Physiological, morphological and anatomical responses of wheat (*Triticum aestivum* L.) to different depths and durations of waterlogging

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Thesis-related publications

Journal publications


Conference abstracts


Declaration

This thesis is my own work; to the best of my knowledge all sources have been acknowledged. The thesis has been completed during the course of enrolment in a PhD degree at UWA and has not previously been accepted for a degree at this or another institution.

Al Imran Malik
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Summary

This study evaluated the physiological, morphological and anatomical responses of young wheat plants (21 d old) to different depths (soil waterlogged at the surface, or to 100 or 200 mm soil depth) and different durations (3, 7, 14, 21 and 28 d) of soil waterlogging. Recovery from these different waterlogging situations was also assessed. The aeration mechanisms of adventitious roots with only the apical few cm exposed to O₂-deficiency were also studied in hydroponics.

During waterlogging, the growth of wheat was reduced proportionally to the depth of waterlogging. Root growth was more reduced than shoot growth in all treatments during waterlogging (6-27% for shoot, 15-74% for roots). During recovery, plants invested more biomass in the root system to restore their original root-shoot ratio. As a result, the relative growth rate (RGR) of roots was faster than that of shoots during the recovery period (shoot RGR 48-84, root RGR 106-110 mg g⁻¹ d⁻¹).

During waterlogging, the seminal root system stopped growing. After draining the soil, seminal root mass could not recover from even short durations of waterlogging of 3-7 d. Seminal roots failed to initiate new laterals once the existing laterals were damaged. In contrast, adventitious roots continued to grow to a maximum length (100-160 mm into the water-saturated zone), and the number of adventitious roots per stem also increased over that of plants grown in drained soil.

Adventitious roots exposed to O₂-deficiency only in the apical few cm in soil and in hydroponic experiments formed aerenchyma along the entire main axis. The functionality of the aerenchyma was demonstrated by measuring radial O₂ loss (ROL) near the root tip. ROL data collected after restricting O₂ entry at the shoot and/or root zone demonstrated the importance of O₂ diffusion into basal, aerobic parts of the roots. This study confirmed that in a waterlogged situation where only the apical portion of roots was exposed to O₂-deficiency, O₂ can diffuse into the aerobic zone of the root and from there longitudinal movement via aerenchyma can occur to provide O₂ to the root tip.

Assessment of recovery from waterlogging is essential in evaluating waterlogging tolerance in crops, as short-term waterlogging can have long-term effects on growth.
Chapter 1

Introduction

Waterlogging of soil is a widespread problem in crop production around the world. It is common not only in flood plains, but also in irrigated cropping systems. One third to one half of the irrigated land of the world has drainage problems (Kozlowski 1984). As a result, after heavy rainfall and/or irrigation the soil can become waterlogged.

Approximately 10 million ha of wheat are at risk from waterlogging (Boru et al. 2001), that is almost 15% of the 70 million ha sown each year (Setter and Waters 2003). In the Indo-Gangetic plains of Northern India and, Eastern and Central Africa (including Ethiopia) about 4.7 million ha of wheat cultivation is affected by waterlogging (reviewed by Setter and Waters 2003).

Sandy duplex soils in Australia often become waterlogged after heavy rainfall, affecting wheat production. Approximately 40 to 60% of the agricultural area consists of duplex soils in Victoria and Western Australia (McFarlane 1990; Fried and Smith 1992). In Western Australia, every 10 mm of August rainfall reduced wheat yields by 150 kg ha\(^{-1}\) (McFarlane and Wheaton 1990).

Waterlogging of soil may last from hours to days and months. It also varies in water table depths (Cox and McFarlane 1990; Setter and Waters 2003). Transient waterlogging is common in both clay and duplex soils in south-Western and south-Eastern Australia (Setter 1999). Despite of the high spatial and temporal variability in waterlogging intensity, most research assessing the effects of waterlogging on crops (including physiological, morphological and anatomical adaptations to waterlogging stress) has been carried out in controlled environments with treatments typically being specific periods of waterlogging to the soil surface. Mostly sand cultures in pots, or hydroponic experiments which employ various means of imposing O\(_2\)-deficient conditions to the plant have been used. Few studies have evaluated the effects of different durations and/or depths of waterlogging, or assessed the recovery of plants after the waterlogging period is over (e.g. wheat: Watkin et al. 1998; lupin: Davies et al. 2000). Very often, only the responses of plants during a waterlogging event have been measured. There are, however, reports on the effects of waterlogging on the yield and
yield components of dryland crops (eg, barley: Leyshon and Sheard, 1974; pea: Jackson 1979; wheat: Sharma and Swarup, 1988; Melhuish et al., 1991). The goal of this thesis was to evaluate the physiological, morphological and anatomical responses of wheat to different durations and different depths of waterlogging and the recovery from this stress. Specific aims of this thesis were:

- To assess the response of wheat to different depths of waterlogging and its subsequent recovery.
- To assess the response of wheat to different durations of waterlogging and the physiological basis of recovery from the stress.
- To study the mechanisms of aeration of wheat roots that only experience low O\textsubscript{2} concentration in the apical few cm while the basal zone remains in aerobic conditions.

References


Jackson MB (1979) Rapid injury to peas by soil waterlogging. *Journal of Science Food and Agriculture*. 30, 143-152.


Chapter 2

Literature Review

Plants need rapid gas exchange to grow and survive (Jackson and Drew 1984). When soil is waterlogged, the gas exchange between the soil and atmosphere is drastically reduced (Armstrong 1979), which leads to a deficiency of O₂ and to the accumulation of gases such as carbon dioxide, methane and ethylene, sometimes to toxic levels (Ponnamperuma 1984). The O₂ deficiency can occur within a few hours or days of waterlogging as soil microorganisms and roots use up the O₂ present in the soil (Ponnamperuma 1984). As a consequence, aerobic respiration in roots is inhibited, which greatly diminishes ATP formation, and can result in reduced plant growth or even death (Drew 1997).

Waterlogging tolerance of plants may be achieved by features that improve gas exchange, thus enabling continued root growth, as well as by inducing pathways of anaerobic carbohydrate catabolism, which help to maintain energy production at least for survival (Armstrong et al. 1994).

2.1 Causes of waterlogging

Waterlogging of soil results from a combination of factors such as excess rainfall, poor soil drainage and water storage capacity of the soil (Cox and McFarlane 1990). Irrigation may also cause waterlogging, depending on soil type. Two soil types are very waterlogging-prone: clayey soils and duplex soils (Cox and McFarlane 1990). In clayey soils, water easily ponds on the surface, and the saturation of the soil starts from the top downwards (Cox and McFarlane 1990). Duplex soils are sandy soils over an impenetrable rock or clay layer. In these soils, excess water seeps through the soil eventually to reach a rock or clay layer, and then accumulates, so that the watertable rises from the bottom. Thus, the watertable of a waterlogged soil can vary with different soil types and amount of rainfall (as demonstrated for a site in the Western Australian wheat belt in Figure 2.1) or irrigation.
2.2 **Assessment of waterlogging intensity**

As soil properties can vary even on a small scale, and as there is great variability in the amount and distribution of rainfall between sites and years, it is difficult to compare the intensity of waterlogging events amongst locations (even at small distances within a field) and years. In the Netherlands in the 1960s, Sieben (1964) developed an index that integrated the time and depth of waterlogging during which the water table in a polder field rose above a threshold value. Sieben (1964) found that the sum of excess water table above 30 cm soil depth (SEW$_{30}$) during the winter months negatively correlated with the yield of a subsequently sown spring crop, due to the effects of winter waterlogging on soil structure. The SEW$_{30}$ index integrates the water table depth and duration of a waterlogging event and gives a single value, for example, if the watertable is at 25, 10 and 5 cm below the surface on three successive days, then the SEW$_{30}$ value will be 50 cm days. Fig 2.2 illustrates that a range of different waterlogging scenarios can lead to similar SEW$_{30}$ values, suggesting similar intensity of these different waterlogging events. More recently, Australian researchers have adopted the concept of SEW indices, and applied it to situations where winter waterlogging affects an autumn-sown crop (reviewed by Setter and Waters 2003). Apart from the arbitrary nature of the set 30 cm limit in this new context, there are other limitations of the use of the SEW$_{30}$ index, as it does not take into account the recovery capacity of a crop as well as other
Figure 2.2 Different durations and watertable depths of soil waterlogging illustrating the same SEW_{30} value of 300 cm. day. Dark colour represents the watertable depth.

Environmental parameters like temperature and light intensity (McFarlane et al. 1989; Setter and Waters 2003). There are no data available that evaluate the recovery of a crop in connection with different SEW_{30} values.
2.3 Effect of waterlogging on soils

A series of physical, chemical, and biological changes take place soon after the onset of a waterlogging event in a dry soil. All these changes occur due to the disappearance of molecular O$_2$ from the soil. The 10,000 times slower diffusion of gases in water compared with that in air results in decreased O$_2$ concentrations in waterlogged soil as roots and soil microbes consume the available O$_2$ in the soil solution (Ponnampetum 1984). The rate of O$_2$ disappearance from the soil can be as short as one day to near depletion, and depends on depth (Drew and Sisworo 1979; Cannell et al. 1985), temperature (Cannell et al. 1980; Trought and Drew 1982), soil type (Cannell et al. 1980), vegetation type and duration of waterlogging (Drew and Sisworo 1979; Trought and Drew 1982; Blackwell 1983; Barrett-Lennard et al. 1986).

Disappearance of O$_2$ and slower gas exchange between atmosphere and water leads to accumulation of the gases such as, carbon dioxide (Drew and Sisworo 1979; Trought and Drew 1980a, 1982) and ethylene (Dowdell et al. 1972; Drew and Sisworo 1979; Trought and Drew 1980a, 1982) in waterlogged soil. The study of waterlogging in relation to soil-borne gases has mainly focused on ethylene. As a gaseous plant hormone, ethylene reduces root growth (Konings and Jackson 1979), inhibits leaf growth and initiation (Jackson et al. 1981) but stimulates adventitious root initiation and aerenchyma formation (Drew et al. 1979; Visser et al. 1996a) (as discussed below).

After the disappearance of O$_2$, the usual aerobic microbial processes are replaced by anaerobic processes (Laanbroek 1990). As a result, waterlogged soils become rich in reduced metal ions like Mn$^{2+}$ and Fe$^{2+}$ as a result of the microbes using the oxidized forms of Mn(IV) and Fe(III) as e$^-$ acceptors. The reduced forms, Mn$^{2+}$ and Fe$^{2+}$ are much more soluble than the oxidised forms, Mn(IV) and Fe(III). Therefore, availability of these two nutrients to plants increases in waterlogged soil, and can even reach toxic levels.

Waterlogged soil may contain 20-50 fold higher Fe$^{2+}$ concentrations compared with drained soil, depending on the duration of waterlogging (Stieger and Feller 1994). The Fe$^{2+}$ concentration in the soil was positively correlated with the concentration in shoots of rice (Yamauchi 1989). The critical toxicity level of Fe in the tissue depends on the species. For example, in wetland species like rice, the toxicity level in leaves is $\sim$500 $\mu$g
g\(^{-1}\) dry weight, and depends on the concentration of other nutrients in the plant (Yamauchi 1989). Regardless of their waterlogging tolerance, *Rumex* species had similar threshold levels of iron toxicity in leaves (~1100-1600 µg g\(^{-1}\) dry weight, Laan et al. 1991). However, when waterlogged, wetland plants might be able to oxidise the upper layer of the soil through radial oxygen loss from the roots and thereby create an aerobic micro-environment, resulting in low Fe\(^{2+}\) concentrations in the rhizosphere (Laan et al. 1991). Thus, the ability of the root to oxidize the rhizosphere in waterlogged conditions confers an ability of the plant to escape Fe toxicity.

Like Fe\(^{2+}\), the concentration of Mn\(^{2+}\) might also increase up to 200-fold in waterlogged soils (Stieger and Feller 1994). Mn can be readily transported from roots to shoots, and as a result, toxicity symptoms occur in the shoot first (Marschner 1997). Critical toxicity levels (200 to 5300 µg g\(^{-1}\) dry weight) of Mn in the shoots vary with plant species (Marschner 1997). The non-wetland species, barley, responded by reduced shoot weight to less than 200 µg g\(^{-1}\) dry weight tissue Mn. In contrast, the wetland species rice did not show any reduction of total shoot dry weight even at tissue Mn concentrations ten times higher than those of barley (Vlamis and Williams 1964). High Mn tolerance of shoot tissue is considered to be an important character for waterlogging-tolerant plants (Marschner 1997).

Within a few days of waterlogging, nitrate disappears from the soil, as the process of denitrification by soil microbes is accelerated (Ponnamperuma 1972). Most of the nitrate is lost in the form of N\(_2\)O and N\(_2\). As a result, waterlogged soil becomes devoid of one of the most important nutrients that affect plant growth (as will be discussed below). However, waterlogging can increase the capacity of soils to fix N\(_2\) biologically, and as a result, the NH\(_4^+\) concentration increases (Ponnamperuma 1972).

### 2.4 Intermittent waterlogging

Soils can become waterlogged for a short period after heavy rainfall or irrigation in non-wetland cropping systems. As a result, the possibility of intermittent waterlogging is more common than continuous waterlogging for non-wetland crop species. However, there are very few data available that report on intermittent waterlogging stress on plants.
Several cycles of intermittent waterlogging of soil can be more harmful for plant growth than a continuous waterlogging event when considered on the basis of the actual number of days of waterlogging. A possible explanation could be derived from the experiment by Waldren et al. (1987) that shows that previously waterlogged compost when re-waterlogged became reduced rapidly, compared with the speed of reduction during the previous waterlogging event. Watson et al. (1976) have documented that intermittent waterlogging and continuous waterlogging reduced grain yield (by ~40 and ~55%, respectively) of wheat, barley and oat. However, in this experiment, intermittent treatment pots received three waterlogging cycles, and in between, pots were allowed to drain, while waterlogged pots received continuous 42 days of treatment. In a more recent experiment by Thomson et al. (1992), two wheat-cultivars and one triticale cultivar were studied where two cycles of waterlogging (each for 7 days duration) were imposed with an interval of 7 days each time and compared with continuously waterlogged plants. Intermittently waterlogged plants were less affected (8 to 20 % in plant fresh weight) than continuously waterlogged plants. However, the total duration of waterlogging was longer (~2-fold) in the continuously waterlogged treatment than in the intermittent waterlogging treatments. There are no data available comparing intermittent and continuously waterlogged crops exposed to the same duration of waterlogging (Setter and Waters 2003).

2.5 Response of shoots to waterlogging

2.5.1 Shoot growth

Waterlogging changes the behaviour of the shoot in many aspects (Jackson and Drew 1984). It affects shoot growth by changing the root-to-shoot communication, mainly by three different ways: i. increased supply of substances from waterlogged roots to shoots (e.g., ABA), ii. decreased supply of substances from waterlogged roots to shoots (nutrients), and iii. accumulation of photo assimilates in the shoot (Jackson and Drew 1984). These substances could be photosynthetic products, hormones or their precursors, nutrients and/or soil-derived toxins. Signals that affect shoot performance under waterlogging will be discussed below.
Shoot growth of non-wetland species with roots exposed to soil waterlogging or stagnant anaerobic conditions decreases (compared with aerobic controls) to a greater extent than that of wetland species. For example, McDonald et al. (2001a) reported a reduction of shoot dry mass of 55 to 70% for wheat, barley and rye subjected to deoxygenated stagnant nutrient solution for 21 days. By contrast, stagnant conditions reduced the shoot dry mass of wetland grasses such as *Lophopyrum elongatum* or *Critesion maritum* by only 6 and 26%, respectively. However, a growth reduction of non-wetland species suffering from waterlogging might not become obvious as reduced dry matter accumulation until several days after the onset of the stress (wheat, Trought and Drew 1980a; barley, Drew and Sisworo 1979). This might be due to a lag in the response of shoot growth to waterlogging or structural growth might be confounded by an increase in the concentration of non-structural carbohydrates in shoots after waterlogging (discussed in further detail in 2.5.7). The reduced shoot dry matter in waterlogged non-wetland grass species is usually the combined result of decreased leaf length, reduced leaf area and decreased tiller number (Trought and Drew 1980a).

### 2.5.2 Leaves

Reduced leaf area is a common response of non-wetland crop species to waterlogging or anoxia. The leaf area of two wheat cultivars (14 days old) was reduced by ~50% after 21 days of hypoxia when compared with plants grown in aerated nutrient solution (Huang et al. 1997a). Sojka et al. (1975) observed an 86% decrease in leaf area in wheat (11 days old at the start of the treatment) grown in an N₂-flushed soil culture system for 25 days compared with plants grown in air-flushed soil. Leaf area of yellow and narrow-leafed lupin (28 or 56 days old at start of treatment) was reduced by 24 to 56%, respectively, after 14 days of waterlogging compared with plants grown in drained soil (Davies et al. 2000a). The extent of the reduction of leaf area varies with species, cultivar and the duration of a waterlogging treatment.

The reduction of leaf area due to waterlogging can be attributed to reduced leaf extension. The leaf extension rate of wheat was reduced by ~50% (leaf 4) in plants that were exposed to soil waterlogging (Trought and Drew 1980a). By contrast, the duration of the leaf extension was hardly affected (Trought and Drew 1980a). As a consequence, waterlogged wheat produces shorter leaves compared with plants grown in drained soil.
There are no data available on the cause of the reduction of leaf elongation rates in waterlogged plants. However, there are reports of the presence of high levels (up to 10 μl l⁻¹) of ethylene in the waterlogged soil (Dowdell et al. 1972; Drew and Sisworo 1979; Trought and Drew 1980a, 1982). Jackson et al. (1981) showed for maize that leaf extension was reduced by ~40% when 5 μl l⁻¹ ethylene was supplied to an aerated nutrient solution. Voesenek et al. (1996) showed a reduced petiole growth of the non-wetland plant *Rumex acetosa* when exposed to increasing ethylene concentrations in the nutrient solution. Interestingly, wetland plants can respond to low doses of exogenous ethylene with faster petiole elongation (e.g., *Rumex palustris*, Voesenek et al. 1996). Apparently, ethylene can act as a signal for either faster or slower leaf elongation depending on the species.

In addition to slower rates of leaf extension, the senescence of older leaves was accelerated when wheat (Trought and Drew 1980b) or barley (Drew and Sisworo 1979) were subjected to waterlogging, thus further contributing to the reduced functional leaf area in plants under waterlogging stress.

### 2.5.3 Tillering

Non-wetland grass species respond to waterlogging by reduced tiller numbers (Table 2.2). Longnecker et al. (1993) for wheat and Birch and Long (1990) for barley demonstrated the positive effect of nitrogen (urea fertilizer) on tiller production in drained soil. As Trought and Drew (1980c) reported, a 50% reduction of total shoot nitrogen in waterlogged wheat compared with plants grown on drained soil, it may be assumed that a waterlogging-induced lower nitrogen status contributes to a reduction in tiller production in waterlogged plants.

### 2.5.4 Nitrogen status

Waterlogging or anoxia decreases the nitrogen concentration in the aerial part of non-wetland crop species. The reduction of nitrogen in the shoot depends on the cultivar and duration of waterlogging. In barley, two days of soil waterlogging reduced the nitrogen
concentration (drained: 3.2, waterlogged: 2.4 mmol g\(^{-1}\) dry weight) in the shoots compared with plants grown in drained soil, and the reduction increased with time; as a result, after 20 days of soil waterlogging, the reduction was 50% compared with plants grown in drained pots (Drew and Sisworo 1979). Similar observations were made by Trought and Drew (1980b,c) in wheat. The initial drop of the shoot nitrogen concentration in barley may have been caused by insufficient uptake or translocation, as there was sufficient nitrogen present in the soil solution (2.5 mM NO\(_3^-\)) for plant growth (Drew and Sisworo 1979).

In wheat, waterlogging or hypoxia affected both the rate of nitrogen uptake by roots (Kuiper et al. 1994) and the rate of nitrogen translocation from the root to the shoot (Buwalda et al. 1988). As a result of this, the shoot nitrogen concentration decreased, and leaf chlorosis occurred in wheat (Trought and Drew 1980c) and barley (Drew and Sisworo 1979). Redistribution of nitrogen from older leaves to newly developed leaves occurred in waterlogged barley, which accelerates the senescence of older leaves (Drew and Sisworo 1979). Trought and Drew (1980c) have demonstrated for wheat that the application of nitrogen to the topsoil of waterlogged pots can inhibit the chlorosis and senescence of older leaves.

Waterlogging affects the availability of nitrogen to plants, as the soil nitrate pool can be rapidly depleted due to denitrification (Ponnamperuma 1984). Huang et al. (1995) demonstrated in sand culture with half-strength and full-strength nutrient solution that the nitrogen concentration in waterlogged wheat leaves was reduced compared with plants grown in drained pots when supplied with half-strength nutrient solution. By contrast, plants in waterlogged pots supplied with full-strength nutrient solution had the same leaf nitrogen concentration than did plants grown in drained pots. Thus, the addition of nitrogen to waterlogged or hypoxic media might alleviate symptoms of waterlogging stress.

### 2.5.5 Photosynthesis

Waterlogging of soil reduces photosynthesis in many non-wetland crop species (Table 2.1). Reduction of photosynthesis rate depends on the species, cultivar, age of the plant at the start of the treatment and duration of the treatment (Table 2.1). Photosynthesis of
Table 2.1 Effect of waterlogging or hypoxia on net photosynthesis and stomatal conductance of six non-wetland crop species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Age of the plant at the start of the treatment (days)</th>
<th>Duration of the treatment (days)</th>
<th>Leaf number and position</th>
<th>Reduction of net photosynthesis % of the control</th>
<th>Reduction of stomatal conductance % of control</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lupinus luteus</em> (Yellow lupin)</td>
<td>28-56</td>
<td>14</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; or 3&lt;sup&gt;rd&lt;/sup&gt; youngest leaf</td>
<td>35-55</td>
<td>33-62</td>
<td>Davies et al. (2000b)</td>
</tr>
<tr>
<td><em>Lupinus angustifolius</em> (Narrow-leaved lupin)</td>
<td>28-56</td>
<td>14</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; or 3&lt;sup&gt;rd&lt;/sup&gt; youngest leaf</td>
<td>45-78</td>
<td>55-60</td>
<td>Davies et al. (2000b)</td>
</tr>
<tr>
<td><em>Triticum aestivum</em> (cv. Bayles)</td>
<td>14</td>
<td>15</td>
<td>Youngest fully expanded leaf</td>
<td>44</td>
<td>70-92</td>
<td>Haung et al. (1994a, 1997a)</td>
</tr>
<tr>
<td><em>Triticum aestivum</em> (cv. Savannah)</td>
<td>14</td>
<td>15</td>
<td>Youngest fully expanded leaf</td>
<td>47</td>
<td>47-54</td>
<td>Haung et al. (1994a, 1997a)</td>
</tr>
<tr>
<td><em>Helianthus annuus</em> (Sunflower)</td>
<td>21-25</td>
<td>4</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; basal leaf pair</td>
<td>30</td>
<td>No difference</td>
<td>Wample and Thornton (1984)</td>
</tr>
<tr>
<td><em>Arachis hypogaea</em> (Peanut)</td>
<td>16</td>
<td>14</td>
<td>4&lt;sup&gt;th&lt;/sup&gt; youngest leaf</td>
<td>19</td>
<td>59-61</td>
<td>Bishnoi and Krishnamoorthy (1992)</td>
</tr>
</tbody>
</table>
the youngest fully developed leaf of wheat grown in hypoxic nutrient solution started to
decline after 9 days of treatment (Huang et al. 1991a). However, when the same cultivar
(Bayles) was grown in waterlogged soils, the rate of photosynthesis started to decline
within three days of the onset of the treatment. In both treatments, the reduction of
photosynthesis after 14 days was approximately 33% compared with plants grown in
aerated solution or drained soil (Huang et al. 1994a). The reduction of photosynthesis in
waterlogged non-wetland crop species might be caused by a combination of the factors
that will be discussed in the following paragraphs.

Reduced photosynthesis in waterlogged plants has been suggested to be caused by
reductions in leaf water potential, stomatal conductance, amount or activity of
photosynthetic enzymes and chlorophyll content of the leaf (Huang et al. 1994a).
However, in waterlogged sunflower, there was no evidence of water stress as leaf water
potential of plants grown in drained and waterlogged soil was similar (-0.6 MPa), while
the rate of photosynthesis was reduced (Table 2.1, Wample and Thornton 1984). In
waterlogged wheat, there was a positive correlation between the reduction of
photosynthesis and stomatal conductance (Huang et al. 1994a, 1997a). However, this
might not necessarily point to a causal relationship between the stomatal conductance
and rates of photosynthesis. In waterlogged yellow lupin and narrow-leaf lupin,
photosynthesis, stomatal conductance and leaf water potential responded independently,
as the intercellular CO₂ concentration in leaves was unchanged by waterlogging (Davies
et al. 2000b). As reductions in photosynthesis usually precede losses of leaf chlorophyll
(Huang et al. 1997a), it is very unlikely that the chlorophyll is the initial cause of
reduced photosynthesis in waterlogged plants. However, reduced leaf nitrogen and
chlorophyll concentrations as they develop during the course of waterlogging might
further reduce leaf photosynthesis. Inhibition of the translocation of photosynthetic
products in waterlogged plants might also reduce photosynthesis (see below 2.5.7).

2.5.6 Stomatal conductance

The reduction of stomatal conductance in response to waterlogging is well documented
(reviewed by Sojka 1992). It varies with species, duration of waterlogging and growth
stage of the plant at the start of the waterlogging (Table 2.1). Reduction of stomatal
conductance is apparently caused by the increased concentration of ABA in the leaves
of non-wetland waterlogged plants. In waterlogged pea, 24 h of waterlogging increased the ABA concentration in leaves by 262% and stomatal conductance was reduced by 87% compared with plants grown in drained pots (Jackson and Hall 1987). However, there is conflicting evidence of the source of the ABA in the leaves of waterlogged pea. Jackson et al. (1988) did not find any increase of ABA in O₂-starved pea roots in their experiment in hydroponics, and suggested that increased ABA concentrations in the leaf (4 to 7-fold higher depending on the duration of waterlogging) originated in the leaves as translocation was halted. By contrast, Zhang and Davies (1987) reported an increased amount of ABA both in roots (10 fold) and leaves (20 fold) of waterlogged peas compared with plants grown in drained soil (compost). In more recent work with tomato, Else et al. (1996) showed that waterlogged roots decrease ABA export to the shoot by 75% within 4 h of the start of the waterlogging and stay at that level at least up to 72 h. However, increased ABA concentration in the leaves was observed (drained: 1.25, waterlogged: 2.0 nmol g⁻¹ fresh weight) transiently at 12 h and 24 h after the onset of the treatment, and then dropped back to the level of the drained plants by 72 h of the treatment (Else et al. 1996). Stomatal conductance of waterlogged tomato started to decrease after 4 h of waterlogging, and after 48 h of the treatment the reduction was 48-67% of the drained plants, and the reduction increased progressively. The stomatal conductance of pea and tomato mutants that are deficient in endogenous ABA was less affected (pea: 3 fold, tomato: 2 fold) by 24 h of waterlogging than that of wild-types (Jackson and Hall, 1987). Else et al. (1996) suggested that for waterlogged tomato plants, an unidentified factor other than ABA is involved in the reduction of stomatal conductance. While there is doubt about the origin and the role of ABA in stomatal closure in the leaves of waterlogged plants, a negative relationship between the ABA concentration and stomatal conductance has been described (Jackson and Hall 1987; Castonguay et al. 1993).

2.5.7 Carbohydrate status

Waterlogging or anoxia reduce assimilate translocation from leaves and initiate the accumulation of carbohydrates. Increased concentration of non-structural carbohydrates (~30% higher than in control plants) in shoots of waterlogged or anoxic wheat (Barrett-Lennard et al. 1988) and barley (Limpinuntana and Greenway 1979) have been documented. In alfalfa, 14 days of waterlogging of soil increased leaf starch.
concentrations 5-fold compared with those in plants grown in drained pots (Castonguay et al. 1993). Similarly, sucrose concentration in the leaves of waterlogged alfalfa reached up to 5.5 mg g\(^{-1}\) dry weight (~2 fold higher than in drained plants) by day 7 of the treatment, and at the same time photosynthesis was reduced by 67% of the control value (Castonguay et al. 1993). Translocation of labelled carbon (\(^{14}\)C) from the leaves of *Phaseolus vulgaris* when the roots were anoxic was reduced by 30-50% compared with that in plants grown in aerated nutrient solution (Schumacher and Smucker 1985). The reduced rate of export of photosynthate from leaves to stems and roots may lead to a feedback inhibition of photosynthesis (Plaut et al. 1987).

2.6 **Response of roots to waterlogging**

2.6.1 **Root growth**

The root system of grasses is composed of two distinct root types, seminal and adventitious roots. Seminal roots develop from the embryo during germination, and adventitious roots form later from the nodes. Both root types support the plant during its entire life (Klepper 1984). Seminal roots are mainly attached to the main stem and help seedling establishment, and mostly support nutrient and water uptake to the main stem (Manske and Vlek 2002). By contrast, adventitious roots mostly form from the tillers when the 4\(^{th}\) leaf of the main stem starts to emerge, and support the tillers. However, the nutrient and water taken up by any part of the root are translocated all over the plant (Manske and Vlek 2002).

In waterlogged soil or under low O\(_2\) concentrations in the root zone, the seminal root growth of non-wetland species is severely affected (Limpinuntana and Greenway 1979, Trought and Drew 1980a, McDonald et al. 2001a). The reduction of dry weight of seminal roots can be as high as 90% in waterlogged and/or stagnant plants compared with plants grown in drained and/or aerated conditions (Trought and Drew 1980a; McDonald et al. 2001a). Waters et al. (1991a) have shown that wheat seminal root elongation ceases soon after exposure to anoxia in solution culture experiments. With intact maize plants, after only two days in N\(_2\)-flushed solution, the increase of seminal root length was reduced by 86% compared with plants grown in aerated nutrient solution (Atwell et al. 1985).
Seminal roots of wheat have little constitutive porosity (Thomson et al. 1990), which strongly limits the diffusion of oxygen when these roots are exposed to a hypoxic or anoxic medium. Thomson et al. (1990) showed in solution culture experiments that young seminal roots of wheat seedlings (5-7 days old and <10 mm long) when transferred to O$_2$-deficient media could elongate as they developed higher porosity than did aerated roots (12.4% in stagnant solution, 3.4% in aerated solution). By contrast, older seminal roots of the same cultivar (cv. Gamenya) that were grown in aerated conditions to a seedling age of 18-23 days and grew 119 mm, failed to elongate when transferred to stagnant conditions and these roots failed to substantially increase in porosity (6.2% in stagnant, 4.8% in aerated). In the field and in most pot experiments, seminal roots failed to grow in waterlogged situations as at the onset of treatment plants were 14 to 21 days old, and at this stage of development the seminal roots were presumably longer than 100 mm (Watson et al. 1976, Trought and Drew 1980a, McDonald et al. 2001a). The importance of higher root porosity for root elongation into O$_2$-deficient media will be discussed later in this review (in section 2.6.3.1).

In waterlogged situations, seminal roots and adventitious roots of non-wetland crop species respond differently. Seminal root growth ceases (as discussed above 2.6.1), and adventitious roots keep growing up to a specific length (Trought and Drew 1980a). Therefore, adventitious roots become the major root part for waterlogged crop plants.

2.6.2 Morphological adaptations

The acceleration of adventitious root formation is a common adaptive response to waterlogging of soil for both non-wetland and wetland plants (Table 2.2). The number of adventitious roots formed depends on the species, cultivar, and duration of the treatment and age of the plant when the treatment starts (Thomson et al. 1992, Visser et al. 1995, Wiengweera et al. 1997, McDonald et al. 2001a, Colmer 2003). Dicotyledonous plants like Rumex species formed increased numbers of adventitious roots while exposed to O$_2$-deficient medium or waterlogging (e.g. non-wetland species R. thyrsiflorus 6-fold and wetland species R. palustris 10-fold higher compared to drained or aerated conditions) (Visser et al. 1995). Monocotyledonous wetland species, like rice, in aerated or drained conditions, form more adventitious roots compared with non-wetland species like wheat, particularly when expressed on a per-stem basis (Table
Moreover, waterlogged or stagnantly grown rice produced 2-3 times more adventitious roots than did plants grown in drained soil or in aerated conditions (Colmer 2003). In non-wetland grasses like wheat, waterlogging often reduces tiller production, thus affecting the total number of adventitious roots. However, the number of adventitious roots, when calculated per stem, in these species still increases under waterlogging (Table 2.2).

The accelerated adventitious root formation in response to O₂ deficiency in the rooting medium can be attributed to the action of the gaseous plant hormone ethylene (Table 2.3). However, the maximum number of adventitious roots that can be formed is determined by the inherent capacity of root formation (Visser et al. 1996a). *Rumex* plants grown in aerated nutrient solution form adventitious roots when exposed to ethylene. The waterlogging-tolerant species *Rumex palustris* produces three times more adventitious roots than the intolerant species *Rumex thyrsiflorus* (Visser et al. 1996a). Both species respond to ethylene in a fashion similar to waterlogged or stagnant conditions (Visser et al. 1996a). The wetland species rice also produced more adventitious roots when the aerated root system was exposed to increased ethylene concentrations (Justin and Armstrong 1991). The non-wetland species maize showed similar responses to ethylene (Table 2.3). Seven to 14 days of exposure to ethylene (1-5 μl l⁻¹) to the aerated root system of maize accelerated adventitious root formation (Jackson et al. 1981). Similarly, wheat grows more adventitious roots when exposed to ethylene (Huang et al. 1997b). In all these species, the internal accumulation of ethylene has been reported for plants grown in waterlogged or stagnant conditions or exposed to exogenous ethylene (rice, Konings and Jackson 1979; maize, Atwell et al. 1988 *Rumex*, Visser et al. 1996a; wheat, Huang et al. 1997b). Accumulation of or exposure to ethylene appears necessary for the formation of a greater number of adventitious roots in waterlogged or stagnant-grown plants.

While ethylene has been shown to stimulate adventitious root formation, Visser et al. (1996b) concluded for *Rumex palustris* that high ethylene increases the sensitivity of root-forming tissues to auxin to induce new adventitious roots. In earlier studies, Wample and Reid (1978, 1979) described a primary role of auxin in the formation of adventitious roots primordia in sunflower. They concluded that ethylene might cause a build-up of auxin in the sunflower hypocotyl which then initiates the adventitious root formation in waterlogged plants.
<table>
<thead>
<tr>
<th>Species</th>
<th>Growth medium</th>
<th>Plant age in days</th>
<th>Number of adventitious roots per plant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-wetland</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triticum aestivum cv. Capelle desprez</td>
<td>Sandy soil</td>
<td>26</td>
<td>na</td>
<td>10</td>
</tr>
<tr>
<td>Triticum aestivum cv. Gaminya</td>
<td>Sandy soil</td>
<td>36</td>
<td>na</td>
<td>14</td>
</tr>
<tr>
<td>Hordeum vulgare cv. Proctor</td>
<td>Nutrient solution</td>
<td>34</td>
<td>5</td>
<td>51</td>
</tr>
<tr>
<td>Hordeum vulgare cv. Chinese spring</td>
<td>Nutrient solution</td>
<td>34</td>
<td>13</td>
<td>54</td>
</tr>
<tr>
<td>Secale cereale</td>
<td>Nutrient solution</td>
<td>34</td>
<td>14</td>
<td>51</td>
</tr>
<tr>
<td>Zea mays cv. LG 11</td>
<td>Nutrient solution</td>
<td>21</td>
<td>na</td>
<td>14</td>
</tr>
<tr>
<td>Wetland</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oryza sativa cv. Omrit 39</td>
<td>Soil</td>
<td>35</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Oryza sativa cv. Calrose</td>
<td>Soil</td>
<td>35</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Lophopyrum elongatum</td>
<td>Nutrient solution</td>
<td>34</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>Lophopyrum elongatum</td>
<td>Nutrient solution</td>
<td>34</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Cricium maritimum</td>
<td>Nutrient solution</td>
<td>48</td>
<td>19</td>
<td>32</td>
</tr>
</tbody>
</table>
Table 2.3. The effect of ethylene on the adventitious root numbers per plant of two non-wetland and two wetland species.

<table>
<thead>
<tr>
<th></th>
<th>Treatment days</th>
<th>Growth medium</th>
<th>Number of adventitious roots per plant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Triticum aestivum</em></td>
<td>26</td>
<td>Nutrient solution: aerated, N$_2$ flushed, ethylene flushed</td>
<td>18</td>
<td>Huang <em>et al.</em> (1997b)</td>
</tr>
<tr>
<td><em>Zea mays</em></td>
<td>11-14</td>
<td>Nutrient solution: aerated, N$_2$ flushed and no aeration, ethylene flushed</td>
<td>13.6</td>
<td>Drew <em>et al.</em> (1979)</td>
</tr>
<tr>
<td><em>Helianthus annuus</em></td>
<td>4</td>
<td>Granite-grift (50:50) w/w, ethrel was applied as solution</td>
<td>0-1</td>
<td>Wample and Reid (1979)</td>
</tr>
<tr>
<td><em>Oryza sativa</em></td>
<td>90</td>
<td>Nutrient solution: aerated, N$_2$ flushed, ethylene flushed</td>
<td>29.1</td>
<td>Justin and Armstrong (1991)</td>
</tr>
<tr>
<td><em>Rumex palustris</em></td>
<td>7</td>
<td>Nutrient solution: aerated, ethylene flushed, deoxygenated agar</td>
<td>4</td>
<td>Blom <em>et al.</em> (1994)</td>
</tr>
</tbody>
</table>

*adventitious root primordia
The adventitious roots formed in waterlogged plants develop aerenchyma that allows low resistance O₂ diffusion to the root tip from the shoot (discussed in the following section 2.6.3). As in most crop species, seminal roots stop growing and functioning when waterlogged (discussed above in 2.6.1) and plants have to rely on the newly developed adventitious roots for uptake of nutrients, water, and continued growth (Jackson and Drew 1984); adventitious root formation in waterlogged plants is therefore an adaptive feature.

2.6.3 Anatomical adaptation

2.6.3.1 Porosity

Porosity of adventitious roots depends on the types of cell packing in the cortex, size of the stele and the formation of aerenchyma (Justin and Armstrong 1987). Two distinct types of cell packing, cubical and hexagonal, in the cortex determine the constitutive porosity of roots. Model calculations show that root cortices with cubical packing of perfectly spherical cells have higher (21%) constitutive porosity than cortices with hexagonal cell packing (9%) (Justin and Armstrong 1987). Wetland species generally have a cubical arrangement of cells; by contrast, non-wetland species have hexagonal cell packing and less constitutive porosity (Justin and Armstrong 1987; Gibberd et al. 2001). However, McDonald et al. (2002) reported from their experiment with three non-wetland and seven wetland species that cubical cell packing is not restricted to wetland species. The constitutive porosity in adventitious roots of wetland species ranged from 14-33% and that of non-wetland species from 6-14% (McDonald et al. 2002). In earlier works, constitutive porosities of roots of non-wetland grass species were reported as 6-9% (Smirnoff and Crawford 1983) and 1-6% (Justin and Armstrong 1987). Roots with a small stele volume relative to the whole root would have a greater cortex volume. As the aerenchyma form in the cortex (discussed in the following section 2.6.3.2), in roots with a small stele, more aerenchyma could be formed compared with roots with a large steles (McDonald et al, 2002). In general, the porosity of roots increases 1.2 to 3.0-fold when exposed to a low O₂ medium (Smirnoff and Crawford 1983; Justin and Armstrong 1987; McDonald et al. 2002). Root porosity allows O₂ to diffuse from the shoot to the growing region of the root. As an adaptive mechanism to waterlogged or hypoxic root environments, large gas spaces formed in the cortex, called aerenchyma, can result in increased porosity (Table 2.4).
Table 2.4 Porosity (% gas volume/tissue volume) in adventitious roots of selected non-wetland and wetland plant species grown in aerated or drained and stagnant or waterlogged condition. Porosity includes intercellular spaces and aerenchyma.

<table>
<thead>
<tr>
<th>Species</th>
<th>Non-wetland</th>
<th>Wetland</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Porosity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aerated or</td>
<td>Stagnant or</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Drained</td>
<td>Waterlogged</td>
<td></td>
</tr>
<tr>
<td><em>Triticum aestivum</em></td>
<td>3-6</td>
<td>13-22</td>
<td>Thomson <em>et al.</em> (1990, 1992), McDonald <em>et al.</em> (2001a)</td>
</tr>
<tr>
<td><em>Hordeum vulgare</em></td>
<td>7</td>
<td>16</td>
<td>McDonald <em>et al.</em> (2001a)</td>
</tr>
<tr>
<td><em>Secale cereale</em></td>
<td>3</td>
<td>9</td>
<td>McDonald <em>et al.</em> (2001a)</td>
</tr>
<tr>
<td><em>Zea mays</em></td>
<td>4</td>
<td>13</td>
<td>Drew <em>et al.</em> (1985)</td>
</tr>
<tr>
<td><em>Critesion marinus</em></td>
<td>14</td>
<td>25</td>
<td>McDonald <em>et al.</em> (2001a)</td>
</tr>
<tr>
<td><em>Lophopyrum turcicum</em></td>
<td>5</td>
<td>16</td>
<td>McDonald <em>et al.</em>, (2001a)</td>
</tr>
<tr>
<td><em>Lophopyrum elongatum</em></td>
<td>8</td>
<td>14</td>
<td>McDonald <em>et al.</em>, (2001a)</td>
</tr>
<tr>
<td><em>Phragmites australis</em></td>
<td>44</td>
<td>52</td>
<td>Justin and Armstrong (1987)</td>
</tr>
<tr>
<td><em>Rumex palustris</em></td>
<td>24</td>
<td>42</td>
<td>Visser <em>et al.</em> (2000)</td>
</tr>
</tbody>
</table>

2.6.3.2 Aerenchyma formation

Aerenchyma in root tissue comprise interconnected longitudinal gas-filled channels connected to the air at the root-shoot junction. Aerenchyma is not restricted to wetland species but is also wide-spread amongst non-wetland species when the root system is exposed to waterlogging (Justin and Armstrong 1987). However, there are non-wetland species that do not form aerenchyma (e.g., *Brassica napus*, Voesenek *et al.* 1999). Aerenchyma formation is a major adaptive response to waterlogging in wetland and non-wetland species (Armstrong *et al.* 1994).

Aerenchyma formation in the root cortex can be lysigenous (partial breakdown of the cortex) or schizogenous (cell separation but not collapse) (Armstrong *et al.* 1994). These two different types of aerenchyma create different amounts of porosity near root tips. For example, *Rumex palustris*, with schizogenous aerenchyma attains ~3 fold higher porosity at 5-10 mm behind the root tip than *Carex acuta* with lysigenous aerenchyma (Visser *et al.* 2000). Lysigenous aerenchyma increases the porosity further away from the root tip (*Carex acuta*, Visser *et al.* 2000; rice, Armstrong 1971; wheat, Thomson *et al.* 1992). There are no data available about the difference in functional
significance of these two types of aerenchyma. However, higher porosity near the tip presumably enhances O$_2$ supply to the root tip (suggested by Visser et al. 2000).

Aerenchyma formation is widespread in wetland and non-wetland species (Justin and Armstrong 1987). Wetland plants like rice possess aerenchyma even in well-aerated conditions, although aerenchyma formation is further enhanced by waterlogged conditions (Armstrong 1971; Colmer et al. 1998; McDonald et al. 2002). In contrast, non-wetland species predominantly lack aerenchyma in well-aerated conditions (Armstrong et al. 1994). However, aerenchyma form when the root system is exposed to soil waterlogging or O$_2$-deficient media (e.g., maize, Drew et al. 1979; wheat, Trought and Drew 1980b and barley, Benjamin and Greenway 1979). Thus, aerenchyma formation is inducible in both wetland and non-wetland species when exposed to waterlogging or O$_2$-deficient media.

Table 2.5 Aerenchyma % at 50 mm behind the tip in adventitious root cross sections of non-wetland and wetland species grown in stagnant agar, N$_2$-flushed solution, or in waterlogged soil

<table>
<thead>
<tr>
<th>Species</th>
<th>Aerenchyma % in root cross section</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stagnant agar</td>
<td>N$_2$ flushed</td>
</tr>
<tr>
<td><em>Triticosecale</em> cv. Muir</td>
<td>13</td>
<td>31</td>
</tr>
</tbody>
</table>

n.d.=not determined

Aerenchyma volumes differ depending on the method of imposing O$_2$-deficient conditions in the root environment (Table 2.5). Wheat grown in waterlogged soil or in stagnant agar nutrient solution formed 3-fold more aerenchyma in adventitious roots compared with plants grown in N$_2$-flushed nutrient solution. A similar response was observed for triticale (Thomson et al. 1992). Rice, which has high constitutive porosity, only doubled its aerenchyma volume when grown in stagnant agar compared with plants grown in aerated conditions (Table 2.5). Aerenchyma development in the adventitious roots of wheat and rice appears to respond similarly in stagnant agar nutrient solution and in waterlogged soil (Table 2.5).
Aerenchyma forms in response to low O₂ in the root environment, and is stimulated by ethylene that occurs endogenously in plant tissue or exogenously in waterlogged soil (Jackson 1985). Exogenous ethylene (0.1 - 5.0 µl l⁻¹) increased porosity in roots of wheat (Huang et al. 1997b) and maize (Konings 1982) by the formation of aerenchyma. The role of ethylene in the formation of aerenchyma was confirmed by the application of an inhibitor of ethylene action, which inhibited aerenchyma formation in maize roots (Konings 1982).

2.6.3.2 Role of aerenchyma

Aerenchyma in both wetland and non-wetland plants allows aerobic respiratory metabolism in the roots to continue by providing O₂ to the growing region when plants are growing in anaerobic soils (Konings and Lambers 1991). It reduces the diffusive resistance to longitudinal movement of gases from shoot to root and the O₂ demand per unit volume (Armstrong 1979). The crucial role of aerenchyma in improving root aeration under waterlogging conditions has been confirmed using mathematical modelling (Armstrong 1979), and was measured with platinum electrodes for selected non-wetland and wetland species (discussed below).

2.6.3.3 Oxygen movement within aerenchymatous roots

Plants growing in drained soil have access to an O₂-rich environment both above- and below-ground. O₂ movement in such plants is mainly by radial exchange in the shoots from the atmosphere and by diffusion from the soil atmosphere to the roots. However, plants that are exposed to soil waterlogging, i.e. where shoots are in the air and roots are growing in the waterlogged soil, have developed different mechanisms for O₂ movement. In such circumstances, the root environment has little or no O₂ available for radial diffusion into roots. When shoots are in the air, roots in waterlogged soil can get O₂ only by diffusion from the shoot by longitudinal gas transport. Inside the root, there are then two types of diffusion, lateral and longitudinal (Armstrong 1979).
Longitudinal diffusion of $O_2$ takes place through aerenchyma and intercellular spaces, and is generally gas-phase diffusion (Armstrong 1979). It is rapid as gas-phase diffusion is $10^4$ times faster than liquid-phase diffusion (Armstrong 1979). The longitudinal diffusion of $O_2$ determines the maximum penetration of roots into anaerobic substrates (Armstrong 1979). However, the diffusion rate depends on the root porosity (% volume of gas space, *i.e.* intercellular space and aerenchyma), respiratory demand by the cells ($O_2$ consumed by cells) and the environment (temperature and microbial activity) (Armstrong 1979). Considering all these parameters, longitudinal diffusion of $O_2$ or the length of a root growing into $O_2$-free media can be calculated using equations developed from Fick's laws of diffusion (Armstrong 1979).

The fundamental differential equation for $O_2$ diffusion is attributed to Fick's second law (Armstrong 1979). The planar diffusion along a tube with a uniform radius is the analogue of the linear gas transport and can be calculated through the following equation (Armstrong 1979):

$$\text{Diffusion rate} = \frac{Q}{t} = DA \left(\frac{C_0-C_i}{l}\right)$$  \hspace{1cm} (eq. i)

Here, $Q$ = moles of $O_2$

$t$ = time in seconds

$D$ = diffusion coefficient of the diffusate in the medium in $m^2 s^{-1}$

$A$ = cross sectional area in $m^2$

$l$ = diffusion path length $m$

$C_0$ = $O_2$ concentration at the source (root-shoot junction) in mol $m^{-3}$

$C_1$ = $O_2$ concentration at the root tip in mol $m^{-3}$

However, in roots, the circumstances are different as the root tortuosity may reduce gas diffusivity. However, with increasing porosity, the value of tortuosity will approach unity (Armstrong 1979). The tortuosity factor is expressed as $\tau < 1.0$ unless in an ideal linear situation, and porosity is expressed as fractional porosity $\varepsilon$ of the cross-section of the root (Armstrong 1979). In addition, there are other factors like radial leakage of $O_2$ to the surrounding environment and respiratory demand by the cells which also limit the $O_2$ concentration inside the root that determine the maximum diffusion path length.
within a root ($l$). From the following equation maximum length of a root in an O$_2$-deficient medium with O$_2$ supply to the apex solely dependent on internal O$_2$ diffusion can be calculated (Armstrong 1979):

$$l = \sqrt{2D_e \tau (C_0-C_1)/M}$$  \hspace{1cm} (eq. ii)

Here, $l$ = maximum length m

$D_e$ = diffusion coefficient m$^2$s$^{-1}$

$C_0$-$C_1$ = difference of O$_2$ concentration between root-shoot junction and root tip mol m$^{-3}$

$M$ = rate of O$_2$ uptake mol m$^{-3}$ s$^{-1}$

The equation (i) assumes no radial leakage of O$_2$ and (ii) uptake of O$_2$ by the cells is uniform along the pathway (Armstrong 1979). The assumptions that are used for the above equation, in fact never apply to a growing situation, i.e. respiration will never be uniform along the root length as apical respiration rates will always be faster than elsewhere, porosity will vary with length and, in O$_2$ free media, there is always some leakage of O$_2$ through the root wall (Armstrong 1979). However, considering the limitations of the equation, it still helps to illustrate the principle of aeration in roots of waterlogged plants.

### 2.6.3.4.2 Lateral diffusion

O$_2$ movement through the individual cell and across porous tissues is mainly via lateral diffusion (Armstrong 1979). A steep radial diffusion gradient is a characteristic feature of the non-porous epidermal layer and stele. By contrast, a shallow profile generally occurs in the cortex (Armstrong et al. 1994). The lateral diffusion of O$_2$ could take place in two ways: 1) the radial diffusion of O$_2$ from soil to root and 2) radial O$_2$ loss from root to soil. The latter situation occurs in waterlogged or hypoxic situations, as there is a steep gradient of O$_2$, high concentrations inside the root and a low concentration in the root environment (Armstrong 1979).
Soil conditions, root porosity, cell wall permeability and respiratory activity are the controlling factors for radial O$_2$ loss (ROL) (Armstrong 1971). O$_2$ in the gas spaces (i.e. intercellular spaces and aerenchyma) in the roots is available for the consumption of the adjacent cells, diffuses towards the root tip (longitudinally), or diffuses to the soil, as in waterlogged soils, a steep O$_2$ concentration gradient exists between roots and the soil. The amount of O$_2$ leakage from the roots largely depends on the internal O$_2$ concentration (as affected by porosity and respiration) (Armstrong, 1971) and the presence or absence of a barrier to ROL (Armstrong 1971; Colmer et al. 1998). ROL in waterlogged soils limits maximum length that roots can attain (Armstrong 1979) as this reduces O$_2$ movement to the root tip. However, ROL helps to reduce phytotoxin accumulation as a result of the O$_2$ deprivation (Armstrong et al. 1994) by creating an aerobic micro-environment in the rhizosphere.

Different methods have been used for the measurement of ROL from the roots, reviewed by Colmer (2003). The available methods for the measurement could be divided into, i. qualitative and ii. quantitative methods. For qualitative measurements, Armstrong and Armstrong (1988) and Armstrong et al. (1992) have described the use of methylene-blue in experiments with *Phragmites australis*, as methylene-blue becomes colourless when reduced and turns blue when oxidised. Data from redox measurements taken from waterlogged pots with plants, and pots without plants, can also give an estimate of ROL from root systems (Justin and Armstrong 1987). For quantitative measurements on individual roots and from particular positions along the roots, root-sleeving O$_2$ electrodes are employed as described by Armstrong (1964, 1979). With the root-sleeving O$_2$ electrodes, measurements are taken keeping the shoot in the air and the root system in 0.05-0.1% (v/v) deoxygenated agar. ROL in plants can vary considerably according to their habitat and the tolerance to waterlogging (as discussed in the following section). It also varies within the same roots of a plant depending on the position along the root (e.g., rice, Armstrong 1971). Therefore, to assess the ROL pattern of a plant, quantitative measurements are essential.

ROL from roots differs between species and even cultivars. In rice, different cultivars had different amounts of ROL in deoxygenated agar nutrient solution (Colmer 2003).
McDonald et al. (2002), working with three dryland grass species and seven wetland grass species found a wide range of variation (<1 to 750 nmol m\(^{-2}\) s\(^{-1}\) at 10 mm behind the root tip) in the amount of ROL from the roots. Amongst the non-wetland crop species, variations in ROL from the adventitious roots have been reported by McDonald et al. (2001a, Table 2.6). The growth medium of the plant also has an effect on the amount of ROL from the adventitious roots (McDonald et al. 2001a). The wetland species *Critesion marinum* showed similar values of ROL (300-375 nmol m\(^{-2}\) s\(^{-1}\) at 10 mm behind the tip) in plants grown in waterlogged soil or in stagnant agar. By contrast, the non-wetland species *Hordeum vulgare* grown in waterlogged soil showed 2.3-fold higher ROL at 10 mm behind the root tip compared with plants grown in stagnant solution (McDonald et al. 2001a).

<table>
<thead>
<tr>
<th>Species</th>
<th>Radial O(_2) loss (nmol m(^{-2}) s(^{-1}))</th>
<th>Distance behind the root tip</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-wetland</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Triticum aestivum</em></td>
<td>81</td>
<td>179</td>
<td>McDonald (2001a)</td>
</tr>
<tr>
<td><em>Hordeum vulgare</em></td>
<td>103</td>
<td>217</td>
<td>McDonald (2001a)</td>
</tr>
<tr>
<td><em>Secale cereale</em></td>
<td>103</td>
<td>101</td>
<td>McDonald (2001a)</td>
</tr>
<tr>
<td><em>Brassica napus</em></td>
<td>0</td>
<td>0-500</td>
<td>Voesenek et al. (1999)</td>
</tr>
<tr>
<td><em>Trifolium tomentosum</em></td>
<td>42</td>
<td>167</td>
<td>Gibberd et al. (1999)</td>
</tr>
<tr>
<td><strong>Wetland</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oryza sativa</em></td>
<td>938</td>
<td>130-360</td>
<td>Armstrong (1971)</td>
</tr>
<tr>
<td><em>Oryza sativa</em></td>
<td>175</td>
<td>&lt;1</td>
<td>Colmer (2003)</td>
</tr>
<tr>
<td><em>Critesion marinum</em></td>
<td>273</td>
<td>24</td>
<td>McDonald et al. (2001a)</td>
</tr>
<tr>
<td><em>Lophopyrum turricum</em></td>
<td>115</td>
<td>198</td>
<td>McDonald et al. (2001a)</td>
</tr>
<tr>
<td><em>Lophopyrum elongatum</em></td>
<td>246</td>
<td>220</td>
<td>McDonald et al. (2001a)</td>
</tr>
</tbody>
</table>

*Primary roots

ROL varies with the position along a root axis. ROL from the roots of wetland species is generally highest near the root tip compared to other regions of the same roots (Armstrong 1967, Table 2.6). By contrast, a high rate of ROL from near the root base was observed for non-wetland species (Table 2.6). A high rate of ROL at the basal
region reduces O₂ available to diffuse to the growing region of the root (Armstrong 1979). Wetland plants like rice develop a barrier to minimise the O₂ loss in waterlogged soil from the basal zone (Armstrong 1971; Colmer et al. 1998). This allows O₂ to diffuse longitudinally towards the tip (Armstrong 1979; Colmer 2002). A high rate of ROL at the basal region of non-wetland plants may be one of the underlying causes of waterlogging intolerance (Jackson and Drew 1984).

2.6.4 Metabolic adaptation

Morphological and anatomical adaptations allow plants to grow in waterlogged soil by maintaining their aerobic metabolism. However, when a waterlogging event occurs, before a change of the morphology and anatomy of the plant takes effect, anaerobic metabolism in plants might maintain the energy level to survive in waterlogged conditions.

Plant survival in anoxic conditions depends on the ability of the root cells to maintain their energy status. In such situations, the ATP production is via glycolysis, which produces only 2 mol ATP per mol glucose converted to pyruvate and only 1 mol ATP per mol glucose converted to malate, which is considered a predominant end-product of glycolysis in plants. In contrast, in aerobic conditions, 24-36 mol ATP per mol glucose are produced, using the TCA cycle and the cytochrome electron-transport chain (Figure 2.3). Therefore, waterlogging-tolerant plants need to have faster glycolytic processes to produce enough ATP for their energy requirements (reviewed by Drew 1997), as long as substrates are available.

The energy (i.e. ATP) required in anaerobic plant tissues is generated in a glycolytic process, mainly through ethanolic fermentation (Armstrong et al. 1994). The activity of two enzymes, pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) determines the rate of production of ethanol (Fig 2.3).

There are no exact data available in the literature to suggest the relationship between PDC and/or ADH activity, and waterlogging tolerance. The wetland species rice and the non-wetland species barley showed contrasting ADH activity in root tissues (rice: 2.5 μmol mg⁻¹ protein min⁻¹; barley: 0.75 μmol mg⁻¹ protein min⁻¹) at O₂ concentrations
near zero (Wignarajah et al. 1976). However, at intermediate $O_2$ concentrations (3-12%) the barley roots showed higher ADH activity compared with rice (rice: 0.7-0.5 $\mu$mol mg$^{-1}$ protein min$^{-1}$; barley: ~1.2 $\mu$mol mg$^{-1}$ protein min$^{-1}$) (Wignarajah et al. 1976). Presence of aerenchyma might have made $O_2$ available to the rice roots and thus reduced the ADH activity in the intermediate $O_2$ concentration.

![Metabolic pathways of anaerobic carbohydrate breakdown](image)

Figure 2.3 Metabolic pathways of anaerobic carbohydrate breakdown, showing key reactions involved in fermentation. In the absence of $O_2$, pyruvate is diverted from the TCA cycle into fermentative pathways. 1. lactate dehydrogenase, 2. alanine aminotransferase, 3. pyruvate decarboxylase, 4. Alcohol dehydrogenase. Dashed lines indicate sections where the metabolic pathway is not given in detail (Modified from Gibbs and Greenway 2003).

Some crop species showed better tolerance to anoxia when a hypoxic pre-treatment was given prior to anoxia, compared with plants pre-treated with aerated solution. Hypoxically pre-treated roots of wheat showed 2-6 fold higher (Waters et al. 1991b) and roots of rice showed 2.5 fold higher (John and Greenway 1976) activity of PDC in the root tips compared with plants pre-treated with aerated solution. In the case of ADH, hypoxically pre-treated wheat showed 3.5-17 fold higher (Waters et al. 1991b), maize approximately 4-fold higher (Johnson et al. 1989) and rice 11.5 fold higher (John and Greenway 1976) activity compared with plants pre-treated with aerated solution.

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when exposed to anoxia. As ethanol is the end-product of the activity of these two enzymes (Fig 2.3), ethanol production was also found 5-fold higher in rice (John and Greenway 1976) and 2-4-fold higher in wheat (Waters et al. 1991b) in hypoxically pre-treated roots compared with untreated roots, during the first 2-4 h of anoxia. The elongation potential of the hypoxically pre-treated roots of wheat (Waters et al. 1991b) and maize (Johnson et al. 1989) were 100% after 20 h of anoxia, while roots aerated during pre-treatment failed to elongate after return to aerated conditions following 20 h of anoxia. The better performance (elongation potential) in anoxic situations by hypoxically pre-treated roots compared with roots grown in aerated solution may be positively influenced by increased PDC and/or ADH activity.

The role of ADH and PDC activity in the waterlogging tolerance of crop species is unclear. Experiments with mutant barley (lacking a functional ADH1 gene product) (Harberd and Edwards 1982), transgenic cotton (over-expression of ADH or PDC) (Ellis et al. 2000) and transgenic tobacco (over-expression of PDC) (Tadege et al. 1998) showed no difference in the waterlogging tolerance between mutant/transgenic and the wild type.

It should be noted that ATP generation via anaerobic metabolism in plants may be limited by the supply of carbohydrates. Plants without large storage organs have limited reserves to fuel anaerobic metabolism and can only rely on this as a survival mechanism until $O_2$ becomes available again so that aerobic metabolism can recommence.

2.7 Recovery from waterlogging stress

As soil may become waterlogged for short periods in the field, it is important to know the recovery ability of a crop after the waterlogging stress ceases. When recovery of crops after waterlogging has been assessed, mostly the final yield of crops has been considered (Watson et al. 1976; Belford et al. 1985; Meyer et al. 1985; Meyer and Barrs 1988; Melhuish et al. 1991). There are only few physiological data published that studied the recovery of crop species after a waterlogging or hypoxic treatment (e.g. wheat, Barrett-Lennard et al. 1988; Buwalda et al. 1988; Kuiper et al. 1994; Huang et al. 1994b; Watkin et al. 1998; lupin, Davies et al. 2000a,b).
In wheat, the ability to recover from waterlogging of soil or hypoxia differs amongst cultivars (Huang et al. 1994b; Setter and Waters 2003). Huang et al. (1994b) observed in wheat after 14 days of hypoxic treatment in hydroponics that seminal and adventitious root growth was reduced, but that after 7 days of recovery, adventitious root growth had recovered significantly. Setter and Waters (2003), in a glasshouse experiment with 14 wheat and 2 oat cultivars subjected to 28 days of soil waterlogging and subsequent recovery for 21 days found a wide variability in their recovery capacity (on the basis of shoot growth).

In a more physiological study on young wheat (15 d old), Kuiper et al. (1994) demonstrated in a hydroponic split-root experiment that at the beginning of recovery (first 6 h) from 11 days of hypoxia, nutrient (K and N) uptake rate increased 1.3 to 2.3-fold, and after 7 days of recovery nutrient uptake rate become similar to that of plants grown in aerated nutrient solution. Buwalda et al. (1988) reported for wheat that 4 days of recovery after 10 days of hypoxia led to nutrient concentrations similar to those of plants grown in aerated condition throughout the experiment. In more recent work with two wheat cultivars and one triticale cultivar (14 days old), Watkin et al. (1998) demonstrated that in 7 days of recovery after 14 days of stagnant agar treatment, seminal roots failed to regrow, but that the relative growth rate of adventitious roots was similar (90 to 96%) to plants grown in aerated nutrient solution. High concentrations (1.2 to 2-fold, depending on the plant tissue) of non-structural carbohydrate in hypoxic wheat, compared with the aerated treatment, might have allowed increased uptake of nutrients and faster growth of adventitious roots as carbohydrate concentrations became similar to those in aerobically grown plants within 24 to 48 h of transfer back to aerated solution (Barrett-Lennard et al. 1988).

Most of the studies on waterlogging have dealt with the effect on crop growth during waterlogging periods. However, waterlogging tolerance of a plant is determined by the ability to change morphologically, anatomically and metabolically during a waterlogging period as well as by its ability to rapidly grow during a recovery period when the stress is released. To date, studies on the physiological recovery of crop from waterlogging stress are scant. As a result, knowledge on physiology of recovery of plants after different durations of waterlogging is limited.
In nature, watertable depth and duration of waterlogging varies; however, there are no comprehensive data available in the literature that studied the response of wheat or any other crops in those situations. The present experiments were designed to study the response of wheat to different depths of the water table, and different durations of waterlogging as well as the recovery from the stress.

2.8 References


McDonald MP, Galwey NW, Colmer TD (2001a) Waterlogging tolerance in the tribe Triticeae: The adventitious roots of *Critesion maritimum* have a relatively high porosity and a barrier to radial oxygen loss. *Plant, Cell and Environment* **24**, 585-596.


Chapter 3

Changes in physiological and morphological traits of roots and shoots of wheat in response to different depths of waterlogging

3.1 Abstract

The growth reduction of wheat (*Triticum aestivum* L.) during and after waterlogging stress depends on the depth of water from the soil surface. In a pot experiment with three-week-old plants, soils were waterlogged for 14 days at the surface, or at 100 mm or 200 mm below the surface, and were then drained to assess recovery. A fully drained treatment kept at field capacity served as control. During waterlogging, the relative growth rate of roots decreased more than that of shoots (by 15 to 74% for roots, by 6 to 27% for shoots), and plant growth was reduced proportionally as the water level was increased. Light-saturated net photosynthesis was reduced by 70 to 80% for the two most severe waterlogging treatments, but was little affected for plants in soil waterlogged at 200 mm below the surface. The number of adventitious roots formed per stem in plants grown in waterlogged soil increased up to 1.5 times, but the number of tillers were reduced by 24 to 62%. The adventitious roots only penetrated 85-116 mm below the water level in all waterlogging treatments. Adventitious root porosity was enhanced up to 10-fold for plants grown in waterlogged soil, depending on water level and position along the roots. Porosity also increased in basal zones above the water level when the younger tissues had penetrated the waterlogged zone. Fourteen days after draining the pots, growth rates of plants where the soil had been waterlogged at 200 mm below the surface had recovered, while those of plants in the more severely waterlogged treatments had only partially recovered. These findings show that depth of waterlogging has a large impact on the response of wheat both during and after a waterlogging event so that assessment of recovery is essential in evaluating waterlogging tolerance in crops.
3.2 Introduction

Waterlogging of soil is a widespread problem in crop production (Kozlowski 1984). Waterlogging influences a series of physical, chemical and biological changes in soil that ultimately inhibit growth of waterlogging-intolerant plants. The gas exchange between soil and atmosphere almost stops as soon as the waterlogging sets in, soil microbes and plant roots use up the oxygen trapped in the soil, and the roots may become exposed to an anoxic situation (Jackson and Drew 1984). Waterlogged soils may also become rich in manganous and ferrous ions, devoid of nitrate and sulphate, and products of anaerobic microbial metabolism can accumulate. The changes reverse when waterlogging eases (Ponnampерuma 1984), although plants may suffer from oxidative stress or drought after the soil has dried (Biemelt et al. 1998).

Roots experience the effects of waterlogging first, and shoots experience the consequences. Death of seminal roots has been reported in wheat (Huang et al. 1994a; Trought and Drew, 1980a; Thomson et al. 1992) and other crop species such as barley (Limpinuntana and Greenway 1979), chickpea (Cowie et al. 1996), and lupin (Davies et al. 2000). In contrast, the formation of aerenchymatous adventitious roots is stimulated by waterlogging in some waterlogging-sensitive (e.g., wheat) and many waterlogging-tolerant species (e.g., rice, Thomson et al. 1992). Nevertheless, in wheat, nitrogen net uptake and transport are reduced by root anoxia (Trought and Drew 1980b, 1980c), resulting in leaf chlorosis and premature death of older leaves (Trought and Drew 1980b). Photosynthesis (Huang et al. 1994b, 1997), dry matter accumulation (Huang et al. 1994a,b) and final yield (Box 1986; Musgrave 1994; Musgrave and Ding 1998) are also severely reduced in waterlogging-intolerant crops like wheat.

The response of wheat to waterlogging has often been studied using drained or fully waterlogged treatments. In reality, the water level in waterlogged soil is not always at or above the soil surface. In field assessments of waterlogging intensity, use of an index has been suggested to integrate the depth of the water table and the duration of waterlogging at particular depths (SEW30, sum of excess water above 30 cm soil depth) (Sieben 1964). Knowledge on the responses of plants to different depths of waterlogging and subsequent recovery is scant. This has prompted us to conduct the present study to evaluate the effect of waterlogging at selected depths on several
physiological and growth parameters of wheat, and the recovery of these after the pots were drained.

3.3 Materials and Methods

Plant Culture

Seeds of *Triticum aestivum* L. cv. Cascades were surface sterilised with 2% commercial bleach for 2 min, washed with deionised water, and placed on moist filter paper in Petri dishes in a dark growth cabinet at 18°C. After 2 days, eight germinated seeds were sown at 10 mm depth in each of 45 pots (height 400 mm, diameter 150 mm) and transferred to a walk-in growth chamber (20 °C /15°C day/night temperature, 12 h photoperiod, light intensity of 375-490 µmol quanta m⁻² s⁻¹, PAR). The pots were made from polyvinyl chloride (PVC) tubes. Each tube was fitted with a PVC base, serving as a bottom, which was filled with a 50 mm layer of gravel. A 10 mm diameter hole was drilled in the side, 25 mm above the bottom, and connected to an open-end, transparent hose with the other end clamped to the top of the pot, so that water depth could be gauged. The pots contained 8 kg of Kojonup sand (a sandy surfaced duplex soil, pH (H₂O) = 5.8 and EC (1:5 w/v soil:water extract) = 17.2 mS m⁻¹) collected from the top 150 mm at the CSIRO Research Station at Yalanbee, Western Australia. This soil type had been used previously as substrate in waterlogging studies by Thomson *et al.* (1992).

Nutrients (µmol g⁻¹ soil): NH₄NO₃, 4.46; KH₂PO₄, 0.44; K₂SO₄, 0.92; CaCl₂·2H₂O, 1.21; MgSO₄·7H₂O, 0.09; ZnSO₄·7H₂O, 0.04; MnSO₄·H₂O, 0.07; CuSO₄·5H₂O, 0.024; H₃BO₃, 0.013; Na₂MoO₄·2H₂O, 0.008 were mixed through the soil prior to planting. While filling the pots, Pt electrodes (Patrick *et al.* 1996) were buried at 50, 150 and 250 mm depth in 4 pots. The pots were watered to field capacity each day and rotated within the growth chamber every second day to minimise the effect of different conditions at different positions. After 7 days, the plants were thinned to four per pot.

Treatments were imposed when the plants were 21 days old and the seminal roots had reached the bottom of the pot. An initial harvest of 5 pots was taken at this time. The four treatments were: 1) water level at the soil surface, 2) water level at 100 mm soil depth, 3) water level at 200 mm soil depth, and 4) fully drained soil watered daily to field capacity (control). Ten pots of each treatment were established, so that five pots
from each could be harvested after 14 days of waterlogging, and the other five pots were drained following the waterlogging and the plants allowed 14 days of recovery. Soil solution was collected after draining the pots and analysed for nitrate; loss through draining was less than 1% of the initial nitrate applied (data not shown). The pots waterlogged at the soil surface had drained to field capacity after five days, and thereafter, the soil in each pot was kept at field capacity during the recovery period.

**Measurements**

Redox potential of the soil was recorded daily with a calomel reference electrode (Eh+245 mV) and Pt electrode connected to a mV-meter. Light-saturated rates of photosynthesis and stomatal conductance were measured every one to two days for the youngest fully expanded leaves on the main stem using a LiCor 6400 photosynthesis system (LiCor, Lincoln, NE, USA), with the red/blue LED light source at an irradiance (PAR) of 2000 μmol quanta m⁻² s⁻¹. Measurements were taken on all four plants per pot, for 3 to 4 pots per treatment. Initially, only measurements of control plants and plants grown in fully waterlogged soil or soil waterlogged at -100 mm were taken. For the second half of the experiment, measurements were taken from plants in all treatments. Relative changes in chlorophyll concentration were measured on the same leaves, 50 mm from the base, using a chlorophyll meter (Minolta SPAD 502, Osaka, Japan). SPAD meter readings were converted to chlorophyll a+b concentrations on a leaf area basis after calibration against measurements of pigments extracted from leaf tissue using methanol (Wellburn, 1994) and determined in a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). Growth of the youngest expanding leaf on the main stem was measured every day with a ruler.

Harvests were taken at the time treatments were imposed (initial), after 14 days of waterlogging and after 14 days of recovery. Tillers on each plant were counted, and harvested plants were divided into leaves, stems and roots. For the first two harvests, roots were divided into seminal and adventitious roots. The lengths of the longest seminal and adventitious roots were measured, and the numbers and lengths of adventitious root main axes were recorded. Leaf areas were determined using a leaf area meter (LiCor 3000, Lincoln, NE, USA). All plant tissues were dried for two days at
70°C, and dry weights were measured. Total nitrogen concentrations of the dried plant samples were determined using an auto-analyser (Leco CHN 1000, St. Joseph, MI, US).

Root porosity

In a second experiment, plants were grown following the above protocol and treatments, but were waterlogged at the specified depths for either 14 or 28 days. Redox potential was measured in three pots. In this experiment, porosity (% gas space/volume) of adventitious roots was determined for different segments of adventitious roots, using the method described by Raskin (1983) and the equations as modified by Thomson et al. (1990). Briefly, adventitious roots were lined up at the tips and cut into 50 mm segments with distance behind the tip to yield different-aged root tissues for a spatial analysis of porosity along the roots. The presence of aerenchyma was confirmed using light microscopy of hand cross-sections taken at selected positions along the adventitious roots.

In a companion experiment using the same protocol, but in which plants were exposed only to control or fully waterlogged treatments, the concentration of total non-structural carbohydrates in the youngest fully expanded leaves was measured using the anthrone method (Fales 1951). Also, carbon-isotope discrimination in the youngest fully expanded leaves (i.e. leaves that had grown during the stress and recovery periods) at days 14 and 28 was determined on a mass spectrometer (Europa Scientific ANCA System, Cheshire, UK).

Calculations

The relative growth rate (RGR) based on leaf area, shoot dry weight, or root dry weight, and net nitrogen uptake rate were calculated over the periods of the experiment from day ‘0’ to day 14 and from day 14 to day 28 using the equations of Radford (1967). Shoot mass ratio (SMR) and root mass ratio (RMR) were calculated as the ratio of shoot or root dry mass to total plant dry mass, respectively.
Statistical analysis

Data were analysed by calculating means, standard errors and one-way analysis of variance (ANOVA), where appropriate, using the SPSS 8.0 for Windows statistical software (SPSS, Chicago, IL, USA). Significant differences were stated where P<0.05.

3.4 Results

Redox potential

Redox potential in waterlogged soil (at various soil depths) dropped from about 600 mV to about 200 mV over the first 14 days and then to 40 mV after 28 days of waterlogging. After draining the pots, the redox potential in the upper soil layers increased almost immediately, whereas the redox potential at 250 mm soil depth only started to increase five days after draining the pots. Redox potential along the soil column recovered to the control value of 600 mV within 10 days of the pots being drained.

![Figure 3.1 Dry mass of different plant parts (leaves, stems, seminal and adventitious roots) of wheat after 14 days of waterlogging at different depths (A) and after 14 days of recovery (B). Drained = fully drained soil; WI-200 = waterlogged at -200 mm soil depth; WI-100 = waterlogged at -100 mm soil depth; WI = waterlogged at the soil surface. At the commencement of treatments, average leaf dry weight was 0.067 ± 0.005 g, average stem dry weight was 0.032 ± 0.002 g, and average root dry weight was 0.042 ± 0.004 g. (total roots, adventitious root had just started emerging). Standard error bars refer to whole plant dry mass (n = 5). Bars with different letters are significantly different (p<0.05).](image-url)
Shoot growth

After 14 days, the plants exposed to the waterlogging treatments had substantially reduced dry matter production when compared with the drained controls (Figure 3.1A). Dry matter was decreased with increasing water level (Figure 3.1A). The reduction of shoot growth was associated with several factors. Firstly, plants grown in waterlogged soil produced fewer tillers. For plants grown in soils waterlogged at the surface or at 100 mm or 200 mm below the surface, the numbers of tillers were reduced by 62%, 45% and 24%, respectively (Table 3.1). Secondly, while the duration of leaf elongation remained largely unchanged, the rate of leaf elongation was reduced where the soil had been waterlogged at the surface or at -100 mm, resulting in shorter leaves (Figure 3.2). Thirdly, senescence of older leaves was greatly accelerated by the waterlogging treatments. In plants grown in soil waterlogged at the surface or at -100 mm, the oldest three leaves had senesced, leaving the plants with only three functional leaves on the main stem, whereas in plants grown in soil waterlogged at -200 mm, only the oldest leaf had senesced (data not shown). At the same time, all leaves on the main stem of control plants were still green and turgid.

Table 3.1 Tiller numbers of wheat after 14 days of waterlogging at different depths and after 14 days of recovery. Drained = fully drained soil; Wl-200 = waterlogged at 200 mm soil depth; Wl-100 = waterlogged at 100 mm soil depth; Wl = waterlogged at the soil surface. The average tiller number per plant at the commencement of treatments was 1.4 ± 0.1. Means are followed by standard errors (n = 5). Values within a column followed by different letters are significantly different (p<0.05.).

<table>
<thead>
<tr>
<th>14 days waterlogging</th>
<th>14 days recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tillers per plant</td>
</tr>
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<td>Drained</td>
<td>7.1±0.3</td>
</tr>
<tr>
<td>Wl-200</td>
<td>5.4±0.3</td>
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<tr>
<td>Wl-100</td>
<td>3.9±0.2</td>
</tr>
<tr>
<td>Wl</td>
<td>2.7±0.2</td>
</tr>
</tbody>
</table>

As older leaves senesced in the plants grown in waterlogged soil, total leaf area was reduced to a greater extent than was leaf mass (Table 3.2). Leaf area of plants grown in soil waterlogged at the surface was reduced by 68%. In plants grown in soil waterlogged at -100 mm or -200 mm, leaf area was reduced by 53% and 33%,
respectively (Table 3.2). Also, the RGR on a leaf area basis was affected more severely than the RGR of shoots on a mass basis (Table 3.3).

After the 14 day recovery period, control plants were still larger than the plants where the soil had previously been waterlogged at the surface or at -100 mm; there was no significant difference in biomass between the control plants and those where the soil had previously been waterlogged at -200 mm (Figure 3.1B). Also, total leaf area of plants grown in soil that had been waterlogged at -200 mm reached control values. The RGR for shoot and root dry matter as well as for leaf area did not significantly differ between control treatments and those waterlogged at -200 mm (Table 3.3). Total leaf area of plants where the soil had been waterlogged at -100 mm or at the surface had not fully recovered after 14 days (Table 3.2).

![Figure 3.2 Cumulative length of leaf 6 of wheat waterlogged for 14 days at different depths and subsequently drained (recovery). Treatments were: drained (○), waterlogged at -200 mm soil depth (●), waterlogged at -100 mm soil depth (▲) and waterlogged at the soil surface (●). Blades of leaf 6 were visible from day 8 after the onset of waterlogging. Symbols represent means ± standard errors (n = 5). During the linear growth phase of leaves, it was possible to calculate daily leaf elongation rates from the slope of the growth curves. Leaf elongation rates (mm d⁻¹) for leaf 6 were: Drained, 30.7; Waterlogged to -200 mm, 30.4; Waterlogged to -100 mm, 22.3; Waterlogged to surface, 18.8.](image)

Root growth

Seminal root death occurred in plants grown in waterlogged soil, as the longest seminal roots in plants exposed to all waterlogging treatments for 14 days were shorter than they were at the beginning of the treatments (Table 3.4). At the beginning of the treatments,
Table 3.2 Leaf area and biomass allocation to shoots (shoot mass ratio, SMR) and roots (root mass ratio, RMR) of wheat after 14 days of waterlogging at different depths and after 14 days of recovery. Drained = fully drained soil; WI-200 = waterlogged at 200 mm soil depth; WI-100 = waterlogged at 100 mm soil depth; WI = waterlogged at the soil surface. At the commencement of treatments, the average leaf area per plant was 24.8 ± 1.7 cm². SMR was 0.70 ± 0.02 g g⁻¹ and RMR was 0.30 ± 0.02 g g⁻¹. Means are followed by standard errors (n = 5). Values within a column followed by different letters are significantly different (p<0.05).

<table>
<thead>
<tr>
<th>14 days of waterlogging</th>
<th>14 days of recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf area (cm²)</td>
<td>SMR (g g⁻¹)</td>
</tr>
<tr>
<td>Drained</td>
<td>87.9±4.9</td>
</tr>
<tr>
<td>WI-200</td>
<td>58.9±2.8</td>
</tr>
<tr>
<td>WI-100</td>
<td>41.4±2.7</td>
</tr>
<tr>
<td>WI</td>
<td>27.7±1.6</td>
</tr>
</tbody>
</table>

Table 3.3 Relative growth rate (RGR) of wheat on a leaf area, shoot dry weight, or root dry weight basis during 14 days of waterlogging at different depths and during 14 days of recovery. Drained = fully drained soil; WI-200 = waterlogged at 200 mm soil depth; WI-100 = waterlogged at 100 mm soil depth; WI = waterlogged at the soil surface. Means are followed by standard errors (n = 5). Values within a column followed by different letters are significantly different (p<0.05).

<table>
<thead>
<tr>
<th>14 days of waterlogging</th>
<th>14 days of recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGR (leaf area) (m² m⁻² d⁻¹)</td>
<td>RGR (shoot) (mg g⁻¹ d⁻¹)</td>
</tr>
<tr>
<td>Drained</td>
<td>0.090±0.004</td>
</tr>
<tr>
<td>WL-200</td>
<td>0.061±0.003</td>
</tr>
<tr>
<td>WL-100</td>
<td>0.036±0.005</td>
</tr>
<tr>
<td>WL</td>
<td>0.007±0.004</td>
</tr>
</tbody>
</table>
Table 3.4 Adventitious and seminal root traits of wheat after 14 days of waterlogging at different depths. Drained = fully drained soil; WI-200 = waterlogged at 200 mm soil depth; WI-100 = waterlogged at 100 mm soil depth; WI = waterlogged at the soil surface. At the commencement of treatments, the longest seminal root was 358 ± 15 mm, the number of adventitious roots per plant was 2.4 ± 0.2, the number of adventitious roots per stem was 1.0 ± 0.0, the longest adventitious root was 72 ± 12 mm, and the total length of adventitious root main axes was 101 ± 17 mm. Means are followed by standard errors (n = 5). Values within a column followed by different letters are significantly different (p<0.05.).

<table>
<thead>
<tr>
<th>Longest seminal root (mm)</th>
<th>Number of adventitious roots per plant</th>
<th>Number of adventitious roots per stem</th>
<th>Longest adventitious root (mm)</th>
<th>Total length of adventitious root main axes (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drained</td>
<td>402a ± 21</td>
<td>14.0b ± 0.9</td>
<td>1.7b ± 0.1</td>
<td>467a ± 20</td>
</tr>
<tr>
<td>WI-200</td>
<td>276b ± 14</td>
<td>11.4b ± 0.6</td>
<td>1.8b ± 0.1</td>
<td>285b ± 13</td>
</tr>
<tr>
<td>WI-100</td>
<td>260b ± 6</td>
<td>11.2b ± 0.5</td>
<td>2.3b ± 0.1</td>
<td>210b ± 8</td>
</tr>
<tr>
<td>WI</td>
<td>242b ± 14</td>
<td>10.4b ± 0.5</td>
<td>2.8c ± 0.1</td>
<td>116d ± 7</td>
</tr>
</tbody>
</table>

Table 3.5 Nitrogen concentrations on a dry weight basis in leaves, stems and roots of wheat after 14 days of waterlogging at different depths and after 14 days of recovery. Drained = fully drained soil; WI-200 = waterlogged at 200 mm soil depth; WI-100 = waterlogged at 100 mm soil depth; WI = waterlogged at the soil surface. At the commencement of treatments, nitrogen concentrations of leaves, stems and roots were 4.6 ± 0.0, 4.1 ± 0.1 and 2.9 ± 0.0 mmol g⁻¹, respectively. Means are followed by standard errors (n = 3). Values within a column followed by different letters are significantly different (p<0.05.).

<table>
<thead>
<tr>
<th>Leaf (mmol N g⁻¹)</th>
<th>Stem (mmol N g⁻¹)</th>
<th>Root (mmol N g⁻¹)</th>
<th>Leaf (mmol N g⁻¹)</th>
<th>Stem (mmol N g⁻¹)</th>
<th>Root (mmol N g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drained</td>
<td>3.5a ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>2.2 ± 0.0</td>
<td>3.5a ± 0.1</td>
<td>2.5a ± 0.1</td>
</tr>
<tr>
<td>WI-200</td>
<td>3.0a ± 0.3</td>
<td>2.3 ± 0.2</td>
<td>2.3 ± 0.0</td>
<td>3.7a ± 0.0</td>
<td>2.9a ± 0.0</td>
</tr>
<tr>
<td>WI-100</td>
<td>2.2b ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>2.4 ± 0.0</td>
<td>3.4b ± 0.1</td>
<td>2.9b ± 0.1</td>
</tr>
<tr>
<td>WI</td>
<td>2.5c ± 0.1</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.1</td>
<td>3.2b ± 0.0</td>
<td>2.9b ± 0.0</td>
</tr>
</tbody>
</table>

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Figure 3.3 Distribution of adventitious roots of wheat into main axis length classes after 28 days of waterlogging at different depths. A: waterlogged at soil surface. B: waterlogged at -100 mm soil depth. C: waterlogged at -200 mm soil depth. Adventitious roots of plants in drained pots were not restricted by the water level and reached the bottom of the pots (data not shown). Error bars represent standard errors (n = 3). Dotted lines indicate the depth of water from the soil surface in the pots.

Plants had few (2.4 ± 0.2) and short adventitious roots; thus most of the adventitious roots emerged during the treatment period. The number of adventitious roots per stem increased with the water level (Table 3.4), but as tiller numbers (and thus stem numbers) decreased (Table 3.1), plants from all treatments had similar numbers of adventitious roots per plant (Table 3.4). The length of the longest adventitious roots decreased with increasing water levels (Table 3.4). The total length of longest adventitious roots
decreased with increasing water levels (Table 3.4). The total length of main axes of all adventitious roots per plant was also reduced by waterlogging; lengths were reduced by 73%, 58% and 39% for plants grown in soil waterlogged at the soil surface, -100 mm or -200 mm respectively (Table 3.4).

The maximum penetration of the adventitious roots below the water level was similar for plants grown at the three levels of waterlogging. Roots penetrated 85-116 mm into the waterlogged zone after 14 days of waterlogging (Table 3.4). The longest adventitious roots observed after 14 days of waterlogging did not increase significantly in length after 28 days of waterlogging. For this reason, only the adventitious root length distributions after 14 days of waterlogging are shown (Figure 3.3). The increase in root numbers in the longer classes for all waterlogged treatments demonstrates that the longest adventitious roots had reached the maximum penetration into the waterlogged zones. After the recovery period, adventitious roots in all treatments reached the bottom of the pots (data not shown).

While increasing water level decreased RGR of both roots and shoots, that of roots was affected 2.7-fold more severely, at least for plants grown in soils waterlogged at the surface (Table 3.3). For this reason, shoot mass ratio (SMR) of plants in waterlogged soils increased and root mass ratio (RMR) decreased during 14 days of waterlogging (Table 3.2). During the recovery phase, root RGR of plants from previously waterlogged treatments exceeded that of the controls. This appeared to be at the expense of shoot growth, for which the RGR was still severely reduced during the recovery phase in plants from previously waterlogged treatments. Thus, by the end of the recovery period, the ratio of dry matter in shoot v. roots of plants previously grown in waterlogged soil had returned to control values (Table 3.2).

Root porosity

Adventitious root porosity increased with increasing water level (Figures 3.4A and B). After 14 days of waterlogging, root porosity was increased for plants grown in soil waterlogged at the surface or at -100 mm, and in the tip region of plants from soil waterlogged at -200 mm (Figure 3.4A). After 28 days of waterlogging, root porosity had increased further in plants grown in soil waterlogged at the surface, but was similar for
the two sample times for plants grown in soil waterlogged at −100 or −200 mm (Figure 3.4B). The porosity of the adventitious roots was increased by the formation of lysigenous aerenchyma (Figure 3.5 and data not shown).

![Image](image_url)

Figure 3.4 Distribution of porosity along the main axis of adventitious roots of wheat (A) after 14 days and (B) after 28 days of waterlogging at different depths. The greatest distance behind the root tip for each treatment is close to the root-shoot junction. Treatments were: drained (○), waterlogged at -200 mm soil depth (●), waterlogged at -100 mm soil depth (▲) and waterlogged at the soil surface (■). Symbols represent means ± standard errors (n = 3).

**Tissue nitrogen concentrations**

Total leaf nitrogen concentration was reduced by 29% in plants grown in soil waterlogged at the surface, by 37% where the soil was waterlogged at -100 mm and by 14% where the soil was waterlogged at -200 mm (Table 3.5). Fourteen days after draining the pots, the plants grown in soil previously waterlogged at -200 mm had fully recovered, but in the plants previously waterlogged at the soil surface leaf nitrogen concentration was still lower than in controls (Table 3.5). Stem nitrogen concentration
was not significantly affected by waterlogging treatments (Table 3.5). Root nitrogen concentration was little affected by the treatments (Table 3.5). The net nitrogen uptake rate per unit root mass was not statistically different for plants in the different waterlogging treatments (data not shown).

Photosynthesis

Within 24 h after waterlogging the pots, net rates of light-saturated photosynthesis in the third leaf (the youngest fully expanded leaf at that stage of plant growth) of plants grown in soil waterlogged at the surface had decreased by 25%, compared with control plants (Figure 3.6A). After five days of waterlogging at the soil surface, photosynthesis in the third leaf had declined to zero, and photosynthesis in the fourth leaf was reduced by 82%, compared with control plants (Figure 3.6A). For plants grown in soils waterlogged at -100 mm, rates of photosynthesis showed a similar response to that of the plants grown in soils waterlogged at the surface. In contrast, even after 14 days of treatments, there was only a small difference between the rates of photosynthesis of plants grown in soils waterlogged at -200 mm or control plants.
Figure 3.6 (A) Net photosynthesis and (B) stomatal conductance of wheat during 14 days of waterlogging at different depths and during 14 days of subsequent recovery. Data are for the youngest fully developed leaves. The readings were taken at saturating light (2000 µmol m\(^{-2}\) s\(^{-1}\)), a leaf temperature of 20°C, leaf/air vapour pressure deficit of 1 ± 0.1 kPa and at ambient CO\(_2\) concentrations. Treatments were: drained (○), waterlogged at -200 mm soil depth (●), waterlogged at -100 mm soil depth (▲) and waterlogged at the soil surface (●). Symbols represent means ± standard errors (n = 3 or 4).

During the first three days after draining the pots, recovery of photosynthesis of the youngest fully expanded leaf of plants previously waterlogged at the soil surface or those waterlogged at -100 mm was relatively slow. However, after 14 days of recovery, photosynthesis of the youngest fully expanded leaf of the plants previously exposed to the three waterlogging treatments had in each case recovered almost to the control value.

Stomatal conductance of the third leaf in plants grown in soil waterlogged at the surface declined by 32% within 24 h of treatment and dropped to approximately 20% of control
values for both the third or fourth leaf by the fifth day of treatment (Figure 3.6B). Where the soil was waterlogged at -100 mm, plants showed a similar response to those from fully waterlogged soil, whereas at the end of the treatments, there was no significant difference between plants grown in soil waterlogged at -200 mm and the control plants.

Recovery of stomatal conductance followed a similar pattern to that of rates of photosynthesis (Figure 3.6B). The effect of stomatal conductance on photosynthesis can be assessed by calculating the intercellular CO₂ concentration (cᵢ). In plants grown in waterlogged soil, cᵢ initially increased and then was maintained near control values throughout the experiment (data not shown). In a companion experiment, we found that

Table 3.6 Carbon-isotope discrimination (δ¹³C) and total non-structural carbohydrate concentrations in the youngest fully developed leaves on the main stem of wheat after 14 days in drained soil (Drained) or waterlogged at the soil surface (Wl) (leaf 4) and after 14 days of recovery (leaf 6). Means are followed by standard errors (n = 3). Values within a column followed by different letters are significantly different (p<0.05.).

<table>
<thead>
<tr>
<th></th>
<th>14 days waterlogging</th>
<th>14 days recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ¹³C (%)</td>
<td>TNC (mg g⁻¹)</td>
</tr>
<tr>
<td>Drained</td>
<td>-33.2 ± 0.1</td>
<td>101 ± 4</td>
</tr>
<tr>
<td>Wl</td>
<td>-29.7 ± 0.5</td>
<td>312 ± 71</td>
</tr>
</tbody>
</table>

Table 3.7 Chlorophyll (Chl a+b) concentration in the youngest fully developed leaves on the main stem of wheat after 14 days of waterlogging to different depths (leaf 4) and after 14 days of recovery (leaf 6). Drained = fully drained soil; Wl-200 = waterlogged at 200 mm soil depth; Wl-100 = waterlogged at 100 mm soil depth; Wl = waterlogged at the soil surface. Means are followed by standard errors (n = 5). Values within a column followed by different letters are significantly different (p<0.05.).

<table>
<thead>
<tr>
<th></th>
<th>14 days waterlogging (g Chl a+b m⁻²)</th>
<th>14 days recovery (g Chl a+b m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drained</td>
<td>0.45 ± 0.01</td>
<td>0.43 ± 0.01</td>
</tr>
<tr>
<td>Wl-200</td>
<td>0.43 ± 0.01</td>
<td>0.43 ± 0.01</td>
</tr>
<tr>
<td>Wl-100</td>
<td>0.38 ± 0.01</td>
<td>0.39 ± 0.01</td>
</tr>
<tr>
<td>Wl</td>
<td>0.25c ± 0.01</td>
<td>0.36 ± 0.01</td>
</tr>
</tbody>
</table>

the δ¹³C value of the youngest fully expanded leaf was higher in plants grown in soil waterlogged at the surface for 14 days, and it remained higher in the newly developed leaves even after a further 14 days of recovery, suggesting a lower cᵢ than calculated from gas exchange data (Table 3.6). In plants from the same experiment, the total non-
structural carbohydrate concentration (TNC) was higher in the youngest fully expanded leaf of plants in soil waterlogged at the surface for 14 days compared with control plants. After 14 days of recovery, the TNC concentrations in the young leaves of plants from waterlogged soil and control plants were similar to each other, but only slightly lower than the TNC concentration after 14 days of waterlogging (Table 3.6).

Chlorophyll concentration in the youngest fully developed leaves was also affected by the various waterlogging treatments. Plants in soil waterlogged at the surface or at -100 mm showed a significant decline relative to control plants during the waterlogging as well as during the recovery phase (Table 3.7). There was no difference between control plants and plants in soil waterlogged at -200 mm throughout the experiment.

3.5 Discussion

Root growth

Waterlogging (present study) or anoxia (Trought and Drew 1980a) severely damaged the seminal root system of wheat. Newly emerged adventitious roots might form the bulk of the functional root system in wheat plants where the soil has been waterlogged for more than several days (Thomson et al. 1992; Watkin et al. 1998; McDonald et al. 2001). The number of adventitious roots produced per stem (main stem plus tillers) increased with waterlogging intensity (Table 3.4). However, as tiller numbers were reduced with increasing waterlogging intensity, plants experiencing waterlogging finally possessed fewer adventitious roots than the drained plants (Table 3.4). Nevertheless, the number of adventitious roots per unit shoot weight compared with that of control plants increased with the level of waterlogging (data not shown).

Porosity of the adventitious roots increased for wheat when grown in waterlogged soil (Figure 3.4 A, B) to values similar to the 15% reported by Thomson et al. (1990) in adventitious roots of wheat grown in N2-flushed solution. Other non-wetland species, like maize, also show higher root porosity (15-20%) under conditions of O2 deficiency compared to well aerated controls (Jackson et al. 1985). Ethylene is reputed to be the signal involved in the induction of aerenchyma, as found for maize by Jackson et al. (1985). In the present study, adventitious root porosity was highest in plants from the pots waterlogged at the soil surface, but porosity also increased in roots from soils that
were partially waterlogged. Porosity tended to be greatest in those parts of the root system that grew into the waterlogged soil zone, but root porosity was also enhanced in those parts of the same roots above the waterlogged soil. Root cross sections confirmed that lysigenous aerenchyma were associated with the increased porosity (Figure 3.5). To our knowledge, there are no other reports on the induction of aerenchyma in adventitious roots where only part of the root system experiences O₂-deficient conditions. Ethylene, or its precursor ACC, produced near the root tips may move back and provide the signal for the formation of lysigenous aerenchyma (Atwell et al. 1988).

In this study, adventitious root penetration was restricted to a depth of approximately 100 mm below the water level. These lengths were very similar to the maximum adventitious root length observed by Thomson et al. (1992) for wheat cv. Gamenya when grown in the same soil type waterlogged at the surface. Theoretically, adventitious roots of wheat with 12-20% porosity (Figure 3.4) should be able to extend 132-171 mm into an anoxic medium at 20°C when solely supported by internal O₂ diffusion, assuming a rate of O₂ uptake of 2.34 mmol O₂ m⁻³ s⁻¹ (Thomson et al. 1992) and not allowing for radial O₂ loss along the root (Armstrong 1979; Thomson et al. 1992; Watkin et al. 1998). The penetration of roots into the waterlogged soil zone by plants in the present experiments were less than predicted using Armstrong’s model (Armstrong 1979). Similar observations were also made for a different cultivar by Thomson et al. (1992). The reasons could be higher respiration rates than assumed, or probably that radial loss of O₂ along the roots was substantial. The slightly deeper penetration of adventitious roots below the water level after 28 days of waterlogging at the soil surface as compared with soils waterlogged at −100 or −200 mm may be ascribed to the increased average porosity in roots growing in fully waterlogged soil. After extended waterlogging (28 days), roots grown in fully waterlogged soil had greater porosity in the tip region than after 14 days of waterlogging (Figure 4). Had the root porosity increased along the whole root length from 12 to 20 %, then this would have theoretically allowed, according to Armstrong’s (1979) model, 44 mm of further root penetration into the soil. However, the basal region of the adventitious roots had the same porosity after 14 or 28 days of waterlogging. Studies of the sources of O₂ in roots growing in partially waterlogged soils (such as the shoot base or the aerobic soil layers above the water level in the pot) would be required to fully understand the restrictions to root growth in partially waterlogged soils.
Leaf growth and tillering

The reduction of shoot growth of wheat as a function of waterlogging intensity was caused by reduced tillering, reduced leaf elongation rates and accelerated senescence of older leaves. Sojka et al. (1975) and Trought and Drew (1980c) also reported reduced tiller production for waterlogged wheat, and Trought and Drew (1980c) also found that the elongation rate of wheat leaves was reduced by waterlogging. As the duration of leaf elongation was little affected (Figure 3.2), slower elongation rate resulted in shorter leaves. There are no data as to whether the reduced leaf elongation rates are caused by decreased cell division and/or cell elongation. Shorter leaves and fewer tillers in plants from waterlogged soils are ultimately reflected in a reduced shoot RGR (Table 3.3).

Nitrogen status

Decreased shoot nutrient concentrations in wheat grown in waterlogged substrate have previously been described by Trought and Drew (1980b). In our experiment, leaf nitrogen concentrations were decreased by 15% to 30% when soils were waterlogged at different depths, but root nitrogen concentrations were not affected (Table 3.5). This indicates that transport of nitrogen from roots to shoots may have been impaired in plants growing in waterlogged soil. Buwalda et al. (1988) observed a decrease of net nitrogen transport to the shoot when wheat was growing in hypoxic nutrient solution, but the plants subsequently recovered after transfer to an aerated solution. Possibly, roots of wheat develop stelar anoxia during waterlogging, which may inhibit loading of nutrients into the translocation stream in the xylem. Stelar anoxia has been described for maize roots (Thomson and Greenway 1991), and the inhibitory effect of this condition on ion transport in the xylem of roots has been demonstrated (Gibbs et al. 1998). As a result of restricted nitrogen supply to the shoots in plants growing in waterlogged soil, older leaves senesced earlier, presumably so that nitrogen and other nutrients could be remobilised and used for the growth of new leaves (Trought and Drew 1980b).

Photosynthesis and stomatal conductance

Net light-saturated rates of photosynthesis in leaves of plants growing in soils waterlogged at the surface were slightly reduced after one day, and severely reduced
after two (or more) days of waterlogging (Figure 3.6A). Reductions in net photosynthesis of the youngest fully emerged leaf of wheat during times of waterlogging have been suggested to be caused by reductions in leaf water potential, stomatal conductance, amount or activity of photosynthetic enzymes and chlorophyll (Huang et al. 1994a,b). As rates of photosynthesis were already severely reduced before decreases in chlorophyll in leaves measured in the present work, it is unlikely that decreased leaf chlorophyll concentrations caused the reduced rates of photosynthesis in the young leaves of waterlogged wheat. In our study, reduced rates of photosynthesis coincided with reduced stomatal conductance. However, intercellular CO$_2$ concentrations in the leaves were not significantly reduced by the waterlogging treatments, and were even increased after prolonged waterlogging. To conclude that consequently, stomatal conductance was not limiting net rates of photosynthesis, one has to assume a uniform distribution of stomatal conductance across the leaf. However, in many cases, stomatal closure as induced by ABA treatments has been shown to be patchily distributed across the leaf (Downton et al. 1988; Terashima et al. 1988), in which case the intercellular CO$_2$ concentration as conventionally calculated according to von Caemmerer and Farquhar (1981) is overestimated. The increased δ$^{13}$C of leaves grown under waterlogged conditions (Table 3.6) suggests that the average intercellular CO$_2$ concentration in these leaves was indeed reduced (Farquhar et al. 1982). Thus, decreased stomatal conductance appears to account at least partly for the reduced photosynthetic rates in our experiment. Alternatively, a reduced CO$_2$ transfer conductance from the substomatal cavities to the sites of carboxylation might be responsible for the increased δ$^{13}$C values of waterlogged leaves (Evans and von Caemmerer 1996), thereby limiting photosynthesis. Such a situation has been observed for salt-stressed spinach leaves (Delfine et al. 1998).

Another possible factor in the reduction of photosynthesis in plants grown in waterlogged soil is the accumulation of carbohydrates in leaves, which could suggest a feedback inhibition of photosynthesis (Plaut et al. 1987). There may have been a lower sink demand for carbohydrates due to the severely reduced rates of growth. In our experiment, non-structural carbohydrate concentration in leaves was greatly increased after 14 days of waterlogging (Table 3.6). This may thus have contributed to the reduction in photosynthesis. Similarly, a rapid increase of leaf carbohydrates after waterlogging has been reported for barley (Limpinuntana and Greenway 1979) and for alfalfa (Castanguay et al. 1993). However, leaves of control and waterlogged plants
after 14 days of recovery in our experiment showed high photosynthetic rates despite relatively high leaf carbohydrate concentrations, suggesting that factors other than merely feedback inhibition may also contribute to the reduction in rates of photosynthesis in wheat grown in waterlogged soil.

Relationship of waterlogging damage at different depths and capacity to recover

Sieben (1964) suggested that waterlogging intensity be measured using the concept of SEW$_{30}$ (sum of excess water above 30 cm soil depth). The SEW$_{30}$ index assumes that crop yield is negatively affected when the water table rises to within 30 cm below the soil surface. To make comparisons between different depths and durations of waterlogging, this concept assumes that the adverse effects of waterlogging on plants increase linearly with water table rise above 30 cm and the number of days of duration of waterlogging. SEW$_{30}$ integrates the water table depth and the time the water table is maintained at a given depth; for example, a soil that is waterlogged for 5 days at the soil surface or for 15 days at 20 cm soil depth both result in a SEW$_{30}$ of 150 cm days. McFarlane et al. (1989) demonstrated the use of the SEW$_{30}$ concept in field situations with transient waterlogging and perched water tables and raised caution about its empirical nature, as the effects of waterlogging with similar SEW$_{30}$ values varied with site and crop species investigated.

Our results suggest that one must apply caution using the SEW$_{30}$ concept. After 14 days of waterlogging, dry matter production of wheat did appear to be reduced proportionally to water depth. However, when 14 days of recovery were allowed, wheat biomass at final harvest was no longer affected proportionally to the previous level of waterlogging. Plants previously in soil waterlogged at the surface or at 100 mm below the surface had similar mass after the recovery period. They could only partially recover after 14 days, as there was a smaller capacity for assimilation in plants from these two treatments due to photosynthesis rates only recovering slowly and fewer photosynthetically active leaves being available at the end of the waterlogging. Also, carbon was preferentially allocated to root growth during the recovery period. In contrast, waterlogging at 200 mm below the soil surface had only minor negative effects on wheat. Carbon assimilation was never as impaired as for the two more severe waterlogging treatments and plants recovered rapidly after the waterlogging event.
Moreover, these plants had lost only a few leaves to premature senescence, and already had a larger root system when compared with plants from the two more severe waterlogging treatments. Although care should be taken when extrapolating from data of vegetative growth to crop yield, we suggest that it is crucial to consider the recovery of plants after waterlogging in experiments that aim to determine the waterlogging tolerance of crop species.

3.6 References


Chapter 4

Short-term waterlogging has long-term effects on the growth and physiology of wheat

4.1 Abstract

The effect of different durations of waterlogging and subsequent drainage is described for three-week-old wheat plants. In a pot experiment, plants were subjected to waterlogging to the soil surface for 0, 3, 7, 14, 21 or 28 days, and then drained to allow recovery for up to 25 days. During waterlogging, the seminal root system stopped growing. Adventitious roots grew to a maximum length of about 150 mm. Leaf nitrogen concentration was severely decreased by waterlogging. When waterlogged pots were drained, seminal root mass did not recover to control values, even when waterlogging lasted only 3 or 7 days, due to death of existing apices and no initiation of new lateral roots. In contrast, adventitious roots resumed elongation after drainage. By the end of the experiment, shoot mass remained 2-3 fold lower in plants from all waterlogged treatments compared with continuously drained controls, due to lower tiller numbers and shorter final leaf lengths in previously waterlogged plants. The results demonstrate that even short periods (e.g., 3 days) of waterlogging have considerable long-term effects on the growth of young wheat plants.
4.2 Introduction

Excess rainfall can lead to waterlogging of soils, the duration of which varies greatly depending on the amount of rain, evapotranspiration and soil structure. During waterlogging, the gas exchange between soil and air decreases, as gas diffusion in water is decreased 10,000-fold. O$_2$ in the soil is rapidly depleted, and the soil might become hypoxic or anoxic within a few hours. Moreover, some waterlogged soils become rich in Mn$^{2+}$ and Fe$^{2+}$, devoid of NO$_3^-$ and SO$_4^{2-}$, and anaerobic microbial metabolites may accumulate. These effects become more pronounced during prolonged periods of waterlogging, lasting weeks or months (Ponnamperuma 1984). Even short-term, transient waterlogging (lasting hours or days) can have considerable effects on growth and yield of dryland crops (Leyshon and Sheard 1974; Jackson 1979; Sharma and Swarup 1988; Melhuish et al. 1991).

The effects on wheat of waterlogging in soil, or of exposure to low O$_2$ concentrations in culture solutions, are well documented. Seminal roots cease to grow, whereas adventitious root formation is promoted (Trought and Drew 1980a), but the final lengths of these adventitious roots are restricted (Armstrong 1979; Thomson et al. 1992; McDonald et al. 2001; Malik et al. 2001). Ultimately, both root and shoot dry mass production are reduced (Trought and Drew 1980a; Huang et al. 1994; Malik et al. 2001). Leaf elongation rates and final leaf size decrease (Malik et al., 2001) and leaf senescence is accelerated (Trought and Drew 1980a). The non-structural carbohydrate concentrations in leaves and roots increase (Barrett-Lennard et al. 1988; Malik et al. 2001), and nutrient concentrations decrease in shoots, but can increase in roots (Trought and Drew 1980b; Huang et al. 1995).

The severity of the effects of waterlogging depend on the growth stage of the plant (Leyshon and Sheard 1974; Watson et al. 1976; Orchard and Jessop 1984; Davies et al. 2000), the depth of the water level (Malik et al. 2001), and the duration of the waterlogging event (Jackson 1979). For wheat, waterlogging for periods as short as one or two days can decrease final yield (Sharma and Swarup 1988; Melhuish et al. 1991), although other authors have reported no yield penalty for wheat after four days of waterlogging (Meyer and Barss 1988). Some dryland crop species tolerate waterlogging for three days in the vegetative stage without any, or with little, yield penalty (e.g., sunflower and sorghum: Orchard and Jessop 1984), while others only recover partially.
from one day of waterlogging (e.g., barley: Leyshon and Sheard 1974; pea: Jackson 1979).

To date, with few exceptions (Thomson et al. 1992; Malik et al. 2001) most experiments investigating the effects of waterlogging on plant growth have evaluated responses during waterlogging, or, in cases when recovery was assessed, only the effects on final yield were considered (Watson et al. 1976; Belford et al. 1985; Meyer et al. 1985; Meyer and Barrs 1988; Melhuish et al. 1991). Our previous experiments have confirmed the importance of growth assessments during a recovery period to determine the performance of plants subjected to waterlogging (Malik et al. 2001). Knowledge of the physiology of recovery after varying durations of waterlogging is scant. Here, we assess the damage caused by different lengths of exposure to waterlogging and the physiological basis of recovery from this stress. The present study evaluates the growth, tissue N and non-structural carbohydrate concentrations in wheat, during waterlogging as well as during subsequent recovery when the soil was drained.

4.3 Materials and Methods

Plant culture

Seeds of *Triticum aestivum* L. cv. Cascades were surface sterilised with 2% commercial bleach for 2 min, washed with deionised water, and placed on moist filter paper in Petri dishes in a dark growth cabinet at 18°C. After 2 days, eight germinated seeds were sown at 10 mm depth in each of 63 pots (height 400 mm, diameter 150 mm) and transferred to a walk-in growth chamber (20°C/15°C day/night temperature, 12 h photoperiod, irradiance of 375-490 μmol quanta m⁻² s⁻¹, PAR). The pots were polyvinyl chloride (PVC) tubes fitted with a PVC base. A 10 mm diameter hole was drilled in the side, 25 mm above the bottom, and connected to an open-end, transparent hose so that pots could be waterlogged from the bottom after which the open end of the tube was clamped to the top of the pot. The pots contained a 50 mm layer of gravel above which 8 kg of Kojonup sand were filled. Kojonup sand is the top soil of a sandy surfaced duplex soil, pH = 5.8 and EC = 17.2 mS m⁻¹ (both determined for 1:5 w/v soil water extract), collected from the top 150 mm at the CSIRO Research Station at Yalanbee, Western Australia. This soil type has been used previously as substrate in waterlogging
studies by Thomson *et al.* (1992) and Malik *et al.* (2001). Nutrients (μmol g⁻¹ soil): NH₄NO₃, 4.46; KH₂PO₄, 0.44; K₂SO₄, 0.92; CaCl₂·2H₂O, 1.21; MgSO₄·7H₂O, 0.09; ZnSO₄·7H₂O, 0.04; MnSO₄·H₂O, 0.07; CuSO₄·5H₂O, 0.024; H₃BO₃, 0.013; Na₂MoO₄·2H₂O, 0.008 were mixed through the soil prior to planting. Pots were watered to field capacity each day, and rotated within the growth chamber every second day to minimise the effect of different conditions at different positions. After 7 days, the plants were thinned to four per pot.

Treatments were imposed when the plants were 21 days old, at which time seminal roots had reached the bottom of the pots. An initial harvest of three pots was taken at this time. Forty-five pots were then waterlogged to the soil surface, while 15 remained as drained controls watered daily to field capacity.

The start of the treatment was considered as day '0'. Harvests were taken after 0, 3, 7, 14, 21 and 28 days. On day 3, three control pots and three waterlogged pots were harvested, and 12 pots were drained to be harvested on days 7, 14, 21 and 28. This increased the number of treatments harvested from two on day 3 to three on day 7. Similarly, after three pots of each treatment were harvested on day 7, nine more waterlogged pots were drained to be harvested on days 14, 21, and 28 and so forth. Thus, the number of treatments increased with each successive harvest, resulting in six experimental treatments at the final harvest on day 28. When draining the pots, the leached soil solution was collected to detect possible nitrate losses due to leaching through drainage, and this was only 1% of the initial nitrate application. The waterlogged pots came back to field capacity within 5 days. These pots were subsequently kept at field capacity.

**Measurements**

The length of the youngest expanding leaf on the main stem was measured every day using a ruler. Tiller numbers and leaf numbers on the main shoot were recorded at each harvest. Harvested plants were divided into individual leaf blades, stems (i.e. leaf sheaths), seminal roots and adventitious roots. Adventitious root numbers and the lengths of main axes were recorded. Leaf area was determined using a leaf area meter (LiCor 3000, Lincoln, NE, USA). All plant tissues were immediately frozen in liquid N₂ and stored at -80°C until they were freeze-dried. The freeze-dried plant tissues were
then placed in an oven at 65°C for 24 hours to permanently deactivate enzymes, prior to
determination of non-structural carbohydrates.

The relative changes in chlorophyll concentrations were measured on individual leaves
of the main stem, 50 mm from the sheath/blade junction, using a hand-held chlorophyll
meter (Minolta SPAD 502, Osaka, Japan). The measurements were taken on the day
before each harvest. SPAD meter readings were converted to chlorophyll a+b
concentrations on a leaf area basis after calibrating against measurements of pigments
extracted from freeze-dried leaf tissue using methanol (Wellburn, 1994) and determined
in a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). From this calibration, we
derived the relationship: \( \text{Chla+b in g m}^{-2} = 0.009 \text{ ABS (SPAD meter reading)} + 0.0021 \) \( (r^2 = 0.74) \).

Total non-structural carbohydrates (TNC) were extracted from leaves, stems and roots
by boiling tissue samples for 1 h in deionised water. The extracts were collected by
filtering through Whatman no.1 filter paper. The TNC concentration (mg g\(^{-1}\) dry weight)
in the extracts was then determined with anthrone reagent following the procedure of
Fales (1951) using a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). Freeze-
dried plant samples were also analysed for total N using an auto-analyser (Leco CHN
1000, St. Joseph, MI, US) against EDTA and rice flour as standards. Data for plant dry
mass and plant N concentrations were calculated on a structural dry matter basis (i.e.
data were corrected for tissue TNC concentrations).

**Root pruning experiment**

In a second experiment, the potential of the seminal roots to branch as affected by
developmental stage and by damage to the apical root region was determined. Seeds of
*T. aestivum* cv. Cascades were surface sterilised as described above, germinated and
transferred to 20L hydroponic tanks under identical temperature and light conditions as
for the soil experiment. The composition of the nutrient solution followed that of
McDonald *et al.* (2001), and was renewed weekly. Briefly, concentrations of
macronutrients were (mol m\(^{-3}\)): N, 1.25; K, 1.0; Ca, 0.38; Mg, 0.1; S, 0.48; P, 0.05.
Each tank contained 12 plants. At three stages of plant development (2.5, 3.5 and 3.7
Haun stage (Haun, 1973); corresponding to plant ages of 13, 17, and 20 days,
respectively), all emerging adventitious roots were removed, and the seminal roots were pruned back to 50 mm length from the root-shoot junction. Nine plants were pruned at each stage. For three plants, all visible lateral roots in the top 50 mm were also removed. The numbers of lateral roots attached to the top 50 mm of each seminal root main axis were counted for unpruned roots of control plants and for the two pruned treatments at the time of pruning and seven days thereafter.

4.4 Results

Seminal root growth

The seminal roots of plants in waterlogged soil stopped growing, and even some loss of seminal root mass was recorded towards the end of the treatment period (Figure 4.1A). The seminal root system of plants grown in waterlogged soils for the shorter periods (3 or 7 days) resumed growth after the pots were drained, but the structural mass of the seminal root system of these plants never exceeded one-third to one-half of that of the fully drained control plants during the 21 to 25 day recovery period (Figure 4.1A). Plants that were grown in waterlogged soil for 14 days or longer lost the ability to resume seminal root growth during a 14 day recovery period, i.e. there was no significant increase in seminal root dry mass after the pots were drained (Figure 4.1A).

Adventitious root growth

At the beginning of the treatments, plants had just started to produce adventitious roots. For plants grown in drained soil, the adventitious root system accounted for one-third of the total root mass at the end of the 28 d treatment period (Figure 4.1B). The longest adventitious roots of plants grown in drained soil exceeded 400 mm in length after 21 days of treatment (Figure 4.2A), whereas waterlogging restricted the maximum length of adventitious roots to about 150 mm (Figure 4.2B). After the waterlogged soil was drained, adventitious roots resumed elongation; the longest adventitious roots increased from 150 mm to 300 mm within one week after draining soil that had been waterlogged for 21 days (Figure 4.2C). At the same time, the length distribution of adventitious roots after 28 days of continuous waterlogging was essentially the same as after 21 days of waterlogging (data not shown). Thus, while the maximum length of adventitious roots
was restricted during waterlogging, these adventitious roots resumed elongation after draining. The dry mass of the adventitious root system of plants previously in waterlogged soil (up to 14 days) subsequently reached values of drained controls after seven days of recovery (Figure 4.1B).

**Figure 4.1** Structural dry mass of (A) seminal roots, (B) adventitious roots and (C) shoots of wheat after different durations of soil waterlogging and subsequent drainage. The treatments are fully drained (— O —), 3 days waterlogged and drained for up to 25 days (—■—), 7 days waterlogged and drained for up to 21 days (—▲—), 14 days waterlogged and drained for up to 14 days (—▼—), 21 days waterlogged and drained for up to 7 days (—●—), continuously waterlogged for 28 days (— ● —). Continuously waterlogged pots and drained pots are represented by solid lines, and the pots that were previously waterlogged and then drained are represented by dotted lines. Symbols represent means ± standard error (n = 3).
Figure 4.2 Distribution of adventitious roots of wheat into main axis length classes. (A) fully drained control after 21 days (average number of adventitious roots per plant: 25.1 ± 0.5; mean of 3 replicates); (B) waterlogged to the soil surface for 21 days (average number of adventitious roots per plant: 15.5 ± 2.0; mean of 3 replicates); (C) waterlogged to soil surface for 21 days, and allowed recovery for 7 days (average number of adventitious roots per plant: 16.4 ± 1.4; mean of 3 replicates). Error bars represent standard errors (n = 3). At day 0, an average of four adventitious roots were present per plant and more than 60% of the adventitious roots were shorter than 50 mm, and the longest adventitious root was 160 mm long. After 28 days, control plants and plants under continuous waterlogging had similar root length distributions as shown in panels A and B, respectively.

The number of adventitious roots per stem increased in plants in waterlogged soils. This increase was evident even after seven days of waterlogging and continued throughout the treatment period (Figure 4.3). However, the total number of adventitious roots per plant was always higher in those grown in drained soil (data not shown). This was due
to up to four-fold greater tiller numbers in plants in drained soil (Figure 4.4). Plants exposed to soil waterlogging for short periods (3 or 7 days) produced new tillers after the soil was drained, but tiller numbers never reached those in the plants grown continuously in drained soil (Figure 4.4).

![Figure 4.3 Number of adventitious roots per stem of wheat after different durations of waterlogging and subsequent recovery. The treatments are fully drained (---O---), 3 days waterlogged and drained for up to 25 days (---●---), 7 days waterlogged and drained for up to 21 days (---▲---), 14 days waterlogged and drained for up to 14 days (---▼---), 21 days waterlogged and drained for up to 7 days (---◆---), continuously waterlogged for 28 days (---•---). Continuously waterlogged pots and drained pots are represented by solid lines, and the pots that were previously waterlogged and then drained are represented by dotted lines. Symbols represent means ± standard error (n = 3).]

**Shoot growth**

Shoot mass of plants in drained soil at the end of the experiment exceeded that of plants in waterlogged soil two- to three-fold (Figure 4.1C). For plants recovering from waterlogging, shoot growth during early recovery tended to be less than that of plants in continuously waterlogged soil, as the plants invested more biomass in root growth during this period; e.g., RGR of shoots during the first 7 days of recovery from 7 days of waterlogging was 0.091 g g⁻¹ d⁻¹, while the shoot RGR under continuous waterlogging for the equivalent period was 0.106 g g⁻¹ d⁻¹. Shoot dry mass did not recover fully even from short periods of waterlogging, even after long periods of drainage (25 and 21 days, respectively). At the end of the 28 days of treatments, the shoot dry mass of plants that had been in waterlogged soil for 3 days was only 43% of the shoot dry mass of plants grown in continuously drained soil (Figure 4.1C). Plants
that were grown continuously in waterlogged soil for 28 days had 72% less shoot dry mass than the plants always in drained soil (Figure 4.1C). Reduced shoot dry mass was due to lower tiller numbers, slower leaf extension rates, shorter leaves and accelerated leaf senescence (see below). Plants grown in waterlogged soil for 3 or 7 days, and then allowed 25 or 21 days of recovery, had approximately 40% fewer tillers than plants in continuously drained soil (Figure 4.4). Plants grown in waterlogged soil for 14, 21 or 28 days had approximately 70% fewer tillers than control plants at final harvest, i.e. after 14, 7 or 0 days of recovery, respectively (Figure 4.4).

![Figure 4.4](image)

**Figure 4.4** Number of tillers per plant after different durations of waterlogging and subsequent drainage. The treatments are fully drained (— O —), 3 days waterlogged and drained for up to 25 days (—■—), 7 days waterlogged and drained for up to 21 days (—▲—), 14 days waterlogged and drained for up to 14 days (—▼—), 21 days waterlogged and drained for up to 7 days (—●—), continuously waterlogged for 28 days (—•—). Continuously waterlogged pots and drained pots are represented by solid lines, and the pots that were previously waterlogged and then drained are represented by dotted lines. Symbols represent means ± standard error (n = 3).

Final leaf lengths and leaf areas of plants grown in waterlogged soil were less than those of plants in continuously drained soil (Figure 4.5, Table 4.1). The duration of leaf elongation was similar for all treatments (Figure 4.5). Also, leaves on the main stem of plants in waterlogged soil appeared later than those of plants in drained soil (Figure 4.5). These effects also occurred for leaves that developed after the waterlogging stress was relieved (Figure 4.5).

Senescence of older leaves did not occur during short periods of waterlogging (3 or 7 days). However, the first two leaves for these treatments senesced faster after four days...
Table 4.1  Maximum leaf area (mm²) reached by individual leaves of wheat after different durations of waterlogging (WL) and subsequent recovery. Values represent means ± standard errors (n=3). Values within a column followed by different letters are significantly different (P<0.05). At the beginning of the experiment, leaf 3 was fully developed, and leaf 4 was elongating.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Leaf 4</th>
<th>Leaf 5</th>
<th>Leaf 6</th>
<th>Leaf 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (drained 28d)</td>
<td>1212 ± 89</td>
<td>1601 ± 91</td>
<td>1819 ± 79</td>
<td>1885 ± 58</td>
</tr>
<tr>
<td>3 days WL (drained 25d)</td>
<td>1116 ± 26</td>
<td>1211 ± 83</td>
<td>1263 ± 83</td>
<td>1175 ± 71</td>
</tr>
<tr>
<td>7 days WL (drained 21d)</td>
<td>1003 ± 71</td>
<td>1192 ± 28</td>
<td>1231 ± 86</td>
<td>1336 ± 30</td>
</tr>
<tr>
<td>14 days WL (drained 14d)</td>
<td>1003 ± 71</td>
<td>1068 ± 17</td>
<td>1026 ± 30</td>
<td>850 ± 16</td>
</tr>
<tr>
<td>21 days WL (drained 7d)</td>
<td>1003 ± 71</td>
<td>973 ± 87</td>
<td>1007 ± 46</td>
<td>624 ± 100</td>
</tr>
<tr>
<td>28 days WL (drained 0d)</td>
<td>1003 ± 71</td>
<td>973 ± 87</td>
<td>887 ± 74</td>
<td>177 ± 46</td>
</tr>
</tbody>
</table>
of recovery than did plants grown in continuously drained soil, as indicated by decreasing leaf chlorophyll concentrations (Figure 4.6, leaves 1 and 2). Continuous waterlogging accelerated senescence of older leaves, as illustrated by the reduced chlorophyll concentrations in leaves of plants growing in waterlogged soil (Figure 4.6). As a result, plants had only three functional leaves on the main stem after 28 days of continuous waterlogging, while control plants had six functional leaves on the main stem.

![Figure 4.5 Cumulative length of leaves 4 to 7 of wheat after different durations of waterlogging and subsequent drainage. Leaf 1 is the oldest leaf on the plant. The treatments are fully drained (--- O ---), 3 days waterlogged and drained for up to 25 days (---■---), 7 days waterlogged and drained for up to 21 days (---▲---), 14 days waterlogged and drained for up to 14 days (--- ▼ ---), 21 days waterlogged and drained for up to 7 days (--- ● ---), continuously waterlogged for 28 days (--- • ---). Symbols represent means ± standard error (n = 3).]

**Dry matter partitioning**

The loss or decrease in seminal root growth resulted in severely decreased total root mass in plants from all waterlogged treatments. Waterlogging reduced the root mass ratio, as root growth was initially more severely affected than was shoot growth (Figure 7). For transiently waterlogged plants, root mass ratio was restored to control values during the first week after relieving the stress (Figure 4.7). During this time, the RGR of
adventitious roots of previously waterlogged plants exceeded root RGR of the drained control plants, irrespective of the duration of waterlogging (Figure 4.8). In contrast, RGR of the drained controls always exceeded that of the waterlogged treatments (Figure 4.8).

*Nitrogen status*

There was a gradual decrease in green leaf total N concentration with age/size in plants continuously grown in drained soil (Figure 4.9). Plants grown in waterlogged soil, however, showed a more rapid decrease in green leaf N concentration (Figure 4.9). Leaf N concentration decreased significantly within three days of waterlogging, and continued to decrease throughout the experimental period (Figure 4.9). Draining the soil resulted in no further decrease in leaf N concentrations in these plants, and values approached those in control plants of similar size during the recovery period (Figure 4.9). The determination of N only in the green leaves is conservative; if the senesced leaves of plants in waterlogged treatments are taken into account, the overall leaf N concentration would decrease even further than that of green leaves (data not shown). The N concentration of stems (data not shown) showed a similar pattern to that of the leaves. In contrast, the N concentration in adventitious roots usually increased relative to controls during waterlogging, while that of seminal roots decreased (Table 4.2).

Table 4.2 Nitrogen concentration (mmol g\(^{-1}\) dry mass) in adventitious and seminal roots of wheat grown in drained or waterlogged soil. Values represent means ± standard errors (n=3). Comparison were made between treatments for the same root type. Values within a row pair followed by different letters are significantly different (P<0.05.).

<table>
<thead>
<tr>
<th>Days</th>
<th>Adventitious root</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drained</td>
<td>Waterlogged</td>
<td>Drained</td>
<td>Waterlogged</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.2(^a)±0.1</td>
<td>2.8(^b)±0.0</td>
<td>3.1(^a)±0.1</td>
<td>2.8(^b)±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3.3(^a)±0.1</td>
<td>3.8(^b)±0.1</td>
<td>2.8(^a)±0.1</td>
<td>2.3(^b)±0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>2.3(^a)±0.0</td>
<td>2.6(^b)±0.1</td>
<td>2.6(^a)±0.0</td>
<td>2.2(^a)±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>2.0(^a)±0.0</td>
<td>2.5(^b)±0.1</td>
<td>2.4(^a)±0.0</td>
<td>2.2(^b)±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>1.6(^a)±0.1</td>
<td>2.3(^b)±0.0</td>
<td>2.3(^a)±0.3</td>
<td>2.2(^a)±0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.6 Chlorophyll a+b concentration of leaves 1 to 5 of wheat in response to different durations of waterlogging and drainage. Leaf 1 is the oldest leaf on the plant. The treatments are fully drained (— O —), 3 days waterlogged to soil surface and drained for 25 days (— ■ —) and waterlogged to soil surface for 28 days (— ● —). Symbols represent means ± standard error (n = 3).

Figure 4.7 Root mass ratio of wheat plants waterlogged for different durations, and subsequently allowed drainage. The treatments are fully drained (— O —), 3 days waterlogged and drained for up to 25 days (— ■ —), 7 days waterlogged and drained for up to 21 days (— ▲ —), 14 days waterlogged and drained for up to 14 days (— ▼ —), 21 days waterlogged and drained for up to 7 days (— ● —), continuously waterlogged for 28 days (— ● —). Continuously waterlogged pots and drained pots are represented by solid lines, and the pots that were previously waterlogged and then drained are represented by dotted lines. Symbols represent means ± standard error (n = 3).
Accumulation of total non-structural carbohydrates (TNC)

During the development of plants in drained soil, TNC concentrations increased gradually for the first three weeks of the experiment, and then doubled during the last week (Figure 4.10) in all plant tissues (data shown for leaves only). In contrast, leaf TNC accumulated soon after plants were waterlogged; leaf TNC concentration was doubled in plants grown in waterlogged soil for 3 days compared with plants in drained soil (Figure 4.10). For plants in waterlogged soil, leaf TNC concentrations continued to increase during the whole experimental period. During recovery, leaf TNC concentration started to decline soon after the waterlogging was removed in plants exposed to relatively short periods of waterlogging (3 or 7 days). Stems, seminal roots and adventitious roots showed similar patterns of TNC accumulation over time and due to treatments as shown for the leaves (data not shown).

Figure 4.8 Relative growth rate of (A) adventitious roots and (B) shoots of wheat plants waterlogged for different durations, and subsequently allowed drainage. The treatments are fully drained (— O —), 3 days waterlogged and drained for up to 25 days (—■—), 7 days waterlogged and drained for up to 21 days (— ▲ —), 14 days waterlogged and drained for up to 14 days (— ▼ —), 21 days waterlogged and drained for up to 7 days (— ● —), continuously waterlogged for 28 days (— ● —). Continuously waterlogged pots and drained pots are represented by solid lines, and the pots that were previously waterlogged and then drained are represented by dotted lines.
Figure 4.9 Nitrogen concentration in green leaves (taken from tillers) of wheat plants after different durations of waterlogging and subsequent drainage. The treatments are fully drained (— O  —), 3 days waterlogged and drained for up to 25 days (— ■ —), 7 days waterlogged and drained for up to 21 days (— ▲ —), 14 days waterlogged and drained for up to 14 days (— ▼ —), 21 days waterlogged and drained for up to 7 days (— ● —), continuously waterlogged for 28 days (— • —). Continuously waterlogged pots and drained pots are represented by solid lines, and the pots that were previously waterlogged and then drained are represented by dotted lines. Symbols represent means ± standard error (n = 3). Values are plotted against structural dry matter of the plants (note the log scale) in order to account for plant size effects. Each point represents the mean for one harvest.

Figure 4.10 Concentration of total non-structural carbohydrates (TNC) in leaves of wheat after different durations of waterlogging and subsequent drainage. The treatments are fully drained (— O  —), 3 days waterlogged and drained for up to 25 days (— ■ —), 7 days waterlogged and drained for up to 21 days (— ▲ —), 14 days waterlogged and drained for up to 14 days (— ▼ —), 21 days waterlogged and drained for up to 7 days (— ● —), continuously waterlogged for 28 days (— • —). Continuously waterlogged pots and drained pots are represented by solid lines, and the pots that were previously waterlogged and then drained are represented by dotted lines. Symbols represent means ± standard error (n = 3). Values are plotted against dry matter of the plants (note the log scale) in order to account for plant size effects. Each point represents the mean for one harvest.
Table 3  Number of lateral roots on the main axis of the seminal roots of wheat at different stages of development. The main axis was pruned at 50 mm from the root-shoot junction at three different stages of development. In one treatment, laterals were left intact, and in a second treatment, all visible laterals were removed. After 7 days growth in aerated nutrient solution, the number of laterals was recorded. Values represent means ± standard errors (n=3). No increase in lateral root numbers was recorded for unpruned control plants (data not shown).

<table>
<thead>
<tr>
<th>Day after sowing</th>
<th>Main stem Haun stage</th>
<th>Seminal root length</th>
<th>Initial</th>
<th>7 days after pruning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Existing laterals</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>left intact when</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>main axis pruned</td>
</tr>
<tr>
<td>13</td>
<td>2.5</td>
<td>160-200 mm</td>
<td>34.0±3.5</td>
<td>33.7±3.3</td>
</tr>
<tr>
<td>17</td>
<td>3.5</td>
<td>260-275 mm</td>
<td>42.0±3.1</td>
<td>40.2±4.8</td>
</tr>
<tr>
<td>20</td>
<td>3.7</td>
<td>300-340 mm</td>
<td>27.1±3.3</td>
<td>28.6±4.4</td>
</tr>
</tbody>
</table>
Root pruning experiment

To assess whether the loss of the seminal root apices, such as may occur if apices die in seminal roots of plants in waterlogged soil, could be compensated for by increased production of lateral roots from the seminal root bases, we conducted a pruning experiment where the apical regions of seminal roots in hydroponically grown wheat were removed at different developmental stages. Pruning of the seminal roots at 50 mm below the root/shoot junction did not affect the number of laterals present on this part of each seminal root main axis; no new laterals were formed by 7 days after pruning. However, the laterals already present had branched and increased in length during the seven days after the main axis was pruned. Moreover, when all lateral roots along the upper 50 mm of the seminal root main axis were also removed at the same time the lower part of the main axis was excised, only very few new laterals were formed during the subsequent 7 days (Table 4.3). This indicates the limited capacity of the seminal roots to overcome the loss of their apical regions.

4.5 Discussion

The present study clearly demonstrated that the longer-term growth of wheat was severely reduced by a short-term (3 d) exposure to waterlogging. During short-term waterlogging, no adverse effects were visible for the shoots, but after-effects of waterlogging on shoot growth were clearly visible during the recovery phase; this included accelerated leaf senescence (Figure 4.6), decreased final leaf lengths (Figure 5), smaller final leaf area (Table 4.1) and decreased tiller production (Figure 4.4); together resulting in decreased shoot mass. At the end of the experiment, plants exposed to waterlogging for 3 days and then allowed 25 d recovery were closer in appearance to plants under continuous waterlogging than to the plants grown in continuously drained soil. The negative effects of waterlogging on leaf function and growth also became more pronounced in the period following waterlogging for some other dryland species (e.g., barley: Leyshon and Sheard 1974; pea: Jackson 1979; and maize: Purvis and Williamson 1972). For wheat, some studies have measured the effect of various durations of waterlogging on yield (Meyer and Barrs 1988; Melhuish et al. 1991). However, studies that closely monitored growth after waterlogging are scarce (Thomson et al. 1992; Malik et al. 2001). Roots experience the direct effects of waterlogging, and
shoots reflect these effects. Here, we will discuss the effects of waterlogging on root growth and possible reasons for the poor recovery of wheat from short-term waterlogging.

**Root growth**

Seminal root growth of wheat ceased soon after waterlogging was imposed (Figure 4.1A). Seminal root mass did not recover over the durations evaluated in this experiment (14 days) when waterlogging exceeded seven days. Similarly, Trought and Drew (1980a) have shown for 11-day-old wheat plants that there was very little increase in seminal root dry mass during 15 days of continuous soil waterlogging. Barrett-Lennard et al. (1988) found little growth of seminal roots during four days of recovery from 10 to 14 days in hypoxic solution culture. When wheat is exposed to anoxia in solution culture, seminal root elongation ceases almost immediately (Waters et al. 1991). For shorter periods of anoxia (up to two days), root tips could maintain their viability, presumably due to the energy produced via glycolysis linked to ethanolic fermentation. Seminal root growth of plants exposed to soil waterlogging in our experiment presumably stopped due to the scarcity or absence of O₂ at the root tip. The seminal roots were longer than 300 mm at the time waterlogging was imposed. Internal O₂ diffusion would have only reached 32-70 mm down the seminal root main axes (calculations based on model of Armstrong (1979) for O₂ diffusion in roots, and assuming a constitutive porosity of 0.8 to 3.4% and rates of respiration in wheat seminal roots as measured by Thomson et al. (1990, 1992)). The maximum diffusion depth of O₂ will vary with root respiration rate. However, even a 30% decrease in root respiration as would be achieved by a 5°C lower root temperature assuming a Q₁₀ of 2, would only allow O₂ transport in seminal roots to a maximum depth of 84 mm. For wheat grown in waterlogged soil for 14 days, Malik et al. (2001) observed a decrease in maximum length of the seminal roots, which indicates death and decay of seminal root apices under these conditions.

For plants waterlogged for 3 to 7 days, some increase in seminal root dry mass after draining the soil was observed (Figure 4.1B), and this could have been achieved by growth of laterals in the basal zones that had survived the waterlogging period. For wheat roots exposed to anoxia, in N₂-flushed solution culture for 10 days, seminal root
apices died, but laterals emerged from the basal root zones during re-aeration (Barrett-Lennard et al. 1988). In the present experiment, seminal root mass in plants exposed to 14 days of waterlogging or longer, did not increase when the soil was drained (Figure 4.1B). The potential for development of new lateral roots may depend on the position along the main axis (ie. access to O₂ diffusing from the shoot), the duration of anoxia as well as the developmental stage of the plant. In order to investigate the influence of plant developmental stage/root age, we simulated apical root death in plants at various developmental stages grown in aerated nutrient solution by pruning seminal roots back to 50 mm. Plants produced very few new laterals from the basal root zones (Table 4.3), although existing laterals could elongate past 50 mm. This indicates a limited ability to initiate growth of new laterals from the basal region of the seminal root main axis in wheat. Moreover, in waterlogged soils, other factors like the accumulation of toxic substances (Ponnamperuma 1984), or at least some degree of tissue O₂ deficiency, may have inhibited even the production of these few new laterals.

Unlike the seminal roots, adventitious roots elongated into the waterlogged soil to a maximum length of ~150 mm (Figure 4.2); presumably due to the aerenchyma allowing O₂ diffusion only to this distance. Similar maximum lengths of adventitious roots of wheat in waterlogged soil have been observed by Trought and Drew (1980a) and Thomson et al. (1992). After reaching the maximum length, adventitious roots did not lose their potential to elongate; during seven days of recovery after waterlogging for 21 days, the existing adventitious roots penetrated deeper into the soil (Figure 4.2). Similarly, Watkin et al. (1998) observed that adventitious roots of wheat could resume elongation during recovery from a 14-day period in deoxygenated stagnant nutrient solution.

After waterlogging, the mass of adventitious roots increased at a faster rate (e.g., 0.119 g g⁻¹ d⁻¹ over the first 7 days after 14 days of waterlogging) than that of the shoots (e.g., 0.019 g g⁻¹ d⁻¹) (Figure 4.8), while there was little to no growth of the seminal roots. This restored the partitioning of dry mass between roots and shoots to values equal to those in drained values within 7 days after draining (Figure 4.7). The growth of the adventitious root system during waterlogging could not compensate for the severe inhibition of seminal root growth. In seven-week-old plants in drained soil, the seminal root system still constituted two thirds of the total root mass (Figure 4.1 B and C); likewise the seminal root system accounted for three quarters of total root mass of 4
week old wheat plants grown in aerated nutrient solution (Trought and Drew 1980a). While the adventitious root system of plants grown in waterlogged soil can recover after draining the soil, to the size in plants grown in continuously drained soil, the severe reduction or loss of growth potential for the seminal root system presumably limits overall plant growth.

Carbohydrate accumulation

Accumulation of TNC in waterlogged plants or those exposed to root-zone anoxia is well documented for wheat (Barrett-Lennard et al. 1988; Huang and Johnson 1995) as well as for barley (Benjamin and Greenway 1979; Limpinuntana and Greenway 1979). In the present experiment, concentrations of TNC increased in all plant parts within three days of soil waterlogging (Figure 4.10; data only shown for leaves). Limpinuntana and Greenway (1979) ascribed the TNC accumulation in barley plants exposed to root-zone anoxia to severely reduced root growth. This appears to be confirmed by the present experiment, as the TNC concentration returned to control values in all parts of the plant within seven days after the waterlogging stress was eased. Initial regrowth of the adventitious roots might have been primarily fuelled by the high levels of TNC. For example, the TNC pool in a wheat plant after 14 days of waterlogging was 167 mg. Assuming construction costs of 1 to 1.6 g C per g dry matter produced for root tissues (Poorter 1994), this would allow the production of 104 to 167 mg root dry matter. The actual increase in adventitious root dry mass during the first seven days after draining the soil was 129 mg.

Nitrogen

N concentrations in leaves, stems and seminal roots decreased with increasing waterlogging duration (Figure 4.9, Table 4.2 and data not shown). However, the N concentration increased in adventitious roots relative to drained controls (Table 4.2). These observations are similar to those for wheat grown in N2-flushed nutrient solution (Buwalda et al., 1988). The higher concentration of N in adventitious roots, as compared to seminal roots, was presumably due to maintenance of the ability for nutrient uptake in the aerenchymatous adventitious roots (Drew and Saker, 1986). In contrast, seminal roots may have a greatly reduced capacity for N uptake presumably
due to an energy deficit, and in the longer term may even experience solute leakage (Greenway et al. 1992), the latter presumably being caused by deterioration of membranes during anoxia.

Shoot N concentrations were reduced markedly in the plants during waterlogging (Figure 4.9). Data on dry mass and tissue N concentrations from a sequence of harvests, allow rates of net N uptake, and of net N translocation to the shoot to be calculated (Williams 1948). During the first week of waterlogging, both these rates were severely reduced, being only 20-50% of rates in drained controls (calculated from Figures 4.1 and 4.9, and Table 4.2). While shoot N concentration decreases during waterlogging, this does not appear due to impaired N transport from the root to the shoot, as N uptake was equally affected. For later periods in the experiment, large differences in size among plants in the various treatments result in difficulties in making interpretations from such calculations, since differences in plant size/growth result in differences in demand for N, so that causality between treatments and N uptake rates are confounded (Watson and Petrie 1940). Nevertheless, the reduced leaf N concentrations show N uptake or translocation to the shoot was impaired in plants in waterlogged soil.

Belford et al. (1985) hypothesised that a lower N concentration in the main stem of wheat in waterlogged soil may contribute to lower rates of tiller production. This suggestion is consistent with results from our study. Tiller production was greatly reduced in the wheat when in waterlogged soil (Figure 4.4). N status is a major determinant of tiller initiation and development in wheat (Longnecker et al. 1993) and other cereals (e.g., barley: Birch and Long, 1990) and can influence the RGR of plants (Glimskär and Ericsson 1999). Furthermore, older leaves of the wheat plants senesced earlier during or after waterlogging, as compared to the drained control (indicated by declining chlorophyll concentrations, Figure 4.6), presumably due to N deficiency. While declining chlorophyll concentrations would ultimately affect photosynthetic rates, photosynthesis of plants during waterlogging is markedly decreased several days before chlorophyll concentrations decline (Malik et al. 2001). After draining the pots, however, the N concentration in the green leaves gradually recovered to levels in those of leaves of control plants of similar size (Figure 4.9), indicating the availability of N in substrate and its absorption by roots during the recovery period.
The magnitude of the effect of short-term waterlogging on the growth of wheat in this experiment was surprising. Where the effects of short-term (1-4 days) waterlogging on final biomass or grain yield of wheat have been assessed in the field, only Sharma and Swarup (1988) reported considerable yield penalties on a sodic soil. In other experiments, either no significant effect of waterlogging was detected (Meyer and Barrs, 1988), or the magnitude of the effect (Belford et al. 1985; Melhuish et al. 1991) on yield was lower than that on vegetative growth in our experiment. What are the possible reasons for this apparent discrepancy? Firstly, the severity of waterlogging effects may depend on the developmental stage of the plant (Watson et al. 1976). Waterlogging at early vegetative stages will affect growth and yield more than waterlogging during the late vegetative or reproductive phase (Watson et al. 1976). Waterlogging treatments for the field experiments of Meyer and Barrs (1988) and Melhuish et al. (1991) were applied to wheat plants more than two months older than those in the present experiment. Secondly, most of the reduction in above-ground biomass in plants grown in the present study was due to decreased tiller production. Under plant densities common in the field, only the first few tillers produced by plants would become ear-bearing (Rawson 1971), so that even the tiller number of plants after waterlogging could be sufficient to maintain yield. Also, a possibly reduced tiller and ear number of wheat after waterlogging could be compensated for by an increased number of grains per ear (Belford et al. 1985). Thirdly, in field experiments, the soil in the 'control' plots is seldom kept at field capacity like it was in our experiment. A waterlogging treatment may cause soil anaerobiosis, but also relieve possible drought stress in otherwise insufficiently watered plants (Melhuish et al. 1991). Fourthly, in field environments, other, late-season, environmental stresses such as heat or limited availability of nutrients may ultimately cap the higher yield potential of plants in non-waterlogged, as compared to waterlogged plots.

4.6 Conclusions

For waterlogging-susceptible wheat, other published studies have shown that the adventitious root system can acclimate, for example by developing aerenchyma, to ensure survival during longer waterlogging periods, albeit at reduced growth rates (Thomson et al. 1992; Malik et al. 2001). However, short-term as well as long-term
waterlogging may severely affect growth and survival of the seminal root system, thereby affecting the balance between root and shoot growth. The cessation of seminal root growth, reduced tillering and leaf growth accounts for the slow recovery and low final mass of 3 week-old wheat plants subjected to 3 to 21 days of waterlogging.

4.7 References


Chapter 5

Aerenchyma formation and radial $O_2$ loss along adventitious roots of wheat with only the apical root portion exposed to $O_2$-deficiency

5.1 Abstract

We studied aerenchyma formation and function in adventitious roots of wheat (*Triticum aestivum* L.) when only a part of the root system was exposed to $O_2$-deficiency. Two experimental systems were used: (i) Plants in soil waterlogged at 200 mm below the surface, or (ii) a nutrient solution system with only the apical region of a single root exposed to deoxygenated stagnant agar solution with the remainder of the root system in aerated nutrient solution. Porosity increased 2-3 fold along the entire length of the adventitious roots that grew into the water-saturated zone 200 mm below the soil surface, and also increased in roots that grew in the aerobic soil above the water-saturated zone. Likewise, adventitious roots with only the tips growing into deoxygenated stagnant agar solution developed aerenchyma along the entire main axis. Measurements of ROL, taken using root-sleeving $O_2$ electrodes, showed this aerenchyma was functional in conducting $O_2$. ROL measured near tips of intact roots in deoxygenated stagnant agar solution, while the basal part of the root remained in aerated solution, was sustained when the atmosphere around the shoot was replaced by $N_2$. This illustrates the importance of $O_2$ diffusion into the basal regions of roots within an aerobic zone, and the subsequent longitudinal movement of $O_2$ within the aerenchyma, to supply $O_2$ to the tip growing in an $O_2$-deficient zone.
5.2 Introduction

Terrestrial plant growth can be severely reduced by soil waterlogging. When the water table rises to under or above the soil surface, roots surrounded by the waterlogged soil suffer O\textsubscript{2}-deficiency (Jackson and Drew 1984). As the O\textsubscript{2} concentration in the rhizosphere decreases, root apices must access O\textsubscript{2} from other sources, or they will eventually die. Aerenchyma in roots provides a low-resistance internal diffusion pathway to supply O\textsubscript{2} to the root apex (Armstrong 1979; Colmer 2003). Aerenchyma formation is widespread amongst wetland and also non-wetland species (Justin and Armstrong 1987). It is inducible in roots of non-wetland species when exposed to low O\textsubscript{2} concentrations, for example in wheat (Trought and Drew 1980a), barley (Benjamin and Greenway 1979), or maize (Drew et al. 1979). In wetland species like rice, aerenchyma formation is constitutive; however, the aerenchyma volume increases when rice is exposed to low O\textsubscript{2} concentrations in the root environment (Armstrong 1971; Colmer et al. 1998).

A rise in internal ethylene concentrations induces the formation of aerenchyma in roots of several species (Drew et al. 2000). In plants, ethylene is produced by oxidation from the precursor, 1-aminocyclopropane-1-carboxylic acid (ACC) (Jackson 1985). External application of ethylene to maize (Drew et al. 1979; Konings 1982) or wheat (Huang et al. 1997) or the application of ACC to maize (Konings 1982; Konings and Lambers 1991) induced aerenchyma in adventitious roots of plants grown in aerated nutrient solutions. Also, nitrogen starvation can induce aerenchyma in maize (Konings and Verschuren 1980). Although the physiology of aerenchyma formation in adventitious roots has been extensively studied, little is known about aerenchyma formation and aeration in roots in partially waterlogged soil (i.e. where the water table remains below the soil surface).

In most experiments assessing plant responses to soil waterlogging or root-zone O\textsubscript{2}-deficiency, with few exceptions (Konings and Lambers 1991; Malik et al. 2001), plants were exposed to waterlogging to or above the soil surface, or hypoxic and/or anoxic treatments were applied to the entire root system. However, in the field water levels may not always reach the surface, so that aerobic layers persist over water-saturated soil. In such cases, only the more apical parts of the root system are exposed to subsurface water tables that vary in depth (McFarlane et al. 1989). Malik et al. (2001)
reported the presence of aerenchyma in the basal portion of adventitious roots of wheat that were waterlogged only at depth. The presence of aerenchyma in root tissues not directly exposed to soil waterlogging prompted us to design the present experiments. The objectives of the experiments described in the present paper were to study the effects of low external O₂ concentrations, applied to part of a root system, on the growth of adventitious roots of wheat, the formation of aerenchyma, and on O₂ transport and radial O₂ loss from adventitious roots.

The development of root porosity in new adventitious roots still above the water-saturated zone was evaluated in soil columns. Experiments in hydroponics were used to investigate root growth and the development and functionality of aerenchyma, when only one adventitious root of an intact plant was exposed to deoxygenated stagnant agar nutrient solution at the tip with the remainder of the root system in aerated nutrient solution.

5.3 Materials and Methods

Soil experiment

Plant culture

Seeds of *Triticum aestivum* L. cv. Cascades were surface-sterilised with 2% commercial bleach for 2 min, washed with deionised water, and placed on moist filter paper in Petri dishes in a dark growth cabinet at 18°C. After 2 d, eight germinated seeds were sown at 10 mm depth in each of 12 pots (height 400 mm, diameter 150 mm) and transferred to a walk-in growth chamber (20 °C /15°C day/night temperature, 12 h photoperiod, light intensity of 375-490 μmol quanta m⁻² s⁻¹, PAR). The pots were made from polyvinylchloride (PVC) tubes. Each tube was fitted with a PVC base, serving as a bottom, which was filled with a 50 mm layer of gravel. A 10 mm diameter hole was drilled in the side, 25 mm above the bottom, and connected to an open-end, transparent hose with the other end clamped to the top of the pot, so that water depth could be gauged. The pots contained 8 kg of Kojonup sand (a sandy surfaced duplex soil, pH (H₂O) = 5.8 and EC (1:5 w/v soil: water extract) = 17.2 mS m⁻¹) collected from the top 150 mm at the CSIRO Research Station at Yalanbee, Western Australia. This soil type had been used previously as substrate in waterlogging studies by Thomson *et al.* (1992)
and Malik et al. (2001, 2002). Nutrients were mixed through the soil prior to planting (composition given in Malik et al. 2001). While filling the pots, Pt electrodes (Patrick, Gambrell and Faulkner 1996) were buried at 50, 150 and 250 mm depths in 3 pots. The pots were watered to field capacity each d and rotated within the growth chamber every second d to minimise the effect of different conditions at different positions. After 7 d, the plants were thinned to four per pot.

Treatments were imposed when the plants were 21 d old, at which time seminal roots had reached the bottom of the pots. The three treatments were: 1) water level at the soil surface, 2) water level at 200 mm soil depth, and 3) fully drained soil watered daily to field capacity (control). Treatments lasted 21 d and there were three replicates of each.

**Measurements**

Redox potential of the soil was recorded daily with a calomel reference electrode (Eh +245 mV) and Pt electrode connected to a mV-meter. In a separate experiment, gas samples were taken after 7, 14 and 21 d with a syringe at 100 mm depth from pots that were waterlogged to 200 mm depth, or fully drained. The samples were analysed for ethylene using a gas chromatograph (GC 8A, Shimadzu, Kyoto, Japan) and for O₂ concentration using a flow-through O₂ sensor (Qubit Systems, Kingston Ont., Canada). An initial harvest of 3 pots was taken when the treatments were imposed, and the final harvest was taken after 21 d of treatments. Plants were divided into leaves, stems, seminal roots and adventitious roots. The lengths and number of adventitious root main axes were recorded. Plant tissues were dried for 2 d at 70°C and dry mass was measured.

Porosity (% of gas space per volume) of adventitious roots was measured using the method described by Raskin (1983), and the equations as modified by Thomson et al. (1990). Adventitious roots from each plant were divided into three length classes (300-400 mm, 150-200 mm and 100-150 mm) and cut into 50 mm segments. The 100-150 mm roots had not reached the waterlogged zone, 150-200 mm roots had just reached the waterlogged zone, and 300-400 mm roots had penetrated into the waterlogged zone. The presence of aerenchyma in these roots was confirmed by light microscopy of hand cross-sections taken at selected positions along the main axes.
Solution culture experiments

Seeds of *T. aestivum* L. cv. Cascades were surface sterilised as described above. Germination was carried out in the dark on a plastic mesh floating over 0.1 strength aerated nutrient solution (see below) for 4 d and then exposed to light, in the same growth chamber with temperature and light conditions as in the soil experiment. Seven-d-old seedlings were transferred to aerated 0.25 strength nutrient solution in 20 l plastic tanks containing 12 plants each. After a further 7 d, the solution was changed to full strength. The composition of the nutrient solution was as given in McDonald *et al.* (2001). Twenty-three d-old plants at 4.5 ± 0.1 Haun stage (Haun 1973), and with 9.0 ± 0.6 adventitious roots (at least one was ~100 mm) were chosen for the experiments.

Growth, aerenchyma development and radial O₂ loss (ROL) of intact adventitious roots experiencing low external O₂ at the tip region were studied. The apical portions of selected adventitious roots were exposed to stagnant deoxygenated solution while the upper part of the same root, as well as the remainder of the root system, was in aerated solution (Figure 1).

![Figure 5.1 Experimental set up for applying a partially stagnant treatment to single wheat roots. The tip (~15 mm) of a single adventitious root (~100 mm length) of wheat is guided through a small hole (1 mm diameter) into a plastic 250 ml pot. The rest of the root system, including the upper portion of the same root, is in aerated solution in the main pot. The large 4.5 l container is filled with nutrient solution and continuously aerated from the bottom through an air stone. The small plastic pot with an airtight lid contains deoxygenated stagnant agar nutrient solution.](image-url)
Individual plants were transferred to a 4.5 l plastic container (height 170 mm, width 200 x 125 mm; covered with aluminium foil) containing aerated nutrient solution (composition as above). A 250 ml transparent plastic container with an air-tight lid (height 90 mm and diameter 67 mm) and containing deoxygenated stagnant nutrient solution with agar (0.1%, Wiengweera et al. 1997), or aerated nutrient solution (control), was affixed to the bottom of each 4.5 l container. An adventitious root of ~100 mm length was carefully guided through a hole (1 mm diameter) in the lid of the 250 ml container, so that ~15 mm of the apical portion was inside.

Preliminary experiments had shown no effect on the growth of the whole plant when a single adventitious root was growing inside the 250 ml container with aerated nutrient solution or deoxygenated stagnant nutrient solution. The growth of adventitious roots while inside the 250 ml container with aerated nutrient solution was also unaffected. For all subsequent experiments we decided to use the 250 ml container only in the treatment where a single adventitious root was exposed to low O₂ at the tip. After 120 h, O₂ concentration of previously deoxygenated stagnant solution (0.1 mg l⁻¹ O₂) inside the 250 ml container increased to only 0.4 ± 0.1 mg l⁻¹ (without root inside) and 0.8 ± 0.1 mg l⁻¹ (with root inside) when affixed to the bottom of 4.5 l pots containing aerated nutrient solution.

We exposed plants to three treatments: 1. the entire root system was grown in aerated nutrient solution (aerated); 2. the entire root system was grown in deoxygenated stagnant solution (fully stagnant condition); or 3. the apical portion (~15 mm) of a ~100 mm adventitious root was exposed to deoxygenated stagnant solution, and the remainder of the root system, including the upper portion of this same root, was in aerated solution in the main container ('partially stagnant' condition). Treatments were imposed for 24 h, 48 h, 72 h and 120 h, with three replicate blocks of each treatment and sampling time.

Aerenchyma measurements

The percentage of aerenchyma was measured in cross-sections taken at 20 and 40 mm behind the root tip, and at 20 mm below the root-shoot junction at several times (see above). The first two positions were chosen to ensure measurements were taken for
locations that had grown inside or were close to the top of the deoxygenated stagnant solution (Figure 5.1). By contrast, the section 20 mm below the root-shoot junction was chosen as it was always aerated. Photographs of the sections were taken, and the percentage of aerenchyma in digitised images were measured using an image-analysis program (SigmaScan Pro, version 5.0.0).

**Radial O$_2$ loss measurements**

In a second experiment using solution culture, roots were treated for 120 h under the same ‘partially-stagnant’ conditions as described above, and radial O$_2$ loss (ROL) from selected adventitious roots was measured in these conditions. Also, plants that were grown in fully aerated or deoxygenated stagnant solutions were transferred into the partially stagnant setup (Figure 5.1) in a temperature-controlled room (20°C). Root-sleeving O$_2$ electrodes (height 5.0 mm, inside diameter 2.25 mm, fitted with guides; Armstrong 1971, 1979; Armstrong and Wright 1975) were used for the measurements. The partially-stagnant-grown adventitious roots were 158 ± 3 mm (i.e. ~100 mm was in the aerated compartment during measurements), fully-stagnant-grown adventitious roots were 86 ± 1 mm (i.e. ~40 mm in the aerated compartment) and aerated-grown roots of ~164 ± 4 mm were chosen (i.e. ~100 mm in the aerated compartment).

Selected adventitious roots were guided through a small hole in the air-tight lid of the 250 ml container of stagnant deoxygenated 0.1% agar with 5.0 mol m$^{-3}$ KCl and 0.5 mol m$^{-3}$ CaSO$_4$. The root tip was then inserted through a root-sleeving O$_2$ electrode and ROL was measured at different positions (10, 20, 40 mm behind the root tip). The O$_2$ concentration around the shoot and basal portion of the root was then manipulated, and ROL at 20 mm behind the root tip was recorded. A clear plastic cylindrical hood (height 320 mm, diameter 55 mm) attached to a high purity N$_2$ cylinder was used to replace the air around the shoot with N$_2$. After recording the new steady state ROL when the shoot was in N$_2$, the hood was removed so that the shoot was again exposed to air. Recovery of ROL following re-entry of O$_2$ was measured. The basal root zone of the adventitious root entering the small container, and all other roots, were then exposed to O$_2$-free conditions by replacing the aerated nutrient solution in the 4.5 l container with deoxygenated solution containing 0.1% agar. ROL 20 mm behind the tip of the root in the small container was then measured.
5.4 Results

Redox, O2 and ethylene in partially waterlogged soil

In soil waterlogged to 200 mm depth, after 21 d, the redox potential declined from 600 mV to 345 ± 47 mV at 50 mm soil depth, and to 228 ± 57 mV at 150 mm soil depth. Both of these measurement points were above the water level. Redox potential at 250 mm depth (below the water level) declined to -40 ± 74 mV by the end of the 21 d treatment. In the drained pots, redox potential remained near 600 mV throughout the experimental period.

The O2 concentration at 100 mm below the surface of soil waterlogged to 200 mm depth had decreased slightly below that in drained pots (150 ± 10 and 190 ± 5 mmol mol⁻¹, respectively). Concurrently, ethylene concentration in the soil above the water-saturated zone increased. After 7 d, the ethylene concentration at 100 mm soil depth in pots waterlogged to 200 mm depth was 5.2 ± 1.7 μmol mol⁻¹ and after 14 d, it was 2.4 ± 0.1 μmol mol⁻¹. Unfortunately, due to a mechanical fault, we could not measure ethylene after 21 d of treatment. In the drained pots, the ethylene concentration was always lower than the detection limit (0.01 μmol mol⁻¹).

Plant growth in partially waterlogged, fully waterlogged, or in drained soil

Compared with the drained controls, total dry mass was reduced by 15% after 21 d of soil waterlogging at 200 mm soil depth, and by 50% after 21 d of waterlogging at the soil surface (Figure 5.2). Fewer tillers and slower leaf development caused the reduced mass of the above-ground portion of the plant (Table 5.1). The reduction of seminal root

<table>
<thead>
<tr>
<th>Soil treatments</th>
<th>Number of leaves on the main stem per plant</th>
<th>Number of tillers per plant</th>
<th>Number of adventitious root per plant</th>
<th>Number of adventitious roots per stem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drained</td>
<td>7.6±0.0</td>
<td>13.3±0.3</td>
<td>31.5±1.7</td>
<td>2.2±0.1</td>
</tr>
<tr>
<td>WI-200</td>
<td>7.0±0.2</td>
<td>10.2±0.8</td>
<td>26.3±0.7</td>
<td>2.4±0.2</td>
</tr>
<tr>
<td>WI</td>
<td>5.8±0.0</td>
<td>6.6±0.3</td>
<td>21.2±0.4</td>
<td>2.8±0.1</td>
</tr>
</tbody>
</table>
dry mass was 85% in plants that were exposed to soil waterlogging to the surface and 50% in the plants where the water level was at 200 mm depth (Figure 5.2). Plants in soil waterlogged to 200 mm soil depth produced 26% more adventitious root dry mass than did drained controls, while plants in fully waterlogged soil produced 35% less adventitious root dry mass (Figure 5.2). Waterlogging to the soil surface stimulated the number of adventitious roots produced per stem, but decreased tiller numbers, leading to fewer adventitious roots produced per plant (Table 5.1).

![Figure 5.2](image)

**Figure 5.2** Dry mass of different plant parts of wheat after 21 d of waterlogging at different depths. Drained, fully drained soil; wl-200, waterlogged at 200 mm depth; wl, waterlogged at the surface. At the start of the treatment average leaf dry mass was 0.12±0.0 g, stem/sheath dry mass was 0.05±0.0 g, seminal root dry mass was 0.07±0.01 g and adventitious root mass was 0.01±0.0. Standard error bars refer to total plant dry mass (n=3).

**Porosity of adventitious roots of plants grown in partially waterlogged, fully waterlogged or in drained soil**

For plants in soil waterlogged at 200 mm depth, adventitious roots that were only 100 to 150 mm in length at the time of harvest, had 2-3 fold higher porosity compared with roots of plants grown in fully drained soil (Figure 5.3A). Roots of 150 to 200 mm length also showed 2-3 fold increased porosity along the entire main axis (Figure 5.3B). It is possible that the tips of these later roots had experienced O₂-deficiency, due to the capillary rise of the water in the soil column. The porosity of the longest roots (300-400 mm) growing into the water-saturated soil layer also had increased by a similar magnitude along the whole main axis (Figure 5.3C). The roots of plants that were...
exposed to waterlogging to the soil surface had 4-5 fold higher porosity than roots of plants grown in drained soil (Figure 5.3A). There were no roots in the two longest classes for plants in soil waterlogged to the surface, as adventitious roots had only penetrated up to 160 mm into the waterlogged soil. The presence of aerenchyma in roots that had increased porosity was confirmed by examining hand-cross sections under the microscope (data not shown).

Figure 5.3 Distribution of porosity along the main axis of adventitious roots of wheat of different length classes. (A) 100-150 mm, (B) 150-200 mm, (C) 300-400 mm, after 21 d of soil waterlogging to different depths. Treatments were: Drained, (open circles); waterlogged to 200 mm soil depth, (closed triangles); and waterlogged at soil surface, (closed circles). Symbols represent means± standard errors (n=3).
Growth of adventitious roots in partially stagnant, fully stagnant or aerated nutrient solution

Adventitious roots (initially ~100 mm in length) in aerated solution grew at 34 ± 2.2 mm d\(^{-1}\) throughout the experiment, while adventitious roots initially ~100 mm in length and transferred to fully stagnant conditions did not increase in length. Roots transferred to partially stagnant conditions grew slowly initially (5 to 13 mm d\(^{-1}\)), and growth rates increased between 48 and 72 h (27 mm d\(^{-1}\)), and then slowed (10 mm d\(^{-1}\), Figure 5.4). After 120 h, adventitious roots of plants grown in aerated nutrient solution were ~105 mm longer than those that experienced partially stagnant conditions.

![Figure 5.4 Cumulative length of adventitious roots of wheat exposed to different treatments for 120 h. Adventitious roots grown in aerated nutrient solution (○); adventitious roots grown in deoxygenated agar nutrient solution, 'fully stagnant' (●) and adventitious roots exposed to deoxygenated stagnant nutrient solution at the tip, 'partially stagnant' (▲). At the start of the treatment, the average length of adventitious roots was 112 mm. Symbols represent means ± standard error (n=3), where missing, error bars are smaller than symbols.](image)

Aerenchyma formation in adventitious roots in partially stagnant conditions

Adventitious roots (~100-260 mm in length) grown in aerated nutrient solution did not form aerenchyma. Adventitious roots growing in partially stagnant conditions started forming aerenchyma along the whole root length between 24 h and 48 h of treatment. The volume of aerenchyma increased with time (Figures 5.5 and 5.6). However, no aerenchyma developed at the position 10 mm behind the root tip. The sections taken 20 mm below the root-shoot junction represents tissue that aged during
Figure 5.5 Aerenchyma formed in adventitious roots that were partially exposed to stagnant conditions. At the start of the treatment, the apical ~15 mm of a 100 mm adventitious root was exposed to, and then grew into, deoxygenated stagnant agar. The cross sections were taken after 48 h (A, B, C), 72 h (D, E, F) and 120 h (G, H, I) at different positions along the root main axis: 20 mm from the root-shoot junction (A, D, G), 40 mm behind the root tip (B, E, H) and 20 mm behind the root tip (C, F, I). The scale bar represents 100 μm.

the experiment, while the sections at 20 mm behind the root tip represent tissues formed while the experiment progressed. However, sections taken at 20 and 40 mm behind the root tips at each harvest during the first 48 h also represent progressively older tissues, as the root extension rate during this initial stage was slower than 20 mm d⁻¹ (see below).
Figure 5.6 Relative proportion of aerenchyma at various distances behind the tip for cross-sectional areas of adventitious roots of wheat sampled with time after exposure to treatments. Sections were taken at different positions along the main axis: 20 mm behind the root tip (●); 40 mm behind the root tip (▲) and 20 mm from the root-shoot junction (O). Symbols represent means ± standard error (n=3).

ROL from adventitious roots in partially stagnant conditions

ROL measurements were taken from the roots of intact plants, with the shoot in air, and the apical ~60 mm of a selected adventitious root positioned in the deoxygenated stagnant solution in a small container while the basal 40-100 mm (depending on treatment) of the same root (and reminder of the root system) were kept in aerated nutrient solution. Roots that had developed in either partially or fully stagnant conditions showed markedly greater rates (2-6 fold) of ROL at 10, 20, and 40 mm behind the tip than roots grown in aerated nutrient solution (Figure 5.7), indicating enhanced diffusion of O₂ through the roots of plants that had formed aerenchyma in stagnant or partially stagnant treatments.

O₂ around the shoot was removed by enclosing the shoot in a chamber and replacing the air with N₂. The rate of ROL at 20 mm behind the root tip was then measured. Roots grown in the partially stagnant treatment prior to being transferred into the measurement system showed only a 10% decrease in the rate of ROL (Table 5.2) when air around the shoot was replaced with N₂. By contrast, ROL from roots of plants previously in fully stagnant conditions prior to being transferred into the measurement system decreased by more than 80%. When air around the shoot was restored, the measured rates of ROL returned to within 5% of the initial values for all three treatments (data not shown).
Figure 5.7 Rates of radial O$_2$ loss (ROL) at selected positions behind the tip of adventitious roots of wheat after 120 h of treatments prior to the measurements. Plants were previously grown in aerated conditions (open circles), partially stagnant (closed triangles) and fully stagnant (closed circles) and then transferred to a partially stagnant system. Aerated-grown roots and partially stagnant roots were 160-165 mm at the time of measurement, while stagnant-grown roots were 85 mm. The apical 50-60 mm was placed inside small containers filled with deoxygenated stagnant agar while the rest of the root system was in aerated nutrient solution. Symbols represents means ± standard errors (n=3).

Table 5.2 Radial O$_2$ loss (ROL) measurements for roots of plants raised under various conditions for 120 h and then transferred into partial stagnant set up (Figure 1). O$_2$ at possible source locations was manipulated. Air at the shoot was replaced with N$_2$, or the aerated nutrient solution at the upper portion of the root system was replaced with deoxygenated stagnant solution containing 0.1% agar. Roots of plants grown in aerated nutrient solution and partially stagnant conditions were 158-164 mm at the time of measurement, while roots of plants from stagnant conditions were 85 mm. The apical 50-60 mm was placed inside small containers filled with deoxygenated stagnant agar while the remainder of the root system was in aerated nutrient solution. Recovery of ROL was measured after the shoot was back in air and the values were within 5% of the initial measurements. Means with standard errors (n=3)

<table>
<thead>
<tr>
<th>Roots raised in</th>
<th>Shoot in air, root in partial stagnant set</th>
<th>Shoot in N$_2$, root in partial stagnant set</th>
<th>Shoot in air, whole root system in stagnant set up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerated</td>
<td>46.4±17.9</td>
<td>26.4±16.1</td>
<td>0</td>
</tr>
<tr>
<td>Partially stagnant</td>
<td>298±19.7</td>
<td>265.2±18.8</td>
<td>4.23±4.23</td>
</tr>
<tr>
<td>Stagnant</td>
<td>202±55</td>
<td>47.8±42.4</td>
<td>28.3±14.7</td>
</tr>
</tbody>
</table>

availability around the entire root system was then eliminated by replacing the aerated nutrient solution with deoxygenated stagnant solution. In such cases, the only way for O$_2$ to enter the root would be via the root-shoot junction. Under these conditions the rate of ROL at 20 mm behind the tip was zero or near to zero for roots that were previously exposed to partially stagnant or aerated treatments (Table 5.2). The adventitious roots
previously exposed to fully stagnant conditions showed a greatly decreased, but still detectable, rate of ROL (Table 5.2).

5.5 Discussion

Soil experiment

During waterlogging growth of the seminal root system of wheat ceases (Thomson et al., 1990, Malik et al. 2002), and new adventitious roots form the bulk of the root system. The newly grown adventitious roots of wheat develop aerenchyma, thereby increasing root porosity to up to 20% (Malik et al. 2001, Figure 5.3 in present study). When roots of wheat grew into water-saturated soil at 200 mm below the surface, porosity was increased by the formation of aerenchyma along the entire root length (Malik et al. 2001, present study). Furthermore, porosity increased in adventitious roots that were still well above the water level (Figure 5.3). This phenomenon begs the question as to what signal triggers the development of aerenchyma in roots or root parts surrounded by aerobic soil, when other regions are in water-saturated soil.

As ethylene is widely accepted as the signalling molecule for the induction of aerenchyma (Jackson 1989, Drew et al. 2000), an increased concentration of ethylene (produced within the roots and/or in soil) may have induced the formation of aerenchyma in the basal parts of roots that only have apical portions in water-saturated soil. The importance of internal diffusion of ethylene or transport of its precursor ACC, will be discussed together with the hydroponic experiment below. Ethylene concentration in the aerobic soil layer above a waterlogged soil zone increased, while the O₂ concentration was only slightly reduced. The presence of ethylene in waterlogged soil has also been found by Dowdell et al. (1972), Drew and Sisworo (1979), and Trought and Drew (1980b, 1982). This ethylene could be produced by plant roots as well as soil micro-organisms in the waterlogged soil layer and diffuse upwards through the soil, thereby acting on plant parts that grow above a water-saturated zone. Exogenous ethylene at concentrations similar to those found in the present experiment promoted formation of aerenchyma in adventitious roots of wheat (Huang et al. 1997), maize (Drew et al. 1979), and rice (Justin and Armstrong 1991) when these species were grown in aerated nutrient solution.
The O₂ available in a waterlogged soil layer would be very little, so that the O₂ supply for those root parts that grow into a water-saturated soil must diffuse within internal gas-spaces from plant parts containing higher O₂ concentrations (Armstrong, 1979). The O₂ sources may be the shoot atmosphere as well as the soil atmosphere above the waterlogged layer. As aerenchymatous adventitious roots grow between 100 and 170 mm into a water-saturated soil irrespective of the position of this water-saturated soil layer (Malik et al. 2001), it is reasonable to assume that a substantial amount of O₂ enters the basal portions of roots growing in the aerobic soil layer enhancing O₂ supply to the tip, either by less internal O₂ being consumed along the diffusion pathway from the root-shoot junction and/or by providing O₂ from the aerobic soil layer. This hypothesis was tested for roots grown in a hydroponic system as described below.

Hydroponic experiments

The results from our soil experiment indicated that there are possible external as well as internal signals acting on roots growing into water-saturated soil at depth that can trigger the development of aerenchyma along adventitious roots. In soil, the boundary between the anaerobic and the aerobic soil layers is not clear-cut. In order to have a clear demarcation between the aerated part of the root and the more apical parts exposed to O₂-deficient conditions, we developed a 'partially stagnant' hydroponic system (Figure 5.1).

Adventitious roots with the apical 15 mm inserted into a deoxygenated medium had begun to develop aerenchyma, even in the basal parts in aerated solution, in less than 2 d (Figures 5 and 6). ACC produced in the O₂-deficient apical part of the root could be oxidised to ethylene in the apical region if a residual amount of O₂ was available and/or exported towards the aerated portion and converted there to ethylene, triggering formation of aerenchyma along the main axis (Atwell et al. 1988, Konings and Lambers, 1991). The appearance of aerenchyma after 2 d of treatment coincides with accelerated adventitious root growth (Figures 5.4 and 5.6). It is important to note that an external action of ethylene is unlikely to have influenced the formation of aerenchyma in the aerated part of the root in the solution culture experiments, as the stagnant medium was clearly separated from the aerated medium, and as no other adventitious roots on the
same plant and growing in the aerated nutrient solution developed aerenchyma (data not shown). This result indicates that for roots growing into an O₂-deficient zone, the internal transport of ACC and/or ethylene would be sufficient to trigger aerenchyma formation. Nevertheless, external ethylene concentrations in soil above waterlogged zones may also enhance aerenchyma formation in root systems in aerobic layers above waterlogged soil.

Roots grown in partially stagnant conditions exhibited high rates of ROL along the portion in the container of deoxygenated solution. Our measurements of ROL after O₂ source manipulations, demonstrated that the aerated basal portion of the root contributes to the O₂ supply of the non-aerated apical portion. This effect presumably results from an aerated basal portion meeting its own O₂ demand locally, and/or it acts as a source of O₂ by providing O₂ via diffusion from the surrounding aerated solution. The importance of O₂ diffusion between the upper part of the root and the aerated portion of the nutrient solution for supply to the apical region in the O₂-deficient medium, was evaluated by replacing air around the shoot with N₂. In the absence of shoot O₂, all root O₂ (measured as ROL near the root tip) had to be sourced from the aerated nutrient solution, and ROL from roots of plants grown in partially-stagnant conditions was only slightly decreased (being 90% of original values, Table 5.2). Under these conditions, ROL near the tip of adventitious roots of plants grown in fully-stagnant conditions prior to being transferred into the partially-stagnant set up was reduced to 25% of the initial value, (Table 5.2).

There are a number of possible reasons that could explain these differences. Firstly, the conductivity for O₂ between the aerenchymatous cortex and the nutrient solution may have been different in roots of plants from partially- and fully-stagnant treatments. The basal zones of roots grown in stagnant conditions can decrease in radial O₂ permeability, although such changes are much smaller in non-wetland as compared with wetland species (McDonald et al. 2001; McDonald et al. 2002) Secondly, the conductivity between the root and the shoot (which may become an additional O₂ sink in a N₂ atmosphere) may have been lower in plants from partially than in fully-stagnant solution. Thirdly, there may have been insufficient uptake of O₂ from the aerated medium by roots of plants previously in fully stagnant solution, as the portion of the root that was exposed to aerated nutrient solution was much shorter in these roots (40 mm instead of ~100 mm). In addition, roots of plants grown in a partially stagnant system developed more laterals in the basal zones (data not shown), and these may have acted as an additional point of O₂ entry from the aerated medium.
When O$_2$ supply to the shoot was restored and the whole root system was exposed to deoxygenated stagnant agar solution, only the roots of plants grown in fully-stagnant conditions showed measurable rates of ROL at 20 mm behind the root tip (Table 5.2). The O$_2$ diffusion pathway from the O$_2$ source (the shoot) was on average 60 mm shorter for roots of plants grown in fully-stagnant solution when compared with roots of plants grown in partially-stagnant solution. In addition, the basal zone of roots of plants grown in fully-stagnant solution had developed fewer lateral roots than the roots of plants grown in partially-stagnant solution. Lateral roots can act as an additional sink for O$_2$, as has been demonstrated for a range of plant species such as pea (Armstrong, Healy and Lythe 1983, Armstrong and Healy, 1984), canola (Voesenek et al. 1999), clovers (Gibberd et al. 1999), and wheat (A.I. Malik unpublished data).

5.6 Conclusions

Adventitious roots of wheat, with only the apical portions exposed to waterlogging in soil or O$_2$-deficient agar solution, formed aerenchyma along the main axis of the entire root (including the basal regions of the root in aerobic conditions). The aerenchyma enabled adventitious roots to grow into water-saturated soil or into deoxygenated stagnant agar solution by providing a low-resistance pathway for O$_2$ diffusion to the tip. ROL near the tip of ‘partially stagnant’ adventitious roots (i.e. tip in O$_2$-deficient media, basal zones in aerated solution) was maintained even when there was no O$_2$ around shoot, showing that O$_2$ can enter the basal portion of the root in an aerated medium and diffuse to the root tip in an O$_2$-deficient medium.

5.7 References


McDonald MP, Galwey NW, Colmer TD (2001) Waterlogging tolerance in the tribe Triticeae: the adventitious roots of *Critesion marinum* have a relatively high porosity and a barrier to radial oxygen loss. *Plant, Cell and Environment* 24, 585-596.


Chapter 6

General Discussion

The previous chapters have shown how the anatomy, morphology and physiology of wheat are affected by waterlogging, and how the variability in depth and duration of waterlogging modifies this response. Wheat is the major crop in many waterlogging-prone areas of the world (Chapter 2), and access to wheat varieties with some tolerance to waterlogging would be of major importance to agriculture (Setter and Waters 2003). Waterlogging firstly affects the gas exchange between soil and air, and that restricts aerobic respiration in the roots; however, it also alters other physical, chemical and biological factors in the root environment (Ponnamperuma 1972). This thesis has assessed key traits affected by waterlogging (i.e. mainly due to O₂ deficiency), and here will be discussed the possibility to modify these traits to improve waterlogging tolerance in wheat. In particular this chapter focuses on traits important for the generation of energy and to maintain O₂ movement in roots exposed to waterlogging or O₂ deficiency.

6.1 Death of seminal roots

In the first weeks to months of plant growth, seminal roots form the bulk of the root system of wheat (Chapters 3 and 4). If a waterlogging event occurs, the response of the seminal root system is therefore of critical importance. Chapter 4 has shown that even short-term waterlogging (3 to 7 d) permanently damages the seminal root system. This corroborated earlier findings that seminal roots longer than 100 mm will stop growing, and roots tips will eventually die when deprived of O₂ in waterlogged soil or anoxic solution culture (Thomson et al. 1990). Once the tips of the main axis of the seminal root are dead, the function of the seminal root system has to rely on lateral roots. At the start of this project, there were no data available on the formation of new laterals on the main axis of seminal roots once the existing laterals and the tip of the main axis have been damaged by waterlogging. A root-pruning experiment in aerobic nutrient solution (described in Chapter 4) showed that the ability of wheat to develop new laterals once the existing laterals are damaged is limited.
The death of seminal root tips is a consequence of insufficient aeration in waterlogged or anoxic conditions. This is due to the low constitutive porosity of seminal roots of wheat (0.8 to 3.9% v/v, Thomson et al. 1990), which, according to the model of Armstrong (1979) would allow O₂ diffusion to a length of 34 to 75 mm. The model explains why the tips of roots longer than this distance would suffer from O₂-deficiency. There is little variation within wheat, barley, rye or their wild relatives in constitutive seminal root porosity, and waterlogging or O₂-deficiency increases this porosity by only 30-50% (McDonald et al. 2001). This hardly increases the maximum length that diffusion of O₂ can occur.

If increased porosity and new lateral root formation (hypothetically) allowed the seminal roots of wheat to continue growth, they would still have to survive the initial anoxia upon the onset of waterlogging, by drawing on energy generated by anaerobic metabolism. In the soil experiments described in the previous chapters, the seminal root system of 21 d old wheat plants (at the onset of waterlogging) had a dry weight of ~50 mg, and a non-structural carbohydrate concentration of 0.03 to 0.05 g g⁻¹. With this amount of carbohydrates, seminal roots would be able to sustain anaerobic carbohydrate catabolism for up to ~2 hours (assuming no translocation of new assimilates to the roots, calculated from Waters et al. 1991b). In hydroponic experiments wheat seminal roots can survive anoxia for at least 10 h (Waters et al. 1991a), indicating translocation of carbohydrates to these roots (Waters et al. 1991a). Nevertheless, anoxia tolerance in wheat roots can be enhanced by adding carbohydrates (glucose, Waters et al. 1991b). There are no data available on the genetic variation in the capacity of ethanol synthesis or carbohydrate reserves in wheat seminal roots. However, it appears that there is limited scope in improving the tolerance of wheat to waterlogging by altering seminal root traits.

6.2 Importance of the adventitious root system

The formation of adventitious roots is an adaptation to waterlogging (Jackson and Drew 1984). The experiments described in Chapters 3 and 4 demonstrate enhanced adventitious root formation of wheat (based on the number of stems per plant) in waterlogged soil, when compared with control plants in drained soil. However, as wheat responded to waterlogging with reduced tiller production, the overall number of
adventitious roots in control plants was always greater. A lesser suppression of tiller development during waterlogging may contribute to enhance adventitious root formation per plant (e.g., wheat cv. Chinese Spring, McDonald et al. 2001).

Even as the formation of adventitious roots is enhanced during waterlogging, the length of these roots is restricted. Chapters 3 and 4 showed that the maximum penetration of adventitious roots into the water-saturated layer of a soil column was ~160 mm. The penetration of the newly formed adventitious roots into the water-saturated zone was aided by enhanced porosity (Chapters 3, 4, 5 and Table 2.4).

Constitutive porosity of adventitious roots of wheat ranges from 2.8 to 6.8%, and increased 3 to 8-fold during waterlogging (Chapters 3 and 5, Thomson et al. 1990, 1992, McDonald et al. 2001). Adventitious root porosity increased due to the formation of aerenchyma, and aerenchyma volume increased with waterlogging intensity, i.e. duration of waterlogging and/or depth of water (Chapter 3). The maximum porosity (20% v/v, Chapters 3 and 5) achieved by wheat roots would allow a maximum O₂ diffusion path length of 171 mm, assuming no radial O₂ loss (ROL), and a respiration rate of 2.34 mmol O₂ m⁻³ s⁻¹ at 20°C (calculated using the equation in Armstrong 1979). This value is very close to the observed maximum length of adventitious roots of 150-160 mm (Chapters 3, 4 and 5). By contrast, the constitutive porosity of adventitious roots of rice (e.g., cv. Calrose) was 22-27%, and in waterlogged conditions it increased to 36-48%, which allows penetration into waterlogged soil up to 289 mm (McDonald et al. 2002, Colmer 2003). There are two major differences in the root anatomy between wheat and rice: firstly, the cortex cells in rice roots are cubically packed with quadrangular intercellular spaces, while wheat roots have a hexagonal packing structure with triquetrous intercellular spaces (Justin and Armstrong 1987). Even in the absence of aerenchyma, cubical cell packing enhances porosity. While there is evidence of cubical packing of cells in non-wetland grass species (e.g., Cenchrus ciliaris and Sorghum bicolor, McDonald et al. 2002), there is no report of this in close relatives of wheat. Secondly, the stele in rice roots occupies a smaller portion of the root volume than that in wheat (at 10 mm from the root-shoot junction 5% in rice (McDonald et al. 2002); at 20 mm from the root-shoot junction 21% in wheat (own unpublished data). This reduces the cortical volume in wheat where aerenchyma could form. There are variations in the stele proportion in roots of wetland species (McDonald et al. 2002).
Enhanced \( O_2 \) diffusion that would allow continued root extension into \( O_2 \)-deficient medium requires greater porosity than achieved by current wheat cultivars.

Along with increased root porosity, the restriction of ROL could enhance the penetration of adventitious roots of wheat into \( O_2 \)-deficient media. The difference between the observed maximum length of adventitious roots into a water-saturated layer and the predicted length (discussed above) is presumably due to higher ROL from basal root zones (McDonald et al. 2001). By contrast, wetland species like rice develop a barrier to ROL in their adventitious roots when exposed to waterlogging, and this restricts ROL at the basal root portion, allowing \( O_2 \) to diffuse towards the tip (Armstrong 1971; Colmer et al. 1998). Ultimately, such roots achieve greater length in a waterlogged soil (Armstrong 1979). In addition to rice, several other grass species develop a barrier to ROL, some of which are more closely related to wheat (McDonald et al. 2001, 2002). Further study is warranted to evaluate the possibility to improve these two traits (root porosity and barrier to ROL) in wheat (Thomson et al. 1992), or in wild relatives of wheat with the view of integrating their adaptive traits into wheat germplasm (Colmer 2003).

### 6.3 Root tips exposed to waterlogged soil or \( O_2 \)-deficiency

Where root tips were exposed to \( O_2 \) deficiency, roots developed increased porosity through the formation of aerenchyma along the entire root length (Konings and Lambers 1991, Chapters 3 and 5). The portion of the root system that remains in aerobic conditions was shown to be crucial for the aeration of the apical portion of a root or root system that extends into \( O_2 \)-deficient media (Chapter 5). Oxygen uptake from the aerobic soil layer enables wheat roots to grow 100-150 mm into a waterlogged zone, irrespective of the position of the water table (Chapter 5). Moreover, adventitious roots that were newly developing in the aerobic soil above a waterlogged zone also developed aerenchyma, presumably triggered by rising ethylene concentrations in the rooting medium (Chapter 5). The ecological importance of this response is that, if the water table rises further, the newly formed roots are already prepared to continue growth into the waterlogged soil zone. Where aerobically grown roots are exposed to waterlogged soil or stagnant de-oxygenated agar, root growth initially ceases and after a lag phase during which aerenchyma is induced, root extension into an \( O_2 \)-deficient medium...
resumes (Chapter 5). Roots with pre-formed aerenchyma would not undergo this lag phase.

While the aerobic part of the root system can sustain roots growing into anaerobic zones, it is unclear what parts the different root portions play in the uptake of water and nutrients by the plant.

6.4 Root-shoot ratio and recovery from waterlogging stress

The previous chapters have shown that both root and shoot growth of wheat are affected by waterlogging. However, as root growth is more reduced during waterlogging, the root-shoot ratio decreases (Chapter 4). This ratio can be altered by a number of different environmental stresses (e.g., light, temperature, nutrients, water) (Reich 2002). However, as the stress is released, plants usually restore their root-shoot ratio (for a given plant size or stage of development), but end up with smaller plant size (Reich 2002). In experiments where part of the root system or part of the leaves of 12 d-old barley seedlings grown in hydroponics were pruned, the root-shoot ratio was restored to the initial value within a week (Brouwer 1962; Poorter and Nagel 2000). However, the final size of the pruned plants was smaller than that of un-pruned plants (Brouwer 1962; Poorter and Nagel 2000). In wheat, with increasing waterlogging intensity (duration and water depth), the root-shoot ratio decreased as the seminal root system was severely affected and adventitious root length was restricted (Chapters 3 and 4). During recovery, wheat first restored the root-shoot ratio by developing new roots and/or increasing the length of the existing roots (Chapter 4). As restoring some “optimum” biomass partitioning between roots and shoots appears to be a general response of plants after a stress is released, it is hypothesised that the size of the root system that survives or develops during waterlogging should determine the speed of recovery of wheat. It may be for this reason that shoot growth during waterlogging is often a poor predictor of the ability of a wheat variety to recover from waterlogging (Setter and Waters 2003). This further emphasises that the key traits to select for when developing wheat varieties with some waterlogging tolerance are likely to be root traits. Shoot traits may be of secondary importance for the improvement of waterlogging tolerance in wheat.

In conclusion, the results of the present work indicate that:
• There is a linear relationship between water-table depth and growth reduction in wheat in waterlogged soil. However, during recovery, the linear relationship does not hold. It is important to investigate the response of wheat during waterlogging as well as during recovery to determine waterlogging tolerance (Chapter 3).

• Short-term waterlogging has long-term effects on young wheat plants. Seminal roots of young wheat did not recover, even after 3-7 d of waterlogging over a period of 25-21 d recovery (Chapter 4).

• In adventitious roots of wheat grown in partially waterlogged conditions, O₂ diffusion from the aerobic portion of the roots to the apex facilitates root extension into the O₂-deficient media (Chapter 5).

Research to improve the waterlogging tolerance of wheat must consider the great variability of possible waterlogging scenarios, and evaluate for traits that allow for sustained root growth during cycles of waterlogging and draining events that are of different intensity.

6.5 References


McDonald MP, Galwey NW, Colmer TD (2001) Waterlogging tolerance in the tribe Triticeae: The adventitious roots of Critesion marinum have a relatively high porosity and a barrier to radial oxygen loss. Plant, Cell and Environment 24, 585-596.


