Chapter 1: General introduction

1.1 Background

Wheat (*Triticum aestivum* L.) is one of the most important food crops in the world and is mainly planted in the semi-arid and semi-humid areas. The world population is currently more than 6000 million and is expected to increase 35% by 2030 (Ali and Talukder, 2008). Simultaneously, the growing population will result in substantial additional demand for food. About 40% of the land in the world is under arid and semi-arid climatic conditions (Gamo, 1999). Efficient use of rainwater and optimisation of crop water productivity are important under these conditions. Therefore, the sustainability of crop production by alleviating either biotic or abiotic stresses is a major issue in managing and improving the productivity of the wheat crop.

Cereal crops are normally subjected to simultaneous multiple stresses. Drought is the most important environmental (abiotic) limiting factor for crop productivity (Fischer and Turner, 1978; Sivamani *et al*., 2000; Suiqi *et al*., 2002) and it is becoming an increasingly severe problem in many regions of the world. The drought in these regions may occur at any time during the growing season (Al-Karaki, 1998). Plant responses to drought are complex in addition to the complexity of drought itself (Passioura, 2007). However, different resistance mechanisms (tolerance, avoidance and escape) are adopted by plants when they are exposed to water stress (Levitt, 1980; Jones, 2004). Understanding the physiological mechanisms which can improve drought resistance may result in higher production of wheat in arid and semiarid areas (Chaves *et al*., 2003).

In addition to drought (abiotic stress), the biotic stress of fungal (soilborne) root diseases such as Pythium and Rhizoctonia root rot, can also reduce crop yield at almost any stage during development (Cook and Veseth, 1991). The pathogens, *Rhizoctonia* and *Pythium*, cause root decay, occlusion of vascular tissue, a decrease in root mass, and brown and sunken lesions in the root cortex resulting in variable crop stands, decreased tiller number, varying maturity date, and yield loss (Huber and McCay-Buis, 1993; Weller and Cook, 1986). Root diseases have been reported to decrease grain yields of wheat (Cook, 1992) and water use (Martin *et al*., 1986; Amir and Sinclair, 1996) resulting in a reduction in water use efficiency (Martin *et al*., 1986). Biotic stresses such as root diseases could have important interactions with drought, but this has not been studied extensively. It is unknown how important control of minor root pathogens is in increasing crop production when water is limiting.

The yield of dry land crops can be considered in terms of water use, water use efficiency (WUE) and harvest index i.e., mass of grain produced per unit mass of total dry matter (Passioura, 1977; Fischer and Turner, 1978). WUE, which is defined as the ratio of grain yield to crop water use, is the major concern in this research thesis which investigates experimentally the interaction between drought and root diseases. Experimental measurements are required to quantify the effect of this interaction on WUE. These measurements include water relations, stomatal conductance, photosynthetic rate and other physiological and yield parameters.

Roots have a crucial role in water uptake. However, roots can be damaged by diseases or mechanical pruning. It has been reported that both diseases and root pruning reduce root water uptake (Amir and Sinclair, 1996; Andrews and Newman, 1968). Therefore, water use or leaf transpiration will be affected substantially by root damage. A better understanding of the

effects of root damage (either by disease or pruning) under drought conditions on the ability of the crop to use available water may lead to increased WUE.

1.2 Research objectives

The objectives of this project were the following:

1. To investigate whether the interactions between root diseases and drought affect dry matter production, grain yield, WUE, and plant water relations and other physiological parameters
2. To identify the mechanisms which affect the ability to use available water in diseased plants during drought
3. To determine whether there are differences between root damage from diseases and root pruning under drought conditions.

Chapter 2: Literature review

2.1 Introduction

Ecological and agronomic research on crop management is important in achieving sustainability in grain production systems and alleviating the limiting factor of water supply and diseases for crop production in arid and semi-arid zones. Cereal production is limited by a number of abiotic and biotic stresses. One general aim is to improve WUE of wheat under drought and disease. However, there is a limited knowledge of the interactions of many of these stresses. Therefore, this literature review has focused primarily on the response of the water relations of *Triticum aestivum* to root diseases. This review describes the effects of root fungal diseases and drought on water use efficiency, harvest index, water relations, yield components and physiological parameters of wheat.

2.2 Wheat

Wheat belongs to the genus *Triticum* which has many species and subspecies, including the wild and primitive wheats that gave rise to modern wheat (Cook and Veseth, 1991). Wheats are divided into three categories, according to the number of chromosomes: one group has only two sets of chromosomes (diploid), another group has four sets of chromosomes (tetraploid), and the most important group has six sets of chromosomes (hexaploid). Among modern wheats, durum is tetraploid, while all common and club-type wheats are hexaploid. However, no economically important diploid wheats are grown today (Cook and Veseth, 1991). In the common hexaploid, *Triticum aestivum*, the older varieties have larger root systems but lower grain yield than modern varieties (Zhang *et al*., 1999).

Wheat is a widely-adapted crop. It is grown under irrigated and dryland cropping systems, in areas that are warm and humid to dry and/or cold environments. Wheat is an annual plant. Wheat can be grown in most areas where precipitation ranges from 250 to 1750 mm (Leonard and Martin, 1963). Optimal production needs a sufficient source of moisture during the growing season, but too much precipitation can cause yield losses from disease and root problems.

Tillers of wheat have the same structure as the main shoot, and these tillers arise from the axils of the basal leaves (McMaster, 2009). Seedling emergence is important in determining the number of shoots because a specific axillary bud which produces a tiller has a short window of time during which it can appear. In most cases, later tillers will senesce under limited resources. This has implications for final yield prediction because tillers and the main shoot are the primary yield-producing shoots. The root system of wheat contains both seminal and nodal (crown) roots. The seminal roots have usually 5-6 roots from one seed and these originate from primordia found in the seed. The nodal roots are produced from primordia developed after germination. Stem elongation occurs when nodes arise above the soil surface and internode elongation begins from the first node formed below the soil surface (McMaster, 2009). Booting is the stage when the spike can be felt within the whorl of leaf sheaths, but is not visible. Heading is the stage when the first spikelet of the spike (head or ear) appears above the ligule of the flag leaf (last leaf formed on the shoot) at the top of the canopy. Anthesis is the stage when anthers appear and pollen grains start to extrude. Meiosis usually occurs synchronously in both anthers and embryo sac mother cell when the ear is about to emerge from the inflated flag leaf sheath (the boot) (McMaster, 2009). The period of anthesis is short and lasts about 3-5 days. Anthesis is the transition between the end of heading and the

beginning of grain–fill. The grain filling starts when fertilisation of the female ovules occurs (Cook and Veseth, 1991). At this stage, about 40-50% of total biomass is deposited into the grains (Zhang and Yang, 2004).

Cook and Veseth (1991) provided a formulation for wheat productivity that is relevant for all crops as the "four A's": Absolute, Attainable, Affordable and Actual yields. The absolute yield depends only on the genetic potential of the crop. The attainable yield is limited by some factors such as water availability, growing-degree days, depth of top soil, and total radiation. The affordable yield is limited by economics. The actual yield is the yield harvested in any given field and is limited by factors such as diseases, weeds and other hazards. Average wheat yields throughout the world approach only 30–60% of maximum attainable yields and global demand for wheat is growing faster than gains in genetic yield potential are being realized, currently a little under 1% per year in most regions (Deng *et al*., 2005).

Wheat yield is limited by the following factors (French and Schultz, 1984): 1) Temperature and water stresses are the main factors delaying the development of leaf area and thus dry matter. However, temperature per se may not delay leaf area development as this depends on the temperature range. Water availability and fertilisation can affect significantly the productivity of grain crops (Fan *et al*., 2005). Crop production is enhanced by the interception of radiant energy and the efficiency of converting this energy to dry matter. In the early growth stages, the interception is limited by the leaf canopy, and it is not until a leaf area index (LAI) of about 3 has been obtained that the crop starts producing dry matter efficiently. 2) A shortage of nutrients may be critical as nutrient-deficient plants use water at about the same rate as a well-balanced plant. 3) The effect of weeds, diseases and pests.

Drought stress is one of the most common environmental (abiotic) factors limiting crop production and yield. The production and yield of wheat are restricted in a climate with high evaporative demand and low rainfall (Musick *et al*., 1994). Plant resistance to drought usually has been grouped into three different mechanisms: escape, avoidance, and tolerance (Levitt, 1980; Jones, 2004). The water status of plants is described commonly by water potential and stomatal conductance measurements. The most popular method for measuring water potential is a pressure chamber and for measuring stomatal conductance (g_s) is with commercially available diffusion porometers (Kirkham, 2005).

Biotic stresses which affect wheat yield and production include fungal root diseases. The pathogens *Rhizoctonia* spp. and *Pythium* spp. cause a decrease in root mass, root decay, and occlusion of vascular tissue, resulting in variable crop stands, decreased tiller number, varying maturity date, and yield loss. Rhizoctonia root rot causes brown, sunken lesions in the root cortex, and serious infection leads to severance of the root, creating the *spear tip* symptom in roots (Weller and Cook, 1986). Pythium root rot has been reported to decrease grain yields of wheat (Cook, 1992).

2.3 Drought effects on plant physiology

Crop plants must avoid or tolerate cell dehydration to survive drought (Turner, 1986). Drought tolerance differs from drought avoidance. Tolerance is the ability to perform well, despite low plant water status, but avoidance is the ability to maintain relatively high plant water status despite lack of water in the environment.

Drought avoidance can best be detected by measurement of plant water status under drought (O'Toole and Chang, 1979). Drought avoidance strategy is used by plants whose tissues are

very sensitive to dehydration and these plants maintain high water potential by reducing transpiration to minimize water loss, or by increasing uptake of soil water to maximize water uptake (Ludlow, 1989). Also, it involves rapid phenological development, leaf rolling, leaf shading, reduced leaf area and increased stomatal and cuticular resistance.

Plants tolerate drought by maintaining sufficient cell turgor to allow metabolism to continue under increasing water deficits (Morgan, 1984; Turner, 1986). Osmotic adjustment is used by plants with a drought tolerance strategy, as well as smaller cells, or cells with more rigid cell walls (Chaves *et al*., 2003).Osmotic adjustment lowers water potential inside the cell to maintain water uptake or allow maintenance of root growth in dry soils (Serraj and Sinclair, 2002). Smaller cells and more rigid walls reduce structural damage due to shrinkage when water content decreases.

The plants with drought escape can complete their life cycle when water is available (Ludlow, 1989). Early flowering of wheat is important for drought resistance through escape effects in dry regions with predictable early-season rainfall.

2.3.1 Yield and biomass production

The yield (Y) of dryland crops can be analysed in terms of water use (WU), water use efficiency (WUE) and harvest index (HI) (Passioura, 1977; Fischer and Turner, 1978). Grain yield was proposed as a partial function of WUE (Passioura, 1996):

$$Y = WU \times WUE \times HI \qquad \text{Eq. [2.1]}$$

The final grain yield is determined by total biomass production and harvest index. Harvest index is defined as how much grain is produced per unit of total dry matter. The production of

high biomass plants under drought depends on finding a compromise between maximization of carbon assimilation (with high leaf area and stomatal conductance), and/or minimization of transpiration for maintenance of high leaf relative water content (RWC) (with low leaf area and stomatal conductance). Improvement in yield of semi-dwarf wheat has generally been associated with increased harvest index and grain yield per square meter (Deng *et al*., 2005). Allocation of biomass affects both growth and water use, and consequently plant water use efficiency. An allocation pattern that is desirable in terms of growth may not be beneficial in terms of water use. A high proportion of biomass in roots, for example, may not be desirable in terms of plant growth under favourable conditions, since roots are an important sink for assimilates (Van den Boogaard *et al*., 1996). For wheat, 10-45% of the total biomass can be below ground, depending on the soil conditions, and 20-50% of total assimilation is used by the roots. Although a large leaf area is associated with a high growth rate under favourable conditions, a reduced leaf area may be more desirable under water stress because it might finally result in a higher yield by saving water for post-anthesis growth (Van den Boogaard *et al*., 1996). The modulation of leaf area is important through its control of radiation interception and water use during the grain-filling period. One way of reducing the incidence of shoots that contribute through their leaf area index (LAI) to the depletion of soil water without increasing yield could be achieved by a reduction in tillering habit. Another way of reducing LAI may be the selection of genotypes characterised by fewer and smaller leaves(Van den Boogaard *et al*., 1996).

2.3.2 Effect of drought on yield components

Grain yield in wheat depends on assimilate produced over the life of the plant and can be divided into three components: 1) dry matter produced after anthesis and translocated directly

to the grain; 2) dry matter produced after anthesis but stored temporarily in vegetative organs before being remobilised to the grain; and 3) dry matter produced before anthesis and remobilised to the grain during grain filling (Pheloung and Siddique, 1991). Dry matter formed before anthesis has been estimated to contribute 3-30% of the grain dry matter at maturity. The contribution of stored dry matter to grain filling may increase compared to current assimilate if there is water stress during grain filling (Pheloung and Siddique, 1991). However, drought treatments reduce grain yield relatively more than total dry matter production, so that the harvest index decreases with drought (Fischer and Maurer, 1978).

Autumn droughts occur during the early stages of vegetative growth, and spring droughts after tillering and leaf production. Autumn droughts often occur and can reduce tillering, leaf production and grain yield (Johnson and Kanemasu, 1982). Spring droughts generally reduce grain yield only. Many studies have identified the stage of development at which plants are most susceptible to water stress. The biggest effect of water stress on grain yield is during meiosis within the developing reproductive organs of wheat plants (Davidson and Birch, 1978). The development of anthers is most susceptible to drought during meiosis of the pollen mother cells, while ovary development and fertility are largely unaffected (Westgate *et al*., 1996). Drought during inflorescence development decreases grain set in *Triticum aestivum* (Westgate *et al*., 1996). Loss of male fertility has been associated with accumulation of abscisic acid (ABA) in the spike. The increase in male sterility in response to exogenous ABA applied to leaves or spikes of control (well watered) plants has led to the conclusion that increased levels of ABA in anthers cause male sterility of plants under drought conditions (Westgate *et al*., 1996).

During a particular development stage, drought stress has been shown to retard the formation of the yield component which is most actively developing at the time of stress (Entz and Fowler, 1988). For example, tiller mortality and a reduction in the number of kernels per spike have been shown to result from stress prior to anthesis and grain number was increasingly reduced as drought severity increased (Fischer and Maurer, 1978). The abortion of kernels is induced also by stress during the reproductive phase, probably by decreasing the supply of carbohydrates (Saini and Westgate, 2000). Reductions in grain weight have been attributed to post-anthesis stress that restricted flag leaf photosynthesis and the translocation of assimilate to the spike (Wardlaw *et al*., 1989). Also, milder drought treatments (less than 50% yield reduction) lead to a greater relative reduction in kernel weight than in grain number (Fischer and Maurer, 1978). Kernel weight is usually negatively correlated with the number of kernels per spike (Fischer *et al*., 1977). The position of the kernel in the spike has a major effect on kernel weight. The lower and middle kernels position are heavier than upper ones (Duggan and Fowler, 2006).

The effect of drought on grain yield and particular yield components in wheat was described by Munir *et al*. (2007). Under drought, there was a positive and significant correlation of grain yield per plant with flag leaf area, tillers per plant, spike length, grains per spike, grain weight per spike and 1000-grain weight (Munir *et al*., 2007).

2.3.3 Yield and water use (evapotranspiration)

Yield may be related to water use during vegetative growth. Wheat yield is sometimes reduced by high soil nitrogen levels because these lead to high moisture usage before flowering (Colwell, 1963). Vegetative growth is increased by early sowing and heavy rates of

sowing, and heavy fertiliser rates. This leads to an increase in evapotranspiration and, as a direct result an increase in plant water stress, as measured by leaf relative turgidity (Fischer and Kohn, 1966). The water status is decreased during both vegetative growth and after flowering, thus grain yield also decreases.

Grain yield and the harvest index of rain-fed crops depend largely on the amount or proportion of water used just before or after anthesis (Richards and Townley-Smith. 1987) rather than on the total water used by the crop, so that modification of the development of leaf area that results in a slower rate or pattern of water use may have a significant effect on yield. Soil water content at anthesis had a significant effect as well (Richards, 1983). Passioura (1983) suggested that the ratio of pre-to post anthesis evapotranspiration (ET) should be 2:1 to avoid excessive consumption of soil water resources prior to grain filling. However, there is no relation between the ratio of pre-to post-anthesis water use and grain yield. Pre-anthesis ET accounted for over 70 percent of total ET in Australia. Soil evaporation in wheat accounted for up to 40% of the total available soil water in Australia (Blum, 2009).

One way of altering the pattern of water use in wheat under drought conditions is through increasing the hydraulic resistance in the seminal roots (Richards, 1983). This should result in reduced early growth and leaf area. The concept has not been adopted by breeders, so while it is feasible, it has not been useful (Richards, 1983). Other more direct ways of influencing water use genetically may be to select for early growth, tillering, the size of individual leaves, or time of flowering. Breeding for increased seedling vigor leads to more transpiration and a reduction in soil evaporation (Richards *et al.*, 2001). Soil evaporation can be reduced by crop structure (Siddique *et al.*, 1990a) and agronomic practices that stimulate early ground cover,

such as application of fertilisers (Oweis *et al*., 1998), early sowing (Oweis *et al*., 1998) and increased plant density (Van den Boogaard *et al*., 1996).

Another factor leading to increased transpiration efficiency (TE) is the increase in the atmospheric CO_2 concentration (Angus and van Herwaarden, 2001). TE is proportional to the CO_2 gradient from the atmosphere to the mesophyll, and there is evidence that TE is increasing as expected from changing atmospheric CO_2 (Angus and van Herwaarden, 2001). Crop water use can be estimated from the rainfall (R) plus the difference between the soil water at sowing (SW_{start}) and maturity (SW_{end}) using:

$$ET = R + SW_{end} - SW_{start} \qquad \text{Eq. [2.2]}$$

The biomass yield can be estimated by the ratio of transpiration to potential evapotranspiration (Blum, 2009) as:

$$B = mT/E_0 \qquad \text{Eq. [2.3]}$$

Where B is crop biomass, *m* is a crop constant, T is crop transpiration and E_0 is free water (potential) evaporation. There is a linear relationship between yield and ET in wheat under semiarid conditions (Campbell *et al*., 1988). Therefore, the effect of crop water stress on grain yield can be expressed as, for example, the difference between actual and potential ET (Mack and Ferguson, 1968). Evapotranspiration deficits are partially influenced by the amount of water available in the soil. For example, wheat roots have been shown to extract all of the available water from the soil profile by anthesis or by harvest (Domitruk, 1996).

Three factors that influence the relative TE before and after anthesis are the proportion of transpiration to soil evaporation (Es), vapour pressure deficit (VPD), and the proportion of soluble carbohydrate that is allocated to grain (Angus and van Herwaarden, 2001).

2.3.4 Water use efficiency

Water-use efficiency (WUE) is defined as a ratio of biomass accumulation, expressed as either carbon dioxide assimilation (A), total crop biomass (B), or crop grain yield (G), to water consumed, expressed as transpiration (T), evapotranspiration (ET), or total water input to the system (I). The time-scale for defining water use efficiency can be instantaneous (i), daily (d), or seasonal (s). So, water-use efficiency can be determined for observations ranging from gas exchange by individual leaves for a few minutes, to grain yield response to irrigation treatments through an entire season (Sinclair *et al*., 1984).

Tambussi *et al*. (2007) summarised the different meanings of WUE (Figure 2.1). WUE is classified into measured and estimated parameters. Measured parameters include both gas exchange and integrated WUE based on total biomass or yield and ET. WUE in terms of gas exchange includes both $WUE_{instantaneous}$ and $WUE_{intrinsic}$. At the leaf level, $WUE_{instantaneous}$ (A/E) is the ratio between net CO_2 assimilated by photosynthesis (A) and transpiration (E) in the same time period (Polley, 2002). $WUE_{intrinsic}$ (A/g_s) is the ratio between A and stomatal conductance (g_s). These WUE parameters are similar. However, $WUE_{intrinsic}$ is not affected by vapour pressure deficit (VPD) which is the driving force for transpiration rate. Therefore, $WUE_{intrinsic}$ is used in comparative studies, where different evaporative demands could be present (Tambussi *et al*., 2007). At the whole crop scale, integrated WUE includes both $WUE_{biomass}$ and WUE_{yield}.

The parameter for estimated WUE is carbon isotope discrimination ($\Delta^{13}C$) which is accepted as an indicator of $WUE_{intrinsic}$ (Tambussi *et al*., 2007). Plants with high WUE should show reduced discrimination against uptake of the heavier ^{13}C isotope (Farquhar *et al*., 1989). However, the relationship between $\Delta^{13}C$ and grain yield may differ from one environment to another. For instance, a positive correlation between $\Delta^{13}C$ and grain yield has been found in bread wheat under Mediterranean conditions with moderate or no water stress, while in 'stored-water' crops (some regions of Australia), a negative correlation between $\Delta^{13}C$ and grain yield has been found particularly with increased water stress (Tambussi *et al*., 2007).

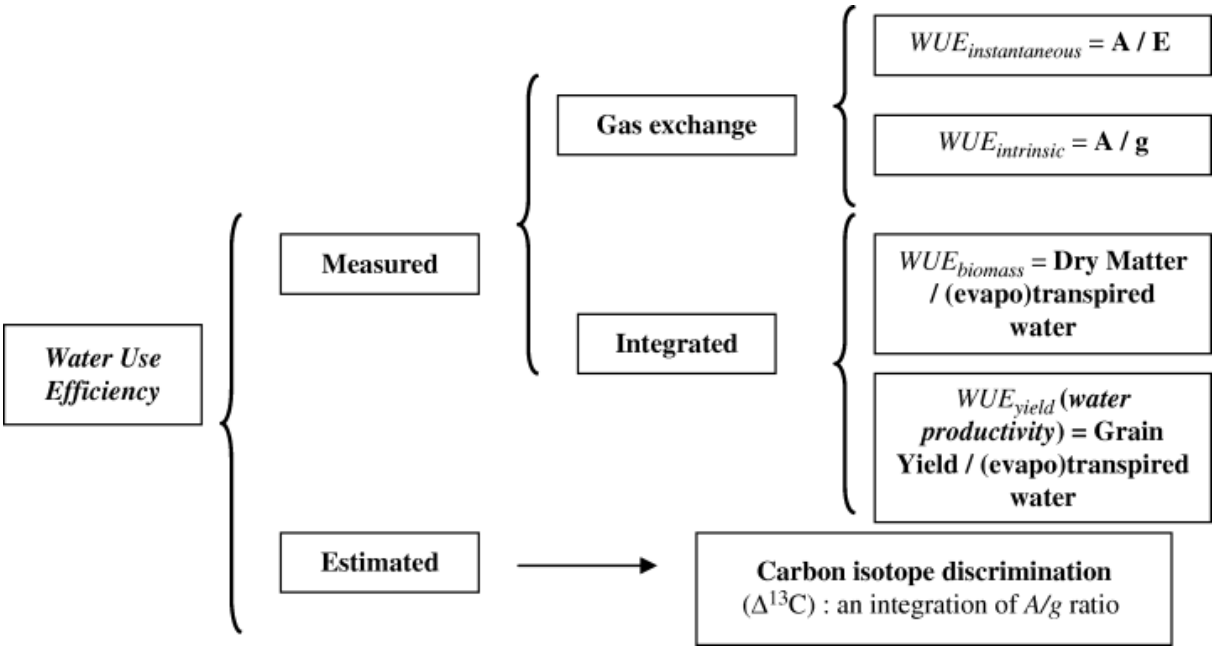

Figure 2.1 The several means of water use efficiency. Source, Tambussi *et al.* (2007)

Water use efficiency (WUE) simplifies the complex mechanisms relating water use and yield (Angus and van Herwaarden, 2001).

Water-use efficiency is strongly influenced by weather conditions which can affect transpiration and assimilation at the leaf, plant, and crop scales differently (Fischer and

Turner, 1978). Some of the most frequently used environmental indexes to explain changes in WUE are pan evaporation, relative humidity, vapour pressure deficit (VPD), and water use (Abbate *et al*., 2004).

Water use efficiency for wheat has been found to be much higher at high production than low production (Zhang *et al*., 1999). Many researchers showed that WUE either increased, did not change, or decreased under drought conditions (Abbate *et al*., 2004). Liang *et al*., (2002) demonstrated that alternate drying and rewatering had a compensatory effect that could reduce transpiration and keep wheat growing so that WUE was significantly higher under drought conditions as osmotic regulation was enhanced. Also, the ratio of root dry weight to shoot dry weight was increased by alternate drying and rewatering. Van den Boogaard *et al*. (1996) concluded that under drought, a lower water use rate due to lower transpiration per leaf area unit linked with high leaf area can improve wheat cultivars performance. For example, the higher WUE in cv. Katya is caused by its lower transpiration and that is related to a higher leaf area per unit plant weight. WUE can be increased without a concomitant reduction in the rate of growth. Moreover, the higher WUE is related to greater partitioning of biomass to leaves and associated with a higher respiration so the growth of two wheat cultivars (Katya and Mexipak) is similar. The higher leaf area can lead to more ground cover which may reduce soil evaporation and thus increase biomass production per unit available water. The study of Zhang *et al*. (2005) has shown that both WUE and grain yield were increased between 1982 and 2002 due to field management practices including selecting better yielding cultivars, reducing soil evaporation and better irrigation scheduling.

The management of transpiration is a possible way to increase WUE and yield potential of dryland crops so that relatively more water is used during the vegetative stage when vapor

pressure deficit (VPD) is low and hence transpiration efficiency (TE) is high. However, depending on budgets of soil water and soluble carbohydrates stored in the vegetative organs and available for retranslocation, this option provides lower TE than conserving soil water for transpiration until grain filling when assimilates are directed to grain (Angus and Herwaarden, 2001). WUE can be increased by growing crops during the time when VPD is the lowest (Fischer, 1979, 1981). In the study of Siddique *et al*. (1990b), modern cultivars had greater WUE_{grain} due to earlier development and flowering that decreases VPD over the life cycle. The improvement of WUE can be achieved by improved biomass production and maintaining a higher harvest index. Condon *et al*. (1993) concluded that to improve WUE of dryland wheat, it may be possible to manipulate stomatal response to post-anthesis drought and hence to maximise dry matter gain during this period. On the other hand, El Hafid *et al*. (1998) found that drought decreases photosynthesis due to reduced stomatal conductance. Water deficit also decreased mesophyll photosynthetic activity. As a result, instantaneous WUE (leaf photosynthesis/transpiration) was reduced by drought.

WUE greatly improved with reduction of irrigation (Zhang *et al*., 1998) because soil drying in the early stage of vegetative growth leads to a relatively deeper root system, a smaller leaf area development and shorter basal internodes. The earlier flowering often associated with lower numbers of leaves may offer further advantages in terms of WUE by reducing the mean VPD during grain filling (Hay and Kirby, 1991). Effect of soil drying during grain filling may lead to a better use of the carbon reserves in the stems and sheaths and therefore an improved HI (Zhang *et al*., 2005). A higher HI is necessary for getting high WUE under drought conditions (Austin *et al*., 1980; Perry and D'Antuomo, 1989).

A higher $WUE_{intrinsic}$ can be attained by lower stomatal conductance or higher photosynthesis or combination of both parameters (Tambussi *et al*., 2007). A higher $WUE_{instantaneous}$ can be achieved by higher specific leaf weight (leaf weight/leaf area) due to higher photosynthetic machinery per leaf area (Morgan and LeCain, 1991). $WUE_{instantaneous}$ can be increased when increase of mesophyll conductance is related to higher photosynthetic rates, without increasing stomatal conductance (Tambussi *et al*., 2007).

Both stomatal and nonstomatal factors are thought to contribute to drought effects on WUE (Martin and Ruiz-Torres 1992). WUE measured as A/E initially increases with stomatal closure due to greater reduction in E than in A. However, WUE then declines because leaf conductance decreases to levels where insufficient heat and gas transfer occurrs (Farquhar *et al*., 1989; Martin and Ruiz-Torres, 1992). Stomatal behavior is therefore important because variation in stomatal conductance (g_s) affects transpiration proportionally more than photosynthesis.

Recent studies were conducted to evaluate the relationship between root: shoot ratio (R/S) and WUE. It has been shown that the WUE of wheat increased gradually as the R/S decreased from diploid to hexaploid plants and from older to modern varieties (Ma *et al*., 2010). WUE of winter wheat under arid and semi-arid conditions was improved by root pruning in pot and field experiments due to improving root efficiency; lowering water consumption and lowering the root biomass in the upper soil layer (Ma *et al*., 2008, 2009, 2010).

Blum (2009) defined effective use of water (EUW) as maximizing soil water capture while diverting the largest part of the available soil moisture towards stomatal transpiration. He argued that EUW is a major target for yield improvement, not WUE, under water-limited

environments. He argued also that selection for high WUE in breeding for water-limited conditions will most likely lead, under most conditions, to reduced yield and reduced drought resistance, while EUW enhances biomass production under drought stress. EUW implies maximal soil moisture capture for transpiration which also involves a decrease in non-stomatal transpiration and minimal water loss by soil evaporation (Blum 2009).

2.3.5 Plant water relations

Water stress can affect water potential and its components, which are considered a reliable measurement of the water status of plant tissue. There is a significant difference in water potential among wheat genotypes under drought stress (Siddique *et al*., 2000). There were reductions in water potential (ψ), osmotic potential (π) and leaf permeability with drought in a study on the water relations of a large set of cultivars of bread and durum wheats, triticale and barley, grown in field plots (Fischer and Sanchez, 1979). Drought stress reduces leaf water potential, with examples of reductions from -0.63 MPa in control plants to -2.0 MPa in droughted plants (Siddique *et al*., 2000) and from -0.4- -0.5 MPa in controls to -1.3 MPa on the seventh day of drying (Liang *et al*., 2002). Plant water potential decreased gradually but slowly (about 0.07 MPa /day) following the onset of drought (Fischer and Maurer, 1978). Water stress reduced ψ_w from -0.84 MPa in control plants to -2.00 MPa (Martin and Ruiz-Torres, 1992).

It has been proposed that the relative water content (RWC) was a better indicator of water status than was water potential (Sinclair and Ludlow, 1985) because RWC through its relations to cell volume may more closely reflect the balance between water supply to the leaf and transpiration. RWC decreased with increasing drought stress and varied among breeding

populations (Schonfeld *et al*., 1988). For example, RWC was reduced from 88% to 45% during plant development (Siddique *et al*., 2000). The same results were obtained by (Schonfeld *et al*., 1988) with no significant differences among populations for ψ, π or presuure potential (P). However, under water stress, RWC differed significantly between populations. The water potential of the wheat grain is less affected by water stress than other parts of the plant (Fisher and Cash-Clark, 2000). For instance, wheat grain ψ was about -1.0 MPa during most of grain filling in well-watered conditions, and vegetative tissues were about 0.5 MPa higher (Barlow *et al*., 1980). Under water stress, grain ψ fell only slightly during the next 10 days, while that in the rest of the plant declined from -3 to -4 MPa. The discontinuity in the xylem at the base of the wheat grain is an important factor in the independence of grain water relations from other parts of the plant (Fisher and Cash-Clark 2000).

2.3.6 Water uptake by roots

The effects of soil water deficits on root growth of crops and on its water uptake have been investigated in several studies (Weir and Barraclough, 1986; Klepper, 1987; Meyer *et al*., 1990; Asseng *et al*., 1998; Xue *et al*., 2003). At the cellular level, water deficits reduce root growth due to suberisation of the apoplast, which affect the water balance by reducing the capacity of roots to take up water; and by decreasing the hydraulic conductivity of root cell membranes possibly due to a closure of water channels in root cell membranes (Steudle, 2000). Water stress changes the anatomy of root tissue because it induces the development of apoplastic barriers for water and ion flow (Steudle, 2000).

Root growth is critical for crops to use soil water and obtain high yield under water deficit conditions (Robertson *et al*., 1993). Therefore, the benefit of a larger root system may be a

higher capacity for water uptake under drought, and thus it may finally result in more biomass (Ehdaie *et al*., 2003). However, Ma *et al*. (2010) argued that a large root system can result in rapid soil water consumption, which may not be favourable in arid and semiarid areas. The breeding of plants with deeply vigorous root systems can increase the yield of wheat (Passioura, 1977). Increased yield should result when plants are able to access more of the available water in the subsoil at anthesis (Passioura, 1977).

The peak size of a root system in terms of biomass or length seems to be genetically controlled and has been reported to be near anthesis (Asseng *et al*., 1998), regardless of dry or wet growing conditions. However, root length density and dry weight decrease after anthesis (Xue *et al*., 2003). Root water uptake per unit length in wheat in well watered conditions is fairly constant until grain filling, only declining shortly before maturity (Asseng *et al*., 1998), whereas it is reduced substantially by a period of drought.

Plants differ in their root:shoot ratios (R/S) in response to environmental factors (Andrews and Newman, 1968). Many researchers have investigated the effects of root pruning on transpiration, e.g Andrews and Newman, (1968). It was concluded that the relationship between root pruning and transpiration is not linear, so that removal of about 25% or 50% of the root system has little or no effect on water use. Gardner (1960) and Cowan (1965) indicated that the transpiration rate is reduced by less than half for 50% root pruning under well watered conditions. They also predicted that root pruning only increases plant resistance at field capacity but under dry soil, both plant and soil resistances are increased. Therefore, a greater decrease in transpiration has occurred as result of drier soil. However, the results of Andrews and Newman, (1968) showed the opposite effects.

Root pruning during the vegetative stage reduced the rate of leaf transpiration and lowered the number of tillers per plant (Ma *et al*., 2010). However, there was no significant difference in transpiration between plants with intact roots and pruned roots at anthesis for plants in pots but the transpiration of plants with pruned roots was higher than those with intact roots under field conditions. Root water uptake is reduced by mechanical root pruning due to reduced soil volume occupied by the roots (Amir and Sinclair, 1996).

2.3.7 Other physiological effects

Drought reduces the carbon balance of the crop by its limiting effects on light interception and radiation use efficiency (Teulat *et al*., 1997). Soil or plant water status affect stomatal conductance (controlling CO_2 flux density) and leaf area index (providing the energy intercepted by the canopy) (Teulat *et al*., 1997). Water deficit can negatively affect plant leaf area by reducing the rate of leaf emergence, the rate of individual leaf expansion and tiller development when soil water potential decreases (Teulat *et al*., 1997). Plant metabolism is also dependent on leaf water status, as measured by RWC (Teulat *et al*., 1997). Drought negatively affects photosynthesis in wheat by reductions in expansion of leaves and stomatal conductance and may ultimately impact primary events in the photosynthetic process (Passioura, 1994).

2.3.7.1 Osmotic Adjustment

Osmotic adjustment (OA) or osmoregulation is defined as a decrease in π because of accumulation of solutes and maintenance of RWC when leaf ψ is reduced (Morgan, 1983; Blum, 1989). OA is the major plant adaptive response to drought at the cellular level (Blum, 2005). OA has two major functions in plant production under drought stress: (a) it enables leaf

turgor maintenance under lower leaf water status, and (b) it improves root capacity for water uptake (Blum, 2009). OA is generally associated with delayed senescence and maintenance of assimilate transfer to the grain, thus increasing harvest index (Teulat *et al*., 1997). There is a positive relationship under water stress conditions between osmoregulation, turgor and shoot growth (Teulat *et al*., 1997).

Osmoregulation is known to vary between wheat genotypes. Genotypes which were selected for higher osmoregulation in the glasshouse have been found to have higher turgor maintenance and grain yields under drought conditions in the field (Morgan and Condon, 1986). Turgor maintenance during reduction in leaf water status due to drought is thought to be a means by which plants maintain metabolic processes and sustain growth and survival (Ali *et al*., 1999). Higher turgor maintenance has been related to maintenance of higher leaf conductances in different species when differences in turgor were induced environmentally (Morgan and Condon, 1986). In addition, genotypes with higher turgor maintenance will maintain higher photosynthesis rates, with higher transpiration rates (Morgan and Condon, 1986).

2.3.7.2 Photosynthesis

Both stomatal and non-stomatal factors affect leaf photosynthesis under drought conditions. The reductions in whole leaf photosynthesis caused by mild drought stress are primarily due to stomatal closure but there is no indication of damage to chloroplast reactions (Inoue *et al*., 2004). Drought decreased photosynthesis by closure of stomata caused by the increase of ABA concentration in the xylem (Liang *et al*., 1997). At more severe drought stress, photosynthesis continues to decrease, while the ratio of intercellular/ambient CO_2

concentration increases significantly to values similar to those obtained in well watered plants (Inoue *et al*., 2004). Thus, the decrease in photosynthesis could result from non-stomatal factors affecting photosynthetic capacity, e.g. reduced activity of some Calvin cycle enzymes, inhibition of photosynthetic electron transport, and impaired photophosphorylation capacity (Inoue *et al*., 2004).

Flag leaf photosynthesis was at one time considered the main source of assimilates for grain filling. However, it is now accepted that ear photosynthesis makes a major contribution to final grain yield, especially in drought, when the ear may be the main photosynthetic contributor to grain filling (Tambussi *et al*., 2005). The net photosynthesis (A), stomatal conductance (g_s) and transpiration (T) of the ear and the flag leaf decrease significantly with water deficit, whereas the A/T and A/g_s ratios increase (Abbad *et al*., 2004).

2.3.7.3 Stomatal conductance

Stomatal conductance measures the changes in water and carbon dioxide through the stomata, in and out of the leaf (Taiz and Zeiger, 2002). The control of water movement through the plant depends on the stomatal conductance of the leaves, which is affected by light, leaf-air vapour pressure deficit, leaf temperature, intercellular carbon dioxide concentrations, and leaf water status (Seaton *et al*., 1977). Cowan, (1965) postulated a critical leaf ψ at which pronounced stomata closure would occur. Seaton *et al*. (1977) examined the concept of critical leaf ψ for stomatal closure. They concluded that there was not a unique relationship between ψ and g_s, and that osmotic adjustment affected the ψ at which stomata closed.

Phytohormone signals produced by the roots and transported to the leaves can decrease stomatal conductance and leaf growth (Ali *et al*., 1999). ABA is the primary signal, even

though many components of the xylem sap changed in response to water deficit. In addition, it has been proposed that root signals could decrease growth and yield in well-watered environments by reducing leaf growth and light interception before full canopy closure occurs (Ali *et al*., 1999). In a growth chamber, it was reported (Blum *et al*., 1991) that although root signals induced by drying top soil layers had been shown to adversely influence leaf area, plant size, biomass and growth duration (earlier heading and flowering), there was no significant reduction in the final yield of droughted wheat plants when they open their stomata for CO_2 uptake, and lose a large quantity of water.

2.4 Root diseases effects

Root diseases can affect wheat at almost any stage during plant growth and development. Their major impact on grain yield is through limiting the number of heads. The root diseases of wheat are caused by many different fungi. The most important in dry soils are caused by *Cochliobolus sativus* (*Bipolaris sorokiniana*) and *Fusarium* species. However, the three root diseases favoured in wet soils are Take-all, caused by *Gaeumannomyces graminis* var. *tritici*; Rhizoctonia root rot, caused by several *Rhizoctonia* species, mainly *R. solani* anastomosis group 8 (AG8) and *R. oryzae*; and Pythium root rot, caused by 10 or more *Pythium* species (Cook and Veseth, 1991).

2.4.1 *Pythium* disease of wheat

Pythium is a large genus of the *Oomycota* including 120 species (Martin and Loper, 1999). *Pythium* species have wide host ranges and mainly cause seed rot, damping off, and root rot of seedlings. *Pythium* root rot occurs commonly on wheat and it is among the other factors which result in plant growth suppression and nutrient deficiency-like symptoms (Cook *et al*., 1980).

Pythium root rot of wheat was first investigated by Vanterpool and workers in Canada in the early 1930s. The disease was called 'browning' root rot because of the scorched, brown appearance of young diseased plants (Waller, 1979). Plants infected with *Pythium* normally have brown, decayed roots with wilted and stunted shoots (Rowe, 1986; Agrios, 1997). *Pythium* species have been reported as important pathogens of wheat and barley in several countries, including Australia (Pankhurst *et al*., 1995). However, wheat infection by *Pythium spp* in South Australia has been considered of only minor importance relative to the damage caused by three other soil borne pathogens of wheat: *Gaeumannomyces graminis* var. *tritici* Walker, *Rhizoctonia solani* Kuhn, and cereal cyst nematode caused by *Heterodera avenae* Woll (Pankhurst *et al*., 1995).

Ten *Pythium* spp were isolated and identified by Chamswarng and Cook (1985) from soils in eastern Washington that were pathogenic to wheat. They found *P.aristosporum*, *P.volutum*, *P.ultimum*, *P.sylvaticum* complex, and *P.irregulare* to be the most virulent among identified isolates. The pathogenicity of four *Pythium* species was assessed on wheat, peas, lentils, and barley (Ingram and Cook, 1990). However, they found *P. ultimum* and *P.irregulare* were the most virulent species to wheat. Moreover, isolates of *P. irregulare* were found by Harvey *et al* (2000) in seven cereal crops throughout South Australia.

One of the most important pathogenic *Pythium* species that is distributed worldwide is *P. irregulare*. This species is distinguished on the basis of oogonium morphology, which has an irregular number (0-5) of projections, and spherical sporangia (Van der Plaats-Niterink, 1981). This fungus species causes severe damage to the roots of wheat and ryegrass and also causes pre-emergent blight and post-emergent stunting of crops and pastures (Harvey *et al*., 2000). This pathogen is adapted to the combination of crop rotations, crop residues and soil

conditions in Mediterranean climates and in cooler, wetter temperate climates (Harvey *et al*., 2000).

Pythium species begin their parasitic invasion of wheat seeds in soil by infecting the embryo within 24-48 hours after planting into moist soil (Hering *et al*., 1987). The infection rate can be as high as 60-70%, but the seedlings are rarely killed and remained stunted and produce small leaves (Cook and Veseth, 1991). However, if embryo damage due to *Pythium* infection after planting is severe, seedlings often fail to emerge when infected with *Pythium* (Fukui *et al*., 1994). There are different forms of *Pythium* inoculum including sporangia, zoospores, mycelia or oospores (Endo and Colt, 1974). Germ tubes are directly formed by germination of some types of sporangia and oospores but the indirect function of these spores are to produce zoospores. Several researchers considered the common units of inoculum are a function of zoospores and mycelia (Endo and Colt, 1974; Stanghellini, 1988).

Pythium root rot of wheat is extremely difficult to control and has been reported to decrease grain yields by up to 25% (Cook, 1992). Infection of roots by *Pythium* resulted in the loss of root hairs and fine rootlets in wheat (Cook *et al*., 1987). Other disease symptoms including severe root browning and stunting, reduced number of tillers and poor growth and grain yields (Wiese, 1987). Some workers have found negative relationships between *Pythium* sp. and grain yield of wheat (Cook, 1992). *Pythium irregulare* has been reported to significantly reduce grain production in southern Australia (Pankhurst *et al*., 1995). Infection with *Pythium* causes a decrease in root mass, which leads to poor nutrient uptake, resulting in variable crop stands, decreased tiller number, varying maturity date, and yield loss (Higginbotham *et al*., 2004). Grain yields of wheat grown in *Pythium*-free soil have been reported to be 15 to 25% higher than those of wheat grown in *Pythium*-infested soil (Cook and Haglund, 1991). High

soil water content enhanced the activity of *Pythium* species (Hendrix and Campbell, 1973). For example, there was higher inhibition in growth of barley seedlings associated with the combination of high soil water and inoculum densities (Bratoloveanu and Wallace, 1985).

The inoculum is distributed in the soil on roots and crop debris which are available for saprophytic colonisation by *Pythium* spp. (Pankhurst *et al*., 1995). The higher number of infection sites on shallow roots rather than on deeper roots is due to high inoculum concentration in the upper soil profile (Hancock, 1985). The distribution of *Pythium* propagules is not uniform but is clustered in the soil. In 39 wheat fields sampled between 1983 and 1986, all had more than 100 propagules of *Pythium* per gram of soil in the top 15 cm (Cook *et al*., 1987). In barley, *Pythium* populations were more abundant in the surface 10 cm rather than in the 10-20 cm soil zone (Bratoloveanu and Wallace, 1985), while Pankhurst *et al*. (1995) reported the maximum population densities for *P.irregular*e are in the surface 10 cm in wheat.

2.4.2 *Rhizoctonia* disease of wheat

Rhizoctonia solani (AG8) is the main causal organism of the 'bare patch' disease of wheat in southern Australia. The disease is generally more severe in sandy soils in Australia (Gill *et al*., 2001). Maintaining the field soil in a 'moist condition' generally results in decrease of root rot caused by *R. solani. Rhizoctonia* hyphae can survive *in vitro* at moisture levels below the permanent wilting point (1.5 MPa) (Gill *et al*., 2001).

The above-ground symptoms of plants affected by *R. solani* including stunting, yellowing, purpling and rolling of leaves, which are usually thought to result from poor nutrition and moisture stress (Hynes, 1937). Direct losses are closely related to the incidence of patches in

crops. AG-8 can infect most legumes and weed species (Wallwork, 1996). The disease increases in incidence and severity when wheat is sown into uncultivated seedbeds by direct drilling (MacNish, 1985; Roget, 1995).

2.4.3 Root damage by fungal diseases

Root infections by fungal diseases result in the loss of root hairs and fine rootlets of wheat (Cook *et al*., 1987). The *Pythium* disease can cause soft rot lesions of a mid to pale brown colour that occur on young roots (Waller, 1979). *Pythium* isolates cause a significant reduction in the number of root tips and root length (Higginbotham *et al*., 2004). Plants infected by *Rhizoctonia* are usually pale and have shorter roots with brown spear tips (Wallwork, 1996). *Rhizoctonia* infects the roots and proliferates in the cortex causing cell collapse (Weinhold and Sinclair, 1996). Root growth of wheat was decreased by high infection with *Rhizoctonia* but not with low levels of infection (Kirkegaard *et al*., 1999). There was loss of root length of over 60% at higher infection levels of *Rhizoctonia*, and this was expected to reduce the capacity for adequate water and nutrient uptake by the plant, and so reduce leaf growth (Kirkegaard *et al*., 1999). However, the reductions in root dry weight at high infection were 40%. The higher decrease in root length compared to root dry weight could result from loss of finer roots as infected by *Rhizoctonia*. The reduction in root activity and/or demand for water by infected plants was responsible for decreased water extraction through the soil profile (Martin *et al*., 1986). The soil-borne fungus *Gaeumannomyces graminis* var. *tritici* (Ggt) has the potential to decrease water uptake (due to Ggt hyphae through the infected root) by reducing either root growth or the efficiency of the root system and causing yield loss due to root dysfunction (Huber and McCay-Buis, 1993).

Some authors explained the mechanisms by which pathogens like *Rhizoctonia* infect and colonise wheat roots (Kirkegaard *et al*., 1999). Root tissue is invaded by penetration at intact surfaces or wounds; the fungus produces enzymes (cytolytic, pectic and cellulolytic) to help in penetration. Host tissue is invaded intra- or inter-cellularly and the hyphae proliferate within the root cortical cells causing browning and collapse of cells and formation of sunken lesions. In *Pythium*-infected root tissues, the fungus has the ability to produce hydrolytic enzymes (Endo and Colt, 1974) and toxic metabolites (Mojdehi *et al*., 1990).

In a large pot experiment, three and six propagules of millet seed inoculum of *Rhizoctonia* induced low and high infections, respectively (Kirkegaard *et al*., 1999). The low infection had no effect on shoot and root growth, but the higher infection reduced water and nutrient uptake by the plant (Kirkegaard *et al*., 1999).

2.4.4 Yield losses from root diseases in wheat

Infection by plant pathogens and yield are linked by epidemiological and physiological processes that may be considered as three major functional relationships. Disease severity is determined by a function of the degree of infection, colonisation, and damage of host tissues. The amount of host development and growth is a function of disease severity, and yield realization is a function of host development and growth (Dawson and Weste, 1984; Gaunt, 1995).

Yield loss due to disease can result from reduced grain number, reduced grain size/weight and reduction of fertile tillers. Slight disease (take-all, eyespot diseases) often has no effect on yield (Clarkson, 1981). Moderate severity of take-all had no effect on the number of ears per plant but significantly reduced grain number per plant, 1000-grain weight and grain dry

weight with more effect in severe disease (Polley and Clarkson, 1980). The effect of take-all on yield and quality of wheat was a result of decreased grain filling by premature ripening (Gutteridge *et al*., 2003). Clarkson (1981) found that moderate eyespot reduced yield per ear, grain number per ear and 1000-grain weight by 10, 8 and 5 %, respectively. Severe eyespot caused corresponding losses of 36, 29, and 15%, respectively.

There was a loss of yield of approximately 70% from the infection of plants by *Cephalosporium gramineum* when compared with the controls due to a smaller number of fertile florets and a smaller seed size produced by the diseased plants (Richardson and Rennie, 1970). Grain yield of winter wheat genotypes was also reduced by *C. gramineum* (Martin *et al*., 1986). *Cephalosporium gramineum* is soil–borne fungus which colonises vascular bundles after entering the host through root wounds. Vascular wilt caused by this fungus results in necrosis and premature senescence of foliar tissue.

2.4.5 Disease and water relations

Rhizoctonia-infected wheat seedlings were investigated by Kirkegaard *et al*. (1999) to determine whether water and phosphorus uptake limit leaf growth in infected plants. To maintain the leaf xylem flow, the pots were pressurized so that leaves were at full turgor. However, the decrease in leaf expansion caused by *Rhizoctonia* was not overcome by pressurisation which indicates that a reduced supply of water to the leaves was not responsible for reduced leaf growth of infected plants. The reason for reduction of growth may be related to growth regulators produced by the fungus or by the plant as a result of the infection.

Fungal root diseases can affect water use. For example, there is a reduction of water use in all winter wheat genotypes affected by the vascular wilt disease Cephalosporium stripe caused by

Cephalosporium gramineum except for one genotype (Martin *et al*., 1986). The most susceptible genotypes showed a reduction in water use and WUE. However, disease reduced grain yield (38%) more than water use (12%). Thus WUE was lower in diseased than non-diseased plants (Martin *et al*., 1986).

Similarly, the transpiration of wheat plants was decreased when soil was infested with Cereal Cyst Nematode (CCN) (Amir and Sinclair, 1996). For example, transpiration rate of wheat was reduced more in the presence of 30 cysts of nematode than 15 cysts. The restricted root depth of infected roots led to less absorbtion of water and as a result the daily transpiration dropped significantly (Amir and Sinclair, 1996). In addition, water loss of plants infested with CCN was compared with root pruning. The results indicated that the effect CCN on restriction of root depth is very similar to those of pruned plants. Therefore, root pruning or restricted root growth is probably the primary cause of damage of CCN rather than nematode feeding or toxic effects.

Rahi *et al.* (1988) found that all plants of tobacco infected by root knot nematodes (*Meloidogyne incognita* and *M. javanica*) had similar evapotranspiration pattern as in controls. Similarly, there were no differences in transpiration of tomato plants infected by *M. javanica* and controls (Meon *et al*., 1978). Cotton plants infected with *Meloidogyne incognita* use water at a similar level or more than controls when the soil is maintained at near field capacity (O'Bannon and Reynolds, 1965). However, plants infected by nematode used about one-half the amount of water used by controls when water fluctuated between 50 and 100% of field capacity. Water use and WUE of nematode damaged sorghum, corn, and potato were lower than those of controls (Chevres-Roman, 1966). The transpiration rate of potato cultivars was not always affected by inoculum density of *Pratylenchus penetrans* (Kotcon *et al*., 1985). The

photosynthesis and transpiration of potato was suppressed by potato cyst nematode at high inoculum densities (Schans and Arntzen, 1991).

2.4.6 Effect of diseases on physiological parameters in wheat

The effect of take-all disease and inoculation with *Gaeumannomyces graminis* var. *tritici* (Ggt) on carbon assimilation rate (A) and biomass production of wheat plants was investigated by Balota *et al*., (2005) under two water regimes. Ggt inoculation affected plant growth and leaf A through a reduction in photosynthetic capacity of the leaves. Ggt significantly reduced A at anthesis over three growing seasons, by 18%, 15% and 12%, respectively. Meanwhile, the number of tillers and production of all plant components, particularly root dry mass and grain mass per plant were decreased. Ggt inoculation negatively affected transpiration ratio (A/E). However, stomatal conductance remained relatively high and intercellular CO_2 concentration increased or did not change which indicated that stomatal control is not limiting for A. The disease reduced the capacity of photosynthesis of leaves by an unknown mechanism not related to water stress. Increasing number of stripes per leaf caused by *Cephalosporium gramineum* reduced net photosynthesis, stomatal conductance, and chlorophyll content of winter wheat (Morton and Mathre, 1980).

Wildermuth and Morgan (2004) tested the differences of genotypes in partial resistance to crown rot which is caused by *Fusarium pseudograminearum* in relation to an osmoregulation gene in wheat. Both crown rot disease and osmoregulation are expressed most when plants undergo drought. They investigated a possible genetic linkage between high osmoregulation and partial resistance to crown rot by using lines which are bred with high osmoregulation (*or* gene) from parents with low osmoregulation and varying resistances to crown rot. However,

there was no relationship between incidence or severity of disease and presence or absence of the *or* gene.

2.5 Drought X pathogen interaction in other plants

Drought and pathogenic fungi are important stressors, among a wide variety of abiotic and biotic factors, affecting plants such as forest trees (Desprez-Loustau *et al*., 2006). The interaction between drought and pathogens has long been recognised (Colhoun, 1973). Most experimental studies used for the measurement of drought-disease interactions have used pots grown in greenhouses subjected to drought with different water treatments, and artificially inoculated. The effect of drought (water stress) has been assessed by comparing pathogen- or disease-related variables (extent of colonisation, lesion length) in water stressed inoculated plants to that in normally watered (control) inoculated plants or by examining the relationship between symptom development and ψ. The variables used therefore commonly refer to severity of disease rather than incidence. Few experimental investigations have been carried out so far including pathogen and abiotic stress with an ecophysiological approach (Desprez-Loustau *et al*., 2006).

The plant-pathogen interactions are affected by duration and timing of drought. A minimum duration of three days at the threshold level (critical water deficit) was required for stress after inoculation of *Betula* with *Botryosphaeria dothidea*. However, tissues could recover resistance 3–5 days after the stress was relieved (Crist and Schoeneweiss, 1975). Johnson *et al*. (1997) working with *Sphaeropsis sapinea* on Scots pines (*P. sylvestris*), demonstrated that saplings subjected to stress levels of –3 to –4.5 MPa were able to confine canker expansion when watering was resumed within a few days after inoculation whereas increased canker development occurred when water was not limiting before inoculation, but withheld during 2

or 3 weeks after inoculation. Differential effects of water stress in relation to timing before or after infection have also been reported for *Lasiodiplodia theobromae* on dogwood, with a more important effect of pre-inoculation than post-inoculation stress on canker development (Desprez-Loustau *et al*., 2006). Paul and Ayres, (1984) reported that a combination of rust (*P. helianthi* Schw.) and drought inhibited the growth of leaf area and total dry weight in sunflower but he made only a single harvest and did not measure tissue water relations. Duniway and Durbin, (1971) observed that beans (*P. vulgaris*) infected by *Uromyces phaseoli* wilted at soil water potentials greater than -0.1 MPa whereas healthy plants did not wilt until soil water potential fell below -0.34 MPa. However, they did not relate these observations to plant growth.

Significant pathogen x water interactions in disease rating and stem dry weight suggest that stem dry weight is less affected by *Verticillium albo-atrum* in alfalfa, and fewer symptoms are present under drought stress than under non-drought stressed conditions. *Verticillium albo-atrum* had no significant effect on stomatal conductance but did alter leaf ψ. The absence of pathogen x water interactions for most of the growth parameters and the decreased effect of the pathogen on stem dry weight under drought stress indicated that resistance to *V. albo-atrum* in alfalfa is stable under drought stress (Pennypacker *et al*., 1991). Clover *et al*. (1999) found no interaction between drought stress in sugar beets and beet yellows virus (BYV) infection. The effects of the disease and the effects of drought occurred at different times of the day and of the season resulting in no interaction between drought and BYV.

So and Thrower (1976) found that rust inhibited net photosynthesis in colonised leaves of *Vigna sesquipedalis* but it stimulated photosynthesis in younger healthy leaves on the same plant. However, drought inhibited the growth of leaf area in both healthy and infected plants

and, when combined with rust, had additive deleterious effects on net photosynthesis. The results of Paul and Ayres, (1984) indicated that the rust impaired the normal increase in WUE of *Senecio* in response to drought. McElrone *et al*., (2003) investigated the effects of the interactions of water stress and infection by *Xylella fastidiosa* on water relations of a host grapevine. The reduced of hydraulic conductivity caused by *X. fastidiosa* infection acts additively with the water limitation imposed by drought stress.

2.6 Conclusion

Both drought and fungal diseases are considered stressors for wheat plants. The production and yield are normally reduced under these conditions in arid and semi-arid regions. From the past until now, there have many investigations and a lot of literature on the effect of drought on WUE of wheat with very limited studies on how root diseases affect water relations in wheat. Also, there is very little done on the interaction of diseases and drought especially in cereals or wheat. Therefore, more work is needed to investigate the interaction between fungal root diseases and drought on WUE of wheat.

Chapter 3: Interactive effects of drought and fungal root diseases on water use efficiency of wheat

3.1 Introduction

Water use efficiency (WUE) is often considered an important determinant of yield under stress and even as a component of crop drought resistance (Blum, 2009). Therefore, improved WUE of crop cultivars is one approach to enhancing grain yield and there has been considerable research effort, particularly in dry regions (Sadras and Angus, 2006; Boyer, 1996; Ehdaie, 1995). WUE provides a simple means of assessing whether yield is limited by water supply or other factors (Angus and van Herwaarden, 2001).

Numerous studies have investigated the effects of drought on WUE of wheat (e.g. Angus and van Herwaarden, 2001; Liang *et al*., 2002; Shangguan *et al*., 2000; Martin and Ruiz-Torres, 1992). The effect of root diseases under drought stress has been investigated previously in wheat for a limited number of diseases (Huber and McCay-Buis, 1993; Martin *et al*., 1986; Balota *et al*., 2005). Interactive effects of drought and diseases have been reported in other plants such as forest trees (Desprez-Loustau *et al*., 2006). However, little has been done on the interactive effects on WUE and harvest-index.

This chapter describes an exploratory experiment on disease-drought interactions. Two root-rotting pathogens (*Pythium irregulare* and *Rhizoctonia solani*), two drought periods at different growth stages (tillering and anthesis drought) and two wheat cultivars were chosen. The two pathogens were chosen to test whether there were differences between them in terms of water use, water relations and root damage. The stages selected for the imposition of

drought were at pre-anthesis and post-anthesis. Stressing the plants at different stages of development is important. Drought at tillering can lead to tiller mortality and reduce the number of grains per spike and can affect meiosis, while anthesis drought is critical for grain fill and can reduce grain set. Post-anthesis is the most common period for drought stress. Two spring wheat (*Triticum aestivum*) cultivars were used: cv. Janz was used as a representative bread wheat cultivar, while cv. Mulgara, which has a gene for osmoregulation (Wildermuth and Morgan, 2004) was used as a cultivar expected to have a different drought response. Both cultivars are semi-dwarf and quick maturing cultivars.

The overall aim of this experiment was to investigate the effect of root diseases on the growth and water relations of wheat under drought. Specifically, this experiment tested a number of hypotheses: that root diseases reduce water uptake (transpiration) and water use efficiency; that root diseases have a more severe effect when plants are water stressed; that the interaction between root diseases and drought depends on the time at which drought is imposed; and that genotypes with different drought responses may differ in the effects of disease on water relations.

3.2 Materials and methods

3.2.1 Inoculum preparation

Pythium irregulare was isolated from a seedling of triticale (X *Triticosecale* Wittmack) at the Laureldale Research Farm of the University of New England (UNE). An isolate of *Rhizoctonia solani* AG-8 was obtained from Dr Stephen Barnett, SARDI, South Australia. Fungal cultures were maintained on potato dextrose agar (PDA).

The inoculum was prepared by soaking millet seeds for 16 hr overnight, draining off water, and autoclaving at 121^0C for 30 minutes on each of three consecutive days. *Rhizoctonia solani* and *Pythium irregulare* cultures grown on PDA were cut into 5 mm cubes, and were inoculated onto autoclaved millet seeds in Petri dishes and incubated at 25^0C for three weeks to allow colonisation of the millet seeds. The inoculum was dried in a laminar flow unit and stored in a refrigerator to be used later for wheat inoculation.

3.2.2 Soil preparation

A pot experiment was conducted in a glasshouse at the University of New England, Armidale (152^0E, 31^0S, elevation 980 m a.s.l.). Sixty pots (20 x 20 cm) were used for this experiment, and each was filled with 3.5 kg of sandy loam soil mixed with peat (3:1) (v/v). The soil pH was adjusted to 6.4 (in 1:5 soil:water) with agricultural lime. Granular N:P:S (14.3:12:10.5) Starter 15 fertiliser was applied to the soil mixture at a rate of 13 g m^{-2}.

3.2.3 Inoculation of wheat plants

Wheat seeds were surface sterilised for 5 minutes with 1% sodium hypochlorite in 10% ethanol before sowing. The pots were filled with soil to about 5 cm below the top. Two infested millet seeds were placed 2-3 cm beneath each wheat seed (Kirkegaard *et al*., 1999). Four wheat seeds were sown per pot and covered with 2-3 cm soil on 17 March, 2009. Five days after emergence (i.e. on 26 March), plants were thinned to three seedlings per pot at the two-leaf seedling stage.

3.2.4 Growth conditions and treatments

The two wheat cultivars (25 grams/line), Mulgara (AUS 29466) and Janz (AUS 24794) were supplied by the Australian Winter Cereals Collection, Tamworth NSW. Temperature in the greenhouse was controlled at 25/18 ^{0}C (day/night). The relative humidity (RH) was maintained at approximately 60% within the greenhouse. The photoperiod was 12 hours on average over the course of the experiment. The soil surface of each pot was covered with plastic beads to a depth of 1.5 cm to reduce soil evaporation. Pots were supplied weekly with Aquasol^{TM} N:P:K (23:4:18) fertiliser from the beginning of tillering stage (7 April). Water stress was imposed by withholding watering for 7 days at tillering (GS 22; D1) or anthesis (GS 65; D2) according to the Zadoks scale (Zadoks *et al*., 1974) on separate sets of plants, and compared with well-watered (WW) plants. D1 was imposed from 23 April to 30 April, 2009 and D2 from 13 May to 20 May, 2009.

The pots were watered to field capacity at the beginning of the experiment, and then re-watered to the same level every 2-3 days. Each pot was weighed prior to re-watering to field capacity to determine the amount of water lost since last watering. The pots were then watered

to excess and allowed to drain overnight. The following morning they were re-weighed. The amount of water lost through soil evaporation was monitored by weighing unplanted pots, with polystyrene bead coverings. Transpiration was determined by subtracting water loss of unplanted pots from that of planted pots.

The experimental design was a randomised complete block with three replicates and three factors: two wheat cultivars (cvs. Mulgara and Janz), three drought conditions (WW, D1 and D2), and three pathogen conditions (control, *Rhizoctonia solani* and *Pythium irregulare*).

3.2.5 Measurements

Growth stages (plant development) for the two cultivars were determined for both diseased and control plants one week after the end of the D1 drought treatment and at the start of the D2 drought treatment.

Water potential and its components

Water relations parameters, leaf water potential and osmotic potential, were measured at the beginning and end of both D1 and D2 and compared with well watered (WW) plants.

Leaf total water potential (ψ) was measured between 2 pm and 5 pm on a fully expanded mature leaf blade at tillering, and on the flag leaf at anthesis. A Soil Moisture Equipment Corporation Scholander type pressure chamber was used to measure ψ. The first 10 cm of the tip of the leaf blade was used to measure ψ. A second portion, approximately 4 cm in length, of the same leaf sample, was used to determine osmotic potential (π) and relative water content (RWC). Sub-samples of about 2 cm^2 from the second leaf portion were chopped and placed into small tubes (Eppendorf tubes) which were plunged immediately into liquid

nitrogen. The samples were thawed after freezing in liquid nitrogen, centrifuged and then π was measured using a VAPRO 5520 vapour pressure osmometer. Values in osmoles (mmol kg^{-1}) were converted to MPa using the formula:

$$\Pi \text{ (MPa)} = - \text{ (mmol kg }^{-1}) * 2.487*10^{-3} \qquad \text{Eq. [3.1]}$$

Turgor pressure (P_t) was calculated as the difference between water potential and osmotic potential ($P_t = \psi - \pi$).

Relative water content

Relative water content (RWC) was determined by excising a 1 cm^2 sub-sample of the mid-leaf from the same sample. The samples were placed into small tubes and transferred within three hours after excision to the laboratory to determine the fresh weights (W_f). Turgid weights (W_t) were obtained after soaking the leaf samples in deionised water in small tubes kept overnight in a refrigerator at 4^oC. The following morning, leaf samples were quickly and carefully blotted with tissue paper, to remove excess water from leaf surface, and re-weighed. Dry weights (W_d) were determined after oven drying the leaf samples for 24 hours at 80 ^oC. RWC was calculated as a percentage from the equation:

$$\text{RWC (\%)} = (W_f - W_d)/ (W_t - W_d)*100. \qquad \text{Eq. [3.2]}$$

Root lesion percentage

A sample of soil was taken from each pot at grain filling stage (GS 70) according to the Zadoks scale (Zadoks *et al*., 1974) by coring to a depth of 5 cm and diameter of 2 cm for each treatment 30 days before final harvest. The soil was washed off the roots on a 2.8 mm sieve.

Percentage of root length with lesions was measured by the gridline intersect method (Giovannetti and Mosse, 1980).

Yield components

At full maturity stage (about 20 weeks after sowing), the number of tillers and heads, and proportion of fertile tillers per pot, were counted. The plants were harvested on 4 August 2009 when plants reached final maturity. The above-ground plant parts were harvested and separated into vegetative and reproductive parts (i.e heads). The grains were separated from heads by threshing and grain weight was determined for each pot. Total dry weight of shoots and head components (awns, glumes, etc) was determined after drying in an oven at 80 ^{o}C for two days. The one thousand-grain weight was determined from the weight of 200-seed samples.

Statistical analysis

Data were analysed by factorial ANOVA. Water relations data were analysed for each drought period independently, comparing droughted and well-watered plants. Tukey's HSD test was used to separate means when appropriate.

3.3 Results

3.3.1 Variation of growth stages

The effects of drought at tillering (D1) and root diseases (*Pythium* and *Rhizoctonia*) on plant development one week after the end of the drought period are presented in Figure 3.1. Growth stages (GS) varied from 47 to 60 on the Zadoks scale for Mulgara and from 32 to 59 for Janz. Mulgara was significantly ($P < 0.01$) less affected (GS 54) than Janz (GS 47) following tillering drought. Well watered plants (WW) had reached a significantly higher GS (plants almost at anthesis stage, GS 50) than those droughted at tillering (between stem elongation and booting, GS 37). Fungal treatment alone had no significant effect. There were, however, significant interactions between fungus and both cultivar and D1.

There was a significant ($P < 0.001$) drought X cultivar X fungus interaction. This was because growth stage was lower in the droughted, inoculated plants than controls in Janz, but not in Mulgara. In Janz, the effect of drought on GS was greatest in the *Pythium* and *Rhizoctonia* treatments, and not significant in the control treatment (Figure 3.1). In Mulgara, the effect of drought on GS was significant for both *Pythium* and control treatments, but not for *Rhizoctonia*. In WW plants, the effect of *Pythium* and *Rhizoctonia* on GS did not differ significantly between cultivars, but the cultivar effect was significant ($P < 0.01$) in the control treatments. In contrast, cultivar had no significant effect on GS of control droughted plants, but the variety effect was significant for diseased, droughted plants ($P < 0.01$).

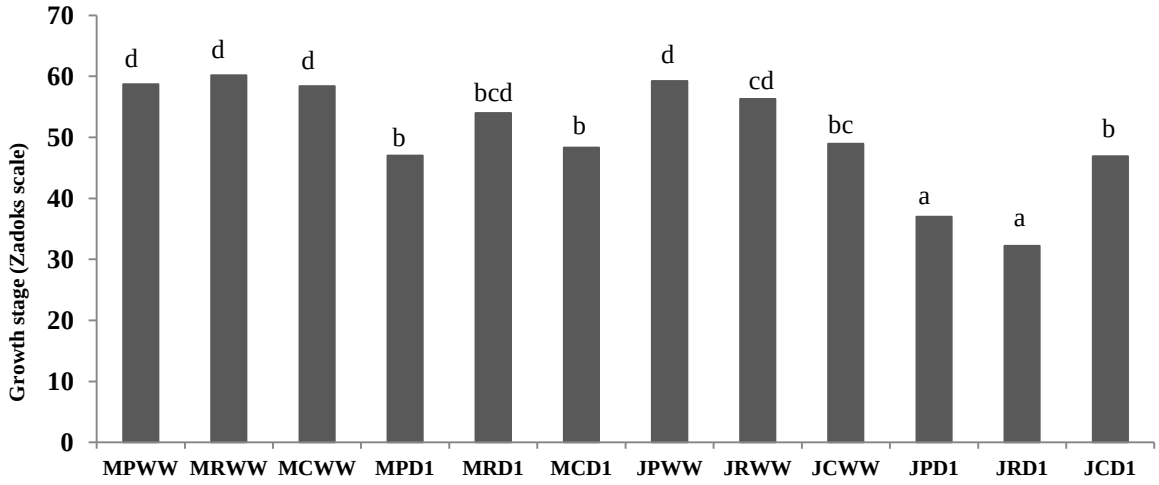

Figure 3.1 Effect of drought at tillering (D1) and disease on growth stage one week after drought treatment for *Triticum aestivum* cvs. Mulgara (M) and Janz (J). WW: well watered, C: controls, P: *Pythium* and R: *Rhizoctonia*. Columns labelled with the same letter are not significantly different at P<0.05.

There were also significant differences ($P < 0.05$) between cultivars for GS at the start of anthesis drought (D2) (Figure 3.2). Mulgara was at half complete anthesis (GS 65) while Janz was just at the beginning of anthesis (GS 63). GS was also affected significantly ($P < 0.05$) by the interaction of cultivar and drought treatments. Growth stage was lower in D1 than in other drought treatments for Janz, but not for Mulgara. There were no other significant effects or interactions.

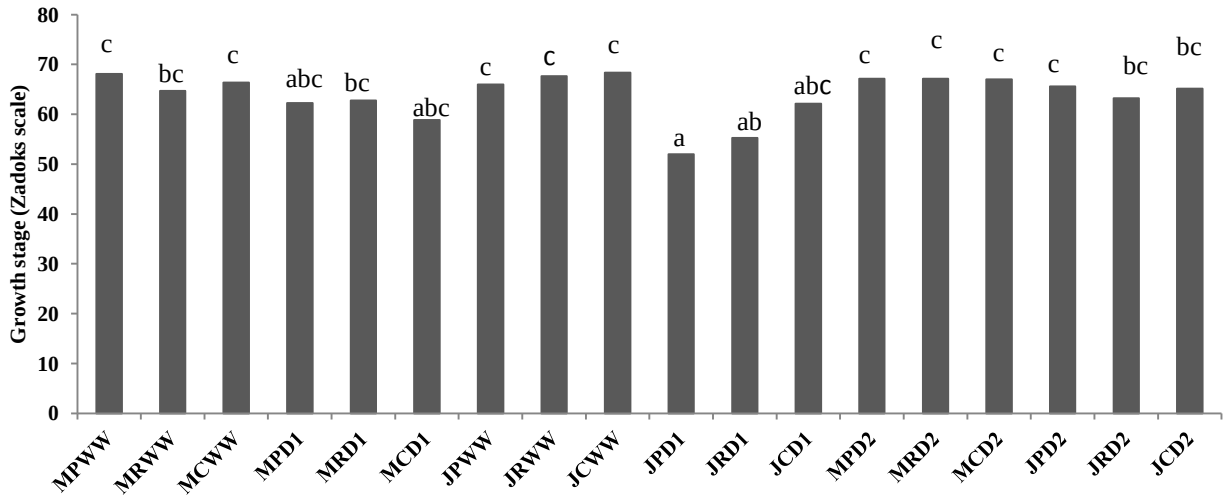

Figure 3.2 Effect of anthesis drought (D2) and diseases on growth stages at the start of the anthesis drought treatment (D2) for *Triticum aestivum* cvs. Mulgara and Janz. WW: well watered, C: controls, P: *Pythium* and R: *Rhizoctonia*. Columns labelled with the same letter are not significantly different at P<0.05.

3.3.2 Water Use (Transpiration)

Cumulative transpiration

Figure 3.3 showed a sigmoidal pattern in cumulative transpiration (CT) for cvs. Mulgara and Janz under different drought and disease treatments. There was no significant difference between Mulgara and Janz in CT. The trend line for the CT for Mulgara was the same for all diseases, and at all stages of growth at WW. There was no difference in CT between controls and diseased Janz in WW treatments until July, which then indicated slightly higher CT for controls, however, the effect was not significant.

The trend of CT in D1 was very similar to that for WW. However, CT was significantly lower ($P < 0.01$) for both cultivars in D1, when compared with WW. In D1, the CT of diseased Mulgara plants was higher than for controls (*Rhizoctonia* > *Pythium* > control). However, controls of Janz had higher CT than infected plants (control > *Rhizoctonia* > *Pythium*).

The trend of CT for D2 plants differed from other treatments (WW and D1). The CT increased rapidly until anthesis (mid May) for both cultivars. After anthesis drought (D2), the CT line steadied until harvest time. The CT of D2 plants was approximately 50% of those of D1.

Overall, drought treatments significantly decreased CT ($P < 0.01$). The interaction between cultivar and fungus for CT was significant ($P = 0.078$) at the 10% level. The total transpiration of Janz in controls (12.7 l) was higher than that for diseased plants (*Pythium* and *Rhizoctonia* were 10.7 and 12 l, respectively).

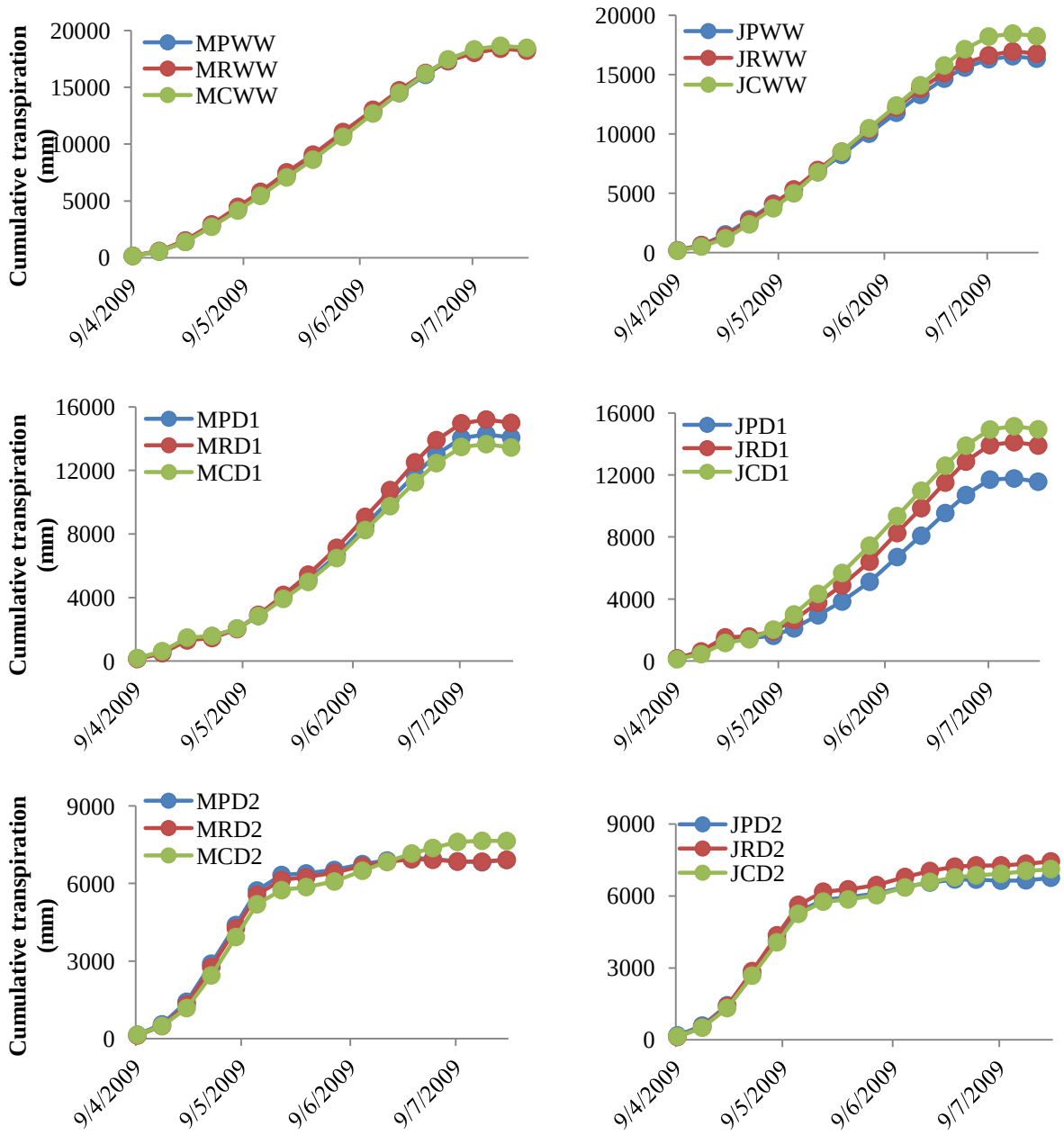

Figure 3.3 Cumulative transpiration (mm) from 9 April (tillering) to 23 July (harvest) for cvs. Mulgara (left) and Janz (right) under diseases treatments: *Pythium* (P), *Rhizoctonia* (R) and control (C) and drought treatments at tillering (D1) and anthesis (D2) Upper: well water, Middle: D1 and Lower:D2. D1 was imposed from 23 April to 30 April and D2 from13 May to 20 May, 2009.

Transpiration per week

The response of transpiration per week (TPW) for cvs. Mulgara and Janz under disease and drought treatments from tillering to harvest is shown in Figure 3.4. The lowest TPW was at the beginning and end of the plant growth period. The highest TPW was in June (at anthesis) for well watered (WW) and tillering droughted (D1) plants, of both Mulgara and Janz, but lowest for anthesis droughted (D2) plants. TPW for D1 plants was reduced to a minimal value at the time of water stress, but then quickly rose to levels almost equivalent to those of WW plants at anthesis. TPW for D2 plants sharply decreased at the time of the drought treatment (anthesis), and transpiration was maintained at minimal levels until harvest.

There were no significant differences in TPW between treatments from 9 April until 23 April. However, TPW was significantly reduced by D1 at 30 April when compared with WW. Cultivars and diseases had no effect on transpiration at that date. In addition to the drought effect, there was a cultivar effect on TPW on 7 and 13 May. Mulgara showed a significantly higher TPW ($P < 0.05$) than Janz. There was a significant interaction ($P < 0.05$) between cultivars and fungal treatment on 20 May where controls of Janz showed significantly higher TPW ($P < 0.05$) than *Pythium* infected plants which had undergone tillering drought. The effect of fungus on transpiration of Mulgara was, however, not significant. Moreover, TPW was significantly affected ($P < 0.05$) by the interactions of fungus and drought on 2 July. TPW of controls was significantly higher ($P < 0.05$) than for diseased plants in the D2 treatment, but not for D1 plants.

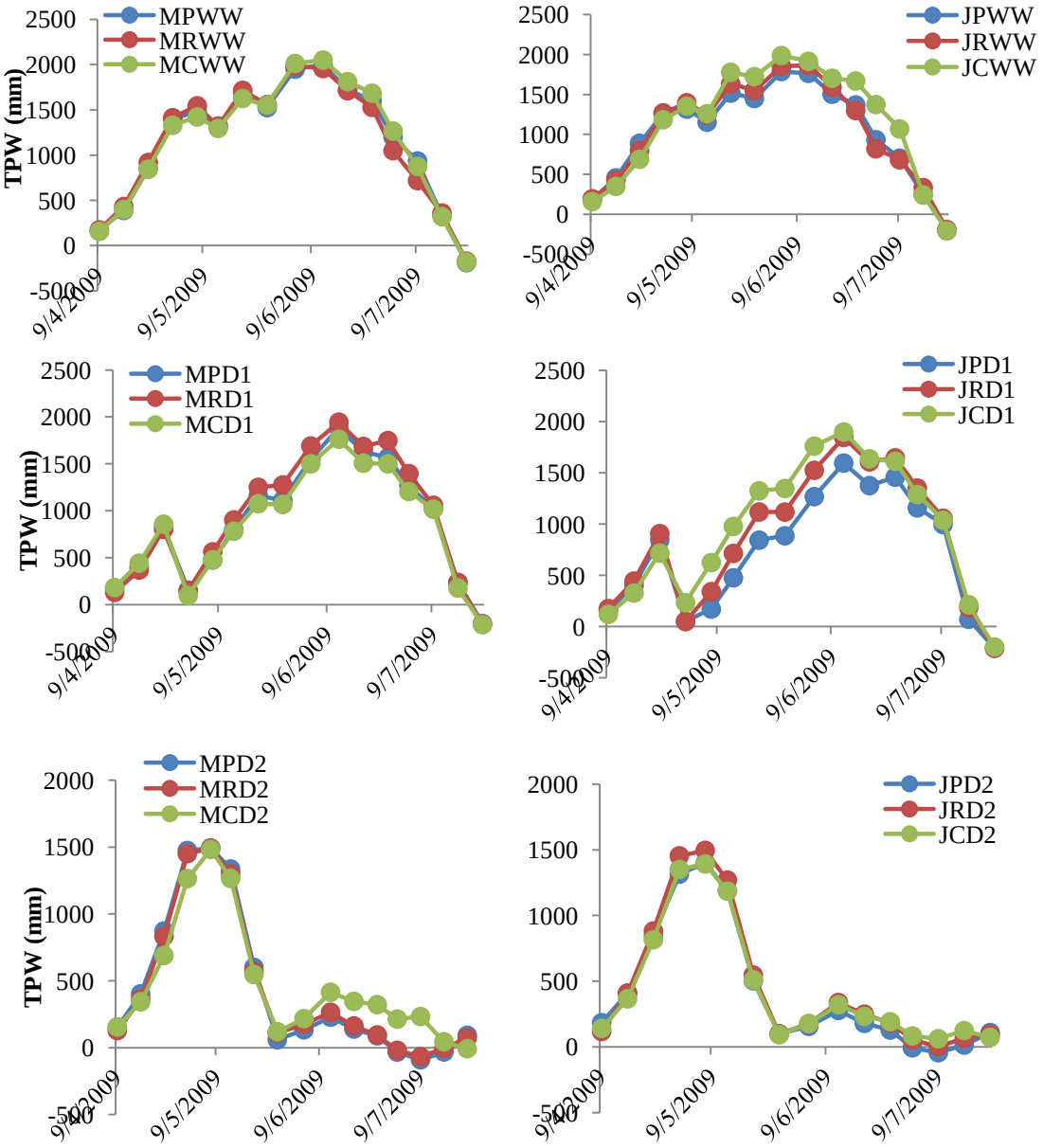

Figure 3.4 Transpiration per week (mm.week^{-1}) from 9 April (tillering) to 23 July (harvest) for cvs. Mulgara (left) and Janz (right) under disease treatments (*Pythium* (P), *Rhizoctonia* (R) and control (C)) and drought treatments at tillering (D1) and anthesis (D2). Upper: well watered, Middle: D1 and Lower:D2. D1 was imposed from 23 April to 30 April and D2 from13 May to 20 May, 2009.

Transpiration during water stress

Cumulative transpiration (CT) during 7 days of water stress at D1 and D2 was determined for both Mulgara (Figure 3.5A and 3.5C, respectively) and Janz (Figures 3.5B and 3.5D). In both drought treatments, cvs. Mulgara and Janz did not differ in transpiration when they were stressed for 7 days. WW plants of Mulgara and Janz had significantly higher CT ($P < 0.01$) than D1 after 4 and 7 days of withholding water. CT of D2 plants remained steady between 2-7 days of water withholding. There was no effect of fungus or any interaction on transpiration during the drought period.

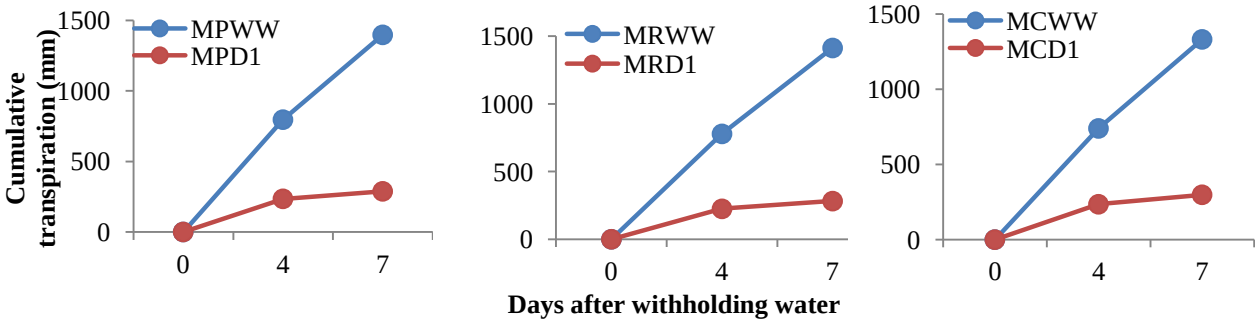

Figure 3.5A Cumulative transpiration (mm) of well watered (WW) and droughted at tillering (D1) of plants of cv. Mulgara under different disease conditions (*Pythium* P; *Rhizoctonia* R; control c) during 7 days of water stress.

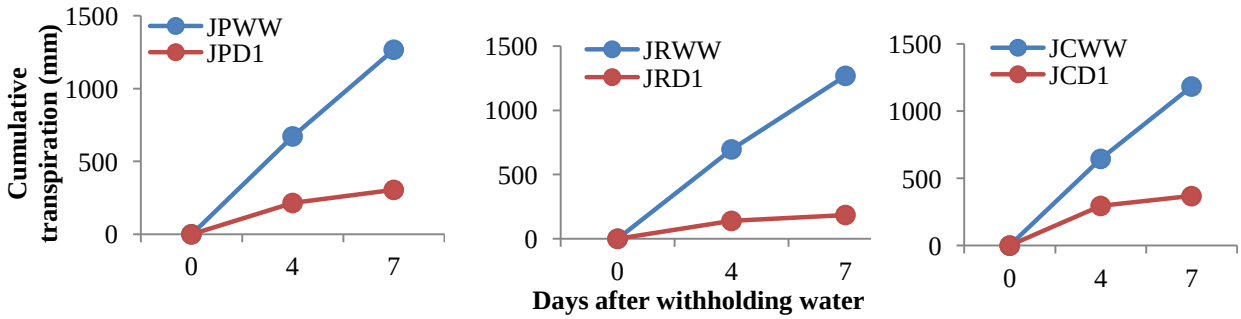

Figure 3.5B Cumulative transpiration (mm) of well watered (WW) and droughted at tillering (D1) of plants of cv. Janz under different disease conditions (*Pythium* P; *Rhizoctonia* R; control c) during 7 days of water stress.

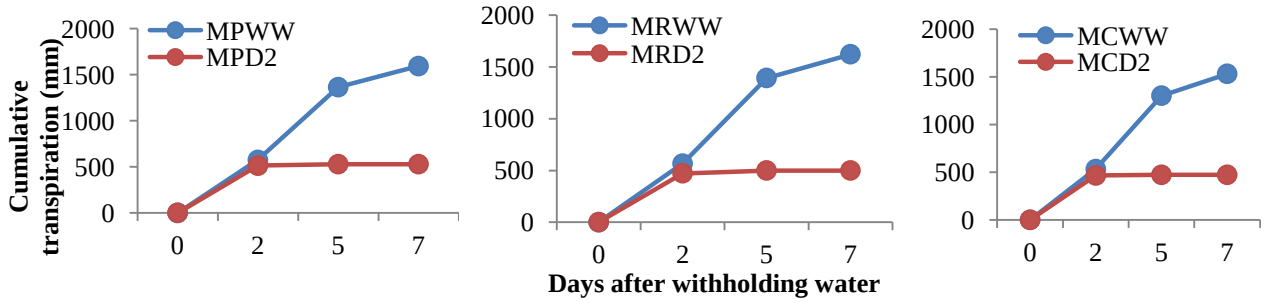

Figure 3.5C Cumulative transpiration (mm) of well watered (WW) and droughted at anthesis (D2) of plants of cv. Mulgara under different disease conditions (*Pythium* P; *Rhizoctonia* R; control c) during 7 days of water stress.

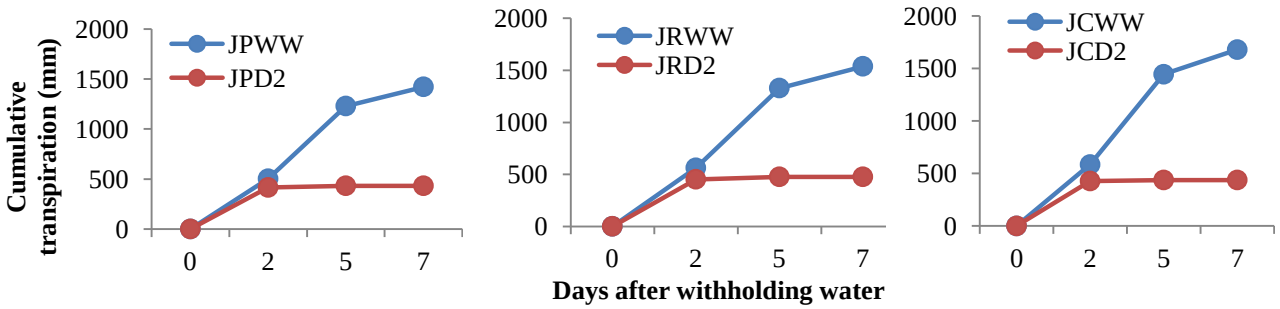

Figure 3.5D Cumulative transpiration (mm) of well watered (WW) and droughted at anthesis (D2) of plants of cv. Janz under different disease conditions (*Pythium* P; *Rhizoctonia* R; control c) during 7 days of water stress.

3.3.3 Plant water relations

Tillering drought (D1)

Water relations (total leaf water potential, osmotic potential and pressure potential) of cvs Mulgara and Janz at the beginning (day 0) and end (day 7) of tillering drought (D1) were represented in Figures 3.6A and 3.6B. Fungus and varieties had no significant effect on total water potential at day 0 of D1. However, osmotic potential was affected significantly ($P < 0.05$) by cultivars. Janz had higher osmotic potential (-1.145 MPa) than Mulgara (-1.192 MPa) at day 0. Pressure potential did not differ between varieties or treatments at day 0.

The effects of drought at tillering on water relations were also compared with well watered (WW) plants after 7 days of water stress for both cultivars under diseases conditions. Drought altered water relations in both cultivars. Leaf water potential (Ψ) reduced significantly from -0.9 MPa for WW to -2.6 MPa for D1 for Mulgara and from -1 MPa for WW to -3 MPa for D1 for Janz at day 7. Both fungus and drought significantly affected osmotic potential (π) at day 7. Osmotic potential was lower in D1 than WW, and was lower in *Pythium* and *Rhizoctonia* infected plants than in control. The fungus by drought interaction on π was close to significant ($P = 0.061$), with *Pythium* and *Rhizoctonia* tending to have a larger effect in D1 plants than in well-watered plants. The variety by fungus interaction on π was close to significant ($P = 0.053$), with *Pythium* and *Rhizoctonia* having a larger effect on Janz than on Mulgara. Only drought affected pressure potential at day 7.

Well watered plants of Mulgara and Janz had similar relative water content (RWC) in all disease treatments (Figure 6C). RWC was significantly higher in Janz than Mulgara at day 0. However, only drought affected RWC at day 7. There was no disease effect on RWC.

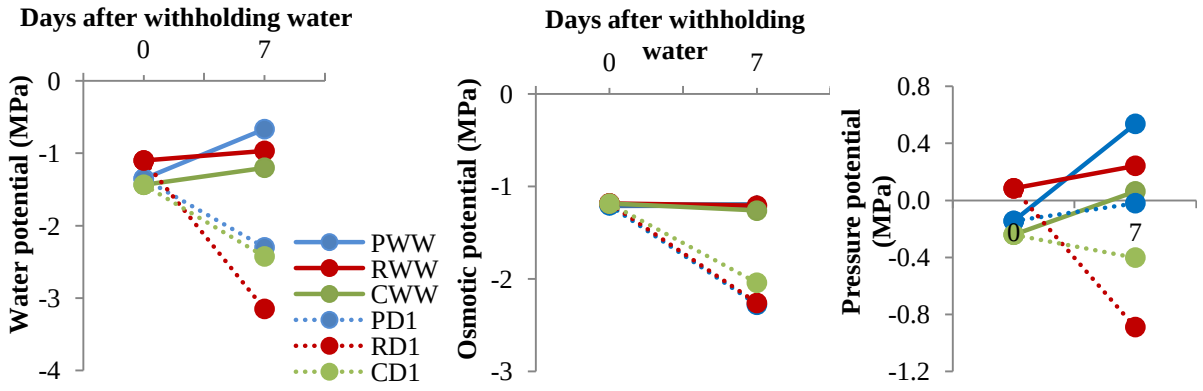

Figure 3.6A Water potential (left), osmotic potential (mid) and pressure potential (right) in cv. Mulgara (M) infected with *Pythium* (P), *Rhizoctonia* (R) and control (C). The measurements were taken at the beginning and end of 7 days of withholding water from plants droughted at tillering (D1) and equivalent time for well watered (WW) plants.

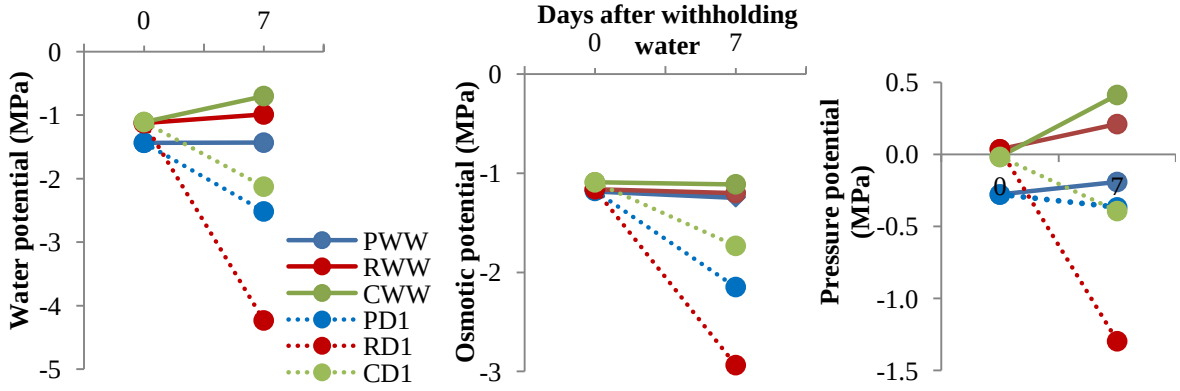

Figure 3.6B Water potential (left), osmotic potential (mid) and pressure potential (right) in cv. Janz (J) infected with *Pythium* (P), *Rhizoctonia* (R) and control (C). The measurements were taken at the beginning and end of 7 days of withholding water from plants droughted at tillering (D1) and equivalent time for well watered (WW) plants.

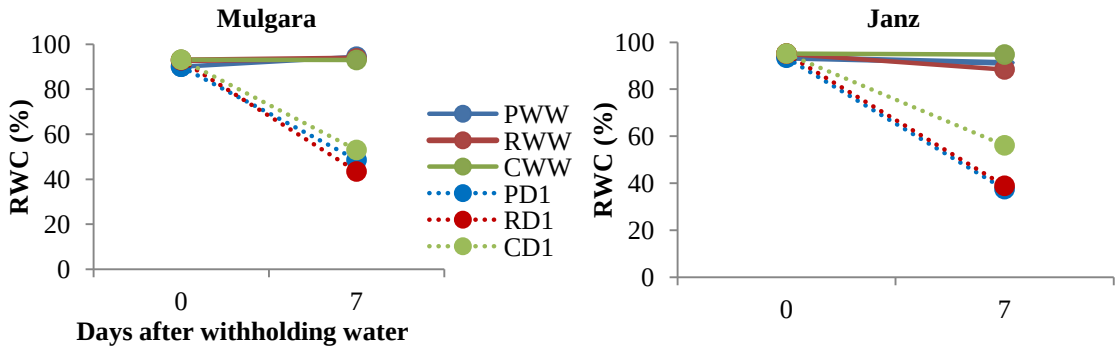

Figure 3.6C Relative water content (%) for WW (0) and D1 (7) after 7 days of withholding water for cvs. Mulgara (left) and Janz (right) infected with *Pythium* (P), *Rhizoctonia* (R) and control (C). The measurements were taken at the beginning and end of 7 days of withholding water from plants droughted at tillering (D1) and equivalent time for well watered (WW) plants.

Anthesis drought (D2)

The diseases had no significant effect on water relations of well watered plants at the beginning (0 day) of anthesis drought (D2) (Figures 3.7A and 3.7B) in either cultivar. However, cultivar had a significant effect on total leaf water and pressure potentials. Mulgara had higher water potential (-0.384 MPa) than Janz (-1.46 MPa) at the beginning of the drought.

At the end of D2 (7 days), droughting of plants at anthesis significantly affected water relations in both cultivars, with water stress being more severe in D2 than in D1. Drought was the only factor or interaction that had a significant effect on Ψ, π or pressure potential at the end of D2. All of these were lower in droughted plants than well-watered plants (Figures 3.7A, 3.7B).

RWC was significantly affected by fungus and drought at day 7 of D2 (Figure 3.7C). RWC was higher in WW plants than D2, and was higher in *Rhizoctonia* and *Pythium* than in controls. There were no other significant effects or interactions.

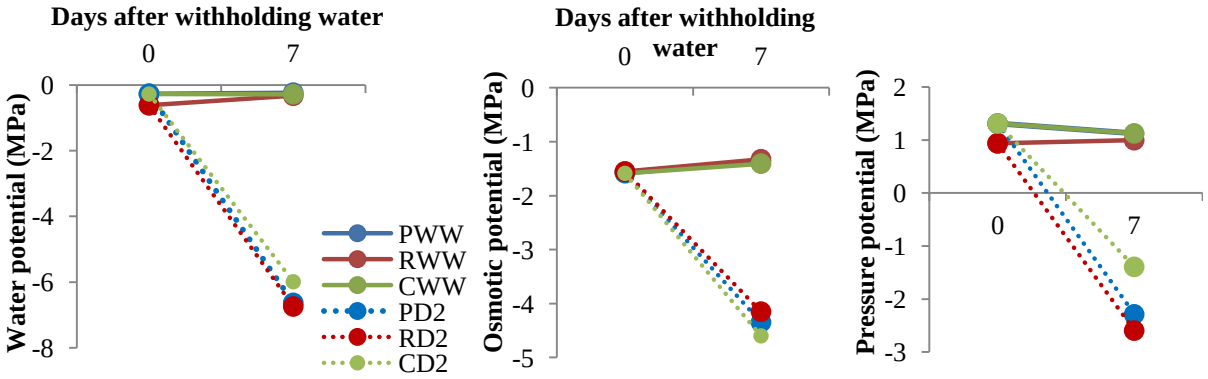

Figure 3.7A Water potential (left), osmotic potential (mid) and pressure potential (right) in cv. Mulgara (M) infected with *Pythium* (P), *Rhizoctonia* (R) and control (C). The measurements were taken at the beginning and end of 7 days of withholding water from plants droughted at anthesis (D2) and equivalent time for well watered (WW) plants.

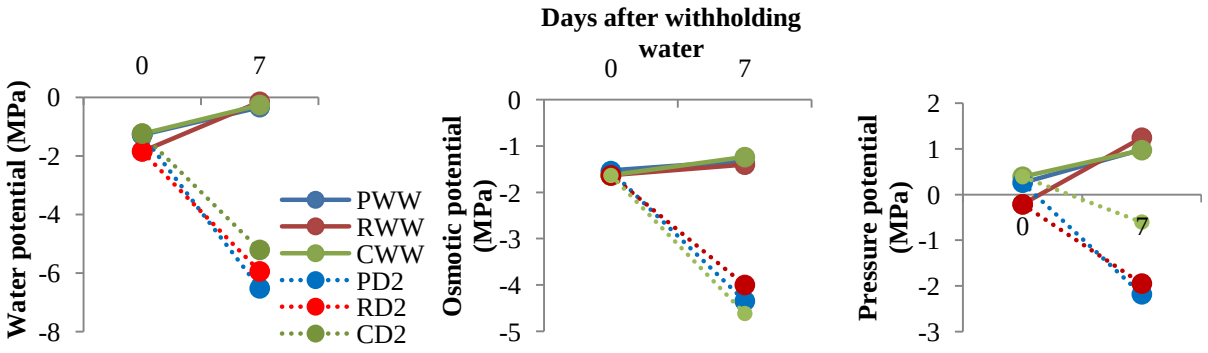

Figure 3.7B Water potential (left), osmotic potential (mid) and pressure potential (right) in cv. Janz (J) infected with *Pythium* (P), *Rhizoctonia* (R) and control (C). The measurements were taken at the beginning and end of 7 days of withholding water from plants droughted at anthesis (D2) and equivalent time for well watered (WW) plants.

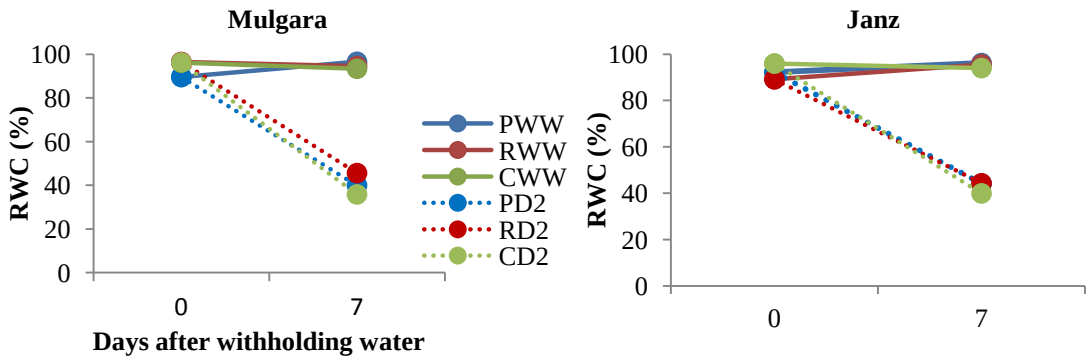

Figure 3.7C Relative water content (%) for WW (0) and D2 (7) after 7 days of withholding water for cvs. Mulgara (left) and Janz (right) infected with *Pythium* (P), *Rhizoctonia* (R) and control (C). The measurements were taken at the beginning and end of 7 days of withholding water from plants droughted at anthesis (D2) and equivalent time for well watered (WW) plants.

3.3.4 Yield components

Yield components for both wheat cultivars were compared between well watered plants and those droughted at either tillering or anthesis under inoculation by *Pythium* and *Rhizoctonia* in Table 3.1. In general, yield components were affected by droughts but not by diseases. Therefore, the data represent the mean values of all disease treatments for both cultivars. The number of heads and tillers varied significantly ($P < 0.01$) between variety and drought treatments. The number of heads and tillers for Mulgara was significantly higher (23.3 and 24.7, respectively) than those for Janz (20.1 and 21.9, respectively). The number of heads and tillers did not differ between well watered (WW) and tillering drought (D1) treatments. However, anthesis drought (D2) reduced the number of heads and tillers approximately 50% compared with WW. The proportion of fertile tillers only varied significantly ($P < 0.01$) in D2. The proportion of fertile tillers in WW and D1 plants was 98% and decreased to 76% in D2 plants.

Number of grains and grain weight were also significantly different for cultivars and droughts (Table 3.1). Mulgara had significantly higher grain weight ($P < 0.05$) and grain number ($P < 0.01$) than Janz. The grain weight was reduced significantly by 21% and 91% at D1 and D2, respectively when compared with WW. However, drought had a smaller effect on grain number than grain weight. There were reductions of 14% and 76% in grain number at D1 and D2, respectively. The results showed a minor interaction effect between fungus and cultivar ($P = 0.095$) on grain number. The healthy plants of Janz had higher number of grains than diseased ones and vice versa for Mulgara. One thousand- grain weight was significantly ($P < 0.01$) reduced by D2. There were no differences between 1000-grain weight of WW and D1.

Total dry weight was affected significantly by drought treatments. Total dry weight was higher in WW plants than those droughted at either D1 or D2. Cultivar and drought at anthesis had significant effects on harvest index (HI). The HI of Mulgara (0.39) was significantly higher than HI of Janz (0.34). There was no difference between HI of WW and D1. HI of D1 was 0.49 and WW 0.48 but HI was reduced to 0.13 by D2.

Table 3.1 Number of tillers per pot (TN/pot), number of heads per pot (HN/pot), grain weight (GW), dry matter weight (DMW), number of grains (GN), 1000-grain weight (1000-GW) and harvest index (HI) for well watered (WW), tillering drought (D1) and anthesis drought (D2) of two wheat cultivars under combined disease conditions. The variety by drought interaction was not significant for all yield components.

Cultivar	Drought	TN/pot	HN/pot	GW(g)	DMW(g)	GN	1000-GW(g)	HI
Mulgara	WW	29.4	29.1	22.0	21.3	613.3	35.96	0.50
	D1	28.6	28.1	16.7	15.6	507.4	33.2	0.50
	D2	16.2	12.6	1.96	11.6	166.3	12.2	0.14
Janz	WW	27.1	26.2	18.9	23.2	519.1	36.8	0.45
	D1	24.8	24.1	15.2	18.4	460.2	33.0	0.46
	D2	13.9	9.9	1.6	11.4	101.7	14.7	0.11
LSD(0.05)		2.9	2.9	3.0	4.9	80.8	4.8	0.05

3.3.5 Water Use efficiency (WUE)

WUE based on grain yield

Figures 3.8A-3.8C show the effects of drought either at tillering (D1) or anthesis (D2) on water use efficiency (WUE_{grain}) in both cultivars infected by *Pythium* or *Rhizoctonia* and controls. There was no difference between WUE_{grain} of D1 and WW plants under all disease conditions. However, WUE_{grain} was decreased significantly in D2 for both cultivars. Mulgara had slightly higher WUE_{grain} than Janz but the effect was not significant ($P = 0.08$). There was no significant disease effect on WUE_{grain}.

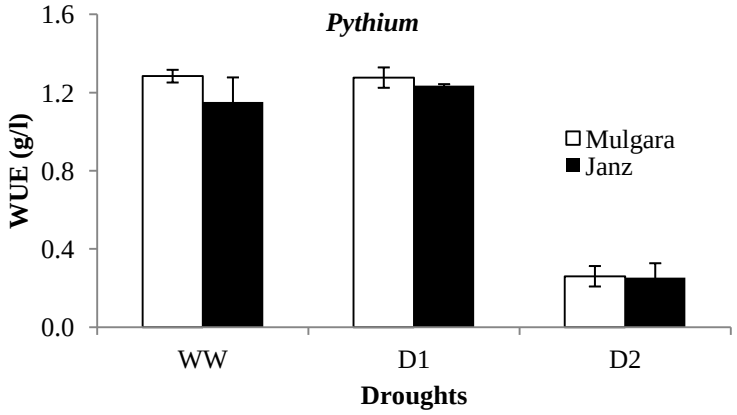

Figure 3.8A Water use efficiency based on grain yield (g/l) for cvs. Mulgara and Janz infected by *Pythium* at either tillering (D1) or anthesis (D2) droughts compared with well watered (WW) plants. Values are means ± s.e., n =3

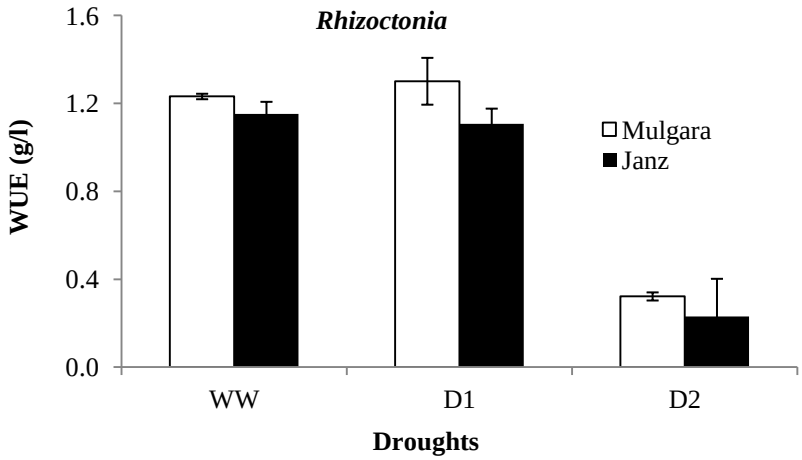

Figure 3.8B Water use efficiency based on grain yield (g/l) for cvs. Mulgara and Janz infected by *Rhizoctonia* at either tillering (D1) or anthesis (D2) droughts compared with well watered (WW) plants. Values are means ± s.e., n =3

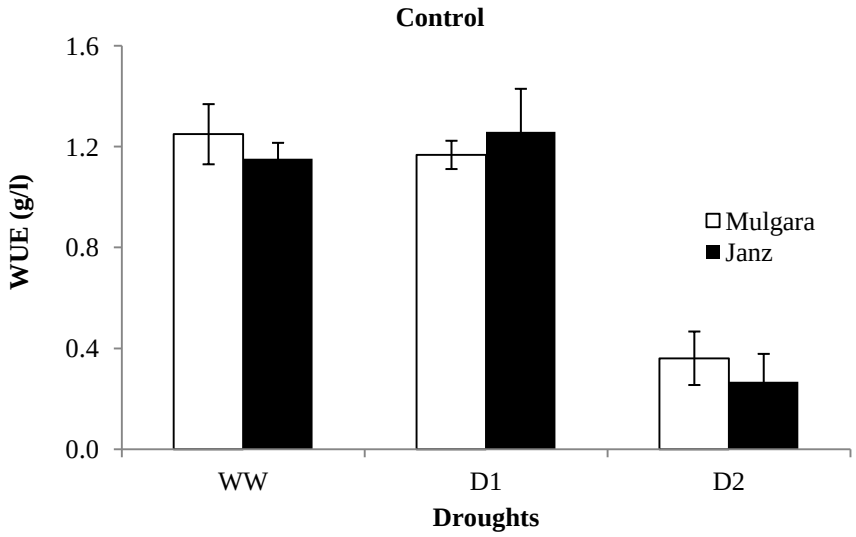

Figure 3.8C Water use efficiency based on grain yield (g/l) for uninfected cvs. Mulgara and Janz at either tillering (D1) or anthesis (D2) droughts compared with well watered (WW) plants. Values are means ± s.e., n =3

WUE based on dry matter

There was a significant drought effect on WUE based on dry matter (WUE_{DM}) but not cultivar or disease (Figures 3.9A-3.9C). WUE was reduced by D2 but not by D1.

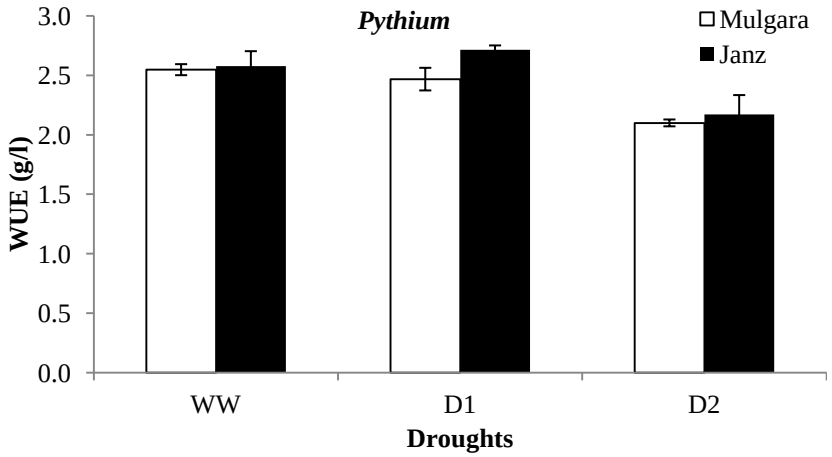

Figure 3.9A Water use efficiency based on shoot dry weight (g/l) for cvs. Mulgara and Janz infected by *Pythium* at either tillering (D1) or anthesis (D2) droughts compared with well watered (WW) plants. Values are means ± s.e., n =3

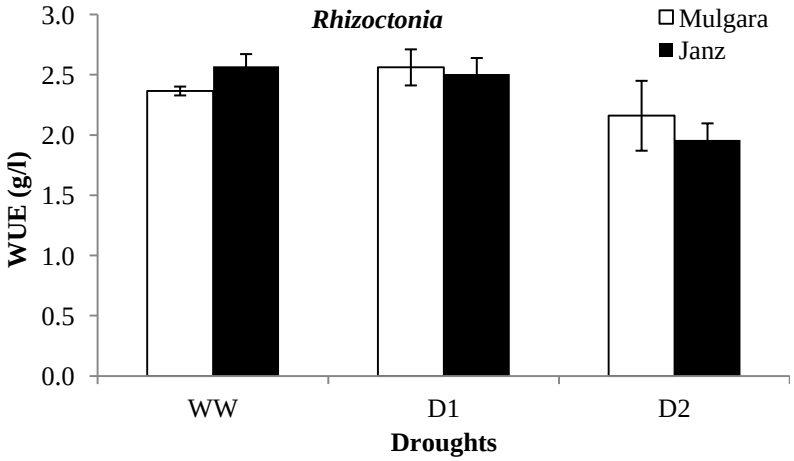

Figure 3.9B Water use efficiency based on shoot dry weight (g/l) for cvs. Mulgara and Janz infected by *Rhizoctonia* at either tillering (D1) or anthesis (D2) droughts compared with well watered (WW) plants. Values are means ± s.e., n =3

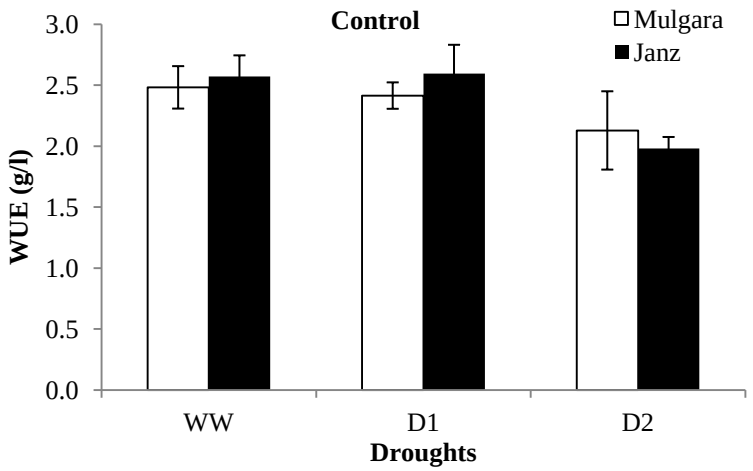

Figure 3.9C Water use efficiency based on shoot dry weight (g/l) for cvs. Mulgara and Janz controls at either tillering (D1) or anthesis (D2) droughts compared with well watered (WW) plants. Values are means ± s.e., n =3

3.3.6 Lesion percentage

The cultivar X fungus interaction had a significant effect on root lesion percentage (Figure 3.10A). The controls (38%) had significantly less lesioned roots than *Pythium* (53.7%) and *Rhizoctonia* (50.3%). *Rhizoctonia* resulted in more lesions on Mulgara (57%) than on Janz (43%). Drought treatments had no significant effect on percentage of root lesioned (Figure 3.10B). Because plants were starting to senesce roots of control plants showed browning, but this was obviously increased by the disease treatment.

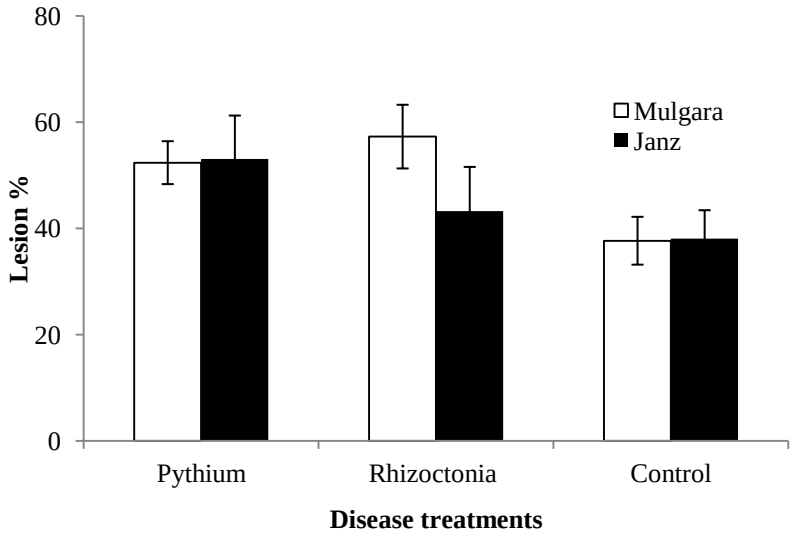

Figure 3.10A Effect of diseases (*Pythium* and *Rhizoctonia*) on lesion % of cvs. Mulgara and Janz. The diseases were compared with controls. Values are means ± s.e., n =3

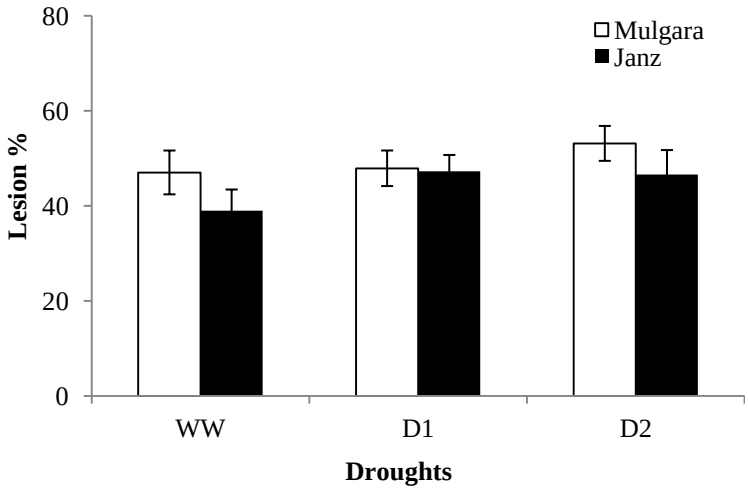

Figure 3.10B Effect of droughts on lesion % of cvs. Mulgara and Janz. Root lesions for 1st drought (D1) at tillering and 2nd drought (D2) at anthesis were compared with well watered (WW) plants. Values are means ± s.e., n =3

3.3.7 Qualitative effects of disease on root systems

The figures below (3.11A -3.11C) showed the effect of drought and diseases treatments on root system of both cultivars. In Figure 3.11A, the roots size of Janz was almost the same under all disease conditions at anthesis drought (D2). However, roots of control Mulgara were larger than those infected by either *Pythium* or *Rhizoctonia*. The roots of plants droughted at tillering (D1) were larger than at D2 (Figure 3.11B). Controls of both cultivars had larger and longer roots than under diseases. There were no obvious differences in root systems of either cultivar between controls and disease treatments (Figure 3.11C).

Figure 3.11A Roots of cvs. Janz (left) and Mulgara (right) from the anthesis drought (D2) treatment. Control roots (left) and roots infected with *Rhizoctonia* (centre) and *Pythium* (right).

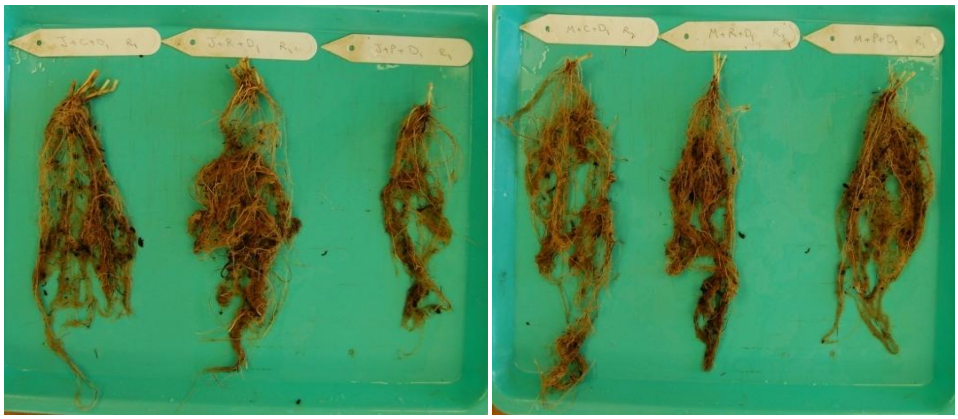

Figure 3.11B Roots of cvs. Janz (left) and Mulgara (right) from the tillering drought (D1) treatment. Control roots (left) and roots infected with *Rhizoctonia* (centre) and *Pythium* (right).

Figure 3.11C Roots of cvs. Janz (left) and Mulgara (right) from the well watered (WW) treatment. Control roots (left) and roots infected with *Rhizoctonia* (centre) and *Pythium* (right).

3.4 Discussion

Inoculation with *Pythium* or *Rhizoctonia* did not significantly reduce transpiration or water use efficiency in well-watered plants. However, there was an indication of reduced transpiration in diseased plants of Janz following tillering drought, and of Mulgara following anthesis drought. Root diseases have been reported to affect transpiration of wheat. Amir and Sinclair (1996) showed a decrease in wheat transpiration as a result of cereal cyst nematode disease and the reduction of root growth was associated with decreased shoot growth. In this study, there was no disease effect on WUE based either on grain yield or vegetative dry matter. However, Martin *et al.* (1986) found lower WUE in wheat plants infected by *Cephalosporium gramineum* disease than non-diseased plants because grain yield was reduced more than water use.

Drought at tillering or anthesis reduced transpiration for both cultivars. Water use was reduced approximately 50% by drought at anthesis compared with drought at tillering. Drought developed slowly at the tillering stage because of the large volume of soil in proportion to leaf area (Frank *et al*., 1973) and developed rapidly at anthesis stage (severe drought) Findings of water use showed that there was no significant difference between Janz and Mulgara This study showed higher transpiration rates during reproductive than vegetative stages Therefore, the soil water supply is more rapidly exhausted at anthesis, which also results in reduction of leaf ψ (Morgan, 1977) Water use and biomass were reduced by the stress treatment (Blum, 2005). Plants at D2 had low water use after anthesis and thus reduced grain yield and HI. Also, Passioura (1977) and Seif and Pederson (1978) indicated that grain yield and the HI of rain-fed crops depends largely on the amount or proportion of water used after anthesis or just before anthesis (Richards, 1983) rather than on the total water used by the crop.

Wheat at anthesis has a large root system that may aid water uptake under drought. However, Ma *et al*. (2010) argued that a large root system can result in rapid soil-water consumption, which may not be favourable in arid and semiarid areas. Under water stress, the reductions in leaf size result in smaller transpiring leaf area (Tardieu, 2005) which has a similar role to stomatal closure and allows the plant to avoid damaging leaf water potential in leaves by reducing the water flow through the leaf surface.

Water use efficiency (g/l) was affected only by drought. There have been numerous studies interested in improvement of yield and WUE under drought e.g. Zhang *et al*. (1998) and Hay and Kirby, (1991). However, Blum (2009) argued that effective use of water (EUW) is the major target in yield improvement. In this study, there was no difference in WUE between D1 and WW. Van den Boogaard *et al*. (1996) and El Hafid *et al*. (1998) found that WUE did not change with drought. However, Sinclair *et al*. (1975) concluded that WUE decreased under drought conditions because of the increase in stomatal resistance that decreased both photosynthetic productivity and WUE. WUE at D2 was reduced greatly because of the reduction of grain yield, although it must be acknowledged that droughting at D2 was more severe than at D1. Zhang *et al*. (1999) found that WUE from stem elongation to milking is reduced when wheat is exposed to stress. However, De Wit (1958) and Zhang *et al*. (1998) found that WUE under stress was higher than WUE for plants with adequate soil moisture.

The effect of disease on reducing water potential and osmotic potential was greater in Janz than in Mulgara. Both drought treatments had significant effects on water relations. These results are in agreement with Fischer and Sanchez (1979), Martin and Ruiz-Torres (1992) Siddique *et al*. (2000) and Liang *et al*. (2002). In this study, ψ and its components and RWC were reduced during D2 more than D1 due to higher transpiration rates during anthesis

(Morgan, 1977). Although there were no differences in transpiration rates between diseased and control plants during one week of drought at both tillering and anthesis, osmotic potentials of diseased plants were reduced significantly more than controls at D1. This can be interpreted as a passive reduction in osmotic potential due to declining RWC or active osmotic adjustment by the diseased plants in response to the drought. It could be that the pathogen itself had an effect on the extent of the reduction in osmotic potential. Controls of both cultivars recovered faster after drought and their transpiration rates became higher than diseased ones.

The reduction of ψ was not consistent with reduction of RWC possibly due to difference in time of ψ measurement (between 2 and 5 pm). It has been suggested that RWC provides a more accurate measure of plant water status as it estimates tissue water content and cell turgor (Sinclair and Ludlow, 1985). Leaf water status, as measured by relative water content (RWC) is important for plant metabolism (Sinclair and Ludlow, 1985); it has been proposed as a selection criterion for drought tolerance in many crops such as wheat (Schonfeld *et al*., 1988). This study showed that well watered plants of Mulgara at tillering had significantly lower RWC than Janz with no effect of the fungus. Drought at either tillering or anthesis reduced RWC. However, RWC of diseased plants was higher than those of healthy plants after one week of withholding water at anthesis (D2). It could be that the higher transpiration rate of control leaves was responsible for reducing RWC more than in infected plants.

The grain yield for both cultivars was greatly affected by drought, particularly at later stages of development (reproductive stage). Blum and Pnuel (1990) found that water stress occurring during the later stages of development caused a greater reduction in grains per spike and in the total number of tillers per plant. Blum *et al*. (1990) suggested that maintaining the potential

grain number per spike under stress is more important than tillering ability. Yield response can vary depending on crop sensitivity for that particular growth stage (Zhang *et al*., 2005). The crop is very sensitive to the timing of a water stress period rather than the total reduction of applied water (Ouda *et al*., 2007).

Drought at later stages of development decreases grain set, and loss of male fertility has been associated with accumulation of abscisic acid (ABA) in the spike (Morgan and King, 1984). The greatest effect of drought on grain yield is during meiosis within the developing reproductive organs of wheat plants (Davidson and Birch, 1978). The development of anthers is most susceptible to water stress during meiosis of the pollen mother cells, while ovary development and fertility are largely unaffected (Saini and Aspinall, 1982; Saini *et al*., 1984). Drought during vegetative time has an effect on tiller mortality and reduction of grain number per spike (Fischer and Maurer, 1978). Findings from this study showed that grain weight was affected more than grain number by drought at either tillering or anthesis. Grain weight was reduced 21% by tillering drought and that was considered to be a mild drought. That agrees with the findings of Fischer and Maurer (1978) in which milder drought treatments (less than 50% yield reduction) led to a greater relative decrease in grain weight than in grain number (Fischer and Maurer, 1978). Also, a decrease in grain weight has been attributed to post-anthesis stress that restricted flag leaf photosynthesis and the translocation of assimilate to the spike (Wardlaw *et al*., 1989).

The disease had no effect on grain yield as the inoculum level in the soil was not adequate to reduce grain yield. The results also indicate that Mulgara had higher grain yield, water use and thus WUE than Janz. Mulgara was selected for its capacity for high osmotic adjustment (OA), however, while this was not manifested in the flag leaves sampled the cultivar may have

yielded better because of this trait (Wildermuth and Morgan, 2004). Morgan (1983) and Blum *et al*. (1999) found that there is a positive relation between osmotic adjustment and higher grain yield of wheat under water stress. OA or osmoregulation in response to water stress is considered an important physiological mechanism enabling plants to tolerate water deficits (Morgan, 2000). This response typically occurs at water potentials between 0 and -2 MPa. A leaf can increase its resistance to dehydration through a reduction in cellular osmotic potential by a net accumulation of cellular solutes. Wildermuth and Morgan (2004) concluded that the expression of high osmoregulation with disease resistance is an effective way of improving yield in environments favouring disease development under water stress.

There was a relationship between root pathogen inoculation and lesion percentage. The lesions that are caused by *Pythium* are normally mid to pale brown colour and occur on young roots (Waller, 1979) and those caused by *Rhizoctonia* are brown spear tips (Wallwork, 1996). The infected plants had higher lesion percentage than controls and lesioning of Janz was higher except for *Rhizoctonia*. It could be that the roots of Mulgara are more susceptible to *Rhizoctonia* than Janz. Root tissue is invaded by penetration at intact surfaces and a fungus like *Rhizoctonia* produces enzymes for penetration. The hyphae proliferate within the root cortical cells causing browning and formation of lesions (Kirkegaard *et al*., 1999). Hydrolytic enzymes (Endo and Colt, 1974) and toxic metabolites (Mojdehi *et al*., 1990) can be produced by the fungus in root tissues infected by *Pythium.*

In conclusion, drought developed slowly at the tillering stage because of the large volume of soil in proportion to leaf area (Frank *et al*., 1973) and developed rapidly at anthesis stage (severe drought). The diseases had minor effects on above-ground physiological parameters but had major effects on the root function. The pathogens affected transpiration at tillering but

not at anthesis when the roots had developed further below the inoculation point. There was no significant drought × fungus interaction in this study. Further research is required to investigate the interaction between drought and disease at the whole plant level Janz showed stronger effects of disease than Mulgara, and so was selected as the most suitable cultivar for follow-up experiments. There was little difference between the effects of *Pythium* and *Rhizoctonia*, so only one pathogen needed to be used. *Pythium* was chosen for future work because it was more representative of general root-damaging pathogens. The effect of disease was greatest during tillering drought. Examination of root systems suggested that by anthesis most of the roots were beneath the point of inoculation and so were relatively unaffected by disease. In addition, the anthesis drought was too severe so later experiments were set up so that the pots dried out more slowly. Inoculation close to the seed only affected the early-formed part of the root system so more uniform inoculation would be required to cause significant root damage on older plants. Many effects were suggested by the data but were not significant because of variability between pots. This suggested that experiments should have fewer treatments and more replicates.

Chapter 4: Effect of *Pythium* on water use efficiency and gas-exchange rates of wheat under drought

4.1 Introduction

Many workers have studied the relationships between water relations parameters and other physiological measures after infection with fungal diseases in different plant species (Bowden *et al*. 1990; Bowden and Rouse, 1991; Balota *et al*., 2005). Photosynthetic activity is one physiological process which is a major component of yield. In plants infected by pathogens, there is a reduction in photosynthetic activity which results from a decrease in the photosynthesizing leaf area and a decrease in the process efficiency (Goodman *et al.*, 1986). The slower rate of carbon assimilation in infected plants is due to reduced stomatal conductance which is linked to a decline in leaf water potential (Bowden and Rouse, 1991). Moreover, photosynthetic activity is affected by changes in the water status of the crop. Expansion of leaves and stomatal conductance are reduced rapidly by drought and the photosynthetic process may be affected eventually (Passioura, 1994). The stomata are closed under conditions of water stress which leads to a decrease in CO_2 concentration in the sub-stomatal spaces, and in the mesophyll cells, and, therefore photosynthetic activity is reduced as well (Buchanan *et al.*, 1981; Watson and Wardlaw, 1981). In wheat, stomatal conductance is positively correlated with photosynthesis, but it also affects leaf water potential through changes in transpiration rate (Farquhar and Sharkey, 1982). It was reported that post-anthesis water deficit in wheat causes early senescence, reduces photosynthetic activity and results in decreased grain weight (Palta *et al*., 1994). The effects of fungal root diseases on water use and other physiological parameters of wheat have been investigated under different water

regimes (Martin *et al*., 1986; Balota *et al*., 2005; Amir and Sinclair, 1996). These authors found reductions in water use, water use efficiency (WUE), carbon assimilation rate (A), and grain yields resulting from root diseases.

In this chapter, cv. Janz was used because it showed better response to disease and drought than Mulgara. Large (deep) pots were used which allowed rooting to depths similar to those in the field and to inoculate the plants root to a greater depth. To reduce the severity of drought, large pots were used to provide a larger supply of plant available water and to extend the dry-down period when watering is stopped.This chapter reports on two experiments. The first experiment tested the same hypothesis, but more simply, than that in Chapter 3. This was that infection with *Pythium* will reduce water uptake and water use efficiency.

It was unknown how much *Pythium* was needed for inoculating the plants. Therefore, different inoculum densities were used in the first experiment. In the second experiment, a few modifications were made due to high variability related to the effects of *Pythium*, therefore, more replicates with a single and higher inoculum dose were used. The watering regime was modified, based on experience with the first experiment, so that all pots received exactly the same amount of water during the course of the experiment, as would occur in the field. The specific hypothesis tested in this experiment was that infected plants would have more water available during post-anthesis drought, and that this would compensate for the effects of the disease on growth and yield.

4.2 Material and Methods

Experiment 1: Effect of different inoculum density of *Pythium* on water-use efficiency and yield components of wheat

4.2.1 Preparation of inoculum

Millet seeds (300 g) were soaked in distilled water for 12 hours and then drained. Sterilised glass Petri dishes (each containing 30g millet seeds) were wrapped with aluminum foil and autoclaved on two consecutive days. Each plate was inoculated with three plugs of *Pythium irregulare* grown previously on Potato Dextrose Agar (PDA) media. One ml of penicillin G (0.01 g per 20 ml of sterilised distilled water) was added to each plate after filtering with a MILLEX®GS (0.22 µm pore size) filter, to inhibit bacterial growth. All the plates were kept in an incubator for 7-10 days at 25 ^{0}C in the dark and then dried for 30 minutes under a laminar flow.

4.2.2 Soil preparation and soil inoculation with Pythium

Pots were made from PVC pipe, (15 cm in diameter X 100 cm in height) and there were drainage holes at the bottom. Pots were placed in a glasshouse bay which was set with an average maximum temperature of 25^{0}C and an average minimum of 18^{0}C on a 12/12 hour (d/n) cycle. Natural light was used with 14 hours of daylight at the start of the experiment, and decreasing to 12 hours at harvest. The average relative humidity (RH) was approximately 60%. Five levels of *Pythium* inoculum were used (0, 0.1, 0.5, 2 and 5 g/pot) and mixed well into the top 20 cm of soil (sandy loam:peat (3:1 V/V)). Soil pH was adjusted to 6.4 with agricultural lime and Granular N:P:S (14.3:12:10.5) Starter 15 fertiliser was applied to the

soil mixture at a rate of 13 g m^{-2}. Six replicates were used for each inoculum density treatment. The original weight of the soil was about 16 kg for each pot or replicate before watering. Pots were watered to field capacity with 6 l of water before sowing. Three surface sterilised wheat seeds cv. Janz were sown in each pot on 18 December 2009, and later thinned to two plants at the two-leaf stage, GS 12 on the Zadoks scale (Zadoks *et al*., 1974). Plastic beads (200 g) were placed on the soil surface to a depth of 2 cm to reduce soil evaporation.

4.2.3 Water regime

Pots were given excess water and allowed to drain. The amount of water evaporated was monitored by weighing six unplanted pots placed between planted pots. The amount of water transpired was determined by subtracting the weights of the unplanted pots from the weights of the planted pots. Pots were watered every 2-3 days. The amount of water added to each pot was about 200 ml at seedling stage, 700-1000 ml between tillering and booting stage, and 1500-2500 ml from booting to the beginning of the anthesis stage (the period where all pots were droughted until harvest).

Droughts were imposed by withholding watering from full anthesis stage (8 February 2010) until harvest on 15 March 2010. Counts were made of heads and tillers and grain and shoot mass was also determined. Harvest index was calculated using grain weight and shoot dry weight, along with water use efficiency on both a grain weight and total shoot dry weight basis.

The experimental design was a completely randomised design. The pots were not arranged within a block. Treatment effects were analysed by ANOVA.

Experiment 2: Effect of Pythium on water relations and other physiological parameters

4.2.4 Soil preparation and treatments

The inoculum of *Pythium* was prepared in a similar procedure to the first experiment. In this experiment, 12 pots were prepared with *Pythium* inoculum and 12 pots for controls. Each pot was filled with 16 kg of soil. The same type of soil and pots, and growing conditions were used as in the first experiment. Daylength was 10.5 hours at the start of the experiment and 11 hours at harvest. Ten grams of *Pythium* inoculum was mixed into the soil to a depth of 30 cm and another 10 g of autoclaved millet seeds were added to uninfected (control) pots. About 6 l (depending on how much water the pots needed to get drainage from the bottom) of water was added to each pot to bring them to field capacity just before sowing. Three sterilised wheat seeds (cv. Janz) were sown in each pot on 1 May 2010. All the pots were thinned to two plants at the two-leaf stage, GS 12 on the Zadoks scale (Zadoks *et al*., 1974), (12 May 2010) and 200 g of plastic beds were added on the surface to reduce soil evaporation. The starting weight of all pots was 23.30 kg.

The evapotranspiration of all pots was measured regularly during the experiment every 2-3 days. Pots were waterd according to the amount of water lost by the treatrment with lowest mean ET, which was the inoculated treatment. This ensured that both treatments were supplied with the same volume of available water during the experiment, as would occur in the field. The amount of water added to each pot was between 100 ml at the two-leaf stage, GS 12 on the Zadoks scale (Zadoks *et al*., 1974) and 750 ml at late anthesis, GS 69 on the Zadoks scale, before the beginning of the water stress period. The amount of water added in this experiment was much less than that added in the first experiment.

Drought was imposed by completely withholding watering from the full anthesis stage, GS 69 on the Zadoks scale (7 July 2010) until maturity or harvest on 18 August 2010 (approximately six weeks). The plants were harvested at maturity (about 14 weeks after sowing) and the above-ground plant biomass, grain yield, and number of heads and tillers determined.

4.2.5 Measurements

Water potential

Water potential (Ψ) was measured once a week for the four week drought period, at two times. Pre-dawn Ψ was measured at 6.30 am and midday Ψ between 11 am and 12 pm, using a Soil Moisture Equipment Corporation Scholander type pressure chamber. Two flag leaves were sampled (one flag leaf for predawn and the other for midday Ψ) from each of three replicates of either treatment (controls and *Pythium*). The leaves were wrapped with aluminum foil in the glasshouse and returned to the laboratory for measurement of water potential. Although destructive sampling could affect growth, each pot was only sampled once and leves were removed from only one tiller of each plant.

Physiological measurements

Photosynthesis (carbon assimilation), stomatal conductance, internal carbon dioxide concentration and transpiration rate were measured using a portable photosynthesis system (LICOR-6400XT). Measurements were taken at the same time as the weekly pressure chamber readings from another plant in the same pot. The measurements were taken between 10:30 am and 11:30 am on days with full sun, except during the last week of drought when there was full cloud cover.

The experimental design in this experiment was completely randomised. Treatments were compared by t-tests.

4.3 Results

Experiment 1: Effect of different inoculum density of Pythium on yield and water use efficiency

4.3.1 Cumulative transpiration

There was no significant effect of inoculum density of *Pythium* on cumulative transpiration (CT) when compared with controls (Figure 4.1) from tillering until harvest. Water was withheld from the full anthesis stage, stage 69 on the Zadoks scale (Zadoks *et al*., 1974), (9 February 2010) until harvest or full maturity (15 March 2010). The rate of increase in CT for all treatments was reduced during the drought period.

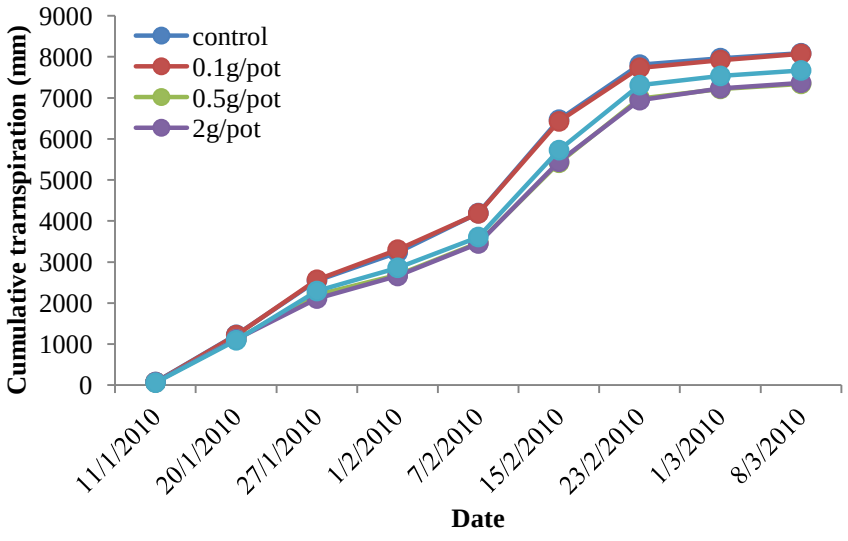

Figure 4.1 Effect of inoculum density of *Pythium* on cumulative transpiration for wheat cv. Janz from tillering (11 January) until harvest (15 March). Drought was imposed from full anthesis stage (8 February 2010) until harvest on 15 March 2010. Values are means of 6 replicates.

4.3.2 Transpiration per week

There was no overall significant effect of inoculum density on transpiration per week (TPW) from tillering until maturity, except in the weeks ending 27 January and 23 February (Figure 4.2). Controls and 0.1 g treatments had significantly higher transpiration ($P < 0.05$) than higher inoculum densities on 27 January, probably because the roots of infected plants were damaged by *Pythium* which caused a reduction in water use. However, controls and 0.1 g treatments had significantly lower transpiration ($P < 0.05$) than other inoculum densities in the week ending 23 February, presumably because they had depleted water more rapidly in the early stage of drought.

Transpiration per week followed the same trend for all the treatments with fluctuations of TPW during different growth stages. The weekly water use ranged from approximately 50 to 1000 ml/week before anthesis. After anthesis, TPW decreased as the plants were increasingly affected by water stress. The TPW reached a minimum (120-130 ml/week) when the plants were mature (i.e. 8 March 2010) and had exhausted all the soil water.

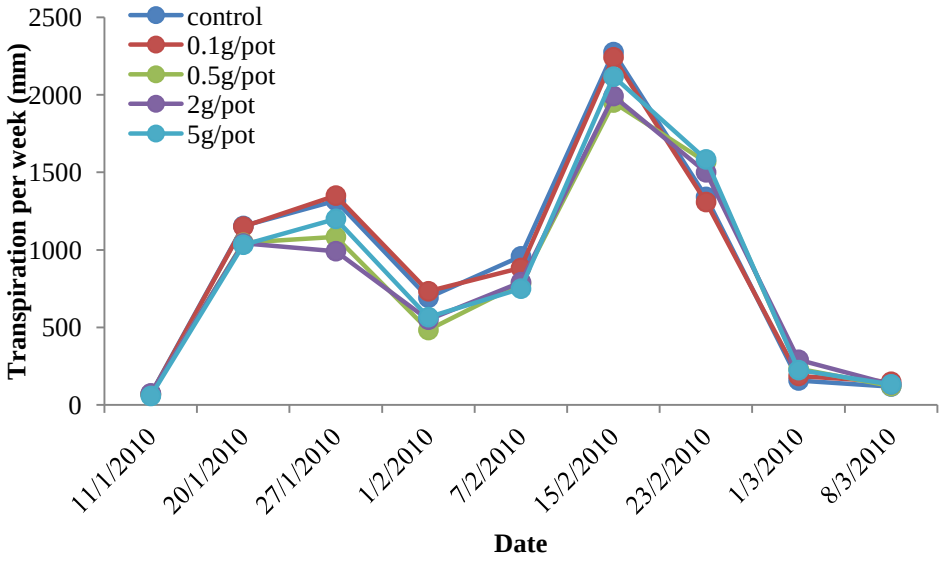

Figure 4.2 Effect of inoculum density of *Pythium* on weekly transpiration rate of wheat cv. Janz from tillering (11 January) until harvest (15 March).Drought was imposed from full anthesis stage (8 February 2010) until harvest on 15 March 2010. Values are means of 6 replicates.

4.3.3 Yield components

The effect of inoculum density (ID) of *Pythium* on yield components of wheat cv. Janz under drought is presented in Table 4.1. There was no significant effect of inoculum density on yield components. Harvest index ranged from 0.34 to 0.37.

Table 4.1 Number of tillers per pot (TN/pot), number of heads per pot (HN/pot), grain weight (GW), dry matter weight (DMW), and harvest index (HI) of wheat cv. Janz wheat following inoculation with different levels of inoculum of *Pythium* (g/pot) (ID). Values are means of 6 replicates.

ID (g/pot)	TN/pot	HN/pot	GW (g)	DMW (g)	HI
Control	26.5	26.3	12.0	20.8	0.36
0.1	27.0	26.4	12.0	22.6	0.34
0.5	24.6	24.6	12.0	20.3	0.37
2.0	25.6	25.2	11.0	19.1	0.36
5.0	27.5	27.0	11.7	20.6	0.36
Level of significance	n.s	n.s	n.s	n.s	n.s

4.3.4 Water use efficiency

The inoculum density of *Pythium* had no significant effect on water use efficiency (WUE) whether calculated on a grain weight or shoot dry weight basis (Figure 4.3).

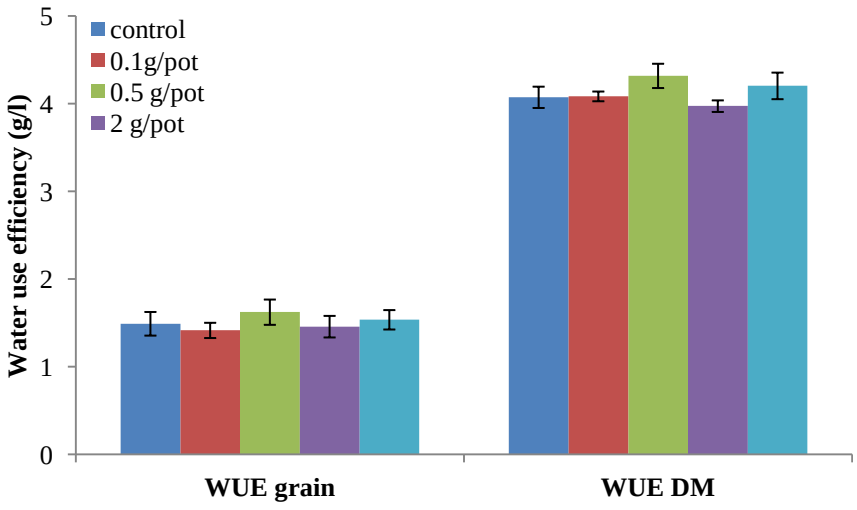

Figure 4.3 Effect of inoculum density of *Pythium* on WUE_{grain} and WUE_{DM}. Values are means ± s.e., n=6.

Experiment 2: Effect of *Pythium* on water relations and other photosynthetic parameters

4.3.5 Cumulative transpiration

The results in Table 4.2 and Figure 4.4 show the effect of an inoculum density of 10 g/pot on cumulative transpiration (CT) of cv. Janz from the three-leaf stage until maturity. There were significant differences ($P < 0.01$) in CT between controls and *Pythium* with CT of controls being higher (4809 mm) than those infected by *Pythium* (4117 mm). The greatest difference in CT between controls and *Pythium* was 1171 mm on 22 July, during grain filling, and under water stress. During the period of water stress from anthesis until harvest, there was no significant difference between treatments in the amount of water transpired. At the conclusion of the experiment, control pots weighed approximately 600 g (equivalent to 600 ml water) less than inoculated pots.

Table 4.2 Cumulative transpiration of wheat cv. Janz for controls and *Pythium* at three growth stages.

Growth Stages	Date	Cumulative Transpiration (mm)		Significance
		Control	*Pythium*	
Three leaf	17 May 2010	17	5	ns
Late anthesis	7 July 2010	2907	2128	0.01
Harvest	4 August 2010	4809	4117	0.0001
Anthesis to harvest		1902	1989	ns

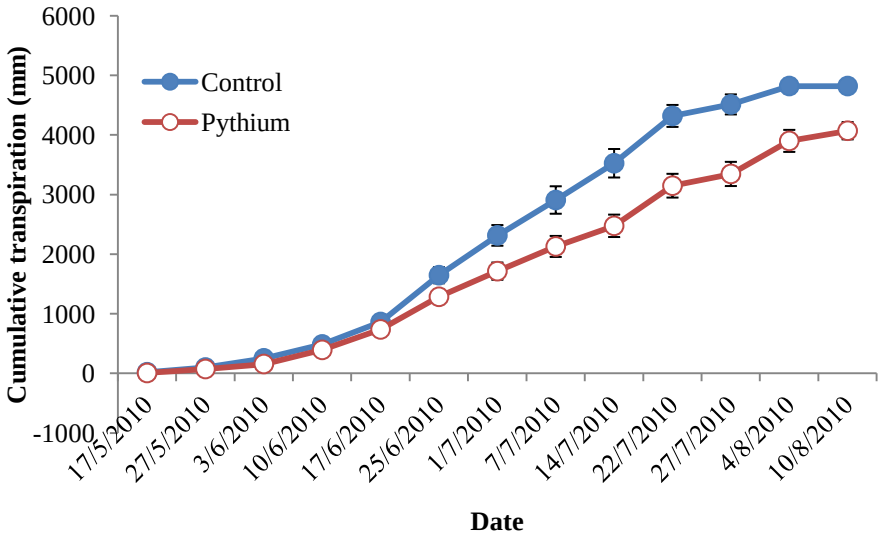

Figure 4.4 Effect of 10 g/pot of *Pythium* on cumulative transpiration of wheat cv. Janz from three-leaf stage (17 May) until harvest (14 August). Anthesis started on 25 June. Water was withheld at late anthesis from 7 July 2010. Values are means ± s.e., n=12.

4.3.6 Transpiration per week

There was no significant difference between treatments in the transpiration per week (TPW) during vegetative growth from 17 May until 17 June 2010 (Figure 4.5). However, TPW was significantly higher ($P < 0.01$) for controls than *Pythium* during reproductive growth from 25 June until 14 July, except on 7 July where $P = 0.056$. There were no significant differences in TPW between controls and *Pythium* from 22 to 27 July. At the late stage of growth, plants inoculated with *Pythium* had significantly higher TPW than controls on 4 August ($P < 0.01$) and on 10 August ($P < 0.05$).

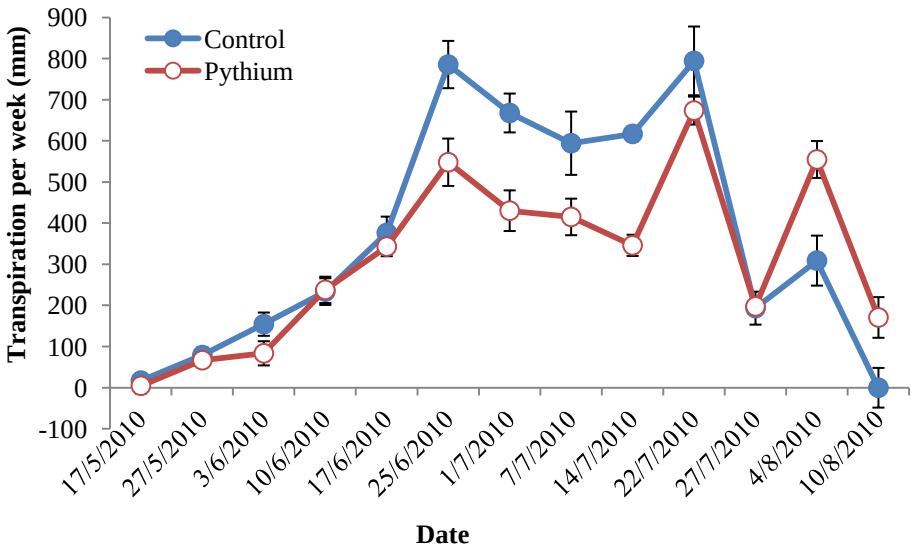

Figure 4.5 Effect of a 10 g/pot inoculum density of *Pythium* on transpiration per week of wheat cv. Janz from the three-leaf stage (17 May) until harvest (14 August). Values are means ± s.e., n=12.

4.3.7 Yield components

The effect of 10 g of *Pythium* inoculum on yield components of cv. Janz under drought is presented in Table 4.3. As for the first experiment, there were no significant differences

between controls and *Pythium* for yield components. In some pots of infected plants there were still some small, green heads on some tillers at the time of harvest. Yield was higher in the *Pythium* treatment, and it was only the very low yield of one replicate that stopped this being significant.

In this experiment the number of heads per pot (13 heads) was reduced 50% compared with the first experiment (26 heads) under all treatments. There were no differences in grain weight for both experiments but shoot biomass in the first experiment was twice as high as in this experiment. These differences improved harvest index (HI) of cv. Janz in this experiment (HI = 0.5) when compared with the first experiment (HI = 0.36).

Table 4.3 The number of heads per pot (HN/pot), grain weight (GW), dry matter weight (DMW), and harvest index (HI) for wheat cv. Janz after inoculation with 10 g of *Pythium* per pot.

Treatments	HN/pot	GW (g)	DMW (g)	HI
Control	12.7	10.3	10.3	0.50
Pythium	12.5	11.2	10.2	0.52
Level of significance	ns	ns	ns	ns

4.3.8 Water use efficiency (WUE)

Plants inoculated with 10 g of *Pythium*/pot had significantly higher WUE ($P < 0.01$) than controls, when based on grain yield, and significantly higher WUE ($P < 0.05$), when based on dry matter (shoot plus grain) (Figure 4.6). WUE_{grain} for controls and *Pythium* was 2.15 and 2.7 g/l, respectively, while, WUE_{DM} of controls was 4.3 g/l and *Pythium* 5.2 g/l.

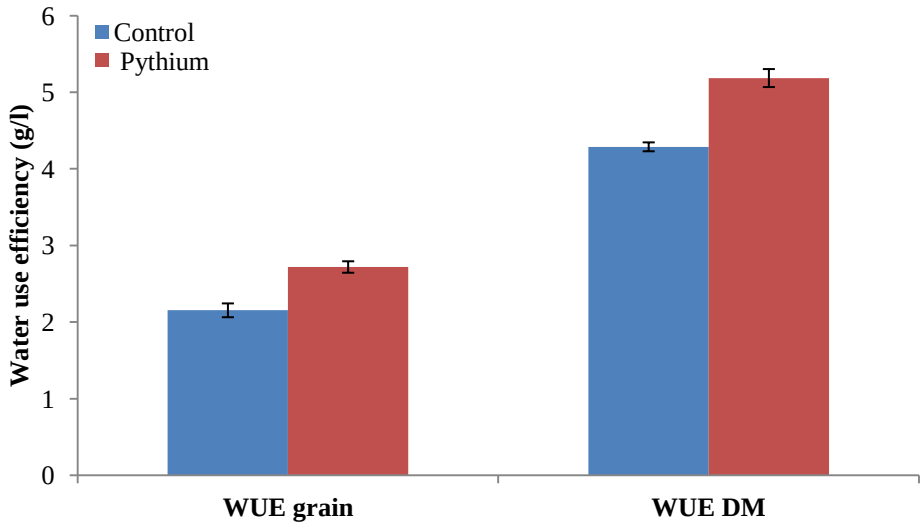

Figure 4.6 Effect of 10g/ pot of *Pythium* on water use efficiency of cv. Janz based on both grain yield (WUE $_{grain}$) and shoot dry matter (WUE $_{DM}$). Values are means ± s.e., n=12.

4.3.9 Plant water relations

The results of predawn water potential (Ψ) (Figure 4.7) showed no significant differences between controls and *Pythium* during 4 weeks of water stress. At the beginning of the drought period (day 0), both controls and *Pythium* had the same predawn Ψ (-0.72 MPa). Predawn water potential was lower at 21 days of stress than earlier stages. It appeared to be lower in the *Pythium* treatment than the control at 21 days but the difference was not significant.

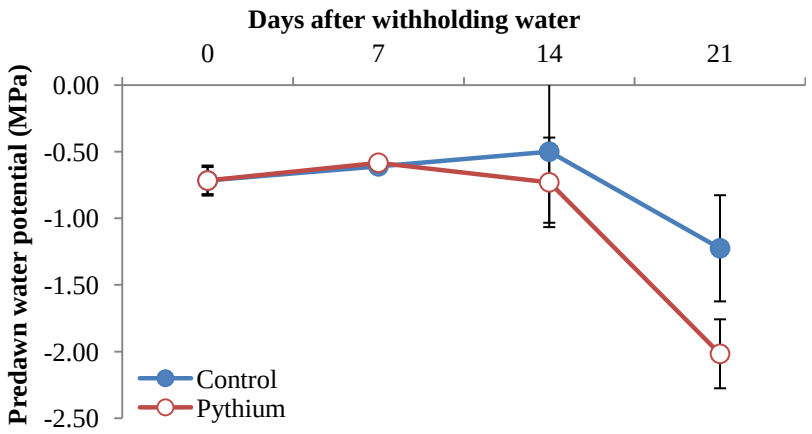

Figure 4.7 Predawn water potential (Ψ) for wheat cv. Janz for both controls and *Pythium* at 0, 7, 14 and 21 days after withholding water. Values are means ± s.e., n=3.

There was no significant difference in midday water potential (Ψ) between both treatments at 0 and 7 days after drought but the difference was significant at 14 and 21 days after withholding water (Figure 4.8). After 14 and 21 days of drought, controls had significantly higher Ψ ($P < 0.05$) than *Pythium*. Midday Ψ of controls at 14 and 21 days after drought was -1.19 MPa and -1.67 MPa, respectively and those infected by *Pythium* -1.69 MPa and -2.43 MPa, respectively. Water potential of plants at the end of drought was lower than at the beginning of stress. In general, the average of predawn Ψ (-1.0 MPa) was higher than midday Ψ (-1.6 MPa) for both treatments and during all the days of measurement.

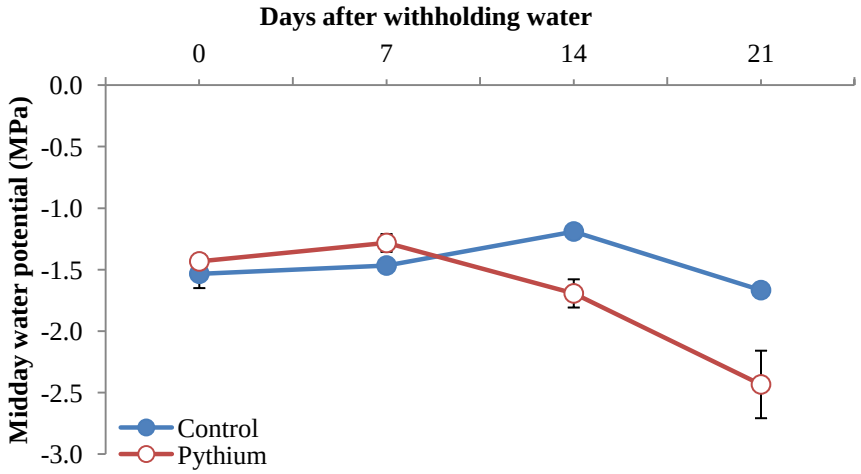

Figure 4.8 Midday water potential (MPa) for cv. Janz for both controls and *Pythium* at 0, 7, 14 and 21 days after withholding water. Values are means ± s.e., n=3.

The relationship between predawn and midday-Ψ is presented in Figure 4.9. Midday water potential was significantly correlated ($P < 0.01$) with predawn water potential in the *Pythium* treatment (Figure 4.9b). However, midday water potential was not significantly correlated with predawn water potential in the control treatments (Figure 4.9a). This suggests that control plants were better able to maintain water potential within a narrow range.

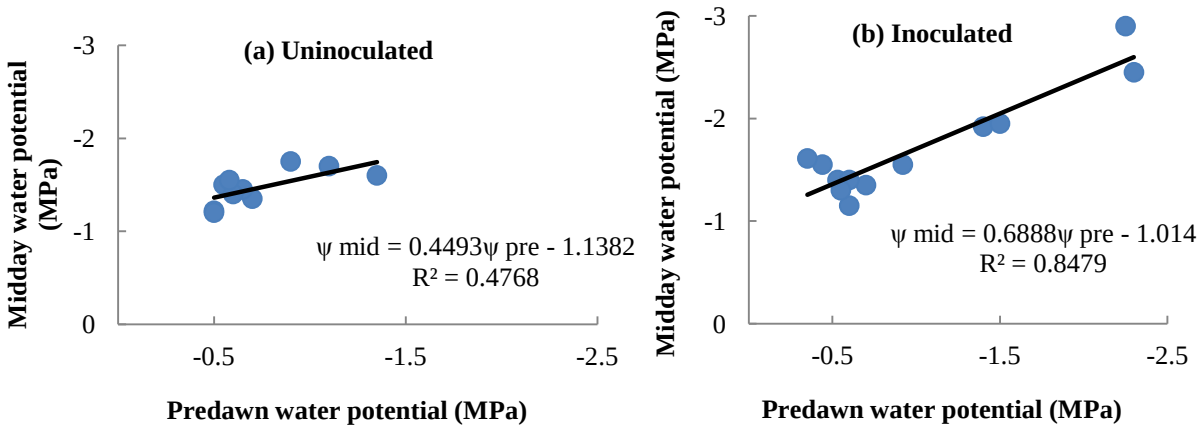

Figure 4.9 Relationships between predawn and midday water potentials for (a) uninoculated and (b) inoculated plants during 21 days of withholding water.

4.3.10 Physiological measurements

Pythium inoculation only had a significant effect on photosynthetic rate (A) ($P < 0.01$) at the beginning of the drought period (day 0 after withholding water). The photosynthetic rate of controls (Figure 4.10) was significantly higher (21.65 µmol CO_2 $m^{-2}s^{-1}$) than *Pythium* (13.62 µmol CO_2 $m^{-2}s^{-1}$). However, there was no significant effect of disease on photosynthetic rate at 7, 14 and 21 days of drought. The photosynthetic rate decreased as the drought period lengthened and reached a minimum (<0 µmol CO_2 $m^{-2}s^{-1}$) after three weeks of stress as the measurement was taken under full cloud cover.

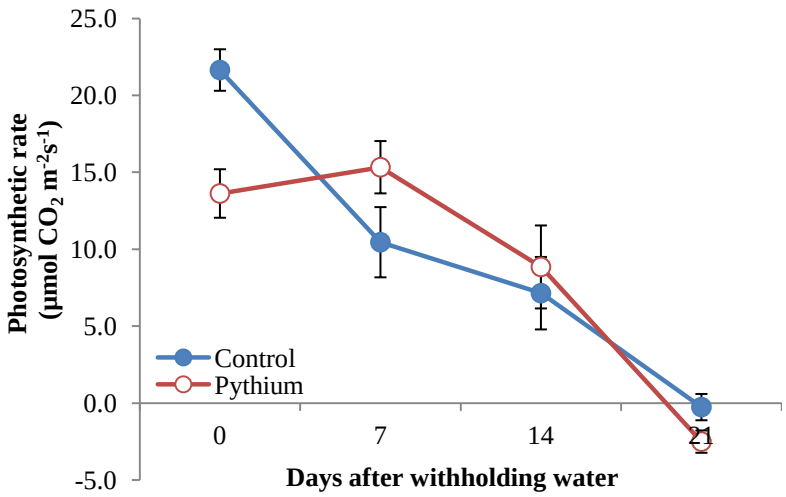

Figure 4.10 Photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$) of controls and *Pythium* of cv. Janz after 0, 7, 14 and 21 days of withholding water. Values are means ± s.e., n=6.

There was no significant effect of disease on stomatal conductance (g_s) at all days of water stress period except 7 days after the drought began, where plants inoculated with *Pythium* had significantly higher g_s ($P < 0.05$) than controls (Figure 4.11). Stomatal conductance was decreased for both treatments after 7 days of drought, as compared with the beginning of stress. The lowest stomatal conductance was 0.03 mol H_2O m^{-2} s^{-1} for *Pythium* inoculated plants and 0.05 mol H_2O m^{-2} s^{-1} for controls at the end of the drought period. Both photosynthetic rate and stomatal conductance exhibited the same trend; as drought length increased, these parameters decreased.

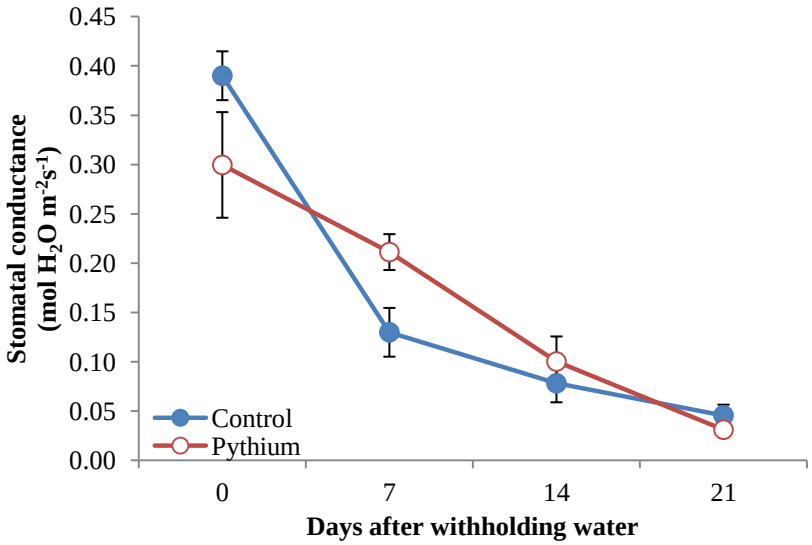

Figure 4.11 Stomatal conductance (mol H_2O m^{-2} s^{-1}) of controls and *Pythium* of wheat cv.Janz after 0, 7, 14 and 21 days of withholding water. Values are means ± s.e., n = 6.

Pythium treatment had no significant effect on intercellular CO_2 concentration $[CO_2]_i$ during the drought period (Figure 4.12). $[CO_2]_i$ ranged from 252 to 279 µmol CO_2 mol^{-1} in controls and from 247 to 306 µmol CO_2 mol^{-1} in *Pythium*-inoculated plants after two weeks of water stress. However, $[CO2]_i$ at the end of the drought period (21 day) increased above ambient levels, presumably due to respiration.

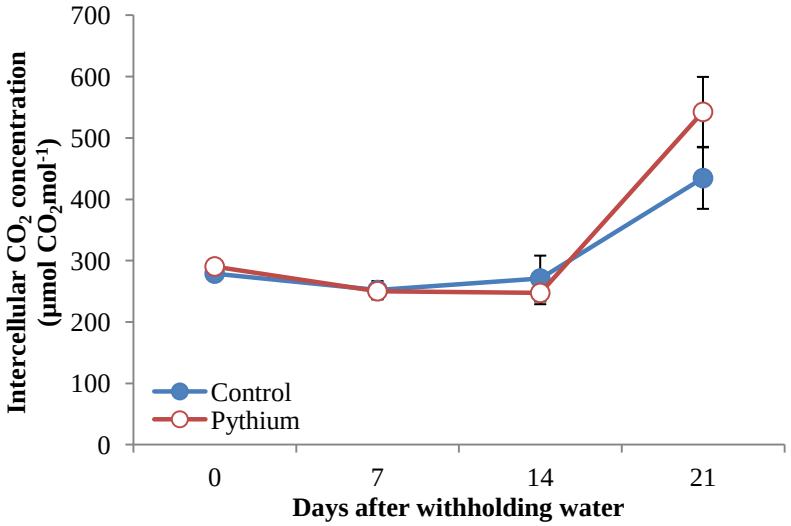

Figure 4.12 Intercellular CO_2 concentration (µmol CO_2 mol^{-1}) of controls and *Pythium* of cv.Janz after 0, 7, 14 and 21 days of water withholding. Values are means ± s.e., n =6

Instantaneous transpiration rate (E) did not differ significantly between both treatments during water stress except after 7 days of drought, when the transpiration rate of *Pythium* (5.9 mmol H_2O m^{-2}s^{-1}) was significantly higher ($P < 0.01$) than controls (3.8 mmol H_2O m^{-2}s^{-1}) (Figure 4.13). Transpiration rate for both treatments was reduced as drought period lengthened.

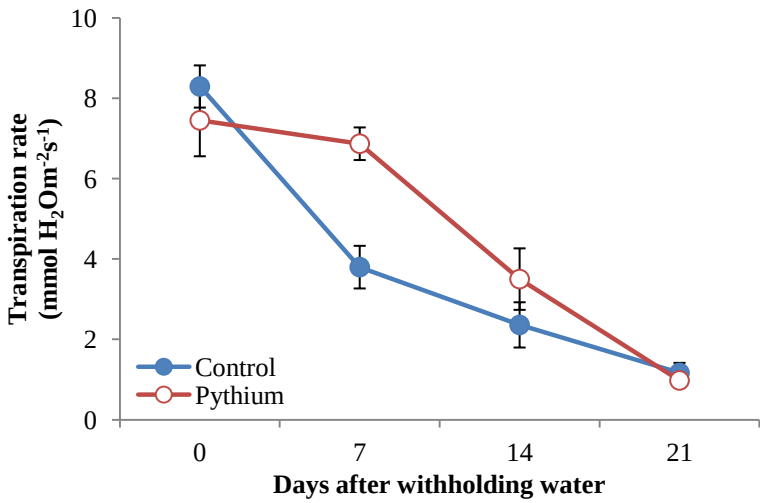

Figure 4.13 Transpiration rate (mmol H_2O m^{-2} s^{-1}) of controls and *Pythium* of cv. Janz after 0, 7, 14 and 21 days of withholding water. Values are mean ± s.e., n =6.

Instantaneous water use efficiency (A/E) did not differ significantly between *Pythium* inoculated plants and controls during stress, except at day 0 of the drought, when control plants had significantly higher values ($P < 0.01$) than *Pythium* (Figure 4.14). Instantaneous WUE varied from 2.2 to 2.6 mmol CO_2/mol H_2O for controls, and from 1.3 to 2.2 mmol CO_2/mol H_2O for *Pythium* inoculated plants. However, A/E was reduced at the end of the drought period to less than 0 mmol CO_2/mol H_2O for both treatments depending on the degree of reduction in carbon assimilation to negative values.

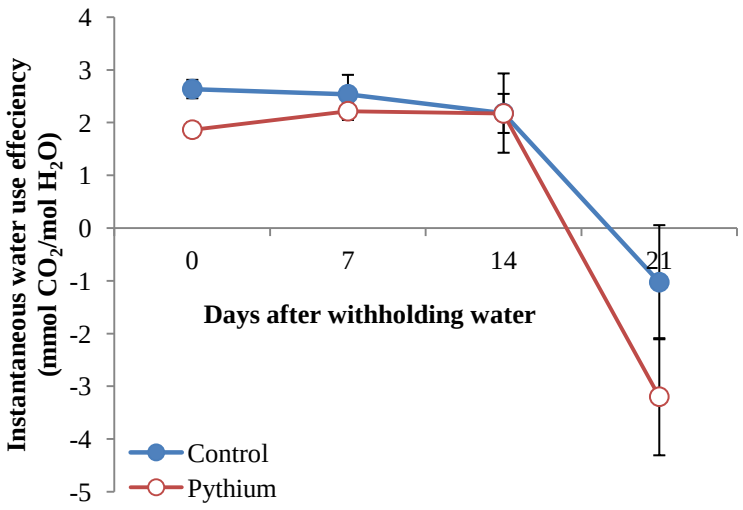

Figure 4.14 Instantaneous water use efficiency (mmol CO_2/mol H_2O) of controls and *Pythium* of cv. Janz after 0, 7, 14 and 21 days of withholding water. Values are mean ± s.e., n = 6.

The relationships between photosynthesis or carbon assimilation (A) and transpiration rate (E) (Figure 4.15) and stomatal conductance (g_s) (Figure 4.16) are presented for both inoculated and uninoculated plants of cv. Janz during 4 weeks of water stress. In both treatments, as E increased, A also increased. Similarly, A increased as g_s increased. At the end of the drought, A was more reduced than g_s for both controls and *Pythium*. The effect of inoculation on the relationships between photosynthesis and transpiration rate was tested using multiple regression. Photosynthetic rate was regressed against ln(transpiration rate) and ln(transpiration rate) × inoculation. A similar analysis was done for stomatal conductance. A significant interaction term indicates that the relationship differs between the two treatments. Regression tables are presented in Appendix 1. Inoculation had a significant ($P < 0.01$) effect on the relationship between photosynthesis and transpiration rate, but not on the relationship between photosynthesis and stomatal conductance. Photosynthetic rate was higher for a given E in controls than in the *Pythium* treatment.

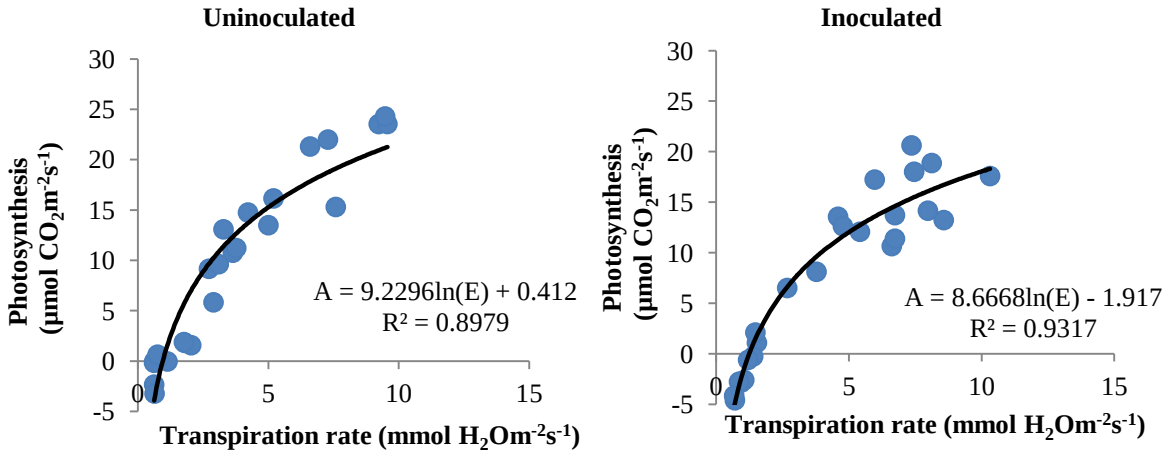

Figure 4.15 The relationship between photosynthesis (µmol CO_2 $m^{-2}s^{-1}$) and transpiration rate (mmol H_2O $m^{-2}s^{-1}$) as affected by *Pythium* inoculation for wheat cv. Janz during the 21 day water withholding period after anthesis. R^2 are significant at $P < 0.01$.

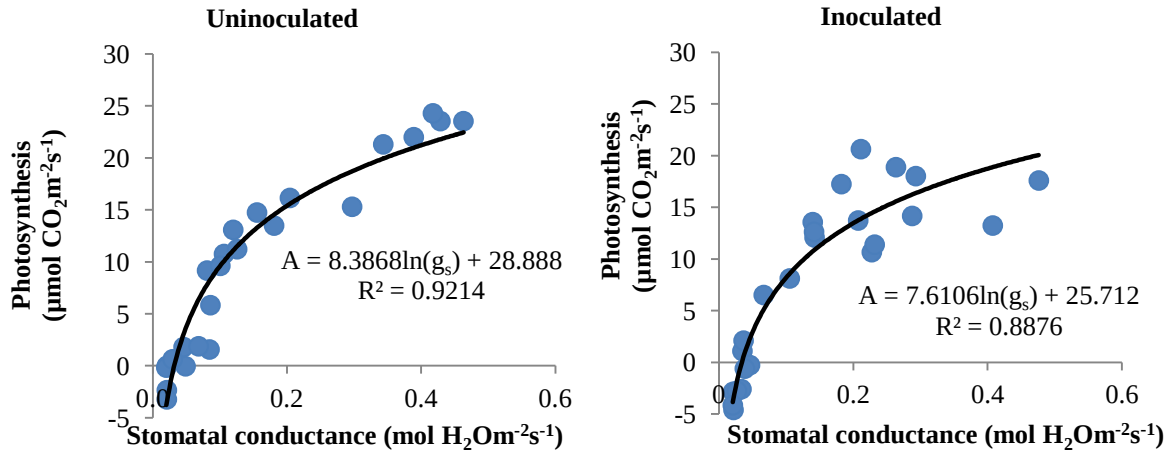

Figure 4.16 The relationship between photosynthesis (µmol CO_2 $m^{-2}s^{-1}$) and stomatal conductance (mol H_2O m^{-2} s^{-1}) as affected by *Pythium* inoculation for cv. Janz during the 21 day water withholding period after anthesis. R^2 are significant at $P < 0.01$

There was a significant relationship between midday leaf water potential and stomatal conductance in the *Pythium* treatment but not in controls (Figure 4.17). Stomatal conductance varied over a wide range of values in controls, while middaywater potential remained in a

narrow range. In inoculate plants, stomatal conductance decreased as midday water potential decreased.

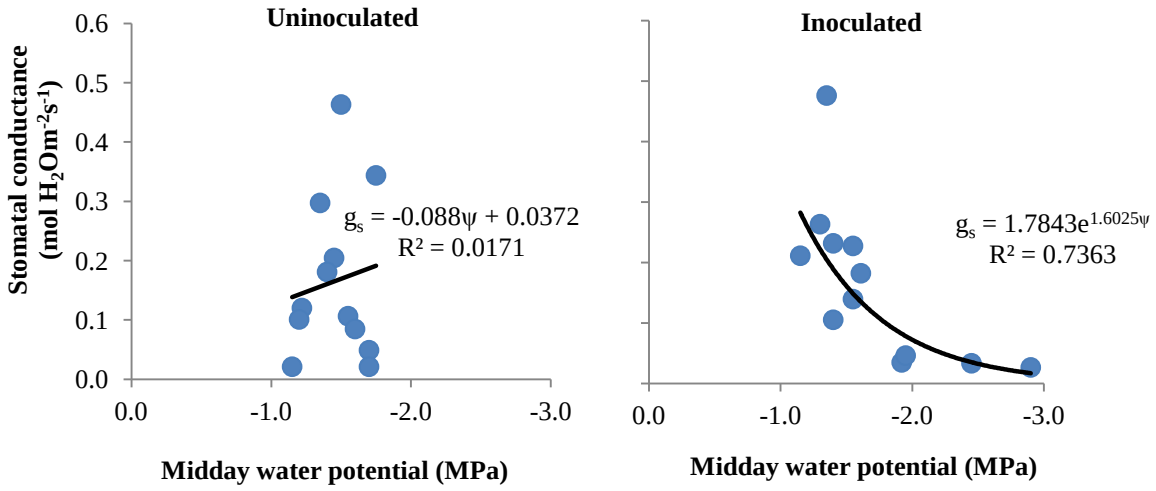

Figure 4.17 Stomatal conductance (mol H_2O m^{-2} s^{-1}) for uninoculated and inoculated plants in relation to midday leaf water potential (MPa) during the 21 day water withholding period after anthesis.

The relationships between intrinsic and instaneous WUE stomatal conductance are showin Figures 4. 18 and 4.19. WUE was negative at very small values of g_s so g_s of 0.1 was chosen as the point at which stomata were sufficiently open to allow normal photosynthesis. There was a significant decrease in intrinsic water use efficiency, or the ratio of net photosynthesis rate to stomatal conductance (A/g_s) as g_s increased and A/g_s decreased significantly ($P < 0.01$) faster in inoculated plants than uninoculated plants (Figure 4.18). There was no significant relationship between instantaneous WUE and g_s (Figure 4.19).

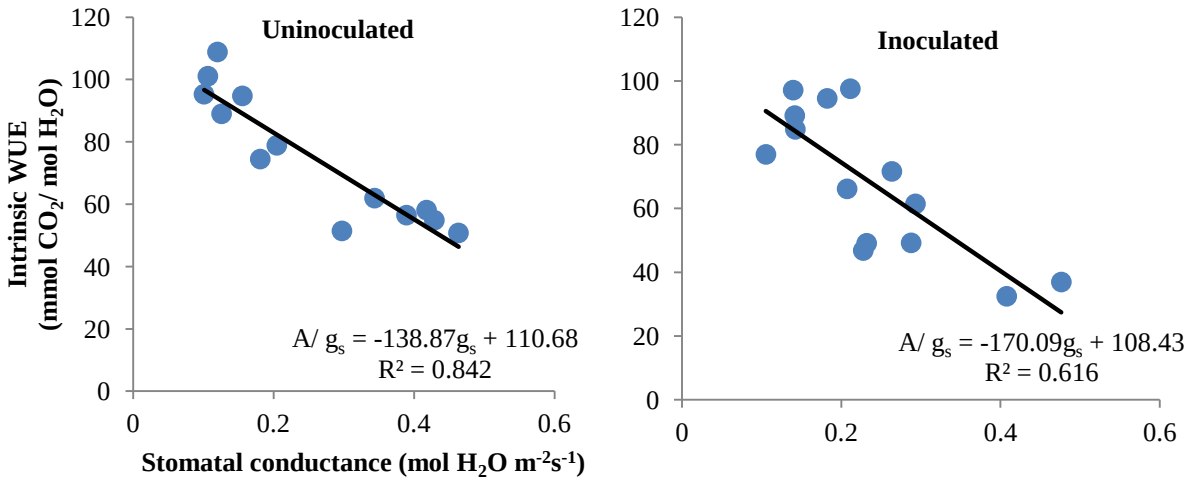

Figure 4.18 The relationship between water use efficiency $_{intrinsic}$ (A/g_s) and stomatal conductance (g_s) for uninoculated and inoculated plants during the 21 day water withholding period after anthesis.

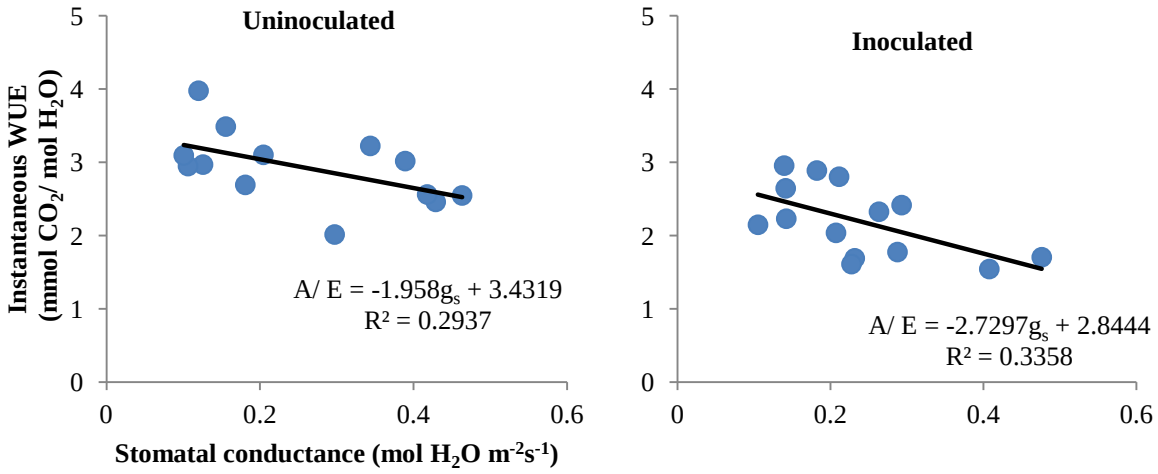

Figure 4.19 The relationship between water use efficiency $_{instantaneous}$ (A/E) and stomatal conductance (g_s) for uninoculated and inoculated plants during the 21 day water withholding period after anthesis.

4.4 Discussion

The results of the first experiment suggested that inoculum densities of *Pythium* up to five grams per pot had no significant effect on total water use under drought conditions from anthesis to harvest although there was a reduction of 6% of cumulative transpiration in all plants infected by *Pythium* when compared with uninfected ones. The different inoculum levels had only a small effect in reducing transpiration prior to anthesis. However, pots infected at the highest levels had the highest transpiration levels mid way through the drought. This could be because they did not deplete water as fast early in the drought. In the second experiment, the cumulative water use of Janz wheat was reduced significantly when infected at higher inoculum density of *Pythium* (10 g/pot). There was a reduction of 14% of total water use in the *Pythium* treatment when compared with controls. There was a clear reduction in transpiration during early growth so that the infected plants entered the drought with significantly more water available. As a consequence, yield was not significantly affected and water use efficiency went up. However, the infected plants were not able to make use of all of the extra water available after anthesis. Generally, infected and uninfected plants in the first experiment transpired approximately twice as much water as in the second experiment at the end of growing season due to a larger amount of water added in the first experiment.

Ploetz and Schaffer (1989) found a reduction in evapotranspiration of avocado as a response to Phytophthora root rot and flooding. The decrease of transpiration could result from pathogen-induced stomatal closure, reductions in air space due to growth of hyphae or decrease in the area of conducting tissue and number of stoma (Duniway and Durbin, 1971; Spotts and Ferree, 1979). These responses relate mostly to leaf diseases, however, reduction in

root mass and blockage of xylem are possibilities for *Pythium*. Therefore, the effect of root diseases on transpiration may indirectly affect photosynthetic productivity.

Grain yield and water use efficiency (WUE) did not change at four levels of *Pythium* inoculum in the first experiment. The potential number of grains is affected by environmental conditions before anthesis but grain set is affected mostly by water stress and other factors at anthesis (Fischer, 1973). Consequent grain growth is sustained largely by current photosynthesis. In addition, the harvest index (HI) was not influenced at all levels of disease and that indicates both total shoot biomass and grain biomass were not affected in either experiment by *Pythium*. Both total DM and grain were lower in Experiment 2. But DM was affected much more by restricting water availability. In experiment 1, the plants would have grown larger before anthesis because more water was available, but grain depends mostly on water available after anthesis. Harvest index was improved in the second experiment as the proportion of grain yield to total above-ground biomass was increased.

These results indicate that under these experimental conditions, wheat plants (cv. Janz) can tolerate low levels (0.1, 0.5, 2 and 5 g/pot) of *Pythium* inoculum. These findings also show that the productivity and yield of wheat under drought from anthesis until maturity was not affected when these inoculum densities were mixed with 3 kg of soil (top 20 cm). The aim of this work was primarily to assess the effect of inoculum densities of *Pythium* on WUE of Janz wheat under drought conditions. However, the results further demonstrate that WUE did not change when inoculum densities of *Pythium* increased up to 50 times per pot (i.e. from 0.1 to 5g/pot). It is possible that the inoculum densities of *Pythium* used in this experiment did not cause a difference from uninfected plants for two reasons: (1) the ratio of *Pythium* inoculum to soil was very low and (2) there was no correlation between infection and soil populations of

oospores (sexual spores) or sporangia (asexual structures). Therefore, the effect of *Pythium* on water use, grain yield and WUE was very minor and inoculum levels may have been insufficient to cause significant effects. In previous work on the effect of inoculum densities of *Pythium* on the growth of barley seedlings, Bratoloveanu and Wallace (1985) found that as inoculum density increased (from 0.5 to 8 g/100 g soil), the dry weight of roots and shoot decreased. In the second experiment, increasing the inoculum density of *Pythium* to 10 g/pot significantly improved WUE under drought conditions because the reduction of water use corresponded with no change of grain yield. It could be that increasing inoculum and infestation of the soil profile down to 30 cm in the second experiment enhanced lesioning of the roots at anthesis which lead to reduced water uptake by infected roots.

There were no significant differences on predawn leaf water potential between controls and diseased plants. However, *Pythium* had a significant effect on midday water potential after 14 and 21 days of water stress. Bowden *et al*. (1990) found that the fungal disease *V. dahliae* reduced leaf water potential in potato. *V. dahliae* blocks the xylem and the symptoms are expressed as plant drought stress.

These results also indicate that increasing drought combined with infected roots significantly reduced midday water potential of diseased plants. Therefore, the effect of *Pythium* under increasing water stress on leaf water potential was greater during midday measurement than predawn. Predawn water potential may reflect the re-establishment of the equilibrium between plant water and soil water during the night (Davis and Mooney, 1986). However, predawn leaf water potential may not reflect soil-water potential (Donovan *et al*., 2001) even under well-watered conditions. Generally in this study, the difference between controls and *Pythium* in terms of water relations was minor. In contrast, Dawson and Weste (1984) found large and

significant differences in water relations between controls and *Eucalyptus* species infected by *Phytophthora cinnamomi*.

There were differences in predawn and midday Ψ between well watered (0 day after stress) and stressed (21 day after water withholding) treatments. However, predawn Ψ of different plant species will come into equilibrium with the wettest portion of the soil in the plant's root zone (Ameglio *et al*., 1999). Thus, the response of plants to soil moisture at midday may differ from that at predawn due to the flux of water occurring while the plant is actively transpiring (Stevens *et al*., 1995).

The values of midday Ψ were always lower than predawn Ψ may be due to predawn water potential being less subject to evaporative conditions than midday water potential except at the end of the drought period where both controls and *Pythium* had almost the same predawn and midday water potentials. In addition, leaf water potential can vary during the day depending on changes of vapour pressure deficit (VPD) and ambient temperature. In addition, both predawn Ψ and midday Ψ represent equally viable methods of assessing the water status. In this study, there was a significant relationship between midday leaf water potential (ψ) and stomatal conductance (g_s) in wheat infected with *Pythium* but not in controls because uninfected plants varied g_s to maintain constant leaf ψ while inoculated plants appear unable to do this (Jones, 1998).

Drought adversely affected photosynthesis (A) and stomatal conductance (g_s) in Janz wheat. As water stress increased, both A and g_s declined. The results indicated that the inoculation of plants with *Pythium* had a significant effect on photosynthesis under well watered (0 day after stress) conditions but not during drought. In contrast, the effect of drought on gas exchange of

potato was similar to the effect of *V. dahliae* (Bowden *et al*., 1990; Bowden and Rouse, 1991). Balota *et al*. (2005) found higher reduction in carbon assimilation under high soil moisture than low moisture in wheat diseased by the soil-borne fungus *Gaeumannomyces graminis*. This shows that diseased plants under high moisture experienced more severe stress from the disease than plants in drier soil. That there was no effect of *Pythium* on photosynthesis under drought, indicates that there was no direct effect of pathogenic mechanisms such as toxins, enzymes, or hormones on the photosynthetic mechanism. A possible mechanism by which the fungus can affect photosynthesis may be mediated by extracelluar enzymes by the fungus and defense reactions of plant (Hornsten *et al*., 2002). Another possible mechanism could be nutrient deficiency (Milroy and Bange, 2003). All these factors may affect plant hormonal balance which consequently may affect carbon assimilation rate (Kirkegaard *et al*., 1999).

In this study, decreases of photosynthesis were observed due to water stress not inoculation. Balota *et al*. (2005) found significant reduction in gas exchange rates, A: E ratio and $[CO_2]_i$ when wheat was infected by *Gaeumannomyces graminis* under different water regimes. The results also showed that there was an increase in $[CO_2]_i$ in both uninoculated and inoculated plants while photosynthesis decreased at the end of drought period i.e 3 weeks after water withholding. This must have been due to negative A, which means that the leaves were respiring and producing CO_2. Stomata were closed so $[CO_2]_i$ rose above ambient levels.

Balota *et al*. (2005) indicated that photosynthesis was not limited by stomatal control but rather by decreased photosynthetic capacity of leaves in plants with take-all because $[CO_2]_i$ was stable or increased while photosynthesis was reduced.

Pythium had no significant effect on g_s during the times of measurement. However, water stress was the cause of low g_s as there was a correlation between stomatal closure and decreased leaf water potential at the end of the drought period under diseased and non-diseased conditions. If stomata were closed by toxin or hormonal effects, then some leaves should have low g_s and high leaf Ψ (Bowden *et al*., 1990). The decreasing of stomatal conductance in wheat can thus be due to the decrease of leaf water potential or turgor pressure (Turner and Henson, 1989). However, carbon assimilation and leaf conductance were not closely coupled to the leaf water potential or leaf turgor pressure in sunflower and oleander (Turner *et al.*, 1985; Gollan *et al.*, 1985).

It appears from this study that the decreasing of CO_2 assimilation at the beginning of drought in plants infected by *Pythium* did not contribute to the decline in stomatal conductance as there were no differences between *Pythium* and controls. It could be that the fungus itself plays a role in a reduction of assimilation at this stage (0 day after water withholding) while decline in soil water content as drought proceeds is thought to be responsible for drought-induced stomatal closure and also for limitations of photosynthesis by decreasing the supply of CO_2 to chloroplasts when stomatal conductance decreases in response to soil drought (Grieu *et al.*, 1988).

Instantaneous WUE measured as A/E was not affected by *Pythium* during water stress. Instantaneous WUE was reduced largely (negative values) for both treatments at the end of the drought period as photosynthetic activity was measured under cloudy conditions. It is well known that the effects of drought on A and WUE were attributed to stomatal and non-stomatal factors. Some authors recognized stomatal limitations to be the major factor reducing photosynthesis (Sharkey and Seeman, 1989). Stomatal behavior is important because variation

in g_s affects E proportionally more than A (E increases linearly with g_s, whereas A levels off at high g_s values). However, other authors emphasise non-stomatal inhibition of A due to reduction in A and WUE in wheat under drought (Farquhar and Richards, 1984; Martin and Ruiz-Torres, 1992). It has been reported that WUE measured as A/g_s will initially increase with stomatal closure due to greater reduction in E than in A, but eventually with a greater decrease in g_s, WUE will decrease rapidly (Farquhar *et al*., 1989). Another reasonable mechanism for increased WUE is improved mesophyll capacity for photosynthesis (the carboxylation efficiency), which allows A to increase while E remains unaffected (Farquhar and Sharkey, 1982).

Ma *et al*. (2008, 2009) found that root pruning of winter wheat decreased the consumption of water due to reduced transpiration in the early growing stage (before anthesis), and so more soil water was saved and supplied to plants after anthesis, which facilitated grain filling and improved the WUE (Li *et al*., 2001). They suggested that lowering the root biomass in the upper soil meant that there were fewer sites of drought-induced signals. Plants with higher upper root biomass were more sensitive to drying topsoil and a possible non-hydraulic root signal than those with less upper root biomass (Blum and Johnson, 1993). The highest WUE was recorded by root pruning of winter wheat at the spring-growth stage in a field experiment (Fang *et al*., 2010). These findings reflected the effect of *Pythium* on improvement of WUE in experiment 2. However, root pruning at stem elongation stage improved photosynthetic rate of winter wheat from anthesis to grain filling stage and that resulted in a higher proportion of photosynthate being allocated to the shoot biomass and an increased HI (Ma *et al*., 2008). Similarly, higher leaf photosynthesis by root pruning was observed by Fang *et al*., (2010). Moreover, the effects of lowering root/shoot ratio on grain yield and WUE were investigated

by Ma *et al*. (2010). They found that lowering the root/shoot ratio improved the grain yield and WUE of winter wheat significantly by lowering its competitive ability and improving root efficiency.

In summary, infected plants had significantly higher WUE for both shoot biomass and grain than uninfected plants when inoculum density of *Pythium* was high enough, although grain yield was not affected. Predawn leaf water potential (Ψ) was not significantly affected by *Pythium* during the drought. However, diseased plants had significantly lower midday Ψ at 14 and 21 days after withholding water, when compared with controls. Instantaneous transpiration rate (E), and g_s of controls, were reduced after 7 days of water stress compared with infected plants. This could be because infected plants entered the drought with more available water due to reduced transpiration during early growth. However, A and instantaneous WUE (A/E), were only higher in controls prior to the drought perhaps due to higher activity of *Pythium* in moist soil. The decrease in A before drought was not due to a decrease in g_s for diseased plants. *Pythium* had no effect on $[CO_2]_i$ at all measurement times. Minor root diseases do not always reduce WUE or yield, depending on how they interact with drought. The insignificant effect of *Pythium* on photosynthetic rate may provide ideas for improvement of WUE of wheat under post-anthesis drought.

In large pot experiments, there was a difficulty in extracting all of the roots from the soil to calculate root dry matter and to estimate root/shoot ratio. Therefore, the experiments in the next chapter used hydroponics to enable manipulation of the root system. The experiments also tested if there was any difference between root damage from *Pythium* and root pruning under induction of water stress.

Chapter 5: Effects of *Pythium* and root pruning on water use efficiency of hydroponically grown wheat under PEG-induced drought

5.1 Introduction

A major concern in closed hydroponic systems is dispersal of root pathogens. Therefore, root diseases caused by *Pythium* species are a big problem in hydroponic systems as these systems provide an ideal environment for root pathogens to infect and spread (Gold and Stanghellini, 1985; Cherif and Belanger, 1992). *Pythium aphanidermatum* has been reported to cause root rots in different plant species in hydroponics (Jenkins and Averre, 1983; Stanghellini *et al*., 1984). *Pythium ultimum* Trow and *P. aphanidermatum* (Edson) Fitzp were extremely virulent in hydroponics because they can destroy a crop within a few days (Ch´erif *et al*., 1994). However, other *Pythium* sp. are usually considered as minor root diseases which decrease plant growth without causing clear symptoms (Cook and Papendick, 1972; Drew and Lynch, 1980). *Pythium* sp. are dispersed as zoospores or as hyphae on fragments of diseased roots (Owen-Going *et al*., 2003; Zheng *et al*, 2000). A surprising aspect of hydroponic crops with Pythium root rot is that the foliage often appears green and healthy even when root rot has become severe. Infected roots, in contrast, develop tip necrosis, expansive browning, and decay.

Roots have an essential function in the maintenance and balance of the growth of the whole plant. Several hypotheses have been proposed for the control of shoot growth and physiology when roots are stressed, including signalling from the roots (Davies and Zhang, 1991). The

major reason for inhibition of plant growth under root impedance is inadequate supplies of water (Bennie, 1991). Restriction of roots (from high soil strength) has been reported to decrease plant growth by reductions, for example, in leaf area, leaf number, plant height and biomass production in various plant species (Canni and Heuer, 1981; Robbins and Pharr, 1988). In a previous study, Bar-Tal *et al*. (1994) found that root pruning (physical removal) of tomato plants grown hydroponically decreased total dry matter (DM) production and transpiration rate. Root pruning caused sharp changes in root size, hormonal effects in the root-pruned plants and altered morphological development (Richards and Rowe, 1977). In wheat, root pruning under hydroponic systems was investigated by Al-Imran *et al*. (2002). These authors found that tillering and leaf growth were reduced by cessation of seminal root growth.

Drought stress in hydroponic systems can be induced by addition of polyethylene glycol (PEG). Water soluble PEGs have been widely used as inert, non-ionic solutes in the study of the water relations of plants including wheat (Lawlor, 1973). Water potential of hydroponic media may be controlled by using PEG if care is taken to ensure that it is not allowed to break down (Davidson and Chevalier, 1987). Effect of PEG (osmotic stress) on water use of wheat was investigated by Morant-Avice *et al*., (1989). They found that the transpiration of wheat in PEG solutions was greatly affected by the relative humidity of air.

Water use efficiency (WUE) of hydroponically grown plants from different species as affected by different physiological factors has been investigated previously (Nagy and Galiba, 1995; Yin and Raven, 1998; Claussen, 2002). However, little has been done on the effect of root pruning or fungal root diseases on WUE under hydroponic conditions. Therefore, the objective of this experiment was to compare the effects of mechanical root pruning and

inoculation of roots by *Pythium* on the WUE, water relations and other physiological parameters such as photosynthesis and stomatal conductance of hydroponically grown wheat (cv. Janz) plants under PEG-induced drought conditions.

5.2 Materials and methods

5.2.1 Plant material and growth conditions

The hydroponic experiments were conducted in a greenhouse at UNE. Day/night (12/12 h) temperature was 20/15 ^{0}C and relative humidity was about 60%. Wheat seeds cv. Janz were surface sterilised with 1% sodium hypochlorite in 10% ethanol for 5 minutes and then were germinated on filter paper for 4-5 days at room temperature and then the seedlings transferred to pots. The pots were made from 10 cm diameter PVC pipe, 15 cm tall, capped at the bottom. Liquid capacity was 1 litre. The pots were lined with a clean plastic bag before adding nutrient solution. Germinated seeds were suspended on fiberglass flyscreen (1 mm mesh) in clear acrylic tubes, 4 cm diameter and 6 cm tall, so that the root system was in contact with the solution. Air was bubbled through thin pipes into the bottom of the pots to provide oxygen. One seedling was grown on the mesh surface in each pot with water only (1 litre) for one week, then in half-strength modified Hoagland nutrient solution.

The nutrient solution contained the following chemicals: 5 mM KNO_3, 5 mM $Ca(NO_3)_2\,4H_2O$, 2 mM $MgSO_4.7H_2O$, 1 mM KH_2PO_4, 0.1 mM NaFe-EDTA. Micronutrient concentrations were: 11.5 µM H_3BO_3, 4.6 µM $MnCl_2.4H_2O$, 0.2 µM $ZnSO_4.7H_2O$, 0.12 µM $Na_2MO_4.2H_2O$, and 0.08 µM $CuSO_4.5H_2O$ (Kerepesi and Galiba, 2000). Iron concentration was increased from that used by Kerepesi and Galiba (2000) because of symptoms of deficiency in preliminary experiments. The pH of the solution was adjusted to between 5.9

and 6.1 with 1M KOH. The nutrient solution was changed twice per week. The measurement of whole plant water loss began after 2 weeks at the 4-leaf stage (stage 14 of Zadoks scale) prior to inoculation of roots by *Pythium*. Pots were placed on an analytical balance to estimate water loss by evapotranspiration. Evaporation from the nutrient solution was estimated by subtracting values for pots with no plants from those with plants.

The hydroponic experiment was repeated twice (Table 5.1). In the first trial, seeds were germinated on 21 June and then seedlings were grown 5 days after germination in the glasshouse. Inoculation of roots by *Pythium* was applied on 31 July at main stem and one tiller stage (stage 21 at Zadoks scale). Root pruning (Rp) and polyethyleneglycol (PEG)-induced drought treatments were applied 3 days after inoculation by *Pythium*. All the treatments were induced for one week, and then fresh solution was added to each pot (end of treatments) on 12 August. One week later, all the plants were harvested between booting and inflorescence emergence stages. In the second trial, seedlings were grown in 24 August. Transpiration was monitored from the 4-leaf stage (9 September). Similarly, roots were inoculated by *Pythium* after 2 weeks. Rp and PEG treatments were applied 5 days after inoculation of roots. At the end of treatments, water relations and other physiological parameters were measured. All the plants were harvested in 11 October.

Table 5.1 Dates of activities for hydroponic experiments 1 and 2

	Date	
Activity	**Experiment 1**	**Experiment 2**
Germination	21 June	19 August
Transfer to hydroponics	26 June	24 August
Inoculation with *Pythium*	31 July	23 September
PEG and root pruning treatments commenced	3 August	28 September
Water relations measurement	-	6 October
End of PEG treatment	12 August	7October
Harvest	19 August	11 October

5.2.2 Root inoculation by *Pythium irregulare*

The *Pythium* inoculation protocol was started by preparation of V8-agar media. The medium consisted of 10 g agar, 0.5 g $CaCO_3$, 100 ml of V8 juice and 500 ml distilled water. The medium was autoclaved for 1 hour. Inoculum for infection of the roots was prepared by growing *Pythium* on V8-agar medium at 25°C for one week. Colonised agar from a plate of *Pythium* was forced through a syringe (20 ml) to create a slurry. Sterilised distilled water (40 ml) was added to the slurry from each plate and stirred well for homogenisation. The number

of colony forming units was determined by dilution plating on PDA: CFU/ml = 6.81×10^7. In the greenhouse, the roots were dipped for 5 hours in plastic bottles (50 ml) containing the mycelium and then the same mycelium (used in root dips) was added to the nutrient solution. 50 ml of mycelium suspension was added to each pot.

Preliminary experiments were carried out to produce zoospores as inoculum. Zoospores were produced by a similar method to Rahimian and Banihashemi (1979) with some modifications. Each isolate was cultured on V8-juice agar medium in Petri dishes for 2 days at 25^0C or grown at 35^0C under continuous light. The colonised medium in each dish was cut into strips 1 cm wide and half of the strips were transferred to empty dishes. The colonies were flooded with 20-25 ml of sterile distilled or filtered pond water in each plate and then incubated at 25 ^{0}C for 48 hours. The water was replaced and then the plates were chilled at 4 ^{0}C for 30 minutes. The colonies were subsequently incubated at 20 ^{0}C under light or dark for 4 hours to stimulate zoospore release.

Another method to produce zoospores was performed using autoclaved mineral salt solution which contained 0.01 M $Ca(NO_3)_2$, 0.005M KNO_3, 0.004 M $MgSO_4$ and deionised water (DI) to make one litre solution. Chelated iron solution (FeEDTA) was prepared using 13.05 g of ethylene-dinitrilotetraacetic acid (EDTA), 7.5 g of KOH, 24.9 g of $FeSO_4.7H_2O$ and DI to make 1 litre and this solution was sterilised by filtration with a Millipore filter (0.22µ pore size). Chelated iron solution was added at 1ml per litre to the autoclaved mineral salt solution. About 20 ml of the solution was added to each colonised dish under the same conditions as above. However, neither method produced enough zoospores for inoculation.

To test whether the roots were colonised by *Pythium* following inoculation, 3-5 roots were cut in lengths of about 5 cm. These roots were surface disinfected by immersion in 50% ethanol for 10 seconds and in 1% NaOCl for 30 seconds, rinsed 3 times in sterilised distilled water and then dried on filter paper. The sterilised roots were cut into segments and placed on cornmeal agar medium amended with ampicillin (250 µg/ml) and rifampicin (10 µg/ml) and plates incubated at 25 ^{0}C for 24 hours (Chatterton *et al*., 2004). Alternatively, roots inoculated with *Pythium* were immersed in distilled water in plates and investigated under microscope to identify lesions as shown in Figure 5.1. Both methods confirmed a high level of colonistaion of roots by *Pythium*.

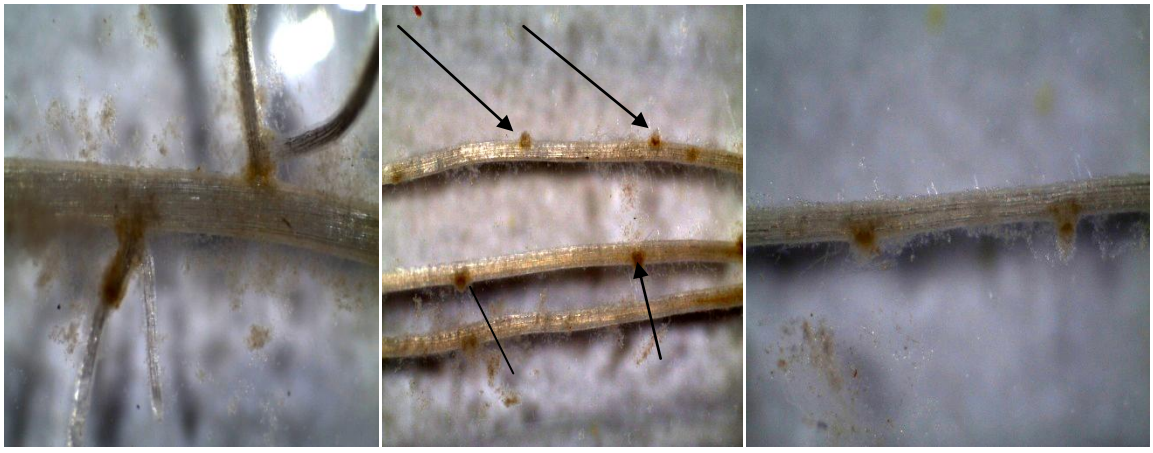

Figure 5.1 Growth of *Pythium* on roots and root lesion (arrows) of wheat inoculated by *Pythium* in hydroponic solution

5.2.3 Root pruning and Polyethyleneglycol (PEG)

Root pruning and PEG were applied 3 days (experiment 1) or 5 days (experiment 2) after inoculation of roots by *Pythium* so that root damage resulting from *Pythium* inoculation would have started at about the same time as other stresses. The treatments for root pruning were

carried out by cutting the roots to a maximum 20 cm length from the base of stem. Roots were maintained at a length of 20 cm for one week. The length of roots was checked every day during the treatment to keep them at the same length.

Osmotic stress (water stress) was imposed at the beginning of the 5^{th} week by application of PEG-6000 (Merck) at a concentration of 10% (100 g PEG/ litre nutrient solution) resulting in osmotic potential of -0.23 MPa in the nutrient solution (measured using a VAPRO 5520 vapour pressure osmometer) while osmotic potential of nutrient solution without PEG was -0.09 MPa.

5.2.4 Measurements

Water loss (whole plant transpiration) was measured every 24 hours for one week of stresses. After one week of plant stresses in the second experiment, predawn water potential was measured by pressure chamber at 6.30 am. Four samples of young expanded flag leaf were taken from each treatment. Mid day water potential was measured at 11.00 am by taking another 4 samples of flag leaf from each treatment. Photosynthesis (A), stomatal conductance (g_s), intercellular CO_2 concentration [CO2]*i* and transpiration rate (E) were measured by LICOR-6400 on the same leaf as mid day water potential. Relative water content (RWC) and osmotic potential (π) were measured also from the same leaf. The leaf was cut into three parts: the first part from the top to measure water potential (ψ), the second part was used for RWC and the third part for π. The procedure for measuring RWC and π was discussed in chapter 3. The plants were harvested at inflorescence emergence stage (stage 50 at Zadoks scale). Shoots and roots were separated to determine both shoot and root dry weight after drying at 80 ^{0}C in an oven for 48 hours.

Statistical analysis

There were 6 treatments for hydroponic experiments:

1. Root pruning
2. Polyethyleneglycol (PEG)
3. *Pythium*
4. PEG X root pruning
5. PEG X *Pythium*
6. Control

The pots were arranged in a completely randomised design, with 8 replicates. Data were analysed by factorial ANOVA, with root treatment (control, *Pythium* and root pruning) as one factor and PEG as the second factor.

5.3 Results

5.3.1 Transpiration per day

There were no treatment effects on transpiration per day (TPD) prior to root inoculation by *Pythium* in the first experiment (Figure 5.2). Two days after inoculation, root treatments had a significant effect ($P < 0.01$) on TPD. Plants inoculated with *Pythium* had lower TPD than controls. Four days after inoculation (1 day after adding PEG and root pruning), PEG ($P < 0.05$) and root treatments ($P < 0.01$) and the interaction between them ($P < 0.05$) had significant effects on TPD. Root pruning with PEG had significantly lower TPD than other treatments. At 6 and 8 days after inoculation, PEG and root treatments and the interaction between them ($P < 0.01$) had significant effects on TPD. Root pruning with PEG had the lowest TPD, while controls without PEG had the highest. The other treatments had significantly ($P < 0.05$) lower TPD than controls without PEG, and significantly higher TPD than root pruning with PEG, but there were no significant differences between them. Similarly, PEG ($P < 0.01$) and root treatments ($P < 0.01$) and the interaction between them ($P < 0.05$) had significant effects on TPD 10 days after inoculation. Root pruning with PEG had significantly lower TPD than all other treatments. TPD of root pruning without PEG and *Pythium* with PEG were significantly lower than TPD of controls without PEG but did not differ significantly from *Pythium* without PEG or controls with PEG.

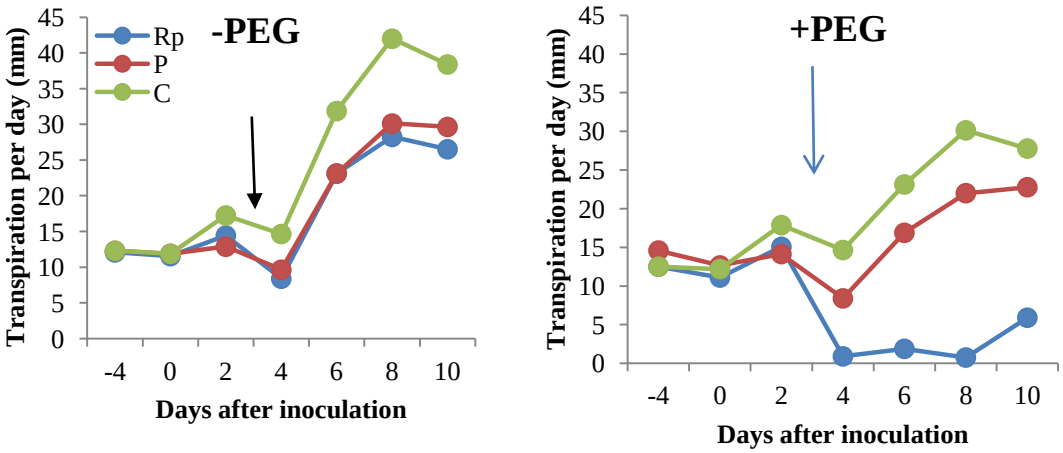

Figure 5.2 Root pruning (Rp) and *Pythium* (*P*) effects on transpiration per day of cv. Janz between 4 days before inoculation and 10 days after inoculation in the absence (-PEG) and presence (+PEG) of polyethylene glycol-induced drought in the first experiment. Arrow shows when PEG and root pruning treatments started.

The effect of root treatments and PEG on TPD in the second experiment were similar to those in the first experiment (Figure 5.3). Four days after inoculation, the *Pythium* treatment had significantly lower ($P < 0.01$) TPD than either root pruning or controls. TPD of root pruning with PEG was significantly ($P < 0.01$) lower than all other treatments 6 days after inoculation (1 day after adding PEG and root pruning). The *Pythium* treatments either with or without PEG had significantly lower TPD than controls without PEG at 6 days after inoculation, but they did not differ significnalty from root pruning without PEG or controls with PEG. At 8 and 14 days after inoculation, the TPD of root pruning with PEG was significantly lower, and the TPD of controls without PEG significantly higher, than all other treatments. TPD of *Pythium* with PEG was significantly lower than for root pruning without PEG, but neither of these treatments differed significantly from controls with PEG or *Pythium* without PEG.

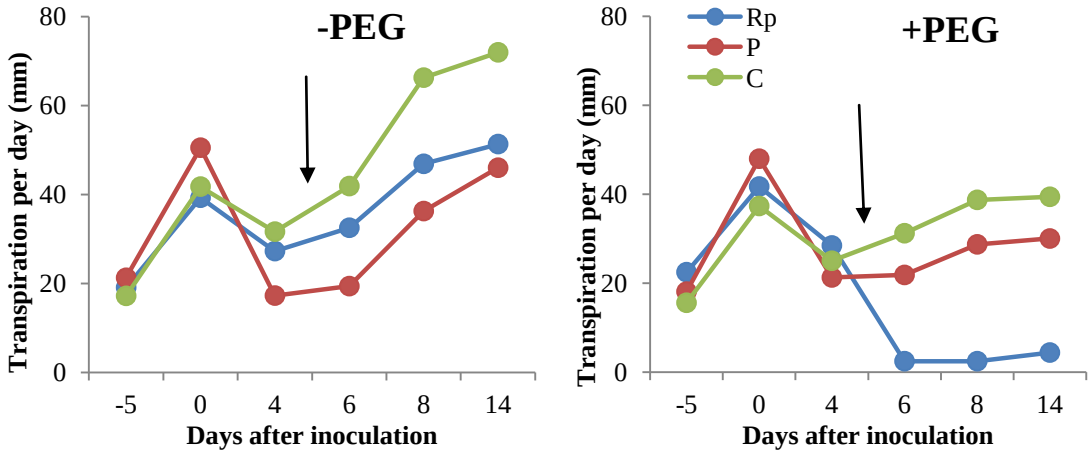

Figure 5.3 Root pruning (Rp) and *Pythium* (*P*) effects on transpiration per day of cv. Janz between 5 days before and 14 days after inoculation in the absence (-PEG) and presence (+PEG) of polyethylene glycol-induced drought in the second experiment. Arrow shows when PEG and root pruning treatments started.

5.3.2 Transpiration per week

Table 5.2 shows the transpiration of root pruned and inoculated plants with different PEG status during 7 days when PEG and root pruning treatments were imposed. In both experiments, PEG, root treatments and the interaction between PEG and root treatments significantly ($P < 0.01$) reduced the transpiration during the week when all treatments were applied.

Table 5.2 Effect of root pruning and *Pythium* in the presence (+PEG) and absence (-PEG) of polyethylene glycol on transpiration of wheat cv. Janz during 7 days in which PEG and root pruning treatments were imposed. Each value represents the mean of eight plants per treatment.

PEG status	Treatments	Experiment 1	Experiment 2
	Control	237.3	471.7
-PEG	Root pruning	171.1	349.2
	Pythium	175.4	276.7
	Control	181.1	271.7
+PEG	Root pruning	24.8	30.4
	Pythium	136.7	209.8
LSD		42.7	79.8

From 7 August (7 days after inoculation) to the end of the experiment, PEG and root treatments significantly ($P < 0.01$) decreased TPW in the first experiment (Figure 5.4). In addition, the interaction between PEG and root treatments was significant ($P < 0.05$) on 7 August and ($P < 0.01$) on 14 August. On 7 August, root pruning in the presence of PEG had significantly lower TPW than all other treatments. *Pythium* with or without PEG, PEG alone and root pruning did not differ significantly from each other. Control plants did not differ significantly from either PEG or root pruning. Similarly, on 14 August root pruning in the presence of PEG had significantly lower TPW than other treatments, and *Pythium* with PEG was lower than the controls without PEG.

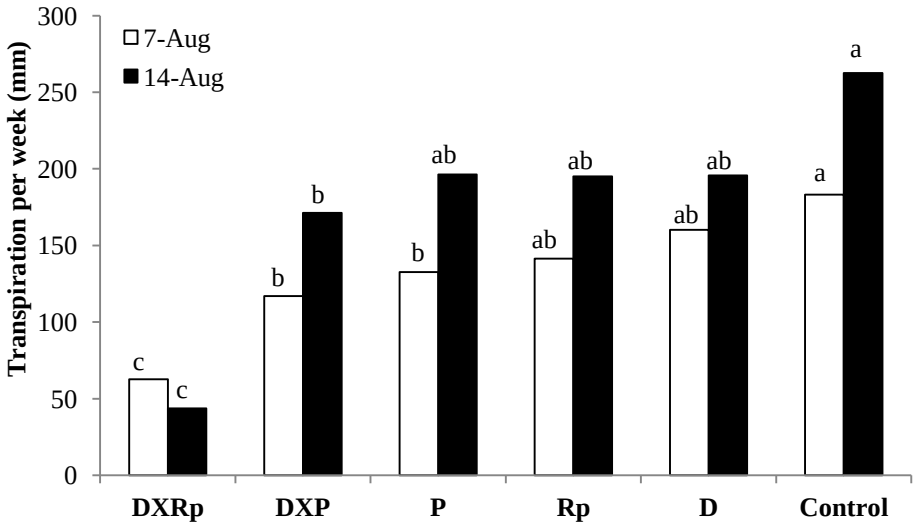

Figure 5.4 Comparison of transpiration per week (TPW) between all treatments on 7 and 14 August (first experiment). Rp= root pruning, P=*Pythium* and D=PEG. Inoculation with *Pythium* was done on 31 July and root pruning and PEG treatments were imposed from 3 August to 10 August. Columns labelled with the same letter are not significantly different at P<0.05.

There was a highly significant effect ($P < 0.01$) on TPW from 2 October until 11 October in the second experiment (Figure 5.5). PEG and root treatments significantly decreased TPW compared with controls and there was a significant interaction ($P < 0.01$) between PEG and root treatments. On 2 October, root pruning with PEG had the lowest TPW (97 mm) but this did not differ significantly from *Pythium* either in PEG or without PEG. Controls had the highest TPW (307 mm) and did not differ significantly from root pruning without PEG. On 9 October, root pruning with PEG had the lowest TPW (35 mm) with the highest for controls (375 mm).

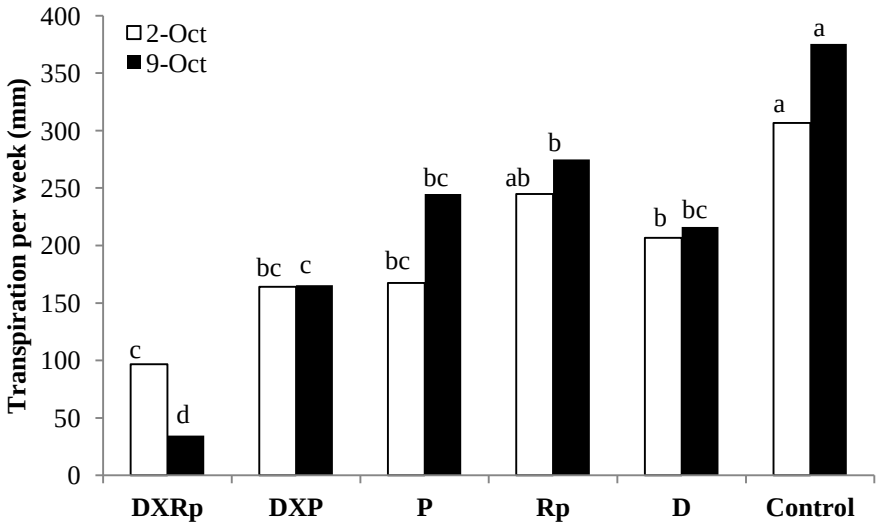

Figure 5.5 Comparison of transpiration per week (TPW) between all treatments on 2 and 9 October (2nd experiment). Rp= root pruning, P=*Pythium* and D=PEG. Columns labelled with the same letter are not significantly different at P<0.05.

5.3.3 Plant Growth

Root treatments had a significant effect on root dry weight in the first experiment ($P < 0.01$) but PEG had no effect. Root dry weight was lower in the root pruning treatments than the other treatments (Table 5.3). PEG and root treatments significantly affected shoot dry weight ($P < 0.01$) and there was a significant interaction between root treatments and PEG ($P < 0.05$). Root pruning plus PEG reduced shoot dry weight compared with other treatments. Root treatments and the interaction between roots and PEG had a significant effect on root/shoot ratio ($P < 0.01$). In presence of PEG, there were no differences in root/shoot ratio for all treatments. In the absence of PEG, *Pythium* showed the highest root/shoot ratio and root pruning the lowest one among the treatments.

Table 5.3 Effect of root pruning and *Pythium* in the presence (+PEG) and absence (-PEG) of polyethylene glycol on root and shoot dry weights, and root/shoot ratio of wheat cv. Janz in the first experiment. Each value represents the mean of eight plants per treatment.

PEG status	Treatments	Root dry weight(g)	Shoot dry weight (g)	Root/shoot ratio
	Control	0.47	1.56	0.299
-PEG	Root pruning	0.29	1.29	0.229
	Pythium	0.44	1.19	0.380
	Control	0.45	1.41	0.327
+PEG	Root pruning	0.17	0.55	0.322
	Pythium	0.39	1.22	0.323
LSD		0.12	0.39	0.056

In the second experiment (Table 5.4), both PEG and root treatments significantly affected root and shoot dry weights ($P < 0.01$) and there was a significant interaction ($P = 0.01$) between PEG and root treatments for shoot dry weight but not for root dry weight. Root and shoot dry weights were highly significantly decreased by root pruning plus PEG when compared with controls. Root pruning also significantly reduced root dry weight in the absence of PEG, and Pythium significantly reduced shoot dry weight in the absence of PEG. Only root treatments had significant effects on root/shoot ratio ($P < 0.01$), with root/shoot ration being increased by inoculation with *Pythium*.

Table 5.4 Effect of root pruning and *Pythium* in presence (+) and absence (-) of PEG on root and shoot dry weights, and root/ shoot ratio of cv. Janz in second experiment.

PEG status	Treatments	Root dry weight(g)	Shoot dry weight (g)	Root/shoot ratio
	Control	0.67	2.76	0.250
-PEG	Root pruning	0.48	2.31	0.213
	Pythium	0.54	1.77	0.302
	Control	0.54	2.08	0.264
+PEG	Root pruning	0.24	0.99	0.251
	Pythium	0.51	1.81	0.279
LSD		0.13	0.59	0.045

5.3.4 Water use efficiency

Water use efficiency (WUE) in the first experiment measured as total biomass (shoot and root dry matter) per litre water use (transpiration) is shown in Figure 5.6A and as above ground (shoot) dry matter per litre water use in Figure 5.6B. PEG had no significant effect on either measure of WUE. There were significant effects of root treatment and the interaction between root treatment and PEG on both $WUE_{(shoot)}$ and $WUE_{(shoot+root)}$.

Both measures of WUE were significantly reduced by the root pruning plus PEG treatment, but did not differ between the other treatments.

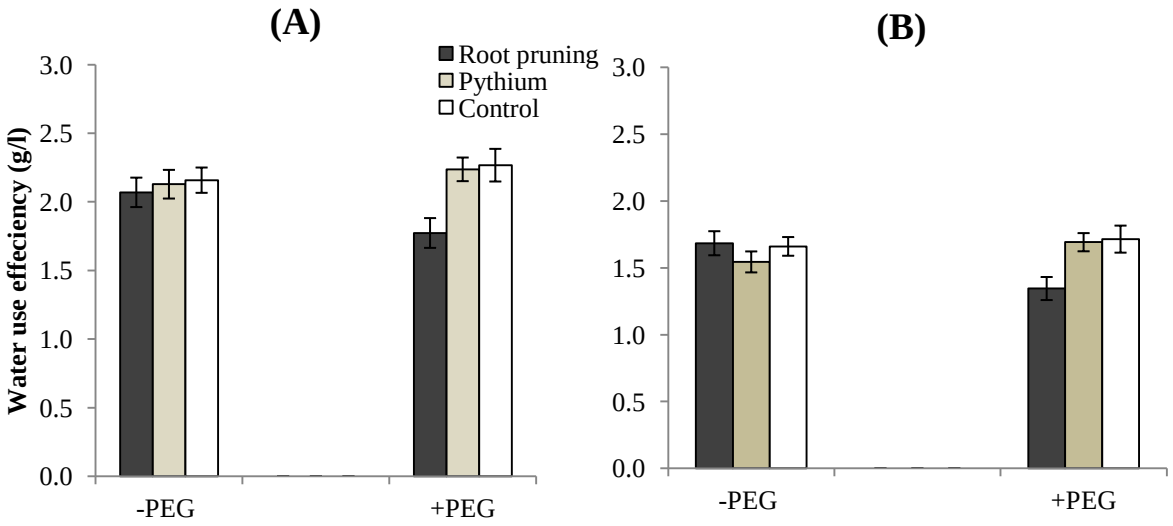

Figure 5.6 Root pruning and *Pythium* effects on (A) WUE $_{(shoot + root)}$ and (B) WUE $_{(shoot)}$ of wheat cv. Janz in absence of (-PEG) and presence (+PEG) of polyethylene glycol-induced drought in the first experiment. WUE was measured in grams dry weight per litre transpired. Each column represents the mean ±s.e for eight plants.

Water use efficiency (WUE) in the second experiment based on total root and shoot biomass per litre water use is presented in Figure 5.7A and based on shoot biomass (aboveground) per litre transpiration in Figure 5.7B. There were no significant effects of PEG, root treatment or their interaction on WUE $_{shoot}$. The main effect of root treatment had a significant ($P < 0.05$) effect on WUE $_{(shoot + root)}$, with WUE being 10% lower for root pruning than controls. This was presumably due to the removal of root tissue by pruning. The interaction between root treatment and PEG was not significant.

The results of WUE in this repeated experiment were very similar to the results in the first experiment. In both experiments, WUE decreased when root pruning was combined with PEG. However, higher WUE was achieved in this experiment. The average of WUE in first experiment based on either total biomass or shoot biomass was 1.85 g/l under all treatments. In this experiment, WUE increased to 2.96 g/l for all treatments.

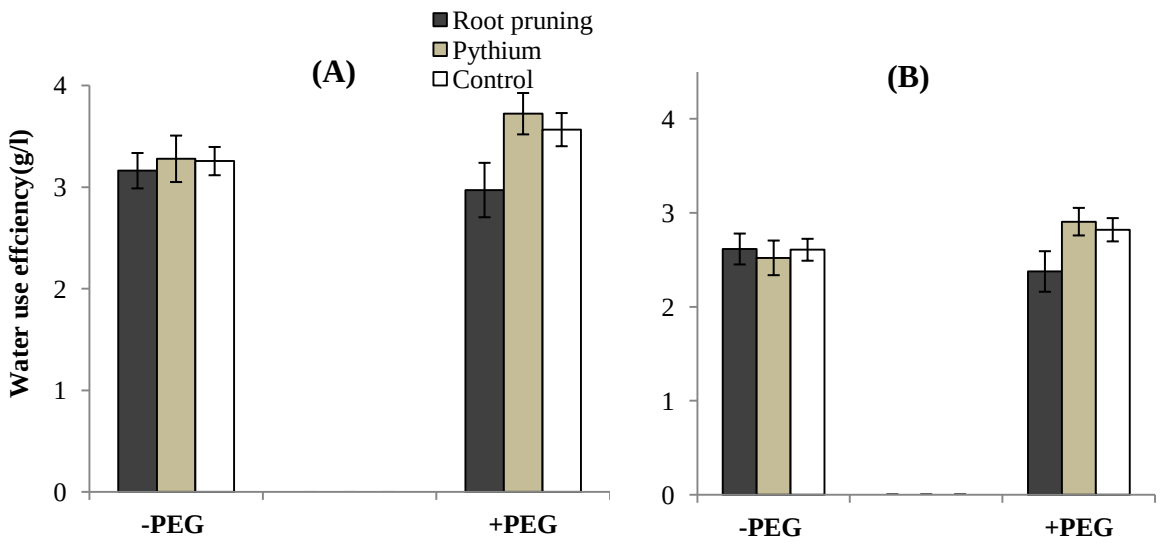

Figure 5.7 Root pruning and *Pythium* effects on (A) WUE $_{(shoot + root)}$ and (B) WUE $_{(shoot)}$ of wheat cv. Janz in the absence (-PEG) and presence (+PEG) of polyethylene glycol-induced drought in the second experiment. WUE was measured in grams dry weight per litre transpired. Each data represents the mean ±s.e for eight plants.

5.3.5 Plant water relations

The effects of root pruning and *Pythium* with and without PEG on components of water potential and relative water content are presented in Figures 5.8 and 5.9. There was no significant effect of treatments on predawn and midday water potentials and pressure potential. However, PEG significantly reduced osmotic potential ($P < 0.01$). Moreover, root treatments and the interaction between roots and PEG had a significant effect ($P < 0.05$) on osmotic potential.

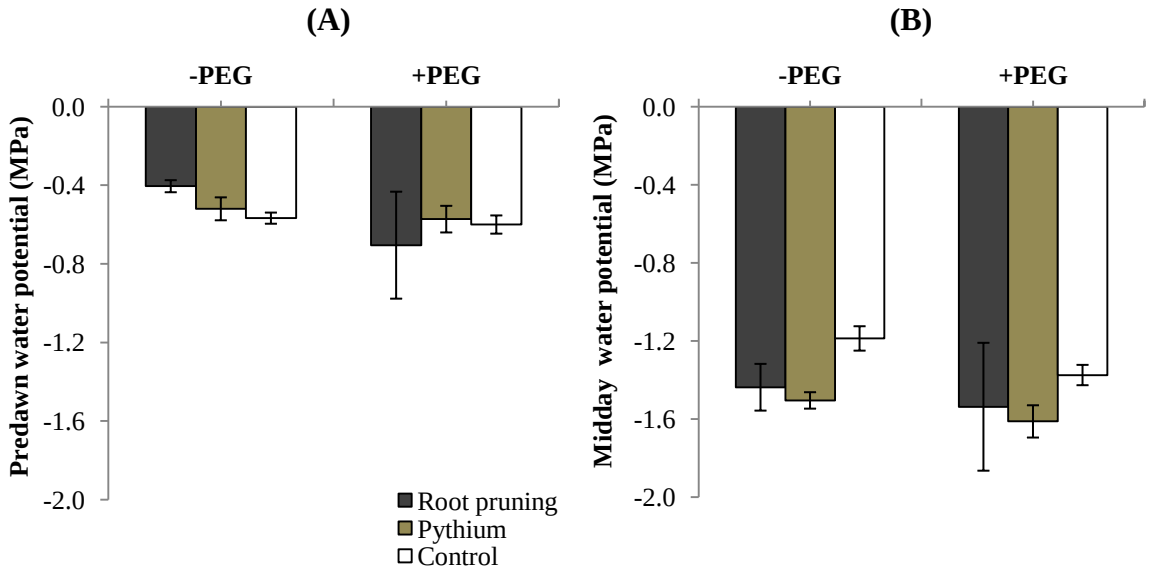

Figure 5.8 Root pruning and *Pythium* effects on (A) Predawn and (B) Midday water potential of wheat cv. Janz in the absence (-PEG) and presence (+PEG) of polyethylene glycol-induced drought. Each column represents the mean ±s.e for four plants.

In the absence of PEG, all root treatments had similar osmotic potential, but in the presence of PEG, osmotic potential was significantly lower by root pruning treatment than in controls. The main effect of PEG by itself had no significant effect on osmotic potential (Figure 5.9A).

PEG was the only significantly effect ($P < 0.01$) on relative water content (RWC). Addition of PEG reduced RWC from 93.9% to 90.5% (Figure 5.9C).

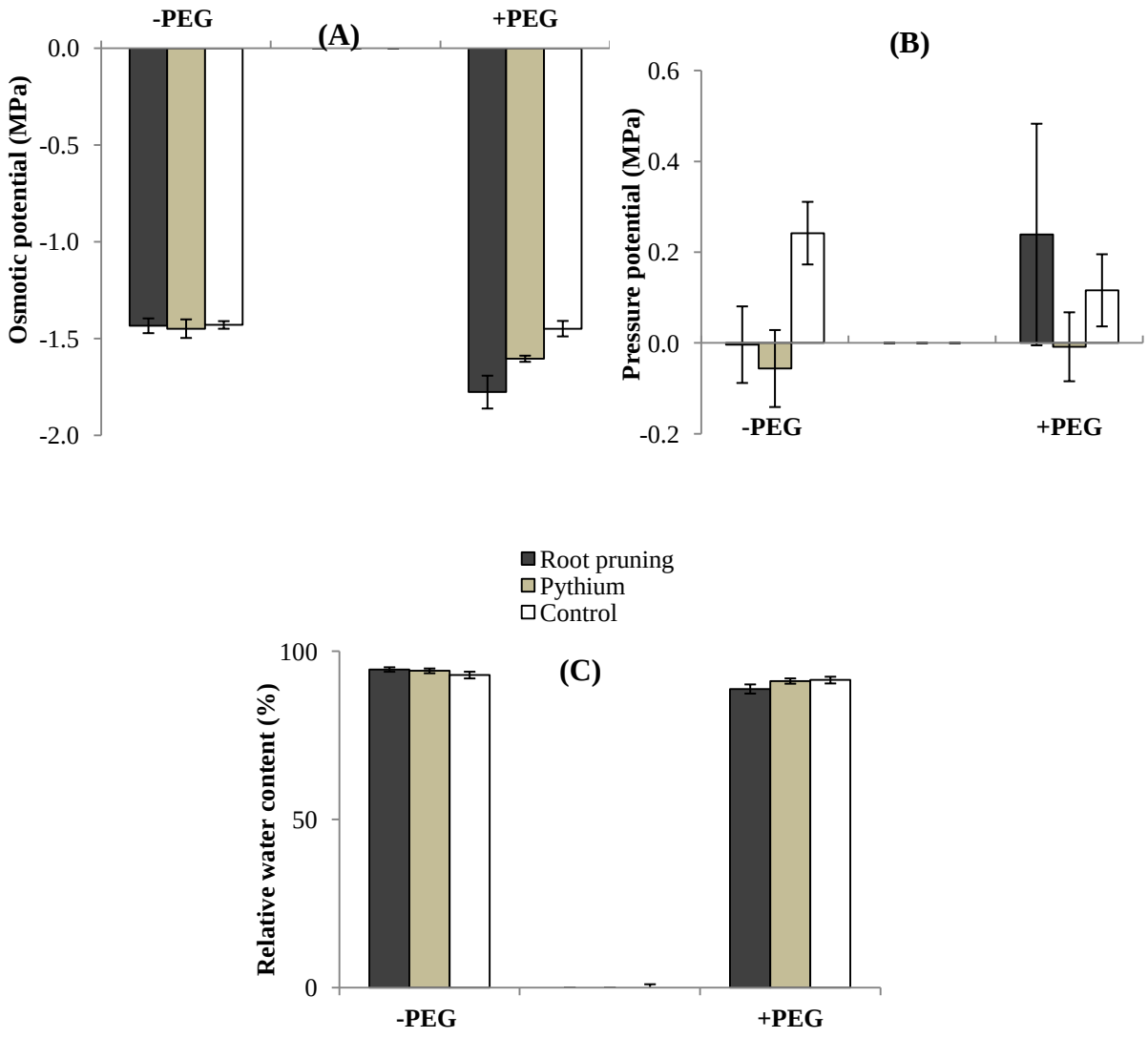

Figure 5.9 Root pruning and *Pythium* effects (A) osmotic potential (B) pressure potential and (C) Relative water content of wheat cv. Janz in the absence (-PEG) and presence (+PEG) of polyethylene glycol-induced drought (+PEG). Each column represents the mean ±s.e for four plants.

5.3.6 Physiological measurments

There was a significant ($P < 0.01$) effect of the interaction between root treatment and PEG on photosynthesis (A). All treatments reduced photosynthesis rate compared with the control

without PEG, but there were no significant differences between the other 5 treatments (Figure 5.10A).

PEG significantly reduced stomatal conductance ($P < 0.01$) and internal carbon concentration ($P < 0.05$) in all root treatments, but there were no significant effects of root treatments themselves or interactions with PEG (Figures 5.10B and 5.10C). Generally, g_s of all plants stressed by PEG was 38% lower than all plants without PEG. Plants stressed by PEG had 7% lower $[CO_2]_i$ than non-stressed plants.

PEG and the interaction between PEG and root treatments had significant effects ($P < 0.01$) on transpiration rate (E). Transpiration rate was significantly higher in the controls without PEG than in the other 5 treatments, which did not differ from each other (Figure 5.10D).

Instantaneous water use efficiency (A/E) was significantly affected ($P = 0.05$) by root treatments (Figure 5.10E). Instantaneous WUE was significantly lower in the root pruning treatments than the controls. There was no significant effect of PEG or interaction between PEG and root treatment.

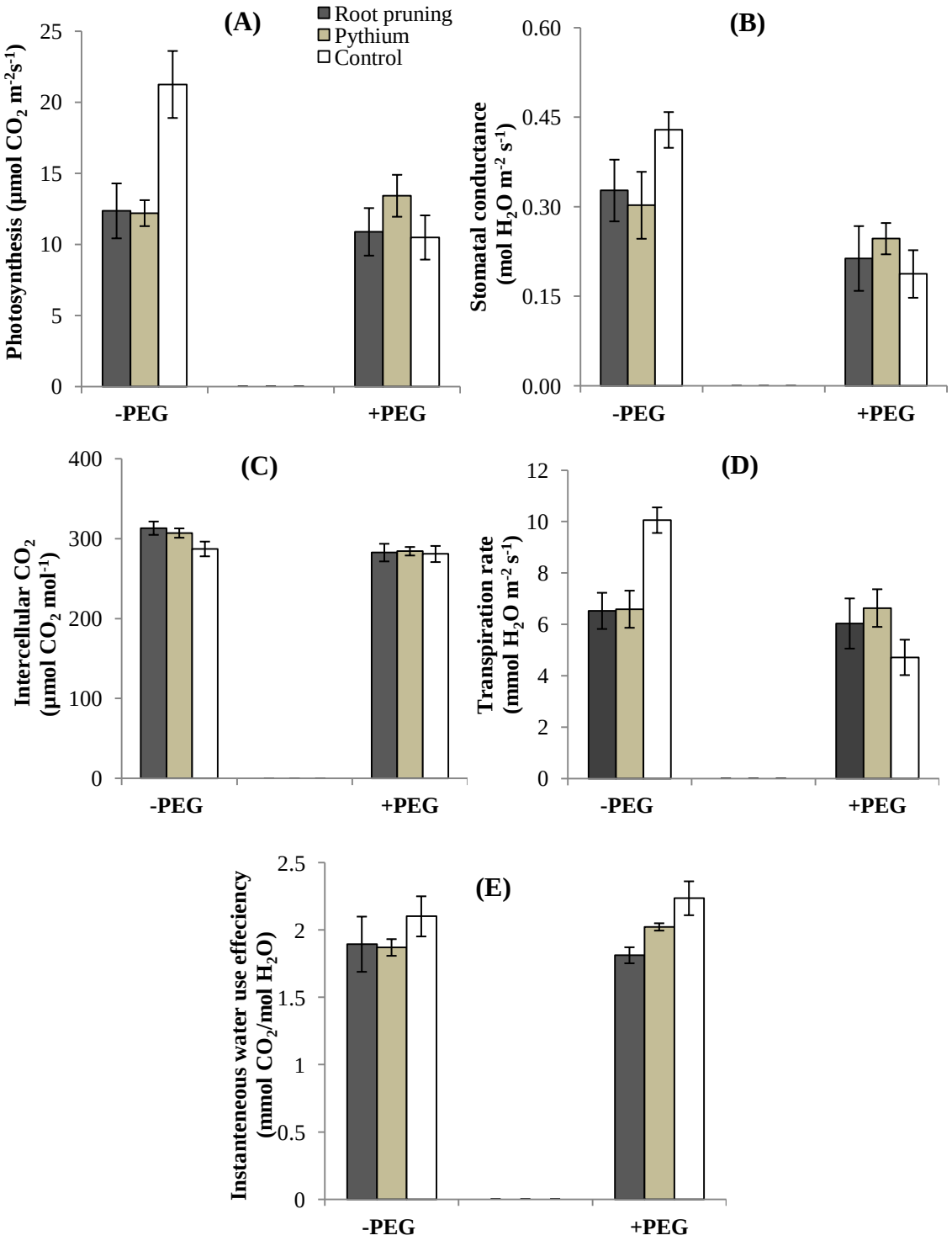

Figure 5.10 Root pruning and *Pythium* effects on (A) carbon assimilation (B) stomatal conductance (C) carbon internal (D) transpiration rate and (E) instantaneous water use efficiency of cv. Janz in absence of polyethyleneglycol (-PEG) and presence of PEG-induced drought (+PEG). Each column represents the mean ±s.e for four plants

5.4 Discussion

Root pruning treatments and PEG concentration were chosen in this hydroponic study based on preliminary experiments to give approximately the same reduction in transpiration as the *Pythium* treatment. Therefore, each treatment was expected to have a similar effect on water uptake. However, any differences between these treatments on other plant measurements would reflect some alternative effect on plant growth. The results from this study indicated that the treatments of root pruning, PEG and *Pythium* had similar effects on transpiration. When root pruning was combined with PEG, transpiration was reduced. However, combining *Pythium* with PEG did not reduce transpiration. Root pruning treatments reduced root dry weight because a large part of the root system was cut off, but *Pythium* did not reduce root dry weight. In addition, root pruning and PEG treatments by themselves did not significantly reduce shoot dry weight when compared with controls. However, *Pythium* in the absence of PEG reduced shoot dry weight and significantly increased root/shoot ratio. Water use efficiency (WUE) based on total root and shoot dry weight was reduced only when root pruning was combined with PEG treatment. Moreover, combining root pruning with PEG reduced osmotic potential and relative water content (RWC) but the effect of other treatments on water potential was not significant. All treatments reduced photosynthesis, stomatal conductance and instantaneous transpiration rate when compared with controls. However, only the root pruning treatment (with or without PEG) reduced instantaneous WUE.

In another study under soil conditions, Ma *et al*. (2009) indicated that leaf water potential of root pruned plants was similar to the controls in well watered plants, which means that water status of the shoot was not affected by root pruning. However, in this study water stress reduced root mass and thus water uptake by roots was unable to match transpiration. As a

result, root pruned plants were unable to maintain leaf water status after root pruning, which resulted in significantly decreased leaf water potential.

In this study, PEG had a fairly small effect on water potential and RWC. The results of Veselov *et al*. (2009) showed that RWC in leaves of barley and wheat plants decreased in response to the addition of PEG to the nutrient solution. Transpiration gradually declined in PEG treated plants due to stomatal closure and was 20–30% slower than in controls after 40 min of treatment (Veselov *et al*., 2009). Davidson and Chevalier (1987) found that the application of PEG to solutions with low water potential resulted in a linear decrease in both leaf water potential and osmotic potential of wheat plants while leaf pressure potential remained fairly constant. Osmotic adjustment within the leaf enabled the plant to maintain a pressure potential favorable to the leaf.

In this hydroponic experiment, PEG had a deleterious effect on plant growth when root size was reduced by mechanical root pruning. WUE, osmotic potential and RWC were all decreased by this combination. PEG may have some effect other than just in reducing osmotic potential. Most experiments with PEG have used intact plants and these show no toxic effects. However, it is possible that when the roots are pruned, the PEG can get directly into the xylem vessels and is transported around the plant. Lawlor (1970) showed that PEG 1000 and PEG 4000 could enter damaged roots of cotton and that this caused a large decrease in transpiration and had other toxic effects. The effects of the combined root pruning plus PEG treatment therefore probably reflect entry of PEG into the xylem rather than additive effects of reduced root mass plus osmotic stress.

The control of root and shoot growth under water stress was investigated in different studies such as Davis and Zhang, (1991); Gallardo *et al*., (1994); Blackman and Davies, (1985); and Sobeih *et al*., (2004). These studies showed that root systems can regulate stomatal conductance and leaf growth in plants exposed to drought via xylem-derived chemical signals which result in optimisation of water use.

Effects of PEG on root growth have been investigated at the cellular level (Chazen and Neumann, 1994; Saab and Sharp, 1989; Carpita *et al*., 1979). PEG-6000 is too big to significantly enter through root cell walls and membranes (Carpita *et al*., 1979). PEG treatment of roots could initially cause leaf changes by osmotically generated hydraulic signals, which means reduced availability of xylem water to growing leaf cells (Kramer, 1988). In addition, osmotic stress applied to the roots might alter the synthesis and upward transport in the xylem of root generated hormonal signals; these could in turn affect leaf growth parameters by increased flux into the growing cells of the growth inhibitor ABA (Saab and Sharp, 1989).

The ratio between root and shoot weights reflects a relationship between growth and development process of the plant and suggests a mechanism that maintains a balance between shoot and root growth (Vysotskaya, 2005). Root/shoot ratio for the treatments in this study did not differ under PEG. Similarly, Shone *et al*. (1983) found no significant effect of PEG on root/shoot ratio in barley and on shoot or root dry weights when PEG (-0.3 MPa) was applied to whole roots. However, Brouwer (1983) and Li *et al*. (1994) found that an increase in root/shoot ratio in response to water stress was due to a shift in the partitioning of dry matter between roots and shoots.

However, *Pythium* in this study significantly increased root/shoot ratio. The plants did not seem more water stressed than for PEG or root pruning treatments, so it is unlikely that water stress would be driving the production of a larger root system in infected plants. What is more likely is that *Pythium* affects the efficiency of the root system so that transpiration per unit of root mass (1358 ml/g dried roots) was decreased compared with controls (1599 ml/g dried roots), which then leads to reduced shoot growth. In contrast, Johnston *et al*. (2005) found that root and shoot dry weights were significantly reduced in plants of bell pepper inoculated with *Pythium aphanidermatum* when compared with controls and that root/shoot ratio was lower in inoculated plants than in noninoculated plants. This may reflect a greater degree of root destruction by *P. aphanidermatum* than by *P. irregulare*. The transpiration expressed at the whole plant level of plants inoculated with *P. aphanidermatum* was reduced minimally, which indicates that the pathogenesis events such as possible cortical or vascular occlusion were not responsible for limiting water availability for leaf expansion (Johnston et al. 2005). The transpiration per unit root mass was higher in *P. aphanidermatum* inoculated plants compared with controls. It could be that the transpiration of inoculated plants was affected by signals that are transferred from roots to the leaves via the transpiration stream.

Roots are a major sink for assimilates and require twice as much assimilate as shoots to produce a unit of dry mass due to high respiration rates (Passioura, 1983). Passioura (1983) suggested that if the roots are small then more assimilates could be available for shoots, which means higher WUE. Partitioning of assimilate between root and shoot was not affected by water stress in barley (Shone *et al*., 1983). However, PEG increased the assimilate partitioning in the roots of rice (Hirai *et al*., 1994). Root growth was activated after parts of roots were excised in different plant species but that requires a redistribution of assimilates in favor of

roots (Biddington and Dearman, 1984; Vysotskaya *et al*., 2001). In this study, root growth was restricted as a result of mechanical root pruning rather than by *Pythium*. However, Amir and Sinclair (1996) indicated that nematode damage was due to restricted root growth rather than toxic effects.

In this study, photosynthetic rate (A), stomatal conductance (g_s) and transpiration rate (E) were reduced in all treatments. However, *Pythium* had no significant effect on instantaneous WUE. This is not the case for root pruning, which had lower instantaneous WUE than controls. Inoculation of capsicum plants with *Pythium aphanidermatum* resulted in reduced whole-plant net carbon exchange rates (Johnston et al. 2005). Infection by *Pythium* did not affect the photosynthetic apparatus directly and the reductions in photosynthesis and growth were not caused by inefficient water transport by diseased roots (Johnston *et al*., 2005). Therefore, the results indicated that water stress was not responsible for reduction of photosynthesis rate by stomatal closure in inoculated plants. It could be that toxins or phytohormones originating in the infected roots have altered the photosynthetic rate. A decrease in photoassimilates available for leaf growth in the infected plants could have arisen from strong sink development in the inoculated roots. Increased sink strength of the diseased roots could be due to demands by the pathogen or other microbes associated with the roots, which in turn increased exudation of carbon compounds into the nutrient solution, or energy demands for production of defense compounds by root cells (Hoffland *et al*., 1998).

Ma *et al*., (2008) indicated that root pruned plants had lower stomatal conductance and transpiration than control plants at the stem elongation stage. Stomatal closure regulated by ABA with increasing water stress is well documented (Cornish and Zeevaart, 1985; Li *et al*., 2000). ABA is a stress hormone and plays an important role during water stress either at the

whole plant level (Davies and Zhang, 1991) or at a cellular level (Straub *et al*., 1994). ABA concentrations in wheat roots increased after root excision treatment (Vysotskaya *et al*., 2003) but with no evidence for any enhanced concentration in xylem sap. PEG induced a significant accumulation of ABA in maize and also induced a decrease in osmotic potential due to decrease in cellular volume (Jia *et al*., 2001). In this study, it could be that a part of the effect of *Pythium* on photosynthetic rate is due to ABA production causing stomatal closure, rather than direct damage to the roots. The water use efficiency of net photosynthesis (A/E) could be increased although stomatal closure under water stress limits both net CO_2 uptake and transpiration of the leaf (Fischer and Turner, 1978).

In conclusion, *Pythium*, PEG and root pruning reduced transpiration to a similar extent, however, the mechanism which affects transpiration differed between the treatments. Reduced hydraulic conductivity of roots caused by disease in the *Pythium* treatment and reduced size of the root system in the root pruning treatment were responsible for decreased transpiration while reduction of stomatal conductance was the main cause for reduced transpiration in the PEG treatment. *Pythium* reduced shoot dry weight and increased root/shoot ratio but had no effect on total or instantaneous WUE. There was a small additive effect of *Pythium* on whole-plant transpiration of plants exposed to drought stress, but there was no evidence of an interaction between *Pythium* and drought on water use efficiency or growth. This suggests that moderate root damage by pathogens has a modest effect on the water relations of wheat plants when compared with other environmental stresses.

In hydroponics, the interaction between root pruning and drought (PEG) differed from the interaction between *Pythium* and drought (PEG). Therefore, the next chapter aimed to compare root pruning and *Pythium* in soil.

Chapter 6: Effect of Root Pruning on Water Use Efficiency of Wheat

6.1 Introduction

Roots can account for 50% or more of the total dry matter production of annual plants (Caldwell, 1979). Poor soil conditions may slow root growth and inhibit root function. Roots may be broken off by soil movement or killed by diseases or other factors. These interruptions of root function can affect the final yield depending on the timing and extent of damage. In general, a large root system is more beneficial to the plant than a small root system for obtaining water (Kramer, 1969). Therefore, selection of progeny with large root systems has been suggested as a breeding strategy for drought-resistance (Hurd, 1974). However, Ma *et al*. (2010) argued that a large root system can result in rapid soil water consumption, which may not be favorable in arid and semiarid areas.

The distribution of root systems is correlated with patterns of soil water uptake and depletion (Clothier and Green, 1994). A decreased root system in the upper soil layer is advantageous to crops if more water was available in a deeper soil layer (Passioura, 1983). Additionally, the decrease of root dry weight in the upper soil contributed to the increase in harvest index (HI) and water use efficiency (WUE) of modern wheat varieties (Siddique *et al*., 1990; Ma *et al*., 2008). Blum and Johnson (1993) found higher sensitivity to drying topsoil for wheat plants with more upper root biomass. Increasing the depth and density of roots in the subsoil is an evident approach to enhance deep water use particularly if roots get access to layers not previously occupied or root densities exceed levels critical for effective water uptake from deeper layers (White and Kirkegaard, 2010).

The relationship between transpiration rate and root pruning has been studied in the past in numerous studies such as Bialoglowski (1936); Parker (1949) and Andrews and Newman (1968). It was found that the relationship between transpiration rate and amount of root is not linear in these experiments. In other words, the rate of decrease in transpiration was less for small amounts of root pruning.

The effects of root pruning in wheat have been investigated for example on plant –water relations (Sharma, 1987), morphological changes (Wiedenroth and Erdmann, 1985) and growth and yield (Ayling, 1989). The most recent studies which have paid attention to grain yield and water use efficiency of wheat as influenced by root pruning include Ma *et al*., (2010, 2009, 2008) and Fang *et al*., (2010). These authors tried to reduce early season water use, so that more water was available for late season stress. Few studies have compared the effects of root diseases and root pruning on plant growth (e.g. Amir and Sinclair, 1996). The present study sought to compare *Pythium* infection with root pruning in a pot experiment on grain yield, WUE and root/shoot ratio of cv. Janz. The hydroponics experiment suggested that the effect of *Pythium* on plant growth was not just due to a reduction in water uptake caused by direct damage to the root system. Therefore, it was possible that root pruning would do something different to *Pythium* in a pot experiment.

6.2 Materials and Methods

A pot experiment was carried out in a glasshouse at the University of New England to compare the effects of root pruning and *Pythium* on grain yield and WUE of Janz wheat. Thirty pots (15 cm diameter X 30 cm tall) were used in this experiment and each pot was filled with 4.5 kg of sandy loam soil mixed with peat (3:1) (v/v). The soil pH was adjusted to 6.4 with agricultural lime. Granular N:P:S (14.3:12:10.5) Starter 15 fertiliser was applied to the soil mixture at a rate of 13 g m^{-2}. For those pots inoculated by *Pythium*, the soil in plastic bags was mixed well with 10 g of *Pythium* inoculum (colonised *Pythium* with millet seeds) prepared from the previous experiment (see chapter 2) and stored in a refrigerator at 4 ^{0}C.

Temperature in the greenhouse was controlled at 25/18 ^{0}C (day/night). The average relative humidity (RH) was maintained at approximately 60% within the greenhouse. The photoperiod was 12 hours on average. There were three treatments (control, *Pythium* and root pruning) and each treatment had 9 replicates. At the end of soil preparation and before sowing, pots were watered with 500 ml each until field capacity (when water escaped from the bottom holes). Wheat seeds (cv. Janz) were surface sterilised and three wheat seeds were sown per pot on 23 August, 2010. Plants were thinned to two seedlings per pot at the two-leaf stage five days after emergence. 200 g of plastic beads were placed on the soil surface in each pot at main stem and one tiller stage. The pots were arranged in a randomised complete block design. Monitoring of water use (transpiration) was started at the 3-leaf stage (9-September 2010) and was achieved by regular watering of the pots (every 2-3 days/week). The amount of water added to each pot was determined in response to average evapotranspiration (ET) of infected pots. Transpiration was determined by subtracting water loss of unplanted pots (3 pots) from that of planted pots. Root pruning was imposed with a stainless steel knife of length 10 cm by inserting the knife

into the soil 4 times around each plant to remove parts of the roots (Ma *et al*., 2010). Root pruning was intended to be done when the *Pythium* treatment started to show significantly reduced transpiration. However, this did not occur so root pruning was done late in the vegetative stage. Root pruning was applied at inflorescence emergence (GS 50) on 7 October, 2010. Plants were left growing until fully mature. Droughting started on 20 October 2010 at the grain filling stage (GS 70) by replacing about half of the water lost by the treatment with lowest evapotranspiration, so the pots were dried slowly. Watering was stopped completely on 4 November 2010. Control and infected plants were harvested on 9 November 2010. Root pruned plants were harvested 2 weeks after both controls and infected plants.

At the fully mature stage, the number of heads per each pot was counted. The above-ground plant parts were harvested and separated into vegetative parts and heads. The shoots were kept in paper bags with heads in envelopes. The roots were extracted gently from the soil and washed with water to remove any remaining materials and then kept in paper bags. Total dry weight of shoots and roots were determined after drying in an oven at 80 ^{o}C for two days. The grains were separated from the heads by threshing and then grain weight and grain number were determined for each pot. In this experiment, root/ shoot ratio, water use efficiency based on grain yield and total dry matter, and harvest index were all determined.

6.3 Results and Discussion

6.3.1 Transpiration

The cumulative transpiration (CT) did not differ significantly between treatments (*Pythium* and root pruning) from 11 September until 2 October (the time where roots were pruned) (Figure 6.1). From 9 October until harvest, CT of root pruned plants was significantly reduced ($P < 0.01$) when compared with either controls or *Pythium*. The largest difference in CT (Figure 6.1) between controls and root pruning was 789 mm 2 weeks after pruning treatment then the difference began to decrease gradually. At harvest, CT difference between controls and root pruning decreased almost 50%.

Transpiration per week (TPW) did not differ significantly between treatments (*Pythium* and root pruning) from 11 September until 2 October (Figure 6.2). On 9 and 16 October, root pruned plants transpired significantly less water ($P < 0.01$) than other treatments. However, there was no significant difference between all treatments on 22 October. From 27 October until 17 November, root pruned plants transpired significantly more water ($P < 0.01$) than other treatments. At the time of harvest (23 November), there was no differences in TPW between all treatments.

In the whole experiment, *Pythium* had no effect on transpiration. Five days after drought was imposed, the results showed reductions of 72% and 76% in transpiration per day (TPD) for controls and *Pythium*, respectively. However, TPD of root pruned plants was less affected by drought and the reduction was 47% compared with other treatments.

The amounts of water in soil either under well watered or water stress conditions play a role in determining water use by the plants. In addition, root characteristics and distribution in soil

have an important role as well. The amounts of water added were the same in all treatments but when the roots were pruned then the water uptake by intact roots from the soil was greatly decreased and that led to decreased transpiration. Therefore, the decrease of water use by root pruned plants contributes to increased water in soil under well watered conditions and when new growth of roots was promoted then the transpiration increases. The highest TPD of controls (139 mm) was at the anthesis stage when more water is transpired because the plants had the longest roots. At that time (anthesis), root pruned plants had TPD of 45 mm. Extra water remaining in the soil for root pruned plants increased the transpiration under water stress while control plants used most water before drought so water availability in the soil decreased which in turn contributed to decreased water use after drought.

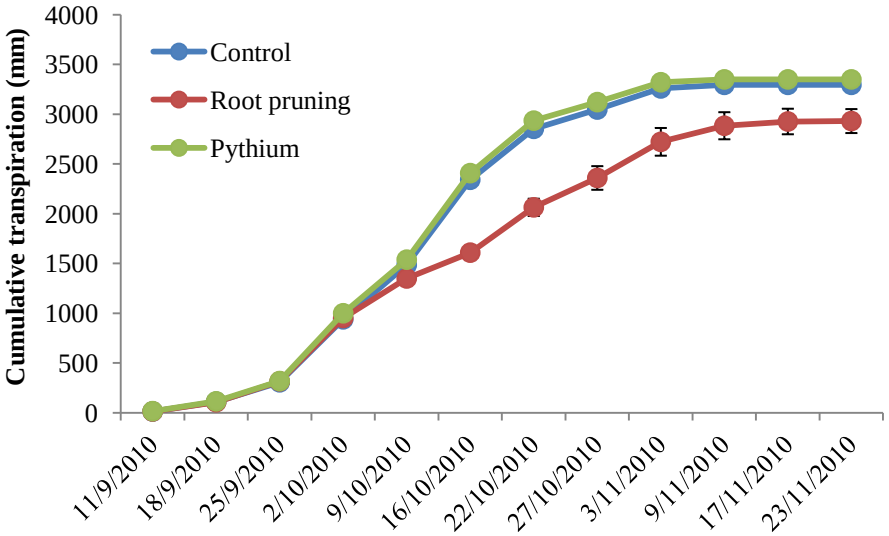

Figure 6.1 Effect of root pruning and *Pythium* on cumulative transpiration (mm) from 3-leaf stage (11-September) until harvest (23-November) in cv. Janz. Root-pruned plants matured 2 weeks after the other treatments. Drought was imposed on 4 November 2010. Values are means ± s.e., n=9.

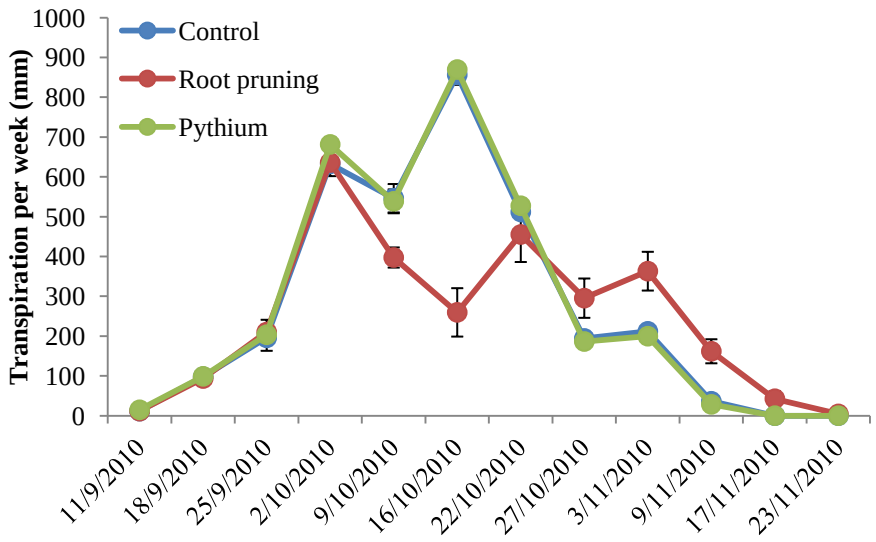

Figure 6.2 Effect of root pruning and *Pythium* on transpiration per week (mm) from 3-leaf stage (11-September) until harvest (23-November) in cv. Janz. Root-pruned plants matured 2 weeks after the other treatments. Drought was imposed on 4 November 2010. Values are means ± s.e., n=9.

Recent studies (Cinnirella *et al*., 2002; Cooper *et al*., 2003) suggest that there was a direct relationship between transpiration and root system size. Ma *et al*. (2010) found that root pruning decreased leaf transpiration rate before heading. However, there was no significant difference in transpiration between plants with intact roots and those with pruned roots in the pots at anthesis. Under field conditions, transpiration of root-pruned plants in the upper soil (0-20 cm soil layer) was significantly higher than that of intact-root plants at anthesis and grain filling stages. In this study, transpiration declined immediately after root pruning and then returned to similar values as controls 15 days after treatment. That was in agreement with the results of Ma *et al*. (2009).

It was suggested that removal of 25% or even 50% of the roots may have little or no effect on transpiration of wheat (Andrews and Newman, 1968), but removal of more roots can lead to

greater decreases in transpiration rate. In most experiments, root pruning can reduce the volume of soil available to the plant and the amount of root per plant. However, Andrews and Newman (1968) showed that soil volume was not affected but root density was reduced. In addition, root pruning can only increase plant resistance at field capacity while soil resistance was negligible. The resistance increased in drier soil due to both soil and plant resistances and this could result in a higher decline in transpiration (Andrews and Newman, 1968).

6.3.2 Grain yield and biomass components

The number of heads per pot was significantly reduced ($P < 0.05$) by root pruning compared with the Pythium treatment (Table 6.1). Root pruning also reduced the number of fertile tillers of wheat in other pot experiments (Ma *et al*., 2008, 2009). However, root pruning increased the proportion of fertile tillers and spike density in a field experiment (Fang *et al*., 2010), so it is possible that the effect of root pruning under variable conditions in the field is more complex than in pots.

The main components of wheat yield are grain number and the average weight of those grains (Acreche and Slafer, 2006). Root pruning treatment significantly decreased ($P < 0.001$) total grain weight and grain number (Table 6.1). The reduction of grain number (51%) for root pruning was greater than for total grain weight (45%). 1000-grain weight was significantly increased by root pruning compared with *Pythium*. However, there was no significant difference between root pruning and controls ($P = 0.07$). The average weight for one grain in root pruned plants was 0.037 g while in controls it was 0.032 g. Slafer *et al*., (1996) found these two components are often negatively associated. There is competition between growing grains after anthesis for limited resources. As grain number increased, each grain can access

less assimilate than needed to maximise growth (Acreche and Slafer, 2006). However, increases of grain number may decrease average of grain weight without any need for competition for assimilates (Miralles and Slafer, 1995; Slafer *et al*., 1996).

Ma *et al*. (2010) found root pruning improved grain yield of winter wheat significantly by improving root efficiency. Similarly, Fang *et al*. (2010) found significant increases in grain yield when roots were pruned in winter compared to control plants. This increased yield occurred despite the lower tiller density that resulted from the root pruning prevented the development of tillers associated with nodal roots that were removed during pruning.

Shoot dry weight and total dry weight of root pruned plants were significantly ($P < 0.01$) reduced (Table 6.1). However, root dry weight and root/ shoot ratio did not differ significantly between treatments. Ma *et al*. (2010) found that root dry weight was decreased by root pruning, while root/ shoot ratio was the same but increased at an earlier stage.

Root pruning significantly reduced ($P < 0.01$) harvest index (HI) (Table 6.1). Harvest index was reduced 20% by the root pruning treatment because the reduction of grain yield was higher than reduction of total biomass production. However, Ayling (1989) found root pruning had no effect on harvest index in winter wheat. Ma *et al*. (2008) found that root pruning increased HI of root pruned plants due to a higher proportion of photosynthate being allocated to shoots.

Table 6.1 Number of heads per pot (HN/pot), grain weight (GW), number of grains (GN), shoot dry weight (SDW), root dry weight (RDW), root/shoot ratio (R:S ratio) , harvest index (HI) and 1000-grain weight (1000-GWt) for controls, infected and root pruned plants of wheat cv. Janz

Treatments	**HN/pot**	**GW(g)**	**GN**	**SDW(g)**	**RDW(g)**	**R:S ratio**	**HI**	**1000-GWt**
Control	9.6	7.3	234.5	7.6	3.0	0.39	0.40	31.56
Pythium	10.2	7.4	259.6	7.8	2.7	0.34	0.41	28.86
Root pruning	8.1	4.0	115.7	6.3	2.1	0.32	0.32	37.03
LSD	1.7	1.0	41.9	0.5	0.8	0.09	0.05	5.98

This study showed higher reduction in grain yield than shoot dry matter as affected by root pruning. Fang *et al*. (2010) found that root pruning of winter wheat in winter resulted in less water use before the stem elongation stage, but root pruning in spring saved more water at the vegetative stage. As a result, root pruning may have exposed the plants to lower water stress during grain filling and improved post-heading accumulation of dry matter. The reason for greater accumulation of dry matter could be due to decrease of carbon consumption by the root system which improves yields if the carbon is re-allocated to grains (Fang *et al*., 2010). Plants root-pruned at anthesis had a higher rate of leaf photosynthesis and lower rate of root respiration, which resulted in a significantly higher grain yield at maturity when compared with controls (Ma *et al*., 2010).

6.3.3 Water Use Efficiency

Water use efficiency (WUE) based on grain yield and total dry matter is presented in Figure 6.3 for infected and root pruned plants compared with controls. Root pruning treatment significantly decreased ($P < 0.01$) WUE either based on grain yield or total dry weight.

The results of Ma *et al*. (2008) and Wang *et al*. (2007) indicated that WUE of winter wheat was improved without affecting grain yield by lowering the root biomass in the upper soil layer at early growing (vegetative) stage and hence reducing competition for water and nutrient uptake. In contrast, WUE of wheat cv. Janz did not improve with root pruning at near anthesis (reproductive) stage.

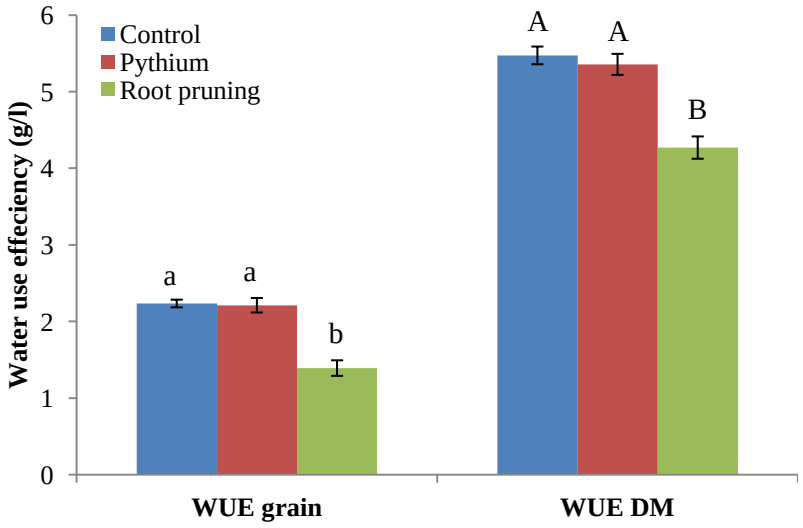

Figure 6.3 Effect of root pruning and *Pythium* on water use efficiency based on grain yield and total dry matter (g/l) in cv. Janz. Values are means ± s.e., n=9. Columns labelled with the same letter are not significantly different at P<0.05.

In conclusion, the root pruning caused a reduction in yield and WUE. It could be that pruning was severe when applied at the time of treatment (inflorescence emergence stage). It seems that root pruning at later stages of growth can lead to reduction of WUE while root pruning at earlier growth stages of wheat can increase WUE (Ma *et al*., 2010). However, the ability to make use of the extra water available after anthesis depends on a number of factors including the rate at which soil dries down. The extra water available in the root pruned pots at the end of experiment was not enough to bring the grain yield and WUE up to controls. There were differences in maturity time between controls and root pruned roots due to soil water availability. Root pruning did more than just restrict the ability to access all the water. This experiment showed no effect of *Pythium* on grain yield and WUE although the inoculum was still viable. However, it may not have been sufficiently viable to establish infection on the roots. There was not enough time to repeat the experiment, and it would still be a good idea to compare the effects of *Pythium* and root pruning during early stages of vegetative growth, as had been done for hydroponics. The experiment did show the importance of the stage at which root damage occurs and how it affects total WUE.

Chapter 7: General Discussion and Future Directions

The main concern of the current project was to investigate the effect of root diseases on water relations and productivity of bread wheat under limited water regimes. Many factors may play a vital role in the response of water relations and physiological growth of wheat to root infection by pathogens. These factors may include growth stage, genotypes, growth media either in soil or nutrient solution, inoculum density and pattern of inoculation, duration and time of water stress and water soil availability to the plant and other factors.

Hypothetically, diseases decrease the ability of the plant to take up and use water (transpiration) resulting in reductions in grain yield, biomass or photosynthetic rate. In this study, grain yield, yield components (number of tillers and heads), biomass and harvest index of wheat were not affected by root diseases either in small or large pots (which reflect wheat growth under field conditions). It has been reported that *Pythium* reduces grain yield in wheat due to reductions in number of tillers and heads which is related to a decrease in root mass, resulting in poor water and nutrient uptake (Weller and Cook, 1986). Severe disease has a large effect in reduction of grain yield due to reduced water uptake (Weller and Cook, 1986) and stomatal conductance or photosynthesis (Goodman *et al.,* 1986) and hence reduced WUE. However, *Pythium* induced at low inoculum levels in this study had no effect on grain yield and shoot biomass. Similarly, Kirkegaard *et al*. (1999) found that low infection by *Rhizoctonia* had no effect on shoot and root biomass, but at higher infection levels, there was loss of root length of over 60% which could be expected to reduce the capacity for adequate water and nutrient uptake by the plant and hence reduce leaf growth. However, the nature of reduced growth is unclear. James *et al*. (1997) showed that the shoot growth of wheat was impaired when 60% of the root length was removed. Root length was not measured during the

course of these experiments but the results of hydroponics showed that the *Pythium* had no effect on root biomass when the plants were harvested at booting stage.

Grain yield depends on water availability at critical times. Grain yield is not just dependent on assimilation rate but also on how much of that assimilate is used for vegetative growth or transported to the grain, and how much is used for other purposes (for example root growth, osmotic adjustment etc). Grain yield is correlated with leaf photosynthesis and translocation of assimilate to the spike. Grain yield is reduced when photosynthesis is restricted under post-anthesis water stress (Wardlaw *et al*., 1989). In the large pot experiment (Chapter 4), photosynthesis was not affected by *Pythium* under post-anthesis drought. Passioura (1977) indicated that the grain yield of wheat depends more on water use after anthesis than on the total amount of water used by the plant. There was no difference between infected and uninfected plants in cumulative transpiration in the second large pot experiment (Chapter 4) between anthesis and harvest, thus grain yield was not affected by disease. *Pythium* reduced shoot dry weight and increased root/shoot ratio in hydroponics. The reduction in shoot biomass may result from impaired photosynthesis in these diseased plants but it is also possible that portions of these reductions resulted from direct or indirect effects of root rot on the host (Whiley *et al*., 1986).

Two possible things occurred when the roots were damaged by *Pythium*: either water or nutrient uptake were reduced, leading directly to reduced transpiration and hence shoot growth; or signaling from the root system reduced shoot growth to maintain root/shoot ratios. This whole area is very poorly understood, but it is possible that plants reduce shoot growth if roots are damaged or missing in order to keep the plant in balance, using hormonal signals from the roots (Davies *et al*., 1994; Davies and Zhang, 1991). If there is a smaller shoot

system, then total transpiration per plant will be reduced because of the reduced leaf area. In hydroponic experiments, the root damage by *Pythium* may have reduced shoot growth by both mechanisms. However, quantification of hormonal signals from the infected roots needs to be tested (Kirkegaard *et al*., 1999).

Pythium increased root lesion percentage on roots. Root infection by fungal disease can result in the loss of root hairs and fine rootlets of wheat. The pathogen has the ability to infect the roots and proliferates in the cortex causing cell collapse. The results of the hydroponic experiment showed that the effect of *Pythium* on the root system was different from those of root pruned plants because *Pythium* did not reduce root mass. On the other hand, 30-40% of the root mass had to be removed by root pruning to achieve the same reduction in transpiration as infection with *Pythium*. Therefore, the primary damage of *Pythium* may be due to reduced efficiency of roots rather than restricted root growth. In other words, the effective size of the root system was reduced. Sharma (1987) investigated the effect of root pruning on wheat water relations and found that root pruning did not reduce leaf dry weight, even when 60% of the root mass was removed. Pillinger *et al*. (2005) suggested that as long as the length of healthy root remains above a certain threshold, root damage by take-all will have little effect on water and nitrogen uptake. This observation suggests that wheat root systems are larger than they need to be for normal function under non-stress conditions. Pillinger *et al.* (2005) suggested that take-all epidemics reduced the effectiveness of the existing roots rather than reducing root growth. The same thing could be happening with *Pythium* where diseased roots were less able to absorb or transport water than healthy ones.

In this study, infection by *Pythium* either decreased or did not change total or cumulative water use. This may depend on inoculum density corresponding to the soil depth. For

example, 10 g of *Pythium*-colonised millet seeds per pot resulted in a significant decrease of total water use when distributed or mixed within the top of 30 cm soil. However, inoculum densities between 0.1 and 5 g of *Pythium*-colonised millet seeds per pot at 20 cm soil depth did not decrease the total water use (Chapter 4). It is not possible to relate these inoculum densities to those occurring in the field, because *Pythium* populations are very difficult to quantify.

Pythium reduced transpiration when water was readily available. This was seen in hydroponics (Chapter 5) and in early growth in pots (Chapter 4). The higher transpiration with *Pythium* (during droughts) was seen when comparing soils with differences in water availability. Presumably at this stage in growth, transpiration would still be lower in the *Pythium* than controls if they had the same water content. The effect of *Pythium* on weekly transpiration rate provided a better indicator for the pattern of water use during the growing stage of the plant. In the first experiment (Chapter 3), *Pythium* reduced transpiration at certain times after vegetative and anthesis drought and this was cultivar dependent. In other words, the effects of disease on water use may differ with response of genotypes to drought. In large pot experiments (Chapter 4), transpiration per week was reduced at early growth stages when the roots were infected by *Pythium* which causes root damage and reduced root efficiency to water uptake. At later growing stages, transpiration of infected plants increased as more soil water was available to plants compared with controls. *Pythium* infected plants had not used all of the available water at the time of harvest. Some tillers still had green heads because there was still water available. As a result, grain yield of plants infected by *Pythium* did not differ from controls but if infected plants were harvested later then grain yield may have increased.

Generally, *Pythium* reduced daily and weekly transpiration of hydroponically grown wheat and this was not due to a decrease of root mass but rather by reduction of root efficiency to take up the solution or possibly due to reduced stomatal conductance, although the effect of *Pythium* on g_s was not significant. Absorption of water by diseased plants may not be utilised efficiently as they lack effective mechanisms to regulate internal water balance (Rahi *et al*., 1988). Generally, midday leaf water potential (ψ) is correlated with active transpiration as stomata open during the day. In deep pot experiments (Chapter 4), stomatal conductance had a relationship (r^2=0.74) with midday leaf ψ of infected plants. However, this relationship was not found in control plants because they varied stomatal conductance to maintain constant leaf ψ while plants infected by *Pythium* seemed to be unable to use stomatal conductance to regulate water potential (Jones, 1998).

The pattern of soil water depletion in winter wheat diseased by *Cephalosporium gramineum* was described by Martin *et al*. (1986). There was a similar pattern in average soil water depletion responses for the control and diseased situation. The differences in soil water depletion between control and diseased plants of wheat increased with time at each depth increment, and these differences became apparent progressively later in the season with increasing soil depth. The infection by *C. gramineum* may have reduced root density throughout the soil profile if it is assumed that soil water depletion is an accurate index of root concentration. However, rooting density may be unaffected and the reduction in soil water extraction may be due to reduced root activity and/or reduced transpiration of diseased plants. The findings from this study could be similar to the results of Martin *et al*. (1986) in that reduced transpiration of plants infected by *Pythium* led to reduced extraction of soil water.

In Chapter 3, root diseases had no effect on total water potential (ψ), pressure potential and relative water content (RWC) under tillering drought (D1). However, diseased plants had lower osmotic potentials (π) than controls after 7 days. This can be interpreted as a passive reduction in osmotic potential due to declining RWC or active osmotic adjustment by the diseased plants, in response to the drought. It could be that the pathogen itself had a major effect on the extent of the reduction in osmotic potential. Under anthesis drought (D2), total leaf water potential and its components did not change due to *Pythium* but diseased plants had higher RWC than controls. This could be due to higher soil water available to diseased plants. In large pot experiments (Chapter 4), *Pythium* had no effect on predawn leaf ψ possibly due to the equilibrium of plant water with soil water during the night (Davis and Mooney, 1986) and equilibrium with the wettest portion of the soil in the plant's root zone (Ameglio *et al*., 1999). The effect of *Pythium* on plant water relations (water potential and its components and RWC) in hydroponics was different when compared with the effect under soil. All parameters of plant water relations including water potential and its components, and RWC were not affected by *Pythium* under hydroponic conditions although transpiration rate and stomatal conductance were decreased. This could be because the level of stress was less than in drying soil. Pre-dawn and midday water potentials in hydroponics were similar to those at the start of drought in the large pots.

The variation in g_s affects transpiration proportionally more than it affects photosynthesis (Martin and Ruiz-Torres, 1992). Transpiration rate and stomatal conductance were increased by *Pythium* after 7 days of water stress compared with controls. This could be explained also by more water available for infected plants at that time of drought. Conversely, there was no reduction of instantaneous transpiration rate and g_s by *Pythium* compared with controls as

water stress progressed, because while the amount of soil water available was being reduced by drought in the *Pythium* treatment it was still relatively higher than those in controls. Therefore, the negligible effect of *Pythium* on transpiration rate may need further investigation and it could be that nonstomatal factors that prevent increase in transpiration.

Pythium had no effect on intercellular CO_2 concentration $[CO_2]_i$ either in wheat grown in soil or solution. The stable $[CO_2]_i$ under *Pythium* inoculation while photosynthesis decreased under hydroponics, indicates that photosynthesis was not limited only by stomatal control but possibly also by reduction of photosynthetic capacity of the leaves. Similar findings were obtained by Balota *et al*. (2005) for the effect of take-all disease on gas-exchange of wheat but under soil pot conditions. Ayers (1978) indicated that plant pathogens may immobilise stomata or lower stomatal resistance resulting in lower WUE of the plant. Often, if assimilation is reduced, the increased $[CO_2]_i$ in the leaf mesophyll results in decreased conductance (Farquhar and Sharkey, 1982). In this study, the reduction of conductance was not related with $[CO_2]_i$. It was not clear whether reduced photosynthesis was the effect or cause of reduced conductance in this work.

The effect of water availability has a major role in WUE. There was a lower instantaneous WUE in inoculated plants in the second large pot experiment prior to drought (Chpater 4) but WUE_{grain} was increased. Although the infected plants are were not using water as efficiently for much of the time, the presence of extra water at critical times late in growth obviously compensated for this. Evrendilek *et al*. (2008) found that WUE of wheat was the lowest during the middle of the day, when temperatures are high and relative humidity low. Transpiration seems to increase faster than photosynthesis during the middle of the day,

leading to lower WUE than in the morning or late afternoon. Differences in WUE may depend on whether plants have stomata open for different periods during the day.

WUE of wheat under drought either increased or did not change as a result of infection by *Pythium* dependent on transpiration and grain yield and/or biomass. Variation in WUE is affected more by variation in water use than in biomass under limited water regimes (Blum, 2005). For example, there was no *Pythium* effect on WUE in small pots (Chapter 3) and large pots with different inoculum density (Chapter 4) because total water use and grain yield or total biomass did not change under these conditions. Similarly, WUE calculated on total root and shoot basis under hydroponic conditions did not change with *Pythium* infection despite the fungus reducing transpiration. WUE increased only when the plants were inoculated with 10 g/pot of *Pythium* as there was a reduction of transpiration with no change in grain yield or total biomass. The most important reason for increased WUE may be the higher soil water available at the grain filling stage of infected plants which may prevent reduction of grain yield.

Pythium in deep pot experiments (Chapter 4) reduced both photosynthesis (A) and instantaneous WUE prior to the drought perhaps due to higher activity of *Pythium* in moist soil. This was also seen by for example by Balota *et al.*(2005) with take-all. Therefore, WUE is reduced by disease in the short term. Instantaneous WUE of infected plants did not differ from controls under post-anthesis drought in seconf large pot experiment. There was a strong correlation between E and g_s in deep pot experiments. The correlation coefficient was 0.98 for controls and 0.96 for inoculated plants, so transpiration is controlled by stomatal conductance rather than by root factors.

There was a significantly different relationship between A and E for infected and control plants (Chapter 4). Photosynthetic rate was higher for a given E in controls than *Pythium*. Since A/E is instantaneous WUE, this is another indication that instantaneous WUE was reduced by *Pythium*. This was also found by Balota *et al.* (2005) for take-all disease Ggt in which the ratio A:E was significantly reduced by inoculation with take-all disease disease Ggt. The difference between *Pythium* and controls decreased as drought progressed.

In hydroponics experiments (Chapter 5), the results were less variable than in soil. *Pythium* did not reduce instantaneous WUE although both photosynthetic rate and instantaneous transpiration rate were reduced. It seems from this study that the differences in the effect of *Pythium* on stomatal conductance, transpiration rate and photosynthesis between hydroponics and the 10 g/pot *Pythium* inoculum experiment can be explained by the hydroponic system being at higher water potential than the soil.

In conclusion, WUE_{grain} and grain yield were not affected by the interaction between drought and root diseases (Chapter 3). Similarly, low inoculum densities of *Pythium* in large pot experiments (Chapter 4) indicated that there was no interaction between drought and disease in their effects on WUE and grain yield. However, WUE was increased when inoculum density of *Pythium* increased to 10 g/pot under post-anthesis drought, with no effect on grain yield, because reduced transpiration during early growth led to greater water availability in the critical period of grain filling. However, diseased plants were not able to access all of the additional water and grain yields were not different from controls at harvest. A similar phenomenon was seen with root pruning (Chapter 6) where more water was available and transpiration was higher in pruned pots during post-anthesis drought, but the plants could not access all of this water. It may be possible to increase grain yield of diseased plants if they are

able access all of the additional water before being harvested. However this may be difficult if disease continues to restrict the function of newly produced roots.

Therefore, this study showed the importance of the interaction between disease at specific levels of inoculum density and drought in increasing WUE. No literature was found with results like this because in general the diseases in preceding studies showed decreased WUE and grain yield particularly with limited water resources. However, there is some literature on root pruning (Ma *et al*., 2010) that showed increased yield when transpiration was reduced at particular times by root pruning.Wheat production in rainfed environments such as Jordan is limited by water availability. Whether the productivity of wheat is either increased or decreased when interacting with biotic stress such as *Pythium* may depend on the severity of disease. This needs to be tested under field conditions.

Another important part of this research was to find if there is a mechanism which affects the ability of infected plants to use available water during water stress. *Pythium* reduced water uptake from the soil and water use (transpiration) of wheat as the roots were damaged by the disease at early (vegetative) stages of growth and prior to drought. It could be that the mechanism of reduced water uptake by diseased plants is explained by a reduced efficiency of roots in extracting water from the soil rather than reduced total mass of the roots. Therefore, when water absorption by the roots is reduced in diseased plants, then more water will be available in the soil at later stages of growth or when the plants are exposed to post anthesis drought. As a result, water uptake may be increased in diseased plants due to higher water availability in the soil rather than increased efficiency of infected roots.

Another objective of this study was to test if there was a difference between root damage caused by disease and mechanical root pruning. It seems that root damage caused by *Pythium* did not reduce root size under drought but reduced the efficiency of roots due to this damage. Conversly, root pruning reduced the size of the root system.

This study investigated how the interaction between root diseases and drought can affect WUE of wheat. The findings of this thesis have, however, provided directions as to further research.

Further work is required on the optimum inoculum density of *Pythium* required for infection. An increase in the inoculum density to 20 -100g colonised *Pythium*/4kg soil should be tested and determinations made of root damage and lesion percentage to be used for disease incidence.

The effect of *Pythium* on root function in the hydroponic study indicated that there was reduced water uptake. This could be either due to reduced hydraulic conductivity of roots or signalling from the root system reducing shoot growth to maintain a constant root/shoot ratio. Further study is, therefore, required to measure changes in hydraulic conductivity and to investigate the role of stress hormones produced in the root on shoot growth.

The comparison of effects between root pathogens such as *Pythium* and root pruning on WUE needs further investigation. Experimentation is required to investigate root pruning at earlier stages and with higher inoculum densities of *Pythium* with some modifications in intensity of root pruning applied. In addition, measurements of water relations and photosynthesis should be done to compare water status and gas exchange rates in both treatments and to understand more of the physiological and biochemical mechanisms.

Root length and distributions within soil profiles should be measured to provide an explanation of soil water extraction patterns under *Pythium* infection and root pruning treatments. Furthermore, infected plants grown in large pots were found not to have utilised all available water up to maturation. It is unclear whether this is due to *Pythium*-damaged roots being unable to access water deeper in the profile or poor hydraulic conductivity. Measurements should, therefore, be made of root length and root distribution with depth along with changes in water content with depth. This would provide an explanation of soil water extraction patterns under *Pythium* infection.

Photosynthesis of infected plants was not affected under drought in the large pot (soil) experiment although the response patterns of stomatal conductance changed in infected plants and photosynthesis decreased prior to drought. There may be biochemical factors relating to hormones, nutrients or carbohydrate dynamics that are causing these changes in photosynthesis and stomatal function and these should be further investigated. However, photosynthesis was reduced by *Pythium* in hydroponic cultures, and it is not clear if this was due to either root pathogen factors or nutrient deficiency in the leaves. Therefore, further experimentation is needed to investigate and analyse the effects of magnesium (Mg) contents in the leaves. It is well known that Mg deficiency has an effect on photosynthesis as it is a basic component of the chlorophyll molecule. This study will also be useful to investigate the effect Mg has on CO_2 uptake in the leaves, that has been attributed to changes in stomatal diffusion resistance (Terry and Ulrich, 1974). Chlorophyll concentration should also be measured. Nitrogen content is another important element to analyse because it has an effect on photosynthesis. Nitrogen analysis would be combined with carbohydrate status as N deficiency causes accumulation of carbohydrates in the leaves, thus inhibiting photosynthesis.

Nutrient deficiencies also cause more carbon to be allocated to roots and thus alter root/shoot ratio (Hermans *et al*., 2006). It would be better to extend the growing period for wheat in hydroponics to late anthesis or harvest by using larger pots and increase concentrations of PEG to investigate the effects of these interactions on WUE.

In this study, a significant correlation was found between midday leaf water potential and stomatal conductance in diseased plants but not in controls, suggesting that movement of ABA from roots to leaves could have an important role in stomatal control of infected plants. This needs further investigation. Experiments are also needed to explore the differing relationships for inoculated and control plants between stomatal conductance and soil water status, evaporative demand and endogenous hormones.

A much better understanding of plant responses to pathogens may lead to improve WUE and grain yield which could be used in breeding programs. Therefore, future work would be necessary at a whole-plant level and in the field, as well as cellular and molecular levels to obtain a better understanding of physiological processes occurring during response to root pathogens under limited water resources.

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Appendix

Chapter 3: Interactive effects of drought and fungal root diseases on water use efficiency of wheat

Growth stage at tillering drought (Fig. 3.1)

Dependent Variable:GS1

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	13.391	2	6.695	0.79	0.461
Variety	628.787	1	628.787	74.173	0
Fungus	0.229	2	0.115	0.014	0.987
GS1drought	1705.387	1	1705.387	201.17	0
Fungus * GS1drought	232.795	2	116.398	13.73	0
Variety * Fungus	151.904	2	75.952	8.959	0.001
Variety * GS1drought	123.82	1	123.82	14.606	0
Variety * Fungus * GS1drought	307.103	2	153.551	18.113	0
Error	313.661	37	8.477		
Total	3457.403	50			

Dependent Variable:GS1

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	13.391	2	6.695	0.79	0.461
GS1treat	3092.745	11	281.159	33.166	0
Error	313.661	37	8.477		
Total	3457.403	50			

Growth stage at anthesis drought (Fig. 3.2)

Dependent Variable:GS2

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	22.443	2	11.222	1.101	0.345
Variety	52.291	1	52.291	5.131	0.031
Fungus	17.34	2	8.67	0.851	0.437
Drought	582.323	2	291.161	28.572	0
Fungus * Drought	21.706	4	5.426	0.533	0.713
Variety * Drought	72.267	2	36.133	3.546	0.041
Variety * Fungus	65.632	2	32.816	3.22	0.054
Variety * Fungus * Drought	77.88	4	19.47	1.911	0.134
Error	315.909	31	10.191		
Total	1191.176	50			

Dependent Variable:GS2

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	22.443	2	11.222	1.101	0.345
Treat	822.375	17	48.375	4.747	0
Error	315.909	31	10.191		
Total	1191.176	50			

Transpiration during the 7-day droughts (Fig. 3.5)

Dependent Variable:Drought1

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	54282.692	1	54282.692	1.678	.210
Fungus	322.738	2	161.369	.005	.995
Water	1.150E7	1	1.150E7	355.507	.000
Block	34710.833	2	17355.417	.536	.593
Variety * Fungus	21720.717	2	10860.359	.336	.719
Fungus * Water	38621.680	2	19310.840	.597	.560
Variety * Water	30185.256	1	30185.256	.933	.346
Variety * Fungus * Water	24843.326	2	12421.663	.384	.686
Error	647139.167	20	32356.958		
Total	3.203E7	34			

a. R Squared = .949 (Adjusted R Squared = .916)

Dependent Variable:Drought2

Source	Sum of Squares	df	Mean Square	F	Sig.
Variety	17336.111	1	17336.111	1.418	.247
Fungus	11772.222	2	5886.111	.481	.624
Water	1.117E7	1	1.117E7	913.057	.000
Block	44272.222	2	22136.111	1.810	.187
Variety * Fungus	55505.556	2	27752.778	2.269	.127
Fungus * Water	22238.889	2	11119.444	.909	.417
Variety * Water	625.000	1	625.000	.051	.823
Variety * Fungus * Water	32616.667	2	16308.333	1.333	.284
Error	269061.111	22	12230.051		
Total	5.542E7	36			

a. R Squared = .977 (Adjusted R Squared = .963)

Water relations at tillering drought (Fig. 3.6)

Dependent Variable:WPD11

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.52	2	0.26	1.329	0.308
Variety	0.062	1	0.062	0.316	0.587
Fungus	0.168	2	0.084	0.428	0.663
Error	1.957	10	0.196		
Total	2.693	15			

Dependent Variable:OPD11

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.001	2	0	0.282	0.759
Variety	0.01	1	0.01	5.77	0.033
Fungus	0.008	2	0.004	2.445	0.129
Error	0.021	12	0.002		
Total	0.04	17			

Dependent Variable:PPD11

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.537	2	0.269	1.257	0.326
Variety	0.021	1	0.021	0.096	0.763
Fungus	0.186	2	0.093	0.435	0.659
Error	2.137	10	0.214		
Total	2.864	15			

Dependent Variable:RWCD11

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	6.32	2	3.16	0.709	0.512
Variety	28.183	1	28.183	6.323	0.027
Fungus	22.318	2	11.159	2.504	0.123
Error	53.487	12	4.457		
Total	110.307	17			

Dependent Variable:WPD12

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	2.186	2	1.093	1.986	0.163
Variety	0.9	1	0.9	1.636	0.216
Fungus	3.069	2	1.535	2.789	0.085
Drought	29.571	1	29.571	53.741	0
Fungus * Drought	3.014	2	1.507	2.739	0.089
Variety * Drought	0.449	1	0.449	0.816	0.377
Variety * Fungus	2.085	2	1.042	1.895	0.176
Variety * Fungus * Drought	0.389	2	0.194	0.353	0.707
Error	11.005	20	0.55		
Total	55.85	33			

Dependent Variable:OPD12

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.19	2	0.095	1.04	0.372
Variety	0.059	1	0.059	0.65	0.429
Fungus	0.685	2	0.343	3.753	0.041
Drought	10.658	1	10.658	116.718	0
Fungus * Drought	0.59	2	0.295	3.229	0.061
Variety * Drought	0.128	1	0.128	1.407	0.249
Variety * Fungus	0.623	2	0.311	3.409	0.053
Variety * Fungus * Drought	0.349	2	0.174	1.909	0.174
Error	1.826	20	0.091		
Total	15.657	33			

Dependent Variable:PPD12

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	1.101	2	0.551	0.671	0.523
Variety	0.496	1	0.496	0.605	0.446
Fungus	1.207	2	0.603	0.735	0.492
Drought	4.72	1	4.72	5.748	0.026
Fungus * Drought	1.364	2	0.682	0.83	0.45
Variety * Drought	0.097	1	0.097	0.118	0.735
Variety * Fungus	0.603	2	0.302	0.367	0.697
Variety * Fungus * Drought	0.234	2	0.117	0.142	0.868
Error	16.423	20	0.821		
Total	27.119	33			

Dependent Variable:RWCD12

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	79.561	2	39.78	0.658	0.529
Variety	30.733	1	30.733	0.508	0.484
Fungus	213.297	2	106.648	1.764	0.197
Drought	18695.33	1	18695.33	309.216	0
Fungus * Drought	76.818	2	38.409	0.635	0.54
Variety * Drought	0.976	1	0.976	0.016	0.9
Variety * Fungus	292.366	2	146.183	2.418	0.115
Variety * Fungus * Drought	105.874	2	52.937	0.876	0.432
Error	1209.209	20	60.46		
Total	20873.7	33			

Water relations at anthesis drought (Fig. 3.7)

Dependent Variable:WPD21

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.289	2	0.144	0.679	0.529
Variety	5.206	1	5.206	24.501	0.001
Fungus	0.862	2	0.431	2.03	0.182
Variety * Fungus	0.06	2	0.03	0.141	0.87
Error	2.125	10	0.212		
Total	8.541	17			

Dependent Variable:OPD21

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.014	2	0.007	0.724	0.508
Variety	0.004	1	0.004	0.418	0.533
Fungus	0.008	2	0.004	0.431	0.662
Variety * Fungus	0.011	2	0.005	0.578	0.579
Error	0.094	10	0.009		
Total	0.131	17			

Dependent Variable:PPD21

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.24	2	0.12	0.613	0.561
Variety	4.919	1	4.919	25.081	0.001
Fungus	0.844	2	0.422	2.151	0.167
Variety * Fungus	0.042	2	0.021	0.107	0.899
Error	1.961	10	0.196		
Total	8.007	17			

Dependent Variable:RWCD21

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	40.048	2	20.024	1.56	0.257
Variety	11.506	1	11.506	0.896	0.366
Fungus	81.741	2	40.87	3.184	0.085
Variety * Fungus	76.005	2	38.003	2.96	0.098
Error	128.38	10	12.838		
Total	337.679	17			

Dependent Variable:WPD22

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Variety	0.751	1	0.751	0.77	0.39
Fungus	1.564	2	0.782	0.802	0.461
Drought	314.235	1	314.235	322.124	0
Fungus * Drought	1.572	2	0.786	0.806	0.46
Variety * Drought	0.645	1	0.645	0.662	0.425
Variety * Fungus	0.4	2	0.2	0.205	0.816
Variety * Fungus * Drought	0.121	2	0.061	0.062	0.94
Error	21.461	22	0.976		
Total	344.706	35			

Dependent Variable:OPD22

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.186	2	0.093	0.516	0.604
Variety	0.017	1	0.017	0.092	0.765
Fungus	0.363	2	0.182	1.005	0.382
Drought	80.941	1	80.941	447.798	0
Fungus * Drought	0.492	2	0.246	1.36	0.277
Variety * Drought	0	1	0	0.001	0.975
Variety * Fungus	0.006	2	0.003	0.017	0.983
Variety * Fungus * Drought	0.064	2	0.032	0.177	0.839
Error	3.977	22	0.181		
Total	86.046	35			

Dependent Variable:PPD22

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	2.821	2	1.41	1.475	0.251
Variety	0.535	1	0.535	0.56	0.462
Fungus	2.974	2	1.487	1.555	0.234
Drought	76.184	1	76.184	79.647	0
Fungus * Drought	3.362	2	1.681	1.758	0.196
Variety * Drought	0.627	1	0.627	0.655	0.427
Variety * Fungus	0.335	2	0.167	0.175	0.841
Variety * Fungus * Drought	0.207	2	0.104	0.108	0.898
Error	21.043	22	0.957		
Total	108.089	35			

Dependent Variable:RWCD22

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	97.601	2	48.8	2.833	0.08
Variety	17.872	1	17.872	1.038	0.319
Fungus	120.309	2	60.154	3.492	0.048
Drought	25655.29	1	25655.29	1489.38	0
Fungus * Drought	51.671	2	25.835	1.5	0.245
Variety * Drought	8.017	1	8.017	0.465	0.502
Variety * Fungus	10.652	2	5.326	0.309	0.737
Variety * Fungus * Drought	20.48	2	10.24	0.594	0.56
Error	378.961	22	17.225		
Total	26360.86	35			

Water use efficiency based on grain yield (Fig. 3.8)

Dependent Variable: WUE g per L

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.254	2	0.127	6.332	0.005
Variety	0.066	1	0.066	3.27	0.08
Fungus	0.009	2	0.004	0.216	0.807
Drought	10.264	2	5.132	255.984	0
Fungus * Drought	0.02	4	0.005	0.248	0.909
Variety * Drought	0.007	2	0.003	0.172	0.842
Variety * Fungus	0.017	2	0.008	0.416	0.663
Variety * Fungus * Drought	0.039	4	0.01	0.491	0.742
Error	0.642	32	0.02		
Total	11.281	51			

Water use efficiency based on total dry matter (Fig. 3.9)

Dependent Variable: WUE dw per L

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	1.46	2	0.73	19.729	0
Variety	0.036	1	0.036	0.964	0.334
Fungus	0.08	2	0.04	1.085	0.35
Drought	2.436	2	1.218	32.918	0
Fungus * Drought	0.013	4	0.003	0.085	0.986
Variety * Drought	0.139	2	0.07	1.882	0.169
Variety * Fungus	0.059	2	0.03	0.801	0.458
Variety * Fungus * Drought	0.132	4	0.033	0.893	0.479
Error	1.184	32	0.037		
Total	5.295	51			

Lesion percentage (Fig. 3.10)

Dependent Variable: Lesion

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.15	2	0.075	9.827	0
Variety	0.024	1	0.024	3.094	0.088
Fungus	0.213	2	0.107	13.965	0
Drought	0.045	2	0.023	2.973	0.065
Fungus * Drought	0.017	4	0.004	0.549	0.701
Variety * Drought	0.022	2	0.011	1.446	0.25
Variety * Fungus	0.062	2	0.031	4.053	0.027
Variety * Fungus * Drought	0.052	4	0.013	1.716	0.171
Error	0.244	32	0.008		
Total	0.836	51			

Chapter 4: Effect of *Pythium* on water use efficiency and gas-exchange rates of wheat under drought

Experiment 1: Cumulative transpiration (Fig. 4.1)

Dependent Variable:CumFeb7

Source	Sum of Squares	df	Mean Square	F	Sig.
Inoculum	3762741	4	940685.2	2.561	0.067
Error	8079667	22	367257.6		
Total	1.18E+07	26			

Experiment 1: Transpiration per week (Fig. 4.2)

Dependent Variable:Jan27

Source	Sum of Squares	df	Mean Square	F	Sig.
Inoculum	597851.852	4	149463	3.11	0.036
Error	1057333.333	22	48060.61		
Total	1655185.185	26			

Dependent Variable:Feb23

Source	Sum of Squares	df	Mean Square	F	Sig.
Inoculum	419250	4	104812.5	3.86	0.016
Error	597416.667	22	27155.3		
Total	1016666.667	26			

Experiment 2: Transpiration per week (Fig. 4.5)

Date	25/6/2010	1/7/2010	7/7/2010	14/7/2010	4/8/2010	10/8/2010
Control	785.27	667.5333	594.0667	616.6667	308.7167	-0.333333
Pythium	547.77	430.0333	414.9	345.8333	554.55	170.5
t test	0.007929	0.002147	0.056143	1.53E-08	0.003617	0.0215917

Experiment 2: T tests for water use efficiency and yield components (Table 4.3 and Fig. 4.6)

Grain weight	WUE grain	Dry wt.	HI	Heads/plant	WUE DW
0.113861504	8.07915E-05	0.910487	0.33096	0.895497961	0.019407

Experiment 2: T tests for Li-Cor (Table 4.10 - Fig. 4.14)

Day	Assimilation	Conductance	Carbon internal	Transpiration	WUE i	WUE (A/gs)
0	0.003125	0.156307	0.319553	0.434489	0.006318	0.488784
7	0.142242	0.034654	0.930989	0.001924	0.482336	0.867339
14	0.643896	0.506166	0.586638	0.257941	0.994176	0.654892
21	0.073195	0.237066	0.185268	0.51178	0.191663	0.178421

Experiment 2: Regression data

Assimilation regressed on transpiration (Fig. 4.15)

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	-2.204	0.763		-2.888	0.006
	Transpiration	3.009	0.178	1.05	16.94	0
	IEinteract	-0.063	0.017	-0.231	-3.724	0.001

a. Dependent Variable: Assimilation

Assimilation regressed on stomatal conductance (Fig. 4.16)

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	0.408	0.948		0.431	0.669
	Conductance	57.929	5.153	0.919	11.241	0
	IGinteract	-0.652	0.588	-0.091	-1.109	0.274

a. Dependent Variable: Assimilation

Midday WP and Gs for controls (Fig. 4.17)

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	0.037	0.312		0.119	0.907
	WPcont	-0.088	0.211	-0.131	-0.417	0.685

a. Dependent Variable: Gscont

Midday WP and Gs for *Pythium* (Fig.4.17)

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	0.46	0.105		4.389	0.001
	WPPyth	0.173	0.059	0.68	2.933	0.015

a. Dependent Variable: GsPyth

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	-1.4	0.116		-12.098	0
	PDCont	0.064	0.099	0.202	0.651	0.53

a. Dependent Variable: WPcont

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	-1.014	0.111		-9.097	0
	PDPyth	0.689	0.092	0.921	7.466	0

a. Dependent Variable: WPPyth

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	109.687	5.389		20.355	0
	Gs	-135.798	20.166	-0.749	-6.734	0
	PyGs	-38.801	17.028	-0.254	-2.279	0.032

a. Dependent Variable: WUEintrinsic

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	3.173	0.198		15.992	0
	Gs	-1.159	0.742	-0.227	-1.562	0.131
	PyGs	-2.744	0.627	-0.635	-4.376	0

a. Dependent Variable: WUEinst

Chapter 4: hydroponic experiments

Experiment 1: Transpiration per week (Fig. 5.4)
Dependent Variable:Week7Aug

Source	Sum of Squares	df	Mean Square	F	Sig.
PEG	18408.33	1	18408.33	16.13	0
Root	40394.79	2	20197.4	17.698	0
PEG * Root	9513.542	2	4756.771	4.168	0.022
Error	47931.25	42	1141.22		
Total	116247.9	47			

Dependent Variable:Week14Aug

Source	Sum of Squares	df	Mean Square	F	Sig.
PEG	78813.02	1	78813.02	27.499	0
Root	97219.79	2	48609.9	16.961	0
PEG * Root	33082.29	2	16541.15	5.772	0.006
Error	120371.9	42	2865.997		
Total	329487	47			

Experiment 1: Plant growth (Table 5.2) and WUE (Fig. 5.6)
Dependent Variable: Root DW

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.072	7	0.01	0.713	0.662
PEG	0.051	1	0.051	3.535	0.068
Root	0.462	2	0.231	16.12	0
PEG * Root	0.022	2	0.011	0.751	0.479
Error	0.502	35	0.014		
Total	1.108	47			

Dependent Variable: Shoot DW

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.827	7	0.118	0.795	0.597
PEG	0.996	1	0.996	6.701	0.014
Root	2.541	2	1.27	8.548	0.001
PEG * Root	1.273	2	0.636	4.281	0.022
Error	5.202	35	0.149		
Total	10.838	47			

Dependent Variable:R/S

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.022	7	0.003	1.224	0.316
PEG	0.005	1	0.005	2.02	0.164
Root	0.046	2	0.023	8.879	0.001
PEG * Root	0.045	2	0.022	8.595	0.001
Error	0.091	35	0.003		
Total	0.21	47			

Dependent Variable: WUE Shoot

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	1.054	7	0.151	4.165	0.002
PEG	0.025	1	0.025	0.69	0.412
Root	0.242	2	0.121	3.349	0.047
PEG * Root	0.533	2	0.267	7.372	0.002
Error	1.265	35	0.036		
Total	3.119	47			

Experiment 2: Transpiration per week (Fig. 5.5)

Dependent Variable:Week2Oct

Source	Sum of Squares	df	Mean Square	F	Sig.
PEG	84168.75	1	84168.75	25.016	0
Root	83626.04	2	41813.02	12.427	0
PEG * Root	43634.38	2	21817.19	6.484	0.004
Error	141312.5	42	3364.583		
Total	352741.7	47			

Dependent Variable:Week9Oct

Source	Sum of Squares	df	Mean Square	F	Sig.
PEG	305602.1	1	305602.1	88.791	0
Root	163240.6	2	81620.31	23.714	0
PEG * Root	51601.04	2	25800.52	7.496	0.002
Error	144556.3	42	3441.815		
Total	665000	47			

Experiment 2: Plant growth (Table 5.3) and WUE (Fig. 5.7)

Dependent Variable: Root DW

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.209	7	0.03	1.714	0.138
PEG	0.214	1	0.214	12.276	0.001
Root	0.482	2	0.241	13.84	0
PEG * Root	0.082	2	0.041	2.358	0.109
Error	0.61	35	0.017		
Total	1.597	47			

Dependent Variable: Shoot DW

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	3.207	7	0.458	1.338	0.262
PEG	5.121	1	5.121	14.958	0
Root	5.408	2	2.704	7.897	0.001
PEG * Root	3.628	2	1.814	5.299	0.01
Error	11.984	35	0.342		
Total	29.348	47			

Dependent Variable:R/S

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.028	7	0.004	2.634	0.027
PEG	0.001	1	0.001	0.675	0.417
Root	0.028	2	0.014	9.123	0.001
PEG * Root	0.008	2	0.004	2.526	0.094
Error	0.053	35	0.002		
Total	0.118	47			

Dependent Variable: WUE Shoot

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Block	3.831	7	0.547	3.81	0.004
PEG	0.172	1	0.172	1.197	0.281
Root	0.507	2	0.254	1.766	0.186
PEG * Root	0.832	2	0.416	2.898	0.068
Error	5.027	35	0.144		
Total	10.369	47			

Experiment 2: Plant water relations (Fig. 5.9)

Dependent Variable: Osmotic

Source	Sum of Squares	df	Mean Square	F	Sig.
PEG	0.209	1	0.209	23.782	0
Root	0.084	2	0.042	4.794	0.021
PEG * Root	0.082	2	0.041	4.664	0.023
Error	0.158	18	0.009		
Total	0.533	23			

Dependent Variable: RWC

Source	Sum of Squares	df	Mean Square	F	Sig.
PEG	70.951	1	70.951	21.617	0
Root	3.843	2	1.922	0.585	0.567
PEG * Root	19.029	2	9.515	2.899	0.081
Error	59.08	18	3.282		
Total	152.903	23			

Experiment 2: Physiological measurements (Fig. 5.1(

Dependent Variable: PS

Source	Sum of Squares	df	Mean Square	F	Sig.
PEG	80.905	1	80.905	6.934	0.017
Root	76.801	2	38.4	3.291	0.061
PEG * Root	158.116	2	79.058	6.776	0.006
Error	210.024	18	11.668		
Total	525.845	23			

Dependent Variable: Stomatal

Source	Sum of Squares	df	Mean Square	F	Sig.
PEG	0.113	1	0.113	14.165	0.001
Root	0.007	2	0.003	0.43	0.657
PEG * Root	0.036	2	0.018	2.263	0.133
Error	0.143	18	0.008		
Total	0.299	23			

Dependent Variable: Carbon

Source	Sum of Squares	df	Mean Square	F	Sig.
PEG	2367	1	2367	8.067	0.011
Root	890.388	2	445.194	1.517	0.246
PEG * Root	611.259	2	305.63	1.042	0.373
Error	5281.449	18	293.414		
Total	9150.097	23			

Dependent Variable: Transpiration

Source	Sum of Squares	df	Mean Square	F	Sig.
PEG	22.333	1	22.333	10.378	0.005
Root	5.143	2	2.572	1.195	0.326
PEG * Root	35.211	2	17.606	8.181	0.003
Error	38.735	18	2.152		
Total	101.423	23			

Dependent Variable: WUE

Source	Sum of Squares	df	Mean Square	F	Sig.
PEG	0.028	1	0.028	0.475	0.5
Root	0.419	2	0.21	3.565	0.05
PEG * Root	0.068	2	0.034	0.579	0.57
Error	1.059	18	0.059		
Total	1.574	23			

Chapter 6: Root pruning experiment

Transpiration (Fig.6.1 and 6.2)

Dependent Variable:Cum9Oct

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	113607.4	8	14200.93	1.295	0.313
Treatment	170696.3	2	85348.15	7.781	0.004
Error	175503.7	16	10968.98		
Total	459807.4	26			

Dependent Variable:Cum16Oct

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	176718.5	8	22089.82	1.524	0.225
Treatment	3536452	2	1768226	121.974	0
Error	231948.1	16	14496.76		
Total	3945119	26			

Dependent Variable:Cum22Oct

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	222733.3	8	27841.67	1.051	0.441
Treatment	4163822	2	2081911	78.554	0
Error	424044.4	16	26502.78		
Total	4810600	26			

Dependent Variable:Cum27Oct

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	390133.3	8	48766.67	1.098	0.414
Treatment	3178689	2	1589344	35.774	0
Error	710844.4	16	44427.78		
Total	4279667	26			

Dependent Variable:Cum3Nov

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	527535.2	8	65941.9	1.118	0.402
Treatment	1951652	2	975825.9	16.551	0
Error	943314.8	16	58957.18		
Total	3422502	26			

Dependent Variable:Cum9Nov

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	544450	8	68056.25	1.269	0.325
Treatment	1166156	2	583077.8	10.873	0.001
Error	858011.1	16	53625.69		
Total	2568617	26			

Dependent Variable:Cum17Nov

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	510546.3	8	63818.29	1.373	0.28
Treatment	953147.5	2	476573.8	10.255	0.001
Error	743537	16	46471.07		
Total	2207231	26			

Dependent Variable:Cum23Nov

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	468550	8	58568.75	1.454	0.249
Treatment	932126.9	2	466063.5	11.574	0.001
Error	644311.1	16	40269.44		
Total	2044988	26			

Dependent Variable:Week9Oct

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	50600	8	6325	0.666	0.714
Treatment	126422.2	2	63211.11	6.655	0.008
Error	151977.8	16	9498.611		
Total	329000	26			

Dependent Variable:Week16Oct

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	80400	8	10050	0.668	0.712
Treatment	2184867	2	1092433	72.587	0
Error	240800	16	15050		
Total	2506067	26			

Dependent Variable:Week27Oct

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	67466.67	8	8433.333	1.19	0.363
Treatment	66422.22	2	33211.11	4.687	0.025
Error	113377.8	16	7086.111		
Total	247266.7	26			

Dependent Variable:Week3Nov

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	54757.41	8	6844.676	0.914	0.53
Treatment	148985.2	2	74492.59	9.942	0.002
Error	119881.5	16	7492.593		
Total	323624.1	26			

Dependent Variable:Week9Nov

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	21340.74	8	2667.593	0.904	0.536
Treatment	100807.4	2	50403.7	17.089	0
Error	47192.59	16	2949.537		
Total	169340.7	26			

Dependent Variable:Week17Nov

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	9051.852	8	1131.481	1	0.473
Treatment	10869.26	2	5434.628	4.803	0.023
Error	18103.7	16	1131.481		
Total	38024.81	26			

Grain yield and WUE

Dependent Variable: Grain wt

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	6.219	8	0.777	0.725	0.668
Treatment	65.637	2	32.819	30.616	0
Error	17.151	16	1.072		
Total	89.007	26			

Dependent Variable: WUE grain

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.724	8	0.091	0.915	0.529
Treatment	4.151	2	2.076	20.965	0
Error	1.584	16	0.099		
Total	6.46	26			

Dependent Variable: Heads

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	9.056	8	1.132	0.409	0.896
Treatment	23.167	2	11.583	4.183	0.04
Error	36	13	2.769		
Total	73.333	23			

Dependent Variable: Grain no

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	8758	8	1094.75	0.622	0.748
Treatment	106328.2	2	53164.11	30.188	0
Error	28177.78	16	1761.111		
Total	143264	26			

Dependent Variable: Root DW

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	2.776	8	0.347	0.583	0.778
Treatment	3.806	2	1.903	3.195	0.068
Error	9.53	16	0.596		
Total	16.113	26			

Dependent Variable: Shoot DW

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	3.077	8	0.385	1.48	0.24
Treatment	11.723	2	5.862	22.553	0
Error	4.159	16	0.26		
Total	18.958	26			

Dependent Variable: R/S

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.073	8	0.009	1.026	0.456
Treatment	0.021	2	0.011	1.202	0.326
Error	0.142	16	0.009		
Total	0.236	26			

Dependent Variable: Total DW

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	3.62	8	0.453	0.349	0.933
Treatment	27.663	2	13.831	10.655	0.001
Error	20.77	16	1.298		
Total	52.053	26			

Dependent Variable: HI

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	0.011	8	0.001	0.521	0.824
Treatment	0.046	2	0.023	8.652	0.003
Error	0.042	16	0.003		
Total	0.099	26			

Dependent Variable: WUE total

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	1.687	8	0.211	1.564	0.212
Treatment	7.923	2	3.962	29.376	0
Error	2.158	16	0.135		
Total	11.768	26			

Dependent Variable:Grain1000

Source	Sum of Squares	df	Mean Square	F	Sig.
Block	177.795	8	22.224	0.622	0.748
Treatment	312.194	2	156.097	4.366	0.031
Error	571.981	16	35.749		
Total	1061.971	26			