STUDIES WITH TRIAZOLES TO ALLEVIATE DROUGHT STRESS IN GREENHOUSE-GROWN MAIZE (*Zea mays*) SEEDLINGS

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
In
Crop and Soil Environmental Sciences

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5 May 2006
Blacksburg, Virginia

Keywords: maize, corn, drought stress, triazoles

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Abstract

In semi-arid environments, dry-land farming often exposes crops to drought stress. Although some plant species are well adapted to drought, most crops are not. Drought can reduce plant populations and limit growth and development in ways that have serious yield consequences. Planting at the beginning of the wet season, when rainfalls are often sporadic and unreliable, can expose young maize seedlings to severe drought. Through the use of plant growth regulators (PGR), maize seedlings can perhaps be altered to elicit responses that mimic drought adaptation mechanisms. A series of studies conducted in the laboratory and greenhouse looked at the response of maize seedlings (two hybrids that differed in their reported drought sensitivity) to severe drought and to PGR applications with or without drought. Results showed that drought stress altered plant morphology and key physiological parameters. Applications of three triazoles (paclobutrazol, uniconazole and tetraconazole) altered morphology and physiology in ways that might impart drought resistance. Paclobutrazol and uniconazole increased root:shoot ratio in laboratory studies and in the greenhouse. When compared to non-triazole-treated controls, uniconazole and paclobutrazol treatments caused water conservation in earlier stages of drought stress, and therefore afforded increased transpiration (and presumably less stress) at later stages. Uniconazole and tetraconazole increased photosynthesis of well-watered plants. Proline content was increased to a greater degree by these same two triazoles under drought stress conditions. It is hoped that knowledge obtained from these studies can be extended to drought-prone areas where maize dry-land farming is practiced.

Dedication

To my parents, Mr. and Mrs. Modikwa, and the rest of my family members; Oaitse, Gaone, Puso, Boago, Opelo, Mosupi, Maipelo, Moakanyi, Ontlametse and Ikanyeng.

To the loving memory of my brother and sister, Nonofo and Latelang.

Acknowledgements

I would like to thank Dr. David J. Parrish for his early ideas and directions regarding this research project. Dr. Parrish believed in my potential when he accepted my application for graduate studies in Virginia Tech, and he really taught me how to do good science, as he promised when we met in May 2004. Drs. Erik H. Ervin and John R. Seiler are thanked for agreeing to serve on my graduate committee and editing the manuscript, as well as their contributions in shaping the project at all stages.

The scholarship from the Botswana College of Agriculture is highly appreciated.

Many thanks are extended to the general CSES faculty, staff members, and graduate students. The following deserve special mention for their individual contribution; Dr. X. Zhang, Sybil Phoenix, Chandra Bowden, Kirill Kostyanosvsky, Patrick O'Boyle, Wynse Brooks, Liz Rucker, John Spargo, and Doug Raymond. Equipment operation training rendered free of charge by John James from Department of Horticulture and Nathan King of the Department of Forestry is highly appreciated. Alice Mutiti-Mweetwa from Horticulture Department contributed a lot of time and effort throughout this project. We also thank Dennis McCoy of Pioneer Hi-Bred International for the provision of seeds in these studies

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Introduction and Literature Review

1.1. Introduction and Rationale

Plant water deficit occurs when insufficient moisture prevents a plant from growing adequately and completing its life cycle. Insufficient moisture can be the consequence of a shortage in rainfall (drought), coarse textured soils that retain little water in the root zone, or drying winds (Swindale and Bidinger, 1981). Depending on the duration and extent of drought stress, a range of plant processes occurring at molecular, biochemical, cellular, and whole-plant levels may be altered. According to Neill and Burnett (1999), drought stress is one of the most important environmental factors limiting the growth and productivity of agronomically important plants.

Because land plants experience constant fluctuations in the availability of water, many have evolved various adaptive features. Many species have evolved in ways that reduce the potential for water stress. These drought ameliorating mechanisms are generally classified as either drought avoidance (developmental and morphological changes that minimize water shortages) or drought tolerance (physiological and biochemical adaptations that permit the plant to function in spite of shortages). By such adaptations, it is possible for some plants to complete their life cycle even in arid regions. In crop production, genetic improvement in drought adaptation is addressed implicitly by selecting for yield stability over locations and years (Nguyen et al., 1997). In addition to conventional breeding, advances in molecular approaches hold promise for physiological and breeding research on drought avoidance and tolerance in crop plants (Nguyen et al., 1997) Furthermore it might be possible to manipulate plants chemically such that they are less drought sensitive.

Plant adaptation to drought stress can involve avoidance mechanisms in which morphological changes in the roots and shoots occur. Reduced shoot growth and increased root development could result in increased water absorption and reduced transpiration, thereby maintaining plant tissue water status. In addition to such avoidance mechanisms, plant responses to water shortages can involve changes in biochemical pathways and expression of genes encoding proteins that contribute to drought adaptation. The proteins could be enzymes involved in the synthesis of osmolytes, antioxidants, or hormones such as ABA and others. Such changes can bring about drought tolerance, whereby plants continue to function at the low water potentials caused by water deficit (Hall, 1993). A central response to water deficit is often increased synthesis of ABA, which in turn induces a range of developmental (avoidance) and physiological or biochemical (tolerance) mechanisms.

Maize is a popular crop in the semi-arid regions of the world where periodic droughts are common. Under water stress, maize exhibits most of the responses mentioned, including both developmental (morphological) and biochemical changes: increased root to shoot ratio; accumulation of proline, glycine betaine, and soluble sugars; and increased antioxidant enzyme activities. Genetic modification of maize plants by breeding to allow growth and yield under water deficits is one approach to dealing with drought stress. This strategy has been employed over the years to produce drought-resistant maize varieties. However, this approach is time consuming and demands sustained effort. Growth regulators such as triazoles (paclobutrazol, uniconazole, tetraconazole) have been used on other plants and reported to affect developmental and physiological characteristics. This study will examine if such methods can be extended to maize under drought stress.

In my home country, Botswana, maize is typically planted at the beginning of the rainy season. However, in some years, the early rainfall pattern is sporadic such that plants will emerge and then be severely stressed by water deficits. In worst-case scenarios, populations are much reduced or plantings are entirely lost. If young maize seedlings could be made less sensitive to early drought stress, they might be able to survive and take advantage of the general rains when they finally do come. This study will look in a limited way at possible genetic differences between maize seedlings in their sensitivity to drought stress, but its primary emphasis will be on examining chemical manipulation of plants to see if that might help them survive early drought stress. An optimal outcome would be finding an easily applied chemical treatment that alters the early-season physiology of plants such that they could better withstand drought.

1.2. Literature Review

1.2.1. Some Key Drought Stress Effects

The ultimate detrimental effect of drought stress is reductions in yield as reported in crops such as rice (*Oryza sativa*) (Brevedan and Egli, 2003), wheat (*Triticum aestivum*) (Cabuslay et al., 2002), soybean (*Glycine max*) (Kirigwi et al., 2004), and chickpea (*Cicer aerietum*) (Khanna-Chopra and Khanna-Chopra, 2004). Various United States Department of Agriculture (USDA) reports have identified drought as the most frequent yield-reducing factor common in arid and semiarid regions, although water deficit may occur even in high rainfall areas (Vamerali et al., 2003). Indian production of cereals and pulses dropped by about 30% in 1971 due to drought (Swindale and Bidinger 1981). In the Sahel in Mauritania and Ethiopia, cereal production decreased by the same magnitude during the same period. The USDA reported

that drought of 1980, 1983, and 1988 significantly reduced U.S. maize and soybean yields (Taiz and Zieger, 1998).

Water stress due to drought can lead to major physiological and biochemical disruptions, such as reduced photosynthesis (Lawlor and Cornic, 2002, Tezara et al., 1999) and marked changes in gene expression (Neill and Burnett, 1999: Pattanagul and Madore, 1999: Romo et al., 2001). Physiological changes under drought stress are often reflected at the transcription level, where the levels of mRNA related to key processes such as photosynthesis are down-regulated (Bartels and Salamani, 2003). When plants experience water deficits, stomatal pores progressively close (Lawlor and Cornic, 2002; Saccardy et al., 1996; Tezara et al., 1999). This process is regulated largely by leaf water potential but can be mediated by ABA. Stomatal closure leads to decreases in photosynthetic CO_2 assimilation due to restricted diffusion of CO_2 into the leaf and altered CO_2 metabolism. Pelleschi et al. (1997) found that reduced CO_2 diffusion during stomatal closure is mainly responsible for the decline in photosynthesis in C3 plants subjected to dehydration. However, Tezara et al. (1999) reported that, in sunflower (*Helianthus annuus*) (C_3 plant) under water stress, the photosynthetic rate is limited more by altered CO_2 metabolism than by reduced diffusion. The lower CO_2 availability inhibits carbon assimilation, and ultimately photosynthetic capacity is lost as a consequence of the reduced stomatal conductance and/or direct damage to carbon metabolism (Bartels and Salamini, 2001: Colom and Vazzana, 2003).

Closure of stomata as result of water deficit and consequent decrease in CO_2 concentration in the leaf mesophyll results in the accumulation of NADPH in the chloroplasts. Under such conditions, where NADP is limiting, O_2 acts as an alternative electron acceptor resulting in the formation of super oxide radical ($O_2 + e^- \rightarrow O_2^-$) (Baisak et al., 1994; Gamble and

Burke 1984; Sairam et al., 1998). The super oxide radical (O_2^-), through a series of univalent reduction reactions, produces hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-), (i.e. $O_2^- + e^- + 2H^+ \rightarrow H_2O_2$, $H_2O_2 + e^- + H^+ \rightarrow OH^- + H_2O$) (Smirnoff, 1993). In addition to these species, super oxide can also generate singlet oxygen ($^1O_2$), through the reactions: 1) $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + {}^1O_2$, 2) $O_2^- \rightarrow e^- + {}^1O_2$, 3) $O_2- + H_2O_2 \rightarrow OH^- + OH^- + OH^- + {}^1O_2$, 4) $O_2^- + OH^- \rightarrow {}^1O_2 + OH^-$, 5) $H_2O_2 + H_2O_2 \rightarrow 2H_2O_2 + {}^1O_2$ (Thomson et al., 1987). The molecules (O_2^- , H_2O_2, OH^-, $^1O_2$), are called reactive oxygen species (ROS). They are highly toxic and can damage important cellular biomolecules such as lipids, proteins, nucleic acids and chlorophyll (Baisak et al., 1994; Fu and Huang, 2001; Li et al., 1998; Moran et al., 1994; Thomson et al., 1987). According to Zhang and Kirkham (1996), the harmful effects of ROS are due primarily to their ability to initiate autooxidative chain reactions on unsaturated fatty acids leading to lipid peroxidation and membrane destruction.

In pea (*Pisum sativum*) plants, levels of lipid peroxidation in leaves increased two to four fold with an increase in drought stress, and this was highly correlated with protein peroxidation (Moran et al., 1994). According to Fu and Huang (2001), production of malondialdehyde (MDA) as a measure of lipid peroxidation, increased due to drought in tall fescue (*Festuca arundinacea*) and kentucky blue grass (*Poa pratensis*). The MDA concentration of rice was reduced by mulching, and this was attributed to reduced production of reactive oxygen species and the subsequent lipid peroxidation (Chao et al., 2003). Similar results were observed in *Phillyrea angustifolia* (Munne-Bosch and Penuelas, 2003). Quartacci and Navari-Izzo (1992) observed that sunflower seedlings under water stress had lower chlorophyll, soluble protein and total and polar lipid content compared to controls. This positively correlated with free radical species (super oxides). However, in this experiment, MDA levels did not change in the stressed seedlings.

1.2.2. Drought Avoidance Mechanisms.

Drought-avoiding plants have the ability to complete their life cycle without severe water deficits developing. Some ephemerals have a very short life cycle that can be completed during a brief rainy season. Other plants exhibit adaptations to increase water uptake and reduce water loss and thereby avoid drought (Bartels and Salamini, 2001). It is an almost universal observation that the root:shoot ratio increases with water stress. Increases in root weight may be due to a greater density or depth of roots (Turner, 1979). Under water stress, new root growth extends into moist soil zones. As water deficits progress, the upper soil layers usually dry first. Thus shallower roots are common in wetter soils as opposed to deeper roots systems in dryer soils layer (Taiz and Zeiger, 1998). Therefore, greater root growth into moist soil can result in drought avoidance.

According to Hall (1993), reduced leaf area, deeper roots, and higher root:shoot ratios account for drought avoidance in most species. Root length, density and diameter help determine the ability of the plant to acquire soil water. Seedlings of *Welwitchia mirabilis*, a desert species, can produce a 3.5-m tap root within 10 weeks of germination (Fitter and Hay, 2002). A well-developed root system that provides for evapotranspirational demand from deep soil moisture is considered a major drought avoidance trait in upland rice (Nguyen et al., 1997). Thick roots persists longer, produce more and longer branches and thereby increase root length density and water uptake capacity (Nguyen et al., 1997). In pea (Chiatante et al., 1999), new lateral roots were produced during prolonged water stress. According to Ervin and Koski (1998), tall fescue was able to avoid drought better than Kentucky bluegrass by developing a deeper and more extensive root system. Thus, under low soil moisture, tall fescue was able to extract deeper soil moisture compared to Kentucky bluegrass.

Development in shoots also plays an important role in water stress responses. At the onset of dry seasons, a desert plant *(Zygophyllum qatarense*) responds to water stress by leaf polymorphism in which it develops unifoliate, xeromorphic leaves. As the dry season progresses, the plant tends to reduce its transpiring surface by substantial leaf loss (Sayed, 1996). Leaves of crops frequently wilt and droop under water stress, and this movement reduces the interception of radiation, thereby counteracting the increase in leaf temperature arising from stomatal closure and preventing further development of leaf water deficit (Turner, 1979). Severe drought stress may result in increased levels of ABA and subsequent leaf abscission, thereby reducing transpirational demand.

These developmental changes within a plant during water stress are important morphological drought-avoiding adaptations that maintain water potential.

1.2.3. Some Biochemical Drought Tolerance Mechanisms.

Drought tolerance is defined as the ability of plants to continue to function at lowered tissue water potentials. Drought-tolerating mechanisms often involve the maintenance of turgor (by accumulation of solutes) and/or desiccation tolerance (by protoplasmic resistance) (Jones et al., 1981). Several classes of compounds have been observed to accumulate under drought stress. Low molecular weight compounds, which would seem to act osmotically, include free amino acids (FAA) like proline in tall fescue (Abernethy and McManus, 1998), common bean (Gonzalez de Mejia, 2003), sugar beet (*Beta vulgaris*) (Gzik, 1995), wheat (Nayyar, 2003), and cotton (*Gossypium hirsutum*) (Sawhney and Singh, 2002: Showler, 2002). In addition to proline, wheat has been reported to accumulate the FAAs arginine, histidine, phenylalanine, threonine, and valine (Showler, 2002). Other plant metabolites altered by water stress and appearing to act as osmoregulators are carbohydrates, such as sugars and sugar alcohols (Pattanagul and Madore,

1999), and quaternary ammonium compounds (QACs), such as glycinebetaine and choline in wheat (Nayyar, 2003).

As soil dries, its water potential becomes more negative. Accumulation of solutes (osmolytes) by plant tissues lowers water potential, allowing plants to maintain turgor to lower water potentials. This process is called osmotic adjustment (OA). By contributing towards OA, osmolytes act as protectants for plants subjected to low water potential (Pandey et al., 2004). Osmotic adjustment maintains cell turgor, which allows cell enlargement and plant growth during water stress; and it can allow stomata to remain at least partially open and CO_2 assimilation to continue at water potentials that would be otherwise inhibitory (Alves and Setter, 2004). Osmotic adjustment by osmolytes has been reported to be a cause of improved productivity in wheat (Flower and Ludlow, 1987), sorghum (*sorghum bicolor*), barley (*Hordeum vulgare*), chickpea, pigeon pea (*Cajanus cajan*) (Khanna-Chopra and Khanna-Chopra, 2004), and rice (Lanceras et al., 2004).

In many plants that can adapt to water stress, a set of genes are transcriptionally activated, leading to accumulation of new proteins in seeds and vegetative organs and greater tolerance of drought. Proteins termed LEA (Late Embryonic Abundant), which were first characterized in cotton, are a set of proteins that accumulate in embryos at the late stage of seed development (Xu et al., 1996). These proteins were induced earlier in embryogenesis when immature embryos were dissected and incubated in ABA solutions.

The LEA proteins have now been detected in plants, not just embryos, of maize, barley, wheat, cotton, sunflower, soybean (Dure, 1993), rice, Brassica (*Brassica napus*) (Baker et al., 1988), and dehydrated resurrection plants (Bartels and Salamini, 2001). These proteins have been assumed to function not as enzymes but to protect the plant cell during dehydration. Their

hydrophilic nature and high solubility indicates that the proteins are maintained in the cytosol. A *lea* gene, *HVA*1 from barley when over-expressed in rice led to improved growth under water stress (Xu at al., 1986). The extent of growth in response to stress correlated with the amount of protein. Baker et al. (1988) observed that, in many cases, the expression of the *lea* genes can be increased by desiccation and/or exogenous ABA at any stage of plant development.

The LEA protein sequences are related to two other groups of proteins, namely; the RAB (responsive to ABA) proteins and the dehydrin (LEA D11 subgroup) proteins (Giordani et al., 1999; Taiz and Zeiger, 1998). An association between tolerance to drought stress and these groups of proteins has been observed in some crop plants. In blueberry (*Vaccinium corymbosum*), the dehydrins were found to accumulate in response to changes in ABA levels during drought stress (Panta et al., 2001). Mundy and Chua (1988) identified a water-stress-inducible gene in rice called *RAB21*. The *RAB* genes have been expressed in osmotically stressed vegetative tissues of barley, wheat, and soybean (Plant and Bray, 1999). A *RAB 17* gene from maize overexpressed in *Arabidopsis thaliana* conferred osmoprotection in the transgenic plant (Figueras et al., 2004).

A common feature of the LEA, RAB and dehydrin proteins is that their production is stimulated by desiccation of seeds or plant water stress. They are water soluble and hydrophilic and have been proposed to function especially in the protection of membranes and other proteins against desiccation damage, possibly by binding water tightly (Taiz and Zeiger, 1998). As suggested by Baker et al. (1988) and Romo et al. (2001), they could be involved in the signal transduction pathway that probably uses ABA as a signal transducer leading to gene expression under drought conditions. Although the specific roles of the LEA proteins remain unclear, it is clear that they are regulated by ABA and cellular water loss.

Another class of proteins that may be important for osmotic balance maintenance in drought tolerance are the water channel proteins (aquaporins), which are present in all organisms (Bohnert and Jensen, 1996). Hydraulic conductivity of cells and tissues could be regulated by levels of the aquaporins translated and/or post translationally by phosphorylation of specific amino acid residues (Johansson et al., 1996). Protein phosphorylation as observed *in vivo* in spinach leaf plasma membrane by Johansson (1996) was dependent on water potential. Although not well understood in relation to other physiological processes, ABA is also reported to be one of the factors that induce the expression of aquaporins (Schaffner, 1998).

In plants, aquaporin proteins localized in the tonoplast are called tonoplastic intrinsic proteins (TIPs), and those in the plasma membrane are called plasma membrane intrinsic proteins (PIPs) (Smart et al., 2001; Tyerman et al., 2002). So far, aquaporins (PIPs and TIPs) have been characterized from *Arabidopsis thaliana,* tobacco (*Nicotiana tabacum*)*,* cowpea (*Vigna unguiculata*), ice plant (*Mesembryanthemum crystallinum*)*,* and spinach (*Spinacia oleracea*) (Schaffner, 1998).

The rate of water flux into or out of a cell is determined by the water potential that acts as the driving force for transport and by the permeability of the membrane. Aquaporins form water-specific pores as an alternative to diffusion through the lipid bilayer, thereby increasing the water permeability of the cell. There is accumulating evidence that aquaporins are involved in plant responses to dehydration stress. Phosphorylation of PIP aquaporin in spinach decreased with decreasing water potential, suggesting closing of plasma membrane water channels in response to water deficits (Johansson, 1996). This protein may be dephosphorylated and water channel inactivated under conditions of dehydration, perhaps to allow water conservation.

In a recent study with tobacco plants, (Aharon, et al., 2003), an over-expressed *Arabidopsis* PIP caused faster wilting under drought stress. The transgenic tobacco plants under non-drought conditions had increased growth, transpiration rate, stomatal density, and photosynthetic efficiency. According to Smart et al. (2001), in *Nicotiana glauca,* a plant well-adapted to arid environments, MIP gene expression is down-regulated under drought stress. In ice plant, as the leaves are rehydrated, transcript levels of the MIP proteins increase (Yamada et al., 1995). However, these observations should be treated with caution, as aquaporin gene expression can be induced by dehydration stress, which should result in osmotic water permeability and loss. In *Nicotiana excelsior*, a moderate decrease in leaf water potential results in down-regulation of PIP gene expression, but more drastic decrease was found after two days of drought stress resulting in up-regulation (Yamada et al., 1997).

Although there may be conflicting reports on the effect of cell water potential on aquaporins, regulating their concentration (expression of genes) and activity (phosphorylation and dephosphorylation) may be additional mechanisms for responding to reduced water availability and to increased drought tolerance.

Plant cells, including photosynthetic cells, are protected against the detrimental effects of reactive oxygen species (ROS) by an antioxidant system that has been associated with stress tolerance in plants. The system is composed of enzymatic and nonenzymatic detoxification mechanisms, which mitigate and repair damage initiated by the reactive oxygen species (Fu and Huang, 2001; Zhang and Kirkham 1996). The enzymatic system is made of the enzymes superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (AP), and glutathione reductase (GR) (Li et al., 1998). Primarily, SOD reacts with the superoxide radicals to generate H_2O_2, H_2O and molecular oxygen (O_2). The H_2O_2 so produced is disposed of by POD

and CAT. Ascorbate peroxidase and GR take part in the ascorbate-glutathione cycle, where H_2O_2 is removed and ascorbic acid is regenerated (Li et al., 1998; Smirnoff, 1993; Thomson, 1987; Zhang and Kirkham 1996). In addition, GR maintains a high ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG), which protects chloroplasts against oxidative damage (Gamble and Burke, 1984). Wheat leaves exposed to mild water stress (-0.5 MPa) exhibited increased activities of SOD, CAT, AP, and GR, which led to reduced lipid peroxidation. However, the system was not effective at severe water stress (-1.5 MPa) (Baisak et al., 1994). These results suggest that the degree of water stress is also important in the systems' protection and that it may represent a drought tolerance response.

The nonenzymatic antioxidants that might improve drought tolerance are lipophilic vitamin E (α-tocopherol), hydrophilic vitamin C (ascorbic acid) and carotenoids. Vitamin E reacts with O_2^- as well as $^1O_2$. Vitamin C can react directly with H_2O_2 in a reaction mediated by AP (Smirnoff, 1993). Glutathione is the substrate for the formation of vitamin C which has the ability to react directly with free radicals such $OH^\cdot$ (Pastori and Trippi, 1992). In addition to their role in energy dissipation in plant tissues, the carotenoids, particularly β-carotene remove singlet oxygen ($^1O_2$). According to Stuhlfauth et al. (1990), total carotenoids increased after one day of stress in leaf disks of two oak species. At low water potential (-2.5 MPa), there was a 25% increase in β-carotene, and this correlated with $^1O_2$ levels, suggesting protection against this species (Stuhlfauth et al., 1990).

In summary, the ROS generated under water deficit can be counteracted in drought-tolerating plants by the activation of the antioxioxidant systems to remove the species from the affected cells. This however, depends of the degree of water deficit, as high levels of stress could cause damage to the antioxidant system itself.

1.2.4. The Role of ABA and other Hormones in Plant Drought Responses

Plants have complex physiological and biochemical sensing and response systems to cope with the changing environment. Growth and development, which can be modified in response to drought, is regulated by endogenous plant hormones. The hormones may be produced to modulate secondary messengers important in cellular protection against stress (Aroca et al., 2003). Ethylene, gibberellins, abscisic acid (ABA), cytokinins, and auxins are regarded as the "classical" five hormones. Abscisic acid is perhaps the most frequently mentioned in drought related studies. When plants are exposed to water stress, there is often an increase in ABA content. Dehydrating plant roots produce increased amounts of ABA, which acts as an important mediator between water-stress imposed changes and physiological responses.

Potassium, which is present within plant cells as the cation K^+, interacts with ABA to play an important role in regulation of the osmotic potential of plant cells (Taiz and Zeiger, 1998). In roots, applied ABA can cause a range of effects on K^+ transport, such as increased efflux or influx, depending of concentration of ABA, cell type, and tissue K^+ status (Ober and Sharp, 2003; Robert and Snowman 2000). The hormone can also influence K^+ flux in guard cells of stomata and hence stomatal apertures.

It has also been reported (Nieves et al., 2001; Vamereli et al., 2003) that proline accumulation in water-stressed plants is under the partial control of ABA. This observation is supported by a report from Carseller et al. (1999) that increased levels of ABA led to proline accumulation. Similar observations were reported in rice (Yang et al., 2000) and in canola (*Brassica napus*) leaf discs (Trotel-Aziz et al., 2003). Glycine betaine has also been reported to increase in drought-stressed pear leaves treated with ABA, and this enhanced drought resistance

in the plants (Gao et al., 2004). In barley plants, glycine betaine synthesis is reported to be an important response to drought and water deficit stress and it is induced by ABA (Jagendorf and Takabe, 2001). These results have been supported through molecular studies on the effect of osmotic stress and ABA on the synthesis of glycine betaine (Gao et al., 2004; Ishitani et al., 1997).

Abscisic acid is involved in the expression of numerous genes and protective proteins during water stress and in tissues undergoing desiccation (Alves and Setter, 2004; Bray et al.1999; Bray, 1988). The role of ABA in the control of gene expression has been demonstrated by using deficient mutants of maize and tomato (Borel et al., (2001). Genes that require ABA for induction have been identified in tomato (Cohen and Bray, 1990), maize (Figueras et al., 2004), and *Arabidopsis* (Lang and Palva, 1992). An mRNA (his1-s mRNA) of a protein structurally similar to HI histone was detected in petioles, stems, and tomato fruit pericarp after being subjected to water deficit and application of ABA to detached leaves (Bray et al., 1999). The H1 histone proteins play a central role in plant drought resistance (Bartels and Iturriaga, 2004). The RAB 17 (responsive to ABA) is a maize LEA protein, inducible by ABA during embryogenesis and also by ABA and water stress in vegetative tissues (Figueras et al., 2004).

In addition to functions related to water stress, it is believed that ABA is involved in other stress responses that induce other endogenous hormones such as ethylene, a major hormonal factor promoting leaf senescence. In plants such as rice (Chen and Kao, 1990), pine (Rajasekarana and Blake, 1999) and maize (Sharp, 2002), ethylene production is substantially greater under water stress than in the control plants. In poplar (*Liriodendron tulipifera*) (Chen et al., 2001) and citrus (*Citrus sinensis*) (Gomez-Cadenas et al., 1996), elevation of ABA concentration promoted ethylene production after the onset of drought or ABA treatment. On the

other hand, Sharp (2002) reported that, under water stress, ethylene production of ABA-deficient maize seedlings was substantially greater, and exogenous application of ABA gave the opposite results. These findings indicate that the concentration of ABA is important in control of ethylene production under water stress. Water stress in rice markedly increased ABA accumulation, whereas drought substantially decreased gibberellic acid (GA) contents in grains (Yang et al., 2001).

Cytokinins promote cell division and can act in concert and in antagonism with ABA. High levels of cytokinins in plants can delay senescence, whereas ABA accumulation has the opposite effect. According to Yang et al. (2003), water stress increased ABA accumulation and reduced cytokinins in wheat. Application of synthetic cytokinins [benzyladenine (BA) and hydroxybenzyladenosine (HBA)] to substrate or sprayed on leaves of sugarbeet exposed to water stress stimulated net photosynthesis rate, transpiration rate, and stomatal conductance (Vomacka and Pospisilova, 2003). However, the recovery of water-stressed sugarbeet plants after rehydration was not markedly and consistently improved by BA and HBA.

These findings suggest that ABA plays an important, perhaps central role in mediating the primary responses of plants to drought stress. In addition, an increase in ABA concentration in response to drought stress may act as a chemical signal to trigger a series of biochemical reactions and physiological responses including the gene expression related to drought tolerance.

1.2.5. Some Maize-Specific Responses to Drought Stress

Maize is grown all over the world under a wide range of conditions. Wherever it is grown, drought can cause yield reductions (Li and Van Staden, 1998; Maiti et al., 1994; Maiti et al., 1996). Genotypic differences have been identified for a range of morphological and physiological characteristics and responses of maize to water stress. An increase in root growth

and increased root:shoot ratio by plants growing in conditions of water stress may be an important adaptive feature (Fitter and Hay, 2002; Hall 1993; Nguyen et al., 1997). At water potentials that completely inhibited shoot growth, the primary root continued to grow in maize (Sharp and Davies 1979; Sharp et al., 1988). In some cases, the roots of drought stressed plants were thicker and more cylindrical (Sharp et al., 1988). Thickened roots could results in lowered resistance to water flow within the roots and between the roots and the shoots (Turner, 1979).

Accumulation of ABA under water stress has been reported to maintain root growth and inhibit shoot growth in maize seedlings (Saab et al., 1992). This observation was supported by Maiti (1996), who showed a significant negative relationship between the rate of leaf expansion and concentration of ABA in xylem of maize under drought stress.

Saccardy et al. (1996) reported in maize, which is a C_4 plant, that inhibition of net photosynthesis by water stress was due more to stomatal closure and reduced CO_2 diffusion than to inhibited CO_2 metabolism. This depended on the speed of dehydration, as rapid dehydration led to inhibition of CO_2 metabolism due to down regulation of the Calvin cycle enzymes namely phosphoenolpyruvate, malate dehydrogenase, and malic enzyme (Saccardy et al., 1996).

Sucrose is the transported sugar in most higher plants. Sucrose, as it cannot be used directly for most metabolic processes, must be cleaved into hexoses (glucose and fructose) by invertase and sucrose synthase (Pelleschi et al., 1997). Maize under water stress has increased invertase activity resulting in hexose accumulation in leaves (Kim et al., 2000; Pelleschi et al., 1997). It has also been observed that invertase (*Ivr2*) gene expression was enhanced by ABA supply in maize leaves (Trouverie et al., 2003). This behavior was also previously noted by Kim et al. (2000). The increase in soluble sugars under water stress has been reported in orchids (Stancato et al., 2001) and coleus (Pattanagul and Madore 1999). In both studies, the sugars were

suggested to play a role in osmotic adjustment. Therefore the accumulation of glucose and fructose in maize experiencing drought may be involved in a signal transduction pathway or an increase in osmotic pressure leading to drought stress tolerance.

Maize often accumulates solutes besides sugars that are important in osmotic adjustment under water stress. Proline, glycine betaine, K^+, and carbohydrates have been reported to accumulate as stress responses in maize. The source of proline production in maize is reported to be mature chloroplasts (Ibarra-Cabalero et al., 1988) and endosperm of germinating seedlings (Raymond and Smirnoff, 2002).

Drought increases proline accumulation in maize (Carceller et al., 1999; Ibarra-Cabalero et al., 1988; Voetberg and Sharp 1991). Proline in maize is important in osmotic adjustment and therefore drought tolerance. According to Ibarra-Cabalero et al. (1988), addition of ABA to maize tissues did not result in proline accumulation. However, Ober and Sharp (1994), working with maize seedlings grown in vermiculite under water stress, concluded that increased ABA is required for proline accumulation in the growing region of maize roots. Similar results were reported in canola leaf discs (Trotez-Aziz et al., 2000; Trotez-Aziz et al., 2003). Wei and Qi (2000) observed that ABA-treated maize had double the amount of proline and increased osmotic adjustment and greater drought tolerance. These findings suggest that, in maize under drought stress, ABA could be required for proline accumulation. Perhaps this might depend on the experimental tissue or its stage of development.

Glycine betaine accumulation has been reported in plants under water stress such as pear (Gao et al., 2004) and barley (Jagendorf and Takabe, 2001), where it has been reported to play a role in osmotic adjustment in tissues and organs experiencing osmotic stress. Glycine betaine has been reported in maize as an osmoprotectant. However, there are significant genotypic

variations, with some genotypes not accumulating glycine betaine at all (Brunk et al., 1989). This variation across genotypes is due to genetic differences, whereby the deficiency is caused by a single gene in the homozygous recessive condition (Brunk et al., 1989). The betaine-accumulating genotypes have been found to be more salt tolerant than the non accumulating lines (Yang et al., 2003).

Like other plants, maize under water stress accumulates proteins. Abscisic acid (ABA) was found to increase synthesis of unidentified proteins in both water stressed and non-water stressed maize seedlings (Heikkila et al., 1984). In the same study, water stress also caused reduction in overall protein synthesis, which recovered to normal levels upon rewatering if the stress had not been severe. Water stress and ABA induced the RAB17 LEA protein in maize embryogenesis and vegetative growth (Figueras et al., 2004). According to Vilerdell (1990), ABA induced the synthesis of RAB17 mRNA and protein in maize calli and embryos, where protein phosphorylation was found only in the maize embryos. Thus maize under water stress has altered protein synthesis, which in this case is the RAB (Responsive to ABA) protein. The other unidentified proteins that were induced under water stress (Heikkila et al., 1984) are likely to be water channel proteins (aquaporins).

Water stress in maize has been found to cause oxidative damage and to elicit the counteractive enzymatic and non-enzymatic antioxidant system. When drought-tolerant (PAN 6043) and sensitive (SC 701) hybrids were exposed to water stress (-0.5 MPa), H_2O_2 and MDA levels increased, and their values were higher in the sensitive than the tolerant genotype (Li et al., 1998). In this study, the activities of the antioxidant enzymes (SOD, CAT, AP, POD, and GR) increased, and the values were higher in the drought tolerant than the sensitive cultivar. The ascorbic acid and carotenoid levels were reduced by water stress. Similar results were reported

by De Longo et al. (1993), where the enzyme activities were higher in the drought tolerant (LIZA) than the sensitive (LG 11) genotype, with apparent damage to chlorophyll and carotenoids in both cultivars. The increased oxidative stress and the enzyme responses exhibited by maize due to water stress (-0.5MPa) are similar to plants such as wheat (Baisak et al., 1994; Sairam et al., 1998), other grasses (Fu and Huang, 2001), and sunflower (Quartacci and Navari-Izzo, 1991). Therefore the enzymatic antioxidant system appears to be one of the most important water stress tolerance mechanisms in many plants, including maize.

In summary, when maize is experiencing a water deficit, morphological, physiological, and biochemical changes occur. In almost all the cases, the changes are mediated by or appear to be related to changes in ABA levels.

1.2.6. The Effects of Some Triazoles on Plants: Development and Potential for Drought Stress Alleviation.

Triazoles are a group of fungicides and plant-growth-retarding chemicals, which contains three conserved nitrogen atoms within a five-member ring. Various of these compounds, including paclobutrazol, uniconazole, tetraconazole, and triadimefon, cause remarkable growth responses in plants. Changes caused by triazoles are mediated through cytochrome P-450 group of enzyme inhibition (Sopher, et al., 1999; Taiz and Zeiger, 1998; Zhu et al., 2004). Plant cytochrome P-450 enzymes catalyze oxidative processes in hormone, sterol, oxygenated fatty acid and phenylpropanoid biosynthesis pathways (Chappel, 1998; Rademacher, 2000).

The primary plant-growth-regulating properties of these chemicals are mediated through inhibition of GA biosynthesis and ABA degradation pathways. Recently, brassinosteroid synthesis inhibition in Arabidopsis seedlings by brassinazole was reported (Asami et al., 2000). In GA biosynthesis, oxidation of *ent*-kaurene to *ent*-kaurenoic acid is inhibited, causing

reduction in GA accumulation (Kalil and Rahman, 1995; Ozmen et al., 2003; Sopher et al., 1998). It has also been shown that triazoles stimulate the accumulation of ABA in plant leaves in a similar way to drought (Asare-Boamah et al., 1986; Ronchi et al 1999; Zhu et al., 2004). This is probably due to the prevention of ABA degradation to phaseic acid by hydroxylation. Fletcher and Arnold (1986) reported that treating cucumber (*Cucumis sativus*) seedlings with triadimefon increased cytokinin levels. These observations were indirectly attributed to higher root growth, and roots are considered to be the predominant site of cytokinin synthesis. This is due to the fact that, there is no metabolic link between triazole action and cytokinin synthsis (Rademacher, 2000). The inhibition of GA and brassinosteroid syntheses, ABA degradation and cytokinin accummulation could cause changes in proportional concentration of these phytohormones associated with regulation of plant development.

The secondary characteristic changes observed in triazole treated plants include morphological changes such as, reduced shoot growth and increased root growth (Asare-Boamah et al., 1986; Ronchi et al., 1999; Zhu et al., 2004). In addition, other biochemical and physiological alterations have been reported, which include; enhanced antioxidant system (Gilley and Fletcher, 1997; Ozmen et al., 2003; Senaratna et al., 1988), increased levels of proline and chlorophyll contents and photosynthetic efficiency (Mackay et al., 2002 and Senaratna et al., 1988). Partial closure of stomata and reduced transpiration has been reported. Treating peas, wheat and soybean with triadimefon caused reduced transpiration (Fletcher and Nath, 1984).

Among the various triazoles developed as plant growth regulators are paclobutrazol ([2RS, 3RS]-1-[4-chlorophenyl]-4,4-dimethyl-2-[1,2,4-triazol-1-yl]pentan-3-ol) and uniconazole ([E-1-[4-chlorophenyl]-4,4-dimethyl-2-[1.2.4-triazol-1-yl]penten-3-ol) (Arteca, 1996; Ronchi et

al, 1987). Tetraconazole (±)-2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazole-1-yl)-propyl,1,1,2,2-tetrafluoroethyl ether) is used as a fungicide (Gilley and Fletcher, 1997).

There is evidence that triazoles applied to plants could be a means to alleviate water stress. In fact, plant responses such increased ABA, proline, antioxidants and stomatal closure observed after triazole treatment appear to mimic some drought avoidance or tolerance mechanisms. This has been reported in wheat (Berova and Zlatev, 2003;Gilley and Fletcher, 1997), tomato (*Lycopersicon esculentum*) (Still and Pill, 2004), perennial rye grass (*Lolium perenne*) (Jiang and Fry, 1998), and silver maple (*Acer saccharinum*) (Marshall et al., 2000).

Maize treated with uniconazole increased the antioxidative system (Li et al., 1998). Tetraconazole produced maize with shorter, thicker leaves, higher relative water content, as well as increased anthocyanin and and secondary metabolite (flavonoid) levels (Ronchi et al., 1997). Paclobutrazol depressed shoots and roots and resulted in more chlorophyll and carotenoids (Sopher et al., 1999; Kalil and Rahman, 1995). In another report, paclobutrazol retarded shoot growth, enhanced lateral root growth, produced darker green leaves, and resulted in a more extensive root system (Lin and Zhang, 1999). Although these characteristics developed, drought stresss was not imposed in most cases. Due to the physiological changes affected by the mentioned plant growth regulators in maize and other plants, especially those mediated by ABA, it might be possible to extend this knowledge and apply it in drought stress situations. This could be a means of crop improvement, as the chemicals can positively affect the biochemical, physiological, and morphological responses to water deficit.

1.2.7. The Effects of Paclobutrazol, Tetraconazole and Uniconazole on Seed Germination and Early Seedling Growth.

During germination and early seedling growth, the stored food reserves such as starch of the endosperm are broken by amylases to soluble sugars. The soluble sugars fuel respiration of the embryo for growth. The cereal embryo regulates mobilization of its food reserves through secretion of GA, which activates amylase synthesis. In addition GAs may be required for activation of the embryo vegetative growth (Taiz and Zeiger, 1998). Absiscic acid has been reported to inhibit the synthesis of amylases that are essential for breakdown of storage reserves (Fosket, 1994; Taiz and Zeiger, 1998). According to Skriver and Mundy (1990), application of ABA to germinating cereal seeds reduces GA gene expression necessary for germination. The roles of GA and ABA on seed germination processes imply that treating seed with triazoles may have effects on germination and early seedling growth. The growth retarding activities of triazoles could also be through repression of enzymes responsible for remobilization of food storage reserves. This is supported by (Hathout, 1995; Prusakova et al., 2004), who found that treating seeds with uniconazole and paclobutrazol inhibit amylase and proteinase activities.

Increasing paclobutrazol concentrations reduced germination in marigold, geranium and tomato (Pasian and Bennett, 2001). Similar results were obtained when paclobutrazol was used on seeds such as silver maple (Marshall et al., 2000), peach (*Prunus persica*) (Gianfagna and Rachmiel, 1986), tomato (Still and Pill, 2004) and maize (Khalil and Rahman, 1995). The germination inhibition by paclobutrazol was accompanied with reduced root, shoot, primary leaf and internode elongation (Gianfagna and Rachmiel, 1986; Marshall et al., 2000; Pasian and Bennett, 2001). In addition to retardated growth caused by paclobutrazol, maize seedlings appeared greener than the controls (Khalil and Rahman, 1995), which suggest that paclobutrazol

treated seedlings had more chlorophyll. Uniconazole has also been reported to reduce germination in rice, as well producing short seedlings (Matsunaga and Yamaguchi, 1993). Uniconazole has also been demonstrated to affect seedling development without any effect on germination. This was found in pea, where leaf number, leaf area, seedling fresh and dry weights were decreased without total germination reduction (Hathout, 1995). Li et al, (1998), also found that maize seed treated with uniconazole had increased antioxidant system responses in a drought tolerant cultivar, and this did not affect the drought sensitive cultivar. The growth regulating activities of tetraconazole are similar to those of paclobutrazol and uniconazole. However, tetraconazole was found to be effective in causing root morphological changes such as shorter root systems and thickened primary and secondary roots in maize (Ronchi et al., 1997). Tetraconazole has also been found not to affect seed germination and seedling height in wheat (Gilley and Fletcher, 1997). This suggests that depending on the triazole type, concentration and plant species, different morphological and physiological developments may be observed on plants.

The triazoles may affect early seedling growth, plant morphology, and physiology in ways that could provide for drought avoidance and/or tolerance. Despite the potential for negative effects on germination, their application might alleviate seedling drought stress when applied at the right concentration in maize.

1.3. Objectives

The initial goals of this work were to:

- examine effects of population and drought on maize seedling growth and survival.
- test for genotypic differences in response to early drought stress.

- examine effects of PGR treatments on germination, seedling growth and drought responses.
- determine the mechanism(s) for drought responses observed.

To these ends, a series of studies were carried out in the laboratory and greenhouse to:

- characterize or describe the epidemiology of drought stress in young maize.
- examine responses of seedlings of two hybrids known to differ in drought sensitivity.
- apply triazoles and examine the effects on seedling growth and drought responses.
- examine some likely physiological/metabolic modes of drought response.

Chapter 2

Materials and Methods

2.1. Two Studies to Characterize Drought Stress in Maize Seedlings

2.1.1. Preliminary study with a drought-sensitive hybrid (to establish protocols, measurement parameters, and an LD_{50})

2.1.1.1. Plant culture and drought treatments

This experiment was conducted in October through December 2004 in a greenhouse with temperatures moderated near 25/20°C day/night. Empty 4-L pots were weighed, filled with grade #3 vermiculite, and reweighed to determine the weight of pots plus vermiculite. Maize (*Zea mays*, hybrid 32W85, Pioneer Hybrid International, Johnston, IA) with a moderately low drought-resistance rating of 5 (Dennis McCoy, personal communication) was germinated in the vermiculite-filled pots. Planting populations were 4, 8, 12, or 16 seeds per pot. At 10 days after planting (DAP) and approximately 3 days after emergence, plants were supplied with a general-purpose fertilizer, 20-20-20, (Scott-Sierra Horticultural Product Co, Marysville, OH) dissolved in water to provide 50 kg $N/P_2O_5/K_2O$ ha^{-1}. Drought stress treatments were randomly assigned in each of the four replications to pots of each planting density beginning at 14 DAP by withholding watering for 0 (controls), 14, 21, 28, or 35 days. The control pots were watered as needed to maintain soil moisture at or near field capacity. At the end of each drought-stress period, before re-watering, the pots were weighed to estimate soil moisture percent and eventually to determine soil water potential (Ψ_{soil}) (see below).

After the 35-day water-stress period ended for that set of pots, all pots received a second fertilizer application (50 kg N/P/K ha^{-1}) and were grown for a further 14 days. At this time (63 DAP), plant height and number of leaves were recorded. Plant height was measured on four

central plants in each pot. The height was measured from the soil surface to the tip of the youngest fully expanded leaf. Plants were harvested at 63 DAP and separated into roots and shoots. The roots were washed free of vermiculite. The materials were dried in a forced-air drier at 65 ^{o}C for 7 days, weighed to determine dry matter, and root to shoot ratio was then calculated.

2.1.1.2. Determination of substrate moisture content and calculation of water potential

Vermiculite moisture content was determined as percent gravimetric water (θg) according to Royo et al. (2001).

$\theta g = TW - [(PW + MDW + SFW)] / (MDW) \times 100$

TW = Total weight of the system (pot + media + seedlings) (g)

PW = Weight of empty pot (g)

SFW = Fresh weight of 12 seedlings (g)

MDW = Dry weight of media in a pot (g)

The percent volumetric water content (θv) was determined by multiplying θg by the substrate's bulk density (Cruz et al., 1992). The vermiculite's bulk density was empirically determined to be 0.1302 g cm^{-3}. This was done with four replicate samples, measuring their volumes in cm^3 and corresponding weights in g. The sample weight was divided by its volume to give bulk density. A moisture release curve (Figure B.1), for grade 3 vermiculite was obtained using a standard pressure plate procedure (Olson, 1979). This was used to estimate water Ψ_{soil} at any θv.

2.1.1.3. Experimental design and statistical analyses

The experiment was a factorial combination of treatments (four planting populations by five drought stress periods), arranged as a randomized complete block (RCB) design replicated

four times. All data sets were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedures of SAS (SAS Institute, Cary, NC). Differences among treatments and their interactions were determined, and treatment means were separated using Fischer's least significant difference (LSD) at 0.05 probability level.

2.1.1.4. Methodology/protocols established by this study and used in subsequent studies

As expected and as will be reported in the results, higher plant populations and longer periods of withholding water caused progressively greater drought stress. Although 16 plants per pot provided high levels of water usage and drought stress, only minimal levels of lethality were observed even after 35 days without watering; and therefore, an LD_{50} was not established. For subsequent studies, we used 12 plants per pot as the standard protocol; and withholding irrigation for at least 28 days was determined to be suitable for inducing severe drought stress.

2.1.2. Study with two hybrids of differing drought sensitivity (two trials in different seasons)

2.1.2.1. Plant culture and drought treatments

This experiment was a greenhouse study carried out in winter 2005 (January and February) and again in summer 2005 (May and June). In both cases, greenhouse temperatures were moderated near 25/20^{o}C day/night. It compared hybrids of differing drought sensitivity. Seeds of two hybrids (Pioneer 32W86 and Pioneer 31R88) were planted in pure stands (12 plants of only one hybrid pot^{-1}) or inter-planted (six plants of each hybrid pot^{-1}) in 4-L pots filled with grade 3 vermiculite. According to Dennis McCoy (personal communication), 31R88 has a drought-resistance rating of 8, more drought resistant than 32W86 (rating of 5).

Fourteen seeds were planted per pot and thinned to twelve seedlings at 10 DAP. The planting pattern in inter-planted pots was such that the seedlings of each hybrid could be identified by their location within the pot. (The hybrids were interspersed, not segregated within each pot). General-purpose (20-20-20) fertilizer was applied at 10 DAP at 50 kg N/P/K ha^{-1}. Fourteen DAP, water was withheld from drought treatments, while watering continued when needed to maintain moisture at near field capacity on the well-watered controls. Thirty-five days after water withdrawal, drought stressed plants were re-watered, another 50 kg N/P/K ha^{-1} was applied, and the soil moisture was maintained at levels equal to the controls for 2 more weeks.

Data collected at harvest (63 DAP) included plant height and number of leaves. The seedlings were separated into roots and shoots. These were dried in moving air at 65 ^{o}C for 7 days to determine biomass and root:shoot ratio.

2.1.2.2. Experimental design and statistical analyses

The experiment used a factorial combination of treatments (two hybrids by two watering regimes by two planting patterns) arranged as a randomized complete block (RCB) replicated four times. The experimental units were one pot in a pure planting pattern and for inter-planting pattern, a pot contained two experimental units. All data sets were subjected to ANOVA and mean separations using the SAS procedures discussed in 2.1.1.2. The experiment was repeated in two seasons (winter and summer) and seasonal effects were tested. There were season by other main factor interactions for all responses (Table A.2), and therefore results from each trial were analyzed separately.

2.2. Triazole Studies: Effects on Maize Germination and Early Seedling Growth

2.2.1. Two studies on effects of triazoles on germination

2.2.1.1. Time-course for imbibition/impregnation with triazoles

In order to establish how long it takes for maize seeds/caryopses to fully imbibe, four replications of 50 seeds each were weighed and then immersed in distilled water. After 1 hour, the seeds were removed from the water and gently pressed between paper towels to remove surface moisture. The seeds were then weighed and immediately placed back into the water. This was repeated at hourly intervals until weight changes leveled off (Figure B.2). As a result, a 16-hour soak was established as appropriate for triazole impregnation.

2.2.1.2. Laboratory studies on effects of triazoles on germination and early seedling growth

These experiments were conducted in August 2005. Two maize hybrids (32W86 and 31R88) were used. Seeds of each hybrid were surface-sterilized in a 10% sodium hypochlorite (NaOCl) solution for 2 minutes, washed in running tap water, and rinsed with distilled water. A Trimmet®2SC formulation of paclobutrazol (PB) supplied by Syngenta Crop Protection, Inc., (Greensboro, NC) was prepared at 0, 50, and 100 mg of active ingredient (a.i) L^{-1}. Two hundred seeds were soaked in 300 ml of each (PB) concentration for 16 hours, washed, and rinsed with distilled water. The imbibed/impregnated seeds were then divided into four replications of 50 seeds each and placed in a 300-ml tray lined with two layers of moist paper towel. Another layer of moist paper towel was put on top of the seeds. The seeds were germinated in the dark at 25^{o}C for 5 days. After 5 days, the study was terminated, the number of germinated seeds were counted, and the seedling axes were dissected from the kernels and into root and shoot portions. The shoot was considered to be everything above the point of attachment to the old kernel. Fresh weights were recorded, and the parts were dried in moving air at 70^{o}C for 48 hours. Dry matter was then measured, and root:shoot ratios were determined.

These germination-testing procedures were repeated using tetraconazole (TC) formulated as Domark 230 ME from Isagro (Morrisville, NC) at 0, 50, and 100 mg a.i. L^{-1}. A third experiment used a Sumagic® formulation of uniconazole (UC) from Valent U.S.A. Corporation (Walnut Creek, CA) at 0, 5 and 10 mg a.i. L^{-1}. (The ranges of concentrations of triazoles tested were based on reports from the literature.)

2.2.1.3. Experimental design and statistical analyses

The experiment used a factorial combination of treatments (two hybrids and three PGR levels) arranged in a completely randomized design (CRD). All data were subjected to ANOVA following the general linear model (GLM) procedures of SAS. Differences among treatments were separated using Fischer's LSD at 0.05 probability level.

2.2.2. Three studies on effects of triazoles on seedling growth and drought responses

2.2.2.1. Paclobutrazol (PB) study

2.2.2.1.1. Plant culture and treatments

This study was conducted in a greenhouse in August and September 2005. Seeds of the two maize hybrids (32W86 and 31R88) were soaked for 16 hours in distilled water or a 50 mg a.i. PB L^{-1} solution (same PB source as previously). The seeds were then rinsed and planted in 4-L pots containing grade 3 vermiculite. In some pots, the PB-treated seeds were co-planted with non-treated seeds in an inter-planting arrangement. Fourteen seeds were planted per pot and thinned to twelve seedlings after emergence was complete (7 DAP). A 20-20-20 fertilizer was applied at 50 kg $N/P_2O_5/K_2O$ ha^{-1} after thinning.

Fourteen DAP, half of the pots of each planting regime were randomly selected and subjected to progressive drought by withholding water. Control plants were watered as needed to maintain the soil near field capacity. Near the midpoint of the drought period, canopy-level

photosynthetic photon flux density (PPFD) was monitored at mid-day on a sunny, clear-sky day with a Licor-250A light meter attached to a Licor-LI-190SA quantum sensor (LICOR, Lincoln, NE). The greenhouse ambient temperature was also recorded at the same time. During this study, the PPFD ranged from 910 to 1150 μmol m^{-2} s^{-1}, and the greenhouse temperature was between 32 and 35^{o}C. The drought-stressed plants were re-watered after 28 days of irrigation withdrawal (42 DAP), and all plants received another 50 kg N/P/K ha^{-1}. Plants were grown for 10 more days under well-watered conditions, and the experiment was terminated at 52 DAP.

2.2.2.1.2. Measurements

Plant height was measured at 14 DAP. Twenty-one DAP (7 days after the drought stress was imposed on some plants), one leaf lamina was removed from four plants per pot in pure plantings or four plants of each treatment in inter-planted pots and frozen in liquid N_2 in zip-lock bags and placed under dry ice. They were then stored at -80^{o}C until proline determination. Proline concentration was estimated using the acid-ninhydrin method (Bates et al., 1973). A 0.5-g sample of the frozen leaf material was ground in liquid N_2 with a mortar and pestle. The ground material was further homogenized in 10 ml of 3% sulphosalicyclic acid, and this was filtered under suction through Whatman #2 filter paper. Then 1 ml of the filtrate was mixed with 1 ml acid-ninhydrin and 1 ml of acetic acid in a test tube. The mixture was placed in a water bath at 100 ^{0}C for 1 hour, after which the reaction was terminated in an ice bath. The reaction mixture was extracted with 2 ml toluene, and the chromophore-containing toluene was aspirated from the aqueous phase, warmed to room temperature. and the absorbance read at 520 nm with a UV-VIS spectrophotometer (Biomate 3, Thermo Electron Corporation, Waltham, MA) using toluene for a blank. Proline concentration was calculated from the absorbance of a set of prepared proline standards.

Drought stressed plants were observed daily for leaf rolling, at which time physiological measurements were determined. Twenty-eight DAP (14 days into the drought stress), gas exchange and photochemical efficiency measurements were made. Four plants pot^{-1}, or four plants $treatment^{-1}$ in inter-planted pots, were measured. Gas exchange measurements were made on the youngest fully expanded leaves using a Licor-6400 infra-red gas analyzer (IRGA) (LI-COR, Lincoln, NE). Irradiance (photosynthetically active radiation, PAR) within the cuvette was 1000 μmol m^{-2} s^{-1} from a LI-6400-02B LED light source (LI-COR). The CO_2 concentration flowing into the cuvette was maintained at 380 μmol mol^{-1}. Temperature was maintained at 30^oC.

Photochemical efficiency (F_v/F_m) was determined by measuring chlorophyll fluorescence with a general fluorescence probe (OS-50, Opti-Sci. Inc., Tynsboro, MA). The ratio of maximum fluorescence at 690 nm (Fv690/Fm690 nm) or F_v/F_m indicates the photochemical efficiency of photosystem II (Zhang and Schmidt, 2000a). Chlorophyll fluorescence was measured on the same leaves used for gas exchange measurements between 1100 and 1400 hours under clear-sky conditions.

Immediately after the gas exchange and chlorophyll fluorescence measurements were made (28 DAP and 14 days into the drought stress), soil water status was determined gravimetrically as described previously.

The number of leaves and plant height were determined at harvest (52 DAP). All height and leaf-count measurements were taken on four plants pot^{-1} in the pure-planting pots or on four plants $treatment^{-1}$ in the inter-planted pots. All 12 plants in a pure-planting pot or all six plants $treatment^{-1}$ in inter-planted pots were harvested and dried in moving air at 65^0C for 7 days to determine biomass, and the root:shoot ratio was calculated.

2.2.2.1.3. Experimental design and statistical analyses

The experiment was a factorial combination of treatments (two hybrids by two watering regimes by two PB levels by two planting patterns) arranged as a randomized complete block (RCB). Each treatment was replicated four times. All data sets were subjected to ANOVA using the general linear model (GLM) procedures of SAS. Differences among main factors and their interaction were determined and treatment means were separated using Fischer's least significant difference (LSD) at 0.05 probability level.

2.2.2.2. Uniconazole (UC) study

2.2.2.2.1. Plant culture and treatments

This study was carried out in a greenhouse in September and October 2005. Seeds of two hybrids (Pioneer 32W86 and 31R88) were soaked for 16 hours in distilled water or 5 mg a.i. UC L^{-1} (same UC source as previously). The experimental setup was as in the PB experiment. Fourteen seeds were planted and thinned to twelve at 8 DAP, when the general purpose 20-20-20 fertilizer was applied at 50 kg N ha^{-1}. Fifteen DAP, drought stress was imposed on half the pots as in the PB experiment. During the drought stress period (28 days without water), the noon time PPFD was 800 to 1100 μmol m^{-2} s^{-1}, and the greenhouse temperature ranged from 30 to 35^{o}C on sunny days.

2.2.2.2.2. Measurements

Plant height was measured at 15 DAP. The number of leaves and plant height were determined at harvest (55 DAP). Plants were harvested and dry matter determined as previously described.

Sampling for the proline content determination was carried out at 22 DAP (7 days without watering for those plants receiving a drought stress) following the procedures previously

described. Proline analysis differed slightly in that the 3% sulphosalicyclic acid filtrate was stored in a refrigerator at 4^{o}C before proline was extracted after 3 days with 1 ml acid ninhydrin, 1 ml glacial acetic acid, and 4 ml toluene (Bates et al., 1973).

At 29 DAP (14 days without irrigation for plants receiving a drought stress), gas exchange and chlorophyll fluorescence were measured as described in the previous study. Immediately after gas exchange and chlorophyll fluorescence measurements, soil moisture content was measured by the gravimetric method as described. In addition, plant water status was measured with a hydraulic leaf press (Campbell Scientific Inc. Logan, Utah) according to procedures described by Nabati (1991). The lamina of the last fully emerged leaf was sampled, and approximately 4 cm of the middle portion was cut and placed in the leaf press chamber. The chamber was activated by a hydraulic pump, and the force (in arbitrary units) that caused water to be exuded uniformly from the cut ends was recorded. The greater the force required to press water from the leaf section, the lower the leaf moisture content (Nabati, 1991; Zhang and Schmidt, 2000).

2.2.2.2.3. Experimental design and statistical analyses

The design of the experiment and data analyses were as in section 2.2.2.1.3.

2.2.2.3. Tetraconazole (TC) study

2.2.2.3.1. Plant culture and treatments

This study was carried out in a greenhouse in October and November 2005. Seeds of Pioneer 32W86 and 31R88 were soaked for 16 hours in distilled water or 50 mg a.i. TC L^{-1} (same TC source as previously). The experimental set up was also similar. However, drought stress was imposed beginning at 17 DAP. The greenhouse noontime PPFD during a sunny day ranged from 600 to 950 μmol m^{-2} s^{-1}, and the temperature range was 25 to 28^{o}C. The drought-

stressed plants were re-watered after 28 days without irrigation, all plants received an additional 50 kg N/P/K ha^{-1}, and all plants were grown for 12 more days under well-watered conditions.

2.2.2.3.2. Measurements

Plant height was measured 17 DAP. At harvest (57 DAP) seedling survival, plant height, and number of leaves were determined. The plants were harvested and dry matter determined as previously described.

Proline content sampling and measurement were carried out at 24 DAP (7 days without watering for drought-stressed plants) as previously described. That is, -80^{o}C frozen-dried samples were ground under liquid N_2, and the 3% sulphosalicyclic acid filtrate was stored at 4^{o}C before proline extraction concentration determination after 3 days in 1 ml acid-ninhydrin, 1 ml glacial acetic acid, and 2 ml (instead of 4 ml) toluene.

At 30 DAP (13 days without watering for drought-stressed plants), gas exchange and chlorophyll fluorescence were measured as described previously, as were soil and plant water status.

2.2.2.3.3. Experimental design and statistical analyses

The experimental design and procedures for data analysis were as in the UC and PB studies just described.

2.2.3. A study to determine the time-course of effects of triazoles on seedling morphology

2.2.3.1. Plant culture and treatments

The experiment was conducted in the greenhouse in January and February 2006. Seeds were soaked for 16 hours at room temperature in solutions of triazoles at the following concentrations: 0 mg L^{-1} (control), 50 mg PB a.i. L^{-1}, 50 mg TC a.i. L^{-1}, and 5 mg UC a.i. L^{-1}. (Triazole sources were as previously described.) The seeds were rinsed with distilled water and

planted (12 seeds pot^{-1}) in 4-L pots filled with grade 3 vermiculite. Each treatment was replicated four times. At 5 DAP and 7 DAP, emerged seedlings were counted. At 7 DAP 50 kg N/P/K ha^{-1} were applied in solution. All plants were well watered throughout this study.

2.2.3.2. Measurements

Sequential sampling and morphological analyses were carried out at 14 and 35 DAP. At both sampling times, plant height was measured. At 14 DAP, half of the pots from each triazole treatment and rep were harvested and separated into roots and shoots. They were then dried at 65^{o}C for 5 days in moving air. Dry matter was determined, and root:shoot ratios were calculated. The number of leaves was also determined at 35 DAP.

2.2.3.3. Experimental design and statistical analyses

The experiment involved a factorial combination of treatments, with two hybrids, two sampling dates, and four PGR treatments (three triazoles and a -PGR control) arranged in a randomized complete block (RCB) design with four replications.

All data were subjected to ANOVA following the general linear model (GLM) procedures of SAS. Differences among treatments and their interactions were determined, and treatment means were separated using Fischer's least significant difference (LSD) at 0.05 probability level.

2.2.4. A final study comparing all three triazoles in inter-plantings

2.2.4.1. Plant culture and treatment

This study was carried out in the greenhouse in February and March 2006. Seeds of the same two hybrids as described previously were imbibed for 16 hours in distilled water (control) or solutions of 50 mg a.i. PB L^{-1}, 5 mg a.i. UC L^{-1}, or 50 mg a.i. TC L^{-1} (triazole sources same as previously). The seeds were then inter-planted in 4-L pots. Fourteen seeds were planted pot^{-1}

(seven triazole-treated seven for control). These were then thinned to six seedlings of each treatment 8 DAP, and 50 kg N/P/K ha^{-1} of general-purpose 20-20- 20 fertilizer were applied in solution.

Beginning 14 DAP, one half of the pots were subjected to drought stress by withholding irrigation. During the drought-stress period, noontime PPFD was 700 to 1020 μmol m^{-2} s^{-1}, and the temperature was 25 to 28^{o}C. The drought-stressed plants were re-watered 28 days after irrigation withdrawal (42 DAP), an additional 50 kg N/P/K ha^{-1} was applied, all plants were watered as needed to maintain moisture near field capacity, and growth continued for 13 more days.

2.2.4.2. Measurements

Plant height was measured 14 DAP and at harvest (55 DAP). Leaf area was also measured on the well-watered plants at 55 DAP with an LI-3100 portable leaf area system (LI-COR, Lincoln, NE). At harvest, the number of leaves was also determined on all treatments. Shoots were clipped at soil level and dried at 65^{o}C for 7 days.

For proline content determination, samples were obtained at 25 DAP (11 days without watering for the drought-stressed plants), and procedures in 2.2.2.1.2 were followed for proline determination.

At 30 DAP (16 days without watering for drought-stressed plants), gas exchange parameters and chlorophyll fluorescence were measured as described in 2.2.2.1.2. Immediately after gas exchange fluorescence measurements, plant water status was measured using the leaf press technique (as in section 2.2.2.2.2).

2.2.4.3. Experimental design and statistical analysis

The experiment was factorial (two hybrids by three triazoles by two triazole levels (0 or 5/50 mg a.i. L^{-1}) by two watering regimes) arranged as a randomized complete block (RCB) design. Each treatment was replicated four times. All data sets were subjected to ANOVA using GLM procedures of SAS. Differences among main factors and their interactions were determined and treatment means were separated using Fischer's LSD at 0.05 probability level.

Chapter 3

Results

3.1. Characterization of Drought Stress in Maize Seedlings

3.1.1 Preliminary study with a drought-sensitive hybrid (to establish protocols, measurement parameters, and an LD_{50})

Analyses of variance (ANOVA) showed that plant population (number of seedlings pot^{-1}) affected root, shoot, and total biomass, root:shoot ratio, and plant height; but leaf number was not affected (Table A.1). The duration of drought affected all the responses measured. There was a plant-population-by-drought-duration interaction for root, shoot, and total biomass; but the two factors did not interact for the root:shoot ratio, plant height, or leaf number.

At four plants pot^{-1}, root dry weight and total biomass were not affected by duration of irrigation withdrawal, while the shoot dry weight was reduced (Table 3.1). Shoot dry weight reduction was greatest when irrigation was withdrawn for ≥28 days. With eight plants pot^{-1}, root biomass was not affected by 35 days' drought, but both the shoot and total seedling biomass were reduced by increasing duration of irrigation withdrawal. The root dry weight at 12 plants pot^{-1} was again not affected by drought, while the shoot and total biomass were reduced by any drought duration of ≥14 days. This trend is similar at the 16-plant-pot^{-1} population, but with root biomass also being reduced, but only after 35 days without watering. There was also even greater reduction of the shoot and total biomass, especially after 21 days without watering. Figure 3.1 shows the expected differences in the rate of dry down (as θv) in pots of differing populations. Figure 3.2 translates the θv data into Ψ_{soil} and predicts the vermiculite water relation as influenced by θv, predicted from the moisture release curve. Figure 3.3 shows the moisture release curve obtained from the pressure plate studies.

Because there were no population-by-drought-duration interactions, data for plant height, root:shoot ratio, and leaf number were pooled across populations (Table 3.2). Plant height was reduced by withholding irrigation for ≥28 days, while leaf number was reduced by any period of irrigation withdrawal, especially 35 days. The root:shoot ratio increased dramatically with increasing duration of drought and was highest after 35 days without irrigation.

3.1.2. Study with two hybrids of differing drought sensitivity (two trials in different seasons)

This experiment was repeated over two seasons (spring and summer 2005), and the ANOVA (Table A.2) shows a seasonal effect, with hybrid and drought or planting pattern interactions. Therefore data were analyzed by season. In the spring trial, there were no hybrid differences; but drought affected shoot dry weight, plant height, and leaf number, while root dry weight and total biomass were unaffected (Table A.3). There were no hybrid-by-drought interactions for these responses. As shown on Table 3.3, there was no difference between planting patterns (pure planting and inter-planted) in shoot dry weights, plant height, and number of leaves for the well-watered controls in the spring trial. However, under drought stress, inter-planted seedlings had lower shoot dry weight, while plant height and leaf number were not affected. In the summer trial, it was observed that the well-watered, inter-planted seedlings had greater shoot dry weights, more leaves, and were taller than the pure-planted treatments. Under drought stress, there were no differences in the shoot dry weight, plant height, and number of leaves between the two planting patterns.

The pure-planted seedlings were further analyzed separately for root growth, total biomass, and root:shoot ratio. In the spring trial, drought did not decrease root dry weight and total biomass, but shoot biomass was decreased (Table 3.4). In the summer trial, drought reduced

the root and shoot dry weight as well as the total biomass. In both seasons, it was observed that, although drought tended to increase the root:shoot ratio, these increases were not significant at P = 0.05 (Table 3.4). Drought reduced Ψ_{soil} in both seasons, and in the summer Ψ_{soil} was as low as -2.7 MPa at re-watering.

3.2. Effects of Triazoles on Germination and Early Seedling Growth

The PB treatments at 50 or 100 mg a.i. L^{-1} did not affect germination or root dry weight of the seedlings at 6 DAP (Tables A.4 and 3.5). Shoot weight and total seedling weight were reduced. There were no hybrid-by-PB-rate interactions for any of these observations. The root:shoot ratio was increased by the PB treatments, with no difference between 50 and 100 mg a.i. L^{-1}.

The UC treatments did not affect germination or early shoot or root weight; but the root:shoot ratio was increased at 10 mg a.i. L^{-1} (Table 3.5). There were no hybrid-by-UC-rate interactions for any parameter examined (Table A.4). The increase in the root:shoot ratio was generally caused by reductions of shoot biomass and increases in root weight, although root and shoot weights did not differ from the 0 UC control.

The ANOVA of the TC experiment (Table A.4) showed that germination, shoot, and total seedling biomass were affected by the triazole treatment. There were no hybrid-by-TC-rate interactions for any parameter. Tetraconacozole reduced germination at both 50 and 100 mg a.i. L^{-1} (Table 3.5). The shoot and total seedling weights were also reduced by the TC, again with no difference between the two concentrations of TC. Shoot biomass was reduced by TC but not enough to significantly affect root:shoot ratio.

3.3. Effects of Triazoles on Seedling Growth and Drought Responses

3.3.1. Paclobutrazol (PB) study

There were hybrid differences for plant height and leaf number at 14 DAP, but hybrids did not differ in shoot dry weight, plant height at 55 DAP, or seedling survival (Table A.8). Drought stress reduced plant height, shoot dry weight, leaf number and seedling survival (Tables 3.6 and 3.7). In the pure-plantings, drought increased root:shoot ratio, while root, shoot, and total weight, as well as survival, plant height, and leaf number were reduced. The PB-treated plants under drought stress had reduced root:shoot ratios compared to non-treated, drought-stressed plants. The PB treatment reduced plant height at 14 DAP, but had no effect on shoot weight, plant height, or leaf number at 55 DAP. There were significant hybrid-by-PGR interactions for plant height at 14 DAP. Paclobutrazol-treated seedlings were shorter than the controls at 14 DAP, but by 55 DAP there were no height differences due to PGR either for well-watered or drought-stressed plants. When treated with PB, hybrid 32W86 was shorter than 31R88 at 14 DAP

Key physiological and biochemical parameters were also monitored in this experiment. The ANOVA indicated that drought stress reduced photosynthesis, transpiration, and soil water content and increased proline content (Tables A.5 and 3.8), while chlorophyll fluorescence was not affected by drought stress. The Ψ_{soil} was -0.01 and -0.8 MPa for the well watered and drought-stressed treatments, respectively, at 28 DAP. The PB treatment main factor did not affect any of these parameters. However, there was drought-by-PB interaction for transpiration. The PB-treated plants exhibited a higher transpiration rate during drought in pure plantings (Fig. 3.4).

3.3.2. Uniconazole (UC) study

The ANOVA of this study showed that hybrids differed in plant height at 14 DAP and number of leaves at 55 DAP (Table A.15). Planting pattern did not have an effect on the

morphological and key physiological parameters measured in this study. The distribution of dry matter in the pure-planted seedlings showed that drought reduced the root, shoot, and total biomass and number of leaves, while the root:shoot ratio increased (Table 3.9). The non-UC-treated, drought-stressed plants had a greater root:shoot ratio. The UC treatments did not affect the shoot weight, but plant height at 15 and 55 DAP and leaf number were affected. There was a PGR-by-hybrid interaction for plant height at 15 DAP but not at 55 DAP. The UC-treated plants were shorter at 15 DAP, and this persisted until harvest (Table 3.10). Uniconazole-treated hybrid 32W86 had more leaves than 31R88 under well-watered conditions but not if drought stressed. The hybrid was also shorter than 31R88 at 14 DAP but not at 55 DAP.

Drought stress reduced photosynthesis, transpiration, chlorophyll fluorescence, Ψ_{soil}, and plant water status and increased proline content (Table 3.10). The Ψ_{soil} was -0.7 MPa in the drought-stressed treatment at 29 DAP (15 days of irrigation withdrawal) (DIW). There were no hybrid differences for these parameters. The UC treatment did not affect fluorescence or proline content; but photosynthesis, transpiration, Ψ_{soil}, and plant water status were increased by the PGR treatment (Table 3.12). There were no significant interactions between any main factors in this experiment. Uniconazole-treated plants had higher photosynthetic rates under both well-watered and drought-stressed conditions. The pure-planted, UC-treated plants under drought stress had higher rates of photosynthesis. When UC- and non-UC-treated plants were inter-planted, the UC-treated plants had higher photosynthesis rates, but not significantly so. This was also observed in transpiration. The triazole-treated plants had significantly higher Ψ_{soil} and plant water status in the pure plantings. When the plants were subjected to equal Ψ_{soil} in an inter-planting system, the triazole-treated plants had higher water status than the non-triazole-treated plants.

3.3.3. Tetraconazole (TC) study

In the TC experiment, drought stress reduced the shoot weight, plant height, number of leaves, and survival at 57 DAP (Table 3.13). Hybrids differed in height at 14 DAP and the number of leaves at 57 DAP. There were no hybrid differences in seedling survival or shoot weight at 57 DAP (Table A.22). The TC treatment increased leaf number and shoot weight. There were no hybrid-by-PGR interactions for shoot weight, plant heights, leaf number, or seedling survival. Tetraconazole increased leaf number in well-watered seedlings but not under drought stress (Table 3.14). The root:shoot ratio of seedlings was increased by drought stress but not by TC treatment (Table 3.13).

Drought stress reduced photosynthesis, transpiration, chlorophyll fluorescence, Ψ_{soil}, and plant water status and increased proline content (Table 3.15). At the time of measurement (16 days without irrigation), Ψ_{soil} was -0.6 MPa for the drought-stressed treatment. There were no hybrid differences for these observations (Table A.19). The TC treatment did not have significant effects on the physiological or soil parameters. However, there was a drought-by-PGR interaction for photosynthesis (Table A.20). In the pure plantings, PGR treatment increased photosynthesis of the well-watered seedlings but not those under drought stress (Figure 3.5).

3.4. Time-Course Effects of Triazoles on Seedling Morphology

This experiment examined the early time-course effects of the three triazoles on growth of well-watered, pure-planted seedlings. Plant age and its interactions with PGR treatment were significant for root weight, shoot weight, total weight, and plant height (Table A.26). At both ages (14 and 35 DAP), there were no hybrid-by-PGR interactions. The data were therefore pooled across hybrids and presented by plant age. The ANOVA showed that the triazole treatments affected shoot weight, root:shoot ratio, and plant height at 14 DAP and emergence at

5 DAP but not at 7 DAP (Tables A.28 and 3.16 and Figure 3.6). Root weight was not affected by treatment with any of the triazoles, but PB reduced total weight. There were no interactions between hybrid and PGR for any of the above observations. At 35 DAP, shoot weight, total biomass, plant height, and leaf number were affected by one or more of the triazoles. Root weight and root:shoot ratio were not affected by PGR treatments at the later sampling time.

At 14 DAP, PB and UC reduced shoot weight, while the TC treatment did not differ from the control (Table 3.16). The total biomass findings were similar to those for shoot weight; except that only PB significantly reduced biomass accumulation, while TC again did not.

At 35 DAP, shoot biomass of the PB- and UC-treated plants was reduced, while the TC treatment did not differ from the control. The PB and UC treatments also lowered total biomass. Paclobutrazol and UC increased the root:shoot ratio at 14 DAP, while TC did not. However, at 35 DAP, there were no significant differences in root:shoot ratio between the control and any of the PGR treatments. All the PGR-treated plants exhibited somewhat higher values than the control, but the difference were not significant at the 0.05 level.

The PB- and UC-treated plants were shorter than the controls at 14 and 35 DAP. There were no height differences between the control and the TC-treated plants at either plant age. The UC-treated plants had more leaves at 35 DAP, while the TC- and PB-treated plants had leaf numbers equal to the control.

3.5. Comparing all Three Triazoles in Inter-Plantings

In this study, triazole-treated plants were inter-planted with non-treated plants, and some pots were subjected to drought stress. The ANOVA revealed no differences between hybrids for shoot weight, plant height at 14 or 56 DAP, or leaf area (for the well-watered controls); but the number of leaves differed between hybrids (Table A.29). There was a drought-by-PGR

interaction and a three-way interaction for leaf number. Drought stress affected shoot dry weight, plant height, and leaf number. The triazole treatments did not affect shoot weight or leaf area, but plant height at 14 and 56 DAP and leaf number were affected by the triazoles.

Drought stress generally reduced shoot weight, plant height, and leaf number at 56 DAP (Table 3.17). At 14 and 56 DAP UC-treated plants were shortest, followed by the PB-treated plants (Table 3.18). Tetraconazole did not reduce plant height at 14 or 56 DAP compared to the non-PGR-treated controls. At harvest (56 DAP), drought stress had not further reduced plant height in PB-treated plants, i.e., the effects were not additive. Within the UC treatments, height did not differ between drought-stressed plants and well-watered plants, i.e., the effects of drought and PGR were likewise not additive. Generalizing, in the well-watered treatments, TC did not reduce plant height at 14 DAP or 55 DAP, while PB and UC reduced it at both dates of sampling. Furthermore, the triazoles had no additional effect on the shortening of plants caused by drought.

The UC-treatments produced more leaves both in well-watered and drought-stressed plants. The other two triazoles had no effect on leaf number. PGR treatment did not affect leaf area of the well watered controls.

Hybrids differed in photosynthesis but not in transpiration, chlorophyll fluorescence, proline content, or plant and soil water status. Drought stress affected all these parameters, and PGR treatments were variable in their effects (Table A.30). Drought reduced photosynthesis, transpiration, chlorophyll fluorescence, Ψ_{soil}, and plant water status, and increased proline content (Table 3.19). The Ψ_{soil} at the time of measurements was -0.1 and -0.8 MPa for the well-watered and drought-stressed treatments, respectively.

There was a PGR-by-drought interaction for photosynthesis and a hybrid-by-PGR interaction for transpiration. When data were pooled across hybrids, the PGR treatments were found to affect photosynthesis, transpiration, and proline content (Table 3.20). The TC- and UC-treated plants under well-watered conditions exhibited higher photosynthetic rates. Although a similar trend was observed in the PB-treated plants, the difference was not significant. The PGR-treated plants also had higher – but not significantly so – photosynthesis under drought stress. The UC-treated plants under-well watered conditions had a higher transpiration rate than the non-triazole-treated controls and PB-treated plants.

Some PGR-treated, drought-stressed plants had increased proline content relative to non-PGR-treated plants. When just the drought-stressed plants are compared, UC- and TC-treated plants had higher proline content than the non-PGR treatments. The PGR treatments did not affect proline levels under well-watered conditions. Somewhat higher proline values were observed for PGR-treated plants in a previous study (section 3.2, data not shown), but they were not significantly different from the non-PGR controls. The PGR-treated plants under drought stress generally exhibited higher water status compared to the non-PGR plants within a pot. However, these were not significantly different.

Table 3.1: Effects of plant population and irrigation withdrawal on greenhouse-grown seedlings of maize 32W86 hybrid. Irrigation was withdrawn for the specified period beginning 14 DAP, then re-watered, and grown with adequate water until all plants were harvested at 63 DAP.

Plant Population	Irrigation Withdrawal	Root DW	Shoot DW	Total DW
pot^{-1}	days	----------g $plant^{-1}$----------		
4	0	1.04	1.16	2.20
	14	1.12	1.05	2.17
	21	1.07	1.01	2.08
	28	1.14	0.76	1.99
	35	1.24	0.66	1.90
	$LSD_{0.05}$	ns	0.10	ns
8	0	0.99	1.22	2.21
	14	0.86	0.97	1.83
	21	0.93	0.83	1.76
	28	0.93	0.79	1.72
	35	1.08	0.58	1.66
	$LSD_{0.05}$	ns	0.20	0.46
12	0	0.79	0.93	1.72
	14	0.68	0.76	1.45
	21	0.90	0.69	1.59
	28	0.71	0.69	1.40
	35	0.68	0.66	1.34
	$LSD_{0.05}$	ns	0.13	0.25
16	0	0.64	0.82	1.46
	14	0.63	0.70	1.33
	21	0.62	0.58	1.20
	28	0.66	0.52	1.10
	35	0.47	0.26	0.73
	$LSD_{0.05}$	0.10	0.07	0.12

DW = dry weight, or biomass; DAP = days after planting

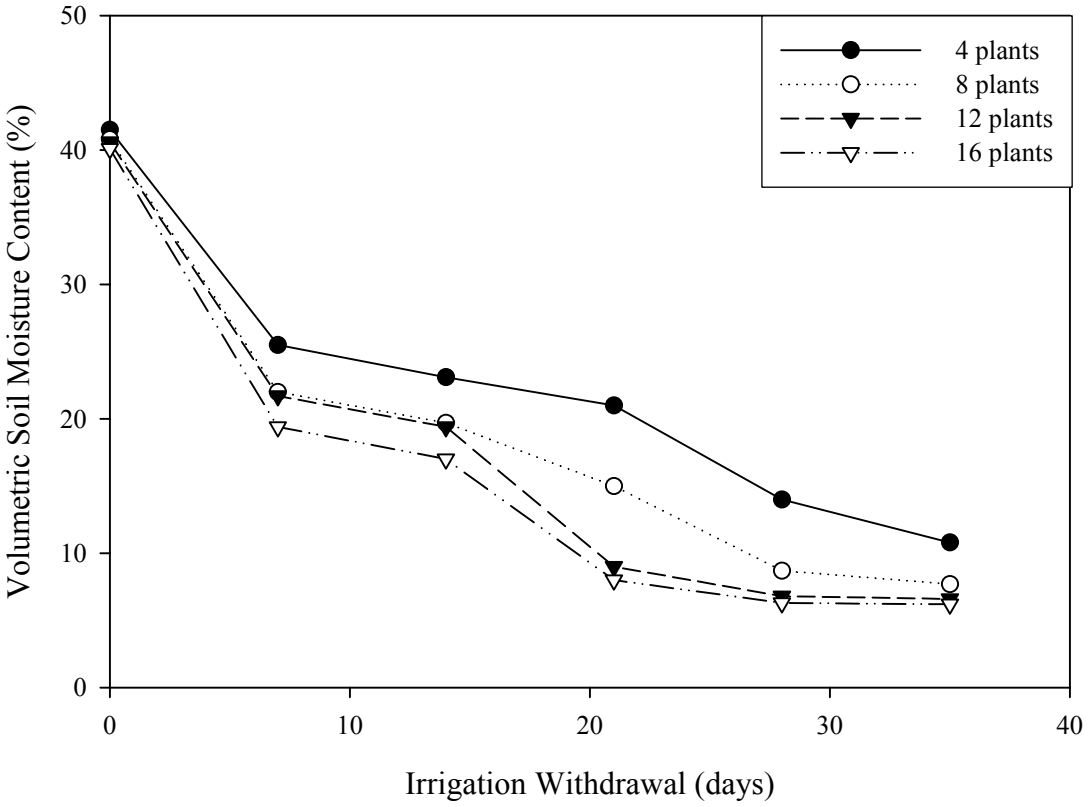

Figure 3.1: The effect of plant population on the change in volumetric soil water content following irrigation withdrawal from pots growing maize 32W86 hybrid

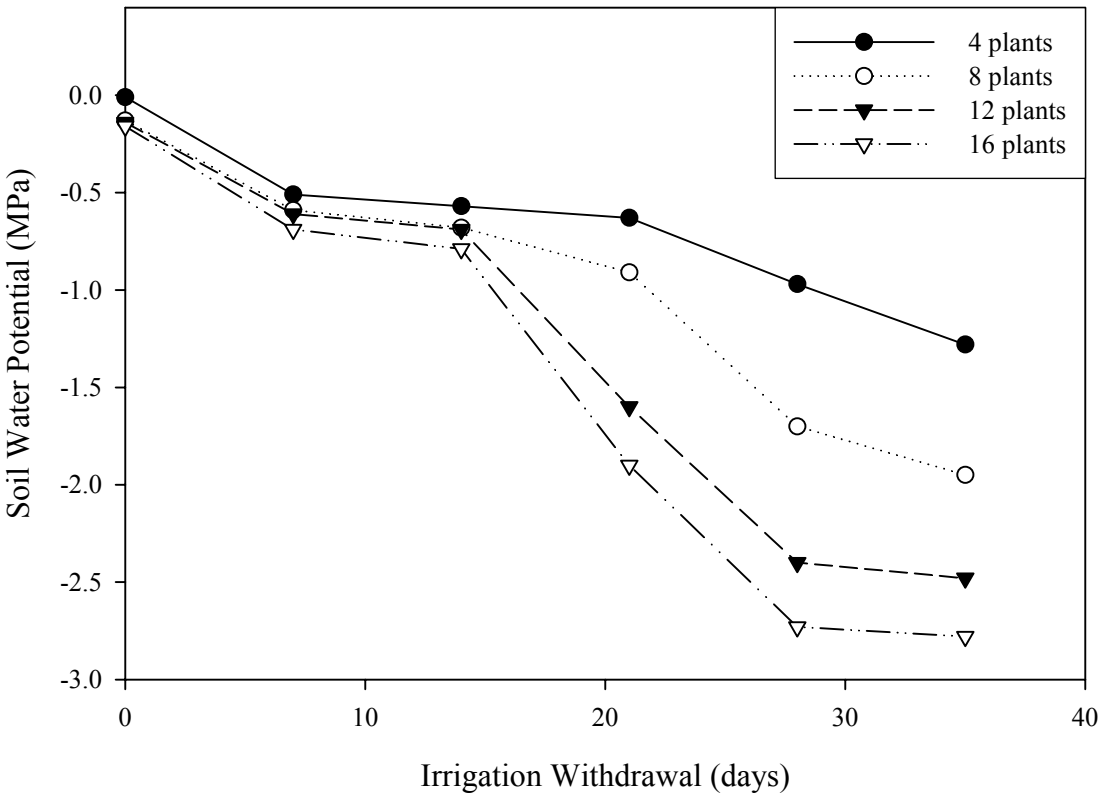

Figure 3.2: The effect of plant population on the change in soil water potential following irrigation withdrawal from pots growing maize 32W86 hybrid

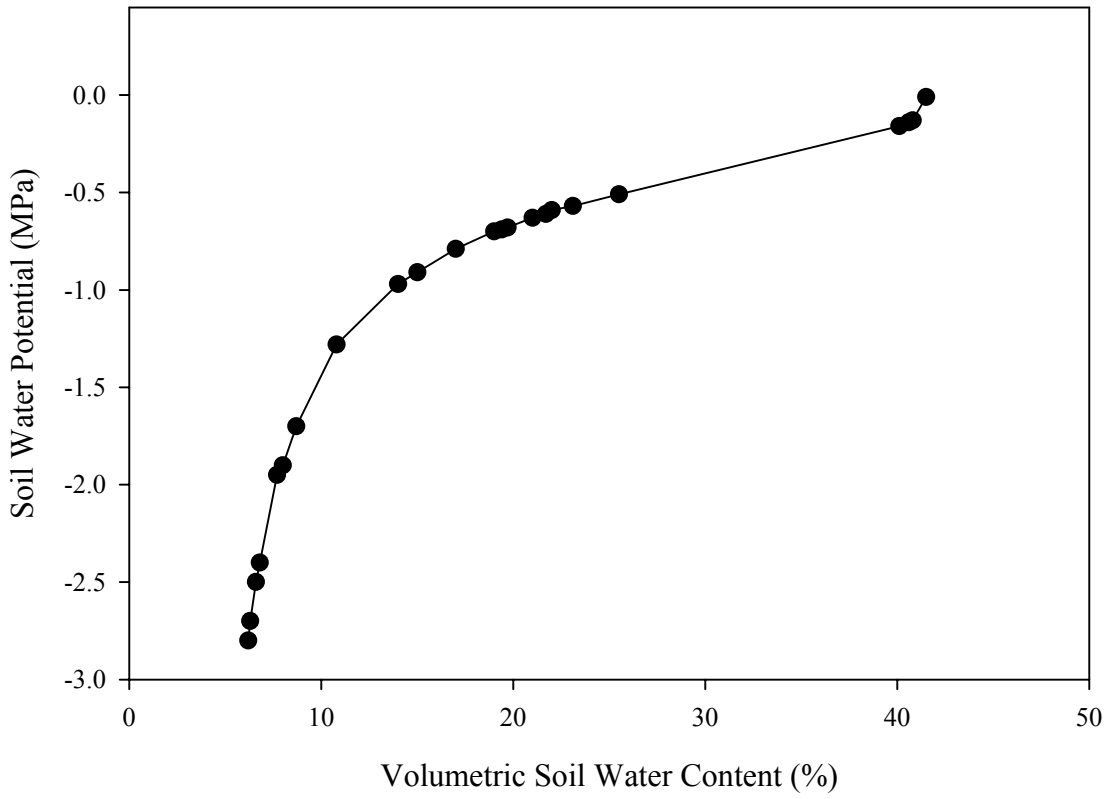

Figure 3.3: The effect of soil volumetric water content on water potential of vermiculite predicted from a moisture release curve.

Table 3.2: Effects of irrigation withdrawal on the greenhouse-grown seedlings of maize 32W86 hybrid. Irrigation was withdrawn for the specified period beginning at 14 DAP, then re-watered, and grown with adequate water until harvested at 63 DAP. Data are pooled across four plant populations.

Irrigation Withdrawal	Plant Height	Leaf Number	Root:Shoot Ratio
days	cm	plant^{-1}	
0	61.63	7.31	0.82
14	58.06	6.81	0.94
21	56.81	6.94	1.14
28	54.08	6.94	1.30
35	49.75	6.50	2.08
$LSD_{0.05}$	5.21	0.36	0.22

DAP = days after planting

Table 3.3: Effects of inter-hybrid competition, season, and drought stress on greenhouse-grown of maize and soil-plant water relations. The seedlings were well watered till 14 DAP, irrigation withdrawn for 35 days (DS), re-watered, grown for 2 more weeks, and harvested at 63 DAP. Data are pooled across two hybrids.

	Spring 05					Summer 05				
Treatment	Shoot DW	Plant Height	Leaf Number	θv	Ψ_{soil}	Shoot DW	Plant Height	Leaf Number	θv	Ψ_{soil}
	g plant^{-1}	cm	plant^{-1}	%	MPa	g plant^{-1}	cm	plant^{-1}	%	MPa
PP/WW	0.75	38.7	6.0	39.5	-0.2	1.79	48.2	8.3	38.8	-0.2
PP/DS	0.58	33.1	5.8	12.3	-1.1	0.72	34.4	6.8	6.4	-2.6
IP/WW	0.79	36.4	5.9	36.8	-0.3	2.49	61.0	8.9	38.0	-0.2
IP/DS	0.47	28.8	5.8	12.9	-1.1	0.71	36.9	6.8	6.2	-2.7
$LSD_{0.05}$	0.06	6.4	0.5	2.8	na	0.47	9.4	0.4	2.1	na

PP = pure planting, one hybrid pot^{-1}; IP = inter-planted, two hybrids pot^{-1}; WW = well-watered throughout; DS = drought-stressed (irrigation withheld for 35 days); DW =dry weight; θv = volumetric soil moisture content at re-watering; Ψ_{soil} = soil water potential at re-watering; DAP = days after planting

Table 3.4: Effects of season and drought stress on biomass distribution in greenhouse-grown maize seedlings (pure plantings). Seedlings were well watered till 14 DAP, irrigation withdrawn for 35 days, re-watered, grown for 2 more weeks, and harvested at 63 DAP. Data are pooled across two hybrids.

	Spring 05				Summer 05			
Treatment	Root DW	Shoot DW	Total DW	Root: Shoot Ratio	Root DW	Shoot DW	Total DW	Root: Shoot
	g plant^{-1}				g plant^{-1}			
WW	1.04	0.75	1.79	1.39	1.26	1.56	2.83	0.82
DS	0.96	0.59	1.45	1.67	0.66	0.72	1.38	0.96
$LSD_{0.05}$	ns	0.06	ns	0.28	0.25	0.29	0.49	ns

WW = well-watered throughout; DS = drought-stressed (irrigation withheld for 35 days); DW = dry weight; DAP = days after planting

Table 3.5: Effects of paclobutrazol (PB), uniconazole (UC) and tetraconazole (TC) on germination and early seedling growth of maize. Germination was determined 5 DAP, and seedlings were harvested at 6 DAP. Data are pooled across two hybrids.

PGR	Rate	Germination	Root DW	Shoot DW	Total Biomass	Root: Shoot
	mg L^{-1}	%	mg plant^{-1}			
PB	0	98.5	11.0	14.60	25.63	0.76
	50	97.5	11.5	8.66	20.17	1.33
	100	95.8	13.6	9.27	22.88	1.45
	$LSD_{0.05}$	ns	ns	2.52	5.25	0.28
UC	0	94.0	8.1	10.30	18.40	0.78
	5	93.1	11.0	8.76	19.79	1.26
	10	91.4	13.8	7.54	21.33	1.82
	$LSD_{0.05}$	ns	ns	ns	ns	0.72
TC	0	96.50	16.1	15.79	31.83	1.02
	50	88.25	12.5	11.34	23.86	1.11
	100	88.75	13.3	9.54	22.82	1.17
	$LSD_{0.05}$	5.17	ns	3.62	7.32	ns

Each triazole trial was a separate experiment. DW = dry weight; DAP = days after planting

Table 3.6: Effects of paclobutrazol (PB) and drought stress on biomass distribution, survival, plant height, and number of leaves of greenhouse-grown maize seedlings at 55 DAP. Drought stress was imposed for 28 days (beginning at 14 DAP), re-watered, and grown for 10 more days. Data are pooled across two hybrids.

Treatment	Plant Survival	Leaf Number	Plant Height	Root DW	Shoot DW	Total DW	Root Shoot
	%	plant^{-1}	cm	----------g plant^{-1}----------			
-PB/WW	100.0	8.4	52.4	1.38	1.37	2.75	1.01
-PB/DS	68.8	7.1	28.1	0.70	0.44	1.14	1.59
+PB/WW	100.0	8.5	50.7	1.19	1.36	2.55	0.88
+PB/DS	84.4	7.8	32.4	0.91	0.71	1.62	1.28
$LSD_{0.05}$	26.8	0.8	8.9	0.24	0.33	0.61	0.24

WW = well-watered throughout; DS = drought-stressed (28 days without irrigation); DW = dry weight; DAP = days after plantin

Table 3.7: Effects of paclobutrazol (PB), planting pattern, and drought stress on plant height and shoot weight of greenhouse-grown seedlings of two maize hybrids. Plant height was measured at the time of drought imposition (14 DAP) and harvest (55 DAP). Drought was imposed for 28 days, re-watered, and grown for 12 more days.

Hybrid	Planting Pattern	Treatment	Plant Height		Shoot DW
			14 DAP	55 DAP	
			----------cm----------		g plant^{-1}
32W86	Pure Planting	-PB/WW	28.9	56.0	1.28
		-PB/DS	28.9	30.0	0.46
		+PB/WW	16.0	52.6	1.44
		+PB/DS	16.1	37.0	0.73
	Inter-Planted	-PB/WW	29.6	43.0	1.28
		-PB/DS	28.7	31.8	0.60
		+PB/WW	15.1	49.0	1.20
		+PB/DS	16.9	30.3	0.78
31R88	Pure Planting	-PB/WW	29.5	49.8	1.47
		-PB/DS	30.3	26.6	0.42
		+PB/WW	22.4	48.8	1.28
		+PB/DS	22.6	27.8	0.69
	Inter-planted	-PB/WW	28.7	42.3	1.31
		-PB/DS	28.1	27.4	0.42
		+PB/WW	23.7	43.8	1.44
		+PB/DS	20.6	28.3	0.66
		$LSD_{0.05}$	2.8	14.1	0.70

WW = well-watered throughout; DS = drought-stressed (irrigation withheld for 28 days); DW = dry weight; DAP = days after planting

Table 3.8: Effects of drought stress on the physiological responses of greenhouse-grown maize seedlings in the paclobutrazol (PB) experiment. Proline content was determined 21 DAP. Photosynthesis and transpiration were measured 28 DAP. Data are pooled across two hybrids, planting patterns, and two PB rates.

	7 DIW	----------14 DIW----------			
Treatment	Proline	Photosynthesis	Transpiration	θ_v	Ψ_{soil}
	mg g^{-1} FW	μmol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	%	MPa
WW	0.039	12.45	9.96	42.1	-0.01
DS	0.140	8.33	6.83	17.6	-0.80
$LSD_{0.05}$	0.098	0.85	0.79	1.9	na

DIW = days of irrigation withdrawal, WW = well-watered throughout, DS = drought-stressed (irrigation withheld for 7 or 14 days); θ_v = volumetric soil moisture content; Ψ_{soil} = soil water potential; DAP = days after planting

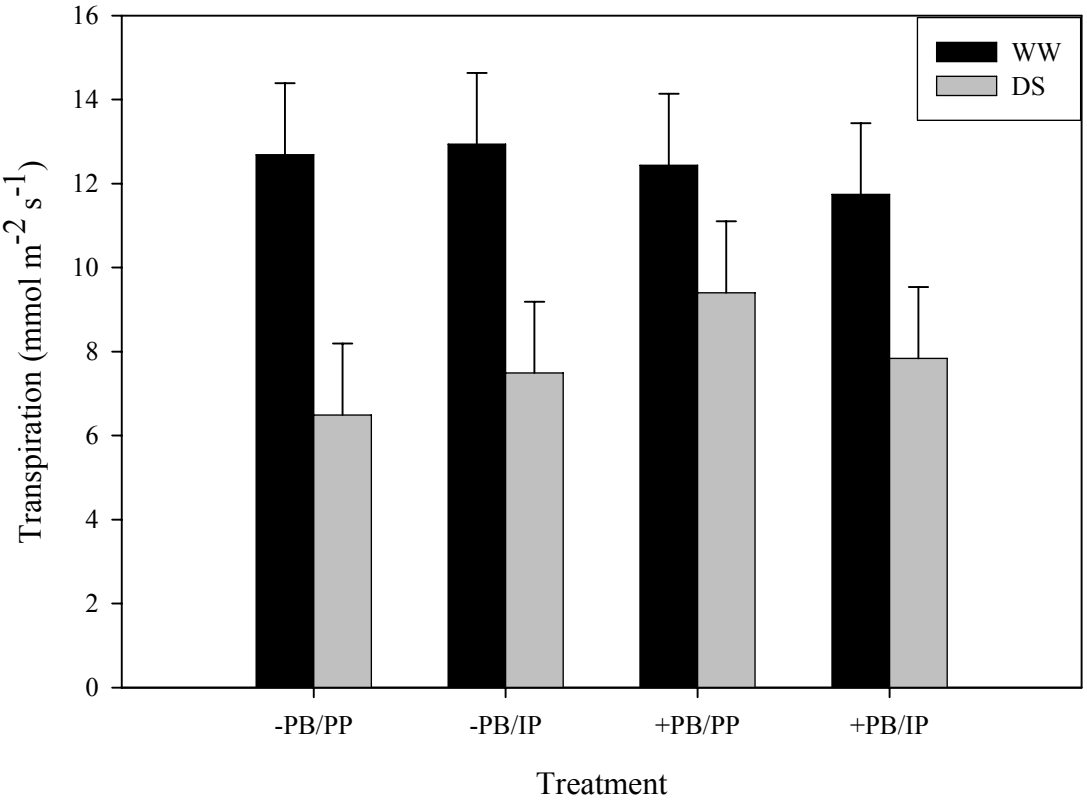

Figure 3.4: The effect of paclobutrazol (PB) and drought on transpiration of greenhouse-grown maize seedlings. Error bars represent the $LSD_{0.05}$ value of 1.69. WW = well-watered, DS = drought-stressed (irrigation withdheld for 14 days). Data pooled across two hybrids. PP = pure planting; IP= inter-planted.

Table 3.9: Effects of uniconazole (UC) and drought stress on biomass distribution, plant height, and number of leaves of greenhouse-grown maize seedlings at 55 DAP. Drought stress was imposed at 14 DAP for 28 days, rewatered, and grown for 10 more days. Data are from pure plantings and pooled across two hybrids.

Treatment	Leaf Number	Plant Height	Root DW	Shoot DW	Total DW	Root: Shoot
	plant^{-1}	cm	----------g plant^{-1}----------			
-UC/WW	7.0	31.8	0.61	0.57	1.18	1.10
-UC/DS	6.9	25.5	0.50	0.33	0.83	1.53
+UC/WW	8.8	18.1	0.60	0.51	1.10	1.18
+UC/DS	7.3	17.0	0.47	0.41	0.88	1.17
$LSD_{0.05}$	1.1	2.6	0.11	0.08	0.16	0.34

WW = well-watered throughout; DS = drought-stressed (irrigation withheld for 28 days); DW = dry weight; DAP = days after planting

Table 3.10: Effects of drought on physiological responses of greenhouse-grown maize seedlings. Proline content was determined 22 DAP. Photosynthesis, transpiration, chlorophyll fluorescence, and plant and soil water status were measured 29 DAP. Data are pooled across two hybrids, two planting patterns, and two UC rates.

	8 DIW	----------15 DIW----------					
Treatment	Proline	Photosynthesis	Transpiration	Chlorophyll Fluorescence	Plant Water Status	θ_V	Ψ_{soil}
	mg g^{-1} FW	µmol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	Fv/Fm[Þ]	press units[¶]	%	MPa
WW	0.060	15.82	5.93	0.60	118.8	41.1	-0.1
DS	1.138	10.56	4.31	0.47	213.8	20.0	-0.7
$LSD_{0.05}$	0.56	1.66	0.78	0.04	5.84	1.82	na

DIW = days of irrigation withdrawal; WW = well-watered throughout, DS = drought-stressed (irrigation withheld for 8 or 15 days);
Þ = variable to maximal fluorescence ratio at 690 nm; θ_V = volumetric soil moisture content; Ψ_{soil} = soil water potential. UC = uniconazole;
¶ = higher values indicate lower water status; DAP = days after planting

Table 3.11: Effects of uniconazole (UC), planting pattern, and drought stress on plant height and number of leaves of greenhouse-grown seedlings of two maize hybrids. Plant height was measured at the time of drought imposition (15 DAP) and harvest (55 DAP). Drought was imposed for 28 days, re-watered, and grown for 12 more days.

Hybrid	Planting Pattern	Treatment	Plant Height		Leaf Number
			15 DAP	55 DAP	
			----------cm----------		plant^{-1}
32W86	Pure Planting	-UC/WW	26.3	31.4	7.5
		-UC/DS	25.8	27.4	7.3
		+UC/WW	9.9	19.5	9.8
		+UC/DS	10.4	17.9	7.5
	Inter-Planted	-UC/WW	24.5	25.7	7.8
		-UC/DS	23.6	24.7	7.5
		+UC/WW	10.5	18.9	8.0
		+UC/DS	10.3	16.5	7.8
31R88	Pure Planting	-UC/WW	27.0	32.1	6.8
		-UC/DS	25.6	23.6	6.3
		+UC/WW	13.4	16.8	7.8
		+UC/DS	13.1	16.1	7.3
	Inter-planted	-PB/WW	25.69	28.4	7.0
		-UC/DS	24.5	23.4	7.0
		+UC/WW	14.4	18.0	8.3
		+UC/DS	13.5	16.1	7.0
		$LSD_{0.05}$	2.3	3.3	1.1

WW = well-watered throughout; DS = drought-stressed (irrigation withheld for 28 days); DAP = days after planting

Table 3.12: Effects of uniconazole (UC), planting pattern, and drought stress on key responses of greenhouse-grown maize seedlings at 29 DAP (15 days without irrigation for DS). Data are pooled across two hybrids.

Planting Pattern	Treatment	Photosynthesis	Transpiration	Plant Water Status	θ_v	Ψ_{soil}
		μmol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	press units¶	%	MPa
Pure planting	-UC/WW	14.06	5.76	130.0	37.7	-0.2
	-UC/DS	6.68	3.93	220.0	17.3	-0.8
	+UC/WW	19.55	7.03	113.5	42.1	-0.01
	+UC/DS	13.13	5.25	206.3	21.9	-0.6
Inter-planted	-UC/WW	14.55	5.56	113.8	41.7	-0.1
	-UC/DS	9.20	3.52	222.3	20.5	-0.6
	+UC/WW	16.00	5.66	117.5	41.7	-0.1
	+UC/DS	11.95	4.23	206.3	20.5	-0.6
	$LSD_{0.05}$	3.07	1.45	10.9	3.5	na

WW = well-watered throughout, DS = drought-stressed (irrigation withheld for 15 days); ¶ = higher values indicate lower water status; DAP = days after planting; θ_v = volumetric water content; Ψ_{soil} = soil water potential

Table 3.13: Effects of tetraconazole (TC) and drought stress on biomass distribution, plant height, and number of leaves of greenhouse-grown maize seedlings at 57 DAP. Drought stress was imposed for 28 days beginning at 14 DAP, rewatered, and grown for 12 more days. Data are from pure plantings and are pooled across two hybrids.

Treatment	Plant Survival	Leaf Number	Plant Height	Root DW	Shoot DW	Total DW	Root: Shoot
	%	$plant^{-1}$	cm	----------g plant----------			
-TC/WW	100.0	6.8	36.3	1.03	0.63	1.65	1.65
-TC/DS	67.6	5.6	24.9	0.70	0.33	1.04	2.23
+TC/WW	100.0	7.2	38.8	1.04	0.74	1.78	1.41
+TC/DS	64.5	6.6	27.1	0.76	0.36	1.12	2.22
$LSD_{0.05}$	29.7	0.6	3.0	0.22	0.09	0.26	0.56

WW = well watered throughout; DS = drought stressed (28 days without irrigation); DW = dry weight; DAP = days after planting

Table 3.14: Effects of tetraconazole (TC), planting pattern, and drought stress on number of leaves and shoot dry weight of greenhouse-grown maize seedlings at 57 DAP. Drought stress was imposed for 28 days, rewatered, and grown for 12 days more days. Data are pooled across two hybrids.

Planting Pattern	Treatment	Leaf Number	Shoot DW
		plant^{-1}	g plant^{-1}
Pure planting	-TC/WW	6.8	0.63
	-TC/DS	5.6	0.33
	+TC/WW	7.3	0.74
	+TC/DS	6.6	0.40
Inter-planted	-TC/WW	6.6	0.58
	-TC/DS	5.9	0.37
	+TC/WW	6.6	0.73
	+TC/DS	6.0	0.37
	$LSD_{0.05}$	0.5	0.13

WW = well-watered throughout, DS = drought-stressed (irrigation withheld for 28 days); DW = dry weight; DAP = days after planting

Table 3.15: Effects of drought stress on some physiological responses of greenhouse-grown maize seedlings. proline content was determined at 24 DAP. Photosynthesis, transpiration, chlorophyll fluorescence, plant and soil water status were measured 30 DAP. Data are pooled across two hybrids, two planting patterns, and two TC rates.

	7 DIW	----------16 DIW----------					
Treatment	Proline	Photosynthesis	Transpiration	Chlorophyll Fluorescence	Plant Water Status	θ_V	Ψ_{soil}
	mg g^{-1} FW	µmol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	Fv/Fm[Þ]	press units[¶]	%	MPa
WW	0.017	12.66	5.78	0.62	145.9	39.8	-0.1
DS	0.257	3.57	4.29	0.45	223.4	20.9	-0.6
$LSD_{0.05}$	0.290	0.90	0.41	0.03	6.1	0.95	

DIW = days of irrigation withdrawal; WW = well-watered throughout, DS = drought-stressed (irrigation withheld for 7 or16 days),
Þ = maximal to variable fluorescence ratio at 690 nm; θ_V = volumetric soil moisture content; Ψ_{soil} = soil water potential;
¶ = higher values indicate lower water status; DAP = days after planting; TC = tetraconazole

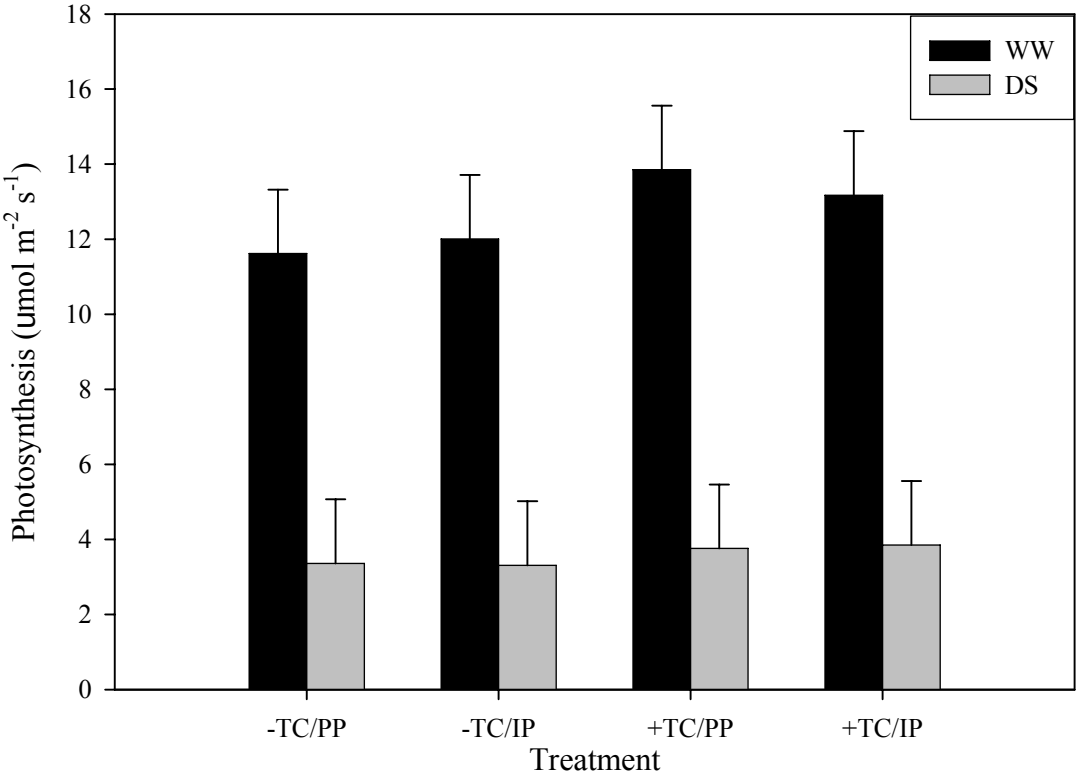

Figure 3.5: Effect of tetraconazole (TC) and drought on photosynthesis of greenhouse-grown maize seedlings. Error bars represent the LSD0.05 value of 1.71.WW = well watered, DS = drought stressed (irrigation withheld for 16 days). Data are pooled across two hybrids. PP = pure plantings; IP = inter-planted.

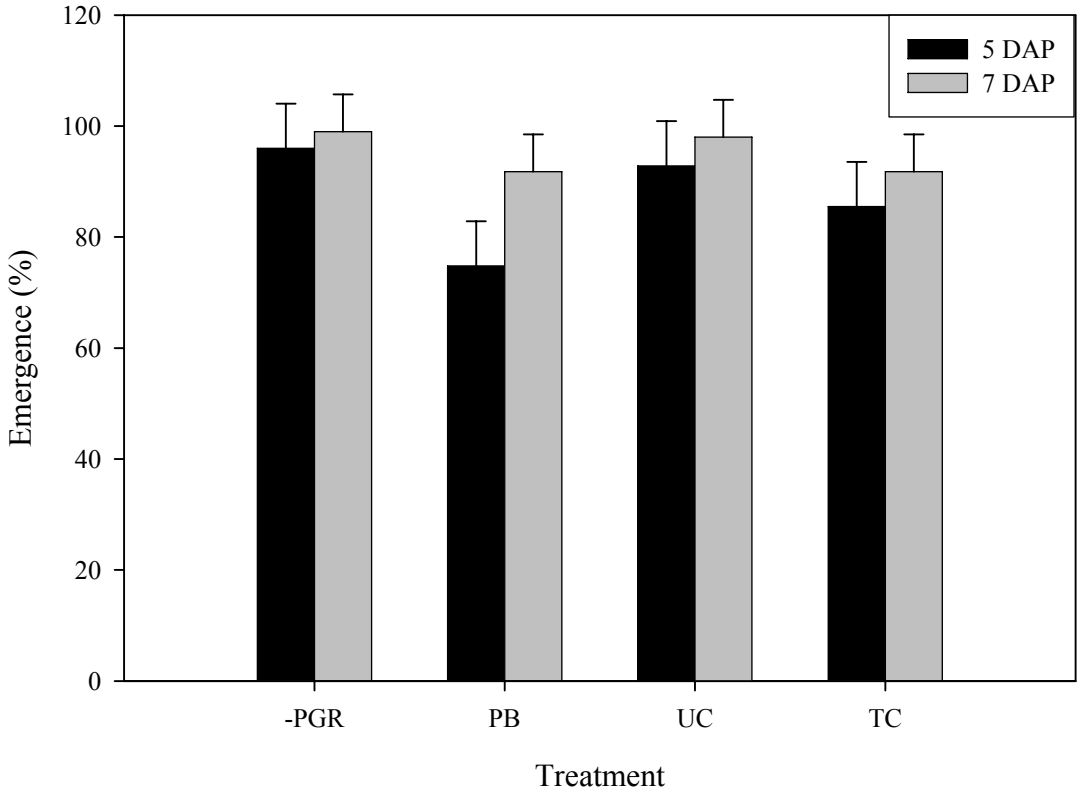

Figure 3.6: Effect of paclobutrazol (PB), uniconazole (UC) and tetraconazole (TC) on greenhouse-grown maize seedling emergence. The error bars represent $LSD_{0.05}$ values of 8.04 (5 DAP) and 6.74 (7 DAP). Data are pooled across two hybrids

Table 3.16: The effect of plant age and triazole treatment on leaf number, plant height, and biomass distribution of greenhouse-grown maize seedlings. Data are pooled across two hybrids.

Plant Age	Treatment	Leaf Number	Plant Height	Root DW	Shoot DW	Total DW	Root: Shoot
			cm	----------g plant^{-1}----------			
14 DAP	-PGR	nd	20.6	0.116	0.076	0.19	1.50
	PB	nd	10.3	0.105	0.061	0.16	1.76
	UC	nd	9.8	0.114	0.065	0.18	1.76
	TC	nd	19.5	0.100	0.070	0.18	1.32
	$LSD_{0.05}$	na	1.6	ns	0.010	0.02	0.36
35 DAP	-PGR	5.9	37.6	0.62	0.55	1.16	1.10
	PB	6.1	23.4	0.58	0.44	0.84	1.31
	UC	6.5	19.8	0.55	0.42	0.91	1.32
	TC	6.0	40.4	0.77	0.62	1.39	1.24
	$LSD_{0.05}$	0.5	3.3	ns	0.09	0.34	ns

nd = not determined; DW = dry weight; DAP = days after planting

Table 3.17: Effects of drought stress on shoot dry weight, plant height, and leaf number of greenhouse-grown maize seedlings at 56 DAP. Drought-stressed plants were grown for 14 days and irrigation was withheld for 28 days, then rewatered, and grown for 2 more weeks. Data are pooled across two hybrids and four PGR treatments.

Treatment	ShootDW	Plant Height	Leaf Number
	g plant^{-1}	cm	plant^{-1}
WW	0.57	27.3	6.3
DS	0.43	22.8	5.7
$LSD_{0.05}$	0.05	2.5	0.3

WW = well-watered throughout; DS = drought-stressed (irrigation withheld for 28 days); DW = dry weight; DAP = days after planting

Table 3.18: Effects of paclobutrazol (PB), uniconazole (UC), and tetraconazole (TC) and drought stress (irrigation withheld for 28 days beginning 14 DAP) on leaf area, leaf number, plant height, and shoot weight of greenhouse-grown maize seedlings. Data are pooled across two hybrids.

Treatment	Leaf Area	Leaf Number	Plant Height		Shoot DW
			14 DAP	56 DAP	
	cm^2 $plant^{-1}$	$plant^{-1}$	----------cm----------		g $plant^{-1}$
Paclobutrazol					
-PB/WW	104.4	6.1	22.5	31.1	0.58
-PB/DS	—	5.6	23.3	24.1	0.44
+PB/WW	92.2	6.1	12.3	25.9	0.59
+PB/DS	—	5.9	12.8	23.3	0.44
Uniconazole					
-UC/WW	104.4	6.5	21.9	28.8	0.57
-UC/DS	—	5.4	22.8	25.3	0.39
+UC/WW	94.6	7.3	10.0	17.6	0.55
+UC/DS	—	6.0	9.4	15.5	0.36
Tetraconazole					
-TC/WW	120.4	6.3	23.1	29.9	0.56
-TC/DS	—	5.8	22.9	24.6	0.45
+TC/WW	113.2	6.1	23.1	30.9	0.60
+TC/DS	—	5.6	23.6	24.3	0.48
$LSD_{0.05}$	37.0	0.7	1.6	4.84	0.14

WW = well-watered throughout, DS = drought-stressed (irrigation withheld for 28 days); DW = dry weight; DAP = days after planting

Table 3.19: Effects of drought stress (irrigation withheld for 11 or 16 days beginning at 14 DAP) on some physiological and biochemical responses of greenhouse-grown maize seedlings. Proline content was determined 25 DAP, and all other parameters were measured 30 DAP. Data are pooled across two hybrids, two planting patterns, andfour PGR treatments.

	11 DIW	----------16 DIW----------					
Treatment	Proline	Photosynthesis	Transpiration	Chlorophyll Fluorescence	Plant Water Status	θ_V	Ψ_{soil}
	mg g^{-1} FW	µmol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	Fv/Fm[Þ]	press units[¶]	%	MPa
WW	0.060	11.33	5.17	0.60	148.3	41.8	-0.1
DS	0.440	1.93	1.40	0.51	232.85	17.8	-0.8
$LSD_{0.05}$	0.060	1.80	0.82	0.03	8.3	2.77	

DIW = days of irrigation withdrawal; WW = well-watered throughout; DS = drought-stressed (irrigation withheld for 11 or 16 days);
Þ = variable to maximal fluorescence ratio at 690 nm; θ_V = volumetric soil moisture content; Ψ_{soil} = soil water potential;
¶ = higher values indicate lower water status; DAP = days after planting

Table 3.20: Effects of paclobutrazol (PB), uniconazole (UC), and tetraconazole (TC), and drought stress (irrigation withheld for 11 or 16 days beginning 14 DAP) on proline content, gas exchange, and plant water status of greenhouse-grown maize seedlings. Proline content was determined at 25 DAP. Photosynthesis and transpiration and plant water status were measured 30 DAP.

	11 DIW	----------16 DIW----------		
Treatment	Proline Content	Photosynthesis	Transpiration	Plant Water Status
	mg g^{-1} FW	μmol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	press units¶
Paclobutrazol				
-PB/WW	0.070	10.75	4.97	150.0
-PB/DS	0.362	1.11	1.39	228.8
+PB/WW	0.050	12.60	5.30	141.9
+PB/DS	0.375	2.18	2.00	223.8
Uniconazole				
-UC/WW	0.080	8.18	3.28	150.0
-UC/DS	0.443	1.52	1.10	236.3
+UC/WW	0.065	19.03	7.45	155.0
+UC/DS	0.613	2.15	1.30	222.5
Tetraconazole				
-TC/WW	0.050	6.60	4.26	148.8
-TC/DS	0.333	1.93	1.26	245.0
+TC/WW	0.054	10.13	5.77	147.5
+TC/DS	0.507	2.67	1.36	235.5
$LSD_{0.05}$	0.147	3.59	1.88	20.4

WW = well-watered throughout; DS = drought-stressed (irrigation withheld for 11 or 16 days); ¶ = higher values indicate lower water status; DAP = days after planting; DIW = days of irrigation withdrawal

Chapter 4

Discussion and Conclusions

4.1. Drought Stress Alters Assimilation, Allocation of Biomass, and Other Processes in Maize.

Findings from this work show that drought stress during the first month or two of a maize seedling's life affects overall biomass production as might be expected; but we also see that it can differentially affect allocation of biomass to roots and shoots. Shoot biomass is more likely to be reduced by drought than is root biomass. In fact, root growth may be increased by drought stress, even while shoot growth is being depressed, and this is indicated in Table 3.1. As a result, a frequent, consistent observation here was a drought-induced increase in root:shoot ratio. Clearly this response can favor survival in a water-limited situation by reducing transpirational surface area while maintaining (or increasing) absorptive root area.

When a plant encounters a severe water deficit, its photosynthesis may decline, leading obviously to a reduction in dry matter accumulation. This reduced photosynthetic output could be due to reduced light interception as leaf expansion is limited or as leaves senesce, and/or to reduced C fixation as stomates close or photo-oxidation damages the photosynthetic mechanisms. Measurements in this work and in Bartels and Salamani (1997) and Pelleschi et al. (1997) confirm drought-induced alterations in transpiration, in photosynthesis, and in chlorophyll fluorescence, which is a gauge of photosynthetic efficiency. Alterations at this level could affect plant assimilation of dry matter and might drive changes in dry-matter partitioning, or allocation, to root versus shoot.

In addition, drought might affect assimilation by altering nutrient translocation and metabolism. Plant nutritional status can affect dry matter accumulation and vice versa. According to Heckathorn and DeLucia (1994), N allocation to shoots decreases during drought in C_4 species (*Spartina pectinata*, *Andropogon gerardii*, *Schizachyrium scoparium*). Other nutrients reduced in shoots due to drought include P and Mg (Huang, 2001). Low shoot N and Mg could lead to loss of photosynthetic capacity and decreased whole-plant C gain both during drought and following rewatering.

Reduction of biomass production, i.e., net photosynthesis, in drought-stressed plants may also be due to nutrition-associated oxidative damage to shoot tissues (Zhang and Kirkham, 1996). When graminaceous species, such as cereals, are subjected to drought, Fe can accumulate and promote lipid peroxidation, or oxidative damage (Price and Hendry, 1991). The accumulation of Fe is more severe in shoots than roots (Price and Hendry, 1991). Therefore, shoots may be more affected than roots by drought-induced Fe accumulation not only in their reduced photosynthetic output but also in their overall metabolic disruption.

An increase in the root:shoot ratio due to drought in maize and other plants is well documented (Chiatante et al., 1999; Nguyen et al., 1997). In maize, the relative increase in root dry matter over that of shoot may be because at water potentials that completely inhibit shoot growth, the primary root continues to grow (Sharp and Davies, 1979; Sharp et al., 1988). Drought has previously been reported to cause a decrease in shoot dry weight and no decrease or even an increase in root dry weight, resulting in increased root to shoot ratio in maize (Schmidhalter et al., 1998). Internal phytohormonal signals, e.g., abscisic acid, might cause a shift in dry-matter partitioning patterns also. Translocation

of N from shoot to root has been reported in perennial C4 grasses under drought stress (Heckathorn and DeLucia, 1994). This could cause the root to grow more than the shoots and lead to an increased root:shoot ratio in drought stressed seedlings. By whatever, mechanisms, a shift in root:shoot ratio that favor roots can be a good drought-stress response or "strategy" for a seedling.

In this study, drought-stressed plants accumulated more proline than well-watered controls. Proline accumulation is a widespread response to low water potential (Abernethy and McManus, 1998; Gonzalez de Meija, 2003; Gzik, 1995; Showler, 2003). The accumulation of proline in maize due to drought has been previously reported (Carceller, 1999; Voetberg and Sharp, 1991). Plants under drought stress accumulate proline for use as compatible, osmotically active solute as discussed in Chapter 1 and Section 4.3 of this chapter.

In one of the experiments reported here, two hybrids that are marketed as differing in drought sensitivity (as determined by grain yield) were compared in side-by-side trials. Those trials included pure plantings (each hybrid in separate pots) and inter-plantings (both hybrids in the same pot). The inter-planted studies were included to investigate more closely differences in drought responses under truly equal soil moisture levels. In studies where plants of differing genotypes are compared only in adjacent pots, it cannot be determined whether response differences are due solely to genotype or to confounding effects of differential soil moisture levels.

In this close study of the two hybrids, there were hybrid differences for root dry weight in the spring trial, but there were no differences in the summer trial for any of the parameters measured (root, shoot, total biomass, root:shoot ratio, plant height, or leaf

number); and there was no hybrid-by-drought interactions for these parameters. Furthermore, in experiments that followed, there were no hybrid-by-drought interactions for the key physiological and biochemical parameters measured. This suggests that the two hybrids at the seedling stage did not generally respond differently to drought stress. It was hypothesized that hybrid 31R88, which is considered less drought sensitive, might respond differently compared to hybrid 32W86. The lack of a difference in response may be due to the stage of development or the greenhouse environment under which these comparisons were made. The drought resistance ratings from Pioneer are based on grain yield and a full life cycle for a planting.

4.2. The Influence of Triazoles on Germination and Seedling Growth

Paclobutrazol (PB) did not inhibit germination in the study reported here where seeds were treated and grown for 7 days in the dark; but in other studies involving maple (Marshall et al., 2001), apple (*Malus domestica*) (Mage and Powell, 1990), celery (*Apium graveolens*) (Pressman and Shaked, 1988), and maize (Khalil and Rahman, 1995), PB at the levels used here has inhibited germination. By contrast, PB administered at comparable levels was not reported to inhibit germination in maize or soybean (Barnes et al. 1989) or wheat (Gilley and Fletcher, 1997). Differences in results may be due to species/cultivar, application method, or experimental conditions. For example, Khalil and Rahman (1995) continuously applied their PGR solutions during the germination stage, while in this study the PGR solution was washed off the seed surface after imbibition.

In these studies, root dry weight of 7-day-old, dark-grown seedlings was not affected by PB treatments, while shoot dry weight and total biomass were reduced. The reduction of the shoot dry weight caused the root:shoot ratio to increase. A similar effect

of PB on reducing shoot biomass without affecting root growth in maize was previously reported (Barnes et al., 1989). Such a shift in root:shoot balance might obviously be favorable in a drought-stressed situation. In fact, it seems to mimic what occurs naturally under drought.

As in the PB experiment, uniconazole (UC) did not inhibit germination or emergence. Although root and shoot dry weights were not affected by UC treatment, the root:shoot ratio of 7-day-old seedlings was increased at 10 mg L^{-1}. As seen in Table 3.5, that UC treatment gave higher root and lower shoot dry weights, although the differences were not statistically significant at the 0.05 level. In loblolly pine seedlings, UC was reported to increase the root:shoot ratio (Barnes and South, 2004).

In the tetraconazole (TC) studies on germination and early seedling growth in the dark, maize germination was slightly reduced. In studies with wheat, TC at 50 mg L^{-1} did not inhibit germination (Gilley and Fletcher, 1997). This difference may be due to species or method of application. As with the other triazoles, TC reduced shoot biomass and total biomass without affecting the root biomass of 7-day-old seedlings.

In a second study (designed to look at growth in the greenhouse), emergence was lower for the PGR-treated seeds at 5 DAP but not at 7 DAP. These results suggest that the PGRs at these rates did not inhibit germination and emergence but just delayed them. The reduced germination percent observed in the laboratory TC experiment may have been due to delayed but not complete inhibition of germination. This could also account for the lower biomass at harvest. This is consistent with findings that triazole treatments delay germination in sunflower and safflower (*Carthamus tinctorius*) over a 5-day period (Kar and Gupta, 1991). Delayed germination in seeds could be due to the fact triazoles

interfere with gibberellin biosynthesis and thereby enzymes responsible for mobilization of seed food reserves. Triazoles have been reported to inhibit α-amylase and protease activities in seedlings of barley (Prusakova et al, 2004) and pea (Hathout, 1999).

4.3. Effects of Triazoles in Combination with Drought Stress on Plant Responses

A series of studies were conducted in the greenhouse to investigate the effect of drought on triazole-treated plants. Throughout these experiments, drought stress reduced plant growth and affected distribution of biomass as observed and discussed in 4.1. While drought stress increased the root:shoot ratio, the PB-treated plants had a reduced root:shoot ratio compared to the non-PGR-treated plants. Similar studies, also with a reduced root:shoot ratio, were reported in well-watered barley and wheat (Khalil, 1995). However, Fletcher et al., (2000) suggest that higher root:shoot ratios are characteristic of triazole-treated plants. To our knowledge, this is the first experiment involving PB-treated, drought-stressed maize seedlings in which root:shoot ratio was assessed. Therefore, the decrease in the root:shoot ratio (relative to the drought-stressed, non-PGR-treated plants) could be due to species-by-triazole-by-drought interaction. (Alternatively, it may reflect the inherent difficulty in managing studies to avoid confounding results due to differing soil water potentials in pots corresponding to different treatments.) This suggests that PB-treated plant's shoots were less sensitive to drought, and as such grew more relative to the non-PGR-treated ones, thereby reducing the root:shoot ratio. Carcellar and Franschina (1986) reported that, although root growth is reduced during drought stress in maize seedlings, its reduction is not as much as in shoots, and on rewatering the shoots initiate growth rapidly, while the roots remain depressed. The PB-

treated plants' shoots may be growing much more rapidly than the non-treated seedling upon re-watering, causing a reduction in root:shoot ratio.

The results from this work also show that the PB effect on plant height was short-lived. Plants that were shortened by PB at 14 DAP were as tall as the no-PGR controls by 40 to 50 DAP. Paclobutrazol lasts for 2 to 3 weeks in wheat tissues but becomes indetectable thereafter (Fletcher et al., 2000).

In contrast, treating plants with UC inhibited plant elongation through the period of observation, which implies that, compared to PB, UC is a more effective inhibitor of gibberellin biosynthesis, or it persists longer in the plant system. The UC-treated plants under drought stress had reduced root:shoot ratios relative to the non-PGR, drought-stressed plants, similar to what was observed in the PB treatment. The UC-treated plants under well-watered conditions had consistently more leaves (Table 3.11, 3.17, 3.20). Uniconazole has been reported to increase tiller number in wheat (Fletcher et al., 2000) and to increase the number of *in vitro* adventitious shoots in carnation (Sankhla et al., 1994). Therefore, it is possible that UC promotes organogenesis, leading to increased leaf number. However, a UC-promoted increase in leaf number was not observed following drought stress.

Tetraconazole did not reduce plant height in any of the experiments reported here, in contrast to PB and UC treatments. Treatment of wheat seed with TC did not reduce plant height (Gilley and Fletcher, 1997). This suggests that this triazole is not an effective inhibitor of gibberellin biosynthesis, which is the presumed mode of action for the other two triazoles. The TC-treated plants under well-watered conditions – but not under drought stress – had increased shoot biomass and total biomass. This may be due to

increased dry matter accumulation as a result of higher photosynthesis or differential partitioning of assimilates.

Key physiological and biochemical parameters such as photosynthesis, photochemical efficiency, plant water status, and proline content were also examined in this research. The results show that, while photosynthesis, photochemical efficiency, and proline content were not increased by PB treatment, transpiration rate was higher in the PB-treated, drought-stressed plants. Previous studies have suggested that, under moisture stress conditions, triadimefon treatment reduced transpiration and increased relative water content in wheat (Sairam et al., 1995) and paclobutrazol reduced water loss in *Prunus serotina* micro propagation media (Eliasson et al., 1994). Perhaps PB-treated plants conserved water, leaving more water in their system at the time of measurement. When inter-planted with PB-non-treated plants (and therefore under truly equal water potential), there was no difference in transpiration either when drought-stressed or well-watered. PB-treated plants in pure-planted pots had higher Ψ_{soil} (-0.8 MPa) than non-treated controls (-1.0MPa), although the volumetric soil moisture content values were not statistically different.

Uniconazole-treated plants had increased photosynthesis, transpiration (under both drought-stressed and well-watered conditions), and plant water status under drought stress. Tetraconazole–treated plants were also found to have higher photosynthesis under well-watered conditions (Figure 3.2). Triazoles are known to cause darker green leaves, possibly because of increase in chlorophyll content per unit leaf area, or more densely packed chloroplasts in a smaller leaf area (Khalil, 1995; Khalil and Rahman, 1995). Triadimefon was reported to induce sun-type chloroplasts in barley and wheat (Khalil,

1995) and radish (Fletcher et al., 2000), which allowed a higher photosynthetic conversion. Therefore, the high photosynthesis rates in UC- and TC-treated plants could be due to the developmental changes that occur in chloroplasts as a result of triazole treatment. It is also interesting to note that, under drought-stress conditions, pure-planted seedlings treated with UC had higher photosynthetic rates; but, when they were inter-planted with non-PGR-treated seedlings, the rates were not significantly different. This is probably a soil moisture effect, as the UC-treated pure planted seedlings had higher soil moisture content and plant water status; whereas, in the inter-planted pattern, the plants were exposed to equal soil moisture content. These transpiration responses under drought stress condition correspond to what was observed in the PB treatment.

Drought increased proline in every case from two to about 18 times the amount of the well-watered controls. Proline accumulation in maize under drought stress is well documented (Carceller et al., 1999; Ibarra-Cabalero et al., 1988; Voetberg and Sharp, 1991). Proline is believed to be important in osmotic adjustment and drought tolerance. The PGR treatment in the first series of experiments did not increase proline content, although consistently higher but not significantly different values were seen. Experimental repetition showed that UC and TC increased proline content under drought stress (Table 3.20). Differences between studies could be due to environmental conditions in the greenhouse. For example, the samples from PB study (section 2.2.1) were obtained during a rainy day in the morning, while in the additional experiment (2.3.1); sampling was during clear-sky mid-day. According to Anguilar et al. (2003), proline content increases as environmental conditions become less favorable to the plants.

Perhaps this determines the extent to which differences between treatments become significant.

Under drought-stress conditions, UC and TC significantly increased proline content in the final experiment. While there is no direct connection between triazoles' mode of action and proline biosynthesis, previous studies have reported that triazoles increased proline content in plants. Under drought-stress conditions, PB increased proline content in black locust (*Robinia pseudoacacia*) seedlings (Shen and Zeng, 1993), and UC increased the amino acid's level in wheat (El-Khallal and Nafie, 2003) and oil-tea (*Camellia oleifera*) trees (PingQing et al., 1998). To our knowledge, there is no information about the effect of TC on proline content in plants, despite the fact that the triazole increased proline in this experiment. It should also be noted that PB did not significantly increase the proline content in this study probably due to species difference and perhaps the level of moisture stress at the time of sample. In rice, a "multi-effect" triazole can delay proline accumulation, which becomes rapid however when zero turgor is reached (Wang and Shen, 1991). Perhaps PB behaves in a similar manner to the triazole used by Wang and Shen (1991).

In drought-stressed plants, proline may accumulate through an increase in its synthesis concomitantly with inhibition of its catabolism (Delauney and Verma, 1993). Therefore, it could be reasoned that the increase in proline due to triazole treatment is due to enhanced synthesis and/or reduced degradation, which could be modulated through key enzymes involved.

4.4. Conclusions

Some of the triazoles used in this study elicited drought resistance mechanisms in the seedlings of the two maize hybrids. The mechanisms included early increase in root:shoot ratio in the laboratory studies, water conservation due to lowered water use, and increased proline content under drought stress. These are some of the drought avoidance and tolerance mechanisms important for crop plants to survive in an environment of uncertain rainfalls, such as Botswana. Lack of germination and emergence inhibition by the triazoles implies that they might safely be used as chemical manipulation to maize for drought resistance. It was also noted that the triazoles can offer an advantage under well-watered conditions, as treated plants had higher photosynthesis. This may increase yield, especially if the effect persists into the reproductive stage. It was also found that, although one triazole (UC) increased photosynthesis, its growth inhibition persisted throughout the experimental period. Therefore, it will be important to grow the seedlings beyond that period to investigate the effect it may have on yield and yield components.

In sum, seed-treatments with triazoles may have some promise for altering maize growth and response to drought such that the seedlings are more likely to survive and thrive when water becomes available. These findings suggest some good avenues of approach for continuing studies.

5.0. Literature Cited

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6.0. Appendices Table of Contents

6.1. Appendix A. (ANOVA Tables)

6.2. Appendix B. (Two Figures)

6.1. Appendix A. (ANOVA Tables)

Table A.1: ANOVA P-values for several morphological variables from the preliminary study of a drought-sensitive hybrid involving differing plant populations and differing drought-stress durations.

	Response Variable					
SOV	Leaf Number	Plant Height	Root DW	Shoot DW	Total DW	Root: Shoot
P	0.1236	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0236**
I	**0.0013**	**<0.0001**	**0.0125**	**<0.0001**	**0.0005**	**<0.0001**
PxI	0.7711	0.0825	**0.0374**	**0.0007**	**0.0112**	0.2640

SOV = source of variation; P = plant population (4 to 16 plants pot^{-1}); I = days of irrigation withdrawal (0 to 35 days); DW = dry weight

Table A.2: ANOVA P-values for several morphological variables from a study involving two hybrids of differing drought sensitivity and two trials in different seasons. Data are from pure and inter-planted seedlings.

	Response Variable		
SOV	Leaf Number	Plant Height	Shoot DW
H	0.7798	**0.0131**	**0.0059**
D	**<0.0001**	**<0.0001**	**<0.0001**
P	0.0578	0.4584	0.1816
S	**<0.0001**	**<0.0001**	**<0.0001**
HxD	0.2864	0.7817	0.3108
HxS	**<0.0001**	**<0.0001**	**0.0019**
DxS	**<0.0001**	**0.0016**	**<0.0001**
NxS	**0.0259**	**0.0104**	**0.0259**
HxDxPxS	**<0.0001**	**0.0049**	**<0.0001**

SOV = source of variation; H = hybrid (two); D = drought (yes or no); P = planting pattern (pure or inter-planted); S = season (spring or summer); DW = dry weight.

Table A.3: ANOVA P-values for several morphological variables from a study involving two hybrids of differing drought sensitivity and two trials in different seasons. Data are from pure-planted seedlings only.

	Response Variable						
SOV	Leaf Number	Plant Height	Root DW	Shoot DW	Total DW	Root: Shoot	θv
Spring '05							
H	0.4202	0.4782	**0.0036**	0.2530	0.2530	**0.0205**	0.5683
D	**0.0013**	**0.0080**	0.2391	**<0.0001**	0.3569	0.0511	**<0.0001**
HxD	0.7731	0.9571	0.1570	0.5121	0.5121	0.3477	0.8093
Summer '05							
H	1.000	0.4072	0.1106	0.1022	0.0747	0.7444	0.5940
D	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.1838	**<0.0001**
HxD	0.2704	0.8448	0.4400	0.5624	0.5624	0.3708	0.9697

SOV = source of variation; H = hybrid (two); D = drought (yes or no); DW = dry weight

Table A.4: ANOVA P-values for several variables from three laboratory studies (one PGR^{-1}) on effects of three triazoles on germination and early seedling growth. Each PGR was examined and analyzed in a separate study.

	Response Variable				
SOV	Percent Germination	Root DW	Shoot DW	Total DW	Root: Shoot
H					
PB	**0.0303**	**0.0137**	**0.0272**	**0.0115**	0.1815
UC	**0.0011**	**0.0023**	**0.0156**	**0.0014**	0.5122
TC	0.1423	**0.0001**	**0.0052**	**0.0021**	0.7315
PGR					
PB	0.2267	0.3393	**0.0001**	**0.0493**	**0.0001**
UC	0.5464	0.3099	0.2904	0.9061	**0.0344**
TC	**0.0116**	0.0894	**0.0471**	**0.0429**	0.2341
HxPGR					
PB	0.9683	0.6462	0.2705	0.9454	0.1065
UC	0.2961	0.4491	0.9112	0.5551	0.9481
TC	0.9194	0.0635	0.6955	0.1955	0.5912

SOV = source of variation; H = hybrid (two); PGR = plant growth regulator (three rates); PB = paclobutrazol; UC = uniconazole; TC = tetraconazole; DW = dry weight

Table A.5: ANOVA P-values for several physiological variables from greenhouse studies on effects of paclobutrazol on seedling growth and drought responses.

	Response Variable				
SOV	Proline Content	Photosynthesis	Transpiration	Chlorophyll Fluorescence	θv
H	0.1742	**<0.0001**	**<0.0001**	0.1908	0.2971
D	**0.0424**	**<0.0001**	**<0.0001**	0.1908	**<0.0001**
P	0.3066	0.6460	0.2916	0.6709	0.5093
PGR	0.9013	0.8445	0.4828	0.9121	0.7675
HxD	0.1188	0.6718	0.4361	0.6154	0.5555
PxD	0.1758	0.2162	0.9896	0.2675	0.7440
HxPGR	0.6819	0.7161	0.6270	0.9880	0.6222
DxPGR	0.7679	0.4441	**0.0191**	0.9924	0.4489
HxDxPGR	0.6159	0.8830	0.6249	0.8927	0.9519

SOV = source of variation; H = hybrid (two); P = planting pattern (pure or inter-planted); D = drought (yes or no); PGR = plant growth regulator (two rates of paclobutrazol); θv = volumetric moisture content

Table A.6: ANOVA P-values for several physiological variables from studies on effects of paclobutrazol on seedling growth and drought responses (pure plantings only).

		Response Variable				
Treatment	SOV	Proline Content	Photosynthesis	Transpiration	Chlorophyll Fluorescence	θv
WW+DS	H	0.1563	0.7417	0.5716	0.1704	0.9353
	D	0.0963	**<0.0001**	**<0.0001**	0.1060	**<0.0001**
	PGR	0.7997	0.9971	0.2268	0.5306	0.3151
	HxD	0.1638	0.8134	0.3153	0.4139	0.8312
	HxPGR	0.7711	0.5678	0.9794	0.7315	0.4921
	DxPGR	0.7082	0.7001	0.1188	0.6471	0.2577
	HxDxPGR	0.6328	0.7759	0.5754	0.4036	0.2577
WW	H	0.8410	0.7225	0.7384	0.0771	0.1596
	PGR	0.5043	0.8067	0.7802	0.8843	0.2331
	HxGR	0.3060	0.5916	0.6613	0.6796	0.6158
DS	H	0.1699	0.9401	0.3075	0.7271	0.3455
	PGR	0.7553	0.7598	0.0810	0.5122	0.4763
	HxPGR	0.7029	0.8174	0.7243	0.4763	0.3971

WW+DS = well-watered and drought-stressed data combined; WW = well-watered plants only; DS = drought-stressed plants only; SOV= source of variation; H = hybrid (two); D = drought (yes or no); PGR = plant growth regulator (two rates of paclobutrazol); θv = volumetric soil moisture content

Table A.7: ANOVA P-values for several physiological variables from studies on effects of paclobutrazol on seedling growth and drought responses (inter-plantings only).

		Response Variable				
Treatment	SOV	Proline Content	Photosynthesis	Transpiration	Chlorophyll Fluorescence	θv
WW+DS	H	0.4344	0.5263	0.0683	0.8327	0.9023
	D	**0.0011**	**<0.0001**	**<0.0001**	0.8900	**<0.0001**
	HxD	0.2063	0.7179	0.6810	0.6210	1.0000
	PGR	0.1097	0.2550	0.9714	0.8870	0.3081
	HxPGR	0.2338	0.1223	0.4416	0.7069	1.0000
	DxPGR	0.4419	0.4261	0.0920	0.6264	1.0000
	HxDxPGR	0.7933	0.4703	0.9465	0.2964	1.0000
WW	H	**0.0147**	0.7596	0.0908	0.8004	0.8004
	PGR	0.5599	0.8041	0.0548	0.9956	0.9956
	HxGR	0.2890	0.1876	0.4969	0.6260	0.6260
DS	H	0.6399	0.1255	0.2806	0.9622	0.2939
	PGR	0.2713	0.3117	0.4533	0.5049	1.0000
	HxPGR	0.4262	0.4447	0.6303	0.3351	1.0000

WW+DS = well-watered and drought-stressed data combined; WW = well-watered plants only; DS = drought-stressed plants only; SOV = source of variation; H = hybrid (two); D = drought (yes or no); PGR = plant growth regulator (two rates of pacloburtrazol); θv = volumetric soil moisture content

Table A.8: ANOVA P-values for several morphological variables from studies on effects of paclobutrazol on seedling growth and drought responses.

	Response Variable				
SOV	Plant Survival	Leaf Number	Plant Height		Shoot DW
			14 DAP	55 DAP	
H	0.5740	**0.0093**	**0.0001**	0.0674	0.8899
D	**0.0049**	**<0.0001**	0.2213	**<0.0001**	**<0.0001**
PGR	0.1701	0.1074	**0.0001**	0.4985	0.6664
P	0.1443	0.1113	**<0.0001**	0.9327	0.9051
HxD	0.5740	0.2942	0.5503	0.8813	0.6096
PxD	0.1330	0.3852	0.9160	0.1888	0.2650
HxPGR	0.7206	0.8480	**0.0001**	0.6422	0.9152
DxPGR	0.1706	0.7017	0.9160	0.7732	0.8272
HxDxPGR	0.7202	0.2942	0.1530	0.7478	0.8480

SOV = source of variation; H = hybrid (two); P = planting pattern (pure or inter-planted); D = drought (yes or no); PGR = plant growth regulator (two rates of paclobutrazol)

Table A.9: ANOVA P-values for several morphological variables from studies on effects of paclobutrazol on seedling growth and drought responses (pure plantings only).

Treatment	SOV	Response Variable				
		Plant Survival	Leaf Number	Plant Height		Shoot DW
				14 DAP	55 DAP	
WW+DS	H	0.9353	**0.0010**	**0.0001**	0.0629	0.9353
	D	**0.0001**	**0.0001**	0.6905	**0.0001**	**0.0001**
	PGR	0.3151	0.0785	**0.0001**	0.6848	0.3151
	HxD	0.8312	0.2234	0.7905	0.8457	0.8312
	HxPGR	0.4921	0.3487	**0.0007**	0.8536	0.4929
	DxPGR	0.2577	0.1357	0.8594	0.3167	0.2577
	HxDxPGR	0.2577	0.2234	0.8248	0.5055	0.4858
WW	H	1.000	**0.0021**	**0.0091**	0.3556	0.9443
	PGR	1.000	0.8224	**0.0001**	0.7433	0.9443
	HxGR	1.000	0.8224	**0.0253**	0.7931	0.4667
DS	H	0.6371	0.1294	**0.0005**	**0.0222**	0.6822
	PGR	0.3788	0.0479	**0.0001**	0.1052	**0.0095**
	HxPGR	0.4983	0.1763	**0.0091**	0.3125	0.9887

WW+DS = well-watered and drought-stressed data combined; WW = well-watered plants only, DS = drought-stressed plants only; SOV = source of variation; H = hybrid (two), D = drought (yes or no); PGR = plant growth regulator (two rates of paclobutrazol); DW = dry weight

Table A.10: ANOVA P-values for several morphological variables from studies on effects of paclobutrazol on seedling growth and drought responses (inter-plantings only).

		Response Variable				
		Plant	Leaf	Plant Height		Shoot
Treatment	SOV	Survival	Number	14 DAP	55 DAP	DW
WW+DS	H	0.7585	0.7274	**0.0042**	0.4371	0.8438
	D	**0.0395**	**0.0041**	0.5407	**0.0007**	**0.0060**
	PGR	0.1330	0.5316	**0.0001**	0.6030	0.9123
	HxD	0.7585	0.7274	0.3814	0.9747	0.6643
	HxPGR	0.3599	0.5316	**0.0001**	0.6662	0.6108
	DxPGR	0.1330	0.3683	0.5757	0.6593	0.6769
	HxDxPGR	0.3599	0.7274	0.1705	0.8973	0.8483
WW	H	1.000	0.6123	0.1412	0.6842	0.7402
	PGR	1.000	0.2746	**0.0001**	0.6023	0.8717
	HxGR	1.000	0.4803	**0.0001**	0.7605	0.7129
DS	H	0.7611	1.0000	**0.0128**	0.3420	0.7290
	PGR	0.1458	0.8511	**0.0001**	0.9243	0.4464
	HxPGR	0.3691	0.8511	**0.0018**	0.7188	0.6428

WW+DS = well-watered and drought-stressed data combined; WW = well-watered plants only, DS= drought-stressed plants only; SOV= source of variation; H= hybrid (two), D= drought (yes or no); PGR = plant growth regulator (two rates of paclobutrazol); DW = dry weight

Table A.11: ANOVA P-values for several morphological variables from studies on effects of paclobutrazol on seedling growth and drought responses (pure-plantings only).

		Response Variable			
Treatment	SOV	Root DW	Shoot DW	Total DW	Root: Shoot
WW+DS	H	0.4709	0.9353	0.9715	0.3929
	D	**0.0001**	**0.0001**	**0.0001**	**0.0027**
	PGR	0.9112	0.3151	0.3902	0.2406
	HxD	0.9444	0.8312	0.8496	0.6244
	HxPGR	0.8020	0.4921	0.5782	0.7539
	DxPGR	**0.0346**	0.2547	0.2324	0.4151
	HxDxPGR	0.8344	0.4858	0.4654	0.4106
WW	H	0.6769	0.9443	0.9048	0.6045
	PGR	0.1952	0.9443	0.8543	0.6045
	HxGR	0.9787	0.4667	0.4972	0.1265
DS	H	0.5369	0.6822	0.8331	0.4797
	PGR	0.0872	**0.0096**	**0.0132**	0.2990
	HxPGR	0.7193	0.9887	0.8098	0.7858

WW+DS = well-watered and drought-stressed data combined; WW = well-watered plants only; DS = drought-stressed plants only; SOV= source of variation; H= hybrid (two), D= drought (yes or no); PGR = plant growth regulator (two rates of paclobutrazol); DW = dry weight

Table A.12: ANOVA P-values for several physiological variables from greenhouse studies on effects of uniconazole on seedling growth and drought responses.

	Response Variable					
SOV	Proline Content	Photosynthesis	Transpiration	Chlorophyll Fluorescence	Plant Water Status	θv
H	0.1968	0.8029	0.8576	0.9992	0.2879	0.4533
D	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
PGR	0.3119	**0.0001**	**0.0432**	0.3579	**0.0006**	**0.0128**
P	0.5626	0.5155	0.0190	0.9503	0.3880	0.3390
HxD	0.2350	0.6806	0.5172	0.2919	0.8309	0.5790
NxD	0.6115	0.5253	0.9314	0.8306	0.2017	0.6214
HxPGR	0.4892	0.6696	0.8601	0.9976	1.000	0.1323
DxPGR	0.5267	0.4426	0.3415	0.5865	0.1387	0.5267
HxDxPGR	0.5487	0.5622	0.3554	0.5175	0.6694	0.5175

SOV = source of variation; H = hybrid (two); D = Drought (yes or no); PGR = plant growth regulator (two rates of uniconazole); P = planting pattern (pure or inter-planted); θv = volumetric soil moisture content

Table A.13: ANOVA P-values for several physiological variables from studies on effects of uniconazole on seedling growth and drought responses (pure plantings only).

		Response Variable					
Treatment	SOV	Proline Content	Photosynthesis	Transpiration	Chlorohpyll Fluorescence	Plant Water Status	θv
WW+DS	H	0.3462	0.3491	0.7887	0.9560	0.7321	0.7592
	D	**<0.0001**	**0.0001**	**0.0167**	**0.0019**	**<0.0001**	**<0.0001**
	HxD	0.4269	0.7106	0.7592	0.8685	0.1786	0.8185
	PGR	0.8426	**0.0001**	**0.0329**	0.1886	0.0004	**0.0029**
	HxPGR	0.0783	0.5860	0.7768	0.7464	0.7321	0.0606
	DxPGR	0.7969	0.5991	0.7768	0.3507	0.7321	0.4196
	HxDxPGR	0.0894	0.2968	0.2406	0.9005	0.4951	0.4094
WW	H	0.1361	0.4079	0.5918	0.8591	0.5116	0.9670
	PGR	0.5443	**0.0057**	0.0854	0.7507	**0.0126**	0.1049
	HxPGR	0.4830	0.7458	0.4000	0.7199	0.5116	0.1545
DS	H	0.3933	0.6544	0.9821	0.9446	0.2012	0.4918
	PGR	0.8214	**0.0003**	0.1591	0.1685	**0.0116**	**0.0068**
	HxPGR	0.0959	0.2240	0.3996	0.9006	0.7914	0.1652

WW+DS= well-watered and drought-stressed data combined; WW= well-watered plants only; DS= drought-stressed plants only; SOV= source of variation; H= hybrid (two), D= drought (yes or no) ; PGR = plant growth regulator (two rates of uniconazole)

Table A.14: ANOVA P-values for several physiological variables from studies on effects of uniconazole on seedling growth and drought responses (inter-plantings only).

		Response Variable					
Treatment	SOV	Proline Content	Photosynthesis	Transpiration	Chlorohpyll Fluorescence	Plant Water Status	θv
WW+DS	H	0.3006	0.4490	0.9356	0.9554	0.0655	0.3714
	D	**0.0077**	**<0.0001**	**0.0008**	**0.0004**	**<0.0001**	**<0.0001**
	HxD	0.3260	0.8239	0.4643	0.1642	0.1209	0.2041
	PGR	0.3019	**0.0418**	0.7111	0.7827	0.1209	1.0000
	HxPGR	0.1357	0.9761	0.9160	0.6850	0.7507	1.0000
	DxPGR	0.3275	0.5343	0.1973	0.7238	**0.0167**	1.0000
	HxDxPGR	0.1661	0.7275	0.9095	0.3705	0.2105	1.0000
WW	H	0.2955	0.7432	0.4724	0.2537	0.8406	0.2765
	PGR	0.2955	0.3679	0.2925	0.9457	0.5900	1.0000
	HxPGR	**0.0416**	0.2925	0.9921	0.6693	0.3243	1.0000
DS	H	0.3230	0.4105	0.6530	0.3801	**0.0149**	0.4167
	PGR	0.3244	**0.0345**	0.3523	0.7047	**0.0057**	1.0000
	HxPGR	0.1630	0.7870	0.9039	0.4349	0.4536	1.0000

WW+DS= well-watered and drought-stressed data combined; WW= well-watered plants only; DS= drought-stressed plants only, SOV= source of variation; H= hybrid (two); D= drought (yes or no); PGR = plant growth regulator (two rates of uniconazole); θv = volumetric soil moisture content

Table A.15: ANOVA P-values for several morphological variables from studies on effects of uniconazole on seedling growth and drought responses.

SOV	Response Variable			
	Leaf Number	Plant Height		Shoot DW
		14 DAP	55 DAP	
H	**0.0004**	**<0.0001**	0.1988	0.1811
D	**0.0027**	0.9848	**0.0174**	**<0.0001**
PGR	**0.0004**	**0.0001**	**0.0001**	0.7385
P	0.7745	na	0.0402	0.3667
HxD	1.0000	0.8699	**0.0066**	**0.0268**
NxD	**0.0103**	na	**0.0486**	**0.0001**
HxPGR	1.0000	0.0024	0.4044	0.3815
DxPGR	0.1490	0.8976	**0.0011**	0.8142
HxDxPGR	0.5326	0.5721	0.3672	0.1260

SOV = source of variation; H = hybrid (two); D = drought (yes or no); PGR = plant growth regulator; P = planting pattern (pure or inter-planting); DW = dry weight

Table A.16: ANOVA P-values for several morphological variables from studies on effects of uniconazole on seedling growth and drought responses (pure plantings only).

Treatment	SOV	Response Variable			
		Leaf Number	Plant Height		Shoot DW
			14 DAP	55 DAP	
WW+DS	H	**0.0019**	**0.0113**	**0.0256**	0.9243
	D	**0.0133**	0.2593	**0.0038**	**0.0001**
	HxD	0.5434	0.7409	0.0989	0.0807
	PGR	**0.0019**	**0.0001**	**0.0001**	0.8867
	HxPGR	0.5433	**0.0253**	0.6716	0.7759
	DxPGR	**0.0331**	0.6674	**0.0001**	**0.0410**
	HxDxPGR	0.0767	0.6674	0.2532	0.3016
WW	H	0.0334	0.1231	0.4072	0.1425
	PGR	**0.0056**	**0.0001**	**0.0001**	0.0841
	HxPGR	0.1751	0.0761	0.0605	0.5418
DS	H	**0.0166**	**0.0449**	**0.0464**	0.2964
	PGR	0.2563	**0.0001**	**0.0003**	0.2269
	HxPGR	0.2563	0.1816	0.6948	0.4124

WW+DS = well-watered and drought-stressed data combined; WW = well-watered plants Only; DS = drought-stressed plants only; SOV = source of variation; H = hybrid (two); D = drought (yes or no); PGR = plant growth regulator (two rates of uniconazole)

Table A.17: ANOVA P-values for several morphological variables from studies on effects of uniconazole on seedling growth and drought responses (inter-plantings only).

Treatment	SOV	Response Variable			
		Leaf Number	Plant Height		Shoot DW
			14 DAP	55 DAP	
WW+DS	H	0.0577	**0.0003**	0.8761	0.1213
	D	0.0577	0.2158	0.3993	**0.0001**
	HxD	0.4014	0.5310	**0.0112**	0.1147
	PGR	0.0577	**0.0001**	**0.0001**	0.7508
	HxPGR	0.4014	**0.0297**	0.3806	0.2164
	DxPGR	0.1674	0.4947	0.2808	0.1084
	HxDxPGR	0.1674	0.6894	0.7422	0.2273
WW	H	0.5390	**0.0022**	**0.0485**	0.0813
	PGR	0.0821	**0.0001**	**0.0001**	0.4449
	HxPGR	0.2299	0.0530	0.3728	0.1668
DS	H	**0.0067**	**0.0367**	0.1094	0.9772
	PGR	0.5254	**0.0001**	**0.0001**	0.0817
	HxPGR	0.5250	0.2418	0.7150	0.9772

WW+DS = well-watered and drought-stressed data combined; WW = well-watered plants only, DS = drought-stressed plants only, SOV = source of variation; H= hybrid (two), D= drought (yes or no); PGR = plant growth regulator (two rates of uniconazole); DW = dry weight

Table A.18: ANOVA P-values for several morphological variables from studies on effects of uniconazole on seedling growth and drought responses (pure-plantings only).

		Response Variable			
Treatment	SOV	Root DW	Shoot DW	Total DW	Root: Shoot
WW+DS	H	0.4806	0.9243	0.5930	0.7630
	D	**0.0033**	**0.0001**	**0.0001**	**0.0196**
	HxD	0.2656	0.0807	0.9564	0.3294
	PGR	0.5006	0.8867	0.4661	0.9929
	HxPGR	0.2791	0.7759	0.3911	0.3557
	DxPGR	0.7967	**0.0410**	0.4793	0.4377
	HxDxPGR	0.5849	0.3016	0.3457	0.2412
WW	H	0.8012	0.1425	0.7378	0.4390
	PGR	0.8012	0.0841	0.3242	0.3731
	HxGR	0.7422	0.5418	0.9513	0.7682
DS	H	0.1287	0.2964	0.6793	0.4849
	PGR	0.4227	0.2269	0.9877	0.6731
	HxPGR	0.1700	0.4124	0.2172	0.2578

WW+DS= well-watered and drought-stressed data combined; WW= well-watered plants only; DS= drought-stressed plants only; SOV= source of variation; H= hybrid (two), D= drought (yes or no); PGR = plant growth regulator (two rates of uniconazole); DW = dry weight

Table A.19: ANOVA P-values for several physiological variables from greenhouse studies on effects of tetraconazole on seedling growth and drought responses.

	Response Variable					
SOV	Proline Content	Photosynthesis	Transpiration	Chlorophyll Fluorescence	Plant Water Status	θv
H	0.1265	0.2845	0.3678	0.9216	0.1060	0.0390
D	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
PGR	0.1623	0.5292	0.9685	0.0654	0.2653	0.1579
P	0.0988	0.8890	0.6430	0.3722	0.1055	0.8320
HxD	0.5984	0.5079	0.4864	0.1777	0.1555	0.5482
HxPGR	0.2010	0.9637	0.5877	0.7980	0.4149	0.3040
DxPGR	0.5918	0.6064	0.5190	0.7084	0.2653	0.5657
DxPGR	0.8165	0.5646	0.3885	0.2295	0.6589	0.1442
DxP	0.8581	0.8581	0.3581	0.0613	0.8573	0.5364
HxDxPGR	0.2192	0.9384	0.6364	0.7379	0.6294	0.5058

SOV = Source of variation; H = hybrid (two); D = Drought (yes or no); PGR = plant growth regulator (two rates of tetraconazole); P = planting pattern (pure or inter-planted); θv = volumetric soil moisture content

Table A.20: ANOVA P-values for several physiological variables from studies on effects of tetraconazole on seedling growth and drought responses (pure plantings only).

		Response Variable					
Treatment	SOV	Proline Content	Photosynthesis	Transpiration	Chlorohpyll Fluorescence	Plant Water Status	θv
WW+DS	H	0.4725	0.2040	0.8364	.4848	0.7073	0.9494
	D	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
	PGR	0.5796	0.1067	0.1682	0.1618	0.1127	0.4683
	HxD	0.4801	0.9522	0.1039	0.4003	0.3841	0.8244
	HxPGR	0.0762	0.8966	0.4754	0.3258	0.9003	0.2178
	DxPGR	0.4308	**0.0240**	0.3278	0.9807	0.5325	0.4876
	HxDxPGR	0.0744	0.5908	0.2153	0.4003	0.5325	0.4219
WW	H	0.9370	0.4293	0.2672	0.2713	0.6458	0.9366
	PGR	0.1509	**0.0308**	0.1639	0.3009	0.3644	0.4789
	HxPGR	0.9370	0.6927	0.2444	0.1959	0.6458	0.3119
DS	H	0.4819	0.2797	0.2242	0.9220	0.4672	0.3945
	PGR	0.5072	0.5175	0.7214	0.3529	0.2014	0.9234
	HxPGR	0.0868	0.7135	0.6442	0.2220	0.6603	0.1946

WW = well-watered and drought-stressed combined; WW = well-watered plants only, DS = drought-stressed plants only; SOV = source of variation; H = hybrid (two), D = drought (yes or no); PGR = plant growth regulator (two rates of tetraconazole)

Table A.21: ANOVA P-values for several physiological variables from studies on effects of tetraconazole on seedling growth and drought responses (inter-plantings only).

		Response Variable					
Treatment	SOV	Proline Content	Photosynthesis	Transpiration	Chlorohpyll Fluorescence	Plant Water Status	θv
WW+DS	H	0.1838	0.7151	0.1078	0.2708	**0.0447**	**0.0009**
	D	**0.0001**	**0.0001**	**0.0004**	**0.0001**	**0.0001**	**0.0001**
	PGR	0.7602	0.6586	0.6786	0.7431	0.1555	1.000
	HxD	0.2331	0.4001	0.0907	0.0582	0.2653	0.0660
	HxPGR	0.6120	0.9657	0.9331	0.3626	0.2653	1.000
	DxPGR	0.8450	0.2397	0.9636	0.5137	0.2653	1.000
	HxDxPGR	0.6600	0.6066	0.5422	0.5583	0.6294	1.000
WW	H	0.0684	0.7636	**0.0430**	0.4662	0.3970	**0.0015**
	PGR	0.1518	0.3171	0.7642	0.7772	0.7747	1.000
	HxPGR	0.3945	0.7273	0.7312	0.2077	0.1689	1.000
DS	H	0.2191	0.3354	0.9448	0.0817	0.0735	0.4811
	PGR	0.8043	0.5464	0.7794	0.5555	0.1426	1.0000
	HxPGR	0.6413	0.7023	0.7023	0.8432	0.7018	1.0000

WW+DS = well-watered and drought-stressed data combined; WW = well-watered only; DS = drought-stressed plants only; SOV = source of variation; H = hybrid (two), D = drought (yes or no); PGR = plant growth regulator (two rates of tetraconazole); θv = volumetric soil moisture content

Table A.22: ANOVA P-values for several morphological variables from studies on effects of tetraconazole on seedling growth and drought responses.

SOV	Response Variable				
	Plant Survival%	Leaf Number	Plant Height		Shoot DW
			14 DAP	57 DAP	
H	0.5759	**0.0406**	**0.0001**	**0.0001**	0.1578
D	**0.0057**	**0.0001**	0.8896	0.8896	**0.0001**
PGR	0.3741	**0.0037**	0.4063	0.4063	**0.0066**
P	0.3119	**0.0201**	NA	NA	0.7390
HxD	0.1762	0.8767	0.0752	0.0752	**0.0260**
PxD	0.3649	0.4757	NA	NA	0.6154
HxPGR	0.4087	0.4875	0.5792	0.5792	0.7498
DxPGR	0.3043	0.2491	0.4886	0.4886	0.1100
HxDxPGR	0.2759	**0.0406**	0.6773	0.6773	0.2284

SOV = source of variation; H = hybrid (two); D = drought (yes or no); PGR = plant growth regulator (two rates of tetraconazole); P = planting pattern (pure or inter-planted); DW = dry weight

Table A.23: ANOVA P-values for several morphological variables from studies on effects of tetraconazole on seedling growth and drought responses (pure plantings only).

Treatment	SOV	Response Variable				
		Plant Survival%	Leaf Number	Plant Height		Shoot DW
				14 DAP	55 DAP	
WW+DS	H	0.3439	**0.0120**	**0.0001**	0.0724	0.7959
	D	**0.0458**	**0.0001**	0.4673	**0.0001**	**0.0001**
	PGR	0.3483	**0.0004**	**0.0365**	**0.0176**	**0.0418**
	HxD	0.3270	1.0000	**0.0028**	0.0724	0.1591
	HxPGR	0.4305	0.5034	0.0772	0.5085	0.7959
	DxPGR	0.3228	0.1869	0.7152	0.8943	0.6257
	HxDxPGR	0.2557	0.5034	1.0000	0.0554	0.3616
WW	H	0.3370	0.1089	0.0001	1.000	0.0927
	PGR	0.3370	0.1089	0.0892	**0.0334**	**0.0155**
	HxPGR	0.3370	1.000	0.2112	**0.0334**	0.4900
DS	H	0.9255	**0.0489**	**0.0009**	**0.0427**	0.5126
	PGR	0.8875	**0.0009**	0.2210	0.1708	0.3667
	HxPGR	0.3411	0.2948	0.2210	0.4341	0.5126

WW+DS= well-watered and drought-stressed combined; WW= well-watered plants only, DS= drought-stressed plants only, SOV= source of variation; H= hybrid (two); D= drought (yes or no); PGR = plant growth regulator (two rates of tetraconazole); DW = dry weight

Table A.24: ANOVA P-values for several morphological variables from studies on effects of tetraconazole on seedling growth and drought responses (inter-plantings only).

Treatment	SOV	Response Variable				
		Plant Survival%	Leaf Number	Plant Height		Shoot DW
				14 DAP	57 DAP	
WW+DS	H	0.0559	0.6782	**0.0297**	0.5717	0.1122
	D	**0.0001**	**0.0001**	0.6248	**0.0001**	**0.0001**
	PGR	0.8196	0.6782	1.0000	0.3354	0.1122
	HxD	0.0559	0.6782	0.5152	0.9354	0.1182
	HxPGR	0.8196	**0.0464**	0.8702	0.8080	0.5016
	DxPGR	0.8196	0.6782	0.5152	0.0610	0.1065
	HxDxPGR	0.8196	**0.0071**	0.6248	0.8080	0.4841
WW	H	1.000	1.0000	0.1079	0.7431	0.0604
	PGR	1.000	1.0000	0.7062	0.1570	0.0567
	HxPGR	1.000	**0.0167**	0.8502	0.8056	0.3945
DS	H	0.0676	0.4811	0.1368	0.2735	0.9819
	PGR	0.8160	1.0000	0.5356	**0.0405**	0.9819
	HxPGR	0.8216	1.0000	0.5356	1.0000	0.9819

WW+DS= well-watered and drought-stressed data combined; WW= well-watered plants only, DS= drought-stressed plants only; SOV= source of variation; H= hybrid (two), D= drought (yes or no); PGR = plant growth regulator (two rates of tetraconazole); DW = dry weight

Table A.25: ANOVA P-values for several morphological variables from studies on effects of tetraconazole on seedling growth and drought responses (pure-plantings only).

		Response Variable			
Treatment	SOV	Root DW	Shoot DW	Total DW	Root: Shoot
WW+DS	H	**0.0001**	0.2418	**0.0001**	**0.0033**
	D	**0.0001**	**0.0001**	**0.0001**	**0.0004**
	PGR	0.5312	**0.0350**	0.1374	0.4480
	HxD	0.7254	0.2264	0.4115	0.2016
	HxPGR	0.3260	0.6530	0.3199	0.4308
	DxPGR	0.6938	0.1388	0.7290	0.4839
	HxDxPGR	0.5879	0.6240	0.8103	0.2361
WW	H	**0.0087**	0.0927	**0.0065**	0.0711
	PGR	0.8905	**0.0155**	0.2799	0.1579
	HxGR	0.7924	0.4900	0.6593	0.0816
DS	H	**0.0011**	0.9783	**0.0048**	**0.0221**
	PGR	0.3561	0.6448	0.3036	0.9730
	HxPGR	0.1755	0.9783	0.2765	0.2693

WW+DS= well-watered and drought-stressed combined; WW= well-watered plants only; DS= drought-stressed plants only; SOV= source of variation; H= hybrid (two; D= drought (yes or no); PGR = plant growth regulator (two rates of tetraconazole); DW = dry weight

Table A.26 : ANOVA P-values for several morphological variables from greenhouse studies on effects of paclobutrazol, uniconazole , and tetraconazole on seedling growth.

	Response Variable				
SOV	Plant Height	Root DW	Shoot DW	Total DW	Root:Shoot Ratio
H	0.5132	0.7772	0.5639	0.9467	0.8213
PGR	**<0.0001**	0.0603	**<0.0001**	**0.0032**	0.2513
A	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
HxPGR	0.0681	0.9442	0.0887	0.8229	0.8556
HxA	0.7001	0.7003	0.5814	0.9038	0.2601
AxPGR	**0.0001**	**0.0492**	**0.0007**	**0.0054**	0.0664

SOV = source of variation; H = hybrid (two); PGR = plant growth regulator (four PGRs; PB, UC, TC and non-treated control); A = plant age (14 DAP or 35 DAP); DW = dry weight

Table A.27: ANOVA P-values for several morphological variables from greenhouse studies on effects of paclobutrazol, uniconazole , and tetraconazole on seedling growth at 14 DAP.

	Response Variable						
	Plant	Emergence		Root	Shoot	Total	Root:
SOV	Height	5 DAP	7 DAP	DW	DW	DW	Shoot
H	0.1565	0.8223	0.8095	0.5361	0.5304	0.9336	0.2510
PGR	**<0.0001**	**<0.0001**	0.0597	0.2855	**0.0006**	0.0994	**0.0494**
HxPGR	0.2007	0.7452	0.4923	0.9234	0.3644	0.6254	0.7623

SOV = source of variation; H = hybrid (two); PGR = plant growth regulator (four: PB, UC, TC, and non-treated control); DW = dry weight

Table A.28: ANOVA P-values for several morphological variables from greenhouse studies on effects of paclobutrazol, uniconazole , and tetraconazole on seedling growth at 35 DAP.

	Response Variable					
	Leaf	Plant	Root	Shoot	Total	Root:
SOV	Number	Height	DW	DW	DW	Shoot
H	0.1155	0.7871	0.7989	0.5438	0.9703	0.6312
PGR	**0.0452**	**<0.0001**	0.0850	**0.0004**	**0.0108**	0.3295
HxPGR	0.2869	0.2400	0.9433	0.1108	0.8300	0.9505

SOV = Source of variation; H = hybrid; PGR = plant growth regulator (four: PB, UC, TC, and non-treated control); DW = dry weight

Table A.29: ANOVA P-values for several morphological variables from studies on effects of paclobutrazol, uniconazole, tetraconazole on seedling growth and drought responses

	Response Variable				
	Leaf	Leaf	Plant Height		Shoot
SOV	Area	Number	14 DAP	56 DAP	DW
H	0.6752	**0.0018**	0.4782	0.2930	0.9941
D	-	**<0.0001**	0.8629	**0.0004**	**<0.0001**
HxD	-	0.0654	0.7443	0.5686	0.7617
PGR	0.5648	**0.0351**	**<0.0001**	**0.0007**	0.2214
HxPGR	0.5612	0.2950	0.7461	0.6467	0.8703
DxPGR	-	**0.0177**	0.9967	0.5695	0.6785
HxDxPGR	-	**0.0100**	0.8169	0.7526	0.9413

SOV= source of variation, H= hybrid (two), D= drought (yes or no), PGR = plant growth regulator (four: PB, UC, TC, and non-treated control); DW = dry weight

Table A.30: ANOVA P-values for several physiological variables from studies on effects of paclobutrazol, uniconazole, tetraconazole on seedling growth and drought responses

	Response Variable					
SOV	Proline Content	Photosynthesis	Transpiration	Chlorophyll Fluorescence	Plant Water Status	θv
H	0.4107	**0.0034**	0.6962	0.9770	**0.0490**	0.5086
D	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
HxD	0.9544	0.2017	0.8038	0.8629	0.1059	**0.0123**
PGR	0.0754	0.1046	0.8813	0.9070	0.2213	0.1970
HxPGR	0.9544	0.2008	**0.0181**	0.1679	0.6172	0.0992
DxPGR	0.1735	**0.0333**	0.7666	0.7677	0.2254	0.8552
HxDxPGR	0.6888	0.9304	0.1894	0.4496	0.1929	0.0592

SOV= source of variation; H= hybrid (two); D= drought (yes or no); PGR = plant growth regulator (four: PB, UC, TC, and non-treated control); θv = volumetric moisture content

6.2. Appendix B. (Two figures)

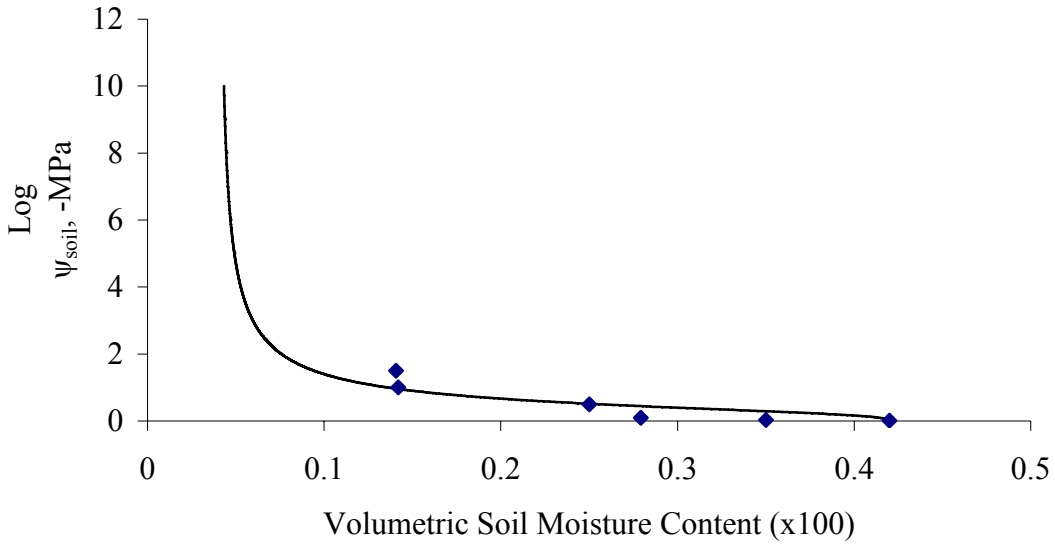

Figure B.1: Grade 3 vermiculite moisture release curve using procedures of Olson (1979).

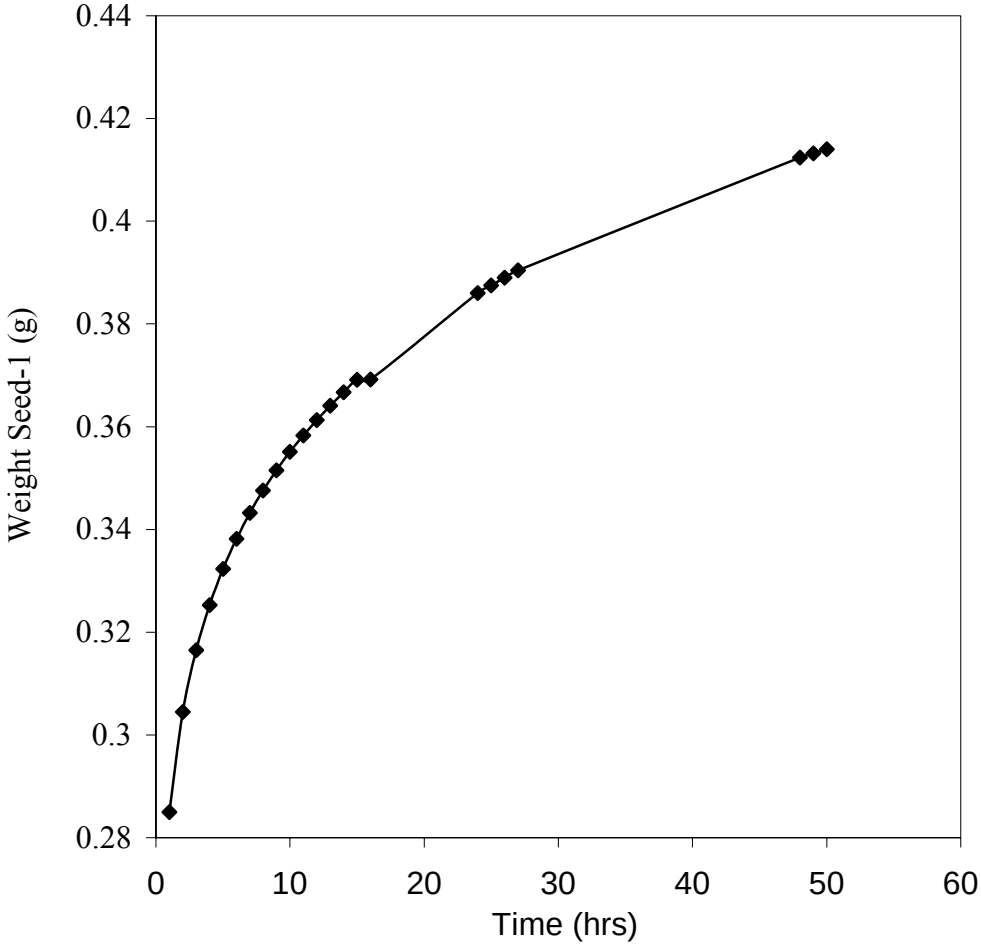

Figure B. 2: The maize hybrid 32W86 imbibition time course. Fresh weight gain of seeds imbibed in distilled water.

Vita

Utlwang Batlang was born October 10, 1968 in Lerala Village, Republic of Botswana to Ngwanyana and Batlang Modikwa. He lived in Lerala during his primary and junior secondary school education. Beginning 1988 he attended Moeng College, where he graduated with a Cambridge Overseas School Certificate in 1989. In 1990 he did a one-year national service with Botswana Government in the Kgalagadi Desert. In 1996 he enrolled for a degree of Bachelor of Science in Biological and Environmental Sciences at the University of Botswana, Faculty of Science, and graduated in 2001. He was employed as an Assistant Tutor in plant physiology at Botswana College of Agriculture, Department of Crop Science and Production in 2002. In May 2004 he was awarded a scholarship by Botswana College of Agriculture to study for the degree of Master of Science in the Department of Crop and Soil Environmental Sciences, 'Plant Physiology Program' at Virginia Polytechnic Institute and State University, Blacksburg, Virginia, from which he graduated in May 2006.