Assessing the drought risk of oilseed rape to target future improvements to root systems

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List of abbreviations

ABA	abscisic acid
DAS	days after sowing
DW	dry weight
ET	evapotranspiration
FC	field capacity
FW	fresh weight
g_s	stomatal conductance
h	hour
MPa	mega Pascal
osr	oilseed rape
PAR	photosynthetically active radiation
PWP	permanent wilting point
RH	relative humidity
RLD	root length density
RWC	relative water content
sem	standard error of the mean
SMD	soil moisture deficit
VPD	vapour pressure deficit
WUE_{grain}	water use efficiency for grain production
WUE_{total}	water use efficiency for total shoot biomass production

Declaration

I hereby declare that this thesis had been composed by me and that the work presented herein is my own, unless otherwise stated. This work has not been submitted for any other degree or professional qualification.

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Assessing the drought risk of oilseed rape crops to target future improvements to root systems

Abstract

The yield of UK's commercial oilseed rape (*Brassica napus*) crops has not increased over the last three decades, while a significant increase in yield has been found in trials that test new varieties before they enter the market. It has been suggested that oilseed rape is susceptible to drought and that this may contribute to the poor yield of some commercial crops. A thorough literature review revealed that there is little information on the water relations of oilseed rape crops and in particular on root growth and function and thus no strong evidence to support the above hypothesis. The aim of this thesis was to investigate root function and water relations of oilseed rape to determine whether it is more sensitive to drought than wheat, a crop species grown in rotation with oilseed rape.

The water relations of wheat (*Triticum aestivum* L. cv. Tybalt) and oilseed rape (*Brassica napus* L. cv. SW Landmark) were compared in a lysimeter experiment conducted in an open sided glass house to test the hypothesis that oilseed rape was more sensitive to drying soil than wheat. Plants were grown with or without irrigation at a population density equivalent to that of commercial field crops. Irrigated oilseed rape crops transpired more water than wheat crops and oilseed rape showed a greater reduction in growth when water was withheld. The onset of drought also occurred slightly earlier in oilseed rape. In a separate experiment the root hydraulic conductance of oilseed rape, measured on a root surface area basis, was about twice that of wheat (113.1 ± 20.0 $ml \cdot m^{-2} \cdot h^{-1} \cdot MPa^{-1}$ for oilseed rape and 53. 5 ± 10.6 for wheat). These results suggest that oilseed rape needs a less dense root system for water extraction than wheat.

In the above experiment plants were grown in relatively loose soil repacked into the lysimeters. It has been suggested that oilseed rape is particularly sensitive to soil compaction, which may be a common occurrence in commercial fields. Therefore the sensitivity of oilseed rape and wheat growth to compaction was compared in an

experiment under well-watered conditions. Plants were grown in a controlled environment chamber in pots packed with soil at four different bulk densities. Although the root length, shoot mass, leaf area and stomatal conductance of oilseed rape were all reduced by soil compaction, oilseed rape was no more sensitive to soil compaction than wheat under these well-watered conditions.

When soil dries it also hardens and high soil strength is known to impede root growth and alter plant-water relations. The hypothesis that oilseed rape is more sensitive to increasing soil strength than wheat was tested in an experiment in which soil bulk density and soil water content were varied to create a range of soil strengths. At low soil strength oilseed rape had a greater stomatal conductance than wheat, but as soil strength increased, stomatal conductance decreased to a greater extent in oilseed rape, indicating a more sensitive response. In dense or strong soil, plants often rely on pores created by earthworms or roots of the previous crop to explore the soil volume. The ability of oilseed rape and wheat to exploit soil pores to penetrate hard soil layers was compared in a pot experiment. A hard layer, comparable to a hard–pan in a cultivated field, was created at twelve centimetre depth of each pot by packing the soil to a bulk density of 1.5 $g \cdot cm^{-3}$ relatively loose soil at a bulk density of 1.1 $g \cdot cm^{-3}$ was present above and below the layer. In one treatment seven pores were drilled through the hard layer; controls had none. Presence of pores in the hard layer led to a significant increase in number of roots in the deeper soil, of 29% for wheat and 54% for oilseed rape.

This project has shown that the physiological response to drought occurred earlier in oilseed rape than in wheat and that stomatal conductance and biomass production of oilseed rape reacted more sensitively to soil drying. However, water use by oilseed rape does not seem to be limited by the ability of its roots to explore the soil and transport water compared to wheat. The growth and distribution of roots under a range of soil conditions was as good as, if not better than, that of wheat. The implications of these findings for the commercial production of oilseed rape in the UK are discussed.

General introduction

Bright yellow fields of flowering oilseed rape have become a familiar site in northern Europe. Oilseed rape is becoming a more widely - grown crop in the world and its area in the UK has increased steadily since the 1970s to 598,000 hectares in 2008 (DEFRA 2009). It is grown for the production of vegetable oil from the seeds and is also used as fodder for cattle. Recently there has been more interest in oilseed rape because of its use in the production of biodiesel. Oilseed rape is also known as *Brassica napus* and swede rape (Scarisbrick 1995). It is grown in cereal rotations as a break crop to prevent the build up of diseases. In England winter oilseed rape is the main crop, while in more northern parts, like Scotland spring oilseed rape is also grown. Oilseed rape yields in the UK have not increased on commercial farms during the last 20 years, despite an increase in recommended list trials. In 2008 the yield per ha was 3.3 tonnes and the yield has fluctuated around 3 $t \cdot ha^{-1}$ since 1984, see also Figure 1.1. In the same time period, the yield per hectare of cereals has increased (Figure 1.1).

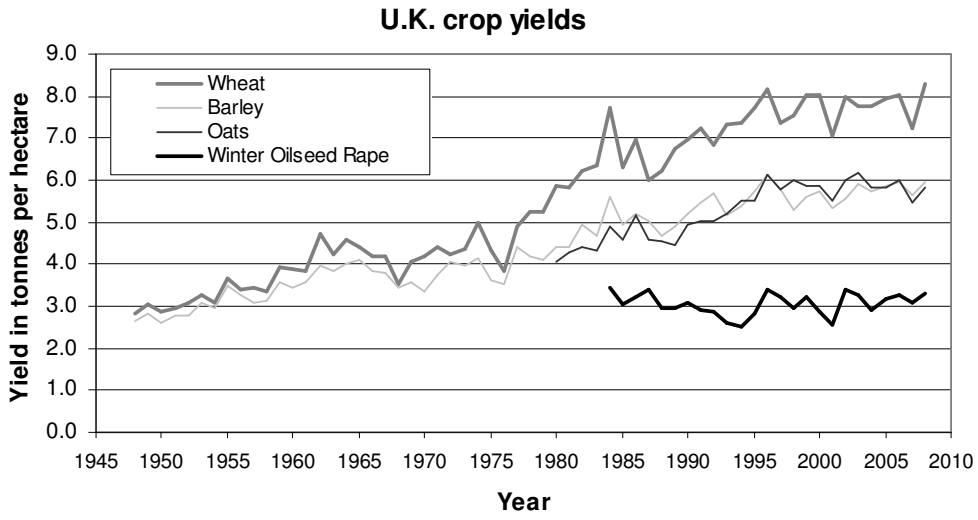

Figure 1.1 Yearly yield of four UK crops (data source DEFRA 2009).

There could be a number of reasons why oilseed rape is not reaching its yield potential, but this project will focus on water-capture. It has been suggested that water could be limiting yield on some fields in the U.K (Berry and Spink 2006). Rape yields from

ADAS Boxworth between 1987 and 1997, for instance, were positively correlated with June rainfall. Furthermore, preliminary results have shown a significant correlation between root length density in the subsoil and yield at the same site (Blake 2006). Data from 11 different commercial crops, suggests that oilseed rape in some instances has a relatively low root length density in the subsoil, compared to a crop like wheat (Blake 2006). Oilseed rape may simply not have enough root length to extract all available water from the soil in some fields. However, if oilseed rape roots are more conductive than wheat roots, like lupins for instance, oilseed rape may not need as dense a root system (Gallardo et al. 1996). At the moment, very little is known about the root system of oilseed rape. In this project oilseed rape water relations and root functioning will be investigated and compared to wheat, a more extensively researched crop often grown in the same fields as oilseed rape.

There are other plant and environmental factors besides root length density which affect plant-water relations. Soil structure is one of those factors; there can be plenty of water in the soil, but it can be out of reach of the plant because it is trapped in clods or under a plough pan (Stirzaker et al. 1996). Compacted soil is hard to explore by plant roots and may limit water uptake by plants. Oilseed rape is thought by some farmers to be particularly sensitive to soil compaction, however there is little evidence in the literature to support this claim (Cresswell and Kirkegaard 1995, Lisson et al. 2007). There are indications that that subsoil constraints and late-season water stress cause underperformance of oilseed rape in New South Wales, Australia (Lisson et al. 2007). Additionally, oilseed rape may just need more water than wheat to complete its life cycle; the high oil content in its seeds does require more energy than the production of wheat seeds (Angadi et al. 2008b). Oilseed rape could also simply be less water-use-efficient.

Overall aim

To investigate the crop water relations of oilseed rape and assess the risk of drought-limitation to yield in the UK. In this thesis I will compare root functioning and drought sensitivity of oilseed rape with wheat. Wheat is used as benchmark because it is a more intensively researched crop species. Additionally, wheat and oilseed rape are grown in the same fields in the U.K .as oilseed rape often functions as a break crop in cereal rotations.

Chapter 1

Background

Plant-water relations

The flow of water through a plant is facilitated by the soil-plant-atmosphere-continuum; the difference between water status of the leaf and evaporative demand of the atmosphere is the driving force for water flow through a plant and a continuous column of water within the plant is replenished at the root surface by soil water.

The flow of water meets resistance on its way from the soil to the atmosphere. At some points, the resistance is negligible; at others it can limit water flow. Major resistances within the plant are the radial resistance of the roots and stomatal resistance.

Driving force for water flow

The difference between the water status of the leaves and evaporative demand of the atmosphere generates the driving force for water flow through a plant. The evaporative demand is the absolute concentration difference in water vapour between leaf and atmosphere. This difference depends on leaf temperature (Taiz and Zeiger 1998).

In the leaves, the xylem vessels branch out into an intricate network of veins; these fine veins supply the leaf cells with water. Water evaporates from a thin film that occurs on the outside of leaf cells walls, into the intercellular spaces of the leaf. The difference in water vapour concentration between the intercellular airspaces in the leaf and the atmosphere determines the rate of water loss to the outside air. The amount of water the air can hold is determined by temperature; warmer air can hold more water. The cuticle of a leaf is almost impermeable to water and the water vapour diffuses from the intercellular airspaces, to the atmosphere via (partially) closed or open stomata (Steudle and Peterson 1998). The number of stomata, the stomatal aperture and also the thickness of the layer of unstirred air adjacent to the leaf surface (boundary layer) determine the resistance water vapour meets when flowing towards the atmosphere (Cowan 1965, Taiz and Zeiger 1998).

The pathway for conduction of water in a plant can be considered as an electrical circuit and can be described by an analogy of Ohm's law: $R = V / I$, where R is the resistance of the plant pathway to water (conductance is the inverse of resistance), V is the potential difference driving the flow and I is the rate of water flow (Kramer 1983). In a plant, far from being a simple series of resistances, the water conducting

system is a parallel network of some complexity (Cowan 1965). The driving force is created by the difference between water status of the leaf and evaporative demand of the atmosphere. Transpiration during the day generates hydrostatic pressure gradients to draw water into the roots and through the xylem of the plant. This hydrostatic pressure gradient is created by the surface tension that develops at the air-water interface in leaves and is transmitted as a negative pressure throughout the water column, where it lowers the water potential of the roots below that of the soil. The negative pressure is equivalent to a tension or pulling force, drawing water upward (Tyree 1997). The water vaporises in the intercellular airspaces of the leaf in the mesophyll and escapes in to the atmosphere via the stomata. The impedance to movement of liquid water in the plant is small to that compared with that encountered by the vapour in the atmosphere and the latter mainly determines the transpiration rate. In moist soils, the critical transpiration rate mainly determines the supply rate of water to the plant, whereas in dry soils, the density of rooting is of greater influence (Cowan 1965).

Pathway of water flow & resistance

When water is in the vapour phase, the greatest resistance to flow occurs at the stomatal aperture. In the liquid phase however, the root system poses the highest resistance to overall water flow (Steudle 2000).

Soil resistance

The ease with which water moves through the soil, the soil's hydraulic conductivity, is determined by many factors. When the soil is saturated, water meets less resistance in soils with larger spaces between particles (sandy soils) than in soils with small voids. Soil compaction and soil structure also affect resistance to water flow. The resistance to water flow by the soil increases as the soil dries. When there is relatively little water in the soil, there is only a thin film of water along the surface of the particles and water flow is restricted to this thin layer, rather than the interstitial spaces (which are now filled with air).

Gardner and Ehlig (1962) estimated that when soil suction was below 0.06 MPa (= matric potential of -0.06 MPa), impedance to water movement was mainly caused by the plant, but when suction was greater than 0.1 or 0.2 MPa, the soil resistance became the

limiting factor. However, the suction at which the soil conductivity becomes limiting depends upon the plant and the texture of the soil, but Gardner and Ehlig (1962) expect many soils of widely different textures to exhibit about the same value of unsaturated conductivity between soil matric potentials of -0.05 and -0.2 MPa.

The contact between roots and soil is important for water flow into plants. When the soil is very dry, both the soil and roots can shrink and as a result of the formation of air pockets between root and soil, the root-soil interface can become highly resistant to water flow. Plants can avoid or overcome this by excreting mucilage around the roots which enhances root-soil contact (Kramer 1983). While root excretions may stabilize soil aggregates in the rhizosphere (Czarnes et al. 2000), there's evidence that the presence of lecithin, an analogue for the phospholipid surfactants present in maize, lupine and wheat root mucilage, reduced water transport to the rhizosphere (lower hydraulic conductivity), resulting in a decrease in water uptake of up to 20% (Dunbabin et al. 2006).

Root resistance

In wet soils, water movement into and through a root system is predominantly influenced by a large resistance to the radial water flux through root tissues outside the xylem (Burch 1979).

The root apex typically has high axial and radial resistances to water flow compared to older parts of the root that have developed xylem (Steudle 2001). Axially (= longitudinally) water can flow through the xylem, which is a tissue of interconnected (dead) cell spaces and therefore axial resistance to water flow is relatively small. Individual plant variation and difference in ages of the individual roots on a plant must both be considered in assessing the axial hydraulic conductivity of any root system (Steudle and Peterson 1998). Root length is not necessarily a good indicator of the length of xylem that is open for conductivity. The vessels of growing maize roots for instance are not open for conduction until at least 15 and sometimes more than 40 cm behind the tips (McCully, 1999).

In the maize root system, the fine roots (diameter < 0.8 mm) are the major sites of water uptake into the mature root system (McCully, 1999). Water that is taken up by the root can flow radially to the stele, where the tracheary elements are located, via

several pathways. The apoplastic pathway involves water movement via the cell walls. In the symplastic pathway, movement is from protoplast to protoplast via plasmodesmata and in the transcellular pathway (cell-to-cell) the water moves from vacuole to vacuole and osmotic gradients are the main driving force for water flow (Steudle and Peterson 1998, Raven et al. 1999, Steudle 2000, Bramley et al. 2007a). Species differ in their use of pathways of radial flow. In wheat roots, water flow occurs by a combination of pathways, while in lupins radial water flow is predominantly apoplastic (Bramley et al. 2007b, 2009).

It is generally thought that the endodermis is a major site of resistance to radial water flow in the root (Steudle and Peterson 1998). The endodermis is a single layer of cells encircling the stele. Its Casparian strips and a continuous layer of suberin restricts apoplastic water flow, water therefore is forced to move symplastically across the plasma membranes and protoplasts of the endodermal cells on its way to the xylem (Bramley et al. 2007a). Ranathunge et al. (2003) measured different components of radial resistance in rice roots and found that the resistance to water flow of the endodermis and stele was 30 times greater than the resistance of the outer root tissue, comprised of rhizodermis, exodermis, sclerenchyma and one cortical cell layer (Steudle 2000). However, there are indications that the greatest resistance to radial water flow does not always occur in the endodermis, at least in cereal roots, where the cortex shrivels with ageing and its resistance increases. Additionally when the water potential of the root decreases, for instance under drought stress, the cortex shrinks. Hence the greatest resistance to radial water flow in the root may occur in the epidermis (Passioura 1988). In young maize roots for instance, the endodermis did not influence radial conductivity (Bramley 2007).

Although water uptake by roots is a passive process, the rate of uptake and/or flow can be regulated by the plant, by either decreasing canopy function (mainly by closing stomata) or at the roots by varying the number of transmembrane water channels (aquaporins), particularly situated around the endodermis, or by varying the rate of water flow through them. Aquaporins can make up to 15% of total membrane protein, indicating that they are important for the cell (Bramley et al. 2007a), but the exact mechanisms of the gating of channels is poorly understood (Ranathunge et al. 2004). Phosphorylation, cytoplasmic pH and heavy metals directly control aquaporin gating, either through conformational changes in the shape of the pore or by direct blockage (Tournaire-Roux et al. 2003, Bramley et al. 2007b). Whereas the importance of

aquaporins in root hydraulic conductivity has been demonstrated, the resulting effect on overall plant conductance, leaf water potential and leaf elongation rate is still poorly studied (Parent et al. 2009).

Plant species differ in how easily water is taken up by the roots and flows upwards to the stem. This difference in hydraulic conductivity in roots does not only occur between species, but also within a single root system (McCully 1999). The seminal roots of cereal root systems are significantly more efficient at supplying water to the shoot than the nodal roots, perhaps this difference is can be attributed to differences in vessel architecture between seminal and nodal roots (Aloni and Griffith 1991).

Hydraulic conductivity changes with root age; as the xylem vessels age they become less resistant to water flow. Different parts of one root system can differ in conductivity, because of variations in xylem diameter and anatomy and possibly aquaporin activity. Root hydraulic conductivity is not only affected by biotic factors, but also by a-biotic factors: gradual soil drying, anoxia, nutrient deficiency, chilling and aluminium can all decrease conductivity (Bramley et al. 2007a).

Shoot resistance

Water flow through the mature xylem meets very little resistance. However, cavitation can cause embolism formation in the xylem and this leads to an increase in resistance to water flow (Holtta et al. 2009). When the tension becomes too high and/or an air bubble is trapped in the water, the air can expand because of the tensile strength stretching the air, this can result in cavitation, thereby hampering water flow and increasing resistance. Cavitation happens when the tensile force in the xylem is relatively high. This is the case in trees with high water columns and when there is drought (i.e. a high atmospheric vapour pressure deficit).

The number of stomata, the stomatal aperture and also the thickness of the layer of unstirred air (boundary layer) determine the resistance water vapour meets when flowing towards the atmosphere (Taiz and Zeiger 1998). Crop boundary resistance is affected by factors like wind speed, the degree of canopy cover, stomatal conductance, crop height, leaf, angle and pubescence. Pubescence of leaves will increase the thickness of the boundary layer and diffusion of water into the atmosphere harder (Jones and Corlett 1992).

Crop water Balance

Soil as a water reservoir

The water content of the soil in the root zone of a crop changes over time. It decreases due to evapotranspiration from soil and crop (and weeds), but water can also leave the root zone by draining to lower soil layers. The soil water reservoir may be replenished by rain or irrigation. When evapotranspiration exceeds rainfall or irrigation, the soil water content will decrease and a 'soil moisture deficit' develops. The soil moisture deficit is usually expressed in millimetres of water and describes the amount of water needed to bring the soil back up to field capacity.

The water holding capacity of a soil depends on several factors: soil type (particle sizes), the porosity of the soil, the compaction of the soil, the soil depth and the soil macrostructure (presence of rocks, clods and hard pans). Clay and silt soils have a high water holding capacity, while sandy soils (large particles, relatively low surface area) can hold less water, because water drains away easily by gravity from the relatively large interstitial spaces. Thus field capacity or water holding capacity is greater for clay and humus rich soils than for sandy soils (Taiz and Zeiger 1998).

Not all water held in the soil is available to plants; some of it is bound too tightly to soil particles to be extracted by plants (Ritchie 1981). Plants can extract water from the soil until the permanent wilting point is reached; this is the point at which plants cannot regain turgor after subsequent watering. This point is usually set at a soil matric potential of -1.5 MPa, but its precise value will also be dependent on plant properties (Taiz and Zeiger 1998, van den Berg and Driessen 2002, Bengough et al. 2006).

The available water capacity of the soil (AWC) is the amount of water held in the soil between field capacity (often arbitrarily defined as the amount of water held at a tension of -0.33 MPa) and -1.5 MPa tension (permanent wilting point). The actual amount of water held within this range depends on soil properties such as organic matter content, particle size and the presence of pores, clods and stones (Teare and Peet 1983, van den Berg and Driessen 2002). How much of that water is actually available to the plant depends on plant characteristics such as rooting depth and root length density.

Water can be redistributed throughout the root zone by hydraulic lift. Hydraulic lift occurs when stomata are closed and the plant is not transpiring and the canopy water potential is too high for a gradient to develop between canopy and roots. Water is

absorbed by plant roots in moist subsoil and transferred to shallow roots in drier topsoil. Here the water, exits the roots and enters the drier soil as the soil water potential is lower than that of the root. The water remains in the shallow soil until stomata open and transpiration lowers the root water potential thus reversing the gradient between root and soil. (Yoder and Nowak 1999). Despite this phenomenon being observed in 30 species so far and its potential importance for water use efficiency and nutrient uptake from dry topsoil, its magnitude and the pathways and resistances of these redistribution processes are still poorly understood (Hinsinger et al. 2009). Typically, hydraulic lift is thought to occur mostly in arid or semi-arid environments, although recently it has been observed in more mesic environments during periods of drought (Young 1998).

Collection of water by root systems

The rate and extent of water uptake from the soil not only depends on soil parameters, but also on plant root system characteristics. Not only are the size, depth, architecture and the anatomy of the root system important for water uptake from soil, but also the distribution of root length throughout the soil profile (Bennett and Doss 1960, Yu et al. 2007). However, the combined influence of these root system parameters on water uptake is not well understood (Ehlers et al. 1991). Root hairs are thought to play an important part in the uptake of nutrients and water from the soil, but root hairs do not increase water and nutrient uptake by increasing the actual root surface area. Instead, they increase water uptake by expanding the apparent diameter of the cylinder that is characterized by the root water potential, because the root hairs mainly absorb water at their tips (Segal et al. 2008).

In most soil profiles, where physical restrictions to root growth are not excessive, root length density decreases exponentially with depth and a high percentage of roots is found in the top 20 cm of soil (Gregory et al. 1978, Hoad et al. 2001, King et al. 2003, Yu et al. 2007). Root length density determines the average distance water molecules have to travel in the soil towards the plant (Maseda and Fernandez 2006).

The critical root length density for water uptake by a crop is defined here as the root length density at which all plant available water can be extracted from the soil. If a crop has root length density less than its critical value some water might not be

extracted by the root system even though the crop is water stressed. Mean root length densities of cereals (0.5–10 cm·cm^{-3}) in the upper layers of most soils are theoretically adequate to access most of the available soil water within the crop's yield-forming period and densities >1 cm·cm^{-3} are associated with only small increases in the total amount of water taken up during this period (King et al. 2003).

The critical root length density of a crop is related to the hydraulic conductance of the root system and shoot. The results from both field and controlled environment experiments suggest that the greater water uptake per unit root length in lupins compared to wheat results from appreciably larger root and shoot hydraulic conductances. The specific root hydraulic conductances were four times greater in lupins than in wheat. Therefore lupins can exhibit the same rate of water uptake as wheat but with a lower root length density (Gallardo et al. 1996). The specific root water uptake (uptake per unit root length) was higher (5 to 12 fold) for lupine in all soils tested. There was also a tendency for root conductivity to increase with depth. The greater hydraulic conductance of lupins might be due to its larger number of root hairs and/or because the diameter of its metaxylem vessels are greater than that for wheat. Dicots tend to have metaxylem vessels with a greater diameter than monocots (Hamblin and Tennant 1987). In field grown wheat there were changes in root diameter with physiological age, the roots having the greatest diameter near the tips (Hamblin and Tennant 1987).

Bennet and Doss (1960) investigated the relationship between root distribution and water extraction by eight cool season forage species grown in soils of three different moisture levels. After irrigation, water was extracted first from the top 15 centimetres where the root length density was the highest. As soil moisture content of the top soil decreased and soil matric potential became lower there, water was extracted from deeper soil layers. However, the rate of water extraction from the soil decreased with increasing soil depth. Plants usually wilted before very much of the moisture was depleted at the lower depths. This means that the root length density or uptake rate per unit root length was insufficient to keep up with the demand of the plant. Bingham (2005) argues on the basis of simple models relating root length density with water uptake and the observation that root length density of wheat crops below 50 cm is often less than 1 cm·cm^{-3}, that wheat crops would benefit from an increase in root length density at depth in years with low rainfall.

From measurements of root systems and water uptake of wheat, barley, lupine, pea, bean and oat crops Ehlers et al (1991) concluded that the uptake rate of water from a certain soil layer increases with increasing rooting density, but that water uptake is also related to the depth of the root system and that the potential water use of crops will depend not so much on rooting density but more on the maximum depth of the root system (Ehlers et al. 1991). However, for maize to cope with shortage of water, the depth of the most densely rooted soil layer was more important than maximum rooting depth (Yu et al. 2007).

Root system architecture and water uptake

Root architecture refers to the spatial configuration of the root system, i.e. the explicit geometric deployment of root axes (Lynch 1995). Fitter (1987) made the link between root system architecture (topology) and function. From modelling of topology and calculation of path-lengths and areas of exploration, it appeared that there is a geometrical conflict in root system architecture. The most efficient systems for exploration of soil, these of high topological index (herringbones, branching principally on main axis), are the least efficient at transporting materials to the shoot system. Root systems of arid zone plants are generally shallow and highly branched (dichotomous) if the plant is active in the wet season and deeper-rooting, herringbone like when active in the dry season.

When anatomical rather than topological features are taken into account, root systems can be divided into tap-rooted and fibrous types. Taprooted systems have a stout main root, the tap root, with a limited number of side-branching roots which can become extensively branched. The taproot facilitates food storage and anchorage of the plant. *Brassica* crops and most other dicots are tap rooted, although there are exceptions. A fibrous root system on the other hand, is often diffuse with many branched roots. Monocotyledonous crops, like cereals have a fibrous system with initial roots (three to five for wheat) emerging from the seed (seminal roots) and subsequent roots emerging from the basal nodes of the stem (nodal or adventitious roots). But some dicots, beans for instance, also have a fibrous root system. The total root length density of monocots is often much greater, but dicots tend to have a higher specific uptake rate, which is

probably facilitated by a higher specific hydraulic conductivity of roots (Bramley et al. 2007a).

Comparisons between tap-rooted and fibrous root system architectures of lupins, in which artificial variations of the root system architecture were induced, showed that the tap-rooted architecture induced a more spatially concentrated uptake zone (near the soil surface) with higher flux rates, but with a xylem water potential at the base of the root system twice as low as the fibrous architecture (Garrigues et al. 2006).

Models of water uptake from the soil by roots generally assume a regular distribution of roots, but in the field roots are rarely distributed evenly and the onset of drought response will occur at higher bulk soil water content, than if roots are distributed uniformly. In a field experiment with maize, clustering was measured and occurred at a centimetre scale, even in parts of the soil that were not disturbed by experimental compaction (Tardieu 1988). When the roots are clumped and by-pass part of the soil layer, root length density becomes a meaningless parameter for predicting water uptake rates from the entire layer because of the distribution of roots is highly non-uniform (Dardanelli et al. 2004).

Growing in strong or non uniform soils

As the soil dries, its conductivity decreases. Gardner and Ehrlig (1962) estimated that at a soil water potential lower than -0.1 or -0.2 MPa, conductance to water movement through the soil is less than the conductance to water by the roots. Whalley et al. (2006) have suggested that at low water content, it is not the low soil water potential that is limiting root growth, but the increase in soil strength of a drying soil mechanically impeding root elongation. The strength of a soil can be measured by a penetrometer. Typically, penetrometer pressures of 2 to 2.5 MPa are sufficient to impede root elongation significantly (Bengough and Mullins 1990). In the UK, the penetrometer resistance of a sandy loam, sandy clay and a clay soil was greater than 2 MPa below 40 cm throughout the year and the top 30 cm of soil was stronger than 2 MPa for most of the spring and summer (Gregory et al. 2007). A correlation between soil strength (measured as cone resistance) and root density has been found in winter oilseed rape grown on a sandy soil (Bonari et al. 1995).

Plant species differ in their ability to penetrate strong soils (Materechera et al. 1993, Clark et al. 2003). Materechera et al (1993) concluded from a study with eight species that dicotyledonous (or at least: legumes) species were in general better at penetrating to depth in both compacted and deep tilled strong soils than monocotyledonous species. The ability of plant roots to grow into and through hard layers of soil is related to how much pressure the root tip can exert, which is correlated with the root diameter (Clark et al. 2003).

Roots tend to grow through pre-existing pores and especially in strong soils, plants rely on pores for soil exploration (Stirzaker et al. 1996). Root soil contact can be a problem for water uptake in pores that have a greater diameter than the root (Bramley et al. 2007a).

Studies with chickpea (Pardo et al. 2000) and maize (Amato and Ritchie 2002). Amato and Ritchie (2002) have shown that the presence of clods in the bulk soil decreased root length density and water uptake from the soil, because plant roots did not explore the clods deeply and consequently could not extract the water that was held in these clods.

Crop responses to water deficit

A plant will experience a water deficit when the rate of water loss from its leaves exceeds the rate at which it can be replenished. Plants often cannot meet the evaporative demand of the atmosphere during drought spells in summer, but also more frequently on a daily basis during midday when solar radiation is high and evaporative demand of the atmosphere peaks.

When the supply of water by the roots of plants cannot keep up with demand for water by the shoot, a number of signalling processes commence. What type of signalling takes place and what type of response it elicits in the plant depends on whether the development of the water deficit is fast or slow and whether it is short or long term. Drought is defined as a prolonged period of abnormally low rainfall; a shortage of water (Malcolm 2009). However drought in plants implies a whole range of different stresses. Heat stress and high light stress also often occur with drought stress. Drought is not a simple, single stress. Research into drought stress and the implications of climate change on crops should take into account stress induced by water limitation, as well as other confounded stresses such as heat, disease, soil strength, nutrient status and hypoxia (Whitmore and Whalley 2009).

Damage caused by water limitation

The severity of the effect of drought depends on the scale of the water deficit and on its duration and timing; water deficit has a different effect on yield depending on the developmental stage of the crop at which it occurs (Passioura 2007). The sensitivity of a growth stage is also dependent on crop type. In Mediterranean climates it is essential to get the timing of flowering right; early enough to avoid late spring and summer periods of high evaporative demand, but late enough to avoid frost damage of plants in early spring. Fertilization and grain set are particularly sensitive to water deficits and frost (Passioura 2007).

The first effect of water limitation is a reduction in leaf expansion, then stomatal closure takes place, with the dual effect of reducing water loss and slowing down the rate of CO_2-capture and hence growth. If drought stress continues, plants will start to

wilt and eventually older leaves will be shed. If the drought perseveres, the plant will desiccate and die (Neumann 2008).

The reduction in leaf expansion can result in a decrease in biomass production through reduced light interception (Passioura 1996, Neumann 2008, Lawlor and Tezara 2009) and eventually yield loss, but it is also an adaptive response, since the area for evaporative loss will be limited. Also energy and metabolic building blocks which were meant to be used for growth can now be utilized for protection of the photosynthetic apparatus and membranes (Neumann 2008). If a plant cannot avoid loss of turgor and its relative water content decreases, potentially damaging reactive oxygen species start to form. If water stress persists, eventually the water molecules that are incorporated in cell membranes get replaced by glucose and sucrose molecules to ensure membrane integrity with dropping water content (Chaves and Oliveira 2004, Moore et al. 2009). There are ways in which a plant can keep turgid even when water is limited, so that membranes are protected and cellular processes can be sustained. For instance most plants are able to osmotically adjust to some degree by accumulating solutes, mainly sugars, organic acids and ions and thereby keep cells turgid at a lower water potential (Taiz and Zeiger 1998).

Primary photosynthetic processes are very resilient to drought (Chaves and Oliveira 2004). The effects of drought on photosynthesis can be direct, by limitation of CO_2 diffusion after stomatal closure and by alterations of photosynthetic metabolism, or indirect by oxidative stress (Chaves et al. 2009). There is debate about the relative importance of the onset of metabolic versus stomatal limitations to photosynthesis and the relative effects of stomatal and metabolic limitations depend on species and conditions of growth and experimentation (Lawlor and Tezara 2009).

Under field conditions, water limitation usually occurs simultaneously with several other stresses, like heat stress and high light stress. Under these circumstances an excess of light energy results in more reducing power than can be handled by the Calvin cycle; this leads to the formation of reactive oxygen species (ROS) that can damage membranes. The excess light energy at photosystem-I however, can be dissipated thermally by the xanthophyll cycle, to avoid formation of ROS. Once ROS are formed, they can be 'disarmed' by glutathione and ascorbate, anti-oxidants which are present in plants and can be up-regulated during stress. There is also evidence that reactive oxygen

species and in particular H_2O_2 act as a local or systemic signal for stomatal closure (Chaves and Oliveira 2004).

Drought avoidance and tolerance

Plants have ways to avoid or tolerate drought stress. Drought avoidance and tolerance strategies are not mutually exclusive and plants can exhibit elements of both. Dehydration avoidance can be accomplished either by minimising water loss from tissues or by maximising water uptake. Water loss can be minimised by closing of stomata, leaf rolling to minimise light and heat absorption, having a dense layer of trichomes to reflect light and act as a barrier to evaporation, decreasing canopy area by either decreasing the leaf angle, so leaves don't intercept as much light, slowing leaf expansion or by shedding of older leaves. Water uptake can be maximised by allocation of biomass to the root system, increasing rooting depth and by recycling water from old leaves that are shed to newly developing leaves (Chaves et al. 2003, Cattivelli et al. 2008, Neumann 2008). Tolerance of drought stress, so a plant can continue metabolic processes, is facilitated by having thick and small leaves. Tolerance to low tissue water potential may involve osmotic adjustment, more rigid cell walls or smaller cells. In areas with extreme drought, plants escape drought altogether by having a very short life cycle and reproducing before the drought season (Chaves et al. 2003, Cattivelli et al. 2008, Neumann 2008).

The most important aspect of drought tolerance in an agricultural context is that the pattern of development of the crop must match the pattern of the water supply in relation to the evaporative demand. The traits controlling this development may often have no direct connection with plant water relations (Passioura 1996).

Sensing drought and signalling processes

To conserve water, a plant can close its stomata even before a loss of turgor has developed in the leaves. Two stomatal strategies are described for plant species: isohydric and anisohydric. In anisohydric plants, such as sunflower, both daytime leaf water status and stomatal conductance decline with decreasing soil water potential. In contrast, isohydric species control gas exchange in such a way that daytime leaf water

status is unaffected by water, in this strategy chemical signalling from root to shoot about the water status of the soil plays a role (Tardieu 1996).

The amount of ABA in the xylem sap is highly correlated with stomatal closure (Davies et al. 1993). But there is uncertainty about where it originates; ABA is probably synthesised in the roots and transported to the shoot. The ABA compartmentation is important for its effect; xylem/apoplastic pH influences ABA compartmentation and consequently the amount of ABA reaching the stomata. Alkalization of xylem sap is a common response to soil drying in some species. A more alkaline pH observed in the xylem/apoplast leads to a decrease in the removal of ABA from xylem and leaf apoplast to the symplast, such that more ABA reaches the guard cells and this increase in apoplastic ABA ultimately results in stomatal closure (Chaves et al. 2009, Davies et al. 2005).

Experiments with alkaline buffers injected into the xylem of plants have illustrated the importance of pH in drought induced stomatal closure and also inhibition of leaf expansion. However, there is very little known about the relationship between xylem pH and the pH of the apoplast of the leaves, and xylem sap pH does not increase in all species as the soil dries (Davies et al. 2005). The response was not found in the majority of the 22 species tested. There was no evolutionary relationship between the species that showed alkalization under drought stress. However, the species that alkalized sap also exhibited good control over internal water status and were the most isohydric species of those tested. None of the species exhibiting anisohydric responses alkalized xylem sap under drought stress (Sharp and Davies 2009).

The effect of ABA on stomata not only depends on the pH of xylem sap, but also on the presence or absence of other factors; it has been suggested that an interplay of ABA and ethylene controls inhibition of shoot growth under water stress (Chaves et al. 2003). Ethylene causes a reduction in stomatal sensitivity to ABA. The effect of ethylene on stomatal closure and leaf expansion is not completely understood at the moment and depends on the presence of ABA, but also on the type of stress and developmental stage of the plant (Wilkinson and Davies 2010).

In addition to ABA and ethylene; cytokinines and carbohydrates may also play a role in signalling. However, the effect of a hormone depends on the origin of the signal. Also the timing and balance between the different hormones is key, with some antagonising each other, while others work in synergy (Chaves and Oliveira 2004). It has

also been suggested that root originated signals other than ABA, also play a role in inhibition of shoot growth, namely hydraulic and peptide signals (Neumann 2008). For instance, H_2O_2 comprises part of a signal transduction chain induced by ethylene in Arabidopsis guard cells to close stomata (Wilkinson and Davies 2010).

ABA accumulation also plays a role in enhancing hydraulic conductance of roots. ABA-induced increases in water flux into the plant over the root membrane and within the plant through the symplast will promote a water replete environment conducive to rapid cellular growth. ABA is also involved in gating of aquaporins in root and shoot cell membranes (Neumann 2008, Wilkinson and Davies 2010).

Adaptations of the root system to drought

Roots play an important role in sensing water limitation in the soil. After rapid osmotic adjustment, they can keep growing, while shoot growth is inhibited by mild water stress (Wu and Cosgrove 2000, Chaves et al. 2003). Plants are often very flexible when it comes to dealing with variability in their environment; roots for instance often preferably grow towards nutrient patches (Wang et al. 2007, Hodge et al. 2009). Not only does the root system exhibit plasticity in response to heterogeneous distribution of nutrients, the Arabidopsis root system exhibits hydrotropism, i.e.: its roots preferably grow towards wetter soil, this trait is assumed to benefit plants in water uptake (Kobayashi et al. 2007).

Drying part of the roots of a plant results in partial stomatal closure, signalled by ABA produced by temporarily dried roots and may improve water-use efficiency. Fruit trees, grapevines and tomato may thus produce similar biomass and especially harvestable yield with significant lower water supply (Wang et al. 2005).

As the soil dries the top soil hardens first and soil strength increases, impeding root extension. The inhibition of root growth due to increased soil strength can lead to nutrient deficiencies. Lack of pores in soils can also inhibit root elongation (Whitmore and Whalley 2009, Wilkinson and Davies 2010). Mycorrhizae have been shown to enhance a crop's tolerance of drought and to improve water uptake of wheat (Al-Karaki et al. 2004).

The hydraulic conductivity of roots tends to decrease under water deficit situations (Neumann 2008). Stressed roots develop a suberised interface between living

tissue and rhizosphere to minimize water loss (Steudle 2000). Aquaporins, which are water channels in the root membranes, can be opened by phosphorylation resulting in increased radial hydraulic conductivity of the root system (Neumann 2008, Wilkinson and Davies 2010). In *Jatropha curcas* (Barbados nut) varieties, drought stress, as expected, negatively affected root hydraulic conductivity, but the drought-resistant variety showed a higher root hydraulic conductivity than the drought-sensitive variety. At the same time, the abundance of aquaporin protein in seedlings of drought-resistant populations clearly increased compared with drought-sensitive populations under water deficit. The abundance of aquaporin protein was induced by heavy drought stress (Zhang et al. 2007).

The pattern of root length density development over time and soil depths of eight crop species measured over three years in Canada, appears to indicate a general principle in soil-plant ecology: that shallow rooted plants can increase fine-root growth as an adaptive response to relative drought, while more deeply rooted plants growing on non restrictive soil profiles do not exhibit this root growth response (Merrill et al. 2002).

Drought development and responses in crops

To understand crop drought response, several crop characteristics rather than single plant characteristics need to be taken into account. A crop consists of many plants grown closely together.

Crop management has an effect of the development of water deficit/timing of drought. The timing of sowing, crop canopy closure, harvest and the rotation sequence all affect how much soil moisture is available in the soil during crop development. Additionally, a crop needs to yield product and the survival of the plant is not necessarily the most important aim. Therefore the strategy of the plant may suit survival of the plant but not be very beneficial for yield production, i.e. the farmer and the plant have different goals.

Water use efficiency or total water availability can be managed by minimizing evaporation from the soil surface by having a high enough seed rate to ensure early canopy closure to cover the soil quickly. The storage/availability of water in the soil also depends on soil properties and on the previous crop (Passioura 2006). The timing of yield production is also important, if a crop flowers too late and experiences drought

during flowering or grain filling it can result in yield loss. Flowering too early however, may mean that the crop has not accumulated enough biomass to covert into grain for an optimal yield. The timing of fertiliser application is important in this respect. Applying too much nitrogen during the vegetative phase of a crop can result in an excessive canopy that uses a lot of water and leaves less water in the soil for the flowering and seed-filling phases. The rotation of crops on a field can also affect water availability of each crop. An appropriate choice of crop sequence can result in better water productivity, reduced disease and weed occurrence and decrease in nitrogen leaching (Passioura 2006).

Aspects which could make crops more effective in using water are: improved water capture from the soil, improved instant water use efficiency (amount of CO_2 captured per amount of water transpired) and to convert more biomass to grain or other harvestable product (Condon et al. 2004).

Oilseed rape water relations

Although oilseed rape has become a more popular crop to grow in the UK and potential yield of newly introduced varieties has increased over the past 25 years in variety trials, its yield on commercial farms has stagnated at about three tonnes per hectare (DEFRA 2009). Water limitation has been suggested as one possible factor contributing to the lack of yield increase. There is also circumstantial evidence that root system functioning could be limiting water-uptake from the soil (Blake 2006, Lisson et al. 2007). There are very few data on total water use and water use efficiency of oilseed rape crops grown in Northern Europe, therefore data from studies into water use by oilseed rape crops grown in other parts of the world will also be considered and oilseed rape will be compared to other crop species to give an indication of its relative water use efficiency and drought sensitivity.

Water use by oilseed rape

There is very little known about water use and drought response of Northern European oilseed rape crops. There are especially very few data on total water use and water use efficiency of oilseed rape crops grown in Northern Europe, but see the studies of Andersen et al (1996), Jensen et al. (1996a, 1996b, 1998), Wang and de Kroon (2005, 2007) , Gammelvind et al. (1996) and Müller (2010) for oilseed rape responses to water deficit.

In Table 1.1, total water use and water use efficiency of oilseed rape and wheat crops is given, but to my knowledge no figures are available for oilseed rape grown in Northern Europe. There have been studies in which water use has been estimated by measuring or estimating water loss from the soil (Andersen et al. 1996, Kappen et al. 2000), however it was not possible to obtain total water use values from these studies, or to calculate water use efficiency.

In the studies conducted outside Europe, such as in Canada and the USA the crops were subjected to a temperate land climate, which is colder than Northern Europe in winter and more arid in summer. To compare oilseed rape WUE and response to water limitation to that of wheat and other crops, some studies comparing water use of

oilseed rape and wheat in a Mediterranean climate (Australia and India) are also included in Table 1.1.

The total seasonal transpiration of oilseed rape crops ranged from 97 to 459.7 mm in Australia and Canada respectively (Nielsen 1997, Robertson and Kirkegaard 2005). Wheat's total water use was similar to oilseed rape's and varied from 176 to 400.2 in the UK and Canada (Foulkes et al. 2001, Angadi et al. 2008a). However care should be taken when comparing water use numbers between studies; the total water use numbers were often not measured directly, but estimates of seasonal water use by taking into account rainfall, stating soil moisture and end of season soil moisture content. Wheat cops in the UK tended to use about 20 mm more water than wheat crops grown in Canada, for irrigated as well as un-irrigated crops (Foulkes et al. 2001, Angadi et al. 2008a).

There are several Canadian (Nielsen 1997, Angadi et al. 2008a, Gan et al. 2009a) and Australian (Zhang et al. 2005, Norton and Wachsmann 2006) studies in which oilseed rape and wheat were compared in the same experiment. Unfortunately these experiments were often not analysed specifically to highlight differences in water use by oilseed rape and wheat and hence little information about statistical significance of differences between crops was given. In the two Australian studies, total water use of oilseed rape and wheat crops was comparable; however in these studies no water limitation treatment was imposed (Zhang et al. 2005, Norton and Wachsmann 2006). Oilseed rape crops that were subjected to water limitation (rain excluded and no irrigation), or were rain fed only, had the same or slightly less transpiration (water use) as wheat crops in two Canadian studies (Angadi et al. 2008a, Gan et al. 2009a). When crops were irrigated, oilseed rape used significantly less water than wheat in one Canadian study (Angadi et al. 2008a), but in another study, irrigated oilseed rape used more or at least similar amounts of water as wheat (Gan et al. 2009).

Judging from the water use data given in the studies described above, there is no consistent trend for one crop using more water than the other. In studies where oilseed rape and wheat seasonal water use were compared in the same study, the total seasonal water use of oilseed rape and wheat was similar. These studies were conducted in Canadian (Angadi et al. 2008a, Gan et al. 2009a) and Australian fields (Zhang et al. 2005, Norton and Wachsmann 2006) and not in the UK.

Table 1.1 Seasonal water use (= transpiration) and water use efficiency of oilseed rape and wheat crops. Difference in letters in subscript means a significant difference ($p < 0.05$) between oilseed rape and wheat.

Study	Location and growth conditions	Crop	Treatment	Total crop water use (transp.) in mm	WUE_{grain} g DW m^{-2} mm^{-1}	WUE_{total} in g DW m^{-2} mm^{-1}
Zhang et al. (2005)	Australia. Spring varieties in field, no drought. Evapotranspiration in mm in brackets.	oilseed rape		245 – 270 (378 – 401)	0.7 – 1.0	2.8 - 3.5
		wheat		213 – 270 (389 – 403)	0.9 – 1.5	3.3 – 4.1
Foulkes (2001)	UK. Six winter wheat cultivars grown in the field in three years. Crop water uptake in mm given.	wheat	unirrigated	176 – 283		4.89 – 6.31
			irrigated	383 – 438		3.72 – 4.43
Nielsen (1997)	Canada. Field study, two years, stage at which irrigation was withheld is given.	oilseed rape	vegetative '93	332.7	0.31	
			reproductive '93	302.3	0.31	
			grainfilling '93	398.8	0.16	
			none '93	358.1	0.26	
			vegetative '94	419.1	0.09	
			reproductive '94	358.1	0.07	
			grainfilling '94	459.7	0.08	
			none '94	396.2	0.10	
Rao and Mendham (1991)	Australia (Tasmania). Summed evapotranspiration for 0-70 cm depth, cv Marnoo	oilseed rape	rainfed	369.0		
			three times irrigated	488.2		
Robertson and Kirkegaard (2005)	Simulation study using data of 42 field crops in Australia.	oilseed rape	Simulated evapotranspiration in brackets	97 - 212 (210 – 520)	0.04 – 0.18	

Table 1.1 continued.

Study	Location and growth conditions	Crop	Treatment	Total crop water use (in mm)	WUE_{grain} g DW m^{-2} mm^{-1}	WUE_{total} in g DW m^{-2} mm^{-1}
Angadi et al. (2008)	Field, semi-arid prairie in Canada. Spring varieties.	oilseed rape	drought	150.0	0.44 [a]	2.07
		oilseed rape	rainfed	340.1	0.51 [a]	2.18
		oilseed rape	irrigated	373.3 [a])	0.58 [a]	2.20
		wheat	drought	155.4	0.77 [b]	2.21
		wheat	rainfed	333.4	0.86 [b]	2.48
		wheat	irrigated	400.2 [b]	0.89 [b]	2.28
Buttar (2006)	Northern India, field. Varied N, seed bed and irrigation.	oilseed rape		343 - 512	0.14 – 0.32	
Norton and Wachsmann, (2006)	Field. Experiments in Australia.	oilseed rape		252.0 - 387.4	0.47 – 0.89	1.72 - 3.12
		wheat		236.6 – 400.6	0.86 – 1.15	1.91 – 3.35
Gan et al. (2009)	Lysimeters in a field in Canada, two years, rainfed and irrigated treatments	oilseed rape	rainfed '06	296	2.80	
		oilseed rape	irrigated '06	395	3.13	
		oilseed rape	rain-fed '07	261	2.98	
		oilseed rape	irrigated '07	389	3.07	
		wheat	rainfed '06	306	5.88	
		wheat	irrigated '06	387	7.00	
		wheat	rain-fed '07	266	5.48	
		wheat	irrigated '07	366	6.97	

Water use efficiency

The amount of dry matter produced for every millimetre of water transpired (WUE_{total}) was generally slightly lower for oilseed rape than for wheat in Table 1.1, but unfortunately no statistical information is available. Water use efficiency for grain production (WUE_{grain}) is 1.2 to 2.2 times lower (significantly) for oilseed rape compared to wheat (Zhang et al. 2005, Norton and Wachsmann 2006, Gan et al. 2009a). The WUE_{grain} range of oilseed rape in table 1 is: 0.14 – 1.0, while wheat's range is 0.77 - 1.5 g DW $\cdot m^{-2} \cdot mm^{-1}$ (Table 1.1).

Oilseed rape's WUE_{grain} is low compared to wheat's in Table 1, but also compared to globally measured WUE_{grain} of wheat (0.6–1.7), rice (0.6–1.6) and maize (1.1–2.7), yet similar to cottonseed (0.41–0.95 g DW $\cdot m^{-2} \cdot mm^{-1}$) (Zwart and Bastiaanssen 2004). This is because the higher oil content of oilseed rape and cotton seeds makes them more costly to produce (Zhang et al. 2005, Angadi et al. 2008a). The range of WUE_{grain} is very large and the variability of WUE_{grain} can be ascribed to: variety, climate, irrigation water management and soil (nutrient) management, among others (Zwart and Bastiaanssen 2004).

Water use efficiency generally increases when less water is available (Zwart and Bastiaanssen 2004). However, the WUE data for oilseed rape in Table 1.1, do not show this trend. The only case where WUE_{total} increased was in UK grown wheat (Foulkes et al. 2001). In other studies there is little effect of water treatment on WUE_{total} (Nielsen 1997, Angadi et al. 2008a), or even a negative effect: the irrigated treatments tended to have a greater WUE than the rainfed treatments in the experiments by Gan et al. (2009a) and Angadi et al (2008a) in Canada. Here WUE_{total} of oilseed rape was less affected by water limitation than wheat's WUE_{total}.

How drought affects oilseed rape

While oilseed rape is generally most sensitive to drought stress during anthesis or stem-elongation, one of the five varieties tested in a field experiment in Australia was most sensitive during pod filling (Richards and Thurling 1978a). Sensitivity to water limitation was defined here as a reduction in yield components (pods per plant and seeds per pod, but 1000 seed weight was generally not decreased by drought) relative to plants that

were not withheld water throughout any growth period. Withholding water during stem elongation led to a significant reduction in pod dry weight, relative water content (41% for stressed plants vs. 71% for unstressed plants) and chlorophyll fluorescence and an increase in osmolarity in pot grown oilseed rape plants (Muller et al. 2010).

Water deficit strongly reduced leaf initiation rates and leaf sizes in oilseed rape (Wang et al. 2005, Qaderi et al. 2006). An alternate watering regime, where the location of watering was switched between two sides of the plant, effectively reduced stomatal conductance, but lead to a higher shoot biomass only under more severe (50%) rather than under milder water deficiency (70% of a well watered control). The plants selectively placed their roots in the wet parts of the pot (Wang et al. 2005), which means the root system of oilseed rape is exhibiting morphological plasticity.

Water limitation not only affects leaf parameters, it can also lead to reduction in seed yield and percentage oil in seeds (Nuttall 1973, Bouchereau et al. 1996, Niknam et al. 2003). In seven rain-fed oilseed rape genotypes in an Australian field experiment; seed yield per ground area was reduced by 2 - 39% depending on genotype compared to when they were irrigated (Niknam et al. 2003). Withholding water from 50% flowering onwards, reduced number of pods per plant and 1000 seed weight in most varieties tested in an experiment in Iran (Norouzi et al. 2008). However, the number of seeds per pod was not reduced in all varieties. Among yield components, the number of pods per plant was affected more by water deficit than others (Norouzi et al. 2008).

The effect of drought on the oilseed rape root system is not thoroughly researched; there are however, some data available. In 14 winter oilseed rape varieties tested in Iran, water stress inhibited shoot dry weight more than root dry weight (Norouzi et al. 2008). Richard and Thurling (1978b) found that drought treatment reduced lateral and tap root weight. However, it was observed that a smaller root weight relative to the above-ground weight and a heavier tap-root relative to the lateral root was associated with a higher seed yield in the five drought treated varieties (Richards and Thurling 1978b). The authors speculate that mobilization of reserves from the taproot to the shoot may have helped seed production; however no evidence for this mechanism is given. The taproot is known to have an anchorage and thought to have an energy storage function (Goodman et al. 2001).

Stomatal response

Ninety percent of soil water absorbed by plants is lost via the stomatal openings (Salisbury and Ross 1991). Plants can open and close their stomata to regulate the rate of water loss from the plant to the atmosphere. Stomatal conductance is a very variable trait; it depends on temperature, time of day, light intensity, leaf position (Rao and Mendham 1991), plant age (Jensen et al. 1996b, Wang et al. 2005, Fanaei et al. 2009), soil strength, watering pattern (Wang et al. 2005) and on the severity of drought stress of the plant (Jensen et al. 1998).

Oilseed rape closes its stomata in response to water limitation (Clarke and McCaig 1982, Andersen et al. 1996, Ali et al. 1998, Jensen et al. 1998, Qaderi et al. 2006). Stomatal behaviour of oilseed rape depends on the rate of onset of drought. When drought onset was gradual in loam soil, the stomata close, but when onset was quick (in sand) the stomata can stay open, even if leaf water potential is low (Jensen et al. 1998). Stomatal closure was induced when leaf water potential was between -0.8 to -1.1 MPa and when leaf turgor pressure was below 0.3 MPa, which is similar to other dicots, like tomato (Jensen et al. 1998).

If oilseed rape stomatal response is not measured in under the same conditions (preferably in the same experiment) as another crop, it is difficult to say whether oilseed rapes stomatal response is particularly sensitive water limitation; i.e. whether it responds earlier (at a lower soil moisture deficit) and/or more severely (by closing stomata more) than other crops.

Osmotic adjustment

Plants can maintain a positive turgor for longer as tissue water potential declines, by accumulating solutes. The maintenance of turgor ensures continuation of cell elongation and division and also of keeping stomata open. Osmotic adjustment promotes dehydration tolerance rather than having a great effect on productivity (Taiz and Zeiger 1998). Osmotic adjustment can occur in the roots as well as the leaves. The decrease in solute potential (the osmotic component of water potential of the plant cell) is typically limited between 0.2 and 0.8 MPa, except in plants that are adapted to extremely dry environments (Taiz and Zeiger 1998).

There is contradictory evidence for oilseed rape's ability to osmotically adjust. This is partly due to the fact that osmotic adjustment depends on the rate of drought onset (Jensen et al. 1996b). When the rate of soil drying was fast the plants did not osmotically adjust, and it is thought that the cues cannot build up to reach the required threshold to start osmotic adjustment (Jensen et al. 1996b). Additionally, oilseed rape's capacity for osmotic adjustment can be influenced by the stage of plant development (Ma et al. 2006). A limited capacity for oilseed rape to osmotically adjust was found in a pot experiment by Müller et al (2010) and in a field based lysimeter experiment by Jensen et al.(1996b). Oilseed rape's tolerance to mild and severe water deficit was compared to *Brassica juncea*'s in an Australian field study. It was suggested that *Brassica juncea*'s superior drought tolerance was correlated with its ability to maintain greater turgor and have greater leaf duration (Wright et al. 1996). In a Canadian field experiment in which crops were irrigated, rain-fed or sheltered from rain, *Brassica* oilseeds were not able to maintain turgor over as wide a range of water stress as pulses and wheat. *Brassica* oilseeds (including oilseed rape) responded to water stress with relatively rigid cell walls and poor osmotic adjustment. In this experiment, wheat responded well to water stress through osmotic adjustment (Cutforth et al. 2009).

From the literature, it appears that oilseed rape has a limited scope for osmotic adjustment. Although Bouchereau et al (1996) found that spring oilseed rape varieties grown in a greenhouse had an osmotic adjustment of -0.8 MPa, it seems that other crop species generally have greater ability for osmotic adjustment and maintenance of turgor under drought stress.

The oilseed rape root system

Oilseed rape has a root system which, in the early stage of growth, is dominated by a single downward-growing tap root from which laterals subsequently develop (Whiteley and Dexter 1982). However, the oilseed rape root system is not well studied and in particular little is known about the response of oilseed rape roots to soil water availability in temperate climates.

As far as I am aware, there is only one published UK study in which root length densities of oilseed rape crops on commercial farms were measured. From it, it can be concluded that root length density varies widely in commercial oilseed rape crops. In a

comparison of eleven commercial crops, the root length density in the top 20 cm of soil varied from 1.37 to 7.43 cm·cm^{-3} and deeper in the profile (80-100 cm depth) a range of 0.72 to 2.09 cm·cm^{-3} was found (Blake et al. 2006). Barraclough (1989) also conducted a study into root length distribution in a UK field. The maximum root length density of autumn sown oilseed rape occurred in the top 20 cm and reached a maximum of 9.4 cm·cm^{-3} in April and declined after flowering. However, below 40 cm the density was never greater than 0.64 cm·cm^{-3}. This is lower than the suggested critical root length density for water uptake of wheat which is 1 cm·cm^{-3} (King et al. 2003). In a Swedish study, maximum average root length density over 100 cm depth was also smaller than 1 cm·cm^{-3}, namely: 0.49 cm·cm^{-3}(Kjellstrom and Kirchmann 1994). Roots were longer and thinner in the dry and warm year than in the wet and cool year, with a 6.5 fold difference in root length between years at a Swedish research farm (Kjellström et al. 1994). In a German field however, the RLD between 30 and 60 cm depth was much greater than 1, namely 3.5 cm·cm^{-3}. The roots in this experiment were measured using mini-rhizotrons, the difference in measurement technique could also have introduced some difference in RLD values between studies (Kamh et al. 2005).

The critical RLD for water uptake for oilseed rape is not known, if it is similar to wheat's and around 1.0 cm·cm^{-3}, in UK and Swedish fields oilseed rape could have too low a root density below 40 cm to make use of all the plant available water stored in the soil. It must be kept in mind that all the previous RLD numbers originate from only several fields and may not be representative of other commercial fields in Northern Europe.

The oilseed rape root system compared to other crops

Considering the lack of knowledge about oilseed rape root system and root functioning, it is useful to compare the crop's root system to other more intensively studied crops, for instance cereals and in several studies direct comparisons have been made. There are very few Northern European studies in which oilseed rape and wheat root systems have been compared in the same experiment, therefore studies from other areas will be included.

While spring oilseed rape root biomass was similar or smaller than wheat under various nitrogen regimes in a container experiment conducted in the Netherlands, its

root length density was about twice as high, the greater specific root length (length per gram DW) of oilseed rape means that spring oilseed rape roots were either thinner or less dense than wheat roots in this experiment (Dreccer et al. 2000).

The Canadian and Australian studies in which oilseed rape and wheat have been compared, do not give a consistent picture. In Australian fields, the RLD of oilseed rape was three times smaller than that of wheat (Zhang et al. 2005), while in Liu et al.'s 2010 study, oilseed rape and wheat had similar root length densities of 1.35 and 1.42 $cm \cdot cm^{-3}$ respectively (Liu et al. 2010).

Withholding water appeared to have a greater negative effect on wheat root mass than on oilseed rape root mass (Gan et al. 2009). In a study conducted in the USA, spanning three years, it was shown that the average depth at which root length is greatest varies between crops and was greatest for sunflower and shallowest for pea. Oilseed rape's maximum density lay at 56 cm while wheat's was deeper at 70 cm. This deeper maximum RLD of wheat was consistent over the years (Merrill et al. 2002).

The soil water distribution patterns over depth and time for oilseed and wheat crops did not differ between rainfed and irrigated treatments in a Canadian field. Additionally the soil water distribution in the profile did not differ, (or only very slightly) between oilseed rape and wheat. Assuming drainage and soil evaporation are similar, the crops used the same amount of water. In this experiment, oilseed crops (oilseed rape, mustard and flax) and wheat used water to below the theoretical wilting point (Gan et al. 2009).

In two out of four experiments in fields in Australia, the top 0.25 m of the soil was drier under safflower and wheat compared with oilseed rape and mustard, whereas there were no significant differences in water extraction among the species in the soil layers down to 1.25m (Norton and Wachsmann 2006). Deep water extraction was important for both oilseed rape and wheat crops, on one site 29% and 20% respectively, of the total water extracted came from below 1m depth. The cumulative change in soil water content (a reflection of total water extraction by the crop), was similar for all crops (oilseed rape, wheat, mustard and linola, except for safflower which extracted significantly more water from the soil) (Norton and Wachsmann 2006).)

The limited amount of data shows no consistent trend of oilseed rape having a lower or higher RLD than wheat. However, it is not known what the critical root length density for water uptake for oilseed rape is. Wheat appears to have some root system

characteristics that could make it less susceptible to drought stress. For instance, wheat depleted the surface soil to a greater extent than oilseed rape in an Australian study (Norton and Wachsmann 2006). And in another study, the maximum RLD was shallower for oilseed rape than for wheat (Merrill et al. 2002), which since shallower soil layers dry out quicker could mean that oilseed rape has its roots distributed less strategically to extract water from the soil when water becomes limiting. On the other hand, both oilseed rape and wheat crops tend to root deeply and take up water from depth, up to 180 cm (Barraclough 1989, Norton and Wachsmann 2006).

Plasticity of the root system

Plants are often very flexible when it comes to dealing with variability in their environment. Roots for instance often (but not always) preferably grow towards and proliferate in nutrient patches (Hodge 2004, Wang et al. 2007, Hodge 2009). Not only does the root system exhibit plasticity in response to heterogeneous distribution of nutrients, the Arabidopsis root system exhibits hydrotropism, i.e.: its roots preferably grow towards wetter soil, this trait is assumed to improve the plant's ability to take up water (Kobayashi et al. 2007).

Research into the plasticity of the oilseed rape root system by Wang et al (2005, 2007, 2009) has shown that the oilseed rape root system is very plastic in response to water distribution. In pot experiments in which small plants were grown under differing watering regimes, oilseed rape roots were able to forage for fixed patches of water by selective root placement. It resulted in about 10% greater shoot biomass compared with uniform watering (Wang et al, 2005). In an experiment with larger containers, root foraging for water was assessed by varying watering patterns as well as groundwater level at the base of the containers (Wang et al. 2009); winter oilseed rape responded vigorously to improved water status as a result of groundwater, but there was little effect of partial root zone drying, which could indicate that oilseed rape plants are very effective at finding (rooting in) and utilising patches of water. However in this experiment, the partial root drying treatment may not have been extreme enough to measure a response in the plant. Another possibility is that its indeterminate growth habit, with short vegetative phase makes the major yield components relatively insensitive to partial root-zone drying (Wang et al, 2009).

Soil structure

Soil structure can affect root growth and soil structure is affected by the type of soil and the tillage treatment (Bonari et al. 1995, Kappen et al. 2000, Becka et al. 2004). The total root length of oilseed rape was lower in conservation tilled (=shallow rotovated) soil than in ploughed soil but deep rooting was not affected (Kappen et al. 2000), this was also the case in a UK field, where ploughing only increased root length density in the top 40 cm or not at all in another field (Blake et al. 2006). While root mass responded to tillage treatment, there was no effect on yield (Bonari et al. 1995) nor did tillage intensity affect the concentration of nutrients in the oilseed rape shoot, while nutrient concentrations in wheat and maize were affected (Mozafar et al. 2000).

The length of the taproot was significantly shorter under minimum tillage, suggesting that oilseed rape root growth is sensitive to soil strength. While the taproot does have a function in anchoring the plant and also storage of energy (Goodman et al. 2001), there is as far I am aware no information in the literature that taproot length is an important indicator of the ability to acquire water and nutrients and final yield.

Oilseed rape can root very deeply, winter oilseed rape roots reached 1 m in November and a maximum depth of 1.8 m later in the growing season in a UK field (Barraclough 1989). In a poorer, sandy soil in Italy, however, roots only reached 0.5 m under minimum tillage (Bonari et al. 1995). Pulse crops had significantly shallower rooting than oilseed rape and wheat (Gan et al. 2009b).

From surveys of fields in three regions of New South Wales (Australia), it was concluded that restriction of the oilseed rape root system occurs in Australian fields due to lack of deep cultivation or the expansion of oilseed rape cultivation to less suitable fields. Water supply and sowing date were suggested to be important drivers for yield. Subsoil constraints and late season water stress were most common factors correlated with underperformance of oilseed rape crops on commercial farms in New South Wales, Australia (Lisson et al. 2007).

Chapter 2

Comparison of the response to drought in oilseed rape and wheat

Introduction

Oilseed rape (*Brassica napus* L.) is an important vegetable oil and fodder crop in many parts of the world. The major oilseed rape producing countries are: China, Canada, India, Germany, France, Poland, United Kingdom and Australia in descending order of rapeseed produced in 2007 (FAOSTAT 2010).

Since the development of varieties which are low in erucic acid and glucosinolates and hence more palatable, oilseed rape's cropping area has increased significantly. Oilseed rape is also used as a break crop in cereal rotations.

Even in temperate climates, such as in the UK, oilseed rape crops are occasionally exposed to periods of water limitation, due to drought spells (Foulkes et al. 2001). It is likely, with the predicted development of climate change that these drought spells will become more frequent and more extreme, therefore it is necessary to assess whether a) oilseed rape crops are sensitive to drought, b) if so, which characteristics are responsible for this and c) what characteristics/aspects of the crop could be improved to enhance its ability to avoid or tolerate drought to at least maintain current yield or even improve future yield.

Water deprivation has multiple effects in crops, depending on how long it lasts, how severe it is and during which stage of crop development it occurs (Passioura 2007). The first effect of water limitation is usually a reduction in leaf expansion, then stomatal closure takes place, with the dual effect of reducing water loss and slowing down the rate of CO_2-capture and hence growth. If drought stress continues, plants will start to wilt and eventually older leaves will be shed. If the drought perseveres, the plant will desiccate and die (Neumann 2008).

Water limitation has a negative effect on oilseed rape yield, especially when it occurs during or just after flowering (Faraji et al. 2009), but a negative effect during stem elongation has also been observed (Mueller et al. 2010). We do not know however, whether it is more drought sensitive than other crops and whether its yield is (more) vulnerable to impending climate change.

Here we will assess whether oilseed rape is prone to drought stress. The onset of drought stress of oilseed rape will be compared to the better studied crop wheat. Wheat and oilseed rape are often grown in rotation, so these crops encounter the same soil and weather conditions. Little is known about relative drought risk of oilseed rape crops in

temperate climates. There is also relatively little known about oilseed rape function and here we will compare root length density, water influx rates as well as water uptake patterns of oilseed rape and wheat plants under water limitation. Another aspect of drought is the effect of 'water-stress' on biomass production and allocation to different plant parts. A species can have an earlier onset of drought, but the effect of water stress can be less severe, if it allocates resources differently or is more efficient in its use of water. Thus the impact of water limitation on oilseed rape and wheat will also be compared in order to establish whether the growth of oilseed rape is affected more by drought than wheat.

The following specific questions will be addressed:

-Is oilseed rape more drought sensitive than wheat?

-If so, which characteristics make it more drought sensitive?

-Does oilseed rape extract water from the soil as effectively as wheat and could oilseed rape benefit from alterations to the root system?

Drought sensitivity was assessed by growing mini-crops in lysimeters in an open sided glasshouse and monitoring canopy function as a function of soil moisture deficit.

Materials and methods

Experimental design and treatments

An experiment involving 24 lysimeters laid out in a randomised block design, with five blocks, was set-up in April 2007 in a glasshouse at Easter Bush, Penicuik, Scotland. The glasshouse was open-sided to encourage airflow and to avoid high temperatures. The lysimeters were constructed of Marley polyvinyl chloride pipes of 30 cm internal diameter and 120 cm height.

A sandy clay loam soil (MacMerry series, (Vinten et al. 1994), sand 55% w/w, silt 25% and clay 25% w/w, was packed into the lysimeters so that at the end of the experiment, the bottom 37 cm was at a mean (± sem*)* dry bulk density of 1.20 ± 0.034 $g \cdot cm^{-3}$, the middle 40 cm section was 1.15 ± 0.031 $g \cdot cm^{-3}$ and the top 28 cm was 1.11 ± 0.025 $g \cdot cm^{-3}$ (Figure 2.1). A five cm diameter access tube for a capacitance probe (Sentek diviner 2000, Kent Town, Australia) was placed into the centre of the lysimeter before packing the soil and the soil was packed around it. *Circa* 200 mm water was added to the top and lysimeters were covered with plastic sheets to prevent evaporation of water from the soil surface and the lysimeters were allowed to drain for three days to achieve field capacity on April 30th 2007 which was the day of sowing seeds. However on this day due to technical difficulties, soil moisture content was only measured from 0 to 50 cm down the soil profile. Therefore the next measurement made at 4 days after sowing (DAS) was taken to be the soil moisture content at field capacity and used as a reference for determining soil moisture deficit. During the first two weeks, all lysimeters were irrigated to get seedlings established.

P, K and S fertiliser was applied before sowing as KH_2PO_4 and K_2SO_4 in solution to supply 60 $kg \cdot ha^{-1}$ P 35 $kg \cdot ha^{-1}$ K and 14 $kg \cdot ha^{-1}$ S The solution was added and mixed with the top 30 cm of soil before it was packed into the lysimeter. Nitrogen (NH_4NO_3) was applied to the soil surface once at a rate of 100 $kg \cdot ha^{-1}$ nitrogen four DAS.

The target density for oilseed rape (spring oilseed rape: *Brassica napus* L. cv. SW Landmark) was eight plants per lysimeter = 113 plants$\cdot m^{-2}$; for wheat *Triticum aestivum* L. cv. Tybalt, the density was 38 plants per lysimeter = 552 plants$\cdot m^{-2}$. These varieties were chosen because they have a similar life-cycle duration. Seeds were sown on April 30 (= 0 DAS), 14 seeds per lysimeter for oilseed rape and 44 seeds for wheat, after two weeks in

the lysimeters the seedling densities were adjusted to eight and 38 respectively by either removing surplus seedlings or sowing extra seeds. See photo 1 for layout of experiment. Additionally there where were four lysimeters without plants, two of which were irrigated and two where water was withheld (non-irrigated treatment). These were included to monitor soil evaporation and for taking samples for determining soil hydraulic properties. Evaporation of soil water from under the canopy was monitored with a greater sample size using micro-lysimeters.

The oilseed rape plants were sprayed twice during the season (DAS 39 & 52) and wheat plants once (DAS 49) with 0.04 $g \cdot l^{-1}$ Bifenthrin (BugFree-Bayer, Bayer Crop Science, Cambridge UK) until run-off to control aphids and flea beetles. The wheat plants were sprayed once on 42 DAS with metrafenone (Flexity, BASF) fungicide to prevent mildew development. On 49 DAS, mesh sleeves (Netlon 3 mm mesh, Conwed Plastics, Genk, Belgium) were put around the canopies to simulate a field situation with shading from neighbouring plants, the oilseed rape plants had four unfolded leaves and wheat plants five at this point in time.

For the plants in the non-irrigated treatment, water was withheld from 14 DAS. The plants in the irrigated treatment were given water once per week to return the soil to field capacity (see below). Air temperature and relative humidity were logged every hour at plant height with a DL3000-8.10 logger positioned at the edge of block two of the experiment (Delta-T Devices Ltd, Cambridge, UK).

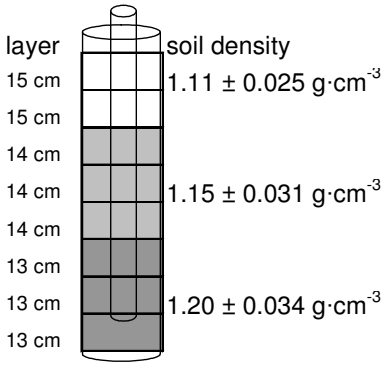

Figure 2.1 Packing of lysimeters, and measured soil bulk density at each different level.

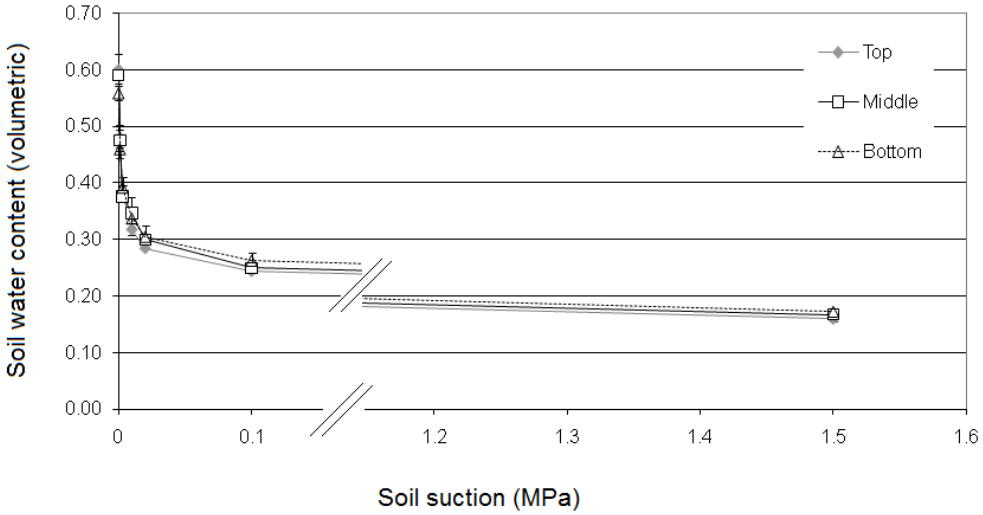

Figure 2.2 Desorption curves of soil cores takes at different lysimeter depths. Sample size is three and standard error of the mean (sem) is represented by the error bars.

Photo 1 The lysimeters with oilseed rape and wheat plants at the day of harvest. The green mesh shading the mini crops during growth was slid down.

Measurements

The height of three plants per lysimeter was measured once a week to the nearest half centimetre, from the soil surface to the tip of highest (outstretched) leaf. The length of an expanding leaf was measured weekly to monitor when plants experienced a decrease in leaf expansion rate as a result of water stress. From 24-31 DAS leaf two of oilseed rape was measured and leaf three of wheat, from DAS 32-52 leaf four and leaf five were measured for oilseed rape and wheat respectively.

Volumetric soil moisture content of the top 80 cm of soil was measured every Thursday and Friday during the experiment with a capacitance probe (Sentek Diviner 2000, Sentek pty ltd, Kent Town, Australia). After the Thursday measurement, the lysimeters in the irrigated treatment were watered to bring the soil back to field capacity, (i.e. the amount of water held in the soil on DAS 4), before measuring again on the Friday.

Evaporation from the soil surface under the canopy was determined by inserting micro-lysimeters in the soil and reweighing them after six days, the difference in weight equalled the water loss from the soil (Boast and Robertson 1982, Daamen et al. 1993). The micro-lysimeters were 100 mm long open-ended centrifuge tubes with an inner diameter of 22 mm placed in the soil under the canopy, about halfway between the edge and centre of the lysimeters. The open ended tube was pushed into the soil and a small soil core was taken out. Micro-lysimeter and soil were weighed, the soil-filled micro-lysimeter was wrapped in plastic and put back in the hole left by the core and re-weighed after a period of six days. Then the micro-lysimeters were put back in the coring holes without the plastic wrapping, the irrigated treatments were irrigated and micro-lysimeters in this treatment were allowed to drain for a day. All micro-lysimeters were then weighed again the next the day at the start of a new six day period. Additionally, there were four unplanted lysimeters to monitor evaporation of water from the soil.

On 67 and 74 DAS, the leaf stomatal conductance (g_s) of the youngest fully expanded leaf in the top of the canopy of one plant per lysimeter was measured around midday with a portable IRGA (ADC-LCA4 Analytical Development Co. Ltd, Hoddesdon, Herts, UK).

On 84 DAS for wheat and 85 DAS for oilseed rape the plants were destructively sampled. After counting total shoot numbers and weighing total fresh weight of leaves, ears and pods and stems, a subsample was taken for further analysis. The subsample was obtained by allocating all the stems of a lysimeter randomly to four separate piles and taking two piles and one pile for further analysis for oilseed rape and wheat respectively. The fresh weight of the subsample was recorded. The leaf, pod or ear and stem area of the subsample were determined with a LI-3100 leaf area meter (Li-Cor Biosciences, Cambridge, UK). The dry weights of the plant parts were determined after drying to constant weight in a fan-assisted oven at 80°C. The total plant DW per lysimeter was calculated after accounting for the sub-sampling, by using the ratio of the fresh weight of the total sample to its subsample.

On the day of harvest, the youngest fully expanded leaves was cut off one of the plants in each lysimeter, put in a sealed plastic bag and stored in a closed plastic container cooled with an icepack for at most an hour. In the laboratory, a rectangular segment was cut from the leaf, avoiding large veins. The fresh weight of this fragment was determined and then the leaf was floated on de-ionised water in a Petri dish for 3-4 hours in a dark room at 21°C to attain full turgid weight (Smart and Bingham 1974). The segment was dabbed dry after 3-4 hours and re-weighed. The segment was dried in an oven at 80°C until the weight was constant, subsequently dry weight was determined. From these weights, the relative water content of the leaf at time of harvest could be calculated: (FW start-DW) / (Turgid Weight – DW).

Root samples were taken after the lysimeters were laid down horizontally and cut open with a saw. Samples were taken at 10 cm depth intervals with a corer of a volume of 209.3 cm^3 and kept at -18°C until root washing took place. The roots of samples from 30-40 cm depth and 70-80 cm depth were washed out with a Delta-T root washer (Delta-T Devices Ltd, Cambridge, UK) and the roots collected on a 0.5 mm mesh. One whole 209.3 cm^3 sample was put in a washing bucket and stirred once an hour. When there were no or few visible roots remaining in the water, washing was terminated and the separated roots were retrieved from the meshes and stored in a flask with water. The roots were kept at 4°C until they were cleaned, sorted and scanned, later the same day or early the next day. Cleaning consisted of picking out debris; sorting consisted of separating old (grass) roots that were already present in the soil prior to the experiment from the new roots of oilseed rape and wheat plants. New roots were

distinguished from old dead roots on basis of their colour. The root samples were immersed in water and spread out carefully in clear plastic trays to minimize overlap of roots and subsequently scanned with a Régent LA1600 scanner, the images were analysed with Winrhizo software (Régent Instruments Inc, Quebec, Canada).

The soil samples from three unplanted lysimeters were used to make a soil desorption curve for the three different soil bulk densities at the three depth zones. Soil cores of a volume of 209.3 cm^3 were extracted from the lysimeters in rings and gauze placed at one end which was secured with a rubber band. The cores were then saturated with water and placed on a tension table filled with a layer of fine silica sand to ensure good contact. The top of the cores was sprayed with 4% formaldehyde solution to prevent seedling growth and to control invertebrates that could be present in the soil. Soil water desorption was determined by increasing water suction in steps and weighing the cores to determine moisture content. At each tension when equilibrium was reached (i.e. no more water was released from the soil cores), the cores were reweighed and placed back on the tension table and the suction was increased; suctions of 3, 10 and 20 kPa were applied. To apply pressures of 100 kPa and 1500 kPa (e.g. permanent wilting point), subsamples from the cores were taken and placed on a pressure plate and pressure applied until water was no longer released by the samples. The soil dry weight was measured after drying the soil at 105°C in an oven for at least 24 hours. The moisture release curves for soil at different bulk densities were plotted (Figure 2.2).

Calculations and statistical analyses

Plant available water is considered to be the mm or ml of water held by the soil between field capacity (FC) and permanent wilting point (PWP). The amount of water held at field capacity is taken to be the amount of water in the soil at DAS 4. The amount of water held at PWP is here assumed to be the amount of water held by the soil when a suction of 1.5 MPa was applied. The first measure (FC) was calculated using soil moisture readings of the lysimeters measured with the capacitance probe, the second measure (PWP) was determined by applying a pressure of 1.5 MPa on soil samples placed on a pressure plate and measuring the amount of water held by the soil.

Evaporation of water from the soil surface was calculated using micro-lysimeter data and deducted from total water loss by soil to give the transpiration rate. Although

soil surface evaporation data from micro-lysimeters is only available for the periods DAS 32-38, 39-45, 46-52 and 60-66; these data for soil evaporation were preferred over evaporation data from unplanted lysimeters, because the sample size from micro-lysimeters is greater (n=5) and because these take into account influences of canopy cover on evaporation.

Cumulative water uptake for each ten cm depth soil layer, as measured weekly with the capacitance probe was plotted. These curves were sigmoid in shape. A straight line was plotted through three points at the linear phase of the curve and the point at which this line crossed the x-axis was taken to be the onset of water uptake from that depth.

For non-irrigated plants, the slope at the linear phase of the cumulative transpiration curve of each 10 cm soil layer was considered to be the maximum influx rate at that layer and from two points at the steepest slope the maximum influx rate in ml per day was calculated for each soil layer.

Root length and root surface area were measured at harvest, DAS 84 and 85. For the inflow rate per unit root length and root surface area, the water transpired by plants between DAS 73 and DAS 80 was used to make calculations about inflow rates into roots.

For all statistical analyses GenStat was used (GenStat 11.1 2008, VSN International Ltd, Hemel Hempstead, UK). For most results, the effect of species and irrigation treatment were tested with a two-way ANOVA test. Data were transformed to obtain normal distribution of residuals where necessary. If transformation did not result in a normal distribution a non-parametric Kolmogorov-Smirnov test was used. A repeated measures ANOVA was conducted when the effect of species and watering regime on a parameter in time (or over depth) was assessed.

Differences between the maximum rates of influx of non-irrigated oilseed rape and wheat plants were ascertained by calculating the 95% confidence interval of the means, by multiplying the error bars (standard error of the mean) by 1.96, if there was no overlap, the species differed significantly in influx rates.

The harvest and root data were analysed with a two-way ANOVA and where necessary, data were log transformed to obtain a normal distribution. Root data were

analysed per depth, at 70-80 cm one sample in the wheat well-watered treatment was an outlier (3-4 times smaller than other sampled in same treatment, while root water influx rates were similar to the other samples; this was one of the first samples analysed and omitted from the root results table and statistical analyses).

Results

Water use and onset of drought

From DAS 20 onwards the potential transpiration rate (irrigated treatment) increased steadily, until it dropped during the interval DAS 45-52 and increased again after this for both oilseed rape and wheat crops (Figure 2.3). Withholding water reduced the transpiration rate of oilseed rape and wheat (Table 2.2). Day 14 was the last watering day and withholding water had a significant effect on plant water use rates from day 42 onwards, e.g. interval 38-45 DAS (Table 2.1). The potential transpiration rate was greater for oilseed rape than for wheat from DAS 42 onwards (irrigated treatments). Later on, during the interval 45-52 DAS, oilseed rape crops transpired more than wheat regardless of treatment. This period coincided with a low atmospheric deficit, following a period of high atmospheric deficit and even the well-watered control plants showed a drop in transpiration rate.

There was no difference in the timing of onset of drought stress between species. In both oilseed rape and wheat the transpiration rate of non-irrigated plants dropped below the potential transpiration rate (irrigated treatments) around day 42 (during the interval 38-45 DAS) (Figure 2.3 & Table 2.1). From day 56 onwards (52-59 DAS interval), withholding water had a significantly greater effect on oilseed rape than on wheat when compared to the irrigated controls. The difference in transpiration rate between non-irrigated and irrigated plants was significantly greater for oilseed rape. The transpiration rates of non-irrigated oilseed rape and wheat were remarkably similar during the last 25 days of the experiment. However, this could have been due to an artefact in soil moisture measurements over this time. Around DAS 52 all plant available water had been extracted from 0 to 80 cm of soil in non-irrigated lysimeters, but the capacitance probe measurements registered a further decline in soil moisture content which could not be completely accounted for by evaporation from the soil surface (water loss by un-planted columns) from this day onwards (Figure 2.4). Possible causes for this are outlined later.

Soil moisture content and hence plant water use was measured weekly, therefore there could have been a difference in timing of drought onset between species, but this at most would have been a few days.

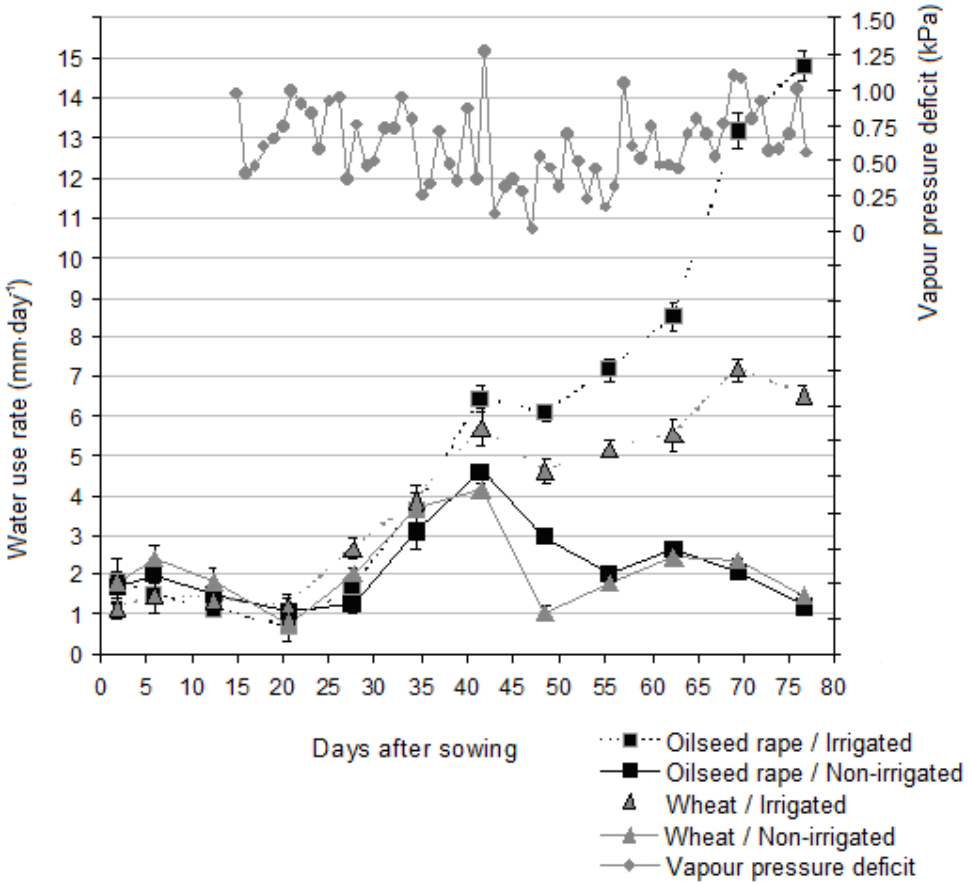

Figure 2.3 Transpiration rates of plants per lysimeter (n=5, sem in error bars). The data point is the mid-point of the interval (usually of 7 days) over which transpiration rate was calculated. Potential transpiration rate (irrigated lysimeters) of oilseed rape (■) and wheat (▲) is represented by symbols connected with intermittent lines. The vapour pressure deficit kPa is also plotted.

Table 2.1 Two-way ANOVA test results (p-values, n=5) for water use rates, data represented in Figure 2.3.

Interval (DAS)	Midpoint interval	Species	Irrigation	Species x Irrigation
0-4	2	0.394	0.426	0.241
4-8	6	0.433	0.002	0.443
8-17	12.5	0.349	0.135	0.879
17-24	20.5	0.77	0.98	0.35
24-31	27.5	0.01	0.07	0.72
31-38	34.5	0.31	0.3	0.64
38-45	41.5	0.094	<.001	0.666
45-52	48.5	<.001	<.001	0.334
52-59	55.5	<.001	<.001	0.002
59-66	62.5	<.001	<.001	<.001
66-73	69.5	<.001	<.001	<.001
73-80	76.5	<.001	<.001	<.001

Table 2.2 Results of ANOVA with repeated measures for testing of effects of species, irrigation (water) and time on transpiration rates, data in Figure 2.3.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	4	4.5341	1.1335	1.23	
Block.Subject stratum					
Species	1	44.0103	44.0103	47.87	<.001
Water	1	393.6449	393.6449	428.2	<.001
Species.Water	1	40.9345	40.9345	44.53	<.001
Residual	12	11.0315	0.9193	2.36	
Block.Subject.Time stratum					
d.f. correction factor 0.5008					
Time	11	761.6389	69.2399	177.77	<.001
Time.Species	11	117.8501	10.7136	27.51	<.001
Time.Water	11	633.8613	57.6238	147.95	<.001
Time.Species.Water	11	114.8726	10.443	26.81	<.001
Residual	176	68.5488	0.3895		
Total	239	2190.927			

Table 2.3 Total plant plus soil water use (e.g. evapotranspiration) and water use efficiency per lysimeter. Statistical test results in Table 2.4.

	Oilseed rape		Wheat	
	Irrigated	Non-irrigated	Irrigated	Non-irrigated
Total cumulative water use (plant + soil) (mm)	497.6 ± 14.93	190.3 ± 2.81	354.6 ± 13.72	193.3 ± 2.91
WUE ($gDW \cdot mm^{-1} \cdot m^{-2}$ ground)	4.13 ± 0.148	5.19 ± 0.130	4.34 ± 0.112	6.30 ± 0.170

Table 2.4 Test results (p-values) of two-way ANOVA tests on water use data presented in Table 2.3.

	Species	Irrigation	Species x Irrigation
Cumulative (total) evapotranspiration (mm)	< 0.001	< 0.001	< 0.001
WUE ($gDW \cdot mm^{-1} \cdot m^{-2}$ ground)	< 0.001	< 0.001	0.011

In Table 2.3 the total cumulative water use (summed evapotranspiration per lysimeter) and water use efficiency per lysimeter are given. Water use efficiency (WUE) is defined as gram above ground dry weight produced per mm water evaporated by the mini-crop per square meter ground area. Due to a possible artefact in the later measurements of water loss from non-irrigated lysimeters (from ~DAS 50 onwards), total water use from the non-irrigated treatments is likely to have been overestimated. Therefore the total water use and WUE values of plants in the non-irrigated lysimeters should be used with care, the WUE of mini-crops in the non-irrigated treatments is likely to be greater than Table 2.3 suggests.

When irrigated, the oilseed rape mini crops had a significantly greater total water use (cumulative evapotranspiration) than wheat mini-crops, while when non-irrigated the total amount of water that was used was similar for oilseed rape and wheat (species x irrigation, $p < 0.001$, Table 2.4). WUE of mini-crops was greater when non-irrigated, and WUE was significantly more increased in non-irrigated wheat mini-crops than in non-irrigated oilseed rape mini-crops compared to the irrigated controls (species x irrigation, $p < 0.011$, Table 2.4).

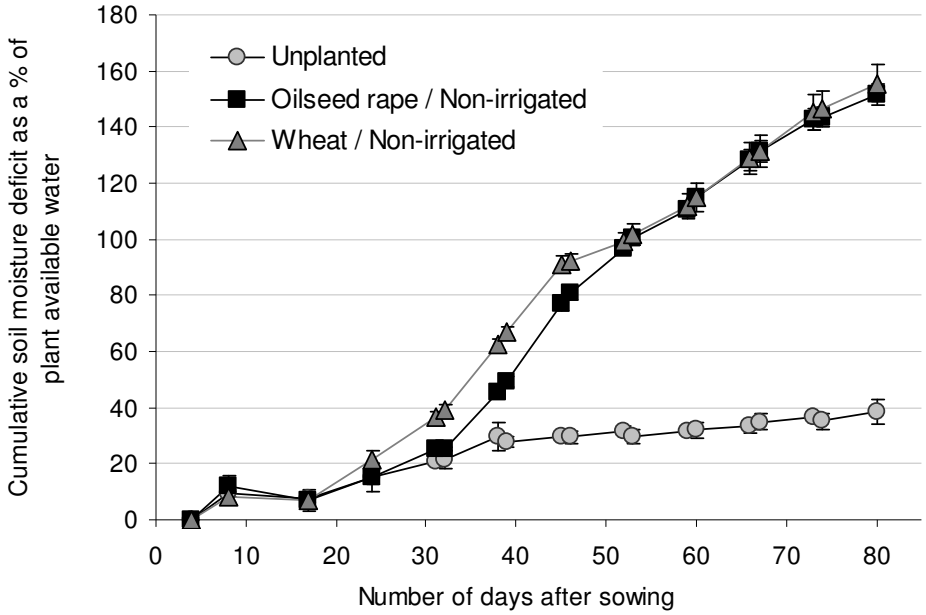

Figure 2.4 Cumulative soil moisture deficit of oilseed rape and wheat plants and unplanted lysimeters, as a percentage of plant available water over the total measured depth of 80 cm depth. Error bars represent sem.

Table 2.5 Results of ANOVA with repeated measures for testing of effects of species and time on cumulative soil moisture deficit measured from 0-80 cm soil depth, data from 0 to 53 days after sowing tested, original data in Figure 2.4.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	399.14	99.79	1.76	
block.Subject stratum					
species	1	1704.16	1704.16	30.05	0.005
Residual	4	226.85	56.71	2.41	
block.Subject.Time stratum					
Time	11	150659.5	13696.32	583.01	<.001
Time.species	11	1510.99	137.36	5.85	0.01
Residual	88	2067.34	23.49		
Total	119	156568			

In Figure 2.4, the cumulative soil moisture deficit of non-irrigated plants over a depth from 0 to 80 cm is expressed as a percentage of plant available water (that was held between field capacity and permanent wilting point). Between DAS 32 and 46, the cumulative soil moisture deficit of soil in lysimeters planted with wheat was significantly greater than that of lysimeters with oilseed rape (Table 2.5).

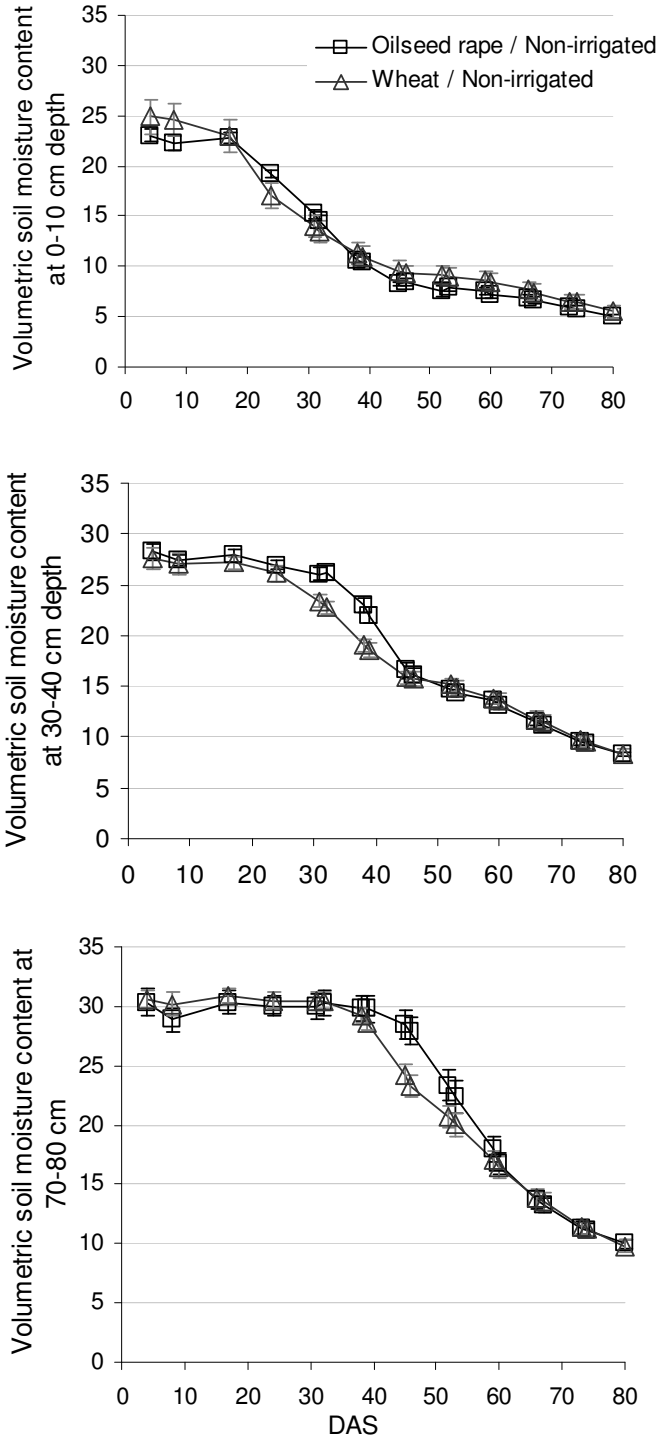

Figure 2.5 The change in volumetric soil moisture content (% v/v) in time at three different soil depths of lysimeters planted with non-irrigated oilseed rape or wheat plants. Sample size was 5, and error bars represent sem. Note that the permanent wilting point is at a moisture content of 16% (see water content of soil in Figure 2.2 at 1.5 MPa suction).

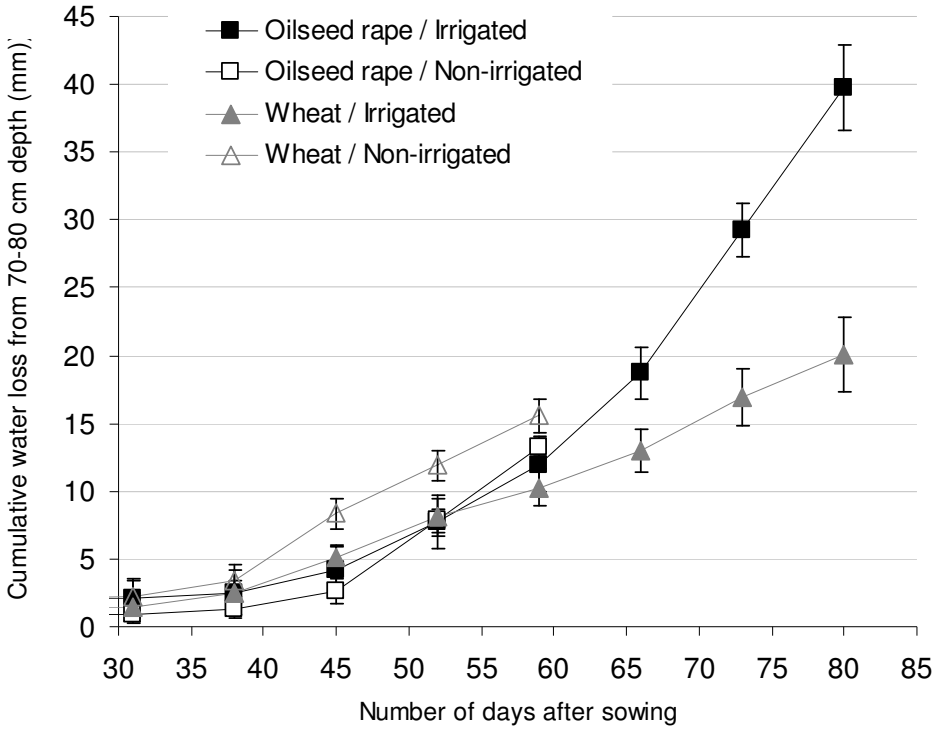

Figure 2.6 Cumulative water loss from soil depth 70-80 cm by plant transpiration. All four treatments shown, error bars represent sem (n=5).

Table 2.6 Results of ANOVA with repeated measures for testing of effects of species, irrigation (water) and time on cumulative water loss from soil depth 70-80 cm on days 31 to 59, data in Figure 2.6.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	213.7799	53.4450	1.75	
block.Subject stratum					
species	1	52.2152	52.2152	1.71	0.215
water	1	32.3972	32.3972	1.06	0.323
species.water	1	68.9285	68.9285	2.26	0.159
Residual	12	366.3465	30.5289	36.31	
block.Subject.Time stratum					
d.f. correction factor 0.1250					
Time	4	1726.3375	431.5844	513.31	<.001
Time.species	4	34.9147	8.7287	10.38	0.003
Time.water	4	45.9636	11.4909	13.67	<.001
Time.species.water	4	7.7227	1.9307	2.30	0.143
Residual	64	53.8102	0.8408		
Total	179	3995.923			

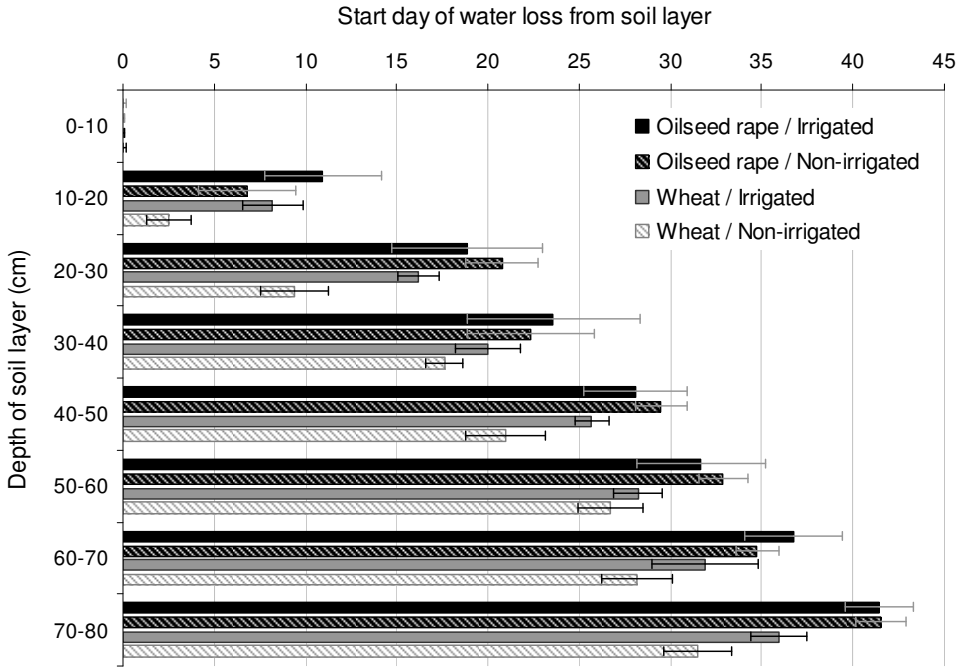

Figure 2.7 Start day of water loss from different soil depths for lysimeters planted with oilseed rape or wheat.

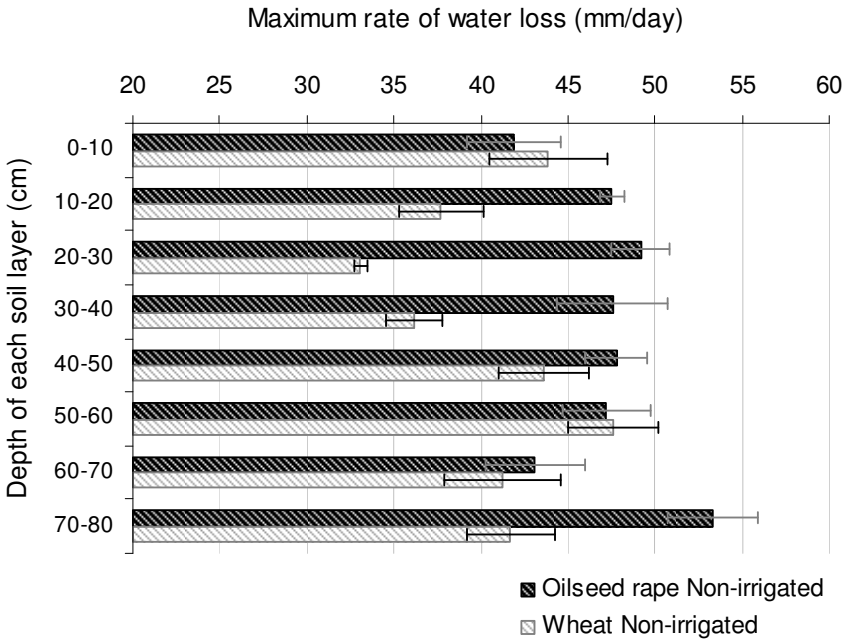

Figure 2.8 The maximum rate of water loss (evapotranspiration) from different soil depths of non-irrigated lysimeters planted with oilseed rape and wheat plants, mean (n=5) and standard error of the mean are given.

Table 2.7 Results of ANOVA with repeated measures testing the effects of species, irrigation (water) and depth on start day of water loss from each soil depth, data in Figure 2.7.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	526.52	131.63	1.78	
block.Subject stratum					
species	1	925.18	925.18	12.49	0.004
water	1	160.71	160.71	2.17	0.166
sp.water	1	108.87	108.87	1.47	0.249
Residual	12	888.74	74.06	5.07	
block.Subject.Time stratum					
d.f. correction factor 0.5523					
Depth	7	23464.6	3352.09	229.25	<.001
Depth.sp	7	199.91	28.56	1.95	0.115
Depth.water	7	85.31	12.19	0.83	0.506
Depth.sp.water	7	76.79	10.97	0.75	0.557
Residual	112	1637.63	14.62		
Total	159	28074.24			

In Figure 2.5 the soil moisture content of three different soil layers is plotted to give an indication of the spatial pattern of water extraction from the soil under non-irrigated oilseed rape and wheat plants. These graphs show that wheat plants tend to take up water from each soil layer earlier than oilseed rape plants, but that the maximum rate of water loss from the soil is greater for oilseed rape plants. It is likely at soil moisture contents below 16%, the access tube for probe measurements lost its even contact with the soil due to soil drying and shrinking and the apparent water loss from the soil after this point (Figure 2.5) was an artefact caused by air gaps. Thus values for water extraction below 16% soil moisture content need to be used with caution and are therefore not shown in Figure 2.6.

The extraction pattern at depth 70-80 cm depth was plotted in Figure 2.6; here can be seen that the pattern of extraction was different for all four treatments, this pattern is less pronounced or different at the shallower depths (not shown). Non-irrigated wheat plants were the first to extract water from this depth (Figure 2.7, Figure 2.6, Table 2.6) and also initially took up water at a greater rate than plants in all other treatments. Non-irrigated oilseed rape plants did not take up water at a greater rate than

irrigated oilseed rape plants and by day 59 had exhausted all the plant available water. After day 59, water extraction by irrigated oilseed rape plants kept increasing and at harvest the total amount of water extracted from this layer was almost twice that of the plants in the other treatments. The onset of water uptake from each soil depth is represented in more detail in Figure 2.7 and the maximum rate of water loss from each soil layer was plotted in Figure 2.8.

Non-irrigated oilseed rape plants started taking up water from each depth, about 6.5 days later than non-irrigated wheat plants (Figure 2.7). Wheat plants took up water from each subsequent layer earlier in time, but there was no significant effect of irrigation treatment (Table 2.7). The lack of statistical significance could have been due to high variance within treatment group. Non-irrigated wheat plants tended to start extracting water from each subsequent layer earlier than plants in the other three treatments as can be seen in Figure 2.7, but the difference was not statistically significant Table 2.7). While oilseed rape plants started depleting soil layers significantly later than wheat, it completed the extraction of available water at about the same time because the maximum rate of extraction from soils planted with oilseed rape was higher (Figure 2.8). At depths 10-20, 20-30, 30-40 and 70-80 cm the maximum rate of water uptake by non-irrigated oilseed rape plants was significantly greater than that of wheat plants.

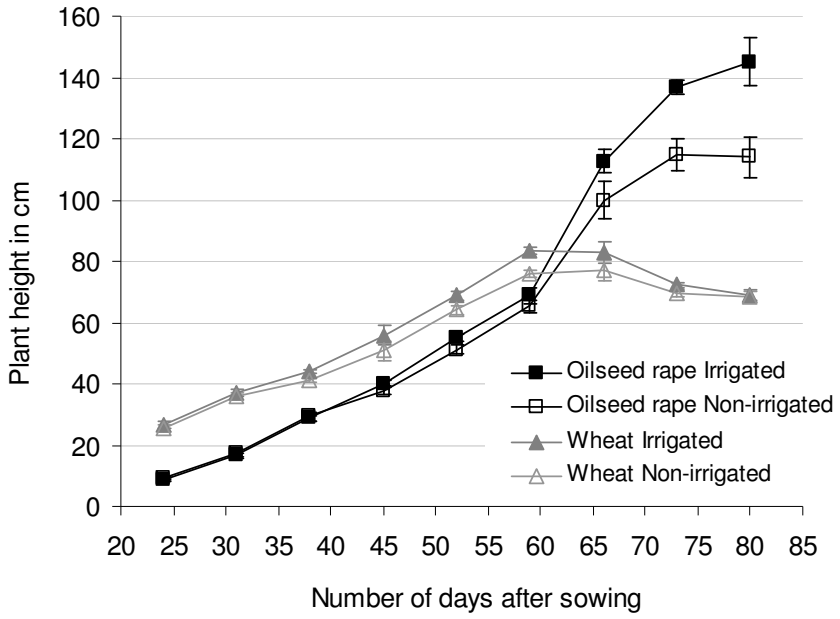

Figure 2.9 Plant height of oilseed rape and wheat as affected by irrigation treatment, error bars depict sem, n=5.

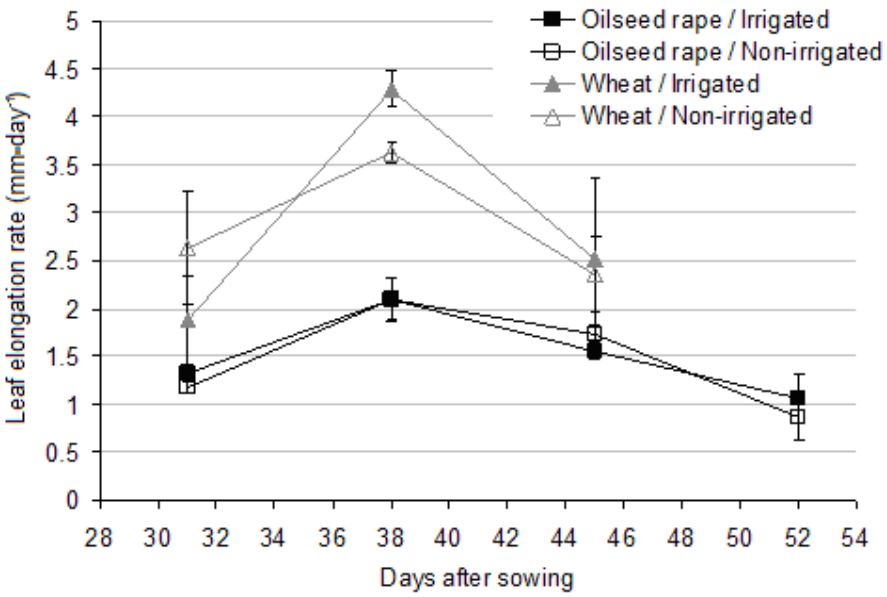

Figure 2.10 The average leaf elongation rate of developing leaves in mm/day; calculated for the week leading up to the day of data point. Leaves two and three measured for data point on day 31 for oilseed rape and wheat respectively, and leaves four and five measured from 31 to day 52.

Table 2.8 Results of ANOVA with repeated measures for testing of effects of species, irrigation (water) and time on plant growth (height), data in Figure 2.9.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	4	476.46	119.11	1.16	
block.Subject stratum					
sp	1	1472.52	1472.52	14.29	0.003
water	1	1531.06	1531.06	14.86	0.002
sp.water	1	258.08	258.08	2.5	0.14
Residual	12	1236.69	103.06	3.56	
block.Subject.Time stratum					
d.f. correction factor 0.3061					
Time	8	158961.3	19870.16	686.61	<.001
Time.sp	8	42652.84	5331.61	184.23	<.001
Time.water	8	1226.39	153.3	5.3	0.006
Time.sp.water	8	1479.23	184.9	6.39	0.002
Residual	128	3704.24	28.94		
Total	179	212998.8			

Table 2.9 P-values of K-S test of effect of irrigation treatment on leaf elongation data of each species in different moments in time. Original data plotted in Figure 2.10.

	Oilseed rape	Wheat
DAS 24-31	0.165	0.261
DAS 32-38	0.449	0.007
DAS 39-45	0.165	0.165
DAS 46-52	0.449	-

Table 2.10 Plant parameters of total number of plants per lysimeter at harvest. Plants were harvested on days 84 and 85 after sowing, the mean of five samples and the standard error are given, result of statistical tests given in Table 2.11.

	Oilseed rape		Wheat	
	Irrigated	Non-irrigated	Irrigated	Non-irrigated
Total above ground biomass (g)	144.9 ± 4.8	69.8 ± 1.9	108.5 ± 4.1	83.1 ± 3.1
Total shoot area (cm^2)	8170 ± 639	3032 ± 136.4	7920 ± 511	3740 ± 171.7
Total leaf area (cm^2)	5364 ± 596.2	1756 ± 88.8	5970 ± 428.2	2484 ± 145.2
Total pod or ear DW (g)	45.6 ± 3.0	21.9 ± 0.8	26.0 ± 0.8	25.1 ± 1.0
Stem or tiller number	9.4 ± 0.51	8.60 ± 0.75	66.0 ± 1.70	50.4 ± 2.50
Relative water content	0.84 ± 0.041	0.68 ± 0.061	0.93 ± 0.008	0.63 ± 0.038

Table 2.11 Two-way ANOVA test results for harvest data.

	Species	Irrigation	S x I
Total above ground biomass (g)	0.095	< 0.001	< 0.001
Total shoot area (cm^2)	0.474	< 0.001	0.181
Total leaf area (cm^2)	0.032	< 0.001	0.457
Total pod or ear DW (g)	< 0.001	< 0.001	< 0.001
Stem or tiller number	< 0.001	< 0.001	0.001
Relative water content	0.552	< 0.001	0.116

Effects of withholding water on shoot growth

Withholding water reduced the above ground dry weight of oilseed rape significantly more than that of wheat (Table 2.10, Table 2.11). The total shoot area (summed area of stems, leaves and pods/ears) of oilseed rape plants was not affected by drought more than wheat, the area of both species was more than halved by drought treatment and the species by irrigation interaction was not significant. Total leaf area was reduced by the

same degree in both species, oilseed rape had a significantly lower total leaf area than wheat in both water treatments.

Withholding water not only reduced dry weight of oilseed rape more than wheat, it also reduced pod dry weight significantly more than the ear dry weight of wheat. While pod dry weight of oilseed rape was halved, wheat ear dry weight was not significantly affected.

In Figure 2.9 plant growth, or more specifically the change in plant height, is plotted. From about day 40 onwards the plant height of non-irrigated plants dropped below that of irrigated plants. Towards the end of the experiment the difference in plant height between irrigated and non-irrigated oilseed rape plants is great, while wheat plant height was affected less by withholding water (Table 2.8). The trends in leaf expansion rate were less straightforward (Figure 2.10), this could have been due to the high variance within treatments of because of the fact that two different expanding leaves were measured for different time-periods. Perhaps differences in leaf expansion would have been observed if a single leaf's complete lifespan (elongation) was followed in time. On most dates there was no significant defect of irrigation treatment on leaf elongation rate, except for day 38 when the leaf elongation of non-irrigated plants was significantly smaller than that of irrigated plants.

The root system and influx rates

At a depth of 30-40 cm, well-watered plants had a significantly greater root length density (Table 2.12). At this depth the root length density (RLD) of oilseed rape was reduced more by withholding water than in wheat (species x irrigation $p < 0.05$). When not irrigated, the RLD of both species was similar, but when irrigated, the RLD of oilseed rape was greater than that of wheat. Although the root surface area of wheat was hardly affected by drought, oilseed rape's surface area was reduced by drought treatment compared to controls albeit not quite significantly (species x irrigation, $p = 0.054$).

At 70-80 cm depth, the RLD and root surface area of oilseed rape plants was smaller than that of wheat, but not significantly so, due to an outlier in the wheat well-watered treatment. Drought reduced the surface area of both species significantly at this depth, but RLD was not reduced significantly. However if the outlier sample 10-8 is omitted, RLD was significantly reduced by drought ($p = 0.016$). At this depth the

measured root characteristics of both species are equally sensitive to drought (e.g. there was no significant species x irrigation interaction; regardless of whether sample 10-8 was included or not).

When irrigated, oilseed rape plants had a greater water influx rate per unit root length and root area, based on root measurements made on DAS 84 and DAS 85 (at harvest) and the change in soil water content between DAS73 and DAS 80 (Table 2.13). At both depths, this difference was significant.

Table 2.12 Root length density and surface area at two soil depths, results of two-way ANOVA given, sample size is five and sem is given.

		Oilseed rape		Wheat		P-values		
	Depth (cm)	Irrigated	Non-irrigated	Irrigated	Non-irrigated	Species	Irrigation	S x I
Root length density ($cm \cdot cm^{-3}$)	30-40	3.85 ± 0.494	2.11 ± 0.217	2.67 ± 0.204	2.38 ± 0.152	0.167	0.006	0.04
	70-80	3.18 ± 0.251	2.31 ± 0.253	3.51 ± 0.600	3.18 ± 0.222	0.166	0.169	0.52
Root surface area (m^2)	30-40	0.154 ± 0.026	0.091 ± 0.011	0.119 ± 0.000	0.117 ± 0.009	0.941	0.047	0.05
	70-80	0.129 ± 0.008	0.110 ± 0.011	0.151 ± 0.026	0.166 ± 0.009	0.042	0.911	0.34

Table 2.13 Influx rate of water at two depths at end of experiment, between DAS 73 and 80. Expressed on a root length and root surface area basis. Irrigated plants only. The mean of five samples and sem are given, result of two-way ANOVA tests given.

		Oilseed rape	Wheat	P-values		
	Depth (cm)	Irrigated	Irrigated	Species	Depth	S x D
Root influx in $ml \cdot m^{-1} \cdot day^{-1}$	30-40	0.52 ± 0.105	0.33 ± 0.055	0.019	0.699	0.527
	70-80	0.54 ± 0.079	0.24 ± 0.077			
Root influx in $ml \cdot m^{-2} \cdot day^{-1}$	30-40	876 ± 195.1	521 ± 88.7	0.012	0.726	0.577
	70-80	908 ± 117.4	381 ± 120.6			

Table 2.14 Stomatal conductance (g_s) in $mol \cdot m^{-2} \cdot s^{-1}$ measured 67 and 74 days after sowing.

	Oilseed rape		Wheat	
	Irrigated	Non-irrigated	Irrigated	Non-irrigated
g_s DAS 67	0.38 ± 0.102	0.14 ± 0.029	0.19 ± 0.003	0.05 ± 0.006
g_s DAS 74	0.40 ± 0.023	0.06 ± 0.002	0.12 ± 0.024	0.03 ± 0.004

Table 2.15 Statistical results of two-way ANOVA tests on stomatal conductance (g_s) data.

	Species	Irrigation	Sp x Ir
g_s DAS 67	0.002	0.020	0.385
g_s DAS 74	< 0.001	< 0.001	< 0.001

When averaged over irrigation treatments the stomatal conductance of oilseed rape was greater than wheat on both day 67 and 74 (Table 2.14). Withholding water resulted in a significant decrease in stomatal conductance in both species on both dates. On the earlier date, drought treatment reduced stomatal conductance to a similar degree in each species (species x irrigation $p > 0.05$), but on the later date, oilseed rape plants responded to drought by closing their stomata more than wheat (species x irrigation $p < 0.001$, Table 2.15).

Discussion

Is oilseed rape more drought sensitive than wheat?

There were several indications that oilseed rape was more drought sensitive than wheat in this experiment. Although the onset of drought happened at about the same point in time for both species, about 40 days after sowing (Figure 2.3, Table 2.1), the effect of water limitation on oilseed rape growth and yield parameters was significantly greater. Additionally, the transpiration rate of oilseed rape was limited at a lower soil moisture deficit (when 45% of the available water had been depleted) than wheat (65% of available water depleted). Which means that oilseed rape became water limited when there was more water left in the soil (Figure 2.4).

Which plant characteristics are associated with the greater drought sensitivity?

Under the current experimental conditions, the oilseed rape plants had a greater water requirement than wheat, when this requirement was not met, there was a greater effect of water limitation on growth (Figure 2.3, Table 2.8). For instance, while wheat ear dry weight was not reduced by withholding water, pod dry weight of oilseed rape was halved (Table 2.10). Additionally, at a depth of 30-40 cm the root length density and root surface area of oilseed rape plants were reduced to a greater extent by withholding water than in wheat. Oilseed rape plants also responded to water limitation by closing their stomata to a greater degree than wheat plants.

The water use efficiency (WUE) of non-irrigated oilseed rape plants was lower than that of non-irrigated wheat. The WUE of oilseed rape plants was also increased to a smaller extent than wheat plants by withholding of water (Table 2.3, Table 2.4). However, due to a possible artefact in soil moisture measurements of non-irrigated treatments the water use numbers of non-irrigated treatments should in Table 2.3 should be used with care; total water use could have been overestimated and hence WUE underestimated in Table 2.3. The lower WUE of oilseed rape plants, compared to wheat, especially when non-irrigated, could have contributed to its greater reduction in total and reproductive biomass when water was limited. The relatively high drought resistance of one of the wheat varieties tested in a field experiment by Foulkes et al

(2001), for instance, was associated with its high WUE when not irrigated. However, the relationship between whole plant WUE and drought tolerance is not straightforward, a high WUE can largely be a function of reduced water use rather then a net improvement in plant production or biochemistry of assimilation. Condon and Richards (1993) found that in field grown cereal crops in Australia, a high WUE could be related to either high or low yield depending on canopy characteristic and environmental factors.

Van den Boogaard et al. (1996) suggested that a relatively high WUE could contribute to drought tolerance, by causing plants to have a slower rate of water uptake during the vegetative phase of growth, which can result in saving water for the grainfilling phase. However, in the current experiment with oilseed rape and wheat, there were no indications that wheat left water in the soil for use later in the season, in Figure 2.5 for instance, wheat started depleting each soil layer sooner that oilseed rape and depleted the soil to the same extent. Possibly the whole plant WUE of wheat was greater than oilseed rape's when water was withheld due to a greater net improvement in plant production or biochemistry of assimilation in non-irrigated wheat plants. The instantaneous water use efficiency (A/E, where A is the photosynthetic rate and E is the transpiration rate measured with an IRGA simultaneously with measuring of g_s on day 74) indicated that this could have been the case (data not shown).

Does oilseed rape extract water effectively from the soil?

Lopes and Reynolds (2010) compared the drought response of eight isomorphic wheat sister lines and concluded that differences in rooting depth explained the superior adaptation to drought of some lines. While there were no indications that oilseed rape had a shallower root system in the current experiment than wheat, the roots of oilseed rape may have reached deeper layers later than wheat, assuming that root distribution can be inferred from water uptake patterns (Figure 2.7). When water was withheld, wheat plants were the first to extract water from the deepest layer measured (Figure 2.6, Figure 2.7) and also initially took up water at a greater rate from this layer than oilseed rape plants.

Unfortunately water extraction from the deeper layers (80-110 cm) was not measured and nothing can be said about water uptake from this depth. However, in all

lysimeters the roots reached the base and there was no difference in maximum rooting depth between oilseed rape and wheat (personal observation). Furthermore, the root length density in the deepest layer measured (70-80 cm) was comparable for wheat and oilseed rape under both irrigated and non-irrigated conditions indicating that oilseed rape is able to generate a large root system in the subsoil. It is possible that the different temporal patterns of water extraction exhibited by wheat and oilseed rape reflects differences in root hydraulic architecture rather than the presence of roots or the root length density in a given soil layer. The high influx rates per unit root length observed in irrigated plants suggests that oilseed rape roots may have a larger hydraulic conductivity than wheat.

Although non-irrigated oilseed plants started to take up water from each soil layer about a week later than wheat plants, the maximum uptake rate by oilseed rape was, for most soil layers, greater than that of wheat (Figure 2.7, Figure 2.8). Thus oilseed rape was eventually able to extract all the potentially available water from each soil depth. Collectively these results suggest that oilseed rape was as effective as wheat in extracting water from the soil and that its greater sensitivity to drought was not the result of inferior root growth and soil exploration. Van den Boogaard et al (1997) concluded from a comparison of ten wheat cultivars in a pot study that a higher leaf area or root weight did not lead to greater water use per plant, as this was counteracted by a decreased specific rate of water use. This also appeared to be true for the plants in the current experiment. While wheat plants had a significantly greater total leaf area than oilseed rape plants (Table 2.10), oilseed rape's total water use (cumulative evapotranspiration) was at least as high as wheat's (Table 2.3). Additionally, the influx rates per unit root length and area were significantly greater for oilseed rape than for wheat root systems (Table 2.13). Differences in water use of the species appeared to be more related to water influx rates per unit root length and stomatal conductance per unit shoot area than on the total root length and canopy area per se.

Relevance to field-grown crops

In this experiment, total water use, measured as the cumulative evapotranspiration (ET) from depths of 0-80 cm was 498 ± 14.9 mm for irrigated oilseed rape (Table 2.3). This was comparable to the total water use (summed evapotranspiration for 0-70 cm depth)

of *Brassica napus*, grown in Tasmania (Rao and Mendham 1991) and for field grown plants in Canada varying between 302 and 460 mm, depending of year and treatment (Nielsen 1997). But the ET measured in the current experiment was greater than total seasonal ET measured in irrigated oilseed rape crops in Canada (395 mm) by Gan et al (2009b) and also higher than oilseed rape grown in a high rain fall environment in Tasmania (401 mm) (Zhang et al. 2005). Moreover, the plants in the current experiment were harvested before grain filling was complete, therefore the cumulative water use given is an underestimate of the total life-cycle water use. In addition, water extraction from the soil below 80 cm was not monitored (80-110 cm). The relatively high total water use by oilseed rape and high daily rates of transpiration in the current experiment might be due to its high stomatal conductance. The stomatal conductance data were consistent with the total water use by the crops in different water treatments. Well-watered oilseed rape plants had a stomatal conductance of 0.38-0.40 $mol \cdot m^{-2} \cdot s^{-1}$ which was significantly greater than that of wheat. When water was withheld, the stomatal conductance of oilseed rape on DAS 67 was 0.14 $mol \cdot m^{-2} \cdot s^{-1}$ which is comparable to the g_s measured in upper leaves of oilseed rape in an experiment by Rao and Mendham (1991). The total leaf area of oilseed rape crops was significantly smaller at harvest than wheat's for both irrigated and non-irrigated crops therefore the greater water use rate of oilseed rape is probably due to the greater stomatal conductance rather than simply a greater leaf area for transpiration. Such high rates of transpiration are unlikely to be found in the field under UK conditions because in closed crop canopies, the influence of stomata on canopy transpiration is less and the contribution of boundary layer resistance greater than in isolated plants or small populations (Jarvis and McNaughton 1986).

The root length densities found in the deep soil layers (*circa* 3 $cm \cdot cm^{-3}$ at 70-80 cm depth) were also greater than might be expected for field crops (Gregory et al. 1978, King et al. 2003, Blake 2006). This may result from the repacking of soil at relatively low soil bulk densities. In addition, the soil that was used was a top soil with relatively large amount of organic matter and no attempt was made in this experiment to recreate a subsoil. However, some comparability between the growth of wheat and rape in the lysimeters and that of field crops was found. The WUE of both irrigated and non-irrigated wheat was within the range reported for UK wheat crops (Foulkes et al. 2001).

Conclusions

The results show that oilseed rape may be more sensitive to drought than wheat. The onset of drought happened at the same moment in time for oilseed rape and wheat, but at a lower soil water deficit for oilseed rape plants. Oilseed rape's water extraction from each subsequent layer lagged about seven days behind that of wheat, but its maximum influx rate was greater. The root length density and influx rate per unit root length did not seem to be limiting water uptake. Thus an inferior root growth and activity does not did not appear be a major factor contributing to the greater drought sensitivity of rape

Oilseed rape's high demand for water and high stomatal conductance may make it a less water use efficient crop than wheat. Additionally the ear dry weight of wheat was hardly affected by water limitation, while oilseed rape's pod dry weight was halved; wheat appeared to allocate resources in order to maintain yield, while oilseed rape responds more conservatively to water limitation by closing stomata more and potentially losing yield.

Since drought spells are going to become more frequent and more severe in future, due to climate change, in order to maintain or increase oilseed rape yield it would be beneficial to select or develop varieties that have characteristics which make them more tolerant to drought stress. Rao and Mendham (1991) suggest using varieties which have an integrated system of adaptation responses, like a high leaf relative water content, an ability to osmotically adjust and a greater soil moisture extraction from deeper layers. The results of the current experiment suggest that enabling the crop to access more water, increasing its water use efficiency and reducing the sensitivity of pod formation and stomata to water stress may be suitable strategies to improve the ability of oilseed rape to avoid and tolerate drought.

Chapter 3

Root hydraulic conductivity

Introduction

In the previous chapter it emerged that when the supply of water was limited, oilseed rape and wheat transpired similar amounts of water, but when the supply was unrestricted, oilseed rape transpired significantly more and at a greater rate. While the root length density of oilseed rape at the two depths measured was not greater than that of wheat, the inflow rate of water was, which suggests that oilseed rape roots could be more conductive to water.

Water flow from the root to the xylem is determined by hydraulic conductivity at three levels; the soil, the root-soil interface and of the root itself (Passioura 1988, Huang and Nobel 1994). When the soil's water content is high, water movement from the soil to the shoots depends mainly on the hydraulic conductivity of the roots themselves (Fernandez et al. 2000). As the soil dries, roots shrink and root-soil contact diminishes, causing a lower root-soil interface conductance. As the soil dries further, soil hydraulic conductance becomes the limiting factor to water flow in the soil-root pathway (Huang and Nobel 1994). Root hydraulic conductivity has a radial component and an axial component. The radial component is determined by the tissues and pathways the water has to cross to reach the xylem at the centre of the root; the axial component addresses the pressure gradient necessary for water movement along the xylem. The relative importance of these two components of root hydraulic conductivity depends on plant species and developmental stage. Axial root conductivity is thought to be less likely to be limiting to water flow in dicots due to formation of secondary xylem (Huang and Nobel 1994). In a study by Bramley et al (2009) the roots of the dicot plant lupine indeed had greater axial conductance than wheat roots, due to greater xylem development. Anatomy played a major role in root hydraulics, wheat roots predominantly absorbed water in a region close to the root tip, but lupine roots absorbed water more evenly along their whole length (Bramley et al. 2009).

King et al (2003) showed that for cereals, mean root length densities of 0.5-1.0 $cm \cdot cm^{-3}$ in the upper layers of moist soils are in theory adequate to access most of the plant available soil water within the crop's yield formation period and that densities over 1 $cm \cdot cm^{-3}$ are associated with only small increases in the total amount of water taken up in this period. The model that was used was a relatively simple one using a only a few

key variables and assuming rooting depth is not restricted by soil physical properties nor influenced by localised heterogeneities in the soil.

If the oilseed rape root system is only as efficient as cereals in extracting water, its root length density could, in some UK fields, be too low to use all the available water in the soil. A survey of oilseed crops in the UK has shown the occurrence of a wide range of root length densities, some with root length densities below 1 $cm \cdot cm^{-3}$ in the upper subsoil layers (Berry and Spink 2006). However, lupins (which are dicotyledonous plants) for instance have smaller root systems than wheat, but take up water from the soil as rapidly, which is facilitated by a higher conductivity to water (Gallardo et al. 1996). The hydraulic conductance of lupine roots varied diurnally and was greater in the afternoon than in the morning, while barley roots measured in the same experiment did not show this diurnal change in conductivity (Passioura and Munns 1984). In monocots, like wheat and barley, without the capacity to adjust xylem dimensions (due to secondary development) axial resistance may dominate root conductance in well-branched root systems (Fernandez et al. 2000).

In order to assess whether oilseed rape root length density for extraction of available water is comparable to that of wheat, or greater as suggested by the results in Chapter 2, the hydraulic conductivity of the oilseed rape roots are in this chapter compared to that of wheat.

Root hydraulic conductance has been measured in various ways; generally there is a water flow induced in the plant and the flow rate and driving force of the water flow are measured. The conductance is calculated as the flux divided by the driving force and expressed on a root fresh weight, length or surface area basis (Fernandez et al. 2000).

Root conductance in intact plants has been measured by varying the transpiration rate and measuring the difference in water potential between the xylem and the soil (Passioura and Munns 1984, Gallardo et al. 1996, Rieger and Litvin 1999). To estimate the xylem water potential, a leaf near the stem base can be wrapped in impervious material to prevent transpiration and allowed to come into water potential equilibrium with the xylem. Measurement of the leaf water potential then gives an estimate of the xylem water potential (Rieger and Litvin 1999).

Using detached root systems water flow can be induced by applying a partial vacuum at the proximate end of the root system, with a capillary attached to register rate

of water efflux. The tension applied to roots is generally less than the tension in the xylem of transpiring plants (Huang and Nobel 1994).

Root conductance can also be measured using positive pressure. An excised root or root system is sealed into a pressure chamber, pressure is applied and the efflux rate of water at the proximate end of the cut root (system) is measured (Henzler et al. 1999). A relatively large positive pressure could force water through apoplastic pathways that are not naturally followed by water and flow can also be forced up through the phloem instead of the xylem, which could increase the exudation to unnatural rates (Huang and Nobel 1994). At low applied pressure (around 0.1 MPa), the osmotic component affecting water movement is not negligible and samples of exudates can be collected for analysis. At high applied pressures (around 0.5 MPa), the osmotic component is negligible, but physical damage to the roots may occur (Fernandez et al. 2000). Intermediate pressures of around 0.3 MPa used in experiment II may be a safe compromise.

The objective of experiments in this chapter was to test the hypothesis that oilseed rape roots have a greater hydraulic conductance than wheat. Root hydraulic conductivity was measured for both winter and spring oilseed rape and wheat varieties in two experiments. In the first experiment, winter varieties were used, in order to provide data to help interpret measurements of drought responses in a field experiment at ADAS. Conductance was determined on intact transpiring plants.

In the second experiment the spring oilseed rape variety SW Landmark and spring wheat variety Tybalt were used for consistency with other work this project and to establish whether differences between species can be observed in both winter- and spring varieties. Additionally, because there was considerable scatter in the data in first experiment, a different technique (root pressurisation) was used to determine hydraulic conductance.

Materials and methods

Hydraulic conductivity of the root system - experiment I

Experimental design and treatments

Winter oilseed rape (*Brassica napus* L. cv. Castille) and winter wheat (*Triticum aestivum* L. cv. Consort) were grown in a Fitotron growth cabinet (model SGC970, Sanyo-Gallenkamp, Loughborough, UK) at 18 ± 1ºC, 16h/8h light/dark, 50 ± 5 %RH, and a PAR intensity of 477 ± 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height, supplied by 22 55W fluorescent lights and four 60W tungsten lights (Philips, Poland). Four seeds per pot were sown in 0.64 litre pots with 370g of John Innes #1 compost (hereafter referred to as soil) (John Innes Manufacturers Association, Theale, UK). Just after sowing, the pots were placed on a tray with a layer of water to let the soil absorb water for two hours, until constant weight (~500 g). Every two to three days, the soil was replenished with water from the pot base, by watering the trays the pots stood on. After seedling emergence the number of seedlings was reduced to one per pot.

Measurements and analyses

The hydraulic conductance of the root system was determined on plants 21 to 22 days after sowing. The lower-most leaf of a plant was covered with aluminium foil the night before the experiment to stop its transpiration and allow its water potential to equilibrate with the xylem water potential of the stem base. On the morning of the experiment, the pot was placed in a plastic bag which was sealed around the stem to prevent evaporation from soil surface. Subsequently, the plant was placed on a balance within a growth cabinet with a designated temperature and light regime and left to acclimatize for one hour. A pilot experiment in which transpiration rate was measured over time following a change in temperature and light regime showed that one hour was enough time to for the transpiration rate to become constant. After acclimatizing, the change in weight of the plant plus pot, which represents water loss in transpiration, was recorded every two minutes for 20 minutes. All transpiration rates were measured within in a time window of two hours before and three hours after midday; as determined by day/night cycle of the growth cabinet.

A variety of temperature and light intensity combinations were used in order to create a range of transpiration rates. The temperature and light treatments were imposed in two growth cabinets (Fitotron, Sanyo-Gallenkamp, Loughborough, UK). In cabinet A (light source: 12 TL80 Philips fluorescent tubes), the plants were exposed to the following temperature and light regimes: 10°C and 28°C at PAR intensity of 19 ± 1.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height; 18°C, 24°C and 28°C at a PAR intensity of 206 ± 2.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$. And in cabinet B (light source: 22 PL-L 55W Philips fluorescent lights): 24°C at 86 ± 1.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$; 14°C and 18°C at 381 ± 1.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$. An oilseed rape and wheat plant that grew up next to each other were measured on the same day, subjected to the same temperature and light treatment.

Immediately after measurement of transpiration, the covered leaf was excised and placed in a moist plastic bag to prevent evaporation and its balancing pressure (i.e. water potential) determined using a pressure chamber (ELE international ltd., Leighton Buzzard, UK) (Turner 1981). The balance pressure at which sap just started to exude from the petiole ($=\psi_{xylem}$) was measured within two minutes after excision.

The soil matric potential was measured with the filter paper method of Hamblin (1981). The filter-paper method has been used to provide a rapid monitoring device for soil-water status. Whatman 42 filter paper placed in close contact with the soil measures matrix potential (Hamblin 1981). A piece of Whatman no. 42 paper (Whatman plc, Maidstone, UK) 70 mm diameter was weighed to the nearest 0.1 mg, placed in a plastic screw cap jar and covered by ca. 80 g of moist soil from the pot in which the plant was grown and transpiration rate measured The jar was placed in a temperature controlled room kept at 18°C and the filter paper allowed to come into moisture equilibrium with the soil. After sixteen hours, the moist filter paper was re-weighed and then dried to constant weight in a fan assisted oven at 107°C. The following equation was used to calculate soil matric potential (ψ_S) in MPa from the fraction of water absorbed by the filter paper:

$$\psi_S = 0.27 \cdot e^{-3.29F} \qquad [1]$$

Where F is the gravimetric water content of the paper, this equation is valid for $F > 0.5$. The equation was derived from the graph in the paper by Hamblin (1981), where the

water content of the filter paper was over 50%. This graph described the relationship between gravimetric water content of the filter paper and the matric potential of the filter paper.

The total shoot area and the area of the covered leaf were measured after the experiment with a LI-3100C area meter (LI-COR, Lincoln Nebraska, USA). After transpiration, water potential and shoot area measurements were taken, the pot with soil and intact root system was kept in a freezer at -4°C. The root systems were washed from the soil over a five and a two mm sieve, organic debris was picked out with tweezers. Due to the large size of the root systems, subsamples were taken: the washed roots were cut to fragments using a scalpel and fragments were mixed, a subsample of a quarter of the total wet weight of roots was scanned and analysed with Winrhizo software (Régent, Quebec, Canada) to determine root length, root area and root DW. The dry weight of both the scanned sample and the remainder of the total root system were determined in order to calculate whole root system properties. Root and shoot dry weights were determined after drying to a constant weight in a fan assisted oven at 70°C.

Root hydraulic conductance was calculated using an equation analogous to Ohm's law:

$$Lp_o = T / |\psi_S - \psi_x| \qquad [2]$$

Where Lp_o is the hydraulic conductance of the whole root system, T is the transpiration rate in ml/h and $|\psi_S - \psi_x|$ is the potential difference between the xylem (ψ_x) and the soil (ψ_S) in MPa (Steudle and Peterson 1998). The root hydraulic conductivity was also expressed by dividing Lp_o by either root length or root surface area after first accounting for the sub-sampling.

Hydraulic conductivity of the root system - experiment II

Experimental design and treatments

In this experiment, hydraulic conductance of the root system of oilseed rape and wheat were measured using a technique in which pressure was applied to the root system and efflux of water measured, instead of making measurements on the shoot as in experiment I.

One spring oilseed rape, (*Brassica napus* L. cv. SW Landmark) seed was sown in a polypropylene tube of with a diameter of 32 mm and a depth of ten cm and lined with a plastic bag (polythene) with perforations at the base to facilitate drainage. The growth medium consisted of a mixture (1:1 v/v) of sharp washed sand and vermiculite (1-3 mm). Spring wheat (*Triticum aestivum* L. cv. Tybalt) seeds were sown in fifteen cm deep tubes at five cm depth to encourage plants to produce a relatively long mesocotyl. This was done to facilitate sealing of the plant into the pressure chamber. Nutrients, in the form of ten ml half strength Hoagland solution were given weekly (Epstein 1972) and water was supplied every other day to keep the growth medium moist. The hydraulic conductivity was measured when plants had two unfolded leaves and a third unfolding, three weeks after sowing. Plants were cultivated in a controlled climate growth chamber, at 18 ± 0.7 °C, 16 hours light, 8 hours dark cycle, 65 ± 9.9 %RH.

Measurements and analyses

Measurements were made within the period one hour before and one hour after the midpoint of the light period, which was eight hours into the light cycle. A wheat and an oilseed rape root system were measured in a pressure chamber (ELE international ltd., Leighton Buzzard, U.K). The shoot was cut just below the first leaves, 30 ml of water was added and the plastic bag including growth medium and root system was placed in the pressure vessel. The base of the stem (oilseed rape) or mesocotyl (wheat) with its cut surface was allowed to protrude through the silicone seal and the chamber lid secured in place forming a seal against the stem base The chamber was pressurised to 0.3 MPa and the root system was left for at least twelve minutes for a constant water flow to establish from the cut surface. A pilot experiment had established that the flow was constant within twelve minutes. The water efflux over a further ten minute period was measured

by absorbing the water expressed out of the xylem at the cut surface of the stem base with a piece of dental cotton roll embedded in a two ml safe-lock Eppendorf tube, see Gallardo et al (1996) for a comparable method. The tube and cotton roll were weighed to the nearest 0.1 mg before use. The weight of water exuded during the ten minute period was determined by re-weighing the tube with the cotton.

After measurement, the root system was washed from the sand/vermiculite spread in a transparent dish containing a film of water and scanned with a Régent scanner (LA1600, Epson expression, 836 XL) and analysed with Winrhizo software (Régent, Quebec, Canada). Leaf area was measured with a leaf area meter (LI-COR, Lincoln Nebraska, USA). After scanning, the root system was dried in a fan assisted oven at 70°C until constant weight and the dry weight determined.

Statistical analyses experiments I and II

All statistical analyses were conducted using GenStat software (GenStat 11, VSN International Ltd, Hemel Hempstead, UK). The significance level for all test results was 0.05.

For experiment I, the effects of light and temperature treatments on transpiration rate of oilseed rape and wheat were tested with an unbalanced ANOVA test. In table 1, the mean transpiration rates per treatment were given, but only for the treatments of which the sample size was two or more.

To obtain values for hydraulic conductance, the transpiration rate of oilseed rape and wheat plants was plotted against the difference in water potential between xylem and soil and linear regression lines were fitted in GenStat, the regression lines were forced through the origin, because it was expected that when there is no potential gradient over the root system, there would be no transpiration. To test for significance of differences in slope (i.e. hydraulic conductivity) between species, a simple linear regression test with groups was used and data were square root transformed to normalize distribution when necessary.

The data from experiment II were tested for significant differences between oilseed rape and wheat with Kolmogorov–Smirnov (K-S) tests due to the non normal distribution of data.

Results

Experiment I

Transpiration rates

A range of transpiration rates were created by varying temperature and light intensity (Table 3.1, Table 3.2). When expressed per unit shoot area, oilseed rape had a significantly higher transpiration rate than wheat over a range of temperature and light combinations ($p = 0.002$). Low light intensity reduced the transpiration rate in both species and transpiration was, in general, increased with an increase in temperature. The effects of temperature were less consistent on wheat than oilseed rape, but there was considerable variation within the data and relatively few replicates for each treatment combination. When expressed on a whole plant basis, variation in size between individual plants obscured, to some extent, the expected effects of temperature and light intensity on the transpiration rate.

The potential difference over the root system (soil matric potential minus xylem water potential) for oilseed rape varied between 0.30 and 0.58 MPa, while for wheat it tended to be smaller and varied from 0.17 to 0.50 MPa (Figure 3.1). At a comparable potential difference (i.e. driving force for water flow), the transpiration rate of oilseed rape plants tended to be greater than for wheat when expressed on a whole plant (Figure 3.1), or root length basis (Figure 3.2). The soil matric potential was low relative to the xylem potential (about a factor 200 lower) and was 2.6 ± 2.00 kPa averaged over all treatments (data not shown).

In Table 3.3 the root and shoot characteristics of the plants are given. Oilseed rape had a significantly greater root length and root surface area per plant than wheat, but the dry weight of the root system was significantly smaller. The specific root length, i.e. the length of root per gram dry weight was significantly greater for oilseed rape. The canopy area of oilseed rape was about three times that of wheat and the shoot: root ratio (canopy area divided by the root length) was twice as high for oilseed rape.

Table 3.1 Transpiration rates of winter oilseed rape and wheat plants that were subjected to different light and temperature regimes. Whole plant transpiration and transpiration rate per unit uncovered shoot area are given. Mean and standard error are given, samples sizes were two to four.

			Whole plant transpiration in ml·h^{-1}		**Transpiration per unit shoot area in ml·h^{-1}·m^2**	
Temp.	Light intensity	in µmol·m^{-2}·s^{-1}	Oilseed rape	Wheat	Oilseed rape	Wheat
10°C	low	19	0.96 ± 0.068	0.26 ± 0.080	83 ± 9.1	38 ± 15.0
24°C	low	86	1.53 ± 0.165	0.58 ± 0.135	122 ± 25.9	81 ± 13.2
28°C	low	19	2.09 ± 0.100	0.22 ± 0.044	166 ± 0.5	61 ± 18.5
14°C	high	381	1.19 ± 0.205	1.53 ± 0.590		
18°C	high	381	2.29 ± 0.308	0.99 ± 0.017	191 ± 21.5	176 ± 21.3
24°C	high	206	2.18 ± 0.288	0.82 ± 0.180	199 ± 28.4	132 ± 6.8
28°C	high	206	2.37 ± 0.191	0.97 ± 0.240	218 ± 27.9	194 ± 33.9

Table 3.2 Results of ANOVA test, testing for effects of species, temperature and light intensity on transpiration (data in Table 3.1), p-values of each parameter and interaction of parameters given. Not all interactions could be tested due to the low number of replications.

Parameter	**Plant transpiration**	**Transpiration per unit shoot area**
Species	< 0.001	0.002
Light	< 0.001	< 0.001
Temperature	0.154	0.030
Species x light	0.086	0.588
Species x temp	0.003	0.306

Table 3.3 Root and shoot characteristics of winter oilseed rape and wheat. The sample size was 15 (the wheat outlier, as plotted in Figures 3.2 and 3.3 was left out of the calculations and tests). P-values of a t-test testing for significant difference between oilseed rape and wheat data are given.

	Oilseed rape	**Wheat**	**P-values**
Root dry weight (g)	0.28 ± 0.007	0.33 ± 0.015	0.006
Root length (m)	163.3 ± 7.10	112.0 ± 5.03	< 0.001
Root surface area (cm^2)	1072 ± 40.9	891 ± 44.9	0.006
Canopy area (cm^2)	169 ± 3.4	59 ± 3.1	< 0.001
Specific root length ($m \cdot g^{-1}$)	587 ± 15.0	347 ± 9.8	< 0.001
Shoot:root ratio ($cm^2 \cdot m^{-1}$)	1.06 ± 0.040	0.53 ± 0.022	< 0.001

Hydraulic conductivity

The linear regression line that was fitted to the oilseed rape data in Figure 3.1 had a greater slope than wheat, which indicated that the hydraulic conductance of oilseed rape root systems was significantly greater than that of wheat ($p < 0.001$) (Table 3.4). The hydraulic conductance of the winter oilseed rape root system was 4.75 $ml \cdot h^{-1} \cdot MPa^{-1}$ and for winter wheat it was 1.76 $ml \cdot h^{-1} \cdot MPa^{-1}$. However, the regression lines had relatively low R^2, due to the scatter of the data points.

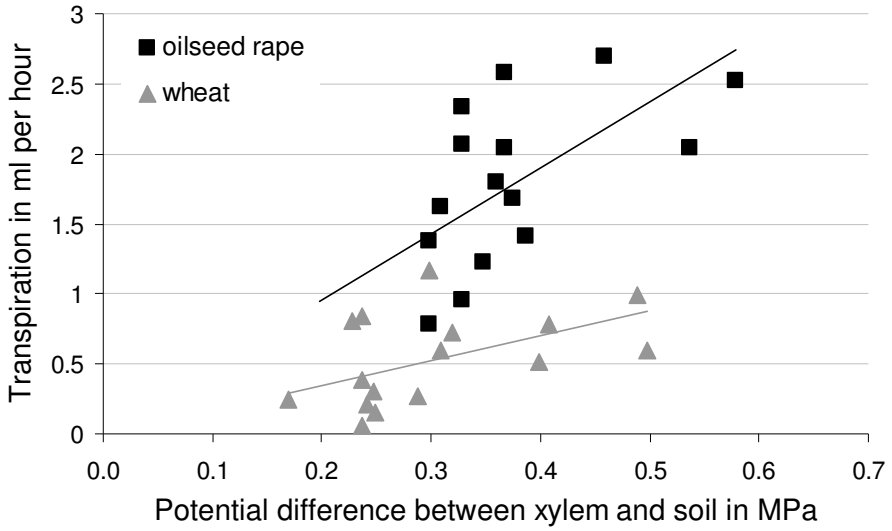

Figure 3.1 The transpiration rate of plants plotted against the potential difference between soil and xylem; the driving force for water flow. The slopes of the fitted lines (linear regressions through the origin) correspond with the hydraulic conductance of the whole root system of each species. For oilseed rape: $y = 4.75x$, $R^2 = 0.27$; for wheat: $y = 1.76x$, $R^2 = 0.21$.

The hydraulic conductivity of oilseed rape roots on a root length basis (Figure 3.2) and on surface area basis (Figure 3.3) was twice as high as that of wheat roots, namely 0.030 for oilseed rape and 0.016 $ml \cdot h^{-1} \cdot MPa^{-1} \cdot m^{-1}$root for wheat. On a root surface area basis the conductivity was 45.5 and 19.9 $ml \cdot h^{-1} \cdot MPa^{-1} \cdot m^{-2}$root respectively for oilseed rape and wheat (Figure 3.3). The slopes of the linear regression lines through the origin were significantly different from zero ($p < 0.001$) and the conductivity (i.e. slope) of oilseed rape was significantly greater than that of wheat ($p < 0.001$) (Table 3.5, Table 3.6). The wheat outlier was not taken into account in the statistical tests. This wheat plant had an extremely small root system compared to all other plants.

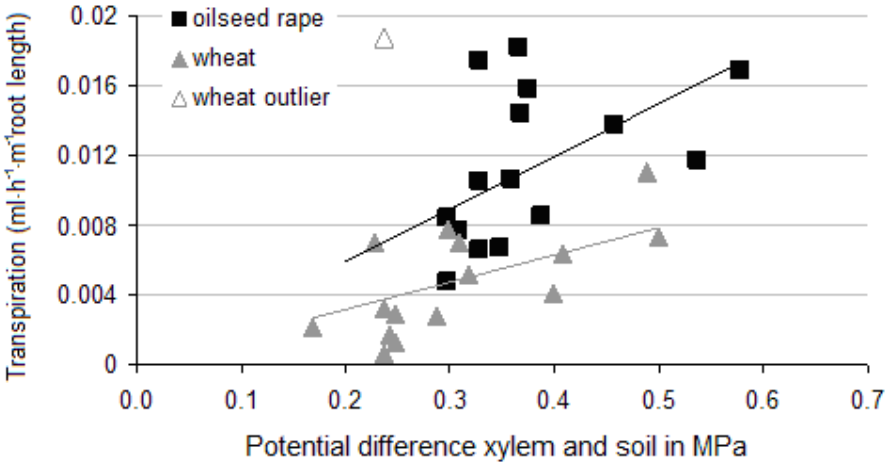

Figure 3.2. The transpiration rate of plants per unit root length plotted against the driving force for water flow. The slopes of the fitted lines (linear regressions through the origin) correspond with the hydraulic conductivity of the roots per meter length of each species. For oilseed rape: y = 0.030x, R^2 = 0.20; for wheat: y = 0.016x, R^2 = 0.45.

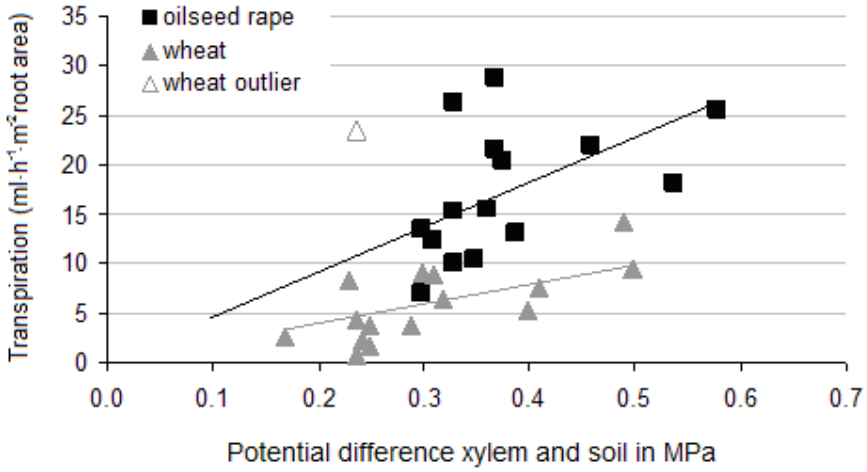

Figure 3.3. The transpiration rates of plants per unit root surface area plotted against the driving force for water flow, e.g. the potential difference across the root system. The slopes of the fitted lines (linear regressions through the origin) correspond with the hydraulic conductivity per m^2 root surface area. For oilseed rape the regression line was described by: y = 45.5x, R^2 = 0. 22; for wheat: y = 19.9x, R^2 = 0.48.

Table 3.4 Regression analysis testing for difference between oilseed rape and wheat in whole root system conductance, data in Figure 3.1.

Response variate:		Transpiration rate			
Fitted terms:		Potential difference + Potential difference x species			
Summary of analysis					
Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	2	55.583	27.7916	172.21	<.001
Residual	29	4.68	0.1614		
Total	31	60.263	1.944		
Change	-1	-8.378	8.3777	51.91	<.001
Estimates of parameters					
Parameter		estimate	s.e.	t(29)	t pr.
Potential difference		4.752	0.268	17.7	<.001
Potential difference.wheat		-2.99	0.415	-7.21	<.001

Table 3.5 Regression analysis on square root transformed data testing for significant difference between oilseed rape and wheat in root length conductivity, data in Figure 3.2.

Response variate:		Transpiration rate per unit root length			
Fitted terms:		Potential difference + Potential difference x species			
Summary of analysis					
Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	2	0.23067	0.1153352	282.87	<.001
Residual	28	0.01142	0.0004077		
Total	30	0.24209	0.0080696		
Change	-1	-0.00386	0.0038603	9.47	0.005
Estimates of parameters					
Parameter		estimate	s.e.	t(28)	t pr.
Potential difference		0.271	0.0135	20.09	<.001
Potential difference.wheat		-0.0648	0.0211	-3.08	0.005

Table 3.6 Regression analysis on square root transformed data, testing for significant difference between oilseed rape and wheat root conductivity per unit root area, data in Figure 3.3.

Response variate:	Transpiration rate per unit root surface area				
Fitted terms:	Potential difference + Potential difference x species				
Summary of analysis					
Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	2	333.06	166.5284	302.52	<.001
Residual	28	15.41	0.5505		
Total	30	348.47	11.6157		
Change	-1	-9.54	9.5415	17.33	<.001
Estimates of parameters					
Parameter		estimate	s.e.	t(28)	t pr.
Potential difference		10.554	0.496	21.29	<.001
Potential difference.wheat		-3.224	0.774	-4.16	<.001

Experiment II

Plant characteristics

As in experiment I, oilseed rape plants were bigger than wheat plants. Spring oilseed rape plants had a significantly greater shoot area and greater root length (Table 3.7). While the root length per plant was significantly greater for oilseed rape plants ($p = 0.002$), the root surface area did not differ significantly ($p = 0.233$), this was because the wheat roots had a greater diameter; the dry weight of the wheat root system was also significantly greater. Oilseed rape's specific root length was about twice that of wheat. The leaf area:root length ratio of oilseed rape plants was greater than that of wheat but not significantly so ($p = 0.069$).

Hydraulic conductivity

On a root area basis, the hydraulic conductance of spring oilseed rape roots was over two times greater than spring wheat ($p = 0.016$, Table 3.7). The conductance per unit root length was also on average greater for oilseed rape roots, but not significantly so ($p = 0.223$). The results follow the same pattern as found in experiment I, but were quantitatively different. The whole root system conductance of oilseed rape plants was two and a half times that of wheat plants in both experiments.

Table 3.7. Root and shoot parameters of oilseed rape and wheat plants measured for root hydraulic conductivity in Experiment II, mean (n=6) and sem are given. In the last column the p-value of a Kolmogorov-Smirnov test for difference between oilseed rape and wheat is given.

	Oilseed rape	**Wheat**	**p-value**
Root DW (g)	0.023 ± 0.002	0.036 ± 0.003	0.016
Root length (cm)	1345.0 ± 93.9	792.9 ± 67.2	0.016
Root surface area (cm^2)	95.7 ± 7.3	85.2 ± 8.0	0.223
Root diameter (mm)	0.224 ± 0.006	0.328 ± 0.007	0.002
Shoot DW (g)	0.090 ± 0.008	0.066 ± 0.007	0.223
Shoot area (cm^2)	19.6 ± 1.2	8.0 ± 0.2	0.002
Specific root length ($m \cdot g^{-1}$)	589 ± 25.0	228 ± 24.9	0.002
Leaf area/root length ($cm^2 \cdot m^{-1}$)	1.50 ± 0.132	1.05 ± 0.0.094	0.069
Total root system conductance ($ml \cdot h^{-1} \cdot MPa^{-1}$)	1.02 ± 0.114	0.44 ± 0.078	0.002
Conductivity ($ml \cdot h^{-1} \cdot MPa^{-1} \cdot m^{-1}$ root length)	0.080 ± 0.014	0.057 ± 0.011	0.223
Conductivity ($ml \cdot h^{-1} \cdot MPa^{-1} \cdot m^{-2}$ root surface area)	113.1 ± 20.0	53.5 ± 10.6	0.016

Discussion

Root systems of oilseed rape plants had a significantly greater conductivity to water flow than wheat root systems. In both experiments, the whole root system conductance was 2.5 times greater for oilseed rape and in both experiments, the root hydraulic conductivity of oilseed rape plants on root area basis was significantly higher and about twice that of wheat. Despite growth conditions, varieties and measurement method being different the same trends could be observed in both experiments. Although the plants in experiment I were much larger than the spring varieties in experiment II, some characteristics were remarkably similar. For example, specific root length for winter oilseed rape was 587 $m \cdot g^{-1}$ compared to 589 $m \cdot g^{-1}$ for spring oilseed rape and for winter and spring wheat was 347 and 228 $m \cdot g^{-1}$ respectively. The greater specific root length of oilseed rape indicates finer roots and in experiment I, where average root diameter was determined, oilseed rape plants indeed had a significantly smaller root diameter. The leaf area: root length ratio was also greater for oilseed rape in both experiments, which is consistent with the high hydraulic conductivity of oilseed rape roots; they can supply a bigger canopy with water using a smaller root system compared to wheat.

Values compared to other studies

The conductivity of the plants in these experiments was comparable to the range found in the literature (Rieger and Litvin 1999). The greater conductivity in experiment I could be due to the fact that root systems were very large. Plant conductance of 30 day old wheat plants was $3.47 \cdot 10^{-10}$ $m^3 \cdot s^{-1} \cdot MPa^{-1}$ for wheat and $6.25 \cdot 10^{-10}$ $m^3 \cdot s^{-1} \cdot MPa^{-1}$ for lupine (Gallardo et al. 1996). This is close to the values observed in experiment I: 4.89 for wheat and $13.2 \cdot 10^{-10}$ $m^3 \cdot s^{-1} \cdot MPa^{-1}$ for oilseed rape. Per unit root length the conductivity was $0.11 \cdot 10^{-10}$ $m^3 \cdot s^{-1} \cdot m^{-1} \cdot MPa^{-1}$ for wheat and $0.41 \cdot 10^{-10}$ $m^3 \cdot s^{-1} \cdot m^{-1} \cdot MPa^{-1}$ for lupine (Gallardo et al. 1996). For experiment II these values were $0.16 \cdot 10^{-10}$ $m^3 \cdot s^{-1} \cdot m^{-1} \cdot MPa^{-1}$ for wheat and $0.22 \cdot 10^{-10}$ $m^3 \cdot s^{-1} \cdot m^{-1} \cdot MPa^{-1}$ for oilseed rape.

The hydraulic conductivity on a root length basis of the wheat plants measured here (0.016 and 0.057 $ml \cdot h^{-1} \cdot m^{-1} \cdot MPa^{-1}$ for spring and winter varieties respectively) was comparable to the value Gallardo et al. (1996) reported for wheat grown in an Australian field (0.040 $ml \cdot h^{-1} \cdot m^{-1} \cdot MPa^{-1}$). But the comparison of hydraulic conductivity

between different studies is not straightforward, due to the use of different growth media, plant growth stages and measurement techniques, as these can all affect the values of hydraulic conductivity. Additionally, some studies express the hydraulic conductivity on a root fresh weight basis instead on a root length or area basis (Clarkson et al. 2000, Matsuo et al. 2009) making comparison with the current findings difficult.

Correlations were found between root conductivity and root diameter by Rieger and Litvin (1999). This suggests that the main resistance does not reside in one layer only (the endodermis), because the longer the path to the xylem, the lower the root conductivity. Thus the cortex could play a significant role in resistance to water flow. This corresponds with the findings in the current experiments. The wheat roots had a greater mean diameter, lower specific root length and also a lower conductivity than oilseed rape roots. In the study of Rieger and Litvin (1999), the relationship between cortical width and conductivity was very much influenced by two extreme clusters of data, suggesting that other factors affect the conductance more. Rieger and Litvin (1999) suggest that the presence/absence of a suberised exodermis affects conductance. It would be interesting to compare oilseed rape and wheat anatomy for possible differences between aquaporin activity. These factors could (partly) explain the differences found in root hydraulic conductivity between species. Bramley et al. (2009) contributed the differences between lupine and wheat root hydraulic conductance to the differences in anatomy of roots. Lupine had a greater axial root conductance than wheat due to greater xylem development. Predominantly apoplastic flow endows lupine roots with the same or superior radial conductance as thinner wheat root, but it provides little ability to adjust root conductance in the short term. For wheat, the bulk water flow was limited to a small region of the endodermis and was controlled by aquaporins (Bramley et al. 2009).

Possible limitations of measurements

It is not clear why there was so much unexplained variation in the data in experiment I. Several factors may have contributed to the variation. In *Lotus japonicus* there is a diurnal rhythm in hydraulic conductivity, causing a three fold change in root length conductivity per unit root length with conductivity; peaking at five to seven hours after dawn (Henzler et al. 1999). It is possible that the time-window that was used for measuring

the conductivity of oilseed rape and wheat in experiment I was too broad, introducing some variability in the conductance data.

The degree of adaptability and variability in conductance of the oilseed rape and wheat root systems is not known, but in lupins, root hydraulic conductance varied diurnally with a higher conductance in the afternoon than in the morning. This diurnal change was not observed in barley in the same experiment indicating that species differ in the variable nature of their hydraulic properties (Passioura and Munns 1984). Changes in conductivity over the course of a day may be related to variation in the rate of transpiration and the contribution that different pathways make to the radial movement of water across the root (Steudle 2000).

For calculating root system conductance of oilseed rape and wheat, in experiment I the regression lines were forced through the origin. The whole plant conductance regression, when not forced through the origin had a positive intercept on the y-axis, which would mean that plant transpired water in the absence of a driving force (e.g. potential difference over the root system), which is not likely. Forcing the line through the origin did not have a great influence on R^2. In most other studies, there is a positive intercept on the x axis in the regression line fitted through the water potential difference vs. transpiration data, implying that there is a minimum potential difference needed to start transpiration off (Passioura and Munns 1984, Gallardo et al. 1996).

Variation in the data in experiment I may have arisen from inaccuracies in estimates of the xylem water potential. One leaf was covered assuming it would come into water potential equilibrium with the xylem. However, the extent to which this occurred may have differed between species. Cereals (monocotyledons) have different vessels compared with dicotyledonous species. Cereals lack a vascular cambium and their vessels are not replaceable, therefore protection against embolisms is essential. Vascular segmentation of the root-shoot junction in cereals may protect them from embolisms. There is a difference in winter and spring wheat vessel architecture. Winter wheat had a greater percentage of adventious roots with (embolism prone) vessels, while in spring wheat the percentage of seminal roots with unsafe vessels was greater (Aloni and Griffith 1991). A difference between wheat and oilseed rape in vessel architecture and connectivity could possibly have affected the equilibration of the covered leaf in experiment I. However, the wheat data did not appear to be any more variable than the

oilseed rape data and the values of hydraulic conductance were comparable with those found in the literature.

Root system conductance and root conductivity measured in the winter varieties in experiment I was about 2.5 times greater (on root area basis) than in the spring varieties in experiment II. It is not possible to determine whether this is the result of differences in growth conditions, variety or measurement technique. In both experiments, plants were measured three weeks after sowing, but in experiment I the plants were grown in larger containers and the plants themselves were larger. There is evidence that winter and spring varieties may differ in hydraulic architecture (Aloni and Griffith 1991). In experiment II, a pressurisation technique was used to determine hydraulic conductivity. Although the magnitude of the pressure applied (0.3 MPa) was comparable to the xylem tension measured in experiment I (~0.2-0.6 MPa), water driven by a negative pressure (suction) in transpiring plants may follow a different pathway across the root to that driven by a positive pressure. When water or very wet soil is used as a growth medium and pressure is applied to the root system, it can cause airspaces in the roots to collapse in the root tips and later the cortex of thin laterals. This could have interfered with the hydraulic conductivity measurements (Passioura and Munns 1984).

Environmental factors and root hydraulic conductivity

In the current experiments, plants were not water limited, but in many species including wheat, root conductance decreases with drought (Trillo and Fernandez 2005). Drought stress reduced root conductivity in soy bean and peach plants, which is possibly due to increased suberisation near the root tips or formation of air lacunae which disrupt water flow across the cortex (Rieger and Litvin 1999). Alternatively is has been suggested that a change in aquaporins could be responsible for reducing conductivity during drought (Rieger and Litvin 1999). In Arabidopsis, a transcriptional down-regulation of aquaporin genes encoding aquaporins of the plasma membrane (PIPs) was observed upon drought stress, but only after at least six days from the start of drought treatment (Alexandersson et al. 2005, Alexandersson et al. 2010). Tournaire-Roux et al (2003) showed in an experiment using hydroponically grown split root plants, that hydraulic properties of the root system can change within 20 minutes. When aquaporins were blocked in one half

of the split root system, the conductance in the untreated half increased and within 40 minutes, the total conductance of the root system was back to the pre-treatment level. It has been suggested that an adjustable root hydraulic conductivity could help plants respond to heterogeneous distribution of water in the soil. The interaction of root system architecture and plasticity and soil and heterogeneity in the soil are at the moment not well understood and need more study (Lynch 1995, Maurel et al. 2010).

Other factors besides root length can play a role in determining the plant's ability to use the water stored in the soil. Water status of the soil and of the plants varies diurnally and seasonally. Root-soil interactions are also very much influenced by soil type and soil structure. Under wet conditions the soil hydraulic conductivity tends to be higher than the radial root conductivity and water uptake tends to be proportional to root length density (Gardner 1964). In drought-prone environments, the influence of root length density is lower and the availability of water depends more on the volume of soil explored. What happens in intermediate conditions is less clear (Draye et al. 2010).

Conclusions

The results support the hypothesis that the root system of oilseed rape has a greater hydraulic conductivity than wheat when the supply of water is unrestricted. The fact that this has been demonstrated using two contrasting techniques and using both winter and spring varieties leads to greater confidence in the general validity of these findings. The greater root conductivity of oilseed rape suggests that the critical root length density for taking up all readily available water from the soil may be lower than that for cereals and that it might be able to 'get by' with a less dense root system, at least when the soil is relatively moist. However it is not known how oilseed rape's root hydraulic conductance responds to drought stress. Oilseed rape might be more sensitive than wheat and suffer more under less optimal conditions.

Chapter 4

The sensitivity of oilseed rape and wheat to soil strength

Introduction

The previous chapters have shown that oilseed rape plants are more sensitive to water limitation than wheat plants. The growth, distribution and hydraulic conductivity of the root system did not appear to contribute to the greater sensitivity under these circumstances. However plants were grown in relatively loose soil, whereas in commercial fields soil is often compacted due to machinery traffic. Compaction problems have been widely encountered in the moist, temperate climatic zones of northern Europe and North America (Soane 1994). There is also increasing evidence of soil compaction problems in humid and dry tropical climates as well as in Mediterranean-type climates (Hamza and Anderson 2005, Chan et al. 2006).

In drying or dense soils, water availability may not be the limiting stress for root growth and plant functioning. In addition to water availability (soil matric potential), aeration and soil strength are factors that can be of importance (Whitmore and Whalley 2008).

When roots extend in soil, they must generate a growth pressure to deform the soil at the root tip. Root extension is governed largely by cell expansion in the root apex which requires the generation of cell turgor and the relaxation of the cell walls leading to irreversible cell enlargement (Wu and Cosgrove 2000). In drying soil, the ability of roots to generate growth pressure is reduced, while the strength of the soil increases (Bengough and Mullins 1990; Whitmore and Whalley 2008). But even well-watered soil can be sufficiently strong to impede root elongation if the bulk density is large. In an experiment on wheat plants by Masle (1998), leaf growth and stomatal behaviour responses of plants could be ascribed to variations in soil mechanical resistance to penetration, rather than changes in water and nutrient availability per se (Masle 1998). Chemical signalling from the roots to the shoot in response to roots encountering mechanical stress is thought to occur (Mulholland et al. 1996, Sauter et al. 2001). The plant hormone abscisic acid (ABA) is thought to play an important role in signalling during drought stress, but changes in xylem pH may also result in stomatal closure (Sauter et al. 2001).

Little is known about the way different stresses interact in soil to influence plant growth, because it is difficult to separate out the effects of soil aeration, matric potential and mechanical impedance. These factors often act simultaneously or in sequence

(Whitmore and Whalley 2008). In some studies the factors have been teased apart to a certain extent. In a sand culture experiment in which mechanical impedance was varied independently of aeration and water status of the sand, mechanical impedance caused a decrease in root and shoot fresh weight and also root length in six rice cultivars (Brown et al. 2006). Similarly, when mechanical impedance and matric potential were varied independently in controlled environments on wheat, plant growth was sensitive to mechanical impedance, but not to small changes in matric potential (Whalley et al. 2006).

Plant species differ in the ability of their roots to penetrate strong soils (Materechera et al 1993; Clark et al 2003). Materechera et al. (1993) concluded from a study with eight species that dicotyledonous species were in general better at penetrating to depth in both compacted and deep tilled strong soils than monocotyledonous species. However, in this study, three out of four of the dicotyledonous species used were leguminous, therefore it is not clear if the ability to penetrate soils well is a characteristic of all dicots or mainly of leguminous dicots. In a laboratory study, the maximum root pressure exerted by dicot seedlings was not greater than that of monocots (Clark and Barraclough 1999).

Brassica crop roots often penetrate 20-30 cm deeper than cereals and 50-60 cm deeper than grain legumes in fields in southern New South Wales, Australia (Kirkegaard, unpublished). It has been suggested that oilseed rape, because it is a tap-rooted species, is good at penetrating plough pans and compacted soils (Chan et al. 2006). However there is no clear evidence for this and Cresswell and Kirkegaard (1995) suggested that perennial plants are better at bio-drilling than tap-rooted annuals like oilseed rape.

Chen and Weil (2010) found that the two *Brassica* species: *Brassica napus* (oilseed rape) and *Raphanus sativus* (radish) and were better at penetrating compacted field soils and taking up nitrogen than the cereal *Secale cereale* (rye). There are indications that these two *Brassica* crops may help ameliorate effects of compaction on agricultural land. Whitely and Dexter (1982) compared the growth of seven species on soils with different tillage treatments for the top 30 cm of soil. Oilseed rape and wheat were two of the species used, but it was difficult to ascertain from these results whether oilseed rape reacted differently to certain tillage practices than wheat.

Chan et al (2006) compared the response of oilseed rape and wheat crops to soil compaction by tractor tracks in the field. Both crops were affected, but oilseed rape's

root, crop and grain biomass were much more affected than wheat's. In fact the grain yield of wheat was not affected at all. The authors speculated that the difference in response to compaction of oilseed rape and wheat could be due to differences in sensitivities of the tap root system of oilseed rape and the fibrous root system of wheat to compaction. However, they did not research those differences in sensitivity of the root systems directly (Chan et al. 2006).

While some authors suggest that a tap-rooted species such as oilseed rape is more sensitive to increase in soil strength (Chen and Weil 2010), others suggest that tap-rooted species are better than fibrously rooted species in exploring strong soil (Chan et al. 2006). Much of the evidence for oilseed rape or tap-rooted species in general being better than species with fibrous root systems, such as wheat, in penetrating hard soils is circumstantial. Therefore the objective of this chapter is to investigate the relative sensitivity of oilseed rape and wheat root growth and canopy function to an increase in soil strength.

In the first experiment, the response of root and shoot growth and water use to increasing soil strength was investigated under well-watered conditions. In this experiment oilseed rape and wheat plants were grown on soils at four different bulk densities without water limitation. In a second experiment a combination of bulk density and soil drying treatments was imposed to create a greater range of soil strength and to test the interactions between compaction and soil drying on the growth and water relations of oilseed rape and wheat.

Material and methods

Experiment I: Bulk density

Soil and packing regime

Sandy clay loam soil from the MacMerry series was collected from Boghall farm, SAC (Penicuik, Scotland UK). The soil pH was 6.7 and the previous crop was spring barley. After air drying for 48 hrs, the soil was sieved with a 5 mm sieve prior to packing into pots. The moisture release curves of this soil packed to a density of 1.2 and 1.3 $g \cdot cm^{-3}$ can be seen in Figure 4.9. For the method of determining these soil moisture release curves, see material and methods section of experiment II.

Pots were constructed of polyvinyl chloride (PVC) pipes (Marley Plumbing and Drainage, Lenham, UK), with an inner diameter of 7.6 cm and 50 cm length. The pots were cut in half lengthwise, bound back together again with silicone sealant and secured with cable ties. This was to facilitate recovery of the soil and root system after harvest. A fine mesh was taped to the base of the pot, to prevent roots growing through the base but permit aeration and water uptake. The base was covered with a plastic bag and water was given in the bag (i.e. from the base of the pot) throughout the experiment. Plants were given water to bring the soil back to field capacity on days 2, 5, 8, 12, 16 and 20 after planting. The amount of water needed was determined by weighing the pots and comparing the weight to the weight of drained pots on the day of planting.

Mineral nutrients $(NH_4)_2SO_4$, KNO_3 and NH_4NO_3 were supplied to give applications equivalent to 120 $kg \cdot ha^{-1}$ N, 83 $kg \cdot ha^{-1}$ K and 30 $kg \cdot ha^{-1}$ S. Nutrients were made up in water and 50 ml of solution was mixed thoroughly with the soil just before packing to ensure even distribution throughout the pot. The above applications were selected after considering potential soil nutrient supply, based on soil analysis and previous cropping and the nutrient requirements of field crops. Soil analysis indicated that the P status was high and hence no P fertiliser was applied.

The bottom 44 cm of each pot was packed with soil to a dry bulk density of 1.1, 1.2, 1.3 or 1.4 $g \cdot cm^{-3}$; the top 4 cm was packed relatively loosely to 1.1 $g \cdot cm^{-3}$ to enable establishment of seedlings. The pots were packed in 10 cm depth intervals, and aliquots of soil of known water content, were packed by hammering with a special device with a rubber end with the same diameter as the pot and a ridge along its edge for a slight

increase of soil bulk density at the edge to discourage roots from growing along the edge of the pot. The pots were watered thoroughly and subsequently drained for two days prior to planting seedlings and determining the start weight. The water content after two days of drainage was taken to equal field capacity of the soil. Unplanted pots packed to a density of 1.3 $g \cdot cm^{-3}$ were used to monitor evaporation of water from the soil surface (n=5).

Experimental design and plant growth conditions

Seeds of oilseed rape *Brassica napus* L. cv. SW Landmark and wheat *Triticum aestivum* L. cv. Tybalt were germinated between two sheets of rolled up filter paper that were stood in a beaker of water to keep seeds moist and to encourage vertical root growth. Seedlings were selected for uniformity of root length and were transplanted to the pots, one seedling per pot, five days after imbibition.

The experiment was laid out in a glasshouse in a randomised block design (n=5). The experiment was conducted in summer (commencing 21 May 2009). Air temperature and relative humidity in the glasshouse were logged with a data logger (DL3000 modular data logger, Delta T device, Cambridge, UK)

The vapour pressure deficit (VPD) was calculated using relative humidity and air temperature at plant height, using an Arden-Buck equation (Buck Research Manual, 1996).

$$Pw = 6.112 \exp((18.678 - T / 234.5)\, T / (257.14 + T)) \quad [1]$$

$$Pws = Pw / \%RH \quad [2]$$

$$VPD = Pws - Pw \quad [3]$$

With T being air temperature in °C, Pw the vapour pressure in hPa and Pws the saturated vapour pressure in hPa. Variation in temperature and VPD are shown in Figure 4.1.

Measurements

The pots were weighed every two to four days to determine plant water use and evaporation of soil water. The stomatal conductance of the youngest expanded leaf was measured with an AP4 porometer (Delta-T devices, Cambridge, UK), on days 12 to 21 after planting.

The leaf length of an expanding leaf was measured on day 14 and re-measured on day 17 to determine its growth rate. The relative growth rate of a leaf was calculated the following way: (ln(length day 17) – ln(length day 14))/3, with leaf length expressed in mm.

At harvest, 22 days after planting, the number of leaves, number of tillers (wheat only), shoot projected area and shoot fresh weight were measured. The shoot area was measured with a LI-3100 leaf area meter (Li-Cor Biosciences, Cambridge, UK). A piece of leaf tissue from the youngest fully expanded leaf was excised for measurement of relative water content and the remaining shoot tissue was dried to constant weight at 70°C in a fan-assisted oven to determine dry weight.

The relative water content of the youngest fully expanded leaves of plants in treatments 1.1 and 1.4 $g \cdot cm^{-3}$ was determined on the day of harvest. A rectangular segment was cut from the leaf, avoiding large veins. The fresh weight (FW) of this fragment was determined to the nearest 0.1 mg and then the leaf was floated on de-ionised water in a Petri dish in a dark room at 21°C to attain full turgid weight (TW) (Smart and Bingham 1974; Jensen et al.. 1996). After 3 hours and 40 minutes, surface water was removed from the leaf segment by gentle blotting and re-weighed. The segment was then dried in an oven at 80°C until the weight was constant, subsequently dry weight (DW) was determined. From these measurements, the relative water content of the leaf at the time of harvest could be calculated:

$$\text{(FW start-DW) / (Turgid Weight – DW)} \qquad [4]$$

The containers with soil and root systems were kept at 4°C for 1 to 16 days, until washing and scanning of roots. The pot was opened length- wise and soaked in a tray of water for 30 min, to soften the soil. The depth of the deepest root was measured and the soil core was cut into four equal parts of twelve cm depth each. The soil was washed

from the root system over a five mm and two mm sieve. Roots were placed in a tray of water and scanned with a Régent LA1600 scanner, the images were analysed with Winrhizo software (Régent Instruments Inc, Quebec, Canada).

Statistical analysis

To test for the effects of species and the effects of increasing soil bulk density (i.e. soil strength) data were tested with two-way ANOVAs in GenStat (GenStat 11.1 2008, VSN International Ltd, Hemel Hempstead, UK). If there was a significant interaction ($p < 0.05$) of the factors species and bulk density on a parameter, it indicated that the species differed in their in sensitivity to soil bulk density.

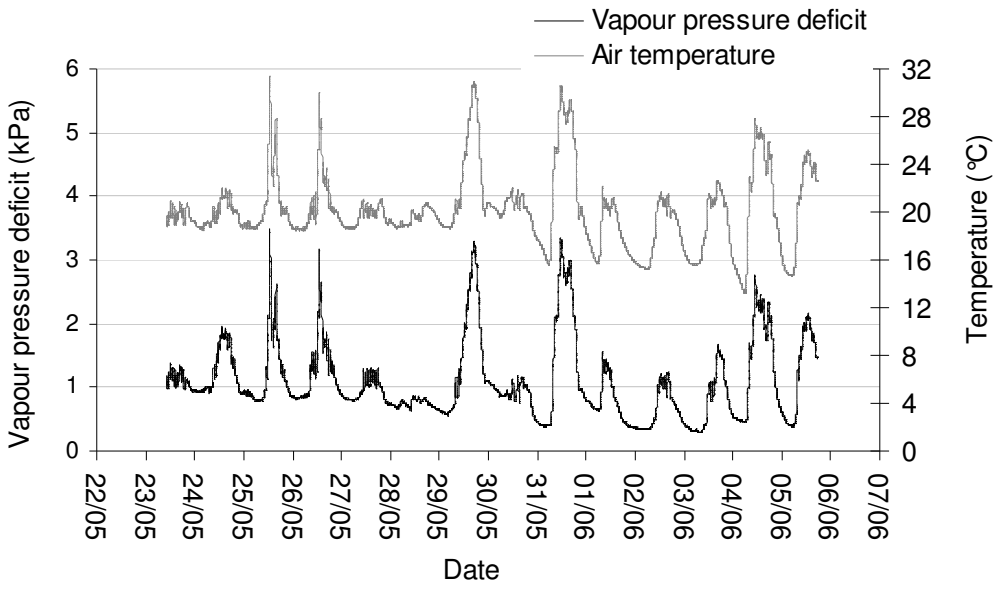

Figure 4.1 Air temperature and vapour pressure deficit at plant height in the glass house during experiment I.

Experiment II: Bulk density plus soil drying

Soil preparation and seed germination

Details of soil preparation, fertiliser application, soil packing and seed germination are as described for experiment I, with the following modifications. Pots were 25 cm high and packed with soil to a depth of 24 cm. Two dry bulk density treatments were prepared, 1.2 and 1.3 $g \cdot cm^{-3}$ and two seedlings were transplanted into each pot, into a hole created with a 1 mm diameter needle of the same depth of the longest seedling root, in the first week the number of seedlings per pot was thinned down to one. The pots were packed in 12 cm depth intervals. Soil was packed by hammering with a special device with a rubber end with the same diameter as the pot and a ridge along its edge. This was to provide a slight increase in soil bulk density at the edge to discourage roots from growing along the side of the pot. Nutrient solution (Hoagland) was mixed in with the soil aliquots before packing to ensure homogeneous distribution of nutrients throughout the soil, see experiment I for details of nutrient solution.

Plant growth conditions and experimental design

The experiment was initially laid out in a glasshouse in a randomised complete block design with two plant species (oilseed rape and wheat), two soil bulk densities (1.2 and 1.3 $g \cdot cm^{-3}$) and two watering regimes (well watered and non-watered) within each of 5 blocks. The experiment commenced with the transplantation of seedlings on the 10th November 2009. Supplementary lighting was supplied by sodium lamps, the light intensity (PAR) at plant height averaged over all blocks was 60 ± 1.50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at midday.

In the glass house, the average minimum daily temperature was 17.9°C and max daily temperature 23.6°C and relative humidity was between 32 and 60%. After 24 days, the pots were transferred to a climate controlled growth room, where light intensity (PAR) at plant height was greater at 175 ± 16.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at midday, averaged over all blocks, additionally the temperature was more stable and lower during the day. Light period in the growth room was 16 hours and temperature was set at 18°C, for the actual temperature and VPD see Figure 4.2. The different watering regimes were imposed after the plants were placed in the climate controlled growth room.

Pots were weighed every two to four days to determine water use. In the well-watered treatment, water was supplied to restore the soil to field capacity. In the non-watered treatment, water was withheld from day 30 (10 Dec 2009) onwards. The treatment was imposed after roots in pots pre-designated for both watering regimes had reached the base of the pot. This was to ensure that plants in each treatment had access to water throughout the whole soil depth before soil strength increased as a result of soil drying and thus minimize the possible differences in water use arising from differences in the depth of soil explored.

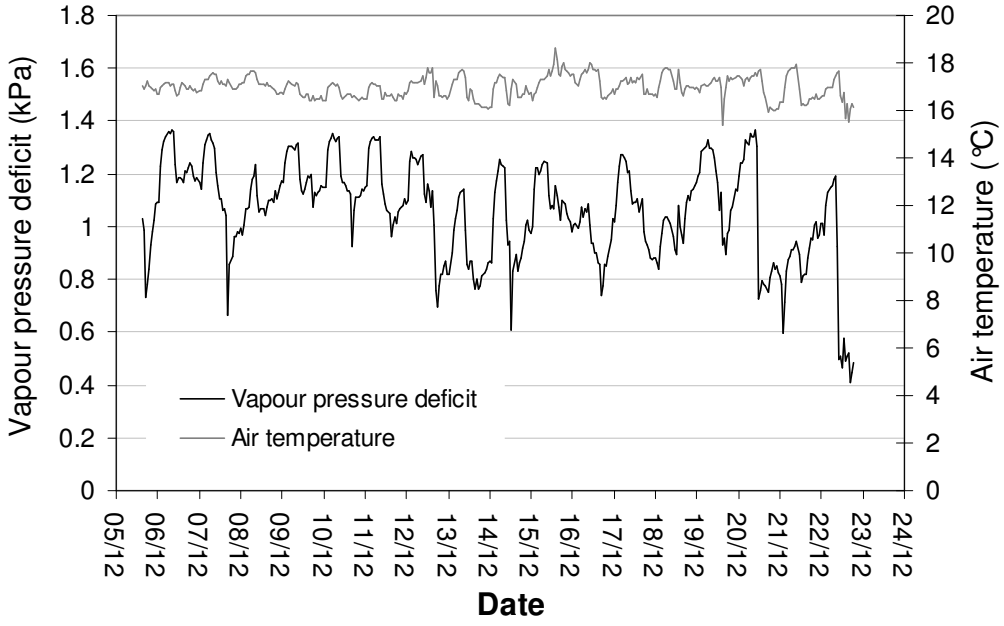

Figure 4.2 Air temperature and vapour pressure deficit at plant height in the climate controlled chamber during experiment II. The x-axis denotes the start of the day at 00.01 am.

Plant measurements

Water use was measured by weighing the pots every two to four days and calculating the weight loss over the time interval. Stomatal conductance (g_s) was determined on the youngest completely unfolded leaf with a portable IRGA (ADC-LCA4 Analytical Development Co. Ltd, Hoddesdon, Herts, UK). Leaf g_s was measured on days 32, 33, 34, 36, 37, 38, 40 and 42 days after transplanting of seedlings.

At harvest (day 43, Dec 22), the shoot area of each plant was measured separately, and for each plant the area of dead (non-green leaves and petioles either still on the plant or shed) and the live (green) parts were measured separately. The shoot area was measured with a LI-3100 leaf area meter (Li-Cor Biosciences, Cambridge, UK). The shoots were dried at 70°C in a fan-assisted oven to constant weight and weighed to determine dry weight.

Directly after harvest of the shoots, the pots with soil and root systems were put in a freezer at -18ºC. The day before root washing, four pots were taken out of the freezer and soaked in a large tray with sodium hexametaphosphate (Calgon solution, 5% by weight), this to loosen the soil and to make it easier to separate soil from roots. After soaking overnight, the soil was washed from the roots with a Delta-T root washer (Delta-T Devices Ltd, Cambridge, UK), the soil in the reservoir was agitated every hour by stirring and when no root fragments floated up, it was assumed that there were no more roots to be washed from the soil sample. The root sample was cut into smaller fragments, mixed up and a subsample of $1/7^{th}$ of the fresh weight of the total root sample taken. The fresh weight was determined after dabbing with filter paper to remove surface water. The roots in the subsample were scanned with a Régent LA1600 scanner, the images were analysed with Winrhizo software (Régent Instruments Inc, Quebec, Canada). After scanning, the dry weights of the root subsample and rest of the sample were determined by drying to constant weight at 70ºC in a fan assisted oven. The total root length and area of roots per pot were calculated using the fraction of dry weight of scanned root sample to the total dry weight of the root system.

Measurement and calculations of soil physical properties

Soil sample rings with a diameter of 5.6 cm and a depth of 4.0 cm were packed with soil to dry bulk densities of 1.2 and 1.3 $g \cdot cm^{-3}$ and put on tension tables at known tension to determine the relationships between soil moisture content, soil matric potential and soil strength. Soil physical properties were measured at SCRI (Scottish Crop Research Institute, Dundee).

The weight of the soil cores was determined after saturating with water and again after equilibrating for 5 to 8 days on a tension table at suctions of 0.5, 2, 10, 25, 50 and 200kPa (n=6). A subsample of each core was taken to measure water content at 1500 kPa using pressure plates. The soil moisture release curves are given in Figure 4.9.

Soil strength was measured on the same cores with a penetrometer with a 1 mm diameter tip after equilibration at suctions of 10, 25, 50 and 200kPa. Soil matric potential and soil strength at each bulk density were plotted against the soil volumetric water content and second order polynomial curves fitted to obtain the relationship between soil water content and soil physical properties for the range of water contents observed in the plant growth experiment. The volumetric soil water content on each measurement occasion during the plant growth experiment was calculated from the measured gravimetric water content and the soil dry bulk density. Matric potential and soil strength was then estimated from the volumetric water content using the standard curves generated above.

The air-filled porosity of a soil can be defined as the total porosity minus the pore volume occupied by water. Field capacity was assumed to be equal to -10 kPa matric potential. The air-filled porosity of the soil at field capacity at bulk densities of 1.2 and 1.3 $g \cdot cm^{-3}$ was determined by calculating the difference between the weight of saturated cores and the weight at field capacity, the difference in water in volume is the air-filled porosity at field capacity. At 1.2 $g \cdot cm^{-3}$ bulk density the air-filled porosity at field capacity was 0.17 ± 0.005 $cm^3 \cdot cm^{-3}$ (17% v/v) and at a bulk density of 1.3 $g \cdot cm^{-3}$ it was 0.12 ± 0.006 $cm^3 \cdot cm^{-3}$.

Calculations and statistical analysis

Statistical analyses were conducted with GenStat software (GenStat 11.1 2008, VSN International Ltd, Hemel Hempstead, UK).

To eliminate the influence of time or plant age on stomatal conductance, on each measuring day, the stomatal conductance of non-watered plants was expressed as a ratio of that of well-watered plants (referred to as the relative stomatal conductance). The stomatal conductance of each non-watered plant was divided by the mean stomatal conductance of well watered plants in the same bulk density treatment on the same measuring day. The ratios that were greater than one at the beginning of the experiment were omitted from this analysis.

To test for the effects of species and the effects of the two soil bulk densities and water treatments (e.g. watered and non-watered) data were tested with three-way ANOVAs for unbalanced data in GenStat.

Results Experiment I

Plant growth

Oilseed rape shoots were generally larger than wheat shoots; their total shoot area and shoot dry weight were significantly greater than those of wheat (Figure 4.3 and Table 4.1). While oilseed rape shoots were larger, oilseed rape root length density, root area, root dry weight and the depth of longest root were not significantly different from wheat when averaged over soil bulk density. The distribution of root length over four soil depth intervals was also very similar for both species at any given bulk density (Figure 4.4).

Soil bulk density had a significant effect on most plant parameters at harvest (Figure 4.3). An increase in soil compaction resulted in a significant decrease in total shoot area, shoot dry weight, specific shoot area, root length density, root area and depth of rooting for both species. The average root diameter of oilseed rape plants was not affected by soil compaction, while the root diameter of wheat plants increased with increasing soil compaction (Figure 4.3 F). The root: shoot ratio (root dry weight/shoot dry weight) increased significantly with increasing soil bulk density for both species indicating that both species allocated proportionately more biomass to the root system when soil was compacted. There was a greater percentage root length in the top soil with compaction (Figure 4.4) and although total root length at high bulk density was smaller, the root length in the top 12 cm of soil was greater in the 1.4 $g \cdot cm^{-3}$ treatments than in the less compacted treatments (data not shown).

For almost all parameters the interactions of the factors species and bulk density was not significant, implying that oilseed rape and wheat responded to bulk density in the same way. However, the average root diameter of oilseed rape plants did not respond in a clear pattern to increased soil compaction, while the root diameter of wheat plants increased with increased soil compaction giving a significant species x bulk density interaction ($p < 0.01$) (Figure 4.3 F).

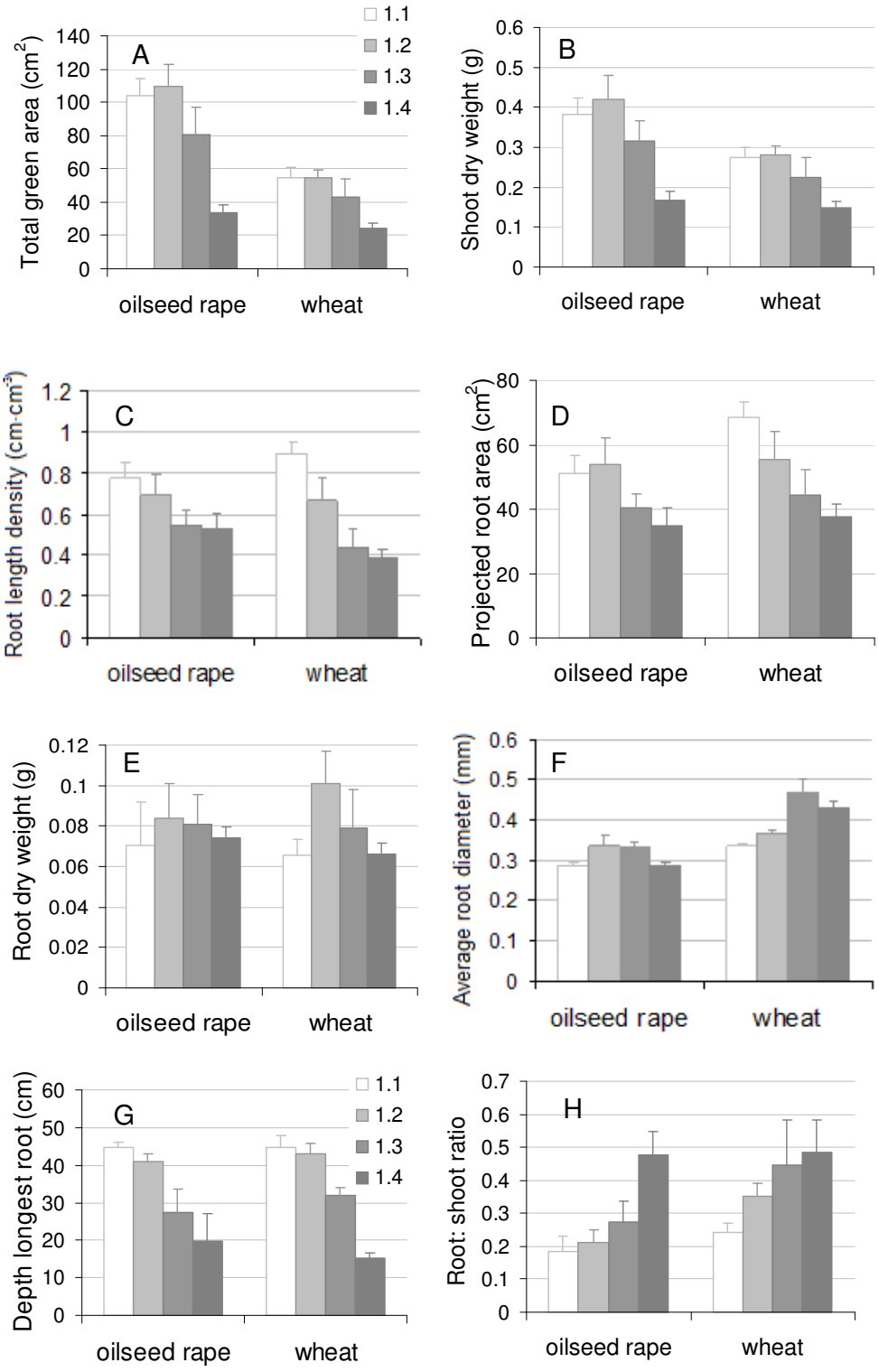

Figure 4.3 Influence of soil bulk density, varying from 1.1 to 1.4 g·cm^{-3} (see key), on root and shoot properties of oilseed rape and wheat plants. Figure H is the dry weight ratio of root to shoot. Results of statistical analysis are presented in Table 4.1.

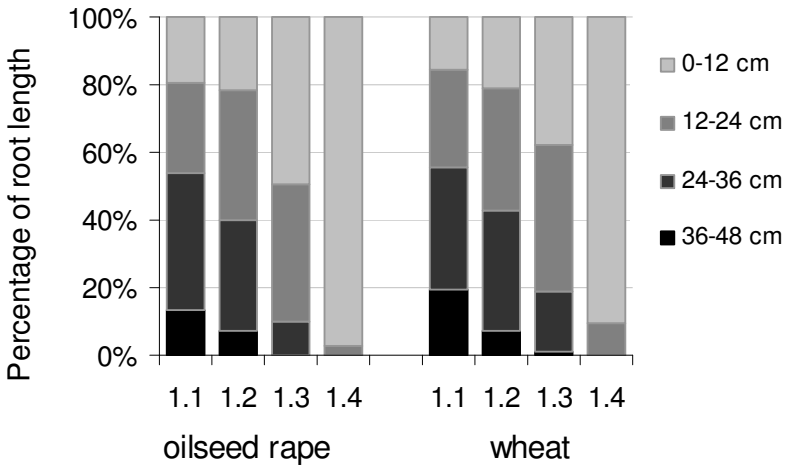

Figure 4.4 Distribution of root length of oilseed rape and wheat over four depth intervals from the soil surface, in soils of four different bulk densities varying from 1.1 to 1.4 g·cm^{-3}.

Table 4.1 Results of two-way ANOVA tests on growth and harvest data, p-values are given for species effect (oilseed rape and wheat), soil bulk density (1.1, 1.2, 1.3 and 1.4 g·cm^{-3}) effect and interaction of those two factors.

Parameter	species	bulk density	interaction
Total green area	< 0.001	< 0.001	0.101
Shoot dry weight	0.002	< 0.001	0.452
Root length density	0.520	< 0.001	0.429
Root projected area	0.186	0.006	0.618
Root dry weight	0.968	0.314	0.819
Root diameter	< 0.001	< 0.001	0.002
Depth longest root	0.834	< 0.001	0.737
Root: shoot ratio	0.055	0.004	0.635
Specific shoot area (cm^2·g^{-1})	< 0.001	< 0.001	0.252
Growth rate leaf	< 0.001	< 0.001	> 0.4
Relative growth rate leaf	> 0.4	> 0.4	> 0.4

Water use

The pots that were planted with oilseed rape lost more water by evapotranspiration than pots planted with wheat (Figure 4.5). The rate of evapotranspiration increased as the plants grew (Figure 4.6). Not only was the total evapotranspiration from oilseed rape

greater than that wheat, its transpiration rate per plant, per unit root area and per unit root length were also significantly greater when averaged over soil bulk density treatments. The transpiration rate per unit shoot area however, was significantly greater in wheat plants (Figure 4.5 C). The transpiration rate was calculated from water use during the last two days before harvest, day 20-22 after transplanting of seedlings to the pots. The relative water content of the youngest completely unfolded leaf of wheat was significantly greater than that of oilseed rape at both bulk densities measured (Figure 4.5 E).

The water use by plants was affected significantly by soil bulk density (Figure 4.5, Table 4.2). The total cumulative evapotranspiration of water, as well as transpiration rates on a unit shoot area and root length basis were significantly reduced by soil compaction, with plants growing in more compacted soil transpiring less water in total and at a slower rate (Figure 4.5). In spite of the lower transpiration rate at high bulk density, leaf relative water content was reduced significantly.

On day 12 there was a clear effect of bulk density on the stomatal conductance (g_s) of oilseed rape, while wheat g_s was affected less. Oilseed rape leaves had a greater g_s than wheat leaves (Figure 4.7). The stomatal conductance of the youngest fully expanded leaf was measured at one or two day intervals, however only the measurements of day 12 were valid. On the other days, according to the specifications of the manufacturer (AP4 porometer, Delta-T devices, Cambridge, UK), the temperature difference between the leaf and the cup of the porometer was too large for the measurements to be accurate.

The rate of leaf expansion between day 14 and 17 was reduced by an increase in soil bulk density in both species (Figure 4.8) wheat had a significantly greater absolute change in leaf length than oilseed rape leaves. While absolute growth in leaf length was affected by species and bulk density, the relative growth rate of leaves was not significantly affected.

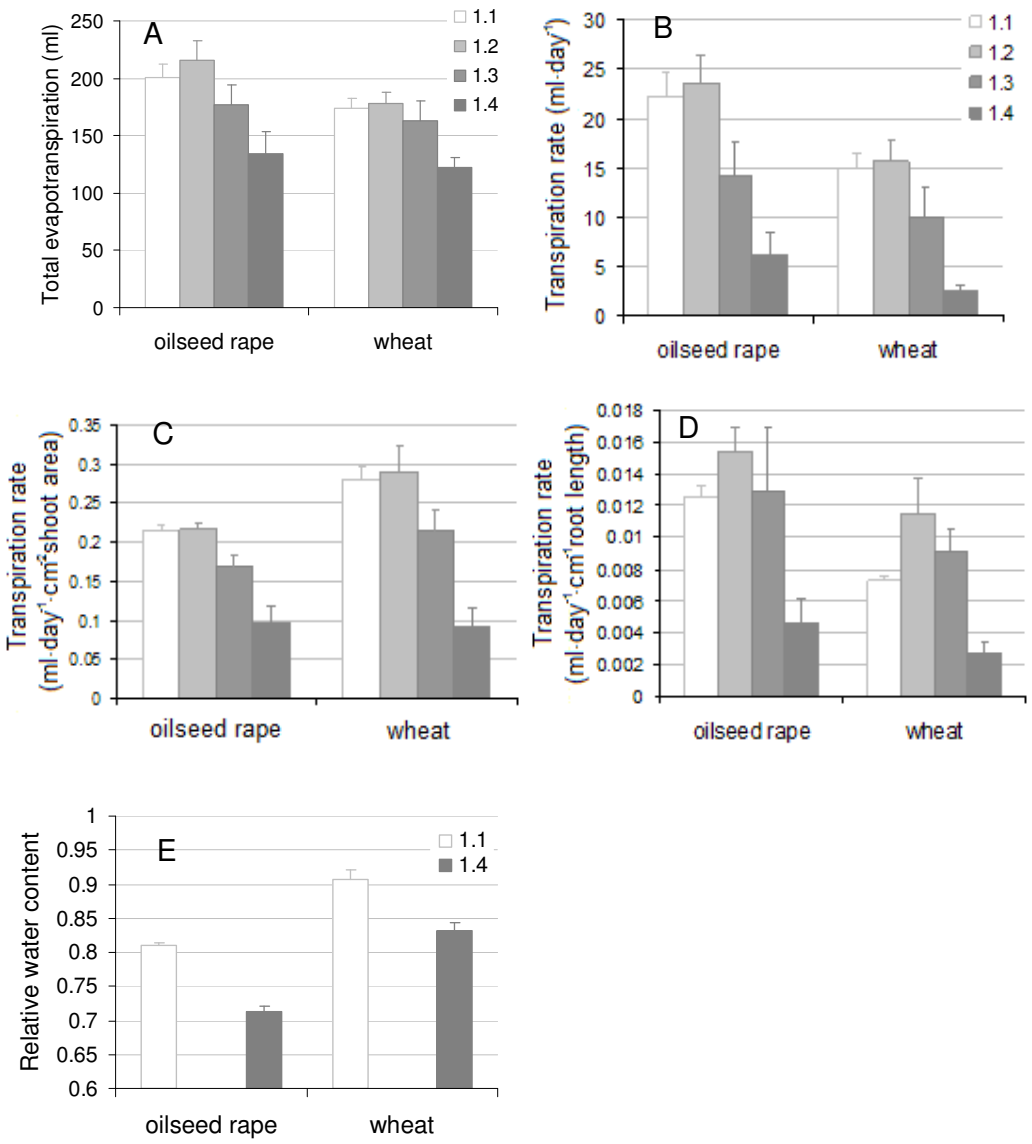

Figure 4.5 The influence of soil bulk density on transpiration and water status of oilseed rape and wheat plants. Sample size was five and the error bar depicts sem. The results of statistical tests for the effects of species and bulk density can be found in Table 4.2.

Table 4.2 Results of two-way ANOVA tests on water use data, p-values are given for species effect (oilseed rape and wheat), soil bulk density (1.1, 1.2, 1.3 and 1.4 g·cm^{-3}) effect and the interaction of those two factors.

Parameter	species	bulk density	interaction
Cumulative evapotranspiration day 22	0.036	< 0.001	0.783
Transpiration rate day 20 to 22:			
Per plant	0.003	< 0.001	0.773
Per unit shoot area. In brackets, an outlier oilseed rape 1.4 was not excluded.	(0.469) 0.007	(0.033) < 0.001	(0.096) 0.187
Per unit root area	< 0.001	< 0.001	0.782
Per unit root length	0.01	< 0.001	0.862
Per unit root length	0.01	< 0.001	0.862
Relative water content leaf	< 0.001	< 0.001	0.389
Stomatal conductance day 12	< 0.001	0.004	0.018

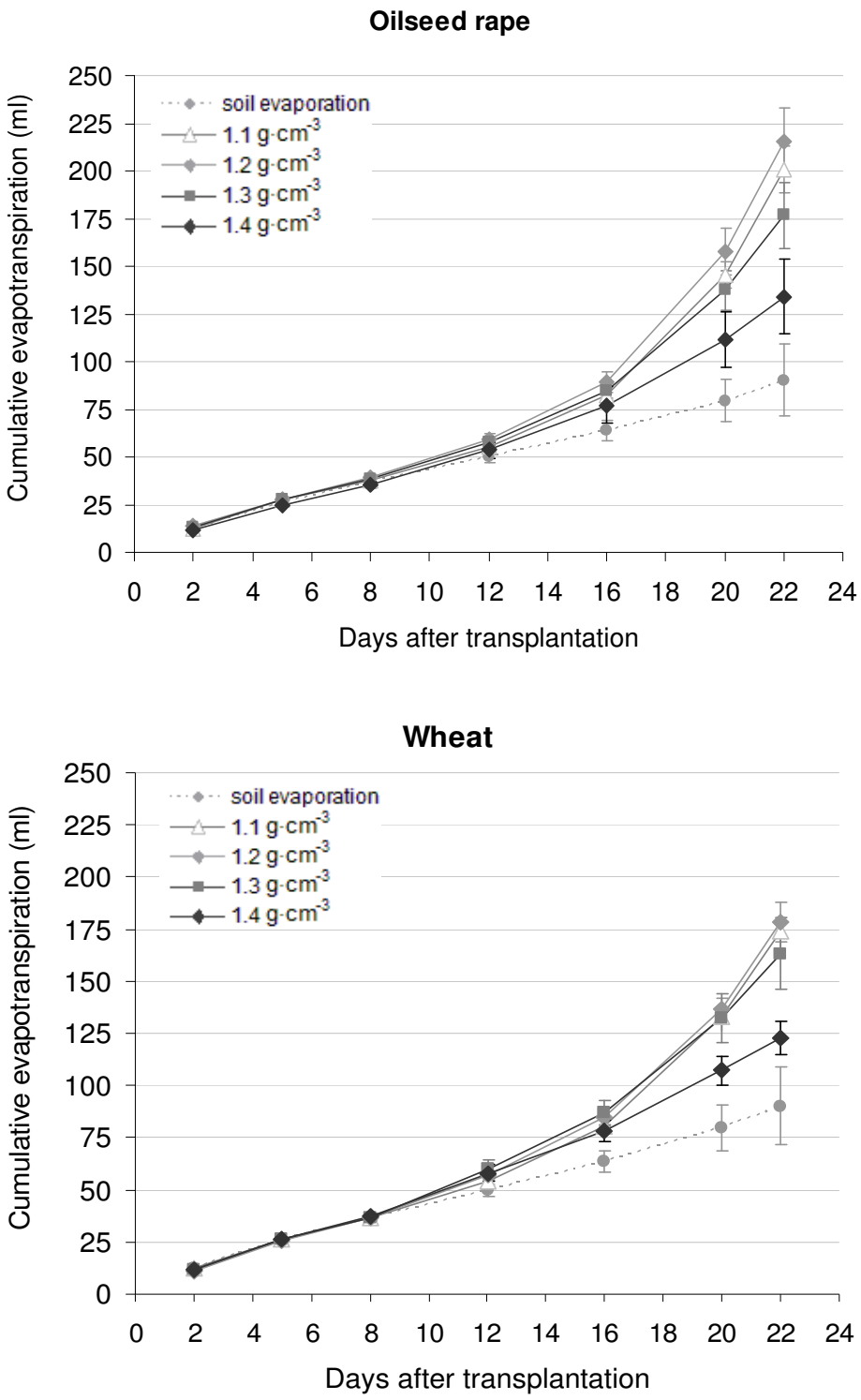

Figure 4.6 Cumulative evapotranspiration per pot for oilseed rape (top) and wheat (bottom), standard error of the mean is given in error bars (n=5). Soil evaporation was measured as water loss from unplanted pots (n=5).

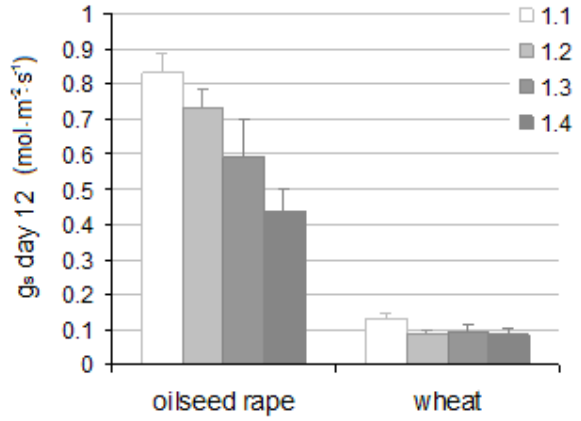

Figure 4.7 Stomatal conductance (g_s) youngest fully expanded leaf of oilseed rape (leaf 1) and wheat (leaf 2) 12 days after transplantation of seedling to pot. The legend refers to the four different soil bulk densities used in $g \cdot cm^{-3}$. The error bar depicts sem (n=5).

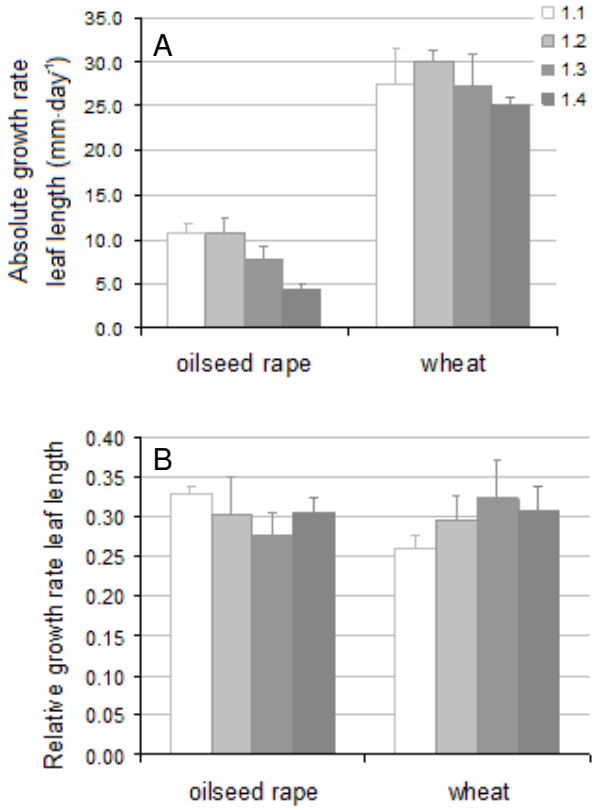

Figure 4.8 Growth rate of leaves between day 14 and 17, determined by measuring length of leaf three of oilseed rape and leaf four of wheat on both days.

Results Experiment II

Soil physical properties

Figure 4.9 shows the moisture release curves of the soil used in both experiment I and II at bulk densities of 1.2 and 1.3 g·cm^{-3}. In Figure 4.10, data from the same cores was used to plot soil strength against the soil volumetric content for the range observed in experiment II. While soil matric potential was not affected much by soil bulk density Figure 4.9), the soil strength was (Figure 4.10); thus by varying soil bulk density and water content, a wide range of soil strengths were created in the experiment. The relationship between soil volumetric water content (x) and soil strength in MPa (y) can be described by the following second order polynomials which were fitted using Excel (Microsoft Office Excel 2003, Microsoft Corporation, Redmond, USA)

$$Y = 92.361x^2 - 83.919x + 19.817,\ R^2 = 0.98,\ \text{for bulk density 1.2 g·cm}^{-3} \quad [4]$$

$$Y = 184.52x^2 - 167.34x + 39.064,\ R^2 = 0.99,\ \text{for bulk density 1.3 g·cm}^{-3} \quad [5]$$

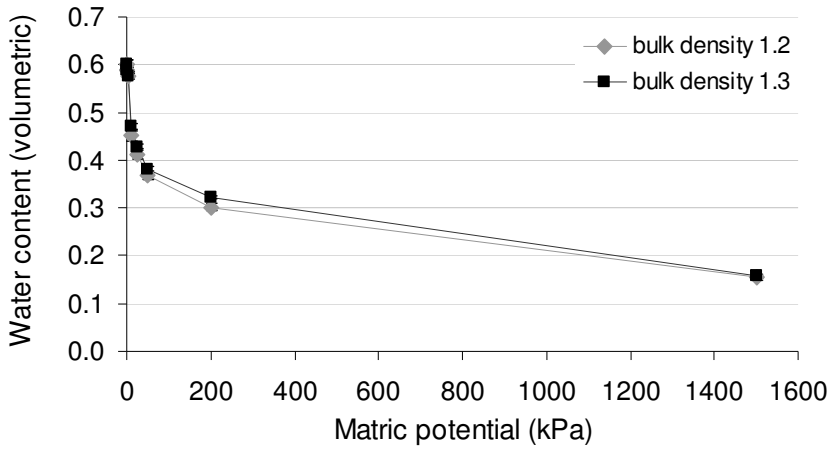

Figure 4.9 Moisture release curve of soil packed to densities of 1.2 g·cm^{-3} and 1.3 g·cm^{-3}. Matric potential is negative pressure, but plotted here as positive. The standard errors of the means fell within the symbols. Measurements were conducted at SCRI.

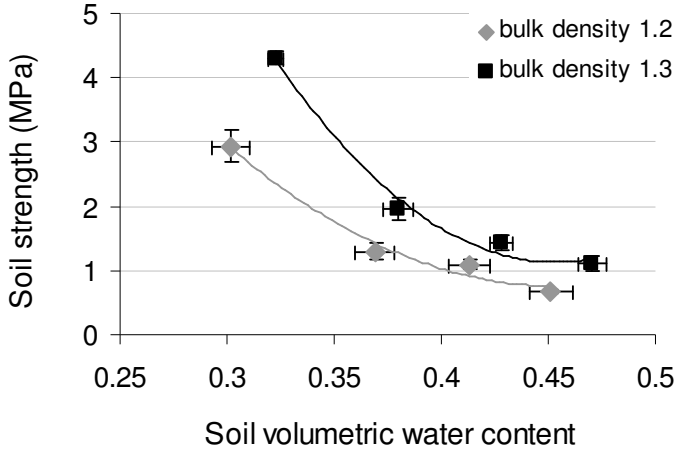

Figure 4.10 Relationship between soil strength and soil volumetric water (in $cm^3 \cdot cm^{-3}$) content of soil at bulk densities of 1.2 $g \cdot cm^{-3}$ and 1.3 $g \cdot cm^{-3}$. Sample size was six and error bars depict standard errors of the mean. The fitted curves are second order. Soil physical properties were measured at SCRI.

Water use and stomatal response

Stomatal conductance decreased over time for plants in both the non-watered and well-watered treatments (Figure 4.11). At the start of the experiment, when plants were unstressed, oilseed rape plants had a greater stomatal conductance than wheat plants (~0.4 and 0.3 $mol \cdot m^{-2} \cdot s^{-1}$ respectively).

The response of the stomatal conductance (g_s) of the youngest fully developed leaf of oilseed rape and wheat to increasing soil strength was plotted in Figure 4.12. Power function curves were fitted to compare the relative gs of oilseed rape and wheat to increasing soil strength. The relationship between relative gs (y) and soil strength (x) can be described by the following equations:

Oilseed rape: $y = 1.572x^{-1.199}$, $R^2 = 0.62$ [6]

Wheat: $y = 0.999x^{-0.427}$, $R^2 = 0.25$ [7]

Both oilseed rape and wheat started reducing their stomatal conductance relative to controls as the soil strength increased. The relative g_s of oilseed rape showed a steep initial decline as soil strength increased. In wheat the decline was less pronounced.

However, for both species there was a lot of variation not accounted for by the relationship (R^2 = 0.62 and 0.25 for rape and wheat respectively).

Relative g_s showed little further response to increases in soil strength above 6 MPa. There were no indications that one species began to respond to soil strength earlier than the other (i.e. at a lower threshold soil strength). Above a soil strength of 6 MPa, oilseed rape plants had significantly lower g_s ratio than wheat (i.e. they closed their stomata more relative to well-watered controls) (Figure 4.12).

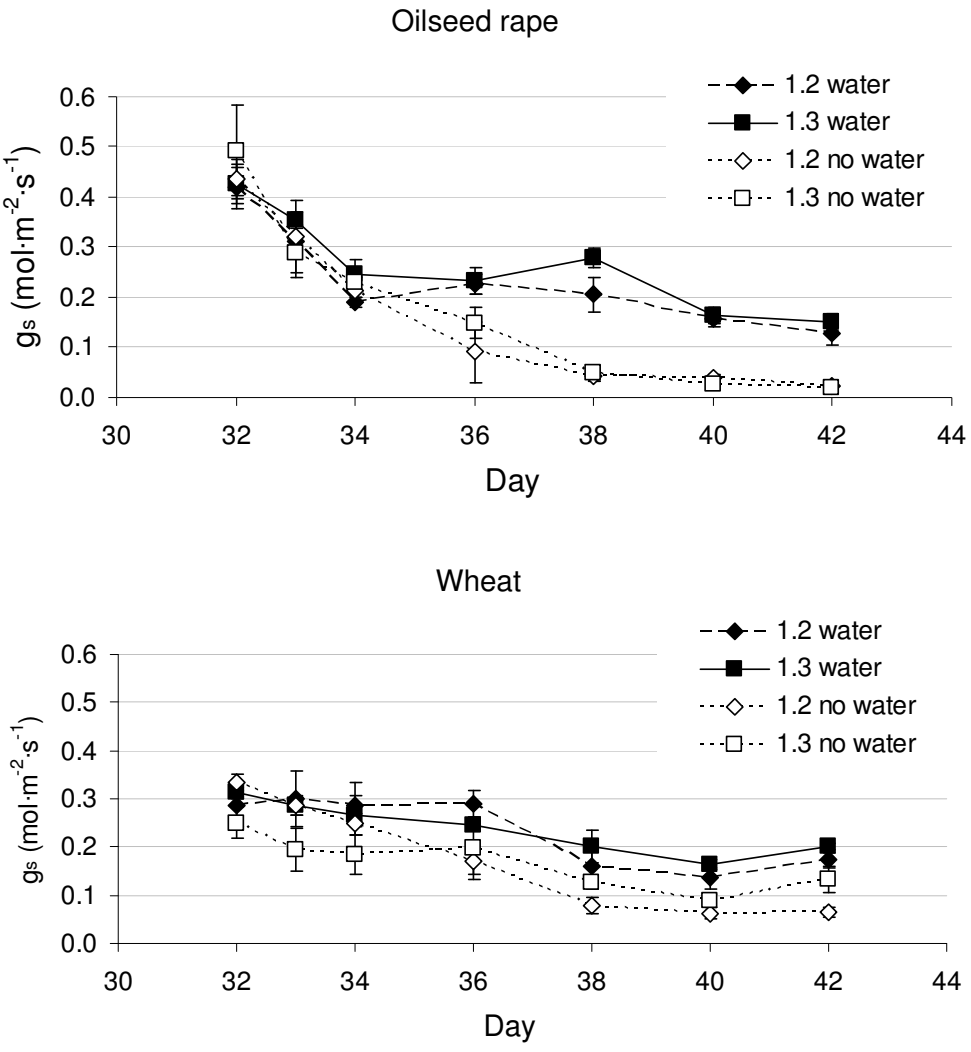

Figure 4.11 Change in stomatal conductance (g_s) in time for oilseed rape (top) and wheat plants (bottom) in the four different treatments, varying irrigation and soil bulk density (1.2 and 1.3 g·cm^{-3}).

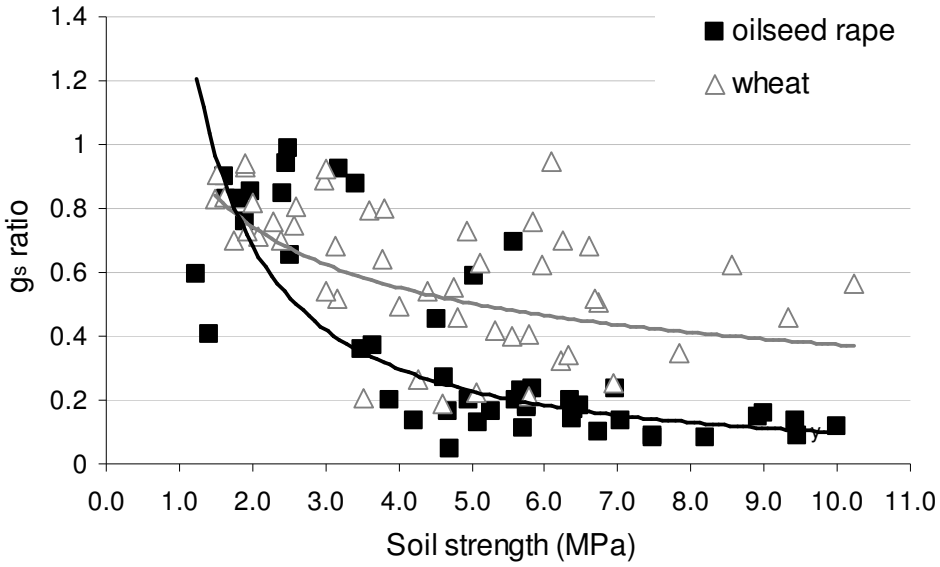

Figure 4.12 The relationship between the relative stomatal conductance of non-watered plants and soil strength. The stomatal conductance of non-watered plants is expressed as a ratio of the average stomatal conductance of well-watered plants in the same bulk density treatment on the same measurement occasion. The fitted curves are power functions, see equations 6 and 7.

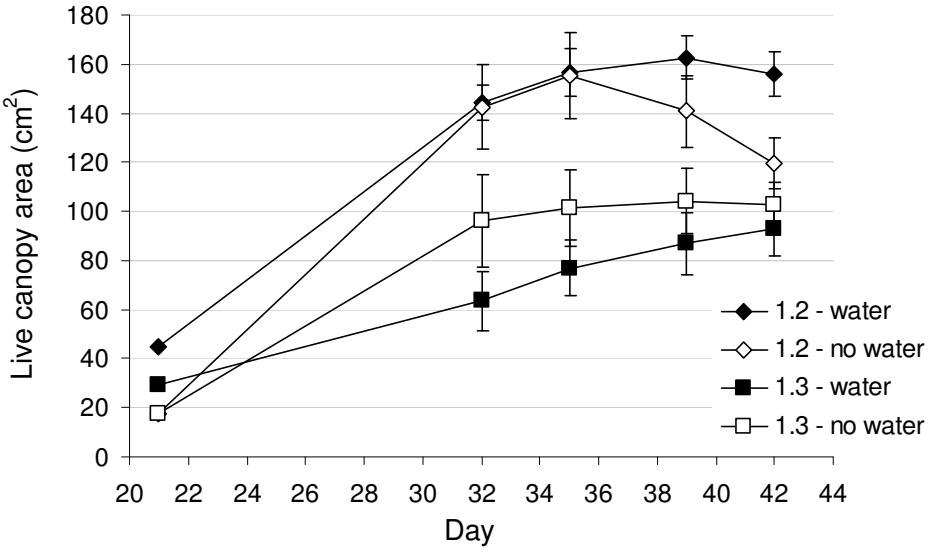

Figure 4.13 Total live (green) shoot area development in time for oilseed rape plants in different treatments.

The effect of soil bulk density and watering regime on shoot and root parameters of oilseed rape and wheat are listed in Table 4.4. Wheat plants had a significantly greater root dry weight and water uptake rate per plant than oilseed rape when averaged across bulk density and watering regimes (Table 4.3 and Table 4.4). For both species an increase in soil bulk density from 1.2 to 1.3 $g \cdot cm^{-3}$ resulted in a significant decrease in dry weight of green (live) leaf area, total shoot dry weight (including stems, petioles and dead leaves), of total leaf area and of root length, root projected area and root dry weight.

When averaged across the other treatments, withholding of water resulted in a significant decrease in dry weight of green shoot area, total shoot dry weight (including stems, petioles and dead leaves), of total leaf area and of root length. While oilseed rape root dry weight was hardly affected by withholding water wheat root dry weight was decreased, but not significantly so ($p = 0.074$ for species x water effect). The uptake rates of water per plant and per unit root length were significantly decreased by withholding water in both wheat and oilseed rape.

Withholding water at soil bulk density of 1.2 $g \cdot cm^{-3}$ had a significantly greater effect on dry weight of live leaves than at 1.3 $g \cdot cm^{-3}$ (Table 4.5). This trend can also be observed in figure 14, where the live canopy development of oilseed rape plants was plotted over time. The same response was seen in root length, which was reduced to a greater extent by withholding water at the lower soil bulk density (Table 4.3).

Table 4.3 Shoot and root parameters per oilseed rape plant at harvest as affected by bulk density (1.2 and 1.3 g·cm^{-3} referred to 1.2 and 1.3 respectively) and water treatment (watered and non-watered). Uptake rate is plant transpiration measured between days 40 and 42, either expressed per plant or per unit root length. Mean and sem are given.

Oilseed rape	1.2 watered	1.2 non-watered	1.3 watered	1.3 non-watered
Shoot	n = 4	n = 5	n = 5	n = 5
DW live leaves (g)	0.92 ± 0.13	0.60 ± 0.07	0.51 ± 0.09	0.52 ± 0.06
Dry weight shoot (g)	1.73 ± 0.20	1.27 ± 0.16	0.82 ± 0.17	0.86 ± 0.14
Total leaf area (cm^2)	171.5 ± 9.21	129.9 ± 7.46	104.6 ± 14.63	105.2 ± 9.91
Roots				
Length (m)	162.6 ± 24.28	102.7 ± 13.90	57.8 ± 13.92	65.69 ± 10.00
Projected area (cm^2)	359 ± 62.4	249 ± 36.4	155 ± 33.6	169 ± 23.4
Dry weight (g)	0.25 ± 0.033	0.23 ± 0.041	0.11 ± 0.020	0.14 ± 0.020
Uptake rate per root length(mg·day^{-1}·cm^{-1})	1.8 ± 0.17	1.2 ± 0.32	5.0 ± 1.04	2.2 ± 0.45
Uptake rate per plant (g·day^{-1})	27.9 ± 1.86	10.7 ± 0.80	24.2 ± 3.32	12.6 ± 0.84

Table 4.4 Harvest parameters for wheat plants, see legend of Table 4.3 for further information.

Wheat	1.2 watered	1.2 non-watered	1.3 watered	1.3 non-watered
Shoot	n = 3	n = 6	n = 2	n = 4
DW live leaves (g)	0.91 ± 0.11	0.65 ± 0.03	0.62 ± 0.07	0.46 ± 0.06
Dry weight shoot (g)	1.80 ± 0.287	1.31 ± 0.0.69	1.28 ± 0.153	0.970 ± 0.163
Total leaf area harvest (cm^2)	174.9 ± 12.08	119.7 ± 6.29	132.5 ± 19.69	87.04 ± 13.61
Roots				
Length (m)	118.2 ± 17.88	91.3 ± 4.47	89.02 ±11.27	63.8 ± 7.28
Projected area (cm^2)	322 ± 56.3	276 ± 12.8	296 ± 37.5	201 ± 24.9
Dry weight (g)	0.39 ± 0.062	0.34 ± 0.018	0.35 ± 0.053	0.23 ± 0.034
Uptake rate per root length(mg·day^{-1}·cm^{-1})	3.2 ± 0.29	1.5 ± 0.07	3.7 ± 0.03	2.1 ± 0.19
Uptake rate per plant (g·day^{-1})	37.3 ± 2.78	13.7 ± 0.55	30.3 ± 3.92	13.6 ± 1.02

Table 4.5 Results of statistical tests (unbalanced three-way ANOVAs) on oilseed rape and wheat shoot and root parameters, results in Table 4.3 and Table 4.4. The shoot dry weight (DW) included dead leaves. Uptake of water was calculated between days 40 and 42 per plant and per cm root length. Root area is the projected root area per plant.

	Species (S)	**Density (D)**	**Water (W)**	**S xD**	**S xW**	**D xW**	**S x D xW**
Shoot							
DW live leaves (g)	0.553	<.001	0.005	0.640	0.503	0.045	0.397
DW shoot (g)	0.147	<.001	0.018	0.599	0.375	0.099	0.617
Leaf area (cm^2)	0.836	<.001	0.001	0.542	0.085	0.137	0.275
Roots							
Length (m)	0.561	<.001	0.035	0.090	0.931	0.033	0.166
Area (cm^2)	0.138	<.001	0.079	0.248	0.796	0.243	0.157
Dry weight (g)	<.001	<.001	0.316	0.936	0.073	0.727	0.238
Uptake rate ($mg \cdot day^{-1} \cdot cm^{-1}$)	0.414	<.001	<.001	0.135	0.893	0.074	0.296
Uptake rate per plant ($g \cdot day^{-1}$)	0.023	0.694	<.001	0.806	0.056	0.077	0.856

Discussion

Effects of soil compaction and drying on plant growth

Increasing soil bulk density through compaction leads to an increase in soil strength. The scale of the effect depends on the soil moisture content as the relationship between volumetric water content and soil strength is non-linear. There is a greater effect of compaction on soil strength at low water content than high (Figure 4.10). Compaction also reduces the air-filled porosity of the soil and hence soil O_2 availability. Consequently at high soil moisture contents soil compaction may affect plant growth through effects of soil strength and reduced aeration, whilst at low soil moisture contents it may influence growth via soil strength and low soil water potential.

Under well-watered conditions compaction resulted in a decrease in root length, root length density and root projected area. It also had a negative effect on the maximum rooting depth of both species. The root dry weight was not significantly affected by compaction. In wheat this was probably due to an increase in root diameter. An increase in root diameter with increased soil bulk density has also been observed in other species (Bengough et al. 1997). However, in the current study there was no effect of compaction on the average root diameter of oilseed rape, even though root length was reduced and root biomass unchanged. This suggests that tissue density might have been increased by compaction in this species.

In dense or dry soils, plant roots can experience mechanical impedance to their elongation. Mechanical impedance stimulates ethylene production and ethylene subsequently could affect root growth by decreasing root elongation rate and increasing root diameter (Clark et al. 2003). It has been suggested that the tap rooted system of oilseed rape might be more sensitive to soil compaction, because compaction reduces soil aeration under wet conditions and oilseed rape tends to be more sensitive to water logging than wheat (Gregory 1998). In the current experiment, the soil packed to a bulk density of 1.3 and 1.4 $g \cdot cm^{-3}$ had an air filled porosity close to 10% when at field capacity, which is relatively low and around the threshold where availability of O_2 may become limiting (Bingham and Bengough 2003). However root growth and distribution of oilseed rape and wheat were equally sensitive to soil compaction as no significant species by bulk density interaction was found other than that on average root diameter. In both species, compaction of the soil resulted in a decrease in green area, shoot fresh

weight and dry weight. As with root responses described above, the shoot growth responses are typical of those reported in the literature (Masle 1998, Bingham and Bengough 2003). While absolute growth rate, measured as the change in leaf length of expanding leaves was significant affected by soil compaction in both species, the relative growth rate (RGR) was not significantly affected (Figure 4.8). This lack of difference could have been due to establishment of differences in RGR prior to day 14, when measurements started. In an experiment by Masle et al (1998) where soil compaction was varied, differences in leaf area of wheat were established five to six days following emergence, after that period, RGR of plants in different treatments were similar. Importantly, there was no significant difference in the sensitivity of shoot growth of oilseed rape and wheat to soil compaction when water supply was unrestricted (experiment I).

Withholding water and allowing the soil to dry elicited comparable plant growth responses to soil compaction. Thus in experiment II soil drying reduced shoot dry weight, leaf area, root length and root projected area in both species. This similarity in response is consistent with the view that the effects of both soil compaction and soil drying are mediated predominantly by the effects of soil strength (Whalley et al. 2008). There was no indication that oilseed rape differed in sensitivity to soil compaction or soil drying (no significant species x density or species x water interaction) which is in contrast to the results of Chapter 2. The possible reasons for this are discussed later.

Plant water relations

As in Chapter 2, oilseed rape plants with unrestricted supplies of water had a greater stomatal conductance and cumulative water use (evapotranspiration) per plant than wheat in both experiments I and II. Soil compaction (experiment I), as well as increased soil strength due to drying (experiment II), resulted in a decrease in leaf expansion and in root length. The smaller canopy may have caused a smaller demand for water and the smaller root system in turn could have contributed to less water being extracted from the soil. However, the transpiration of water per unit leaf area and unit root length were also reduced, suggesting a change in physiological properties of the plant. Radial compression or restriction of roots can lead to metaxylem vessels with a narrower diameter and as hydraulic conductivity of vessels is proportional to the vessel diameter;

this could have implications for the ease of uptake of water from the soil by plants (Bengough et al. 1997). Stomatal closure occurred with soil compaction and this together with the decrease in leaf expansion could explain the lower rates of water use of plants growing in strong soils even when well supplied with water.

The relative water content (RWC) of both oilseed rape and wheat plants was reduced by an increase in soil compaction, but one species was not affected any more than the other. A relatively low RWC can indicate water stress, as RWC is linearly related to leaf water potential (Millar et al. 1968, Rao and Mendham 1991). The RWC of oilseed at the relatively low soil bulk density of 1.1. $g \cdot cm^{-3}$ was 0.81 ± 0.005, which is close to the average RWC of 0.806 reported for 14 oilseed rape varieties when unstressed (Norouzi et al. 2008). In the current experiments oilseed rape plants grown at the high soil bulk density of 1.4 $g \cdot cm^{-3}$ had a RWC of 0.71 ± 0.009. The average RWC of water stressed plants reported by Norouzi et al. (2008) was 0.629. In the current experiments the reduction in RWC with soil compaction was associated with a reduction in stomatal conductance and transpiration which suggests that stomatal closure was insufficient to maintain the leaf water status. A decrease in relative water content can indicate a loss of turgor (due to water limitation) resulting in a limited water availability for cell extension (Norouzi et al. 2008). However, it is conceivable that soil compaction may modify cell wall elasticity altering the relationship between leaf water potential, water content and turgor. Cell wall elasticity was not measured in the current experiment, therefore, it is only possible to speculate about cell turgor. Oilseed rape and wheat tend to have equally rigid cell walls (Cutforth et al. 2009) and the scale of reduction in RWC of oilseed rape in response to soil compaction was comparable to wheat. But RWC of wheat was significantly greater at both high and low soil bulk density.

In an experiment by Masle and Passioura (1996) in which a range of soil strengths were created by varying both bulk density and water content of soils, leaf area and shoot and root dry weights of young wheat plants, were negatively correlated with soil strength. The effects were the same whether variations in soil strength were brought about by changes in water content or in bulk density. They concluded that limiting water and nutrient supply were unlikely explanations for the *onset* of the effects of soil strength and suggested that growth of the shoot was primarily reduced in response to some hormonal message induced in the roots when they experience high soil strength. In an

experiment by Ternesi et al (1994) in which sunflower root systems were confined, the synthesis of a chemical signal, possibly ABA in the roots of plants subjected to mechanical stress, could have been responsible for the inhibition of plant growth. In addition to ABA, pH, cytokinins, a precursor of ethylene, malate and other unidentified factors have all been implicated in root to shoot signalling under drought (Atkinson 1991, Davies et al. 1993, Dodd 2005). However, the identity and relative contribution to signalling of these root sourced chemicals remains controversial. This controversy may be due to differing responses between species, the different intensities of stress treatments applied, the time at which samples were collected during the imposition of drought and/or the different methods used for xylem sap extraction (Schachtman and Goodger 2008). The effects of the signals on the leaves are various. They may affect stomatal conductance, cell expansion, cell division and the rate of leaf appearance. Generally, though not always, they act to harden the plant against falling water status (Passioura 2002).

As water supply was unlimited in experiment I (the soil moisture content was maintained at field capacity), hydraulic signalling from root to shoot for stomatal closure was unlikely. Additionally the pots were supplied with plenty of nutrients, therefore, nutrient signalling is unlikely too. But from the data available from these experiments, there is too little information to make unequivocal statements about which cues caused the stomata to close at high bulk density. Additionally, as the plants were watered from the base of the pot in experiment I it is possible that the topsoil may have dried to some extent, and therefore the possibility of hydraulic signalling cannot be completely be ruled out. Possibly in this experiment a combination of signalling mechanisms occurred. In wheat there appear to be two signalling mechanisms working in the response to drying soil. Stomatal conductance may be affected by hydraulic signalling due to the lower hydraulic conductivity of the soil, perhaps in combination with chemical regulators, whilst shoot growth may be under independent control which is more responsive to mechanical impedance (Whalley et al. 2006).

Typically, penetrometer pressures of 2–2.5 MPa or more are sufficient to impede root elongation significantly (Bengough and Mullins, 1990). But some species are more sensitive than others, the root elongation of *Pinus radiata* seedlings for instance was half its maximum when soil strength was 1.3 MPa, when soil matric potential was kept constant and soil air filled porosity was over 20% (Zou et al. 2001).

In the current experiments it was difficult to ascribe plant reactions unequivocally to increases in soil strength, as soil air filled porosity and water content also decreased depending on the treatment combination. However, in comparing the relative sensitivity of these species to soil strength, it is an appropriate set-up and is representative of the combinations of physical stresses that would be found in the field.

Implications for crops

The experiments were conducted on young plants for a short period of time. It is conceivable that the species may differ in sensitivity to high soil strength in other stages. Oilseed rape, for instance, is believed to be most sensitive to drought stress during anthesis and pod filling (Champolivier and Merrien 1996). This may be the explanation for why there was no difference in the sensitivity oilseed rape and wheat growth to soil drying (in contrast to Chapter 2) even though there were apparent differences in sensitivity of stomatal conductance to an increase in soil strength. It is likely that the relatively short duration of the experiment in the current chapter and rapid depletion of soil water meant that species differences in stomatal response had insufficient time to translate into effects on growth. In Chapter 2, large differences in water use between irrigated oilseed rape and wheat only became apparent at later growth stages i.e. after the start of rapid stem extension.

Additionally we do not know what the effects of increased soil bulk density and soil strength is on final yield of plants; this would require longer duration and more elaborate experiments. In Chapter 2 it emerged that although leaf area of oilseed rape and wheat were decreased to the same extent by water limitation, the pod weight of oilseed rape was affected much more than the ear weight of wheat. There could be a difference in allocation strategies between plants, which makes ultimate yield of oilseed rape more vulnerable to soil stresses than the results of the above experiments indicate. For instance in one report, the root mass density of oilseed rape was decreased by 70% in a compacted part of the field and the crop biomass by 60%, while wheat crop biomass was not affected by compaction and root biomass at 5-20 cm depth was only reduced by 36% (Chan et al. 2006).

Conclusions

When water supply was unrestricted, oilseed rape and wheat responded to an increase in soil compaction in the same way. When soil compaction was combined with soil drying, Oilseed rape responded more sensitively than wheat to an increase in soil strength by closing its stomata to a greater extent relative to controls. Thus oilseed rape plants appear to be more conservative and crop yield could therefore be affected more by soil hardening, as a result of drying of the soil. Additionally the more severe stomatal closure in strong soils could mean that oilseed rape stops taking up water from the soil and its growth becomes water limited, while there is actually water in the soil left to be used. Although the stomatal response of oilseed rape to increased soil strength was greater than wheat's, almost all other growth and water use parameters were equally sensitive to soil compaction within the time frame of the experiment.

Chapter 5

The ability of oilseed rape and wheat to use pores in dense soil

Introduction

In the previous chapter the effect of homogeneously compacted soil on oilseed rape and wheat root growth and water relations was investigated. It was shown that both oilseed rape and wheat plants were negatively affected by soil compaction. Root growth was reduced to a greater extent in the deeper soil layers leading to an altered distribution of root length down the soil profile. The relative distribution of oilseed rape and wheat roots responded in a remarkably similar way to an increase in soil compaction. However, field soils are rarely uniform in bulk density.

The seed bed is often made up of relatively loose soil, with harder, more compacted untilled soil underneath. In tilled soils, a hard plough pan can develop anywhere between a depth of 10 and 50 cm (Ehlers et al. 1983, Floyd 1984). As roots tend to follow pathways of low mechanical impedance, plants often rely on pores or cracks to explore compacted soil or to reach the soil below a plough pan (Ehlers et al. 1983, Dexter 1986, Meek et al. 1992, Masle 1998). Cylindrical biopores are formed mainly by earthworms and by the roots from previous crops. Earthworm tunnels are mainly in the range of 1-10 mm diameter and root channels from agricultural crops are mainly of 0.1-2 mm diameter (Dexter 1986).

When the root system of a plant cannot grow to depth because a hard or compacted layer restricts its penetration into the subsoil, it may become drought stressed, even when there is plenty of water in the subsoil. Additionally if plant roots become clustered in cracks (places of low mechanical impedance), the soil water is depleted locally and plants can experience drought stress, even when the average root length density is high (Floyd 1984, Stirzaker et al. 1996).

Whitely and Dexter (1984) compared the ability of oilseed rape and wheat roots to cross horizontal gaps (cracks) in the soil. Narrower cracks were more likely to be crossed and a lower proportion of roots crossed the gaps when soil strength was increased. Wheat roots were much more successful in crossing one and three mm gaps than oilseed rape roots. However, root parameters were not measured in this particular experiment, so it was unclear what characteristic led to more successful crossing of gaps. Stirzaker et al (1996) investigated the ability of a monocot (barley) and a dicot (pea) to explore hard soil though artificially made pores. The presence of pores gave roots access

to the full depth of the pot and pores were occupied by roots more frequently than expected by chance alone. This resulted in increased plant growth in experiments where the soil was allowed to dry. Their experiments suggest that large biopores were not a favourable environment for roots in wet soil; barley plants grew better in pots containing a network of narrow biopores made by lucerne and ryegrass roots, and responded positively to biopores being filled with peat. Some pea radicles died in biopores.

In this chapter the ability of oilseed rape and wheat to grow through two millimetre diameter pores in a compacted layer of soil were compared in a controlled environment experiment. Soil above and below the compacted layer was comparatively loose. Additionally the effect of the presence of pores on plant water relations and shoot growth were also measured. The following specific hypotheses were tested.

Hypotheses

Wheat will locate more roots in artificial pores (referred to as (bio)pores) within the compacted layer than oilseed rape because monocotyledonous species generally produce a larger number of roots in the top soil than dicotyledonous species (Brereton et al. 1986, Rose et al. 2009).

The species with the greater number of roots in (bio)pores in the compacted layer will have the largest number of roots in the looser soil below.

The species with the greater number of roots in the (bio)pores will be able to exploit finite soil water and nutrient reserves more effectively, delaying the onset of drought and leading to greater shoot growth.

Material and methods

Soil and packing regime

Sandy clay loam soil from the MacMerry series was collected from Boghall farm (SAC, Penicuik, Scotland UK), the previous crop was spring barley and the soil had a pH of 6.7. After air drying for 48 hours, the soil was passed through a five mm sieve. Prior to packing into pots, 100 ml of 5x strength Hoagland solution per pot was mixed in with the soil aliquots, this corresponded to the equivalent fertiliser application of 120 kg/ha N, 55 $kg \cdot ha^{-1}$ P, 25 $kg \cdot ha^{-1}$ K, and 25 $kg \cdot ha^{-1}$ Mg. To prevent manganese deficiency, each pot was given 25 ml $MnSO_4$ solution to the equivalent of 2.5 $kg \cdot ha^{-1}$ Mn, twelve days after transplanting seedlings.

Pots made of polyvinyl chloride (PVC) pipes, with a height of 31 cm and an inner diameter of 10.2 cm were used. The twelve cm layer of soil below and above the compacted layer in the centre of the pot was packed to a dry bulk density of 1.1 $g \cdot cm^{-3}$. The five cm thick compacted layer in the centre had a density of 1.5 $g \cdot cm^{-3}$. Initially, the pots consisted of two sections. Firstly the lower five cm of the top the section was packed in two layers of 2.5 cm, to a dry bulk density of 1.5 $g \cdot cm^{-3}$ using a hydraulic press. With a power drill and a two mm drill bit, seven holes (pores) were drilled in an even pattern, see Figure 5.1 through the compacted layer of half of the number of pots. The 12 cm deep bottom section was then filled with three layers of soil of four cm depth to a bulk density of 1.1 $g \cdot cm^{-3}$, this was done by pressing the soil (by hand) with a device with a disc at the end which fitted snugly in the pot. The two pot sections were then glued together to form a single pot with a compacted layer at the centre at a depth of at 12 to 17 cm from the upper rim. Finally, the twelve cm section above the compacted layer was packed to a density of 1.1 $g \cdot cm^{-3}$ as described for the bottom section. The pots without pores in the compacted layer were used as the control treatment.

After packing of the pots, the soil was calculated to have a volumetric moisture content of about 33%. Each pot was given 200 ml water at the soil surface and pots were left to drain for 44 hours to reach field capacity prior to planting seedlings and determining the start weight. Unplanted pots were used to monitor evaporation of water from the soil surface throughout the experiment (n=3).

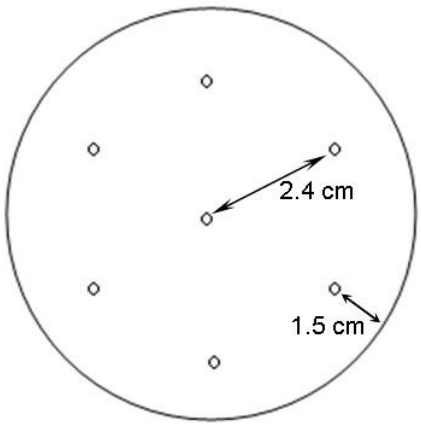

Figure 5.1 Location of the seven 2 mm diameter pores in the cross-section of the compacted layer. Roots that touched the pot edge and roots that were within two mm of the pot edge were counted as 'edge' roots.

Plant growth conditions

Seeds of oilseed rape *Brassica napus* L. cv. SW Landmark and wheat *Triticum aestivum* L. cv. Tybalt were germinated (on 14/9/2009) between two sheets of rolled up filter paper, stood in a beaker with water to keep seeds moist and to encourage vertical root growth. Seedlings were selected for uniformity of root length and four were transplanted to each of the pots four days after imbibition. Three days later the number of seedlings was thinned down to two per pot. The experiment was laid out as a randomised block design (n=6) within a Fitotron growth cabinet (model SGC970, Sanyo-Gallenkamp, Loughborough, UK). Conditions within the cabinet were 16h/8h light/dark and a day/night temperature regime of 18 ± 0.4/14 ± 0.3ºC and relative humidity of 56 ± 5. 1 /57 ± 1.9 %RH (Figure 5.2). The light intensity (PAR) was 753 ± 48.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at initial plant height, measured at the centre of each block, supplied by 22 55W fluorescent lights and four 60W tungsten lights (Philips, Poland). Temperature and relative humidity were logged with a data logger (DL3000 modular data logger, Delta T device, Cambridge, UK).

The surface of each pot was covered with a plastic bag to minimise evaporation of water, with slits cut in the bag to allow stems to grow through. Unplanted pots were treated in the same way. From regular measurements of the weight of unplanted pots it

was concluded that the pots were losing water from their base, therefore the bases of all pots (planted and unplanted) were covered with a plastic bag from 8/10/2009 onwards, which was 20 days after transplanting the seedlings. The weight change of planted pots could be almost entirely attributed to plant transpiration from this day (8/10/2009) onwards.

The pots were watered from the top on 6, 12, 15 and 20 days after transplantation to bring the soil back up to field capacity, by calculating the loss of weight and re-supplying that amount of water.

On day 20 the plants were stood in trays with water for three hours to make sure the bottom soil layer was replenished. After the three hours the pots were also watered from the soil surface with 200 ml water and pots were stood on capillary matting and allowed to drain for two hours. Then the base of the pot was sealed with a plastic bag and elastic band and from this point onwards the plants grew on stored water only. The experiment was terminated when all plants had lost turgor, as shown by visible wilting, 30 days after transplantation.

Measurements

The pots were weighed at least once every three days and daily towards the end of the experiment, to determine water loss from the soil and hence transpiration rate of the plants.

The length of the youngest unfolding leaf of one plant in each pot was measured to the nearest millimetre every 1-2 days from day 19 onwards. To ensure measurements were made on the youngest unfolding leaf, a different leaf was measured on days 24-29 than on days 19-22. For oilseed rape the length from the base of the leaf blade to the tip was measured, for wheat it was the length from the leaf axil to the tip.

Stomatal conductance of the youngest fully expanded leaf was measured with an IRGA on days 23, 25 and 28 after transplantation (ADC-LCA4 Analytical Development Co. Ltd, Hoddesdon, Herts, UK).

At harvest time, 30 days after transplanting seedlings, the oilseed rape plants were at growth stage 1.4 to 1.7 (leaf production stage, 4^{th} to 7^{th} leaf exposed) (Letham-Shank-Farm 2010) and the wheat plants were at Zadoks growth stage 2.9 (late tillering) (Zadoks et al. 1974). The projected area of the shoot (petioles, leaves and stems) was

measured separately for each plant with a LICOR leaf area meter (Li-Cor Biosciences, Cambridge, UK) and the shoot area divided into live (green) and dead (yellow or brown leaves and petioles). The dry weight per shoot was determined after drying to constant weight in a fan assisted oven at 70ºC. For each pot the mean of the two plants per pot is presented.

After harvesting of the shoots, the pots with soil and root systems were frozen at -18°C. Three cross-sections were cut though the frozen pots with an industrial band saw. The first was through loose soil 2.5 cm above the compacted layer (named 'top' from here onwards), the second through the centre of the 5 cm thick compacted layer ('compacted') and the third through loose soil 2.5 cm below the compacted layer ('bottom').

The position of root-ends at the interfaces of the three cross-sections were viewed under a dissection microscope, traced on paper and counted. Additionally, the number of roots directly within 2 mm of the pot-edge and roots within the 2 mm pores were recorded. In the control pots, the roots that were in the corresponding area of where pores were situated in the pore-treatment were counted. This was done by overlying a template of the pore positions on the cross-section. Because of time constraints, measurements were made on all six replicate compacted layers per treatment, but only four of the six replicates from the top and bottom layers.

Analyses and statistical tests

There were five unplanted pots to monitor evaporation of water from the soil surface over the first 19 days. The difference in water loss from unplanted pots with pores (n=3) and without pores (control, n=2) was tested with a K-S test. For each day the p-value was over 0.54, therefore there was no difference in evaporation rate introduced by the pore treatment and the mean water loss of all unplanted pots could therefore be deducted from the water loss (evapotranspiration) of all planted pots in each treatment to obtain plant transpiration rates (Figure 5.4). On day 20 two unplanted pots were destructively sampled to check the moisture content the layer of soil below the compacted layer. The three unplanted pots that remained (two with pores and one without) were treated the same way as the planted pots. The mean water loss from these pots was assumed to be equal to the rate of evaporation from the soil surface of the

planted pots and the value deducted from evapotranspiration rates to give the transpiration rates.

All statistical tests were conducted in GenStat and the results are presented in separate tables. (GenStat 11.1 2008, VSN International Ltd, Hemel Hempstead, UK). The effect of species and pore treatment on most parameters were tested in a two-way ANOVA test. The cumulative transpiration and evapotranspiration rate were tested with repeated measures ANOVAs.

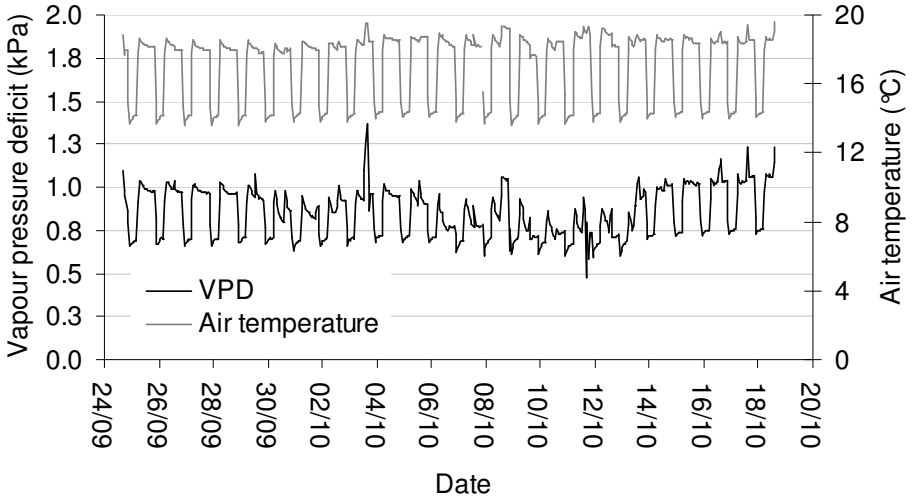

Figure 5.2 Air temperature and vapour pressure deficit (VPD) in the controlled climate cabinet. Water was withheld from 8/10/2009 onwards; 20 days after transplanting seedlings.

Results

Evapotranspiration and leaf expansion

There was a significant loss of water from unplanted pots where the upper soil surface was covered from day 1 to day 19 (Figure 5.3). On day 20 the base of the pots were sealed to reduce evaporation of water from the lower soil surface and no more water was supplied. As a result, from day 20 onwards loss of water from unplanted pots was negligible (3.1 ± 2.9 $ml \cdot day^{-1}$ between days 20 and 30 after transplanting) compared to the transpiration rate of plants within this period (Figure 5.3).

Plants that grew on soil where the compacted layer was perforated with pores had a tendency to transpire more water regardless of species, however this effect of pores on water use was not quite significant ($p=0.07$) (Figure 5.4). The transpiration rate of plants in all treatments increased from day 20, which was the last watering day (Figure 5.5). From day 20 onwards the plants grew on stored water only and the transpiration of plants in all treatments except the oilseed rape control ones started to decline from day 23 after transplantation onward. The oilseed rape plants in the control treatment seemed to transpire less water around day 23, but its transpiration rate was not significantly lower than the other treatments, probably due to high variance of data (one oilseed rape pore treatment plant had a very low transpiration rate for instance) (Figure 5.5).

The decline in transpiration rate (Figure 5.4), after withholding of water corresponded with a decrease in stomatal conductance (Figure 5.6). On day 23 the transpiration rate of oilseed rape controls was the lowest amongst the treatments and its stomatal conductance was half that of plants in the other treatments (Figure 5.6, Table 5.2). The stomatal conductance of plants in all treatments decreased as the soil dried. On day 25 oilseed rape plants had a significantly greater stomatal conductance than wheat, which again corresponded with a significantly greater transpiration rate (Figure 5.5). Three days later, towards the end of the experiment, the transpiration rates had declined to only 20 ($ml \cdot pot^{-1} \cdot day^{-1}$ for all treatments.

As the stomata closed and the transpiration rate declined, the leaf elongation rate of plants in all treatments also decreased significantly (Figure 5.7), but there was no effect of pore treatment on leaf elongation rates (Table 5.5).

Table 5.1 Statistical test results for cumulative evapotranspiration from the soil surface.

	Pores (n=3)	**Control (n=2)**	**K-S p-values**
Day 3	74 ± 9.7	65 ± 4.3	0.766
Day 6	133 ± 8.5	128 ± 12.0	0.549
Day 8	169 ± 11.1	162 ± 20.0	0.549
Day 11	228 ± 20.4	225 ± 40.5	0.549
Day 12	250 ± 28.0	254 ± 55.3	0.549
Day 14	298 ± 50.9	313 ± 92.8	0.549
Day 15	323 ± 62.9	345 ± 113.0	0.549
Day 17	400 ± 99.2	432 ± 161.3	0.549

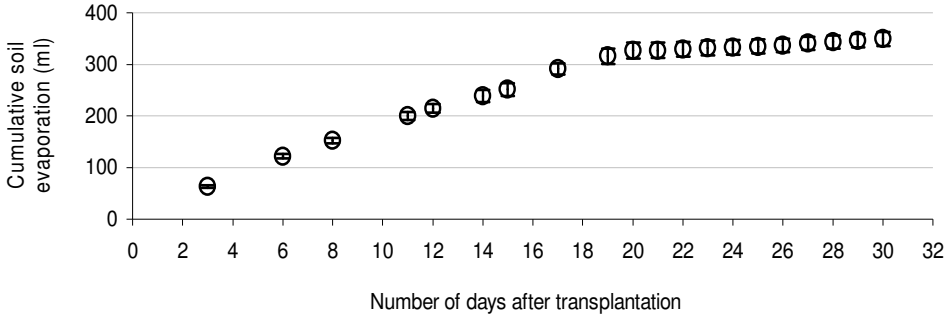

Figure 5.3 Rate of water loss from the soil by evaporation. Mean of three unplanted pots and sem are given. Water was withheld from day 20 onwards and the base of all pots was sealed from day 20 onwards to reduce the rate of evaporation.

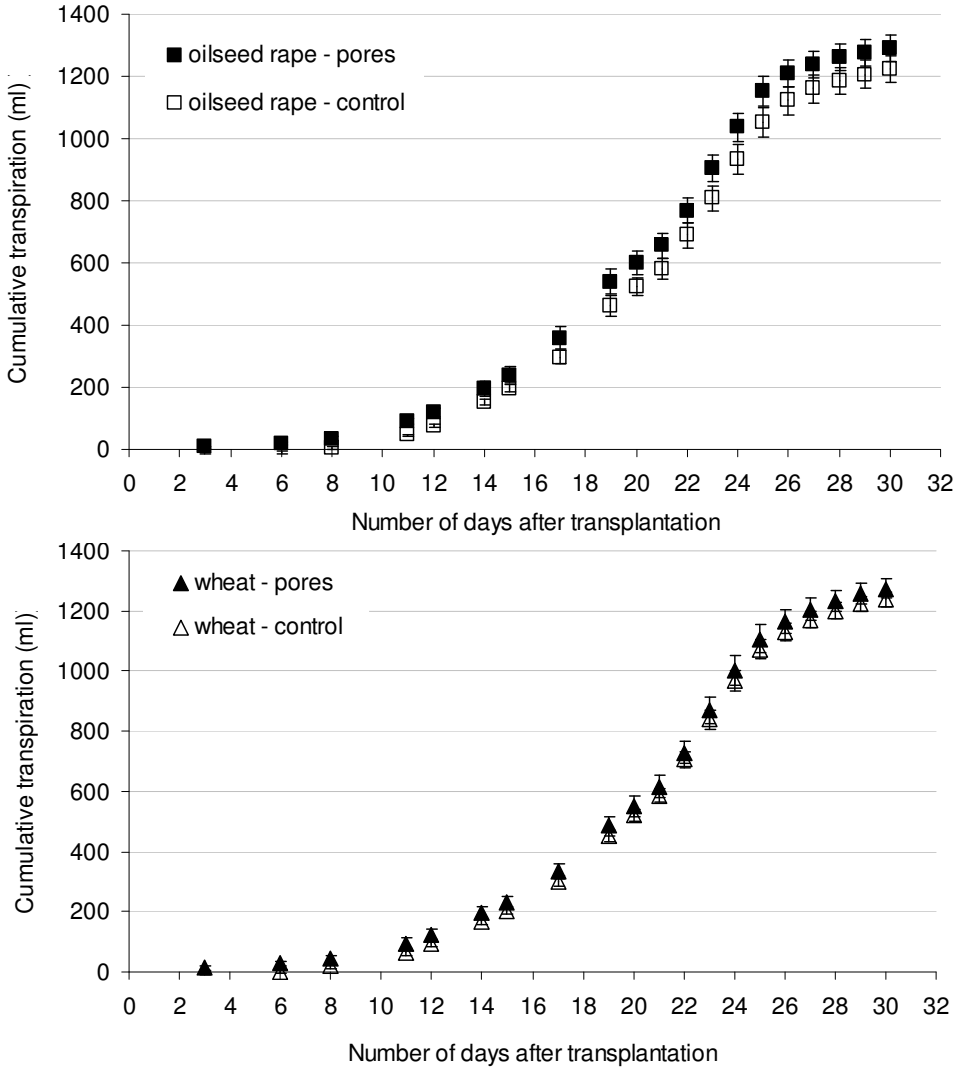

Figure 5.4 Cumulative amount of water transpired per pot (two plants per pot) in ml, of oilseed rape (top) and wheat plants (bottom). Water was withheld from day 20 onwards.

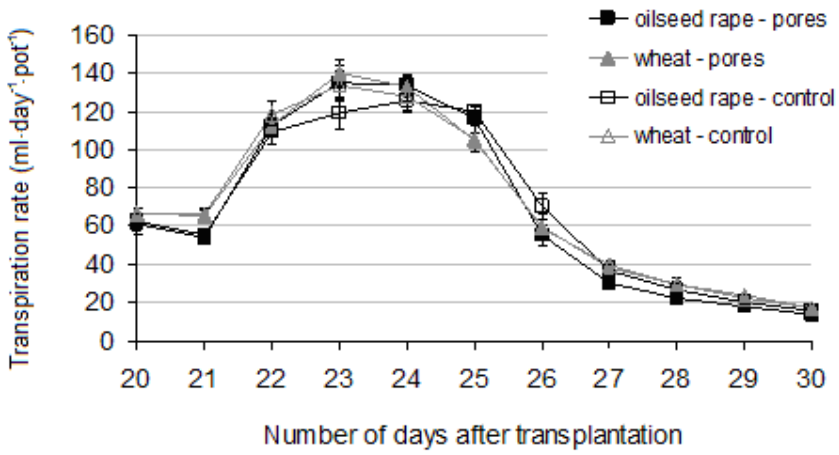

Figure 5.5 Transpiration rate from day 20 onwards, when water was withheld. Values are means of 6 replicates, error bars represent sem.

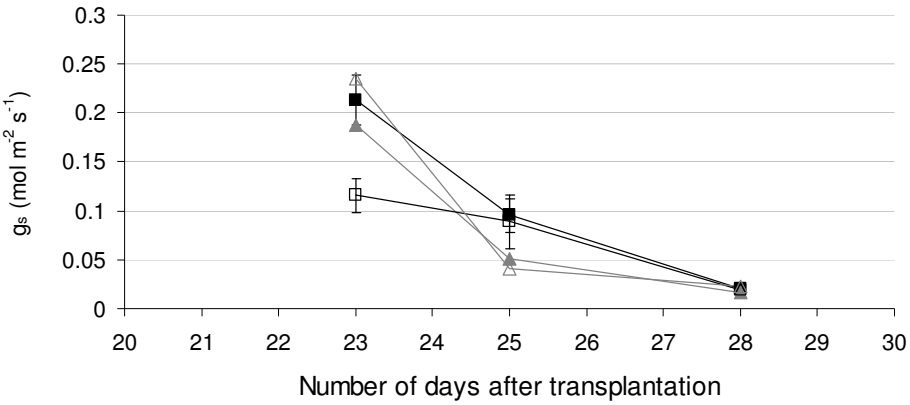

Figure 5.6 Stomatal conductance (g_s) of the youngest completely expanded leaf of oilseed rape and wheat. See figure 5.5 for key to symbols. Values are means ± sem of 6 replicates

Table 5.2 Statistical test results (p-values) of two-way ANOVA of stomatal conductance (g_s) rates shown in Figure 5.6.

	Species	Pores	Species x pores
Day 23	0.097	0.359	0.015
Day 25	0.020	0.642	0.913
Day 27	0..905	0.356	0.213

Table 5.3 Statistical test results of repeated measures ANOVA on cumulative transpiration data in Figure 5.4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
species	1	2750	2750	0.04	0.844
pores	1	269753	269753	3.95	0.066
species.pores	1	35673	35673	0.52	0.481
Residual	15	1025490	68366	40.55	
Time	19	10404977	5E+06	3248.4	<.001
Time.species	19	17898	942	0.56	0.502
Time.pores	19	22339	1176	0.7	0.445
Time.species.pores	19	13992	736	0.44	0.561
Residual	380	640615	1686		
Total	479	10668910			

Table 5.4 Statistical test results of repeated measures ANOVA on transpiration rates results in Figure 5.5.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
species	1	538.4	538.4	9.6	0.007
pore	1	2.2	2.2	0.04	0.846
species.pore	1	26.7	26.7	0.48	0.500
Residual	15	841.1	56.1	0.42	
Time	10	470725.1	47072.5	350.81	<.001
Time.species	10	2601.8	260.2	1.94	0.160
Time.pore	10	1497.8	149.8	1.12	0.335
Time.species.pore	10	760.9	76.1	0.57	0.563
Residual	200	26836.7	134.2		
Total	263	503873.7			

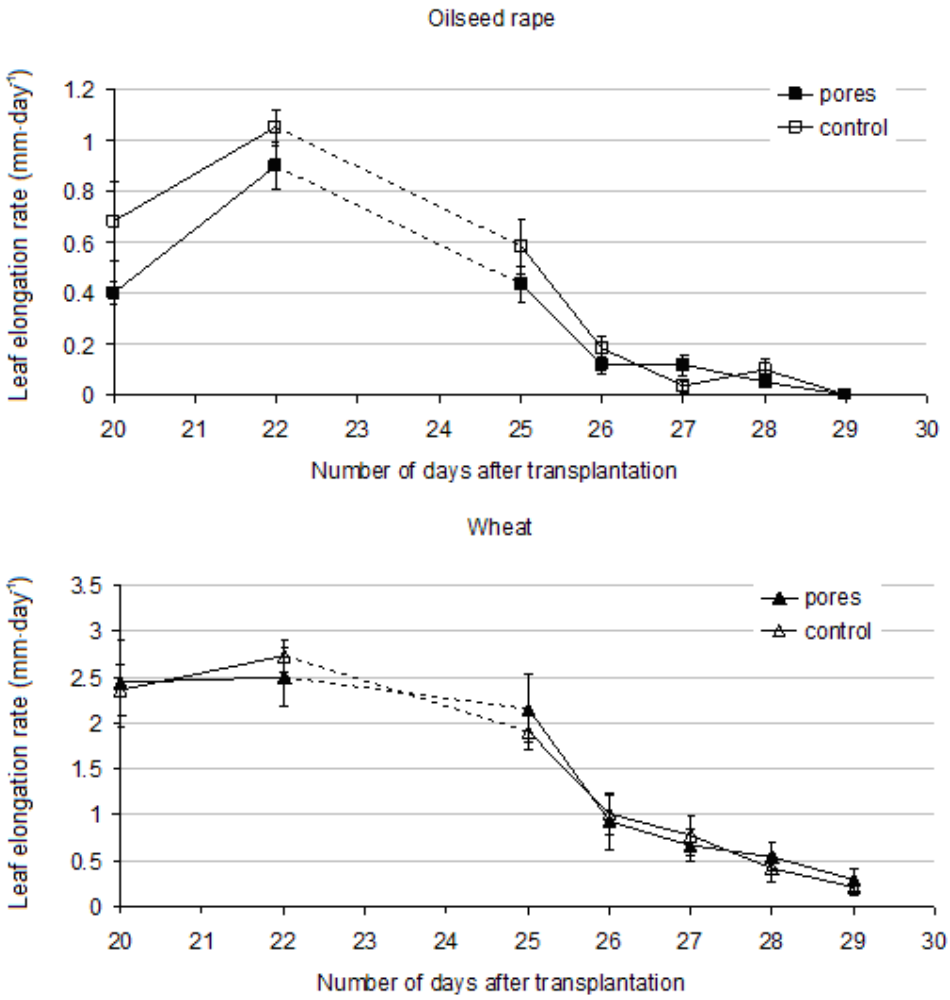

Figure 5.7 Leaf extension rate of the youngest emerging leaf of oilseed rape plants (top) and wheat plants (bottom). The values are the leaf elongation rate over the previous 24 hours (or 48 on day 22). Rates for days 20-22 are for leaf 3 (wheat) and 4 (oilseed rape) and from days 25-29 for leaf 4 (wheat) and 5 (oilseed rape). The vertical error bars represent sem.

Table 5.5 Two-way ANOVA results (p-values) for leaf elongation rates in Figure 5.7, n=6.

	Species	Pores	Species x pores
Day 20	< 0.001	1.000	0.584
Day 22	< 0.001	0.820	0.820
Day 25	< 0.001	0.739	0.564
Day 26	< 0.001	0.870	0.366
Day 27	< 0.001	0.890	0.479
Day 28	< 0.001	0.862	0.438
Day 29	< 0.001	0.827	0.427

Number of roots

Oilseed rape had more roots in the bottom layer and significantly fewer roots in the top layer than wheat. The presence of pores resulted in more roots in the bottom layer, for both oilseed rape and wheat (Figure 5.9, Table 5.6).

In Figure 5.10 the root count of each layer is given excluding the roots growing directly along the edge of the pot, but the general pattern of root distribution is comparable to when the roots at the edge are included in the total (Figure 5.9). The increase in the number of roots in the bottom layer in the presence of pores was significantly more with oilseed rape plants than wheat ($p < 0.05$ for species x pore interaction when edge roots excluded, Table 5.6).

Oilseed rape plants had significantly more roots in the cross-section of the compacted layer (Figure 5.11, Table 5.7). The presence of pores increased the number of roots in the compacted layer for both species, but not significantly so ($p=0.085$, Table 5.7).

A large proportion (55-68%) of the total number of roots in the compacted layer was found at the edge of the pot (Figure 5.12). Wheat plants had a greater percentage of roots growing along the edge of the pot than rape (Figure 5.12; Table 5.7). In the absence of pores (control) in the compacted layer, a significantly greater number of roots grew along the edge of the pot, but this was only at the cross-section of the compacted layer. In the top and bottom layer there was no such effect of pore absence on the number of roots growing along the edge of the pot (Table 5.6).

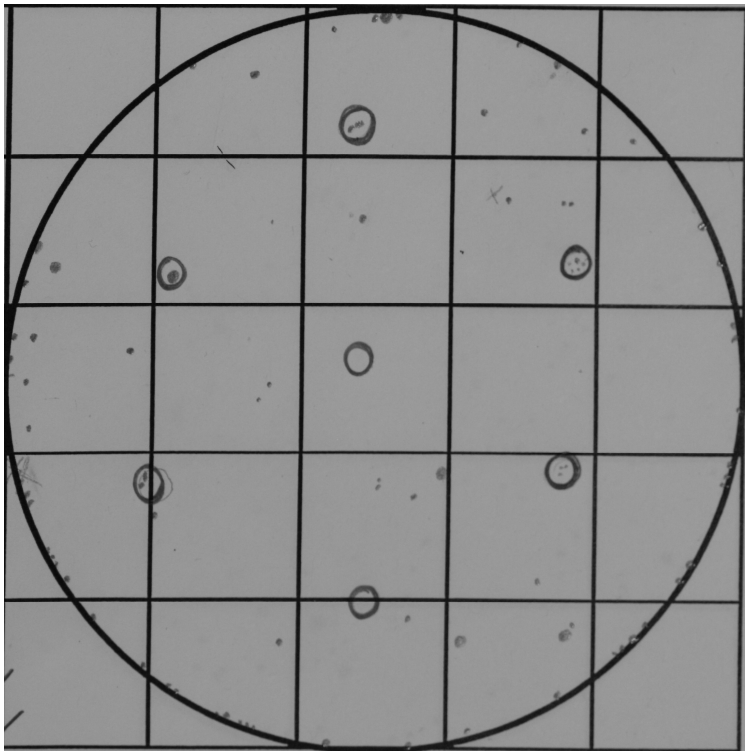

Figure 5.8 Diagram of location of oilseed rape root ends in a cross-section of a compacted layer with pores, pores are highlighted by the drawn circles.

Both species had roots growing in the pores. In Figure 5.8 is one example of a root-count template. Here there were roots growing in five out of the seven pores. Oilseed rape had a greater number of roots growing in pores than wheat, both in absolute and relative terms (Figure 5.13). Almost 19% of oilseed rape roots in the compacted layer (roots growing along the edge were excluded from the count) were situated in pores, while for wheat this was only 10%. In the control treatments 2.6% and 1.5% of the total number of roots for oilseed rape and wheat respectively were situated in the areas where pores were drilled in the corresponding pore-treatment. On the basis of mere chance, one would expect 0.3% of the roots to be growing in the pore area, since the cross-sectional area of the seven pores accounted for only 0.3% of the total cross-sectional area of the compacted layer. In both species a large number of roots were found in the compacted layer not associated with (bio)pores as shown by the difference in values between Figure 5.11 and Figure 5.13, indicating that roots of rape and wheat were able to grow into this dense layer.

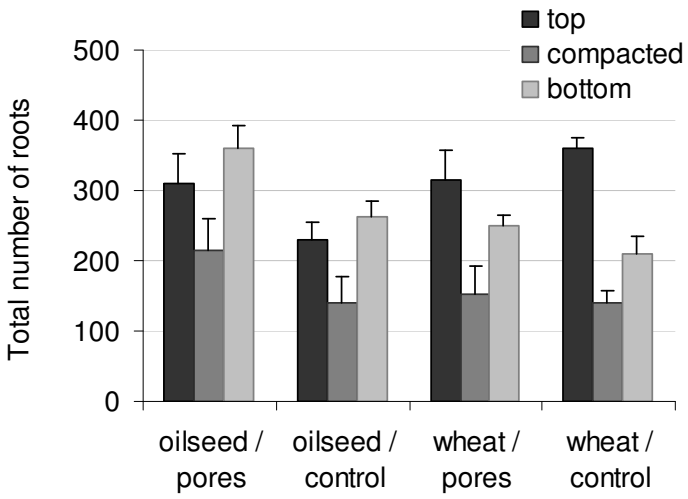

Figure 5.9 The total number of roots in a cross-section of the top, compacted (middle layer) and bottom layer of pots planted with oilseed rape or wheat. The roots growing along the edge of the pot were included (n=4).

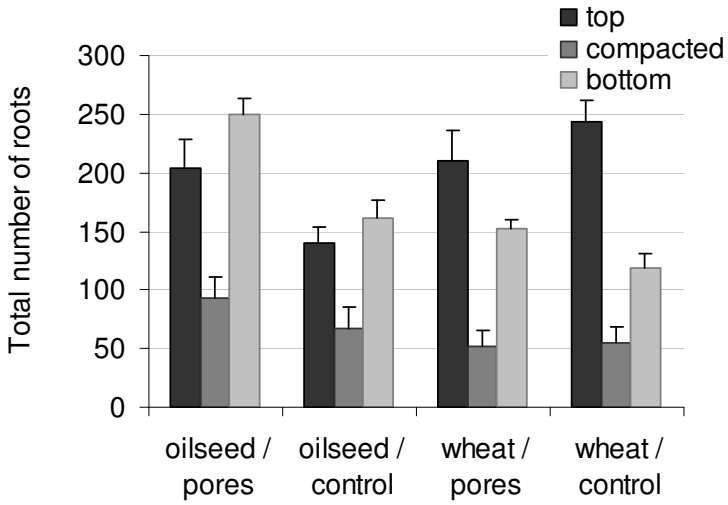

Figure 5.10 The number of roots the cross-section taken at different depths, excluding the roots growing along the edge of the pot (n=4). Vertical bars are sem.

Table 5.6 Results (p-values) of statistical test testing for effect of species, presence of pores and the interaction of those factors on the number of roots in the cross-section of each layer (n=4), data plotted in Figure 5.9 and Figure 5.10.

parameter	Species	Pores	Species x pores
All roots			
Top	0.056	0.582	0.076
Compacted	0.187	0.088	0.213
Bottom	0.002	0.005	0.148
Roots along edge excluded			
Top	0.04	0.518	0.061
Compacted	0.065	0.386	0.296
Bottom	< 0.001	<0.001	0.018
Roots along edge only			
Top	0.433	0.894	0.424
Compacted	0.640	0.028	0.210
Bottom	0.451	0.626	0.870

Table 5.7 Results (p-values) of two-way ANOVA testing for the effects of species and pores on the number of roots in the compacted layer and in the pores (n=6), see Figure 5.13.

	species	pores	Species x pores
Total root count (excl. roots at edge)	0.001	0.085	0.317
% of roots along edge of pot	0.001	0.535	0.943
Number of roots in pores	0.002	< 0.001	0.008

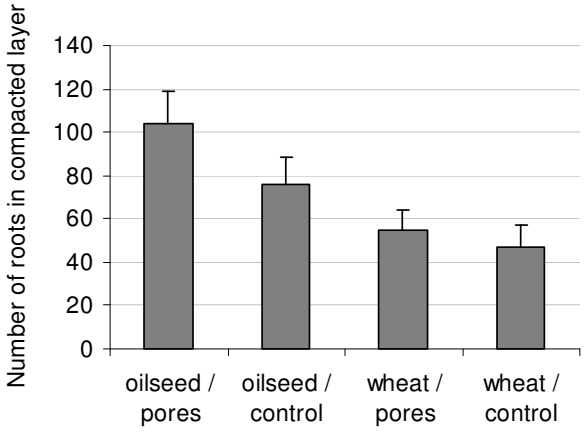

Figure 5.11 Number of roots in the cross-section of the compacted layer, excluding the roots growing along the edge, (n=6). Vertical bars are sem.

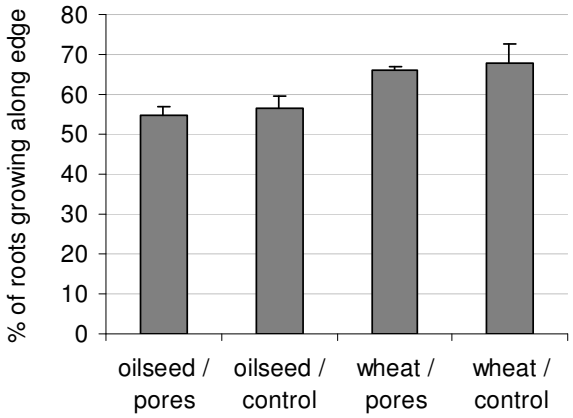

Figure 5.12 The percentage of roots of the total number of roots in the compacted layer growing along the edge of the pot (n=6). Vertical bars are sem.

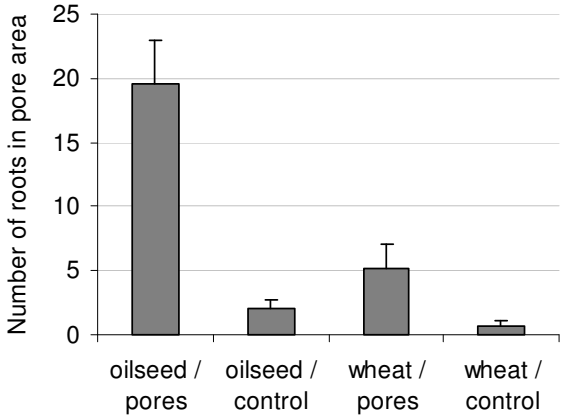

Figure 5.13 The number of roots in pores, or the in the corresponding area in control pots (n=6). Vertical bars are sem.

Oilseed rape plants had a greater shoot dry weight than wheat plants and the presence of pores had no significant effect on shoot dry weight (Table 5.8, Table 5.9). Oilseed rape plants had a significantly greater live shoot area and the area of dead leaves was also significantly greater than that of wheat. The plants in the control treatments had a significantly greater shoot area than the plants in treatment with pores when averaged across species. The area of dead canopy was significantly greater in treatments with pores, regardless of species. There were no significant species x pore interactions in any of these plant characteristics indicating that the oilseed rape and wheat responded in the same way to the presence of pores.

In Figure 5.14 the relationship between the number of roots in the bottom cross section and total water use (transpiration) was plotted. A higher root count in the bottom layer was correlated with a greater total transpiration. Treatments with pores had more roots in the bottom cross section and a greater cumulative transpiration of water. Pore treatment seemed to have a greater positive effect on the number of roots and water use of rape than of wheat. There was no significant effect of pore presence on the number of leaves of oilseed rape neither at harvest, nor on the number of tillers on wheat plants.

Table 5.8 Plant properties at the end of the experiment, 30 days after transplanting. Values are means per plant of six replicate pots (n=6).

	Oilseed rape		**Wheat**	
	pores	**control**	**pores**	**control**
Dry weight shoot (g)	2.06 ± 0.032	2.09 ± 0.081	1.91 ± 0.060	1.95 ± 0.057
Number of unfolded leaves	5.4 ± 0.24	4.9 ± 0.08		
Number of tillers per plant			15.1 ± 1.06	17.0 ± 0.89
Live plant shoot area (cm^2)	181.7 ± 4.68	194.7 ± 5.95	171.2 ± 2.45	184.3 ± 3.76
Total shoot area (cm^2)	224.8 ± 4.84	227.8 ± 5.34	193.4 ± 1.28	203.3 ± 4.43
Dead shoot area (cm^2)	43.2 ±1.25	33.2 ± 4.56	22.2 ± 3.35	19.0 ± 1.28
Percentage dead area (cm^2)	19.5 ± 0.69	14.2 ± 1.91	11.5 ± 1.60	9.6 ± 0.44

Table 5.9 Results of statistical tests (p-values two-way ANOVA) on harvest data of oilseed rape and wheat plants (n=6), results in Table 5.8.

P-values	species	pores	Species x pores
Dry weight shoot	0.003	0.488	0.907
Number of unfolded leaves (oilseed rape only)		0.076	
Number of tillers per plant (wheat only)		0.434	
Live plant shoot area	0.036	0.011	0.989
Total shoot area	< 0.001	0.173	0.415
Dead shoot area	< 0.001	0.029	0.232
Percentage dead area	< 0.001	0.013	0.209

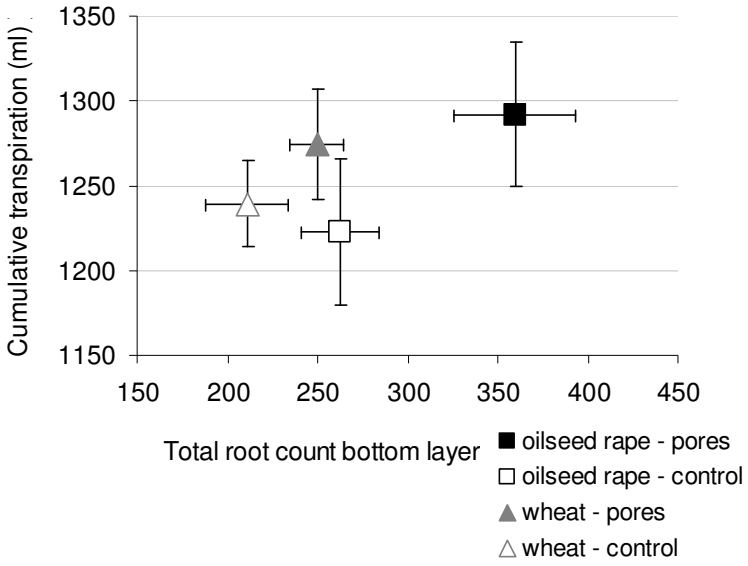

Figure 5.14 The relationship between the total number of roots in the cross section of the bottom layer and the total amount of water transpired at the end of the experiment (n=4). Horizontal and vertical bars are sem.

Discussion

The difference in root distribution of oilseed rape and wheat over soil depth was striking. Oilseed rape had a greater number of roots in cross sections of the bottom layer than the top layer, whilst wheat had more roots in sections of the top layer than the bottom (Figure 5.9). By contrast, in the uniformly compacted soil of Chapter 4, the distribution of root length with depth of oilseed rape and wheat was remarkably similar. The results suggest that the species differ in the ability of their root systems to respond to heterogeneity in soil physical properties.

It is not clear what mechanisms are responsible for the different root distributions, but there are a number of possible factors. It may relate in part to the different root architecture of wheat and oilseed rape. Wheat is a cereal species and tends to produce a greater root length density in the upper 20 cm of soil than many dicotyledonous crops (Brereton et al, 1986; Rose et al, 2009). Wheat produces in the order of five seminal roots and later can develop many nodal axes; each of these axes in turn may branch (Gregory et al. 1978). Oilseed rape on the other hand is a tap rooted species, which produces a single downward-growing seminal root and numerous lateral root branches. The average diameter of oilseed rape roots is smaller than that of wheat and the length per unit dry weight greater (Chapters 3 and 4). The narrow diameter of rape roots may facilitate their penetration into small pore spaces in the soil. Oilseed rape had a larger number of roots in the compacted layer than wheat, both within and out-with the artificial (bio)pores. It is possible that deflection of seminal or nodal axes from the surface of the compacted layer (Clark et al. 2003) contributed to the greater root numbers of wheat roots in the top layer and fewer roots in the compacted layer. However when the ability of wheat and pea roots to locate pores was compared by Dexter (1986), there appeared to be no significant difference in behaviour of pea and wheat in spite of their different nominal root diameters.

Although oilseed rape plants had a similar (with pores) or smaller (controls without pores) number of roots in the top layer than wheat, it had more roots growing in the (bio)pores in the compacted layer. Therefore the hypothesis that the species with the greatest number of roots in the soil overlying a compacted layer is most successful at placing roots in pores in the compacted layer must be rejected. It suggests that some mechanism other than mere chance may be involved in roots locating pores.

Semchenko et al (2007) found that the root growth responses to an obstruction in the soil disappeared in the presence of activated carbon. These results suggest that the ability to avoid obstructions in the soil is dependent on the sensitivity of the roots to their own exudates accumulating in the vicinity of obstructions. A similar mechanism might be involved in the location of (bio)pores. It is also possible that the greater number of roots of oilseed rape found within (bio)pores does not result from a larger number of roots locating and then extending through the pore, but instead from a greater degree of branching of roots once within the pore.

The presence of pores was correlated with a greater number of roots in the bottom layer for both species and suggests that the presence of pores in a compacted layer could benefit the exploration of deeper soil layers by roots (also see: Brereton et al. 1986, Rose et al. 2009). More specifically oilseed rape had a greater number of roots growing in the (bio)pores of the compacted layer and also a greater number of roots in the underlying soil layer, thus supporting the second hypothesis (see introduction to chapter).

It was expected that the species that was best able to bypass the compacted layer by utilizing biopores as channels for root growth and generating roots in the deeper underlying soil would be more effective at exploiting the available soil water and nutrient reserves (hypothesis three). In general, however, the results did not provide strong support for this hypothesis. The total amount of water transpired did not differ significantly between species and pore treatments and shoot growth was not affected by the presence of pores. However, there was some indication of a beneficial effect of pores especially for oilseed rape. There was a broad correlation between the amount of water transpired and the number of roots in the bottom layer which is consistent with the hypothesis. Furthermore, dead shoot area of oilseed rape was greater in the pore treatment compared to controls and its stomatal conductance and rate of transpiration on day 23 greater. These results are consistent with the idea that in the presence of (bio)pores, oilseed rape began to exploit water reserves in the bottom layer earlier than when pores were absent, but then exhausted the finite supply sooner leading to earlier and more pronounced senescence of the leaf tissue by the time the experiment was terminated. The experiment was ended when all treatments showed signs of water stress. Had the design of the experiment provided a replenished supply of water to the

bottom layer, then differences in shoot growth between pore treatments may have been found as the greater root number in the presence of pores may have conferred a larger advantage.

Two other factors may also have reduced the potential benefits of the pores for exploitation of water and nutrient reserves. Firstly, the interface between the soil and the side of the pot provided a pathway of relatively low mechanical impedance for root growth to bypass the compacted layer in addition to the (bio)pores. Secondly plants may be able to meet their demands for water and nutrients with relatively few roots. Thus even in the absence of pores the plant may have had sufficient roots in the bottom layer to capture the available resources. Thus as long as one or two roots are able to bypass the compacted layer and proliferate some roots in the underlying soil, the shoot may be able to grow unrestricted. Biopores may not always confer advantages expected of them. Stirzaker et al (1996) observed in a controlled environment experiment, that while bio-pores did give the plant access to water and nutrients from deeper in the soil, some pea radicles died in large bio-pores. They also suggested that there could be difficulties in securing water and nutrients by roots which are poorly distributed and in poor contact with the bio-pore wall and highlighted the possible effect of inhibitory signals emanating from roots dangling in bio-pores or from lateral roots impeded in the compacted bio-pore walls.

Conclusions

The presence of artificial pores in the compacted layer increased the number of roots in the soil below the compacted layer. Oilseed rape had more roots growing in the compacted layer, both within and out-with the pores and its root number in the deeper soil layer was increased to a greater extent by the presence of pores than was found with wheat. Oilseed rape may, therefore, be better able to exploit spatial heterogeneity of soil than wheat and where adequate supplies of subsoil water exist, access these supplies more effectively in relatively poorly structured soil

Chapter 6

General discussion

Different strategies of water use by oilseed rape and wheat

When grown as individual plants or small populations, oilseed rape transpired at a faster rate than wheat when supplies of water were unlimited. This was found consistently across experiments incorporating plants at different growth stages and under different growth conditions (open-sided and closed glasshouses and controlled environment chambers). However, when the supply of water was restricted and the soil allowed to dry oilseed rape responded more sensitively than wheat. Stomata closed more rapidly as the soil dried and soil strength increased (Chapter 2 and 4) and where the duration of the experiment was long enough (Chapter 2), there was a greater reduction in growth relative to irrigated plants than was found in wheat. Thus, oilseed rape may be considered to be profligate in its use of water when the supply is ample, but more conservative than wheat when confronted with a restricted supply. Wheat by contrast appears to be less wasteful in its use of water when the supply is unrestricted, but also less sensitive to soil drying. Stomatal conductance was less responsive to changes in soil water content and soil strength and water appeared to be accessed from deeper soil layers sooner than by oilseed rape. Wheat may also allocate more energy to reproduction in response to water stress, since its ear dry weight was hardly reduced in non-irrigated treatments, while oilseed rape pod dry weight was halved (Chapter 2).

Oilseed rape can be seen as having an opportunist strategy of water use. When water is readily available its high stomatal conductance will permit high rates of photosynthesis, but at the expense of a high transpiration rate and large total water use and thus low WUE (Chapters 2, 3 and 4). Oilseed rape's indeterminate development may facilitate this opportunism (Wang et al. 2009).

As the soils dries, closure of stomata and conservation of water could allow the plant to survive and complete its lifecycle, but at the expense of growth and yield. There was evidence that stomatal conductance was reduced (transpiration rate reduced below the potential rate) at a lower soil moisture deficit than in wheat (Chapter 2). Relative to oilseed rape, wheat is perhaps better-adapted to normal field conditions and slight water limitation by metering out finite supplies of water for longer through the season, but cannot profit as well from extremely fortunate conditions. Thus wheat may have a safer strategy which enables it to survive in adverse conditions and suffer less yield loss.

The strategy of oilseed rape is reminiscent of that of lupins. Lupins are opportunistic in relation to available soil water; when soil water is available they have high rates of photosynthesis and transpiration (Turner and Henson 1989). However, when water is limited, they suffer large reductions in leaf conductance and photosynthetic rate. In contrast, wheat utilizes water more sparingly when it is freely available and has a more gradual decrease in photosynthesis and stomatal conductance when water deficits develop (Turner and Henson 1989).

Differences in hydraulic conductance between lupins and wheat may be related to their different physiological strategies to water availability with the high hydraulic conductance of lupine facilitating high rates of transpiration via a relatively small root system (Gallardo et al. 1996, Bramley et al. 2009). Oilseed rape was also found to have a greater root hydraulic conductivity than wheat, at least when water supplies were unlimited (Chapter 3), facilitating higher rates of inflow per unit root length. Other aspects of the physiology of oilseed rape are consistent with the apparent opportunistic strategy of water use by this species. From the literature it is known that oilseed rape has a limited capacity for osmotic adjustment and this may contribute to the sensitivity of pod growth and development to drought (Jensen et al. 1996b). Wheat, on the other hand, is a relatively good adjuster (Cutforth et al. 2009), which could have contributed to the less severe reduction in canopy area of wheat when water was withheld in the lysimeter experiment in Chapter 2. Osmotic adjustment could have caused the leaves of wheat to remain turgid when water supply was limited and facilitate leaf expansion.

Root growth and response to soil physical conditions

Although water stress (low soil water potential) is the most intensively researched physical stress to root growth, field data show that it alone may not be the critical factor. Additional factors including hypoxia and mechanical impedance, may all act on the roots in combination or sequence with low water potential as the soil water content or soil structure change (Whitmore and Whalley 2009). In tilled and untilled soil, soil strength (mechanical impedance) appeared to be the main soil physical factor controlling root growth of oat plants (Ehlers et al. 1983). Soil strength varied predominantly via changes in soil water content and, in this experiment, bulk density seemed to be of minor importance for root growth.

However, there is plenty of evidence that compaction and poor soil structure can interfere with the growth of oilseed rape root systems in the field. Bonari et al (1995) found that the presence of subsurface compacted soil layers as the result of continuous minimum tillage caused a progressive worsening of soil conditions for oilseed rape root growth and, consequently, a reduction of root system mass and tap-root length compared with ploughed plots (Bonari et al. 1995). Reduced elongation of rapeseed tap-roots, because of the presence of superficial compacted layers has also been noticed with reduced tillage (Vez and Vullioud 1971). However, there was no evidence from the current study that the root system of oilseed rape was any more sensitive to adverse soil conditions, or any less effective at exploiting soil water reserves than wheat. Uniform soil compaction reduced total root length and altered its distribution down the soil profile to a similar extent in both oilseed rape and wheat (Chapter 4). In each case compaction lead to a shallower root system as reported for field grown plants. For example the total root length of oilseed rape and wheat plants growing in soil of a bulk density of 1.3 $g \cdot cm^{-3}$ was about equal to that of plants growing in soil of 1.4 $g \cdot cm^{-3}$ bulk density. But at 1.3 $g \cdot cm^{-3}$ about half the root length was situated at a depth of 12-24 cm and the other in the top 12 cm, while at the higher bulk density almost all the roots were situated in the top 12 cm of soil.

In the relatively loose well watered soil in Chapter 2, oilseed rape was able to generate root length densities deep in the subsoil (70-80 cm) equivalent to those of wheat. Differences were observed in the temporal and spatial pattern of water extraction by oilseed rape and wheat when grown on stored water. Oilseed rape extracted water from each soil layer later than wheat, but at a faster rate so that all the available water was extracted by about the same time (Chapter 2).

However, field soils are not uniform in structure or in the availability of water and nutrients. Plasticity of root growth and physiological activity is mechanism that enables plants to acquire resources from heterogeneous soil. Oilseed rape is an effective forager for water as well as for nutrients. In a pot experiment in which water and nutrients were supplied in patches, root densities were on average 4.6 fold higher in the watered quadrant if the pot than in the dry quadrant (Wang et al. 2007). However, as oilseed rape's response was not compared to that of another species, it is not known whether it is relatively more or less plastic than average. In the current work, soil drying reduced root length density of oilseed rape (relative to irrigated controls) in the upper

soil (30-40 cm) to a greater extent than in wheat (Chapter 2). By contrast there was no significant effect of soil drying on root length density at 70-80 cm. This difference between species may reflect a greater plasticity of oilseed rape root distribution in response to spatial variation in water availability. The capacity of root axes simultaneously experiencing different soil conditions (e.g. wet/dry, loose/compact) to respond with compensatory growth in favourable areas may be important in not only maintaining the total root length of the plant but also determining subsequent root growth responses (Montagu et al. 2001).

Chapter 5 provided evidence that oilseed rape may also be better able to exploit spatial variation in soil structure. When a compacted layer was present in the soil, analogous to a plough pan in the field, oilseed rape benefited more (more opportunistic) from artificial biopores in the layer than wheat. Oilseed rape had a greater number of roots growing in the pores and more roots growing in the soil layer below the compacted layer. In this experiment, there was little benefit in terms of water extraction from having these extra roots in the subsoil, but the experiment was of short duration and the supplies of water were finite. If oilseed rape is also able to exploit pores in the field more effectively than wheat, greater differences in growth might be expected to occur over the course of the season, especially if there is adequate water available in the deep sub soil layers.

Currently little is known about the extent of genotypic variation in root plasticity of oilseed rape and whether this might be improved through breeding. Variation in root plasticity has been reported for wheat. Song et al (2010) compared the drought tolerance of modern and old wheat cultivars and came to the conclusion that modern cultivars have a greater plasticity in root morphology and have the ability to develop thinner roots when water is scarce.

Implications for crop growth

The opportunist strategy and extravagant use of water by individual or small populations of oilseed rape plants is not something which was obvious from the literature relating to field crops (Table 1.1). The total 'seasonal' water use observed in the lysimeter experiment of Chapter 2 was on the whole greater than that expected for field-grown oilseed rape in a temperate climate. An attempt was made to mimic field

conditions, by creating mini-crops at realistic population densities in deep lysimeters, by shading the edge plants with mesh to reduce boundary effects and by using an open-sided glasshouse to maintain the climate as close as possible to that experienced in the field in the field. Nevertheless, the growth conditions were not exactly the same as in a field crop. Although the mini-crops were surrounded by shading mesh, they were very exposed because they were grown in tall lysimeters and this could have affected airflow and boundary layer resistance around the canopy. Additionally, the soil temperature was likely to be higher than in the field. In field crops with closed canopies, the stomata exert less control over transpiration than in isolated plants or small populations because there is less turbulence in air flow over the canopy. The canopy boundary resistance, radiation receipt and temperature are factors which have more influence on transpiration in a crop. Additionally, there is a feedback within the crop. The vapour pressure deficit of air within a canopy depends on the climatic conditions above it and on the total stomatal conductance of all the leaves in the canopy (Jarvis and McNaughton 1986). Thus the differences between wheat and oilseed rape in terms of profligacy of water use are likely to be smaller in the field.

Suggestions for further research

The present study has suggested that oilseed rape plants are profligate in their use of water when in good supply, but sensitive to soil drying when water is restricted. However, it is not known to what extent this behaviour occurs when oilseed rape is grown as a crop. Future research is needed to compare this aspect of the water relations of wheat and rape under field conditions to determine whether rape is a) at greater risk from drought through excessive water use and b) more responsive to soil drying.

Root hydraulic conductivity was measured under well-watered conditions and the conductivity oilseed rape was significantly greater. Under water limited conditions, however, root-soil contact can decrease due to soil and root shrinkage and root conductance can decrease (Neumann 2008). Future work could focus on whether oilseed rape's water use might be limited by decreased root hydraulic conductivity as the soil dries in order to identify whether improvements to the root system need to be made.

In both Chapter 2 and Chapter 5 there were indications that root distribution over depth differed for oilseed rape compared with wheat. In Chapter 2 when water was withheld, wheat roots may have grown to depth quicker than oilseed rape roots and extracted water from these layers sooner. There was also some indication that the root growth of oilseed rape was more plastic. In Chapter 5 there was a difference in the ability to use biopores to bypass a compacted layer. It would be interesting to follow root length development in time and space to determine whether oilseed rape and wheat respond differently to environmental cues. It would also be useful to establish whether these differences in plasticity and ability to utilise biopores are found under field conditions. A greater understanding of the mechanisms responsible for these differences might enable improvements to be made in the ability of oilseed rape crops to grow in less well structured soils.

There is little known about genetic diversity of oilseed rape root growth. If there is variation in rate of root system development and in maximum rooting depth, it may be possible to select for greater access to subsoil water thereby increasing the crop's ability to avoid drought. Additionally crops may benefit if canopy function could be maintained for longer as the soil begins to dry i.e. by having a less conservative stomatal response and or a greater degree of osmotic adjustment. This could ensure a lower loss of yield when water supply is limited.

In conclusion, oilseed rape appears to exploit the soil just as well or better than wheat. However its demand for water may be greater and oilseed rape's shoot is more sensitive to water limitation and soil hardening. As UK summers are expected to become dryer and warmer, oilseed rape, like wheat, could possibly benefit from deeper rooting. Additionally a less conservative stomatal response may result in a less severe reduction in growth and yield production.

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