants had open canopies as can be observed from fig. 5.3.2, which permitted greater light penetration through the canopy space thereby causing higher values of underground measurements particularly in the droughted tratments in Expt.2.

In Polytunnel Expts. 1 and 2 grain yield, grain number, thousand grain weight, stalk weight, biomass and harvest index were not significantly affected by the antitranspirant. Since, the current project is the first to consider the effect of the film antitranspirant Vapor Gard on grain yield and yield components of droughted sorghum, no reports known to the author are available to contextualize the results of the current study. Nevertheless, there are reports from using other antitranspirants, stomata closing type Phenylmercuric acetate (PMA) and Atrazine as well as the film-forming antitranspirant Folicote that has the same mode of action as the Vapor Gard used in the current study, applied to sorghum under limited irrigation in field conditions showing grain yield increases in droughted plants due to the effect of the antitranspirant (Fuerhing, 1973 a, b). The differences in the results of the aforementioned works and the current study could be attributed to the type of antitranspirant used as well as the rates and time of application, the water stress regime and the environmental conditions. However, the lack of significant effects of the antitranspirant on the grain yield and yield components of sorghum was contrary to expectations. Because the lower transpiration induced by the antitranspirant was expected to decrease plant water loss and increase plant water content as reported in the literature (del Amor, *et al*., 2010), whereas the greater green area index should have increased the amount of light intercepted and led to more photosynthetic activity and a significant increase in yield and yield components in the sprayed compared with the unsprayed treatments. In Polytunnel Expt.1, there could have been the negative effect of shading which must have reduced light interception and photosynthesis in the sprayed compared with the unsprayed plants. Although the high canopy density in the sprayed treatments affords an advantage in terms of greater potential for higher yield than in the unsprayed treatments.

For both Polytunnel Expts.1 and 2, this lack of significant effect of the antitranspirant on yield and yield components could also be adduced to the time of spraying the antitranspirant, as in Polytunnel Expt. 1, the spray was carried out at 51 days after emergence, whereas in Polytunnel Expt. 2, spraying was carried out at 49 days after emergence. As can be observed from the Figures 5.4 and 5.5 in section 5.1 in Polytunnel Expt.1, the plants had reached the half bloom stage at the time of antitranspirant application, at which stage the plant would have accumulated nearly half of its dry matter and any limitation in plant growth caused by water stress, can be compensated for once favourable conditions exist (Besancon, *et al*., 2003). Whereas in Polytunnel Expt. 2, it can observed from Figure 5.19 in section 5.5 that most of the plants were at booting so the effect of water stress on grain yield could have been pronounced and give room for the detection of significant antitranspirant effect. However, in both experiments instead of the antitranspirant to counter any reduction in grain yield and yield components caused by the drought imposed, the yield compensatory mechanism in sorghum may have been activated to ensure that any potential grain yield loss caused by drought, is compensated for via an increase in other yield components. This will limit any improvement in grain yield and yield components due to the antitranspirant. For instance in Polytunnel Expt. 2, grain yield decreased from 175 g in the irrigated to 141 g in the droughted unsprayed and grain number concomitantly decreased from 5737 in the irrigated to 4261 in the droughted unsprayed, but thousand grain weight increased from 30.90 g in the irrigated to 33.90 g in the droughted unsprayed. Another reason for the lack of significant effects of antitranspirant on grain yield in both Polytunnel Expts. 1 and 2 could be due to the differences between the droughted unsprayed and sprayed treatments being too small to be detectable as the experimental design was not sensitive enough to measure very small significant differences. For instance, Polytunnel Expt. 2 involved a one-way factorial design with 3 treatments, for which 4 replicates were performed. This number of replicates was sufficient for detecting very large differences between treatments such as difference between irrigated and droughted as observed from the results, but may not be adequate to guarantee the detection of small differences between treatments which can be observed from large co-efficient of variation indicating a lot of variability in the yield data. However, there was a numerical but not significantly greater grain yield, grain number, weight per grain and biomass produced by the sprayed than the unsprayed treatments in both Polytunnel Expts. 1 and 2, which indicates a potential for the antitranspirant to increase yield of sorghum under drought.

5.6. Conclusions

The results from these experiments showed that in both Polytunnel Expts. 1 and 2 drought treatment caused a significant reduction in volumetric soil moisture content and transpiration. Volumetric soil moisture content came closer to the permanent wilting point in Polytunnel Expt. 2 than in Polytunnel Expt.1, but could not create a significant plant water deficit and reduction in the grain yield and yield components of droughted sorghum. The antitranspirant did not conserve significant soil moisture but induced a significant reduction in transpiration and increased green area index in the droughted sorghum. However, the decrease in transpiration could not cause an increase in plant water content to significantly increase grain yield and yield components under drought in Polytunnel Expt. 1, but could increase grain number in Polytunnel Expt. 2, and the increase in green area index in both experiments did not induce significantly greater yield production in the droughted treatments under antitranspirant. The absence of a significant effect of antitranspirant under significant drought effects on grain yield and specifically in decreasing grain number as in Polytunnel Expt.2 could be attributed to the capacity of sorghum to undertake yield component compensation under water stress. This compensatory mechanism could have ensured that yield components being significantly reduced by the drought are compensated for by an increase in other yield components principally the weight per grain or thousand grain weight. Therefore, any gains obtained from antitranspirant activity is masked by the yield compensatory effect.

CHAPTER 6

General discussion

6.1. Introduction

As presented in the general introduction in chapter 1, this study investigated the potential of increasing sorghum yield and yield components under drought stress using film antitranspirant for a possible understanding of the role(s) of antitranspirant in reducing drought induced yield loss in sorghum.

The investigation involved both glasshouse and field experiments where sorghum was grown under full irrigation and water stress, with and without antitranspirant spray while imposing the drought and applying the antitranspirant at certain sorghum growth stages.

6.2. The effect of drought

The drought treatment decreased volumetric soil moisture content, transpiration, green area index, grain number and increased weight per grain on most occasions in the study. Decrease in transpiration was indicated by significant reduction in stomatal conductance probably mediated by the action of ABA in reducing the stomatal aperture in response to drought. Reduction in green area index by the drought treatment could have been as a consequence of decrease in the individual leaf area, although there was no correspondence between reduced green area index and the leaf area perhaps due to differences between how the two parameters were measured. Grain yield did not decrease in response to the drought whereas grain number reduced and weight per grain and thousand grain weight increased. Reduction in grain number is an adaptive mechanism to perhaps a decrease in photosynthesis caused by water stress. Experimental evidence shows that grain sorghum used in this study is source limited with regards to grain yield (Fischer and Wilson, 1975), but the sorghum grain yield is not limited by the storage capacity of the grain or by the transport system involved in moving material from the stem to the grain. Results from the current study conforms to Fischer and Wilson (1975) and Muchow and Wilson (1976) as the drought effect reduced number of grains set but could not decrease assimilate supply to the developing grains. Decrease in grain number and increase in weight per grain shows the negative correlation between grain number and weight per grain which frequently occurs in sorghum (Heinrich, *et al*., 1983; Ross and Hookstra, 1983; Blum, 1970). The drought applied may have reduced the ‘source’ for a decrease in grain number to have occurred but failed to reduce assimilate translocation into the grain ‘sink’ at the time of grain filling. This led to the compensation of decreased grain number by increased weight per grain.

6.3. The effect antitranspirant

In Experiments 1 and 2 included in Chapter 3, the effect of antitranspirant on transpiration, growth and yield of sorghum droughted at the booting to flowering growth stage and thereafter re-watered was evaluated using two different sorghum cultivars. Antitranspirant reduced transpiration, but growth and yield did not increase in droughted sorghum with the antitranspirant. The lack of significant increase in yield from the antitranspirant was attributed to either the drought not being severe enough to reduce yield or the antitranspirant concentration being too low to relieve the adverse effects of drought on the plants. Thus, in experiment 3 reported in chapter 4 the effect of increasing concentrations of antitranspirant above 1.0 L/ha on transpiration and yield of sorghum droughted from booting to flowering growth stage and thereafter re-watered was carried out. The results indicated that increasing concentrations of antitranspirant above the previous 1.0 L/ha rate decreased transpiration but yield of droughted sorghum did not increase significantly with increasing antitranspirant rates. This agrees with results from experiments 1 and 2. Therefore, the severity and duration of the drought stress was increased further in experiment 4 included in chapter 4 with the drought period being initiated at the 3- leaf growth stage and lasting to harvest without re-watering. The results showed that transpiration and yield were significantly decreased by drought at all growth stages, with a greater reduction occurring at the 3-leaf growth stage. Having established the appropriate sorghum growth stages to impose the drought treatment, in experiment 5 drought was initiated at the 3-leaf growth stage and lasted to harvest without re-watering and the antitranspirant was applied at the booting to flowering stage at a rate of 1.0 L/ha. The antitranspirant increased volumetric soil moisture content, decreased transpiration and did not increase the growth of droughted sorghum. The result was not conclusive in terms of grain yield, as grains were not harvested. But, with the increase in volumetric soil moisture content and decrease in transpiration, an increase in plant water content and yield in the sprayed over the unsprayed droughted plants was expected. Polytunnel experiments 1 and 2 were carried out to determine the responses of sorghum drought tolerance to film antitranspirant under field conditions and the results showed that the antitranspirant decreased transpiration, increased green area index but no significant increase in yield of droughted sorghum occured. The results validates a role by the film antitranspirant in decreasing transpiration and increasing green area index in droughted sorghum.

The antitranspirant may have decreased transpiration by reducing the size of the stomatal opening thereby decreasing the rate of diffusion of moisture vapour from the leaves. Although the size of stomatal openings were not measured in this study, decrease in stomatal conductance by the antitranspirant may indicate reduction in size of stomatal aperture by the antitranspirant solution, since stomatal conductance is influenced by stomatal size, density and distribution among others (Fanourakis, *et al*., 2014). Antitranspirant sprays caused a 30 – 37 % reduction in stomatal aperture in tomatoes (Rao, 1986) and Vapor Gard in particular caused a significant decrease in transpiration and led to the maintainence of a higher leaf water potential for a longer duration in droughted sprayed than unsprayed pea plants (Aldasaro, *et al*., 2019). To have increased green area index, the antitranspirant must have reduced the effect of water stress on plant water status and conserved soil and plant moisture contents thereby extending the period of time before growth is seriously limited.

However, the decrease in transpiration by the antitranspirant did not lead to higher grain yield in the droughted sprayed over the unsprayed sorghum. This contrasts with the results of Fuerhing (1973) which showed significant increase in the yield of sorghum sprayed with antitranspirant under dryland conditions. In the application of antitranspirants to plants it is widely recognized that the antitranspirant reduces transpiration and photosynthesis concurrently since stomata regulates both water vapor loss and carbon dioxide intake and so it is not desirable for arable crops where photosynthesis is important for yield. In the early 1970's, Fuerhring (1973) argued against this position that although photosynthesis is reduced, it is the potential photosynthesis and not the actual photosynthesis that is impaired by the antitranspirant. And that it is possible to reduce the potential photosynthesis without decreasing the actual photosynthesis hence no significant reduction in yield may be recorded. However, later experimental evidence from wheat (Kettlewell, *et al*., 2010) indicating reduction in photosynthesis by antitranspirant counters this position. To this Kettlewell (2014) added that although photosynthesis could be reduced in reality by the antitranspirant, the reduction is momentary and should diminish during grain filling particularly in wheat when sprayed at the stage of meiosis. The position that antitranspirant reduced photosynthesis and so should not be applied on food crops is not continually defensible as the choice is between the possibility of losing a crop during its most vulnerable period or salvaging it at the expense of a momentray reduction in photosynthesis.

Yield component compensation might have had a modifying influence on the effect of drought and consequently overall response of yield and yield components of sorghum to antitranspirant treatments throughout this study. One reason for the success of cereals including sorghum is their capacity for yield component compensation; that is when changes in one component is associated with changes in the opposite direction in another component (Evans and Wardlaw, 1976; Egli, 2017). Such that the later-determined component of yield compensates for earlier losses or restriction of development to take advantage of favourable conditions later in the crop life cycle (Evans and Wardlaw, 1976). So grain number was reduced by the drought treatment in Polytunnel Expt. 2 for instance and any moisture probably saved by the antitranspirant during the grain number setting period would have been used by the plant to generate more materials for grain filling at a later stage. But the antitranspirant induced no significant increase on weight per grain perhaps due to the confounding effects of the yield component compensation mechanism. In order to reduce the confounding effect of the yield component compensating effects, timing of the drought and antitranspirant spray to coincide with when the plant water content is very low could enable the antitranspirant to reduce further water loss from the plant and permit the production of more or heavier grains, despite the yield component compensatory effects. Therefore, the timing of the different growth stages is critical in determining the response of the yield components to drought and antitranspirant treatments. Manjareez-Sandoval, *et al*., (1989) showed that when water stress was imposed prior to anthesis, there was a 31 % decrease in grain number while grain size remained constant. Thus, there is a growth stage at which drought imposed could lead to reduction in grain number without any compensatory increase in weight per grain. In this direction Kettlewell *et al*., (2010) suggested that antitranspirant be sprayed on wheat at the stage of meiosis of pollen mother cells since at this stage drought damage causes floret abortion and eventually reduces grain number. The results from subsequent works in this area validates this growth stage as appropriate for application of antitranspirant to droughted wheat. Weerasinghe *et al.,* (2016) found increased pollen viability in wheat upon antitranspirant application and showed for the first time an underlying mechanism for yield increase in droughted wheat by antitranspirant. Abdallah, *et al*., (2015) showed decreased loss in grain number in wheat sprayed with antitranspirant just before the most drought sensitive stage. However, for sorghum there is no consensus as to the drought most sensitive growth stage except the booting to flowering stage identified in Lewis *et al*., (1974), Fuerhing (1973) and Inuyama (1978) among others. Till date the mechanism by which drought stress reduces grain yield in sorghum droughted at booting to flowering has not been elucidated. But, since yield compensation does not happen when drought is applied prior to anthesis stage as in Manjareez-Sandoval, *et al*., (1989) both drought and antitranspirant applications could be targeted to this growth stage, to ensure crop yield is not confounded by yield component compensatory effects.

6.4. Effect of Vapor Gard and Neem Oil

Neem Oil is being tested as an antitranspirant for the first time in this study and compares favourably with Vapor Gard as both chemicals were effective in decreasing transpiration but did not improve grain yield and yield components of droughted sorghum. The success of Neem Oil in reducing transpiration in sorghum for the first time is a step toward exploring its properties and potentials as antitranspirant vis-à-vis other known antitranspirants like Vapor Gard used in this study. However, to the resource poor farmers in parts of Africa and Asia where drought threatens food production the most compared with other continents, Neem Oil may hold a greater promise as an antitranspirant because it is potentially more accessible and comes at a lower cost than Vapor Gard. A comparison between the cost of the Vapor Gard and Neem Oil required to spray one hectare of sorghum in Nigeria can be made using the price of the Vapor Gard in the USA and the Neem Oil in Nigeria. Given that the price of the film antitranspirant Vapor Gard was £20 ($26) per litre (as in Kettlewell, 2011) and that of the Neem Oil in Nigeria was £67 ($88) per litre, and the antitranspirant was sprayed at the rate of 1 litre per hectare it means that the farmer requires only £20 ($26) and £67 ($88) for spraying 1 hectare of sorghum with Vapor Gard and Neem Oil respectively. This calculation is based on the assumption that other yield limiting factors did not significantly increase/decrease the yield. Although, it is obvious in the foregoing example that for 1 litre, Vapor Gard costs less than Neem Oil, if the cost of shipping Vapor Gard, for instance from the USA to Nigeria are factored into the costs, the cost of Vapor Gard may be greater than Neem Oil. In addition, the neem tree (*Azadiractha indica*) from where the Neem Oil was derived is abundant in Nigeria and India and low cost technologies exist for oil extraction from the neem seeds, which will also considerably reduce the cost. Since, results from the current study (Chapter, 4, Expt. 6) indicated Vapor Gard and Neem Oil can be substituted as antitranspirant, using the Neem Oil may be more cost efficient for farmers in Nigeria and probably India. Nonetheless, in this study (Chapter, 4, Expt. 6) the Neem Oil applied was formulated according to its use as an insecticide and cosmetic not as an antitranspirant. And it was mixed with only tap water and no adjuvant was added to enhance contact between the leaf surface and the antitranspirant, whereas the Vapor Gard with which it was compared was specially formulated as an antitranspirant. There may be need to formulate the Neem Oil solution in the form of an antitranspirant before comparing it with Vapor Gard so that the antitranspirant properties of both solutions is the basis for comparison. In addition, this experiment with the Neem Oil solution was carried out only once and needs to be repeated to further ascertain the results.

6.5. Effects of growth environments

During this study, sorghum was grown in the glasshouse and the polytunnels, these are artificial environments designed to simulate the natural growing conditions of sorghum, and these may have imposed limitations to the growth of the plant (Caddel and Weibel 1971). Any constraint in these growth environments could be interpreted by the plant as a stress factor. The implication of this is that sorghum which is by nature a drought tolerant plant could switch to one of its drought coping mechanisms that is osmotic adjustment, by accumulating organic solutes in its cells as means of maintaining cellular turgor to withstand what it identifies as stress imposed from the artificial growth conditions. Osmotic adjustment is reported to have maintained stomatal conductance and photosynthesis, reduced flower abortion, improved root growth and increased water extraction from the soil under water deficits in plants (Turner, *et al*., 2001). Therefore, sorghum grown in the polytunnels may have adjusted to water stress via osmotic adjustment and this could have played a key role in the lack of significant effects of drought on yield and yield components in the current study.

With regard to the glasshouse, growing sorghum in the summer under long day lengths and in mid-winter under low ambient light levels as well as possible exposure of the plants to incoming light from other sources within or outside the glasshouse for a longer period than required, may delay development and/or alter onset or termination of the various growth stages. Light intensity and duration likely influenced sorghum phenology because it is a short day plant and aspects of its development are controlled by photoperiod (Doggett, 1988). Also, as explained in section 3.6.1 stress develops too rapidly in plants grown in pots under experimental conditions because the roots densely permeate the soil, the soil dries uniformly and induces stress as capillary conductivity limit water supply to the plant. Thus, results obtained under these conditions can serve as a basis for further investigations in field conditions. For instance, stomatal conductances in many of the glasshouse experiments are very low whilst those in the polytunnel experiments are more consistent with published values. The reason for this could be that the plants grown in the glasshouse must have experienced greater levels of stress from the glasshouse enclosure causing higher temperature, higher humidity and lower vapour pressure defecit, in addition to the reduced water availability to the droughted plants. Also, water stress is known to affect stomatal development, as water stress from drought decreased stomatal number in Johnson grass (*Sorghum halepense*). Thus, these stress factors as well as reduced stomatal number must have resulted to the lower conductances in the glasshouse than in the polytunnel grown plants. Furthermore, since the probable cause for lower conductances in the glasshouse than in the polytunnel grown plants was reduced stomatal number rather than aperture, further reductions in conductaces by antitranspirant are possible under glasshouse conditions, since the stomatal aperture remains wide enough to accommodate a partial coverage by the antitranspirant.

To improve air circulation, increase vapour pressure deficit and stomatal sensitivity a standing fan was switched on and placed in the glasshouse during glasshouse experiments 4, 5 and 6.

To improve seedling germination, establishment and growth under both glasshouse and polytunnels, sorghum was grown in nurseries in the glasshouse and later transplanted to pots in the glasshouse and polytunnels in the field. This practice improved the establishment of the sorghum seedlings and stands and was adopted after failures in establishment of 1 glasshouse and 1 polytunnel experiments. Data on germination percentage and seedling establishment were not taken prior to the failures of the experiments, so a comparison cannot be made between transplanted and directly sown plants.

6.6. Contributions to knowledge

This research has made the following contributions to knowledge:

1. The first study to show the benefits of film antitranspirants application to droughted sorghum in terms of increasing green area index.
2. The first study to demonstrate the benefits of antitranspirants to transpiration and growth of droughted sorghum, since the work of Fuerhing in 1973.
3. The first study to successfully show that Neem Oil reduces transpiration in droughted sorghum, thus has the potential of an antitranspirant.
4. The first study to establish a rationale for further research to demonstrate the benefits of antitranspirants to sorghum production in Nigeria.

6.7. General conclusions

To gain an understanding of the role of antitranspirant application as an agronomic tool for improving drought tolerance in sorghum, this research was carried out to evaluate the response of sorghum drought tolerance to antitranspirant. It was hypothesized that antitranspirant do not improve drought tolerance in sorghum. The evaluation was done using the film antitranspirant di-1-p-menthene and sorghum varieties Pen 110, SAMSORG-40 and BirdGO Grain Sorghum (Bright Seeds, UK) grown in the glasshouse and polytunnels in 11 experiments carried out from February 2015 – January 2017.

Growing sorghum in the nurseries and thereafter transplanting led to successful establishment of the plants and shows for the first time the development of a clearly defined methodology for imposing defined water stress on different varieties of sorghum under two different experimental conditions: pot-grown and field environments. Antitranspirant increased green area index indicating an influence towards improving growth in sorghum under drought and a possible contribution of the antitranspirant in increasing leaf moisture content leading to increased green area index. The antitranspirant failed to register significant increase in yield and yield components, although numerical increases were recorded. This was not because the antitranspirant was ineffective in increasing sorghum yield under drought but it could be ascribed to the confounding effects caused by the drought treatment on the antitranspirant effect whereby reduction in grain number often leads to increase weight per grain, which could have reduced the antitranspirant effect. Although the yield difference between the sprayed and unsprayed droughted treatments were not statistically significant, the numerical differences was substantial enough in practical terms to show the potential benefits that can be derived from antitranspirant usage and to validate further research.

6.8. Practical application

In Nigeria sorghum is the third most cultivated cereal crop and is a staple food in the northern part of the country where drought is prevalent, with erratic rainfall distribution and volume. Till now sorghum farmers in northern Nigeria have adopted mixed cropping systems, mulching, early planting, high yielding hybrids and researchers have aimed at producing drought tolerant varieties as strategies to manage drought stress. However, none of these has overcome the challenges of low yield of sorghum under climate change. In northern Nigeria sorghum is being produced under conditions of deteriorating soil water due to early cessation of rainfall which causes terminal drought resulting in little grains being harvested (Flower, 1996). Film antitranspirant can be readily sprayed unto the plants at the flowering stage during the growing season, especially when drought is forecast, without any concern of its being washed off by the rains. Farmers in northern Nigeria are conversant with mixing and spraying herbicides with knapsack sprayers, which are readily available and accessible on a hire basis to spray herbicides. Therefore, the antitranspirant can be sprayed manually using the knapsack sprayer.

7. Further work

There has been renewed interest in the performance of droughted field crops sprayed with antitranspirants wheat (Weerasinghe, 2013; Abdallah, *et al*., 2015; Weerasinghe *et al.,* 2016) and rapeseed oil (Farralli, 2017) since the work of Kettlewell (2010). A number of further studies can be made to optimise the use of antitranspirant in improving the yield of droughted sorghum and other field crops.

Further studies should involve an investigation into the spray characteristics of the antitranspirant with respect to the sorghum leaf. Sorghum leaves are amphistomatous, having stomata on both sides of the leaf (Jones, 2014) with more stomata on the abaxial (lower) than on the adaxial (upper) surface (Liang *et al*., 1975). Turner (1970) reported a stomatal density of 7,400 and 11,300 stomata cm^{-2} on the adaxial and abaxial leaf surfaces respectively in sorghum. Therefore in a spray regime as in the current study only the stomata on the adaxial surface, ~40% are covered by the antitranspirant film, leaving the entire abaxial stomata, ~60% uncovered and sustaining a great degree of transpiration under stress. There is a need to carry out an investigation with the antitranspirant applied on both the adaxial and abaxial leaf surfaces to ensure greater coverage of the stomata which will enable a better assessment of the impact of the antitranspirant film on transpiration. Further investigation into whether or not the sunken stomata could contribute to reducing drought severity in sorghum and how this feature relates to the effectiveness of the antitranspirant should be carried out. In addition, the sorghum leaf has a waxy covering which could impede contact between the antitranspirant and the leaf surfaces. There is possibility that the antitranspirant particles may drift along the leaf after application. Therefore, the duration of the antitranspirant on sorghum leaf needs to be studied and compared with other crops under both glasshouse and field environments. Another aspect of the antitranspirant spray technology to sorghum that needs to be studied is the number of times to spray to achieve significant yield benefits under water stress. It may be necessary to evaluate spraying more than once or at least twice: at the point where grain number is set and when the individual grain weights are determined. This may reduce or eliminate the effect of yield component compensation.

In sorghum the plant body and the leaves also possess a waxy covering, these are considered as an adaptive trait to reduce water loss under stress (Manavalan and Nguyen, 2017). Further studies are required to determine the effect of antitranspirant on wax and bloom deposition, to ascertain whether or not the antitranspirant decreases wax deposition as an indication of reduced water stress on the sorghum plant. An assessment of the responses of photosynthesis and biochemical events like proline accumulation which increases under limited water supply needs to be carried out to ascertain their responses to antitranspirant under drought.

Molecular events such as gene expression under drought and antitranspirant needs to be studied to ascertain which genes are down regulated and which upregulated. This will provide information about the underlying molecular mechanisms suspended, sustained or activated in sorghum under drought and/or antitranspirant. Of particular interest here is the expression of the dehydrin gene induced by various stress factors that can cause cell dehydration, for example drought.

Testing different types as well as formulating plant extracts into antitranspirants and carrying out dose response studies is essential to broaden the scope of the various plant extracts that can be applied to sorghum as antitranspirant and at what doses. An example of such plant extracts are oils from shea tree (*Vitellaria paradoxa*), which is abundant and grows and thrives well in the wild in many tropical countries facing drought. In addition it is not yet part of the main cropping systems so does not compete with other crops in terms of food production. Thus getting the oil may be cheap in comparison with other plants.

There is also a possibility that sorghum drought tolerance could be improved by antitranspirant in combination with other agronomic practices like mulching, therefore further studies may involve applying antitranspirant and muching to sorghum at the most drought sensitive stage. Perhaps the interplay of soil water conservation via mulching and plant water improvement by the antitranspirant could lead to improvement in sorghum drought tolerance.

In addition yield component compensatory effects on the response of sorghum to antitranspirant can be reduced by designing the research to be more sensitive to detect the small differences often observed between droughted sprayed and unsprayed treatments. In Polytunnel Expt. 2, four replicates were performed; this number of replicates was sufficient for detecting very large differences between treatments, for example difference between irrigated and droughted for certain variables, however differences between droughted unsprayed and sprayed were too small to be detectable so were not significant. With the current experimental design a more sensitive arrangement can be achieved by increasing the replicates by dividing the plots into smaller portions with fewer plants, and taking data from all the plants on a smaller plot instead of from a few plants on a few large plots.

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