ASPECTS OF SOIL ACIDITY AND THEIR EFFECT ON PLANT GROWTH

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Abstract

The effects of low pH, Al, organic and phenolic acids on the growth of naturally occurring plant species were determined. The amelioration of Al toxicity by Si and organic acids was also investigated.

Plants were grown from seeds in nutrient solutions simulating the ionic composition of soil solutions from five soil types ranging from acidic peat to calcareous soil. Soil solutions were extracted and analysed using centrifugation, with and without an immiscible displacent (1,1,1-trichloroethane), at both low (4000 rpm) and high speed (12000 rpm).

Races of *Holcus lanatus* L. and *Betula pendula* Roth. from acidic soils (FM and SMM) grew better in low pH solutions (pH< 4.0). In acid-sensitive races Ca absorption was inhibited at low pH.

Races of *B.pendula* from strongly to moderately acidic soils (FM, SMM, KP) were Al-tolerant and effectively excluded Al from shoots. Root elongation and leaf expansion were inhibited by all Al concentrations in races from calcareous soils (KR).

Low concentrations of Al stimulated growth in some races of *B.pendula* (2 and 5 mg Al l^{-1}) and *Anthoxanthum odoratum* L. (1.3 and 2.7 mg Al l^{-1}).

Al (25 and 35 mg l^{-1}) inhibited root and shoot growth in *H.lanatus*. Si (1500 and 2500 µM $Si(OH)_4$) addition to nutrient solutions alleviated Al-damage and restored nutrient uptake to values similar to those in plants grown with neither Al or Si. The ameliorative effects of Si were possibly achieved through Al/Si co-deposition in the root cell walls and maintenance of Golgi activity. Si at 1500 µM was beneficial but inhibited growth at 2500 µM. Al and hydroxyaluminosilicates at pH 5.6 were not toxic.

Formic and tartaric acid ameliorated Al toxicity by reducing its availability. These organic acids on their own stimulated growth in *H.lanatus* and *Deschampsia flexuosa* (L.) Trin.

Phenolic acids stimulated growth of *H.lanatus* in acidic solutions (pH 4.0) but not near-neutral solutions, particularly in races from soils high in phenolics. Addition of plant residue to acidic peats increased the growth of races from calcareous and acidic mineral soils.

Contents

Chapter 1

General Introduction

The nature of soil acidity, together with its implications for plant growth, are discussed in this introduction. There are several causes of soil acidity. Soil parent materials may be acid and initially low in basic cations (granite, base-poor sands) (Fitter & Hay 1991, Frageria *et al.* 1990), or these elements may have been removed from the soil profile by leaching or harvesting of crops. More recently soils have acidified through anthropogenic effects including nitrogenous fertilisers and acid deposition.

1.1 Soil acidification

1.1.1 Endogenous soil acidifying factors

Soil acidification is a slow process when only endogenous factors are operating (de Klein *et al.* 1997, Helyar & Porter 1989). Living soil organisms and plants respire producing CO_2, which dissolves in soil water to give carbonic acid, H_2CO_3, which dissociates to HCO_3^- and H^+. The hydrogen ion does not exist as a free proton (H^+) in solution but rather is combined with at least one molecule of water forming the oxonium or hydronium ion, H_3O^+. For simplicity the H_3O^+ ion will be referred to as the H^+ ion. H^+ ions are discharged from plant roots when cation uptake exceeds anion uptake, thus maintaining charge balance. In addition, both H^+ ions and humic residues (organic and phenolic acids) are released as the products of plant litter and soil organic matter decomposition (Drever 1994, Drever & Stillings 1997, Rowell 1988, Rowell 1995, Tan 1998, Thomas & Hargrove 1984). The mineralization of organic matter produces nitrate, sulphate, and phosphate. Production of NO_3^- (discussed later) involves the nitrification of NH_4^+ and concurrent production of H^+ ions.

The phenolic and carboxylic acids of soil organic matter are not only sources of H^+ ions but are also involved in soil pH buffering which is discussed later. Organic acids have been shown to reduce Al toxicity (Foy *et al.* 1990, Gerke 1994, Harper *et al.* 1995, Hue *et al.* 1986, Kerven *et al.* 1991, Ostatek-Boczynski *et al.* 1995, Ownby & Popham 1989, Slattery & Morrison 1995, Suhayda & Haug 1986, Suthipradit *et al.* 1990) by chelating Al in non-toxic forms (Table 1.1). Evans & Kamprath (1970) found less exchangeable Al in organic soils compared with mineral soils despite the low pH of both. Phenolic acids have also been implicated in allelopathy (Rice 1984) and have been shown to be toxic to plants in mixtures of low concentrations (Vaughan *et al.* 1993). More recently, phenolic acids in plants were

suggested to be adaptations to soil acidity (Northup *et al.* 1995). The effects of organic and phenolic acids on plant growth are investigated in Chapters 8 and 9.

Weathering of primary minerals occurs via physical disintegration and chemical processes (Brady 1990) which is primarily governed by leaching soil water and its dissolved salts and acids (sources listed above) and climatic conditions. Hydrolysis, hydration, and oxidation reactions operate simultaneously converting primary minerals into secondary silicate minerals. Products of these reactions, basic cations (including Ca^{2+}, Mg^{2+}, Na^+, and K^+), can be leached out, remain in solution, or form part of the crystal lattice of new minerals (Nortcliff 1988). As the leaching of basic cations continues, they are replaced by acidic H^+ and Al^{3+} ions on their exchange sites (McLean & Brown 1984). The ratio of Al:Ca+Mg+K in the soil solution will increase parallel to the increased ratio on exchange sites (Rowell 1988). Weathering does not cease after secondary minerals are formed but continues to form more stable chemical states such as kaolinite and quartz, and under extreme weathering, oxides of aluminium (Al) and iron (Fe). The composition of primary minerals influences their susceptibility to weathering. In general acidic rocks show much less chemical change than more basic rocks (Nortcliff 1988).

1.1.2 Exogenous soil acidifying factors

1.1.2.1 Acid Rains

Rain water is naturally acidic (pH 5.6) as a result of dissolved CO_2 and its dissociation. When impurities such as SO_2, NO, and NO_2, and their reaction products HNO_3, H_2SO_4, SO_4^{2-}, and NO_3^-, are present the acidity increases. Acid deposition became an increasingly important factor in soil acidification in Europe after the industrial revolution, and then more recently, with the expansion of livestock production and resulting ammonia emissions (Goulding & Blake 1998). Acid rain is a simplified term to describe all possible routes for the deposition of acidifying pollutants from the atmosphere (Marsden 1993). It has been implicated in most environmental damage in the northern hemisphere (De Graaf *et al.* 1997, Marsden 1993, Raubauch *et al.* 1998), particularly the decline of forests in Central Europe and North America (Hahn & Marschner 1998, Ingerslev 1997, Kreutzer & Weiss 1998, Matzner & Murach 1995).

A decline in soil pH values has been found in several countries during this period. Ahokas (1997) compared topsoil pH measurements, of the southern coast of Finland, measured in 1995 with those of the mid 1930s. There was a mean decline of 0.57 ±0.11 pH units which he attributed to long-distance air pollution carried via prevailing southwest winds. Pederson (1993) estimated that 40-65 % of total acidification in Danish forests was a result of air pollution.

Matzner & Murach (1995) summarised the effects of acid deposition :

- increased rate of soil acidification
- loss of base cations from exchange sites
- decrease in soil pH
- release of Al^{3+} ions into the soil solution

1.1.2.2 Nitrogen based fertilisers

The regular use of ammonium-based fertilisers to supply N to crops has strong acidifying influences on the soil (Adams 1984, Frageria *et al.* 1990, Rowell 1988). Ammonium-based fertilisers include anhydrous NH_3, $(NH_2)_2CO$ (urea), NH_4NO_3, and $(NH_4)_2SO_4$. The nitrification (or microbial oxidation) of NH_4^+ to NO_3^- occurs within a few weeks in agricultural soils via the following reactions :

$$NH_3 + 2O_2 \rightarrow H^+ + NO_3^- + H_2O \qquad \text{(nitrification of ammonia)}$$

$$(NH_2)_2CO + 4O_2 \rightarrow 2NO_3^- + 2H^+ + CO_2 + H_2O \qquad \text{(nitrification of urea)}$$

$$NH_4NO_3 + 2O_2 \rightarrow 2H^+ + 2NO_3^- + H_2O \qquad \text{(nitrification of ammonium nitrate)}$$

$$(NH_4)_2SO_4 + 4O_2 \rightarrow 2NO_3^- + 4H^+ + 2H_2O + SO_4^{2-} \qquad \text{(nitrification of ammonium sulphate)}$$
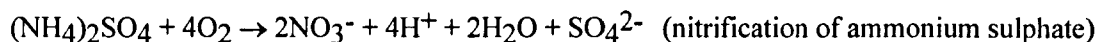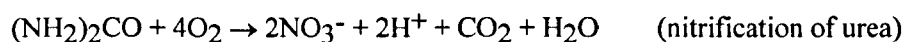
Therefore the fertilisers themselves are not acid but they are acid-forming. The net reaction of ammoniacal N addition, nitrification, and subsequent uptake and assimilation of NO_3^- by plants would be neutral. However, N inputs are generally greater than those assimilated by biota and OH^- ions, released in return for NO_3^- uptake, do not completely neutralise generated acidity (Barak *et al.* 1997, Russell 1988). The leaching of NO_3^- from the root zone causes permanent acidification by uncoupling the proton balancing system (Bouman *et al.* 1995).

Soil acidification through exogenous factors is usually offset, in agricultural land, with the application of lime (de Klein *et al.* 1997), a practice which has been used for thousands of years. Current pollution however requires increasingly greater amounts of lime which, together with the intensification of agriculture, are not economically feasible (Goulding & Blake 1998). Lime addition is discussed in Chapter 4.

1.2 The nature of soil acidity

The inherent pH in any soil is a product of the interaction of several processes resulting in soil development. Consequently soil acidity involves more than just the pH of the soil solution. As soils become more acidic, according to the factors described above, there are associated changes in the soil solution :

- availability of essential plant nutrients, in particular Mo, P, Ca, and Mg, become limited to potentially deficient concentrations
- solubilities of rhizotoxic species including Al^{3+} and Mn^{2+} are elevated
- solubility and availability of Fe^{3+} is increased

These manifestations of soil acidity may act (to a certain extent) independently but far more often act in conjunction to affect plant growth. It is therefore evident that establishing the mechanisms by which H^+ ions, or indeed other rhizotoxic species such as Al^{3+} ions, are directly toxic to plants is complex. The same challenge has faced researchers in the field of soil acidity for the past 30 years (Ritchie 1989). The effects of elevated H^+, and Al^{3+} concentrations on plant growth are investigated in Chapters 4, 5, 6, and 7.

1.2.1 Soil pH and its measurement

The pH of a soil is an estimate of the acidity or alkalinity of the soil solution (Figure 1.1). An acid dissociates in water to H^+ ions and an alkali dissociates to yield OH^- ions. In other words pH measures the concentration (activity) of hydrogen ions, H^+, and hydroxyl ions, OH^-, in the soil solution (Brady 1990). The soil solution is the water held within the soil pores at various tensions which depend upon both the pore size (micropores, mesopores, and macropores) and the amount of water present (Brady 1990).

The pH of a solution is defined as $-\log(H^+)$, where (H^+) is the activity of hydrogen ions in solution. In dilute solutions the activity is roughly equal to the concentration, $[H^+]$, in mol l^{-1}. Therefore pH is defined as :

$$pH = -\log[H^+]$$

(Brady 1990, Rowell 1995). Each unit change in pH represents a tenfold change in the activity of H^+ and OH^- ions. From the equation, 10^{-7} mol l^{-1} $[H^+]$ is equivalent to pH 7 or neutrality. At pH 7 H^+ ions are balanced by OH^- ion. Above pH 7, OH^- ions are in excess, and solutions are alkaline. Below pH 7, H^+ ions dominate over OH^- ions, and solutions are acidic. The range of pH in soil solutions is from about 3 to 10 (i.e. 10^{-3} to 10^{-10} mol H^+ l^{-1}) (Rowell 1995, Figure 1.1).

A knowledge of soil pH is useful, and is frequently used to assess the extent of soil acidification, to estimate the susceptibility of soil to further acidification, and to evaluate the toxicity of elements to plant growth (Courchesne *et al.* 1995, Gillman 1991). For example, most plants grow best in slightly acid soils (pH 6-7, Figure 1.1) where the majority of plant nutrients are available in optimal concentrations (Tan 1998). However, pH is also an arbitrary unit since soil also involves exchangeable H^+ as well as solution H^+ (Rowell 1995, Thomas & Hargrove 1984).

The most common laboratory procedure adopted today, in the measurement of soil pH, involves a combined glass electrode and calomel electrode where the voltage difference between the two is measured by a millivoltmeter (pH meter). The two electrodes are first calibrated in buffer solutions of known pH (often pH values 4 and 7). In order to make electrical contact in the soil, a paste or suspension is prepared. Often an analysis of a 1 : 2.5 (g soil : ml distilled water) suspension is used (Allen 1989).

The measured soil pH is affected by the treatment of soil (drying/storage) between sampling and suspension preparation, and by the choice of solution used to prepare the suspension e.g. distilled water or electrolyte.

Courchesne *et al.* (1995) determined the effects of air-drying on the pH of nine podzolic soil profiles in southern Quebec, Canada. Soil pH measurements were made in H_2O (pH_{H_2O}) and 0.01 M $CaCl_2$ (pH_{CaCl_2}). Drying generally resulted in soil acidification for all horizon types, and the pH change was greater and more consistent for H_2O than $CaCl_2$ (up to 0.5 and 0.25 pH units). The decrease in pH after air-drying was attributed to an increase in organic matter solubility (dissociation of carboxyl groups and phenolic groups) and extractable Al (and its subsequent hydrolysis).
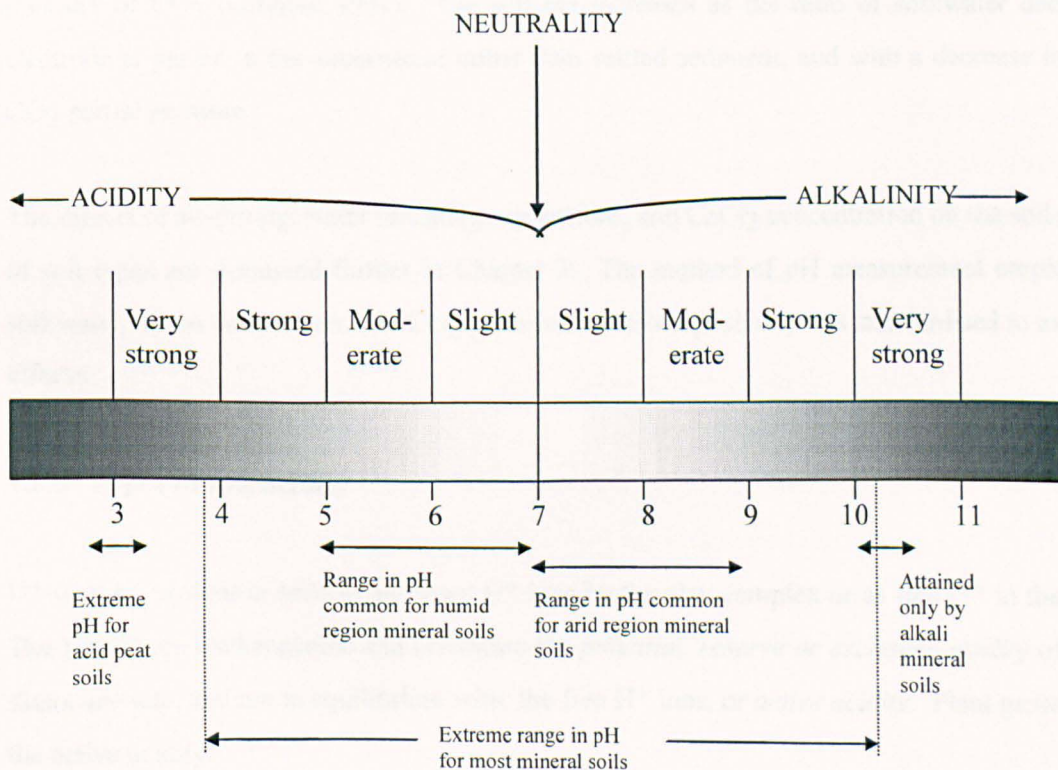
Figure 1.1. Soil pH ranges (Brady 1990).

Addition of distilled water to soils containing predominantly 2:1 and 1:1 clays (negatively charged) decreases the concentration of H^+ ions in the soil solution and hence, in acid soils, causes an increase in the pH relative to the true soil solution in undisturbed soil (the dilution effect) (Gillman 1991, Rowell 1988). A 2:1 clay has two silica sheets (linked Si tetrahedral units) sandwiching one alumina sheet (linked Al octahedral units), and a 1:1 clay has one Si sheet and one dioctahedral gibbsite sheet $(O_2(OH)Al_2(OH)_3)$ (Brady 1990).

In an attempt to avoid the "dilution effect" a dilute electrolyte solution (0.01 mol l^{-1} $CaCl_2$ or 0.1 mol l^{-1} KCl) is used to prepare the soil suspension. The dilution effect is counteracted since the added cation displaces H^+ from the exchange sites into the solution, resulting in a soil pH about 0.5 units lower than that obtained with distilled water. However in acid soils, containing exchangeable monomeric Al^{3+}, the addition of an electrolyte is reported to cause the displacement of Al, the hydrolysis of which results in a lowered pH also not representative of the undisturbed soil pH (Gillman 1991, Thomas & Hargrove 1984). The effectiveness of electrolytes in lowering soil pH values increases with the valence of the cation and electrolyte concentration (Black 1968, Thomas & Hargrove 1984).

Other factors also affect the measured soil pH: the time of contact between soil and solution, the ratio of soil:distilled water, the position of the electrode in the suspension, and the temperature and partial pressure of CO_2 (Gillman 1991). The soil pH increases as the ratio of soil:water decreases, if the electrode is placed in the supernatant rather than settled sediment, and with a decrease in atmospheric CO_2 partial pressure.

The effects of air-drying, water or $CaCl_2$ suspensions, and $CaCl_2$ concentration on the soil pH of a range of soil types are discussed further in Chapter 3. The method of pH measurement employed (ratio of soil:water, room temperature and CO_2 pressure, electrode position) was standardised to avoid any other effects.

1.2.2 Types of soil acidity

H^+ ions are present in soils as adsorbed H^+ ions by the clay complex or as free H^+ in the soil solution. The former are exchangeable and constitute the *potential, reserve or exchange acidity* of soils. These dissociate into, and are in equilibrium with, the free H^+ ions, or *active acidity*. Plant growth responds to the active acidity.

The greater the soil cation exchange capacity (CEC) the greater the potential acidity, and the greater the resistance to change in soil pH (buffer capacity). In effect soils buffer H^+ concentration through ion exchange of the clays, sesquioxides, and organic matter, thereby retaining an equilibrium between the

potential and active acidity (Daji 1970, Rowell 1995). Organic matter increases the CEC in soils and therefore the buffer capacity. Humic acids contain acidic functional groups, such as carboxyl and phenolic hydroxyl groups, that preferentially absorb H^+ ions. Exchangeable Al in the soil also acts as an effective buffer and is discussed below.

1.2.3 The chemistry of aluminium

A knowledge of the behaviour of aluminium in acid soil solutions is essential in any rhizotoxic study. Al speciation is intricately linked to its bioavailability and therefore toxicity in biological systems (Birchall 1992). The toxic species are generally assumed to be the inorganic monomeric fraction of Al, Al^{3+} and its derivatives (Table 1.1). Stable organo-Al (*e.g.* Al-citrate) and Al-F complexes are not considered phytotoxic (Fernández-Sanjurjo *et al.* 1998, Table 1.1).

Total soluble Al (Al_T) is usually between 0.27-9.44 mg l^{-1} in soil solutions, with concentrations >27.0 mg l^{-1} occurring in exceptional circumstances such as acid sulphate soils (Ritchie 1989). However Al does not solely exist in solutions as the free aqueous Al^{3+} ion. Instead it undergoes a series of hydrolysis reactions (Table 1.1) dependent upon the concentration and type of ligands present, ionic strength, and pH of the solution (Chow 1992). The Al ions and their hydrolysis products are the primary sources of H^+ ions in soils (pH< 6.0) for both active and potential acidity (Tan 1998).

Martin (1986), using solutions of Al and pure water, described the species distribution of Al between pH's 3-8, where the only ligand present was hydroxide. He stated that, no matter what other ligands were present, an understanding of the state of Al^{3+} in any aqueous system demands an awareness of the species that Al^{3+} forms at different pH values with the components of water.

In solutions more acidic than pH 5.0, Al^{3+} existed almost solely as the octahedral hexahydrate, $Al(H_2O)_6^{3+}$, commonly abbreviated to Al^{3+}. As the solution H^+ concentration dropped and pH increased, Al^{3+} underwent a series of successive deprotonations to yield $Al(OH)^{2+}$ and $Al(OH)_2^+$. At neutrality, pH 7.0, $Al(OH)_3$ precipitated, re-dissolving in more basic solutions still to form the tetrahedral aluminate, $Al(OH)_4^-$. Polynuclear species could also be present but their compositions were time dependent. These equilibria among mononuclear (monomeric) Al^{3+} species in aqueous solutions are described by reactions 1-3 in Table 1.1 and Figure 1.2. Al^{3+} can be seen to dominate at pH <5.0, a mixture of species are present from pH 5-6.2, and the tetrahydroxo $Al(OH)_4^-$ species dominates at pH >6.2. According to Martin (1986), regardless of any other ligands present, the species distribution in Figure 1.2 prevails in aqueous solutions.

Table 1.1. Possible reactions of aluminium in the soil solutions. L, organic ligand *e.g.* citrate.

Aluminium Reactions

$$Al^{3+} + H_2O \rightleftharpoons Al(OH)^{2+} + H^+$$

$$Al(OH)^{2+} + H_2O \rightleftharpoons Al(OH)_2^+ + H^+$$

$$Al(OH)_2^+ + 2H_2O \rightleftharpoons Al(OH)_4^- + 2H^+$$

$$Al^{3+} + SO_4^{2-} \rightleftharpoons Al(SO_4)^+$$

$$Al^{3+} + 2SO_4^{2-} \rightleftharpoons Al(SO_4)_2^-$$

$$Al^{3+} + F^- \rightleftharpoons AlF^{2+}$$

$$2Al^{3+} + 2OH^- \rightleftharpoons Al_2(OH)_2^{4+}$$

$$3Al^{3+} + 4OH^- \rightleftharpoons Al_3(OH)_4^{5+}$$

$$13Al^{3+} + 28OH^- \rightleftharpoons Al_{13}O_4(OH)_{24}^{7+} + 4H^+$$

$$Al^{3+} + L^{3-} \rightleftharpoons AlL$$
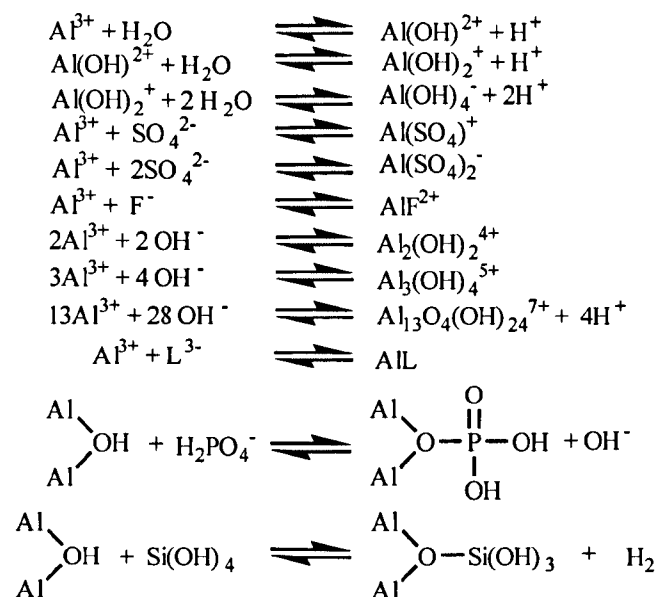
Several different mineral phases are considered sources of soil Al: aluminosilicates of different degrees of crystallinity (e.g. kaolinite); gibbsite (Al(OH)₃) or alternative Al-hydroxides which lack a crystalline structure (Al(OH)₃ (amorphous)); and aluminosulphate minerals such as jurbanite (Fernández-Sanjurjo *et al.* 1998). The Al minerals closest to equilibrium with the soil solution composition are considered those which control the Al solubility and ultimately the amounts, equilibria, and distribution of monomeric Al species. Control of soil solution Al by alumino-sulphate minerals is not usual in natural soils but has been found in acid mine soils. According to Arp & Quimet (1986) total Al solubility increases in the order: kaolinite < gibbsite < Al(OH)₃ (amorphous). We cannot however assume that the least-soluble Al-containing mineral will be controlling the amount of Al^{3+} in solution (Ritchie 1995). Helyar *et al.* (1993) showed soil solution Al activity was within the range of solubility of the main soil minerals present.

Significant quantities of amorphous Al(OH)₃ minerals are present in soils and these, rather than crystalline minerals (gibbsite), are most often regarded as the primary controls over Al solubility. In most soils of the Sor watershed in Galicia, northwest Spain, Fernández-Sanjurjo *et al.* (1998) found Al solubility was controlled by amorphous Al-hydroxides. Similarly, Sjöström (1994) showed Al solubility in the soils of Halland County, southwest Sweden were governed by amorphous Al-hydroxides. Aluminosilicate minerals (illite, smectite, and halloysite) did not exert a strong control on Al solubility.

The absolute amounts of soluble Al permitted by dissolution of gibbsite (crystalline phase of $Al(OH)_3$) compared with those predicted from a representative amorphous $Al(OH)_3$ (non-crystalline) are shown in Figure 1.3. The solubility of Al with respect to amorphous $Al(OH)_3$ is up to 100-fold greater than solubility with respect to gibbsite. The solubility curve of $Al(OH)_3$ (amorphous) is described as representative since a wide range of amorphous Al-hydroxide minerals exist. The minimum solubility in both curves occurs at pH 6.2.
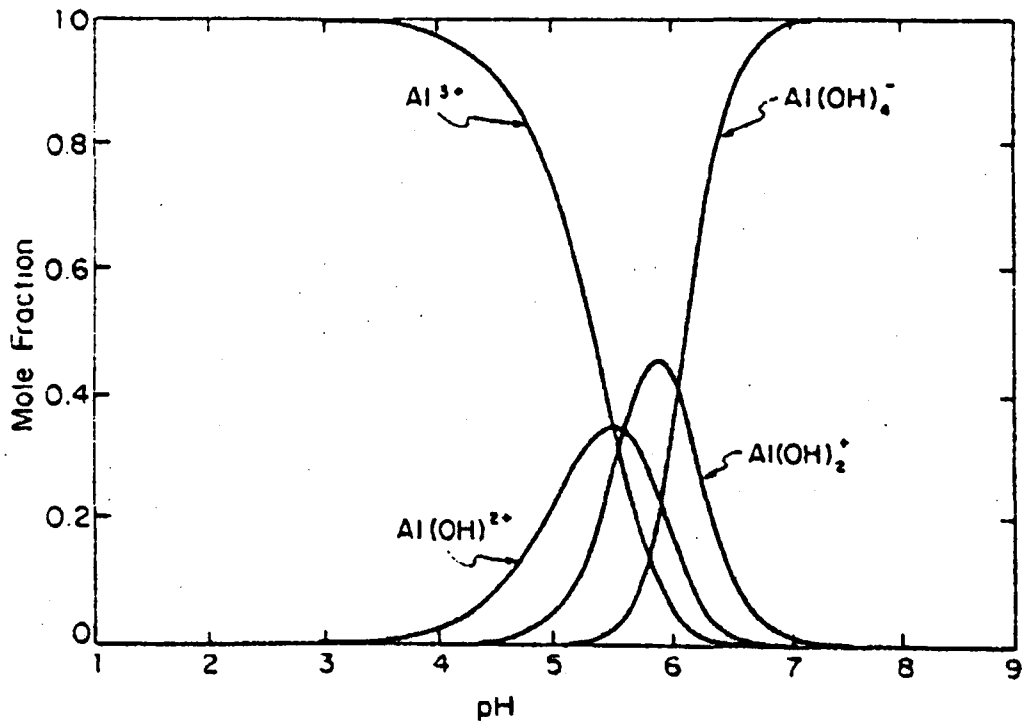
Figure 1.2. Distribution of soluble, mononuclear aluminium ion species in aqueous solutions (Martin 1986).

Finally, the solubility of Al-minerals exert the main, but not exclusive, control over Al in natural solutions. Al distribution is also controlled by pH, organic acids (e.g. citric acid), metal ions, and inorganic ligands (e.g. F^-, PO_4^{3-}, Table 1.1) (Arp & Quimet 1986, Helyar et al. 1993, Marsden 1993).

Al in acid soil also acts as a pH buffer. Free Al^{3+}, released by exchange of adsorbed Al on clay minerals into the soil solution, yields H^+ upon hydrolysis. When these H^+ ions are not readsorbed on the clay complex, but instead are neutralised (liming) or leached from the soil profile, the Al hydroxy ions are precipitated as insoluble $Al(OH)_3$. However, more H^+ ions can be produced through further release of Al from exchange sites to replace that which was lost. The soil pH then remains unaffected (Kennedy 1986, Tan 1998). In this way exchangeable Al, like exchangeable H^+, contribute to the soil potential acidity.
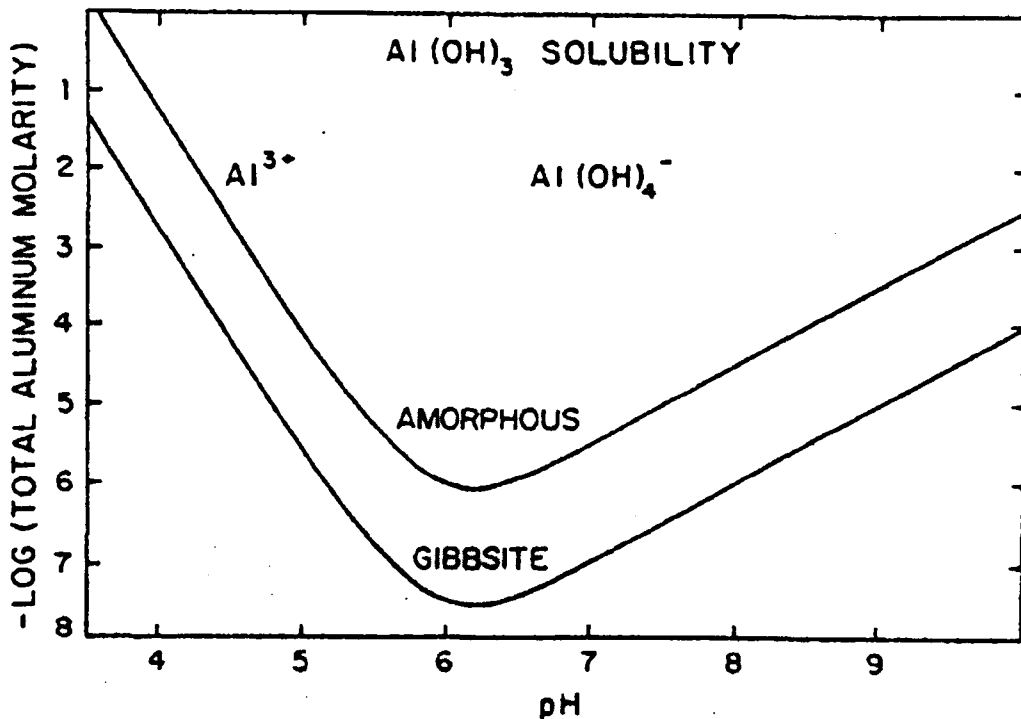
Figure 1.3. Negative logarithm of total molar concentration of aluminium allowed by Al(OH)$_3$ solubility versus pH (Martin 1986). Lower curve represents true equilibrium solubility from gibbsite. Upper curve depicts representative solubility from amorphous Al(OH)$_3$. Al^{3+} is the predominant soluble aluminium species at pH < 5.0 and Al(OH)$_4^-$ at pH >6.2, where the minimum solubility occurs for both curves. Between pH 5 and 6.2 there is a mixture of soluble species, as shown in Figure 1.2.

1.3 The implications of soil acidity for plant growth

1.3.1 H$^+$ ion toxicity

Early research attributed poor plant growth on acid soils to low pH and in particular to H$^+$ ion activity. Arnon & Johnson (1942) showed growth of lettuce, tomato, and Bermuda-grass, was completely inhibited in nutrient solution at pH 3.0. Proposed mechanisms of this growth inhibition included H$^+$-inhibition of nutrient absorption. In low pH nutrient solutions, absorption was inhibited and cations previously absorbed tended to leak out of plants into solutions. H$^+$ was proposed to compete with cations at cation-selective sites on the plasma membrane. Further evidence for this was provided from growth experiments with increased Ca concentrations. Arnon & Johnson (1942) showed growth inhibition in lettuce at low pH was alleviated by increasing external Ca concentrations. Similarly, Rains *et al.* (1964) found uptake by barley roots of the radioisotope Rb (taken up via the same carrier site as K) at pH 3.9 was significantly greater in the presence of Ca than in its absence. It was concluded that Ca functioned in reducing injury by H$^+$ ions to the selective nutrient absorption mechanism.

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Subsequent research, contrary to Arnon & Johnson (1942), frequently showed plants grew well in nutrient solutions at pH values which were not tolerated in soils (Black 1968). Furthermore investigations of the pH of tissue fluids showed plant tissues were able to tolerate acidity greater or equal to that commonly found in acid soils. These observations indicated that H^+ activity in most acid soils was not specifically toxic to plants (Black 1968). H^+ ions are now considered to have a direct toxic effect when present in very high concentrations (pH< 4.0), such as in strongly acidic mine spoils (pH< 3.0) or acid sulphate soils (Foy 1992, Tan 1998). In organic soils, such as acid peats (Figure 1.1), where toxic concentrations of soluble Mn and Al are negligible, H^+ activity is believed to be responsible for poor plant growth (Evans & Kamprath 1970).

1.3.2 Al toxicity

Evidence implicating Al as the primary growth-limiting factor in acid soils came from the following areas:

- the addition of Al, as low as 1 mg l^{-1}, to culture solutions reduced plant growth
- concentrations of Al in soil solutions of soils with pH values < 5.0 were frequently in the range in which Al toxicity was found in culture solutions
- the Al concentration of displaced soil solutions, used as culture solutions for growing plants, were toxic (Vlamis 1953)

Early ecological work was carried out by Rorison with naturally occurring species which differed in their affinity for acid and calcareous substrata. Species were selected on the basis of their relative frequency of occurrence over a range of surface soil pH's. Rorison (1960a) grew two calcicoles: *Scabiosa columbaria* and *Asperula cynanchica* and two calcifuges: *Galium saxatile* and *Holcus mollis*, on calcareous (pH 7.6) and acid (pH 4.8) soils with and without P, K, and Ca additions. Both calcicoles thrived on calcareous soils despite different physical properties but failed on the acid soil. The calcicoles responded positively to acid soil treated with $Ca(OH)_2$. Rorison (1960a) suggested that the pH rise after Ca addition precipitated soluble Al rendering it non-toxic. Rorison (1960b) continued this work further by growing the same species in inorganic and soil water cultures to test the effect of pH, Ca, and Al. Water cultures confirmed that pH and a range of Ca concentrations were not the factors inhibiting the growth of *Scabiosa* and *Asperula*, and that the toxic factor excluding these two species from the acid soil was ionic Al.

The calcifuge, *Deschampsia flexuosa*, is normally restricted to acidic soils and was shown by Hackett (1965) to be tolerant of relatively high concentrations of Al, Mn, and NH_4-N when their concentrations were varied singly in nutrient solution (Rorison 1985). Rorison (1985) compared its growth response to Al and N (NH_4^+-N or NO_3^--N) with that of *Bromopsis erecta* which is normally restricted to habitats

with circum-neutral soils, and *Holcus lanatus* which was most common in soils of pH 5-6. NH_4^+-N is dominant in acid soils and NO_3^--N in calcareous soils. *Deschampsia* was tolerant of 2.7-5.4 mg l^{-1} Al when N was supplied as NH_4^+-N. *Holcus* grew better in solutions with NO_3^--N rather than NH_4^+-N. It showed classical symptoms of Al toxicity (reduced root and shoot growth, and stunted roots) in the presence of NO_3^--N.

Grime & Hodgson (1969) determined the Al concentration required to cause a 50 % inhibition of root growth in common grass species. The results showed a close correlation between the field distribution and Al sensitivity of the different species. *Deschampsia flexuosa*, *Nardus stricta*, and *Holcus mollis* were very resistant to Al (0.54-0.81 mg l^{-1}), and all three were common in acid soils. In contrast *Sanguisorba minor*, *Centaurea nigra*, *Briza media*, and *Leontodon hispidus*, species generally restricted to calcareous soils, were very sensitive to Al (0.14 mg l^{-1}). Furthermore both acid and calcareous races of wide-ranging species, such as *Festuca ovina*, showed considerable resistance to Al.

Rorison (1958) also investigated the effects of Al on calcicolous legumes in acid soils. *Medicago sativa* and *Onobrychis viciifolia* were grown in water culture (which simulated soil solutions) at pH 4.5 with combinations of Al, Mn, and Ca, and their growth was contrasted with that of the calcifuge *Ornithopus sativus*. After four weeks growth in solutions with Al (50 mg l^{-1}) both *Medicago* and *Onobrychis* were dead. *Onobrychis* was strongly affected by Al but not by Mn, whereas both Al and Mn were toxic to *Medicago*. The yield of *Ornithopus* was unaffected by any of the treatments. Tap root and lateral elongation in the two calcicoles had ceased after only a few days in Al solutions. Further experiments with *Onobrychis* alone showed that under toxic conditions of Al (at least 40 mg l^{-1}) the inhibition of growth at pH 4.5 occurred simultaneously with a rapid uptake of Al into the young seedling root. Rorison (1958) suggested Al might inhibit cell division by antagonising active Fe uptake, thus depriving the nucleus of Fe necessary for cell division. The uptake of Fe was inhibited by Al in *Onobrychis*, the degree of inhibition varied according to Al concentration. In agreement, Clarkson & Sanderson (1969) believed cell division to be the primary effect of Al and went on to suggest that Al disturbed the mitotic cycle during DNA synthesis in the S period possibly by inhibition of nucleic acid synthesis. Both Naidoo *et al.* (1976) and Matsumoto *et al.* (1976b) proposed Al accumulated in the nuclei of snapbean, cotton, and pea, and bound to esteric P in nucleic acids.

Clarkson (1966) found, in some plants, low concentrations of Al enhanced growth. The two grasses *Agrostis curtisii* and *Agrostis capillaris*, both calcifuges, grew better in solutions with Al at concentrations of 2.7-10.8 mg l^{-1}. Root lengths relative to those in solutions with no Al were between 104-175 %. Grime & Hodgson (1969) suggested Al occupied metabolically inactive sites which would otherwise be occupied by Fe, and by displacing this Fe, Al prevented Fe deficiency.

Rorison (1958) showed aluminium in anionic form (aluminate, $Al(OH)_4^-$ or AlO_2^-), soluble in solution between pH 6.0-7.5, was taken up at a steady state by legumes but did not cause toxicity. Jones (1961) also found the aluminate anion was accumulated in large quantity by barley cultivars (cv. Herta and Sacaramento) from fly-ash deposits at high pH values (up to pH 9.1) with no apparent toxicity. In a more recent study, Tyler (1994) showed leaf Al concentrations of plants (*Geranium sanguineum* and *Quercus robur*) growing on limestone soils (about pH 8.0) are not very different from those found in plants on acid silicate soils, despite the significantly lower soil concentrations. Since the aluminate anion ($Al(OH)_4^-$) has some structural similarity to $H_2PO_4^-/HPO_4^{2-}$, Tyler suggested aluminate may be taken up by the same mechanism as phosphate.

Davies & Snaydon (1971) found populations of *Anthoxanthum odoratum* from acid soils were more tolerant of high Al concentrations in culture solutions than populations from calcareous soils. Foy *et al.* (1978) associated Al tolerance with a plants' ability to resist Al-induced nutrient deficiency and contain lower concentrations of Al in roots or shoots. The roots of tolerant cultivars of wheat, barley, soybean, and snapbean contained less Al than sensitive cultivars (Foy *et al.* 1978). Tolerance of *Rhododendron spp.*, *Vaccinium oxycoccos*, rice, rye, and alfalfa to Al correlated with lower concentrations of Al in the tops and entrapment of excess Al in the roots (Foy *et al.* 1978). Al tolerance of cultivars of wheat, barley, soybean, and snap bean was explained by the plants' ability to resist Al-induced Ca deficiency. Similarly, certain cultivars of wheat and tomato were P-efficient (Foy *et al.* 1978). Jones (1961) suggested that, oxalic and citric acid in roots of Al-tolerant species chelate Al, preventing Al-P precipitation and P deficiency. More recent research has shown a unique interaction between Al and Si suggesting a potential role for Si in reducing the bioavailability and hence toxicity of Al in biological systems (Birchall *et al.* 1996, Hodson & Evans 1995). This role of Si in ameliorating Al toxicity has been shown in animals (Birchall *et al.* 1996) but contradictory results have been found in higher plants (Hodson & Evans 1995).

Recently, with the amplification of soil acidification, the importance of selecting for Al-tolerant crops and sustaining productivity has led to a wealth of studies using crop species, particularly wheat. The experiments included in this thesis used naturally-occurring species and races present in a range of soil environments (described in Chapter 2). The early studies (described above) used natural species but few studies used populations of the same species. Much research has focused on the amelioration of Al toxicity by elevated Ca concentrations (Kinraide *et al.* 1994), and to a lesser extent Mg. This has followed from the Ca-homeostasis hypothesis which suggests Al disrupts membrane integrity by preventing Ca from binding to Ca membrane proteins. Examples of these studies include Kasran *et al.* (1995), Rengel & Robinson (1990), and Wheeler & Edmeades (1995), who found Ca prevented Al toxicity in groundnut (cv. Matjam), ryegrass, and wheat. Less research has been carried out on the

amelioration of Al toxicity using Si as silicic acid (see above) and organic acids (Section 1.1.1) and this is discussed in Chapters 6, 7, and 8 of this thesis.

A deficiency of many metal-toxicity studies, highlighted by Davies (1991), concerns the chemical background solution in which the metal is supplied. Hydroponics, or nutrient solutions, are widely used since they overcome the complexities of field soils. Hydroponics were defined by Jensen (1997) as a technology for growing plants in nutrient solutions with or without the use of an artificial medium to provide mechanical support. However many studies have tested Al toxicity while using background solutions of 0.5 M $Ca(NO)_2$ which may in itself ameliorate the effects of Al and its concentrations is therefore critical in toxicity experiments (Rorison 1958). The nutrient solutions used here simulated the ionic composition of extracted soil solutions (Chapter 3), were monitored on a daily basis, and their speciation was predicted using the ionic speciation program, GEOCHEM. This was to ensure a good knowledge of the chemical conditions that the plants were actually experiencing rather than that desired (De Rijck & Schrevens 1997).

1.4 Thesis aims

The aims of this thesis were :

- To gain more insight into the way chemical soil factors, associated with soil acidity, both organic and inorganic, influence plant growth.

- To use seeds from populations of naturally occurring species which cover a range in habitat from acidic soils (organic and mineral) to calcareous soils.

- To determine whether or not the growth responses of different populations to aspects of soil acidity are in accordance with their ecological distribution.

- To investigate aspects of soil acidity using hydroponic nutrient solutions which simulate actual soil solutions.

- To determine the effects of extraction method and centrifugation speed on the soil solution ionic composition and pH from fresh and air-dried soils.

- To investigate the effects of high H^+ concentrations in nutrient solutions with no added Al on the growth and root anatomy of *Holcus lanatus* L. and *Betula pendula* Roth. The reasons for selecting these study species are given in Chapter 2, Section 2.2.

- To investigate the effects of increasing concentrations of Al^{3+} on plant growth, root anatomy, and cell ultrastructure of *Betula pendula* Roth and *Holcus lanatus* L.

- To determine the toxicity of inorganic monomeric Al species present in solution at pH 5.6 to *Holcus lanatus* L.

- To establish whether or not silicic acid ameliorates Al-induced growth inhibition in *Holcus lanatus* L. and *Anthoxanthum odoratum* L.

- To determine whether or not silicic acid ameliorates growth by reducing Al bioavailability through production of hydroxyaluminosilicates.

- To determine whether or not organic acids ameliorate Al-induced growth inhibition in *Holcus lanatus* L.

- To investigate the growth response of *Holcus lanatus* L. and *Deschampsia flexuosa* L. Trin. to organic acids and phenolic acids.

Chapter 2

Study sites and species

2.1 Study sites

Soils and seeds were collected from five areas within Central Scotland in February and July 1995 on the dates given in Table 2.1: East Flanders Moss (NS 639973), Kippenrait Glen (NS 794994), Kinloch Rannoch (NN 717574), and Sheriffmuir (NN 830025 and NN 832025). Figure 2.1 shows site locations within Scotland.

East Flanders Moss (FM) (6.1 km^2) is located in the Carse of Stirling, 16 km west of Stirling at the head of the Forth Valley (Figure 2.1). It is the largest single, intact raised bog in the British Isles. Drier areas of the bog are dominated by *Calluna vulgaris, Erica tetralix, Vaccinium oxycoccos* and *Eriophorum vaginatum. Sphagna*, especially *Sphagnum magellanicum* and *Sphagnum papillosum* are more frequent in wetter areas. *Betula pendula* and *Betula pubescens* are encroaching onto the moss from the margins. The acidic peat should be low in total Al concentration (Al complexed) and H$^+$ ions the dominant growth-limiting factor. Samples for this thesis were collected from the south-west corner owned by Scottish Wildlife Trust (SWT). Access to the site was via East Polder farm.

Kippenrait Glen (KP) lies 2 km north of Bridge of Allan, near Stirling at an altitude of about 100 m (Figure 2.1). The predominant geology of the Glen is Lower Old Red Sandstone, and the soil type is Brown Forest soil with patches of boulder clay (Soil Survey of Scotland 1982). *Quercus robur* and *Acer pseudoplatanus* are frequent in the mixed woodland canopy. The ground flora has abundant *Mercurialis perennis, Oxalis acetosella, Pteridium aquilinum*, and several grass species including *Holcus lanatus, Anthoxanthum odoratum* and *Agrostis* spp.

The sample site at Kinloch Rannoch was near Lochan an Daim east of the village of Kinloch Rannoch (Figure 2.1). Soils and seeds were collected from the calcareous heath (KR), the soils of which are derived from the Loch Tay series of limestones. The vegetation is predominantly *Calluna vulgaris* and *Erica tetralix* with several grasses including *Holcus lanatus, Anthoxanthum odoratum, Agrostis* spp. and *Deschampsia flexuosa*. Patches of *Betula pendula* surrounded the sample site. The nutrient status and near-neutral pH of KR soils imply plants do not suffer from acidity problems.

(b)

Kinloch Rannoch

Loch Rannoch

Dunalastair Water

Lochan an Daim

N

1000 m

Schiehallion
1083

(a)

Kinloch Rannoch (b)

Flanders Moss (c)

Stirling (d)

Glasgow

Edinburgh

(c)

Flanders Moss

Blaircessnock

Goodie Water

Easter Polder

River Forth

South Flanders

N

1000 m

National Nature Reserve

(d)

Sheriffmuir

Dunblane

Kippenrait Glen

Allan Water

River Forth

Bridge of Allan

N

5000 m

Stirling

Figure 2.1. (a) The locations of the study sites within central Scotland; (b) Kinloch Rannoch (KR); (c) Flanders Moss (FM); (d) Kippenrait Glen (KP) and Sheriffmuir (SMB and SMM). ★, sites of sample collection.

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Samples were collected from both an area of blanket peat (SMB), and from the acid mineral soils (SMM) surrounding the peat on Sheriffmuir (Figure 2.1). Both areas had a south-westerly aspect at about 200-300 m and were grazed by sheep at low density. The blanket peat was dominated by *Calluna vulgaris* and *Vaccinium oxycoccos* with infrequent *Erica tetralix*. The mineral soil is classified as a Low-Gley soil (Soil Survey of Scotland 1982), derived from Old Red Sandstone sediments. The peat soil is classified as blanket peat (Soil Survey of Scotland 1982). The Al species distribution described in Chapter 1 would be expected in the acidic mineral soils.

Table 2.1. Dates of seed and soil collection from study sites in 1995 and 1996. Samples were collected from Flanders Moss (FM), Sheriffmuir (SMB and SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR).

Site	*Anthoxanthum odoratum* L.	*Betula pendula* Roth	*Deschampsia flexuosa* L. Trin.	*Holcus lanatus* L.	Soil Samples
1995					
FM	30 Jun	1 Sep	1 Aug	1 Aug	12 Feb/6 Jul
SMB	30 Jun	1 Sep	1 Aug	1 Aug	12 Feb/6 Jul
SMM	30 Jun	1 Sep	1 Aug	1 Aug	12 Feb/6 Jul
KP	30 Jun	5 Sep	5 Aug	5 Aug	13 Feb/6 Jul
KR	7 Jul	25 Aug	3 Aug	3 Aug	15 Feb/7 Jul
1996					
FM	7 Jul	5 Sep	10 Aug	10 Aug	-
SMB	7 Jul	5 Sep	10 Aug	10 Aug	-
SMM	7 Jul	5 Sep	10 Aug	10 Aug	-
KP	10 Jul	10 Sep	12 Aug	12 Aug	-
KR	5 Jul	7 Sep	15 Aug	15 Aug	-

2.2 Study species

Throughout this thesis British plant species (including the study species) will be referred to using Latin names, and agricultural crops using Common names. Both Latin and Common names are given in Appendix 1.

The study species were selected to represent grasses and one woody species. Seeds were collected from populations which naturally occurred over the range of soil types from acid to calcareous. Seeds of the following species were collected in 1995/96 (Table 2.1), when present, from each of the five areas: Silver Birch (*Betula pendula* Roth), Sweet Vernal Grass (*Anthoxanthum odoratum* L.), Wavy Hair-grass (*Deschampsia flexuosa* L. Trin), and Yorkshire-fog (*Holcus lanatus* L.). *Betula pendula*, a fast-growing deciduous tree, is widely distributed but most frequent and abundant below pH 5.0. *Deschampsia flexuosa* is slow-growing, and normally restricted to acidic soils. *Holcus lanatus* is a fast growing species represented in grasslands of widely differing soil pH (pH 4.5-8.0) but most

common in the intermediate range pH 5-6. *Anthoxanthum odoratum* is relatively slow-growing, and covers the full range of soil pH but is most common in the range pH 4.5-6.0.

Chapter 3

Extraction and analysis of soil solutions

3.1 Introduction

The mineral nutrients are transported to the root surface via the soil solution and their supply is dependent on their concentration within this solution (Marschner 1995). Soil solution cations, if absorbed by plants are replenished from exchangeable cations held by the negatively charged surfaces on soil particles (Falkengren-Grerup *et al.* 1995, Rowell 1995).

Soil solutions vary in composition with soil moisture and depth, pH, CEC, redox potential, organic matter content, microbial activity, leaching, cation uptake by plants and microorganisms, fertiliser application, and finally, the season of the year (Marschner 1995). Using lysimetry techniques Jones & Edwards (1993a) showed ion concentrations in soil solutions remained relatively constant through time. However using the same techniques Zabowski & Ugolini (1990) found peaks in ion concentrations in both the winter and summer months.

A knowledge of the soil solution composition provides valuable information on the availability of both nutrients and toxic ions to plants (Giesler *et al.* 1996, Lorenz *et al.* 1994, del Catilho *et al.* 1996). The extraction techniques not only produce very small volumes of solution but need large amounts of soil. Moist soils contain on average between 10 and 30 % water, and the extraction techniques most commonly used only remove about 30 % of this. Therefore a 100 g soil sample gives only 3 - 10 ml of solution (Rowell 1995). Edmeades *et al.* (1985) believed about 20 ml of solution were required to carry out a full analysis, and provided the soil was sufficiently wet about 400 g of air-dried soil were required. Wolt & Graveel (1986) found that a vacuum displacement (using 75 g soil) and centrifugation (using 750 g soil) technique produced on average volumes of 3.1 - 7.4 ml and 12.7 - 49.0 ml soil solution. Soil collected from the field is not always sufficiently moist and often deionized water is added to the soil which is then extracted after a period of equilibration. Sometimes soils are air-dried and then rewet before extraction. The wetting methods influence the soil solution as will be discussed later.

No single technique has been shown to produce a solution which is considered to be exactly the same as the *in situ* soil solution and the method and time of extraction, storage and preparation of soil samples, must be considered. The techniques range from laboratory extraction of soil samples, the

acquisition of solutions from the soil profile to the extraction of soil samples by lysimetry techniques (zero-tension or low-tension) (Giesler & Lundström 1993, Giesler *et al.* 1996, Jones & Edwards 1993a). Lysimetry is attractive because not only is the solution sampled *in situ* and therefore might be expected to resemble the "real" soil solution but also temporal monitoring of solutions is possible (Zabowski & Ugolini 1990). However lysimetry techniques have been criticised because the solutions tend to be altered as they pass though the collecting ceramic cups (Jones & Edwards 1993b). They are also only effective for wet soils.

3.1.1 Methods for soil solution extraction

The most commonly used laboratory techniques involve extraction by: low-speed and high-speed centrifugation (Adams *et al.* 1980, Dahlgren 1993, Elkhatib *et al.* 1987, Giesler & Lundström 1993, Lorenz *et al.* 1994, Ross & Bartlett 1990); centrifugation using immiscible displacement (Adams *et al.* 1980, Dahlgren 1993, Elkhatib *et al.* 1987, Kinniburgh & Miles 1983, Mubarak & Olsen 1976, Phillips & Bond 1989, Whelan & Barrow 1980); column displacement using compression, syringes, or solutions such as H_2O or 0.5 % KCNS to push the soil out of the column (Adams *et al.* 1980, Dahlgren 1993, Lorenz *et al.* 1994, Ross & Bartlett 1990, Wendt 1992, Wolt & Graveel 1986); or water (soil:water ratios of 1:1 or 1:2) with centrifugation and filtration (Dahlgren 1993). Smethurst *et al.* (1997) using the last technique estimated the actual solute concentrations of the soil solution using a model which incorporated the change in water content and a different soil-liquid partition coefficient (Kd) of the soil for each solute. There were no significant differences in ionic composition of solutions where equilibration times were increased to 16 hours.

3.1.2 Centrifugation of soils

Extracting soil solutions by centrifugation was first described by Davies & Davies (1963). It is now probably the most popular technique used although it is slow: soils must be carefully weighed into centrifuge tubes and subsequent filtration of the extracted solutions can be very slow. However it is suitable for all soil types and samples can be re-used for further analyses.

The time and the speed of centrifugation differ among researchers and the effect of varying either time or speed on the ionic composition of the extracted solutions is inconsistent. Neither time nor speed of centrifugation affected the concentration of cations in soil solutions extracted by Edmeades *et al.* (1985). Ross & Bartlett (1990) compared centrifugation of soils at 5000 rpm with 10000 rpm. They reported that the high-speed centrifugation increased concentrations of F^-, Cl^-, and SO_4^{2-}, but NO_3^- was unaffected. Elkhatib *et al.* (1987) believed that the force and vacuum present in high-speed centrifugation may be the cause of changes in extracted soil solutions. However when Zabowski &

Ugolini (1990), and Edmeades *et al.* (1985) compared low and high-speed centrifugation there were no differences between the centrifuged solutions. The former authors used centrifugal speeds of 1000 and 10000 rpm, and the latter used speeds of 2000 and 15000 rpm.

3.1.3 Centrifugation of soils with immiscible liquids

Displacing soil solutions using an immiscible displacent was first used by Mubarak & Olsen (1976). The displacent is usually an organic liquid, mostly a hydrocarbon. Since the displacent is insoluble and of a greater density than water the soil solution is displaced upward to where it can be collected for analysis. Sufficient displacent is added to prevent expelled solution from re-entering the soil when centrifuging stops. Any air which is present in macropores is either dispelled or dissolved in the displacent. Several immiscible liquids are commonly used: carbon tetrachloride (d=1.6 g ml^{-1}), 1,1,2 trichloroethane (d=1.4 g ml^{-1}), trifluroethane (d=1.57 g ml^{-1}), 1,1,2 - trichloro - 1,2,2 - trifluroethane (d=1.58 g ml^{-1}), ethyl benzoylacetate (d=1.12 g ml^{-1}), and perchloroethylene (d=1.62 g ml^{-1}). The major problem with this method is the toxic vapour from these organic liquids. They also attack most plastics. Trifluroethane and ethyl benzoylacetate are less toxic and do not attack polypropylene centrifuge tubes but are very expensive. Perchloroethylene, suggested by Whelan & Barrow (1980), has low toxicity and is cheap but it requires nylon centrifuge tubes.

Centrifuging soils however with or without an immiscible liquid is more rapid than column displacement (Walworth 1992) which is slow and depends upon the skilful packing of the column. Sandy soils are not suitable since they are difficult to pack tightly enough into the column (Walworth 1992, Wendt 1992). However Adams *et al.* (1980) recovered more solution per gram of soil (except in sandy soils) using column displacement than with either centrifuge method.

3.1.4 Effect of extraction method on solution composition

Ross & Bartlett (1990) noted differences in Cl$^-$, NO$_3^-$, SO$_4^{2-}$, and Al^{3+}, as well as significant increases in pH and F$^-$ in solutions extracted by centrifugation compared with column displacement. They used a relative centrifugal force (rcf) of 9700 m s^{-2} (corresponding to 10000 rpm at the bottom of the sample) and pH was on average 0.7 units higher. The lower pH values found with column displacement were in the same range as soil pH's in H$_2$O and CaCl$_2$. Elkhatib *et al.* (1987) also reported higher pH's in solutions extracted by high-speed centrifugation (48000 m s^{-2}) compared with immiscible displacement. Dahlgren (1993) again reported increased pH's in centrifuged solutions. pH was on average from 0.5 to 3 units higher than in solutions extracted with either displacement solutions or vacuum displacement. Solute concentrations were ranked centrifugation > immiscible displacement > vacuum displacement. Similarly Wolt & Graveel (1986) found a general reduction in

major ion concentrations in solutions obtained by vacuum displacement compared with centrifugation. However both Adams *et al.* (1980) and Wolt & Graveel (1986) compared miscible displacement and vacuum displacement with centrifugation and found little difference between methods. The variation between soil solutions obtained by each method was not significantly different to invalidate either method for collecting unaltered soil solutions. It should be noted that they used low rcf of 1070 m s^{-2} or less. Elkhatib *et al.* (1987) found a discrepancy between estimated activity values for Al species after centrifugation and immiscible displacement. They suggested that centrifugation alone should be used when Al determination and speciation were of importance.

3.1.5 Effect of storage on solution composition

There has been much discussion on the storage, length of time of storage, and preparation of soil samples before extraction takes place. Solutions can be displaced from field moist soils, wetted-up field moist soils, field moist soils frozen and thawed, and re-wetted air-dried soils. Moist soils are more difficult than air-dried soils to homogenise for representative subsampling (Ure 1996). It is generally recommended to extract field moist soils within 24 h of collection (Edmeades *et al.* 1985, Jones & Edward 1993b, Ross & Bartlett 1990, Walworth 1992). Edmeades *et al.* (1985) showed differences in solutions after 1 day of soil storage at 4 °C in solutions displaced by centrifugation. NO_3^-, Ca^{2+}, Mg^{2+}, Na^+, and to a lesser extent K^+, increased in concentration. This effect of storage was thought to be due to an increased rate of net mineralization of organic N to NO_3-N thereby inducing an increase in cations to balance the charge. In agreement, Ross & Bartlett (1990) showed large increases in solution NO_3^- after 24 h storage at 3 °C. After 4 days storage, NO_3^- instead of SO_4^{2-} was the dominant anion in solutions from all horizons. pH was more slowly affected by storage and started to decline after 7 days.

Equilibration of wetted soil samples prior to extraction has also been shown to affect the ionic composition of solutions displaced. Jones & Edwards (1993b) added water to field moist samples and allowed a 24 h equilibration period. Solute concentrations were in general lower after equilibration. Walworth (1992) also found that the composition of soil solutions from rewetted soils did not approach that of field soils after incubation. SO_4^{2-} and Ca^{2+} concentrations decreased and those of Na increased.

Similar changes in the composition of solutions have been found in dried soils rewetted and extracted by vacuum displacement (Qian & Wolt 1990). Concentrations of Ca, Mg, K, Na, Al, NO_3, SO_4, together with EC and pH, were all shown to change. Jones & Edwards (1993) showed an increase in ion concentrations (especially SO_4^{2-}, Al, Fe, and PO_4^{3-}) upon rewetting of air-dried soil. They suggested that during drying Al^{3+} may move from clay lattices to exchange sites and exchangeable K^+

to non-exchange sites. Air drying may also increase the solubility and oxidising ability of soil organic matter, with associated changes in the chemistry of N and P.

3.2 Aims

- To analyse the ionic composition of soil solutions extracted from five soil types in February and July 1995.

- To determine the effects of centrifugation with and without an immiscible liquid on the ionic composition of soil solutions.

- To determine the effects of centrifugation speed on the ionic composition of soil solutions.

- To determine the effects of air-drying on the ionic composition of soil solutions.

- To determine the effects of air-drying, water and electrolyte suspensions on soil pH.

- To compare soil pH values with soil solution pH values.

3.3 Methods

Soils were sampled from the five study sites FM, SMM, SMB, KP, and KR which were described in Chapter 2.

3.3.1 Soils sampled in February 1995

Twenty soil samples, to a depth of 10 cm, were collected randomly from each site in February 1995 (Chapter 2, Table 2.1). The soils were transported to the laboratory in sealed plastic bags. Ten samples were then stored at 5 °C in the same sealed bags (FR). The remaining ten samples were air-dried at room temperature for seven days, ground using a pestle and mortar, and sieved through a 2-mm mesh (AD). The air-dried soils were placed in trays and slowly saturated with deionized water over a period of two days. Trays were then covered in 'cling film' and left to drain under gravity for 48 h. The macropores of soil (>50 μm) allow rapid drainage of water and once these pores are emptied drainage becomes very slow. The soil is then said to be at "field capacity".

3.3.1.1 Centrifugation of soils (Cen)

From each soil sample, subsamples of 25 g of fresh or rewetted air-dried soil were loosely packed into eight polypropylene 50 ml centrifuge tubes. Following the recommendations by Jones & Edwards

(1993) soil solutions from FR soils were extracted within 24 h of collection whenever possible in order to limit differences in solutions caused by the effects of storage. The soil was centrifuged for 30 minutes at 4000 rpm in a MSE Bench Centrifuge. The extracted solutions were poured into a measuring cylinder and the total solution extracted per sample recorded. Solutions were filtered using Whatman No.42 (Elkhatib *et al.* 1987). del Castilho (1996) recommended membrane-filtration over super-centrifugation of solutions to remove colloidal material. The pH of the extracted soil solutions were determined immediately after extraction using a Corning Eel Model 7 pH meter. Solutions were then diluted to 50 ml with deionized water and frozen until further analysis.

3.3.1.2 Centrifugation with an immiscible liquid (Imm)

The immiscible liquid used was 1,1,1-trichloroethane (d=1.33 g ml^{-1}) which had to be used with Teflon centrifuge tubes (Teflon Sigma 50 ml Naglene, OakRidge) since it distorts polypropylene ones (Whelan & Barrow 1980). Subsamples of 25 g of fresh and rewetted soil were packed into eight centrifuge tubes. 20 ml of 1,1,1-trichloroethane (TCE) were added to reach the neck of the tube in a fume cupboard. This was necessary since the centrifuge tubes are liable to collapse during centrifugation if not full. Also, sufficient liquid had to be added to ensure that after centrifugation, the extracted solution was not in contact with the soil surface, which would have led to it being reabsorbed into the soil. The displaced soil solution was withdrawn from the surface of the displacent with an automatic pipette. Any displacent inadvertently taken up into the plastic tip of the pipette was easily discarded owing to the sharp water-displacent interface. The volumes of extracted soil solutions were recorded and pH measured immediately before dilution to 50 ml and freezing.

3.3.2 Soils sampled in July 1995

Twenty samples of soil, to a depth of 10 cm, were randomly collected from FM, SMB, SMM, KP, and KR in July 1995 (Chapter 2, Table 2.1). The samples were transported to the laboratory in sealed plastic bags. As in February ten samples were air-dried and ten samples were stored at 5 °C and extracted within 24 h. Fresh and rewetted soils were extracted using centrifugation alone. Samples from FM, SMB, and SMM were extracted with both low (4000 rpm) and high-speed (12000 rpm) centrifugation, those from KP and KR were extracted at high-speed only in a MSE High Speed Centrifuge.

3.3.3 Analysis of soil solutions

Concentrations of Ca and Mg were measured using a Varian AA-575 S atomic absorption spectrophotometer (AAS) with a nitrous oxide-acetylene flame. An air-acetylene flame was used to determine Na, K (flame emission) and Fe concentrations. Total Al and Si were measured with a Pye Unicam SP9 Atomic Absorption Spectrophotometer fitted with a Unicam GF90 furnace and FS90

furnace autosampler. Unicam 919 series atomic absorption software was used. Monomeric aluminium was determined with the short-term reaction Pyrocatechol violet (PCV) method after Kerven *et al.* (1989). Absorbance was measured after 60 s at 585 nm with a LKB Novaspec Spectrophotometer. Three ml of soil solution were filtered through a 0.22 μM membrane filter, 0.50 ml of iron interference reagent was added (100 mg 1,10-phenanthroline and 500 mg ascorbic acid), followed by 0.20 ml of PCV reagent and 1.0 ml of hexamine buffer.

The anions F, Cl, SO_4, and NO_3 were measured using ion chromatography. A Dionex QIC analyser fitted with Dionex AI450 software connected to a Dionex ACI with a Dionex AS40 autosampler were used. The columns used were both 4 mm versions: Dionex IonPac AG4 guard column and Dionex IonPac AS4A analytical column.

P and NH_4 were measured on a Tecator FIAstar 5010 flow-injection auto-analyser.

3.3.4 Soil pH

The pH of both fresh and air-dried soil samples was measured in deionized water, 0.01 M $CaCl_2$, and 0.002 M $CaCl_2$. The procedure used 10 g of fresh or air-dried soil with 25 ml of liquid. Suspensions were stirred, left to sit for 1 h, re-stirred and the pH measured with a Corning Eel Model 7 pH meter.

3.4 Results

3.4.1 Centrifugation with and without 1,1,2-trichloroethane

3.4.1.1 Soil and soil solution pH

Table 3.1 shows pH values for fresh (FR) and air-dried (AD) soils, and their extracted soil solutions, from samples collected in February 1995 from each of the five study sites. Soil pH was measured in H_2O, 0.01 M $CaCl_2$, and 0.002 M $CaCl_2$. The highest pH values recorded were in KR soil samples. Soil solutions had a mean pH of 6.1 both FR and AD, and soil pH ranged from 5.5 - 6.2 depending on the method of measurement. The lowest pH values were found in the peaty soils FM and SMB: soil solutions ranged from pH 3.3 - 3.6 in FM and averaged pH 3.6 in SMB, and soil pH ranged from 2.5 - 4.1 (FM) and 2.8 - 4.2 (SMB) depending on the method of measurement.

Soil solution pH values between sites were significantly different (Table 3.2). However there was no significant difference between pH values of solutions extracted from either FR or AD soil, or using Imm or Cen (Figure 3.1 and Table 3.2).

Soil pH values measured in H_2O or in a weak solution of $CaCl_2$ (0.002 M) accurately reflected the pH of the extracted soil solution. Soil pH_{H_2O} and $pH_{CaCl_2(0.002\ M)}$ were not significantly different from any equivalent soil solution pH in any soil type. $pH_{CaCl_2(0.01\ M)}$ was significantly different from soil solution pH values (df = 195, T = 2.45, P < 0.05) in all sites and was consistently shown to be significantly lower than the extracted soil solution pH (Table 3.1).

Table 3.1. Mean pH values of extracted soil solutions (using centrifugation alone), and fresh (FR) and air-dried (AD) soils measured in H_2O, 0.01 M $CaCl_2$, and 0.002 M $CaCl_2$ from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR). Ranges are given in parentheses. * denotes significantly different pH values between soils and solutions at the same site, $p < 0.05$.

Site	Fresh/ Air-dried	Solution pH	pH_{H_2O}	pH_{CaCl_2} (0.01M)	pH_{CaCl_2} (0.002M)
FM	FR	3.6*	4.1	3.2*	3.5
		(3.2-4.0)	(3.9-4.4)	(3.0-3.3)	(3.2-4.0)
	AD	3.3*	3.2	2.5*	2.9
		(3.1-3.5)	(3.1-3.3)	(2.4-2.6)	(2.8-3.1)
SMB	FR	3.6*	4.2	3.2*	3.5
		(3.3-4.0)	(4.0-4.3)	(2.8-3.5)	(3.1-3.7)
	AD	3.6*	3.3	2.8*	3.1
		(3.5-3.7)	(3.2-3.4)	(2.7-3.0)	(2.9-3.3)
SMM	FR	4.3*	4.9	4.3*	4.7
		(3.6-5.7)	(4.6-5.3)	(4.0-4.9)	(4.4-5.3)
	AD	4.3*	5.1	4.7*	4.8
		(3.8-4.6)	(4.7-5.3)	(4.2-5.0)	(4.4-5.2)
KP	FR	5.3*	5.3	4.8*	5.1
		(5.1-5.3)	(5.2-5.6)	(4.7-5.2)	(5.0-5.3)
	AD	5.4*	5.5	5.0*	5.3
		(5.2-5.8)	(5.0-5.8)	(4.6-5.4)	(4.8-5.7)
KR	FR	6.1*	6.0	5.5*	5.8
		(5.1-6.5)	(5.2-6.6)	(4.8-6.1)	(4.9-6.3)
	AD	6.1*	6.2	5.6*	5.8
		(5.5-6.9)	(5.7-6.8)	(5.1-6.3)	(5.2-6.3)

Table 3.2. Statistical analyses for soil solution analyses extracted in February 1995 from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR) using centrifugation with and without 1,1,1-trichloroethane (TCE), and from fresh and air-dried soils. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, n.s, not significant.

	Site (df=4) F	p	FR/AD (df=25) F	p	Imm/Cen (df=25) F	p
Solution pH	617.86	***	1.05	n.s	1.33	n.s
Extracted volume	4.16	*	3.13	**	3.13	**
Cations :						
NH_4	78.82	***	6.09	***	1.71	n.s
K	8.84	***	3.23	**	1.46	n.s
Na	12.18	***	0.91	n.s	2.44	*
Ca	168.42	***	1.29	n.s	0.47	n.s
Mg	115.57	***	2.23	*	0.66	n.s
Fe	21.71	***	1.93	n.s	1.97	*
Anions :						
NO_3	154.11	***	2.44	*	0.43	n.s
Cl	6.86	***	0.45	n.s	0.56	n.s
F	37.77	***	5.05	***	2.91	**
SO_4	80.79	***	0.46	n.s	0.87	n.s

3.4.1.2 Volume of extracted soil solution

Figure 3.2 shows the mean volumes of soil solution extracted from fresh and rewetted air-dried soil from each site, using each of the two methods (Cen or Imm). There was a significant difference in
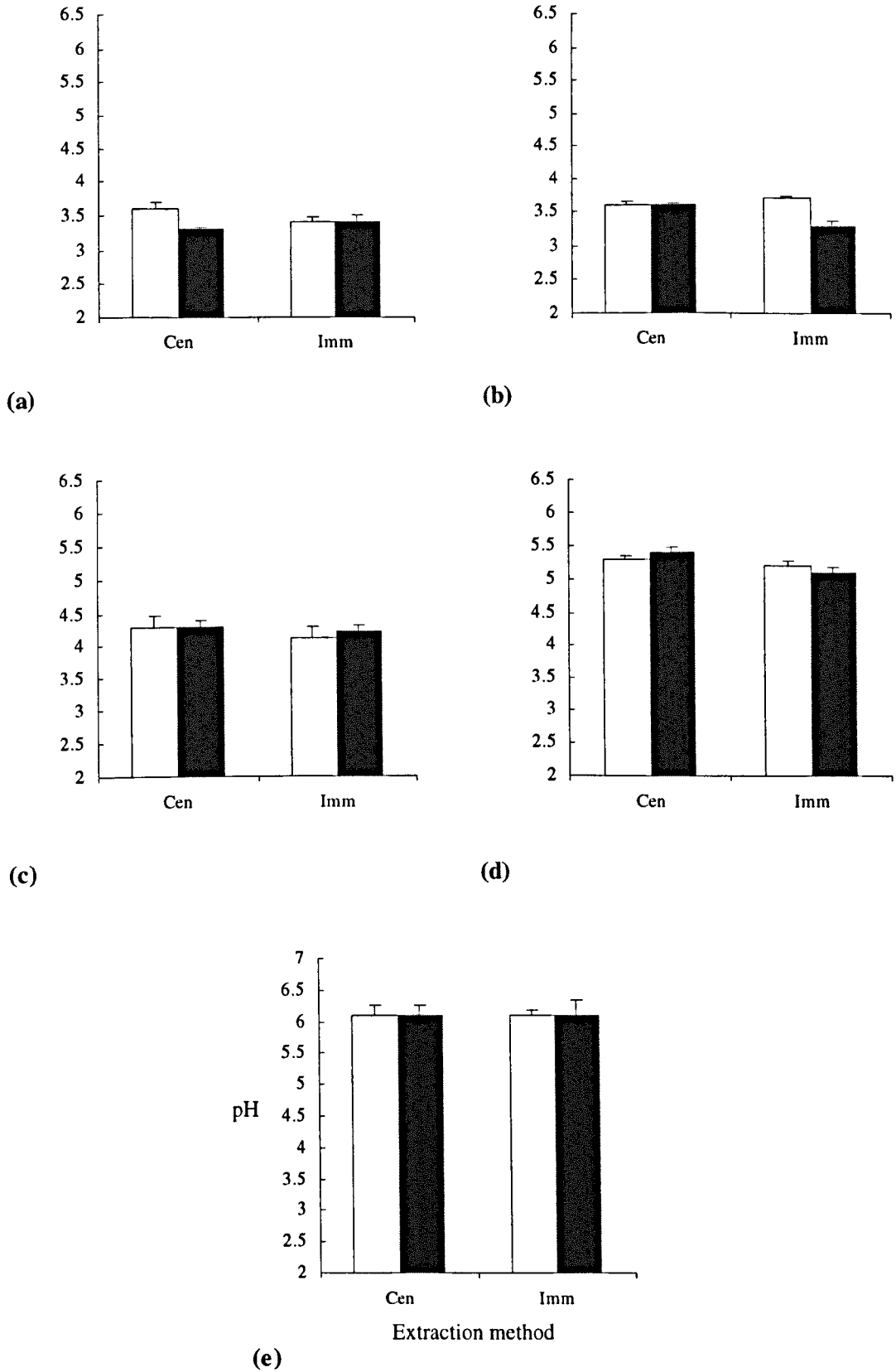
Figure 3.1. Mean pH values (± 95 % C.L.) of soil solutions from fresh (□) and rewetted air-dried (■) soils using centrifugation alone (Cen) and centrifugation with an immiscible liquid (Imm) from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR).
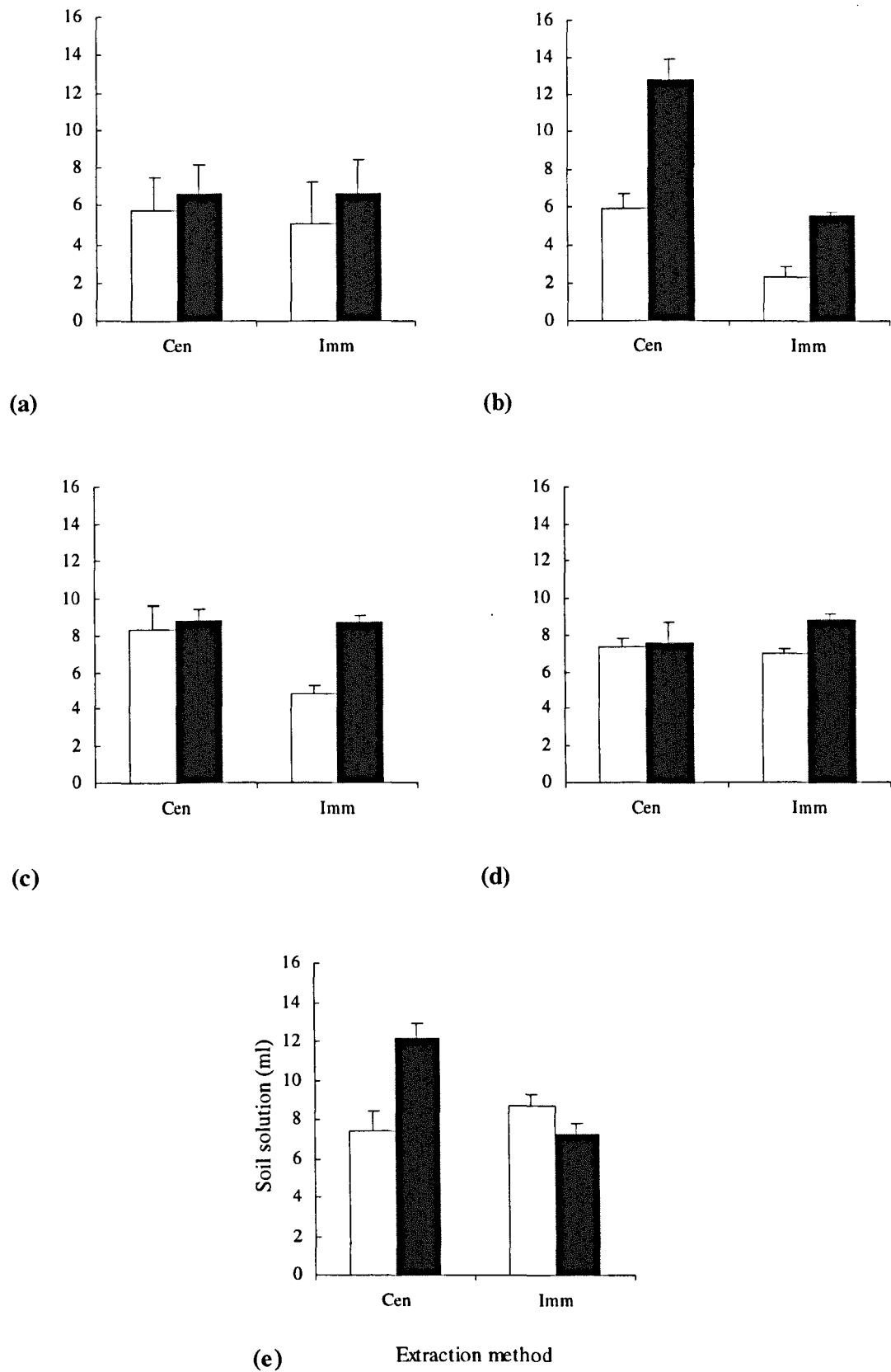
Figure 3.2. Mean total volumes (± 95 % C.L.) of extracted soil solutions from fresh (□) and rewetted air-dried (■) soils using centrifugation alone (Cen) and centrifugation with an immiscible liquid (Imm) from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR).

the volumes of solution extracted between sites (Table 3.2). The lowest volumes were generally extracted from FM soil and the highest from KR. There was also a significant difference in volumes extracted from FR and AD soil (Table 3.2). More solution was extracted from AD soil compared with FR soil.

The extraction method (Cen or Imm) also significantly affected the volume of solution obtained (Table 3.2). The volume of soil solution extracted was either significantly lower when using Imm or not significantly different.

3.4.1.3 Ionic composition of soil solutions

Table 3.3 gives the concentrations of cations analysed in the extracted soil solutions from February collections. Concentrations of all cations were significantly different between the five sites (Table 3.2). Orders of increasing element concentrations between sites were not consistent between FR and AD soil, or between extraction methods. The highest concentrations of Ca were found in KR, and the lowest concentrations in SMB and FM. The lowest concentrations of Mg were found in KP and KR, and the lowest levels of Na in KR. Na and K concentrations were highest in SMB, and SMM or SMB, depending on the method of extraction. NH_4 was highest in the peaty soils, FM and SMB. Al was greatest in the acid mineral soil, SMM.

There were significant differences in concentrations of NH_4, K, and Mg between FR and AD soils, irrespective of the extraction technique (Table 3.2). Concentrations of these cations were significantly greater in soil solutions of AD soil. Differences of 46, nine, and three-fold were observed for NH_4, Mg, and K. Al concentrations were also greater in solutions from AD soil compared with FR soil.

Only the concentrations of Na and Fe were significantly by the extraction technique (Table 3.2). Na concentrations were significantly lower in solutions from FM, SMM, and KP AD soil extracted with TCE. Fe concentrations increased in solutions from both FR and AD soil after extraction with TCE.

Table 3.4 shows the analyses of anions in February extracted soil solutions. Concentrations of NO_3, Cl, F, and SO_4 were significantly different between sites (Table 3.2). The limited data on P concentrations excluded this element from statistical analysis. NO_3 was highest in solutions extracted from SMM and lowest in those from FM. F was lowest in SMM solutions and highest in KR. The highest concentrations of Cl and SO_4 were found in FM and KP (Cl), and SMB (SO_4) soil solutions, and the lowest of both in KR.

Table 3.3. Mean cation concentrations (± s.e) in soil solutions extracted from fresh (FR) or air-dried (AD) soil in February 1995 collected from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR) using centrifugation alone (Cen) and centrifugation with an immiscible liquid (Imm). n.d, no data.

Extraction Method	NH$_4$		K		Na (mg l^{-1})		Ca		Mg		Fe		Al (µM)	
	FR	AD	FR	AD	FR	AD	FR	AD	FR	AD	FR	AD	FR	AD
FM														
Cen	5.91	19.70	6.47	14.27	7.78	9.24	1.86	35.70	0.64	2.47	0.31	0.08	133.93	235.73
	±3.46	±2.18	±2.36	±1.62	±2.95	±1.95	±0.63	±10.5	±0.11	±0.36	±0.10	±0.03	±12.94	±61.99
Imm	2.95	29.21	2.86	8.83	8.30	8.09	13.5	13.00	0.22	1.96	1.39	0.10	n.d	n.d
	±0.18	±2.35	±0.42	±1.96	±2.06	±2.15	±1.91	±1.94	±0.08	±0.25	±0.17	±0.03		
SMB														
Cen	11.58	21.53	9.31	27.05	15.90	15.60	3.08	25.46	0.94	2.54	0.36	1.79	166.42	231.16
	±3.29	±4.07	±1.93	±1.66	±12.7	±3.92	±0.74	±4.08	±0.31	±0.41	±0.10	±0.63	±31.72	±32.48
Imm	3.48	15.32	9.05	24.95	6.53	8.14	6.30	13.81	1.79	2.06	0.93	2.46	n.d	n.d
	±1.94	±1.20	±4.51	±4.92	±2.53	±1.00	±5.57	±2.80	±0.20	±0.75	±0.08	±0.26		
SMM														
Cen	2.70	2.50	10.81	13.76	3.93	5.23	9.41	30.07	2.87	7.30	1.43	1.21	378.80	460.61
	±1.25	±0.87	±2.01	±2.35	±1.43	±0.72	±1.92	±9.79	±1.33	±2.89	±0.45	±0.12	±84.50	±19.13
Imm	0.86	1.17	20.39	15.02	7.65	4.95	7.67	15.75	5.76	10.56	2.20	1.46	n.d	n.d
	±0.48	±0.26	±6.83	±4.88	±1.52	±0.80	±3.69	±2.61	±2.27	±5.60	±0.22	±0.05		
KP														
Cen	0.14	6.43	10.88	20.07	15.14	11.02	15.74	7.14	0.35	1.03	0.80	0.63	n.d	n.d
	±0.02	±2.35	±2.14	±3.17	±10.5	±3.95	±14.3	±2.01	±0.12	±0.22	±0.72	±0.41		
Imm	0.14	4.37	14.61	17.42	13.72	6.83	6.94	17.27	1.21	0.57	5.31	1.32	n.d	n.d
	±0.01	±1.28	±2.60	±2.49	±13.1	±1.42	±1.50	±6.66	±0.98	±0.36	±1.88	±0.92		
KR														
Cen	1.05	4.29	6.82	11.30	3.66	4.55	27.21	92.71	0.95	0.52	2.68	0.41	n.d.	n.d.
	±0.17	±1.71	±2.82	±2.43	±1.31	±0.67	±9.12	±16.7	±0.77	±0.17	±1.48	±0.13		
Imm	0.22	2.64	12.42	16.60	5.65	4.45	72.44	84.48	1.95	0.82	4.33	3.16	n.d	n.d
	±0.08	±1.94	±2.24	±12.9	±1.04	±0.74	±21.0	±9.27	±0.34	±0.33	±2.39	±1.22		

Table 3.4. Mean concentrations of anions (± s.e) in soil solutions extracted in February 1995 from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR) using centrifugation alone (Cen) and centrifugation with an immiscible liquid (Imm). †, below detection level (< 0.15 mg l⁻¹).

Extraction Method	NO$_3$		P		Element Cl mg l^{-1}		F		SO$_4$	
	FR	AD	FR	AD	FR	AD	FR	AD	FR	AD
FM										
Cen	2.60	2.60	0.36	2.12	63.8	37.5	0.06	0.10	53.4	65.5
	±1.25	±2.17	±0.05	±0.84	±47.5	±17.1	±0.04	±0.10	±37.5	±46.6
Imm	0.42	0.31	0.16	3.02	54.2	35.8	0.76	0.52	23.2	65.5
	±0.18	±0.20	±0.03	±3.38	±8.60	±11.6	±0.12	±0.23	±2.30	±30.9
SMB										
Cen	3.43	0.42	5.13	3.91	33.6	22.8	0.22	0.10	87.8	81.2
	±2.60	±0.31	±0.74	±1.46	±16.4	±23.8	±0.08	±0.07	±52.9	±40.3
Imm	11.5	3.09	1.82	2.51	34.3	35.1	0.36	0.32	190.1	188.7
	±6.90	±2.02	±0.43	±0.36	±7.98	±16.2	±0.05	±0.46	±1.75	±111
SMM										
Cen	81.6	207.2	1.67	0.42	35.5	42.0	0.27	0.09	28.5	38.0
	±43.5	±78.9	±0.33	±0.05	±24.2	±25.4	±0.18	±0.01	±13.6	±30.6
Imm	131.4	191.4	†	†	40.0	18.8	0.09	0.08	34.1	121.1
	±32.6	±95.0			±10.2	±12.1	±0.03	±0.05	±11.1	±24.4
KP										
Cen	13.4	32.7	0.36	5.47	34.8	185.5	0.10	1.79	52.8	348.4
	±3.01	±13.8	±0.20	±0.29	±14.4	±19.1	±0.04	±1.45	±2.86	±95.6
Imm	27.8	8.20	0.55	3.38	52.9	22.9	0.10	3.29	116.3	28.2
	±1.89	±4.77	±0.19	±0.08	±29.3	±1.58	±0.05	±1.25	±78.3	±9.56
KR										
Cen	3.59	72.29	†	†	20.22	44.52	0.11	0.34	21.4	16.8
	±2.30	±38.2			±7.96	±23.4	±0.03	±0.53	±14.6	±6.64
Imm	30.21	34.57	†	†	27.57	21.73	1.66	1.59	23.2	17.1
	±6.89	±13.0			±7.53	±6.34	±0.92	±0.53	±11.8	±7.78

The soil condition (FR or AD) only significantly affected the concentrations of NO$_3$ and F (Table 3.2). In SMB and KP (Imm) concentrations of NO$_3$ were significantly lower in AD soil compared with FR soil. Whereas in SMM (Cen), KP (Cen), and KR (Cen) concentrations were significantly greater (up to 50 fold). In KP concentrations of F were significantly greater in AD soil compared with FR irrelevant of the extraction method (33 fold). However in SMM (Cen), concentrations were significantly lower in AD soil (three-fold).

F alone was significantly affected by the extraction method (Table 3.2). Extraction with TCE resulted in increased concentrations (up to 15 fold).

3.4.2 Extraction using low- and high-speed centrifugation

3.4.2.1 Soil solution pH

Similar to February extractions, soil solution pH values were significantly different between sites (Table 3.5). Mean pH values were lowest in FM (pH 3.1), and SMB (pH 3.7), followed by SMM (pH 4.3), KP (pH 5.3), and KR (pH 6.3). The pH values of soil solutions extracted from fresh or re-wetted air-dried soil were not significantly different when using high-speed centrifugation.

Table 3.5. Statistical analyses for soil solution analyses extracted in July 1995 from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), and Sheriffmuir mineral soil (SMM) using centrifugation at low-(4000 rpm) and high-(12000 rpm) speed, and from fresh (FR) and air-dried (AD) soils. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$, n.s, not significant.

	Site (df=2)		FR/AD (df=1)		High/Low (df=1)	
	F	p	F	p	F	p
Solution pH	95.74	***	4.42	**	2.84	*
Extracted volume	6.40	*	2.51	*	29.38	***
Cations :						
NH_4	121.92	***	0.81	n.s	0.59	n.s
K	12.80	***	2.67	*	0.67	n.s
Na	1.19	n.s	10.07	***	26.01	***
Ca	105.20	***	0.22	n.s	1.19	n.s
Mg	0.30	n.s	56.67	***	58.83	***
Al	11.29	***	307.03	***	31.17	***
Fe	73.07	***	5.88	*	8.16	**
Si	103.32	***	212.61	***	276.00	***
Anions :						
NO_3	149.70	***	0.49	n.s	1.04	n.s
P	58.66	***	2.89	*	1.46	n.s
Cl	2.30	n.s	5.27	***	1.58	n.s
F	2.49	n.s	0.61	n.s	0.97	n.s
SO_4	45.64	n.s	1.67	n.s	0.97	n.s

Extracted soil solutions from FM, SMB, and SMM using both low- and high-speed centrifugation were also compared. Both the centrifugation speed and the soil condition had a significant effect on the soil solution pH (Table 3.5 and Figure 3.3). Mean pH values were higher in solutions extracted using low-speed centrifugation with the exception of SMB fresh soils. A difference of up to 0.5 pH units was found in solutions from FM air-dried soil (pH 3.2 vs. pH 3.7).

3.4.2.2 Volume of extracted soil solution

Figure 3.3 shows the volumes of extracted soil solutions from FM, SMB, and SMM FR and AD soil using low- and high-speed centrifugation. There was a significant difference in the volume of solution extracted between sites (Table 3.5). Using high-speed centrifugation the largest volumes of solution were from SMM soil. There was less of a difference in volumes extracted using low-speed centrifugation. However in all cases significantly greater volumes of solution were extracted using high-speed
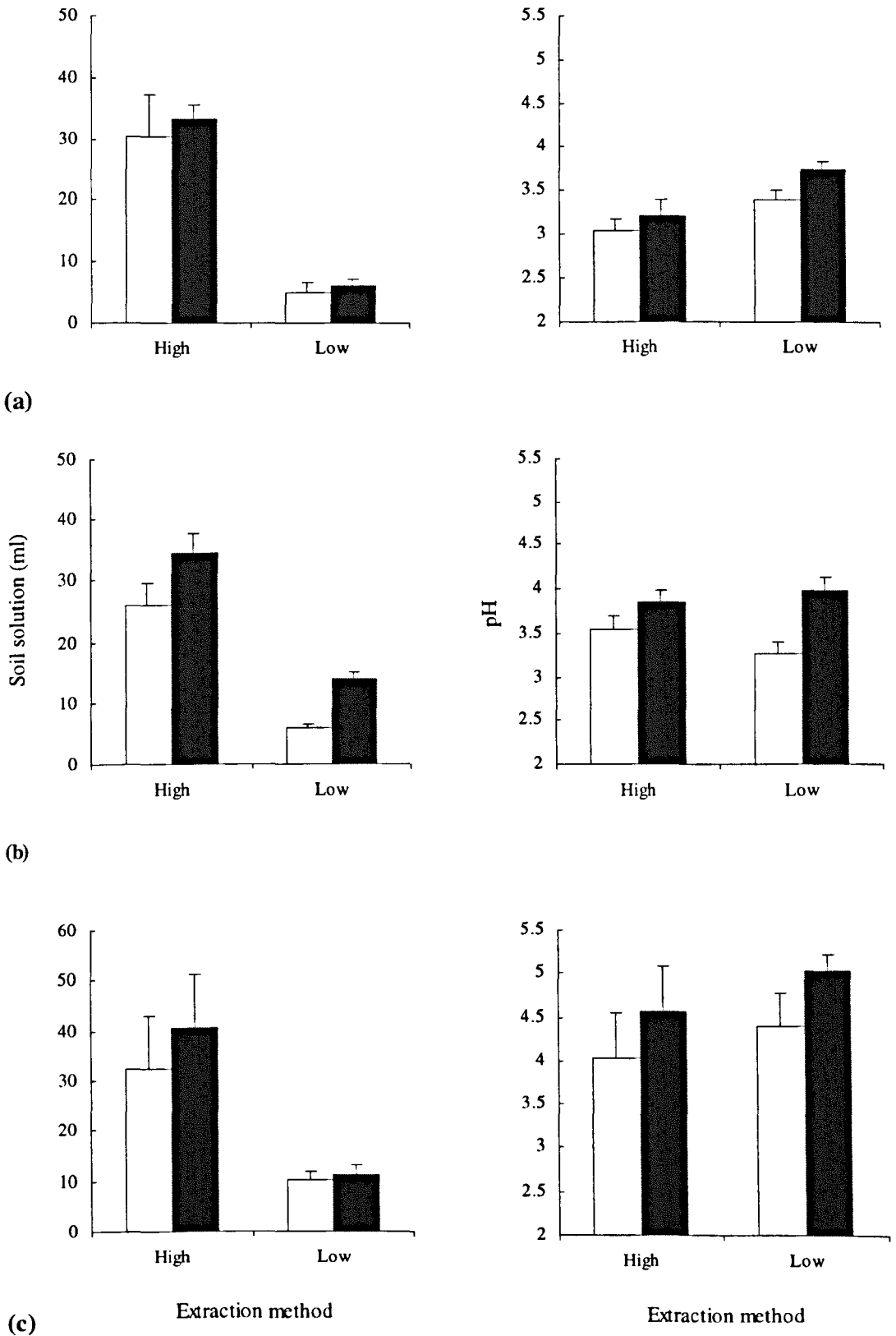
Figure 3.3. Mean total volumes and pH values (± 95 % C.L.) of extracted soil solutions from fresh (□) and rewetted air-dried (■) soils using high-speed (High) and low-speed (Low) centrifugation from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), and (c) Sheriffmuir mineral soil (SMM).

centrifugation (Table 3.5). Differences of up to 29 ml were found (40.7 ml compared with 11.3 ml of solution extracted from SMM AD soil).

Extracted volumes of soil solution were dependent upon soil condition and greater volumes of soil solution were obtained from AD soils (Figure 3.3 and Table 3.5).

3.4.2.3 Ionic composition of soil solutions

Table 3.6 gives the concentrations of cations analysed in the extracted soil solutions from July collections. Soil solutions were not extracted from either KP or KR using low-speed centrifugation. Concentrations of NH_4, K, Ca, Al, Fe, and Si were significantly different between sites (Table 3.5). NH_4 and K were highest in solutions from the peaty FM and SMB soils, and lowest in KR solutions. In contrast Ca concentrations were greatest in solutions from KR and lowest in those from SMB and FM soils. Al concentrations were greatest in the solutions extracted from the acid mineral soil SMM and lowest in the calcareous soils, KR. Fe was highest in KP and lowest in FM. Finally, Si concentrations were highest in solutions of KP, KR, and SMM soils, and lowest in those from SMB.

Concentrations of K, Na, Mg, Al, Fe, and Si were significantly greater in AD soil solutions compared with FR soil solutions (2-11 fold differences) (Table 3.5).

The concentrations of Na, Mg, Al, Fe, and Si were also significantly affected by the speed of centrifugation. Centrifugation at high-speed increased ionic concentrations (3-10 fold increase).

Table 3.7 shows the concentrations of anions in the soil solutions extracted from FR and AD soils using
high- and low-speed centrifugation. Again there were no analyses for soils from KP and KR using low-speed centrifugation. Only NO_3 and P were significantly different between the 3 sites. NO_3 concentrations were greatest in KR and lowest in FM solutions. P concentrations were highest in FM solutions.

Concentrations of P and Cl alone were significantly different between solutions extracted from either FR or AD soil (Table 3.5). Concentrations were greater in solutions from AD soil compared with FR soil and the difference was more pronounced in solutions extracted at high-speed.

The concentration of anions was not significantly affected by the speed of centrifugation (Table 3.5).

Table 3.6. Mean concentrations of cations (± s.e) in soil solutions extracted in July 1995 from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR) using low-speed (Low) and high-speed (High) centrifugation. n.d, no data.

| Extraction Method | NH$_4$ | | K | | Na | | Ca | | Mg | | Fe | | Al | | Si | |
| | | | | | mg l^{-1} | | | | | | | | | µM | | |
	FR	AD	FR	AD	FR	AD	FR	AD	FR	AD	FR	AD	FR	AD	FR	AD
FM																
Low	28.53	14.14	4.74	8.03	7.36	8.94	4.29	3.30	1.61	1.97	0.53	0.21	15.67	35.67	88.59	537.40
	± 2.53	± 2.93	± 0.39	± 0.39	± 0.68	± 0.82	± 0.37	± 0.54	± 0.12	± 0.27	± 0.04	± 0.01	± 1.66	± 1.77	± 7.47	± 56.27
High	2.55	22.48	1.48	24.28	15.32	29.69	0.17	7.34	1.43	6.53	0.34	0.45	23.63	136.80	998.75	1653.59
	± 0.30	± 1.35	± 0.67	± 0.63	± 2.12	±11.5	± 0.02	± 3.53	± 0.27	±0.91	± 0.09	±0.14	± 9.31	±2.95	±50.14	±162.65
SMB																
Low	1.10	19.67	7.15	13.32	3.70	8.75	1.91	0.43	1.23	1.85	1.99	0.81	107.30	50.35	88.97	637.91
	± 0.05	± 1.75	± 0.47	± 1.50	± 0.25	± 0.62	± 0.29	± 0.06	± 0.19	± 0.31	± 0.41	± 0.03	± 6.08	± 6.98	± 5.44	± 43.89
High	2.31	5.98	8.50	27.34	21.62	43.36	0.31	9.82	2.31	5.54	0.75	8.60	25.59	258.04	508.41	865.70
	± 0.40	± 0.42	± 3.78	± 4.74	± 7.78	± 8.87	± 0.14	± 2.14	± 0.92	±1.22	± 0.23	±1.08	± 6.80	±27.9	±38.83	±54.61
SMM																
Low	10.10	17.35	5.06	11.25	4.15	9.05	22.03	11.91	1.04	1.99	1.41	0.61	133.51	99.87	425.74	1413.5
	± 2.16	± 1.95	± 0.88	± 0.85	± 0.24	± 0.33	± 0.94	± 1.60	± 0.17	± 0.22	± 0.08	± 0.04	± 6.82	± 7.22	± 9.41	± 89.14
High	2.13	14.78	7.29	13.78	15.71	30.83	0.43	9.31	2.42	4.35	0.69	0.96	26.13	780.19	1320.00	1743.34
	± 0.09	± 1.43	± 4.18	± 6.08	± 3.26	± 12.1	± 0.10	± 2.32	± 1.26	±0.30	± 0.54	±0.28	± 1.37	±51.5	±52.25	±159.83
KP																
Low	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
High	6.70	15.62	8.11	15.34	23.21	36.08	1.01	12.42	3.18	4.96	2.52	3.42	48.94	195.96	n.d	5731.33
	± 0.97	± 2.20	± 1.19	±0.58	± 3.95	± 12.2	± 0.33	± 5.50	±0.88	±0.72	±0.40	±0.46	±11.1	±69.2		±1588.9
KR																
Low	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
High	1.58	8.08	6.67	10.29	16.05	38.52	0.88	43.80	1.71	2.20	1.37	0.55	29.96	72.06	n.d.	2178.81
	± 0.14	± 0.54	± 1.57	± 2.10	± 1.79	± 9.95	± 0.29	± 17.2	±0.27	±0.49	±0.57	±0.22	±1.82	±20.5		±345.37

Table 3.7. Mean concentrations of anions (± s.e) in soil solutions extracted in July 1995 from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR) using low-speed (Low) and high-speed (High) centrifugation. n.d, no data.

Extraction Method	NO_3 FR	NO_3 AD	P FR	P AD	Element Cl FR (mg l^{-1})	Cl AD	F FR	F AD	SO_4 FR	SO_4 AD
FM										
Low	1.49	2.63	21.5	37.0	23.3	32.2	2.44	4.42	23.6	38.0
	±0.17	±0.13	±3.36	±5.94	±1.05	±4.88	±0.61	±0.93	±1.53	±3.84
High	1.74	2.73	2.80	42.7	6.18	31.1	2.10	0.11	37.2	40.5
	±0.24	±0.27	±0.49	±1.73	±0.35	±5.18	±0.64	±0.00	±3.60	±6.61
SMB										
Low	0.21	1.89	11.6	22.4	15.4	26.8	1.46	3.51	23.9	49.7
	±0.05	±0.19	±1.30	±1.87	±1.30	±1.87	±0.30	±0.12	±3.96	±4.55
High	1.48	0.50	4.85	7.20	14.7	25.4	0.41	2.26	41.5	47.3
	±0.38	±0.08	±0.44	±0.17	±2.63	±2.63	±0.10	±0.90	±4.71	±5.30
SMM										
Low	1.23	3.07	10.4	22.1	20.6	33.2	0.44	2.03	16.6	16.1
	±0.06	±0.52	±1.73	±2.74	±1.81	±4.92	±0.15	±0.45	±2.02	±1.55
High	12.1	3.22	1.64	5.87	10.3	27.4	4.32	0.53	15.8	39.8
	±1.37	±0.14	±0.16	±0.40	±2.03	±4.16	±0.70	±0.09	±2.12	±4.39
KP										
Low	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
High	4.71	44.9	5.28	9.28	17.1	29.1	7.90	1.16	37.8	61.1
	±3.28	±1.52	±0.59	±0.49	±1.16	±5.67	±1.13	±0.12	±4.65	±8.23
KR										
Low	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
High	2.28	17.8	4.44	23.3	9.86	16.9	0.40	0.04	11.6	14.8
	±0.34	±2.52	±1.75	±1.74	±2.16	±3.20	±0.17	±0.01	±0.94	±2.30

3.5 Discussion

The effects of soil condition (FR or AD), extraction technique (Imm or Cen), and speed of centrifugation (Low and High) were compared. There were no differences in the soil solution pH values between FR or AD soils extracted at high-speed. Similarly no differences were found in the February samples extracted at low-speed. However pH values of July samples were higher in solutions from AD soils extracted at low-speed. Contrary to the findings of Dahlgren (1993) centrifugation with an immiscible displacent did not increase the soil solution pH. However the speed of centrifugation did influence solution pH. Contrary to Elkhatib et $al.$ (1987), pH values were up to 0.5 units higher in solutions extracted at low-speed.

All soil solution pH values were most similar to the soil pH_{H_2O} or soil pH measured in a weak $CaCl_2$ solution (0.002 M). Gillman (1991) found that soil pH measured in solutions of ionic strength appropriate to the soil most accurately reflected the soil solution pH. He suggested that soil pH could be standardised by using 0.002 M $CaCl_2$ which had an ionic strength (0.006) close to the average ionic strength of the soil solutions from weathered soils in North America, pasture soils in New Zealand, and agricultural soils in West Australia.

Whether soils were extracted at low- or high-speed, using an immiscible displacent or not, a greater volume of soil solution was acquired from AD soil samples. There was no difference in soil solution volumes between the two extraction techniques. However significantly greater volumes were obtained from all the soil types spun at high-speed. Contrary to expectation less solution was obtained from the boggy soil samples (FM or SMB) compared with the mineral soils (SMM, KP, or KR).

Whether samples were spun at low- or high-speed, or extracted with or without TCE, concentrations of cations were generally greater in solutions from AD soil samples. Results were not consistent among all soil types. NH_4, K, Mg, and Al were all higher in concentration in solutions from AD soil extracted at low-speed. K, Na, Mg, Fe, and Si were also higher in AD extracted soil at high-speed. There were no clear differences in the concentrations of Cl, F, SO_4 or NO_3 between FR and AD at low-speed. At high-speed, solutions from AD soil had significantly greater concentrations of Cl. Jones & Edward (1993b) also found increased ion concentrations upon rewetting of air-dried soils. Orders of increasing ion concentrations between sites were not the same in solutions from both FR and AD soil samples.

The extraction technique had little effect on the ionic composition of the soil solution. Only Fe, Na, and F were significantly different between the extraction methods. For solutions from FR and AD soil, concentrations of F were greater when extracted with TCE. Na concentrations were lower in TCE extracted AD soil and higher in TCE extracted FR soil. Adams et al (1980) and Wolt & Graveel (1986) also found miscible displacement, vacuum displacement, and centrifugation produced soil solutions of no difference. Dahlgren (1993) however extracted fresh-moist soil with immiscible displacement and the extracted solutions had lower solute concentrations compared with solutions extracted by centrifugation alone. Dahlgren used the displacent 1,1,2-trichloro-1,2,2, triflouroethane at high-speed (20000 x g).

The ionic composition of the extracted soil solutions were influenced to a certain extent by the speed of centrifugation. Concentrations of Na, Mg, Al, Fe, and Si were all greater in solutions extracted at high-speed. The speed of centrifugation had no significant effect on the concentration of anions. In contrast SO_4, F, and Cl concentrations were greater in solutions spun at 10000 rpm compared with 5000 rpm by Ross & Bartlett (1990). Zabowski & Ugolini (1990), and Edmeades et al (1985) found no differences at all between solutions spun at 1000 rpm and 10000 rpm, and 2000 rpm and 15000 rpm.

The ionic composition of soil solutions were also dependent upon the time of soil sampling (February or July). An increase in soil temperature during the summer months should in turn increase microbial activity and decomposition of organic matter. In agreement, the concentration of NH_4 in soil solutions extracted from SMM, KP, and KR, and P in all five soil solutions, increased between February and July by as much as ten-fold, but both NO_3 and SO_4 concentrations were reduced. Elements such as K, Ca, and Mg, are primarily released into the soil solution through the dissolution of soil minerals (aluminosilicates) which also increases with temperature. Concentrations of both Na and Mg were lower in July solutions extracted from the organic soils (FM and SMB) but increased in SMM, KP, and KR soils. In contrast K and Ca concentrations were significantly reduced, by as much as 50 %, in July soil solutions from all five soil types. The observed reduction in exchangeable cations in organic soil solutions is not surprising since these soils are not rich in weatherable minerals. Furthermore the month of July falls midway through the growing season and reductions in NO_3, K, and Ca as a result of plant uptake is similarly expected.

In summary there were few significant differences recorded in the soil solutions extracted using the different techniques and at the different centrifugal forces. However the differences in solutions from FR or AD soils were large and appear to be consistent amongst authors. It is recommended that soil solutions be extracted from fresh soil samples using centrifugation at a low-speed without an

immiscible displacent. Furthermore when using fresh soils, extraction should be within 24 h of collection.

3.6 Conclusions

- Soil solution pH values from fresh and rewetted air-dried soils were not significantly different.

- Centrifugation with an immiscible displacent did not affect solution pH.

- Soil solution pH values were greater using low-speed centrifugation.

- Soil solution pH values more closely resembled conventional soil pH measurements made in H_2O or 0.002M $CaCl_2$.

- Extraction of rewetted air-dried soils, and high-speed centrifugation, produced greater volumes of solution.

- Soil solutions from rewetted air-dried soils had significantly greater ionic concentrations than those from FR soils.

- Centrifugation with or without 1,1,1-trichloroethane did not affect solution ionic compositions.

- High-speed centrifugation increased the concentration of some cations in the soil solutions.

Chapter 4

Differences in low pH tolerance by races of *Anthoxanthum odoratum* L., *Holcus lanatus* L., and *Betula pendula* Roth.

4.1 Introduction

4.1.1 Low pH and soil acidity

Acid soils (pH <5.6) constitute about 1.17×10^9 ha of nonirrigated arable lands of the world (Polle & Konzak 1990). Factors determining soil acidity were described in Chapter 1. Simultaneous chemical interactions occurring in acid mineral soils include: increased solubility of mineral elements, such as Al, Fe, and Mn, to potentially toxic concentrations, and reduced availability of essential nutrients such as P, Ca, Mg, and Mo. Hence the hydrogen ion (H^+) activity, and direct H^+ toxicity, is no longer considered as the growth limiting factor associated with acid soils of pH >4.0 but rather the toxicity or deficiency of these other elements (Adams 1984, Bell 1996, Foy 1992, Frageria *et al.* 1990, Kamprath 1984). The conclusion reached by Wilkinson & Duncan (1989) that H^+ toxicity was the major limiting factor of sorghum growth in the acid soils of Georgia (pH <4.8), was later contradicted by Shuman *et al.* (1990) who showed Al toxicity was primarily reducing sorghum growth in these soils (Foy 1992). Similarly, Osaki *et al.* (1997) found that the effect of pH on plant growth was less conspicuous than the effects of Al. However H^+ toxicity may restrict the survival and activity of rhizobia and other soil microorganisms, and could be a significant growth-limiting factor in strongly acidic mine spoils (pH <3.0) or acid sulphate soils (Foy 1992). In organic soils where toxic concentrations of soluble Al and Mn are negligible, H^+ activity may be responsible for poor plant growth. Evans & Kamprath (1970) showed a sharp reduction in corn growth in an organic soil with pH< 4.0 which they attributed to H^+ toxicity.

Excess H^+ competes with other cations for root absorption sites, interfering with ion transport and uptake, and causing root membranes to become leaky. Prolonged exposure results in a diminished capacity for nutrient absorption and an increased nutrient, especially Ca, requirement by the plant (Foy 1992). Lund (1970) showed a higher Ca requirement by soybean taproots when grown in nutrient solution at pH 4.5 compared with those grown at pH 5.6 (Foy 1992). Hussain *et al.* (1954) showed a significant loss of K, Ca, P, and soluble N in barley roots after exposure to pH 3.0 (Foy

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1992). Their subsequent abilities for K absorption were also reduced. Excess H^+ ions have also been shown to decrease the uptake of Mg, Cu, Mn, and Zn (Foy 1992).

Nutrient solutions or sand cultures offer a means of distinguishing the direct effects of excess H^+ from the indirect effects mentioned above (Frageria *et al.* 1990). Both Arnon & Johnston (1942) and Islam *et al.* (1980) showed reduced root growth in plants grown in culture solutions at pH< 4.0. Arnon & Johnson (1942) showed H^+ was toxic at pH 4.0 to tomato but not to Bermuda-grass. Islam *et al.* (1980) grew six species including cassava, French bean, wheat, and maize in nutrient solutions adjusted to pH's 3.3 to 8.5. All species produced maximal or near-maximal yields within the pH range 5.5-6.5. At pH< 4.0 lateral root development was suppressed and in some cases root tips were necrotic. The roots were a discoloured brown or dull grey. Minimal growth of sorghum occurred at pH 3.7 and growth increased with increasing pH values (Guerrier 1982). Yokota & Ojima (1995) found root elongation in alfalfa was inhibited after 20 h treatment in nutrient solutions at pH 4.0, with a loss in root surface cell viability after only 4 h. The tap roots of soybean (cv. Ransom) grown in solutions at pH< 4.6 had symptoms of H^+ injury: roots were brownish, stunted, and had limited lateral growth (Sanzonowicz *et al.* 1998). Brunet (1994) grew two woodland grasses: *Bromus benekenii* and *Hordelymus europaeus*, characteristic of less acidic fertile soils, in solutions at pH's 3.9, 4.0, and 4.3. There was a sharp decrease in root growth below pH 4.0. New roots were thickened and discoloured.

Differences in tolerance to low pH between species or races has been shown in sunflower, soybean and subterranean clover. 90 % of maximum total dry matter yield was obtained by four sunflower cultivars at cultivar-specific solution pH's between 4.0-5.0. A medium pH of 3.5 was lethal to all four cultivars (Blamey *et al.* 1982). Handreck (1992) showed differential tolerance of low pH in ferns (*Asplenium* and *Adiantum* spp.). Ferns reputed to need alkaline growing media were intolerant of low pH values (pH <5.0).

4.1.2 Soil acidity amendment through lime applications

In most cultivated soils, periodic treatments with lime can correct for mineral element deficiencies or toxicities associated with low pH (Baligar *et al.* 1990a, Foy 1992). This use of liming materials to increase soil pH and root growth is an old and common practice (Frageria *et al.* 1995, Sharpley *et al.* 1992). The obvious direct effects of lime addition are, an increase in both soil Ca concentration and pH, resulting in increased P and Mo availability, reduced exchangeable Al, and lower availability of Cu, Fe, Mn, and Zn (Frageria *et al.* 1990, McLean & Brown 1984, Stevens & Laughlin 1996). Therefore overliming may lead to micronutrient deficiencies. Liming also improves microbiological activities of acid soils, which in turn increases N fixation by legumes thus liberating N from incorporated organic materials (Frageria *et al.* 1995). The bulk of agricultural lime comes from

ground limestone but many other materials are now used : ground marl and chalk, slag from iron and steel making, flue dust from cement plants, refuse from sugar beet factories and paper mills (Thomas & Hargrove 1984). Linz-Donawitz slag, a by-product of the Fe and steel making industry, was successfully used as a liming agent by Besga *et al.* (1997). The soil pH and exchangeable Ca and Mg increased, while exchangeable Al decreased.

Frageria *et al.* (1995) showed increased dry matter yields in both bean and corn when grown in a limed oxisol. Dry matter yields of perennial pasture and perennial ryegrass also increased after liming a clay soil (0-12 t ha^{-1}) in County Antrim (Stevens & Laughlin 1996). Substantial increases in the grain yield of wheat were consistently obtained over a 12-year period after one single lime application (2.5 t ha^{-1}). Yields increased by 79 % in an acid-sensitive (Oxley) wheat cultivar (Coventry *et al.* 1997). Finally, low-level liming of an ultisol (increased soil pH from 4.76 to 4.95) increased shoot mass, shoot N uptake, number of nodules, and root mass of white clover inoculated with *Rhizobium leguminosarum* bv. *trifolii* (Staley & Morris 1998).

In most soils however, conventional liming of the plough layer is insufficient to neutralise subsoil acidity (where root development is needed for greater resistance to drought and more effective use of subsoil nutrients), and mixing lime throughout the entire soil volume is not economically feasible (Barceló *et al.* 1996, Foy 1992, Foy 1996, Sanzonowicz *et al.* 1998, Sharpley *et al.* 1992). The normal limit of lime incorporation using conventional farm machinery is about 10 cm. Liming an acid soil to a depth of 1 m neutralised Al, increased soluble Ca concentrations, stimulated root development in subsoils, and increased alfalfa yield by 50 % (Sumner *et al.* 1986). Incorporation of lime to 20 cm compared with 10 cm also increased yields of barley and wheat. However there was no further advantage in liming to a depth of 40 cm (Scott *et al.* 1997).

An alternative to liming, or indeed in combination with liming, is the use of acid-tolerant crops or cultivars which can improve productivity in acid agricultural soils (Dwivedi 1996, Foy 1996, Scott *et al.* 1997, Sharpley *et al.* 1992). Foy (1996) limed Al-toxic Tatum subsoil with 0.75 or 4 mg CaCO$_3$ kg^{-1} soil to reach final soil pH values of 4.4 and 5.7. Relative shoot dry weights averaged 28.6 % for the acid-tolerant and 14.1 % for the acid-sensitive barley cultivars. The dry matter yields of rice and wheat were not much improved with the addition of 4 g kg^{-1} lime. This was attributed to their acid soil tolerance (Frageria *et al.* 1995). Dwivedi (1996) compared the effects of liming an acid soil (Inceptisol) in the Himalayan region on the growth of different crops. Lime application decreased foliar concentrations of Al, Fe, and Mn, and increased grain yields, in all crops except buckwheat, rice, bean, horse gram, and amaranth which were all acid tolerant.

4.2 Aims

- To investigate the effects of low pH on the growth of naturally occurring races of *Holcus lanatus* and *Betula pendula*. (The reasons for the choice in study species were given in Chapter 2, Section 2.2.)

- To investigate the possibility of differences between races in response to low pH.

- To investigate the effects of low pH on nutrient absorption.

- To investigate the effects of low pH on root cell anatomy and race-specific differences.

- To determine whether or not lime addition improved yields of naturally occurring *Anthoxanthum odoratum* and *Holcus lanatus*.

4.3 Methods

4.3.1 pH tolerance in nutrient solutions

4.3.1.1 Experiment 1

Seed material of *Holcus lanatus* was collected in August 1995 from FM and KP (Chapter 2, Table 2.1). Seeds were stored in dry and dark conditions until the start of the experiment. Seeds were germinated in August 1995 in Petri dishes on seed test paper (Whatman Grade 181) with acid-washed sand in the Stirling University growth rooms. The Petri dishes were kept under a photoperiod of 16 h light and 8 h dark with a PAR of 200 μmol m^{-2} s^{-1}. Temperature was 20°C during the day and 15°C during the night. Seeds germinated after 3-4 days. Seedlings were kept in Petri dishes for seven days following germination and watered with dilute culture solution (10 times dilution). They were then carefully threaded through thin glass tubes using deionised water. The glass tubes were suspended from the lids of 600-ml beakers into an initial culture solution identical in ionic composition to that described below but at pH 5.6.

The composition of the culture solutions is shown in Table 4.1. Stock solutions of 100-strength of NH$_4$OH, Na$_2$SO$_4$, NH$_4$H$_2$PO$_4$, KFeEDDHA, Ca(NO$_3$)$_2$.4H$_2$O, CaCl$_2$.6H$_2$O, Mg(NO$_3$)$_2$.6H$_2$O, H$_3$BO$_3$, KH$_2$PO$_4$, and MES buffer, and 1000-strength of MnSO$_4$.4H$_2$O, ZnSO$_4$.7H$_2$O, CuSO$_4$.5H$_2$O, and (NH$_4$)$_6$Mo$_7$O$_{24}$.4H$_2$O were made up and diluted appropriately. The culture solution was based on soil solution analyses of fresh and air-dried soils extracted from Flanders Moss (FM) samples in February

1995 by centrifugation (Table 4.2) and micronutrients were based on concentrations used by Johnston & Proctor (1981) (1/10 those of Hoagland & Arnon 1950). MES buffer was used to keep the culture solution constant at pH 5.6 before treatments began. KFeEDDHA was used instead of NaFeEDTA following cautions by Chaney & Bell (1987) about the possible confounding effects of NaFeEDTA in micronutrient experiments. GEOCHEM predicted 63.8 % of Fe^{3+} in solutions remained bound to EDDHA compared with 11.9 % using EDTA. Beakers were covered in tinfoil to prevent algal growth. Solutions were stirred daily and the pH corrected where necessary to 5.6 using 1M NaOH or 1M HCl. Culture solutions were changed twice per week.

After two weeks growth in the initial culture solution seedlings of similar size were separated into beakers each holding two seedlings. The plants were grown in culture solutions at pH 2.0, 3.0, 4.0, 5.0, and 5.6. There were ten replicate seedlings per treatment per site. Solutions were changed every three days. Solutions were buffered at pH 2.0 using NaCl, citric acid, and HCl; at pH 3.0 using Na citrate and citric acid; at pH 5.0 and pH 5.6 using MES Buffer (Table 4.2). Nutrient solutions were stable at pH 4.0 without the addition of a buffer.

Table 4.1. Chemicals and their rate of application used in culture solutions. *, 1000 μM MES buffer is 2(N-morpholino)ethanesulphonic acid used to buffer solutions at pH 5.6 and at pH 5.0. **, Na citrate/citric acid used to buffer solutions at pH 3.0. ‡, NaCl/citric acid/HCl used to buffer solutions at pH 2.0.

Chemical used	Concentration in culture solution (μmol l^{-1})
NH_4OH	776
Na_2SO_4	350
$Ca(NO_3)_2.4H_2O$	74
$CaCl_2.6H_2O$	74
$Mg(NO_3)_2.6H_2O$	58
$NH_4H_2PO_4$	56
KH_2PO_4	22
KFeEDDHA	9.9
H_3BO_3	4.6
$MnSO_4.4H_2O$	0.91
$ZnSO_4.7H_2O$	0.076
$CuSO_4.5H_2O$	0.032
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.0074
MES buffer	1000 *
Na citrate/citric acid	1000/2000 **
NaCl/citric acid/HCl	610/300/82 ‡

The number of roots and their lengths, the number of tillers and blades and their lengths, were recorded before seedlings were put into treatments and thereafter every five days until harvesting after 15 days of treatment (14 Sep-29 Sep 1995). At harvest roots and shoots were separated, rinsed in deionised water, and dried in an oven at 60 °C for 48 h and the dry weights recorded. Root:shoot ratios were determined.

Between 100 and 300 mg of oven-dried shoots and roots were digested in a sulphuric acid-hydrogen peroxide mixture (Allen 1989) in a block digester at 330 °C. Digested plant material was filtered through a No.44 Whatman filter paper and made up to 100 ml. Concentrations of Ca and Mg were measured using a Varian AA-575 S atomic absorption spectrophotometer with a nitrous oxide-acetylene flame. An air-acetylene flame was used to determine K (flame emission) and Fe concentrations.

P was measured on a Tecator FIAstar 5010 flow injection auto analyser using the stannous chloride-ammonium molybdate method.

Ten terminal 1 cm sections of roots from both KP and FM seedlings, and grown at each pH, were embedded in paraffin. Samples were fixed in FAA (13 ml formaldehyde: 5 ml glacial acetic acid: 200 ml 50% ethanol). Root sections were then dehydrated with graded ethanol (diluted with deionised water) and embedded in wax. Cross-sections were cut using an ultramicrotome and stained with safranin and light green. All the sections were observed under an optical Zeiss light microscope.

For electron microscopy terminal 10 mm root portions were embedded in Spurr's resin following the procedure outlined in Chapter 7, Section 7.3.2.

Table 4.2. Concentrations (mg l^{-1}) of each ion analysed in soil solutions from Flanders Moss (FM) and compared with their equivalent concentration in culture solutions. FR, soil solutions extracted from fresh soil. AD, soil solutions extracted from rewetted air-dried soil.

Element	Soil FR	Soil AD	Added through	Concentration in culture solution
			mg l^{-1}	
NH_4	5.91	19.70	$NH_4H_2PO_4$, NH_4OH	15.16
NO_3	2.60	2.60	$Mg(NO_3)_2.6H_2O$, $Ca(NO_3)_2.4H_2O$	16.40
PO_4	0.36	2.12	KH_2PO_4, $NH_4H_2PO_4$	7.35
K	6.47	14.27	KH_2PO_4, KFeEDDHA	6.75
Na	7.78	9.24	Na_2SO_4	15.95
Ca	1.86	35.70	$Ca(NO_3)_2.4H_2O$, $CaCl_2.6H_2O$	5.86
Mg	0.64	2.47	$Mg(NO_3)_2.6H_2O$	1.41
Fe	0.31	0.08	KFeEDDHA	0.54
SO_4	53.36	65.47	Na_2SO_4	33.89

4.3.1.2 Experiment 2

Seeds of *Betula pendula* were collected in August/September 1995 from SMM, KP, and KR (Chapter 2, Table 2.1). The seeds were stored in the same conditions as those of *Holcus lanatus*. Seeds were germinated in 1 % agar (DIFCO Laboratories 1995) in March 1996 in the Stirling University growth rooms with the same photoperiod and temperature regime as in Experiment 1. The agar was poured into sterile Petri dishes in a hood. Seeds germinated after five days and were kept in agar for 1-2 weeks

before being transferred into culture solution. At the first leaf stage they were removed from agar and carefully threaded through thin glass tubes with deionised water. The glass tubes were suspended from the lids of 600-ml beakers in an initial culture solution (prepared as in Experiment 1) at pH 5.6 (Figure 4.1).

Figure 4.1. *Betula* seedlings at initial transfer into solutions. Seedlings were transferred at the first leaf stage and suspended from glass tubes into "initial" nutrient solutions.

After four weeks growth in culture solution at pH 5.6 seedlings of similar size were separated into beakers each holding two seedlings. At this stage seedlings had an average of 3-6 roots of 40-80 cm total length, and 7-11 leaves of 16-38 cm^2 total area. The seedlings were grown in culture solutions at pH 3.0, 4.0, 5.0, and 5.6. There were five replicate seedlings per treatment per site. Solutions were changed every three days.

The number of roots and their lengths, the number of leaves and their length and maximum breadth, and the height of the seedlings were recorded before seedlings were put into treatments and at harvest. To determine the leaf area expansion over the treatment period a relationship between actual leaf area and measured values of length and breadth was established. One hundred leaves, collected from separate birch seedlings, which were grown alongside experimental seedlings, were scanned and their areas measured using NIH 5b Image. A regression equation between leaf area and leaf maximum breadth and length was then determined and used to estimate the leaf area of experimental seedlings before treatments began and at harvest.

Seedlings were harvested after four weeks growth in the +Al treatments (17 Apr-15 May 1996). Roots and shoots were separated, rinsed in deionised water, and dried in an oven at 60 °C for 48 h and the dry weights of leaves and roots recorded. Root:shoot ratios were determined.

Between 100 and 300 mg of oven-dried leaves and roots were digested and analysed for P, K, Ca, Mg, and Fe using the same digestion procedure and analytical techniques as in Experiment 1.

4.3.2 Pot experiments

Seeds of *Holcus lanatus* and *Anthoxanthum odoratum* were collected from FM, SMB, SMM, KP, and KR in June/August 1996 (Chapter 2, Table 2.1), and were germinated in acid-washed sand on Seed Test Paper in July 1997 and September 1997. Seeds germinated after 3-4 days (*Holcus*) and 7 days (*Anthoxanthum*).

$Ca(OH)_2$ was added to moistened, air-dried soil to raise the soil pH_{CaCl_2} to 5.6, at the following rates: 13.16 g kg^{-1} (FM), 9.4 g kg^{-1}(SMB), 2.73 g kg^{-1} (SMM), and 3.99 g kg^{-1} (KP) based on recommendations by Rowell (1995). The required $Ca(OH)_2$ was added to polythene bags containing 100 g samples of <2 mm air-dried soil. Deionised water was added to bring the soil to 40 % of its holding capacity. The soil was thoroughly mixed and left to equilibrate for two weeks in the bags which were loosely folded. The bags were occasionally shaken to ensure good aeration (Rowell 1995). At the end of the incubation period the pH of each soil sample was measured. The limed soils had a pH of 5.6 ±0.1. $Ca(OH)_2$ was not added to KR soil.

Seedlings from each site were potted (78-mm pots) in limed and unlimed soil from each of the five sites. There were five replicates of *H. lanatus* and ten of *A. odoratum* per treatment.

After eight weeks growth seedlings were harvested. *Holcus* were harvested on 1 Sep 1997, and *Anthoxanthum* were harvested on 2 Nov 1997. Roots and shoots were separated, washed in deionised water, and dried in an oven at 60 °C for 48 h and the dry weights recorded.

4.4 Results

4.4.1 pH tolerance in *Holcus lanatus*

4.4.1.1 Root elongation and number

Figure 4.2 shows the rate of root elongation (RER) and increase in mean number of roots (per plant) over 15 days of growth at pH 2.0-5.0. pH had a significant effect on root growth (Table 4.3). Root elongation was significantly reduced in pH 2.0, 3.0, and 4.0 in *Holcus* originating from KP. After 5 days in solution at pH 2.0 elongation was completely inhibited and did not resume. Elongation at pH 3.0 was also severely reduced after only 5 days. In solutions at pH 4.0 there was a small increase between 5 and 10 days, thereafter elongation was inhibited. In contrast, rates of root elongation increased almost linearly with time in seedlings grown in solution at pH 5.0 and 5.6. There were no differences in root growth between these two pH values.

There were significant differences in root elongation between sites (Table 4.3). Like KP seedlings, root elongation in FM races was reduced at pH 2.0 and 3.0, but at a lower rate than in KP races. However both pH 5.0 and pH 5.6 also had an inhibitive effect on root elongation. Rates of elongation slowly declined over the 15 days of treatment. In contrast, at pH 4.0 root elongation increased significantly with time and after 15 days rates were more than twice those at pH 5.6 (Figure 4.2).

Solution pH also had a significant effect on root number (Table 4.3). Like root elongation, root numbers did not increase in KP seedlings at pH 2.0 and 4.0, and only slightly at pH 3.0 (Figure 4.2). In contrast root numbers increased in all three treatments in FM seedlings throughout the full 15 days of treatment. At both pH 5.0 and 5.6 root numbers increased with time in KP seedlings. There were only significant increases in root numbers of FM seedlings at pH 4.0. Numbers increased more than two-fold between the start and end of treatment (Figure 4.2).

There were toxicity symptoms in the roots of seedlings from both sites grown at pH < 4.0, and these were more pronounced in KP seedlings (Figure 4.3). Root tips were swollen and black in *Holcus* from KP grown in solutions at pH 3.0. The same roots of FM plants were less swollen and only slightly discoloured.
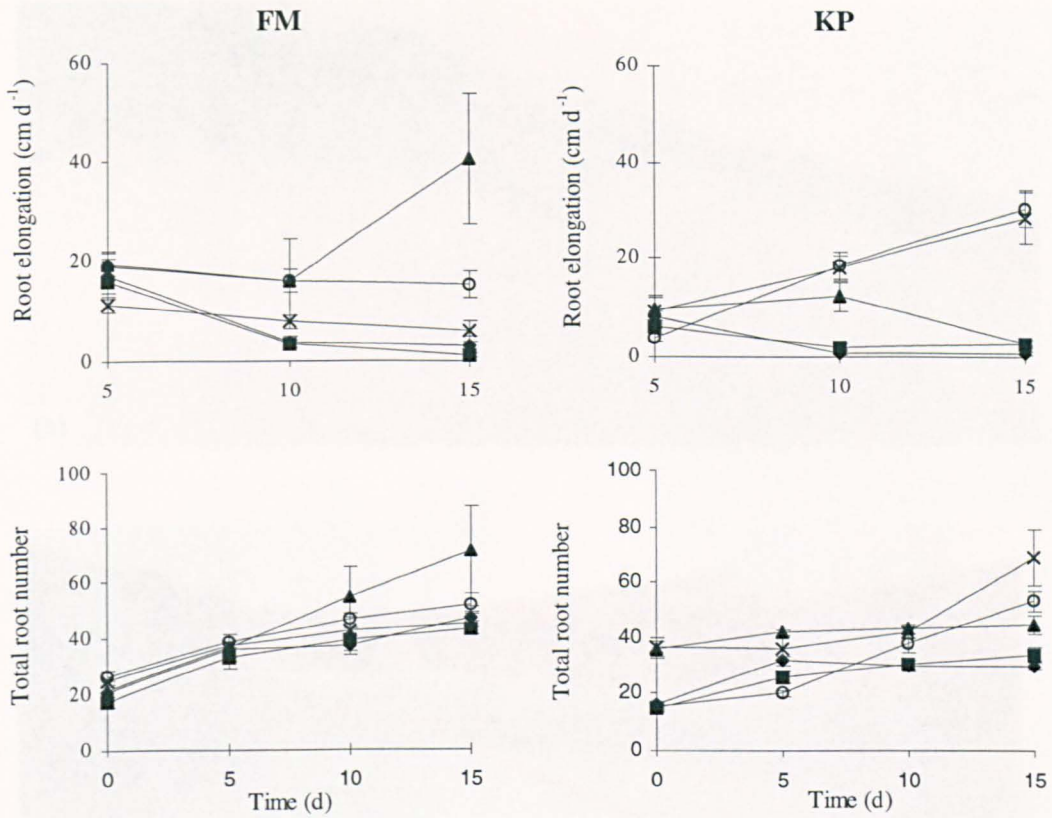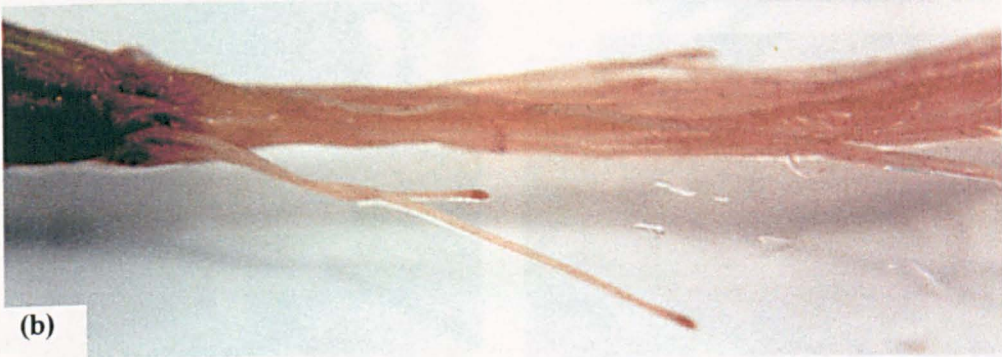
Figure 4.2. Mean root elongation (cm day^{-1}, ± s.e) and increase in mean total root number (± s.e) in *Holcus lanatus* originating from Flanders Moss (FM) and Kippenrait Glen (KP). Seedlings were grown in nutrient solutions at pH 2.0 (◆), 3.0 (■), 4.0 (▲), 5.0 (×), and 5.6 (O). Treatments began on day 5 and lasted 10 days.

Figure 4.3. Roots of *Holcus lanatus* from Kippenrait Glen (KP) at pH 5.6 (a) are undamaged. Roots of Flanders Moss (FM) at pH 3.0 (b) have some tip necrosis but are healthier than those of KP at the same pH (c).
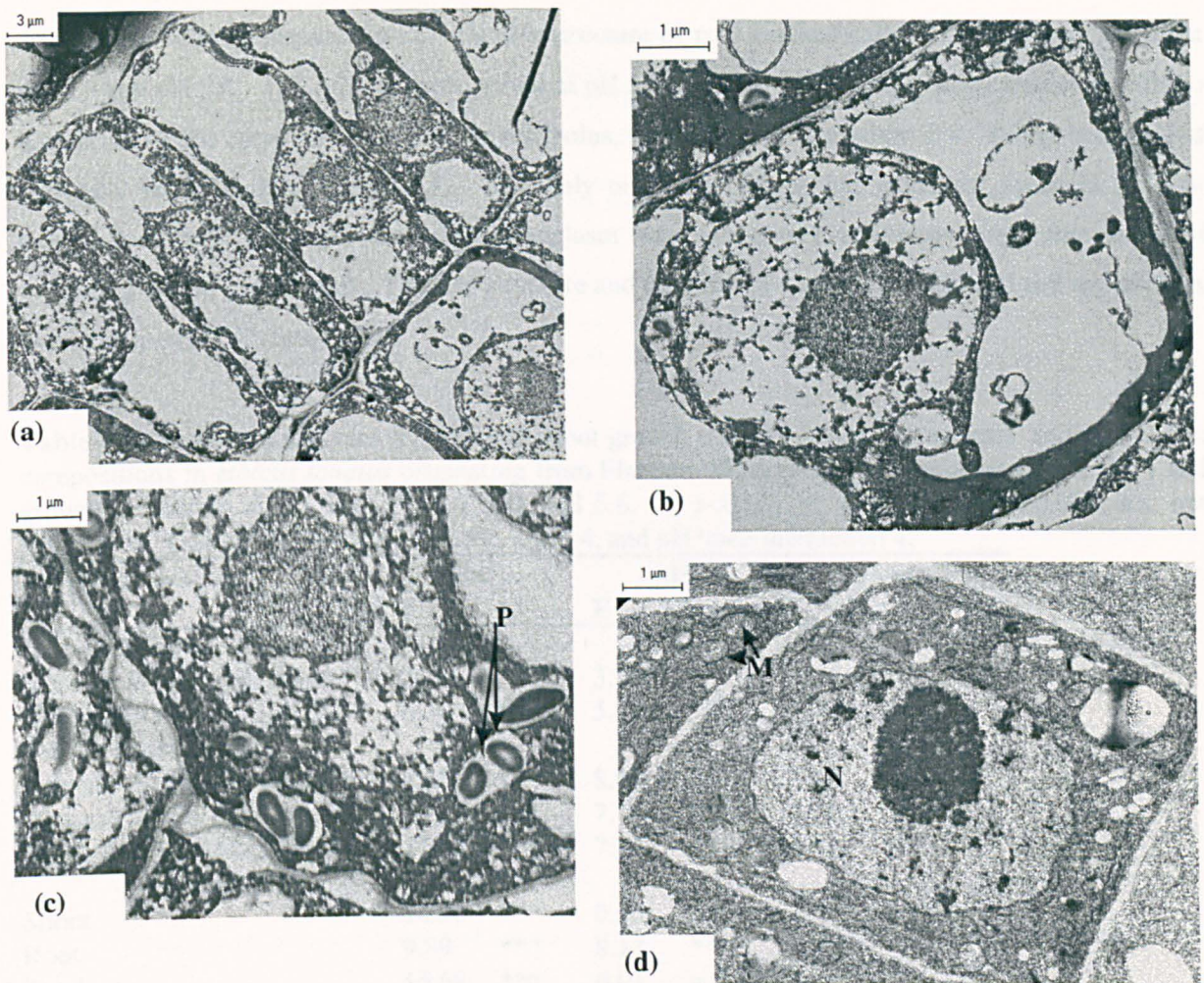
Figure 4.4. TEM micrographs of cortical cells of longitudinal root tip sections of *Holcus lanatus* grown in (a), (b), & (c) pH 3.0, and (d) pH 5.6 nutrient solutions. M = mitochondria, N = nucleus, P = plastid.

4.4.1.2 Root anatomy and ultrastructure

The differences in root anatomical traits between races of *Holcus* at each pH treatment are shown in Table 4.4. The outer cortical cells and epidermis of KP races were often disintegrated in roots treated at pH< 5.6. The root diameter, and number of cortical cells, of FM races did not change greatly between pH treatments, with the exception of pH 3.0 where diameter and whole-root area were substantially enlarged. At pH 3.0 there was also an increase in cortical cell number (about 30 cells more). In contrast the whole-root diameter and area of KP *Holcus* decreased when acidity increased beyond pH 5.0. Neither the diameter nor area of stelar tissue changed greatly in either of the two races. At pH 3.0 the stele was enlarged in FM races and it also occupied the lowest percentage of whole-root area. In KP seedlings the % area occupied by the stele increased with increasing acidity. The % area occupied by cortical cells was greater in FM *Holcus* compared with KP *Holcus* irrelevant of pH. This area also remained consistent despite increasing H^+ concentration in FM but decreased substantially in KP.

There were large differences in the cell ultrastructure of root cortical cells between plants grown at pH 3.0 and pH 5.6. The cells of roots grown at pH 5.6 retained the normal cellular structure with no disruption to the cytoplasm, nucleus, or nucleolus, and contained abundant E.R., Golgi bodies, and immature mitochondria (Figure 4.4). The only organelles which had remained intact in pH-3.0-treated root cells were plastids. The cytoplasm was completely withdrawn from the cell wall indicating severe plasmolysis. The fine structure and integrity of nuclei, nucleoli, and cell membranes were lost in pH-3.0-treated roots.

Table 4.3. Statistical analyses for root and shoot growth measurements, dry weights, and plant ionic compositions in *Holcus lanatus* originating from Flanders Moss (FM) and Kippenrait Glen (KP) and grown in solution at pH 2.0, 3.0, 4.0, 5.0, and 5.6. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; n.s, not significant. Degrees of freedom are: race 1, pH 4, and pH*race interaction 4.

Measurement	Race		pH		pH*race interaction	
	F	p	F	p	F	p
Root growth						
Increase in total root length	23.91	***	3.12	*	3.62	**
Increase in number of roots	6.34	*	5.78	***	1.48	n.s
Tops growth						
Increase in total shoot length	43.35	***	8.01	***	1.42	n.s.
Increase in total tiller number	76.11	***	7.74	***	5.74	***
Increase in total blade number	124.2	***	7.84	***	2.62	*
Dry weights						
Shoot	14.96	***	0.50	n.s	4.07	**
Root	9.89	***	8.17	**	3.86	**
Total	15.68	***	0.01	n.s	4.49	**
Root:shoot ratio	4.59	**	30.32	***	0.22	n.s
Ionic composition						
Shoots						
P	23.30	***	17.95	***	6.90	***
K	4.84	*	5.94	***	7.57	***
Ca	3.55	n.s	6.69	***	0.98	n.s
Mg	12.62	**	7.85	***	4.28	**
Fe	0.04	n.s	13.70	***	4.67	**
Roots						
P	23.30	***	33.73	***	3.37	*
K	27.92	***	6.92	***	1.71	n.s
Ca	0.01	n.s	10.13	***	2.37	n.s
Mg	4.08	*	3.00	*	0.46	n.s
Fe	0.00	n.s	7.42	***	0.74	n.s

Table 4.4. Mean root diameter and area, stele diameter and area, cortex area and number of cortical cells (± s.e) in *Holcus lanatus*, originating from Flanders Moss (FM) and Kippenrait Glen (KP), grown in nutrient solutions at pH 5.6, 5.0, 4.0, 3.0, and 2.0. The mean proportion of whole-root area occupied by vascular tissues and cortex are given in parentheses. †, no data available. Areas were estimated from diameter measurements.

Treatment	Root diameter (mm)	Root area mm²	Stele diameter (mm)	Stele area mm²	Cortex area mm²	Cortical cell number
FM						
pH 5.6	0.26 ±0.03	0.80 ±0.09	0.09 ±0.009	0.29±0.03(35.5)	0.52±0.07(64.5)	60.67 ±6.94
pH 5.0	0.29 ±0.02	0.90 ±0.07	0.09 ±0.004	0.28±0.01(31.4)	0.62±0.07(68.6)	60.33 ±7.99
pH 4.0	0.23 ±0.02	0.72 ±0.08	0.09 ±0.001	0.28±0.03(38.6)	0.44±0.06(61.5)	†
pH 3.0	0.37 ±0.02	1.16 ±0.08	0.11 ±0.003	0.34±0.01(29.4)	0.82±0.08(70.6)	89.50 ±5.40
pH 2.0	0.21 ±0.03	0.66 ±0.08	0.07 ±0.007	0.22±0.02(32.4)	0.45±0.06(67.6)	59.67 ±2.06
KP						
pH 5.6	0.23 ±0.02	0.72 ±0.05	0.09 ±0.006	0.28±0.02(38.9)	0.44±0.04(61.1)	79.33 ±2.33
pH 5.0	0.27 ±0.03	0.86 ±0.09	0.11 ±0.006	0.35±0.02(40.3)	0.51±0.08(59.7)	†
pH 4.0	0.19 ±0.07	0.59 ±0.04	0.08 ±0.004	0.26±0.01(43.4)	0.33±0.08(56.6)	†
pH 3.0	0.17 ±0.01	0.52 ±0.04	0.09 ±0.005	0.27±0.01(51.2)	0.26±0.03(48.9)	†
pH 2.0	0.19 ±0.01	0.59 ±0.04	0.10 ±0.009	0.30±0.03(51.1)	0.29±0.04(48.9)	†

4.4.1.3 Shoot growth

Rates of shoot elongation (SER, cm day^{-1}) are shown in Figure 4.5. Shoot growth increased over the ten days treatment in seedlings from KP grown at both pH 5.0 and 5.6, and in FM at only pH 4.0. Shoot growth decreased with time in KP seedlings at pH 2.0, 3.0, and 4.0. SER only decreased at pH 5.6 in FM seedlings and remained constant in other treatments. Effects of pH on shoot growth and differences between sites were statistically significant (Table 4.3).

The shoots of plants grown at pH 5.6 or 5.0 were green, turgid, and healthy in appearance (Figure 4.6). With increasing acidity the shoots became severely chlorotic and wilted. This was again far more pronounced in KP seedlings (Figures 4.6 c and d) compared with FM seedlings (Figure 4.6 e).

Solution pH also had a significant effect on tiller production and number of blades with significant differences between the sites (Table 4.3). Both tiller and blade number increased throughout the 10 days in seedlings of FM at all pH values, although only slightly at pH 2.0 and 3.0. In contrast increases were only seen in KP seedlings at pH 5.6 and 5.0 (Figure 4.5).
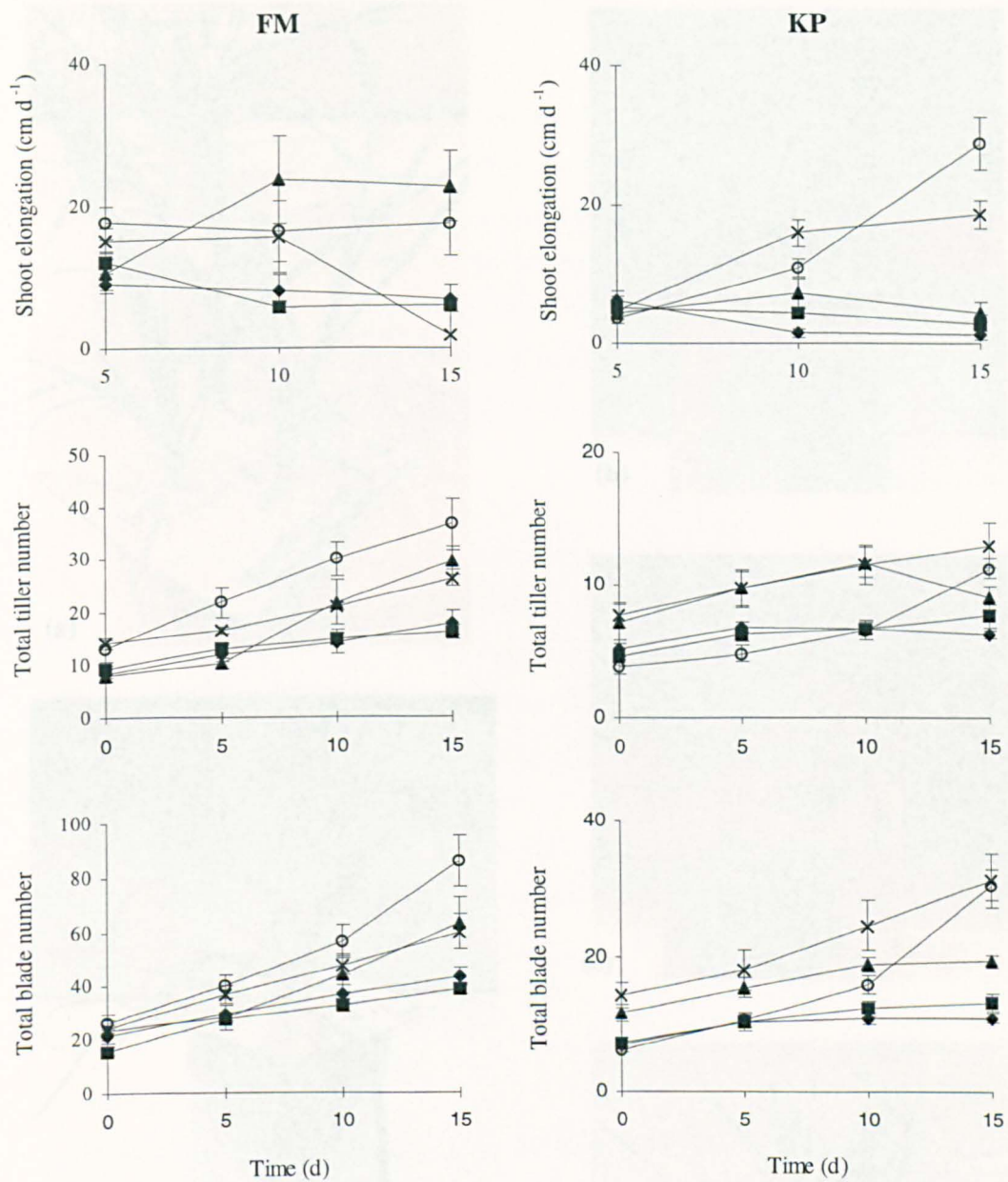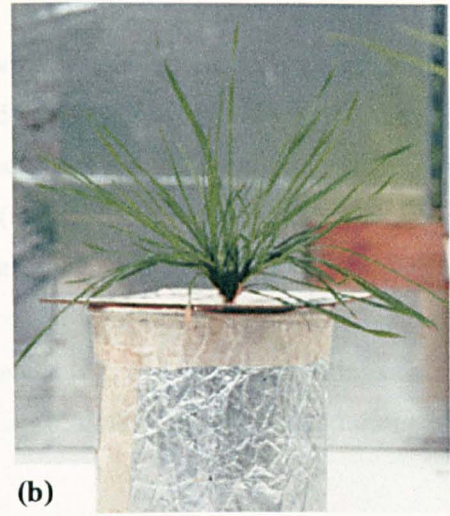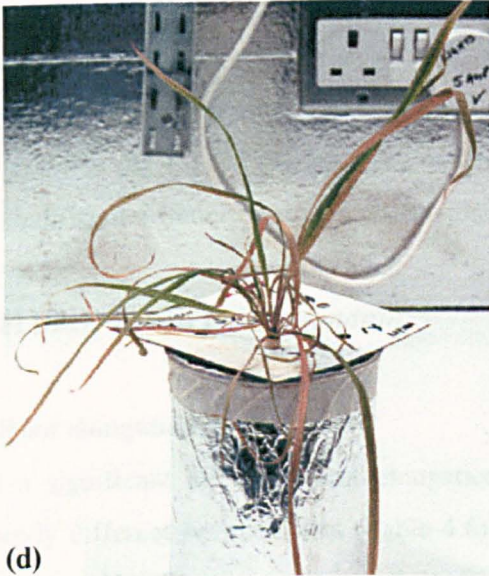
Figure 4.5. Mean shoot elongation (cm day^{-1}, ± s.e), and increase in total tiller and blade number (± s.e) in *Holcus lanatus* originating from Flanders Moss (FM) and Kippenrait Glen (KP). Seedlings were grown in nutrient solutions at pH 2.0 (◆), 3.0 (■), 4.0 (▲), 5.0 (×), and 5.6 (O). Treatments began on day 5 and lasted 10 days.
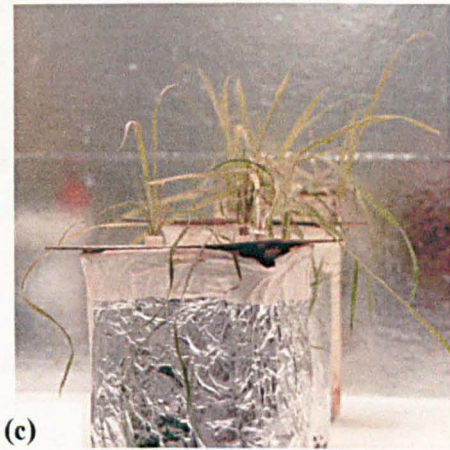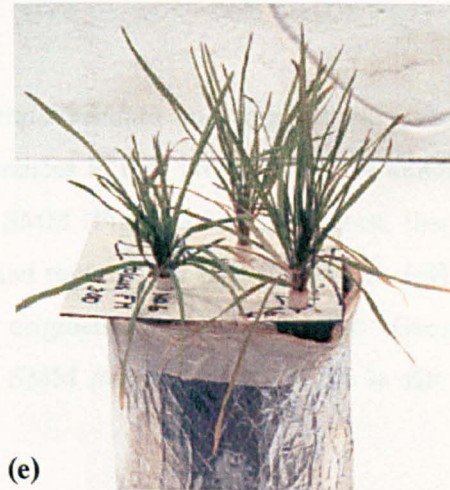
Figure 4.6. *Holcus lanatus* from (a) Kippenrait Glen (KP) and (b) Flanders Moss (FM) grown in nutrient solutions at pH 5.6. After 15 days growth at (c) pH 3.0 and (d) pH 2.0 KP seedlings wilted and leaves were extremely chlorotic. In contrast shoots of FM after the same growth period at pH 3.0 remained turgid with little chlorosis (e).

4.4.1.3 Plant tissue ionic composition

Root K concentrations dropped in FM seedlings only between pH 3.0 and 2.0, while progressively decreasing in KP seedlings from pH 5.6 to 2.0 (Table 4.5). Differences between sites and pH values were significant (Table 4.3). There were no major trends in shoot K concentrations. Mg root and shoot concentrations decreased with increasing acidity (although not consistently), and to a greater extent in KP seedlings. Root Ca concentrations on the other hand increased with decreasing pH, especially in KP seedlings. Like Ca, Fe uptake by roots and translocation to shoots increased with increasing acidity (again not consistently). Differences in Ca, Mg, and Fe concentrations between pH values were significant (Table 4.3). KP seedlings had significantly greater concentrations of K and Mg in both the roots and shoots (Table 4.3).

4.4.1.4 Plant dry weights

Figure 4.7 shows mean total, shoot and root dry weights, and root:shoot ratios for *Holcus lanatus* from FM and KP at each of the five pH treatments. Both dry weights and root:shoot ratios were highest in KP seedlings at pH 5.6 and 5.0, and dry weights were lowest at pH 2.0. Root:shoot ratios increased again at pH 2.0. A similar pattern in root:shoot ratios was seen in FM but ratios were consistently greater, between 1.5 and two-fold, indicating a greater contribution to yield from roots than shoots. There were no significant differences in total, shoot, and root dry weights between *Holcus* grown at pH 4.0 and pH 5.6. Dry weights were highest at these two pH values. There were also no significant differences among dry weights of the remaining three treatments.

4.4.2 pH tolerance in *Betula pendula*

4.4.2.1 Root elongation and number

pH had a significant effect on root elongation in *Betula pendula* and rates of elongation were significantly different between sites (Table 4.6). Differences in root growth and root number were least between pH values in seedlings originating from SMM (Figure 4.8). In contrast, there was a large and significant difference in both root elongation and root number between the low (pH 3.0 and 4.0) and high pH values (pH 5.0 and 5.6) in seedlings originating from KP and KR. Overall, root elongation and root number were highest at pH 5.6 in SMM *Betula*, and at pH 5.0 in KP and KR seedlings.

4.4.2.2 Leaf area expansion and number

Figure 4.9 shows the mean total leaf area and number in *Betula* from each site grown at each of the four pH values. Leaf area and numbers were highest in SMM seedlings and increased with pH. However they were not significantly different between pH values 4.0, 5.0, and 5.6. Leaf area and numbers also increased with pH in both KP and KR and the differences between pH values were more pronounced. Increasing the solution pH from 4.0 to 5.0 caused a significant increase in leaf production and area in KP seedlings which thereafter did not change greatly. Whereas increasing pH

from 5.0 to 5.6 caused a further increase in leaf number and area in KR seedlings. Both numbers and area of leaves were consistently lowest in KR seedlings. Total leaf area was significantly different among sites, and pH significantly influenced both the leaf area and number (Table 4.6).

Table 4.5. Mean ionic composition (mg g^{-1} dry weight, ± s.e) of shoots and roots of *Holcus lanatus* grown in nutrient solutions at pH 5.6, 5.0, 4.0, 3.0, and 2.0. *Holcus* originated from Flanders Moss (FM) and Kippenrait Glen (KP).

| Treatment	P		K		Ca mg g^{-1}		Mg		Fe	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
FM										
pH 5.6	3.82	4.19	7.63	4.62	2.00	1.66	1.08	0.51	0.25	1.46
	±0.19	±0.11	±0.33	±0.77	±0.17	±0.20	±0.12	±0.04	±0.05	±0.03
pH 5.0	7.33	3.85	14.43	5.56	2.23	1.69	1.37	0.58	0.20	1.66
	±0.54	±0.20	±1.59	±0.64	±0.18	±0.41	±0.10	±0.05	±0.06	±0.44
pH 4.0	3.60	2.77	9.89	4.04	2.18	1.97	1.05	0.36	0.40	4.36
	±0.21	±0.23	±0.62	±0.78	±0.11	±0.45	±0.16	±0.04	±0.08	±0.64
pH 3.0	3.64	2.28	9.54	4.88	1.87	2.89	0.96	0.32	0.52	3.18
	±0.08	±0.09	±0.59	±0.27	±0.04	±0.21	±0.04	±0.03	±0.04	±1.07
pH 2.0	3.73	1.02	8.01	1.95	2.41	4.72	0.98	0.48	0.55	1.36
	±0.23	±0.14	±0.20	±0.24	±0.10	±1.09	±0.04	±0.18	±0.06	±0.33
KP										
pH 5.6	3.93	6.24	9.74	12.56	2.18	1.79	0.96	0.76	0.04	1.04
	±0.50	±0.26	±0.71	±1.96	±0.11	±0.08	±0.09	±0.09	±0.01	±0.45
pH 5.0	3.81	3.81	10.45	8.95	2.34	1.79	1.01	0.63	0.23	1.07
	±0.41	±0.57	±0.12	±1.84	±0.31	±0.09	±0.07	±0.12	±0.06	±0.31
pH 4.0	2.99	3.68	12.34	6.48	2.18	3.94	0.89	0.57	0.68	5.03
	±0.18	±0.56	±1.15	±0.93	±0.32	±1.18	±0.03	±0.07	±0.22	±1.27
pH 3.0	2.36	3.61	9.60	8.48	1.94	0.26	0.67	0.38	0.52	1.93
	±0.11	±0.19	±0.37	±2.83	±0.11	±0.12	±0.05	±0.03	±0.06	±0.72
pH 2.0	4.07	2.81	13.40	4.61	3.03	5.37	1.21	0.48	0.37	2.39
	±0.24	±0.35	±0.43	±0.39	±0.08	±0.59	±0.05	±0.04	±0.02	±0.71

FM

KP
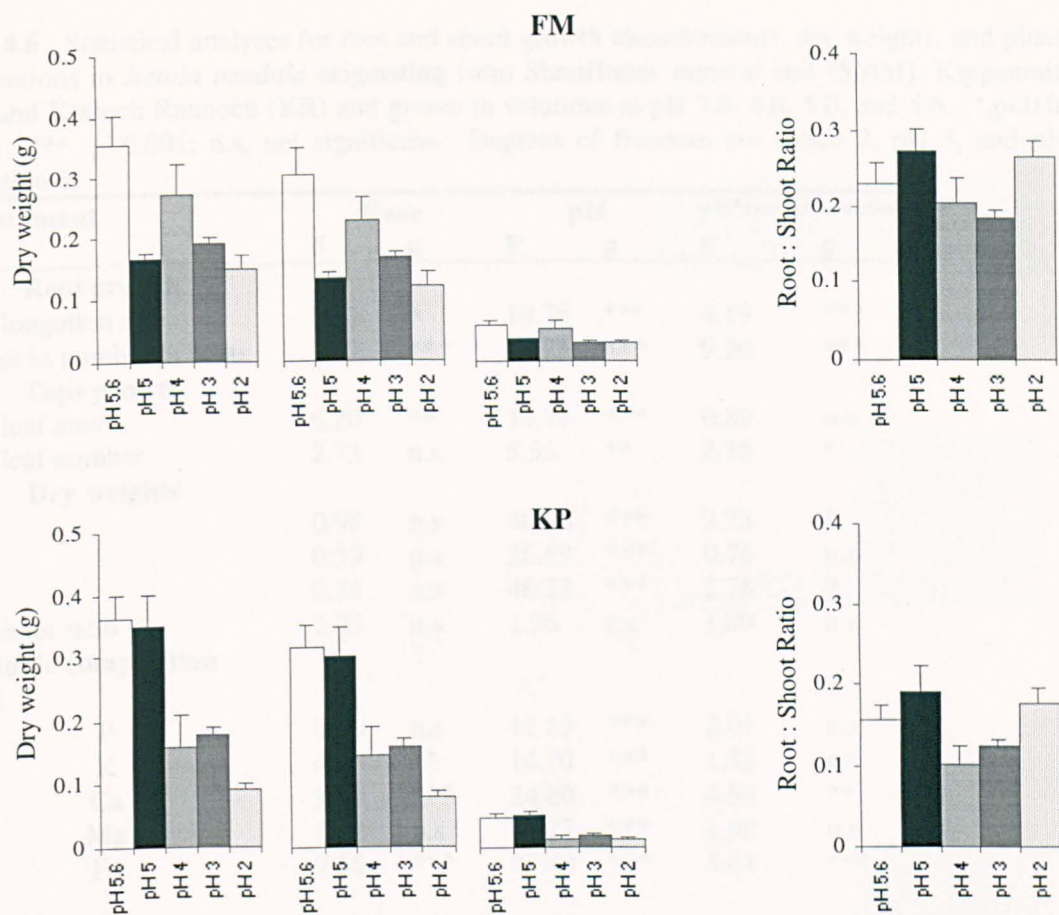
Figure 4.7. Mean total, shoot, and root dry weights (from left to right) (g, ± s.e), and root:shoot ratios (± s.e) of *Holcus lanatus*, originating from Flanders Moss (FM) and Kippenrait Glen (KP), when grown in nutrient solutions at pH 5.6 (□), pH 5.0 (■), pH 4.0 (▨), pH 3.0 (▨), and pH 2.0 (▨).

Table 4.6. Statistical analyses for root and shoot growth measurements, dry weights, and plant ionic compositions in *Betula pendula* originating from Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR) and grown in solutions at pH 3.0, 4.0, 5.0, and 5.6. *,$p<0.05$; **, $p<0.01$; ***, $p<0.001$; n.s, not significant. Degrees of freedom are : race 2, pH 3, and pH*race interaction 6.

Measurement	Race		pH		pH*race interaction	
	F	p	F	p	F	p
Root growth						
Root elongation rate	3.72	*	19.76	***	4.19	***
Increase in number of roots	8.13	***	34.78	***	9.20	***
Tops growth						
Total leaf area	6.20	**	14.78	***	0.89	n.s
Total leaf number	2.73	n.s	5.55	**	2.55	*
Dry weights						
Shoot	0.98	n.s	40.96	***	2.73	*
Root	0.59	n.s	26.59	***	0.76	n.s
Total	0.36	n.s	46.23	***	2.78	*
Root:shoot ratio	2.03	n.s	1.36	n.s	1.80	n.s
Ionic composition						
Shoots						
P	0.16	n.s	12.13	***	2.01	n.s
K	4.25	**	14.70	***	1.32	n.s
Ca	9.33	***	24.80	***	4.50	**
Mg	1.92	n.s	29.27	***	1.90	n.s
Fe	9.56	***	22.40	***	5.61	***
Roots						
P	6.10	**	12.89	***	16.34	***
K	6.72	**	3.11	*	5.78	***
Ca	10.81	***	4.85	**	6.11	**
Mg	3.73	*	5.66	**	1.46	n.s
Fe	20.30	***	15.82	***	10.04	***

4.4.2.3 Birch dry weights

Total, shoot, and root dry weights decreased with decreasing solution pH, pH having a significant effect on dry weights (Table 4.6). Unlike root elongation and leaf expansion there was a large difference in shoot dry weights between pH 5.0 and 5.6 in SMM seedlings (Figure 4.10).

4.4.2.4 Foliar ionic composition

The ionic composition of *Betula* leaves and roots is given in Table 4.7. pH had a significant effect on both root mineral uptake and translocation to shoots (Table 4.6). With the exception of shoot P and Mg, concentrations were significantly different between plants originating from the three sites (Table 4.6). The uptake of P, K, Mg, and Fe by roots tended to increase with increasing acidity. This was most pronounced in SMM seedlings, and least in KP seedlings. Root Ca concentrations increased with increasing acidity in SMM seedlings but decreased in KP and KR races. Subsequent translocation of P, K, Ca, Mg, and Fe to the shoots was significantly increased with a decrease in pH. This was most pronounced in KR races.
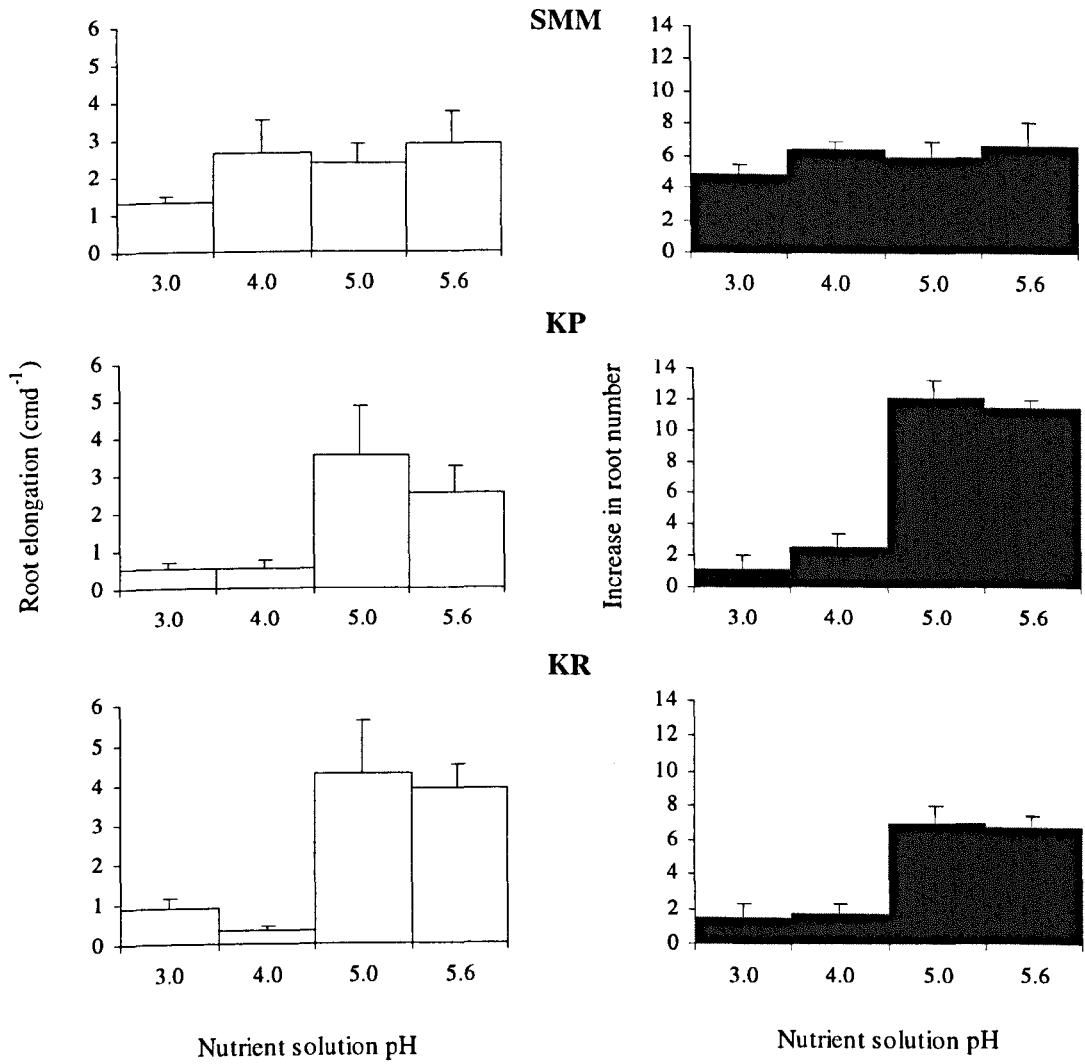
Figure 4.8. Effects of increasing solution pH on the mean rates of root elongation (cm day^{-1}, $\square$, ± s.e) and increase in root number ($\blacksquare$, ± s.e) of *Betula pendula* seedlings originating from Sheriffmuir (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR). Seedlings were grown at pH 3.0, 4.0, 5.0, and 5.6.
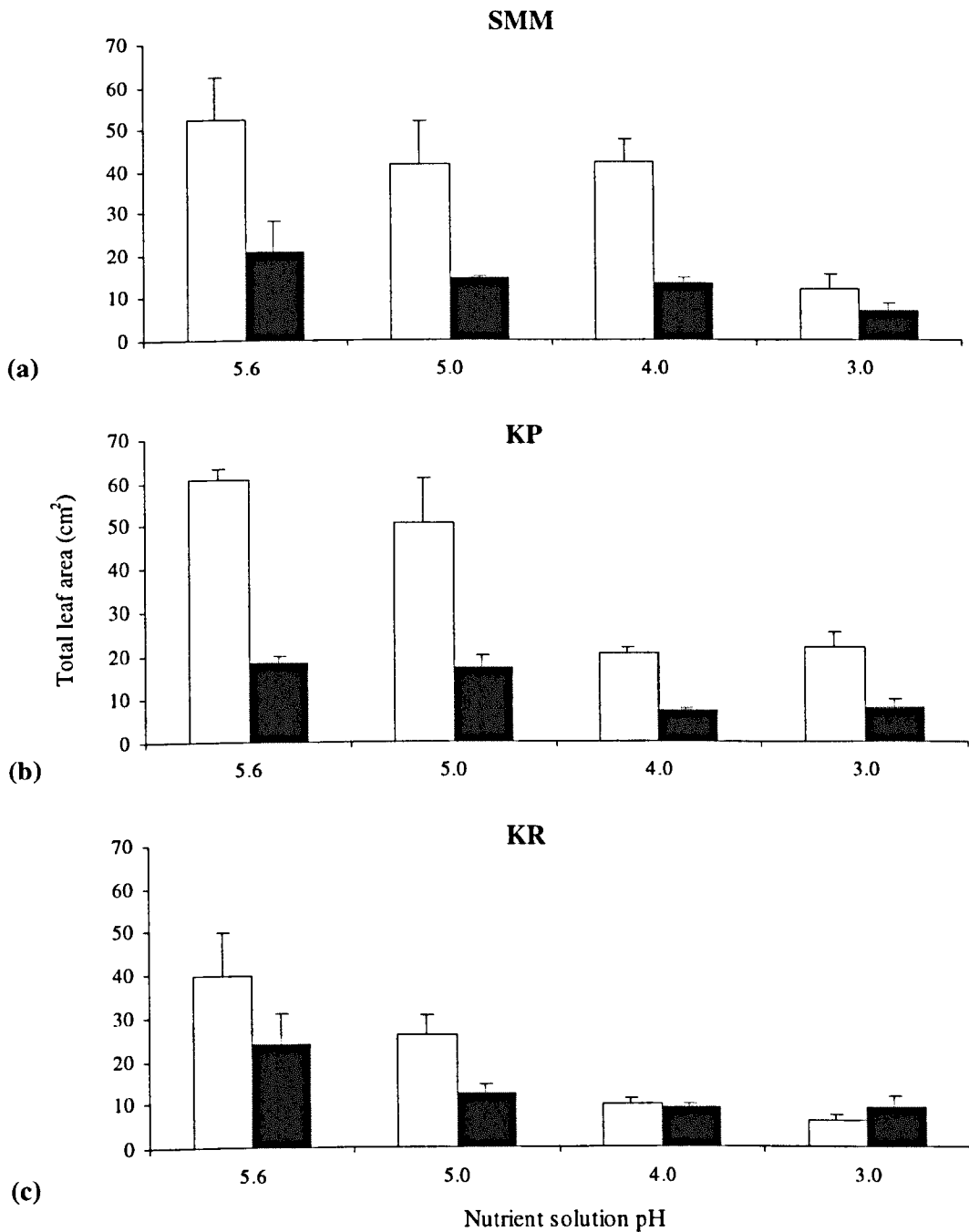
Figure 4.9. Mean total leaf area (± s.e) (□), and total leaf number (■, ± s.e) of *Betula pendula* seedlings after treatment at pH 3.0, 4.0, 5.0, and 5.6. Seedlings originated from (a) Sheriffmuir (SMM), (b) Kippenrait Glen (KP), and (c) Kinloch Rannoch (KR).

4.4.3 Soil pH increase with $Ca(OH)_2$

4.4.3.1 *Anthoxanthum odoratum*

Figure 4.11 illustrates the dry weights of *Anthoxanthum* after growth in FM, SMB, and KP soil with and without $Ca(OH)_2$. With the exception of KP soil, the addition of $Ca(OH)_2$ increased the dry weight yield of *Anthoxanthum* originating from FM and KP. There was no significant difference in
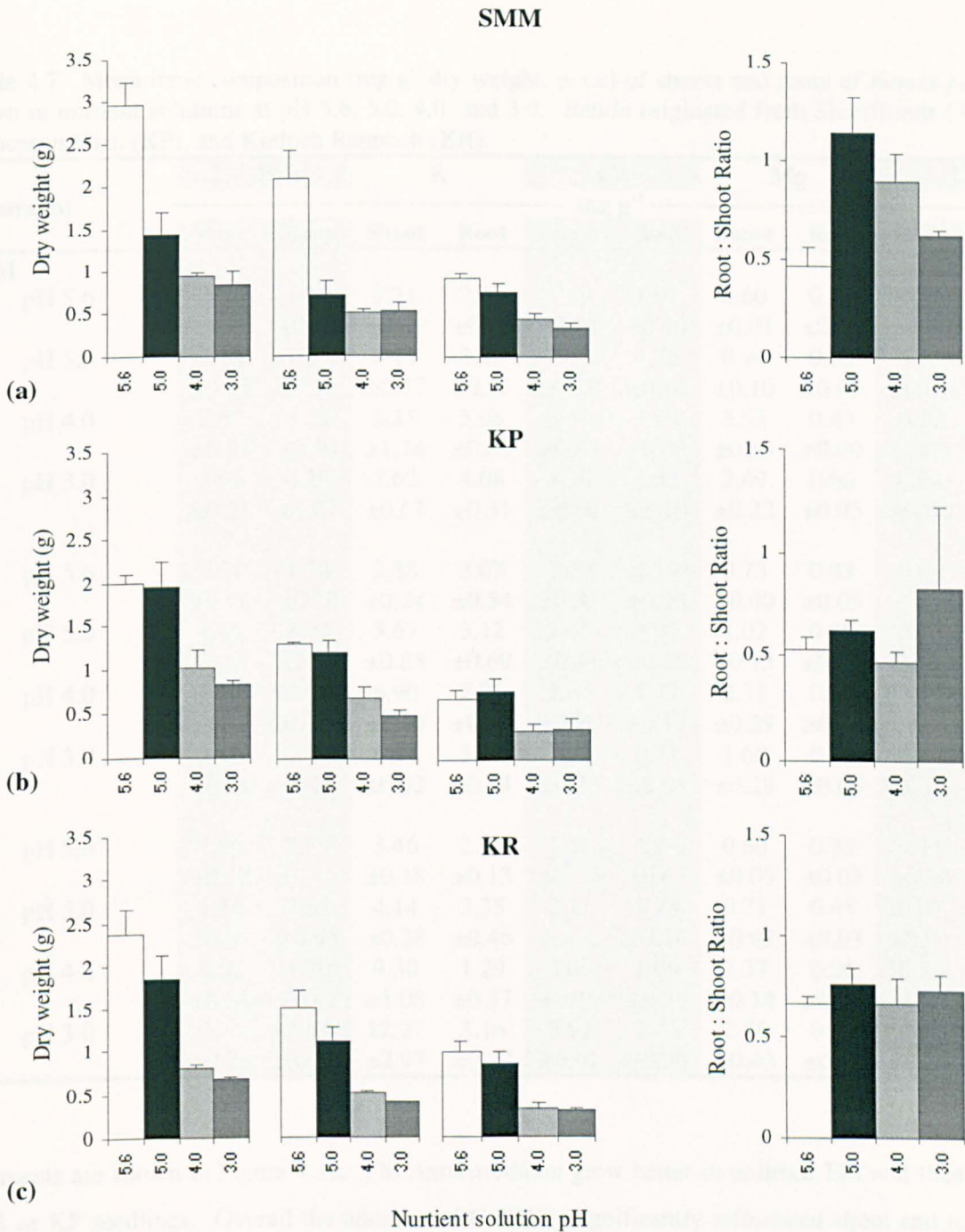
SMM

KP

KR

Nutrient solution pH

Figure 4.10. Mean total, shoot, and root (± s.e) (left to right) dry weights, and root:shoot ratios (± s.e) of *Betula pendula* originating from (a) Sheriffmuir (SMM), (b) Kippenrait Glen (KP), and (c) Kinloch Rannoch (KR) grown in nutrient solutions at pH 3.0 (■), 4.0 (■), 5.0 (■), and 5.6 (□).

the yields of FM seedlings in limed vs. unlimed KP soil. Similarly KP seedlings grew equally well in KP soil whether $Ca(OH)_2$ was added or not. Shoot dry yield was significantly greater in KP soil without $Ca(OH)_2$ added. There was a large and significant increase in dry weight yields of SMB seedlings grown in limed FM soil compared with unlimed. However there was no significant difference between yields of SMB *Anthoxanthum* from SMB soil with or without $Ca(OH)_2$. Yields were also greater in KP soil which had no added $Ca(OH)_2$. The differences in *Anthoxanthum* among

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Table 4.7. Mean ionic composition (mg g^{-1} dry weight, ± s.e) of shoots and roots of *Betula pendula* grown in nutrient solutions at pH 5.6, 5.0, 4.0, and 3.0. *Betula* originated from Sheriffmuir (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR).

Treatment	P Shoot	P Root	K Shoot	K Root	Ca Shoot	Ca Root	Mg Shoot	Mg Root	Fe Shoot	Fe Root
SMM										
pH 5.6	0.76 ±0.11	1.13 ±0.10	3.21 ±0.41	2.27 ±0.17	1.89 ±0.34	1.97 ±0.40	0.60 ±0.01	0.32 ±0.01	0.05 ±0.00	0.28 ±0.02
pH 5.0	2.10 ±0.25	1.35 ±0.31	4.11 ±0.77	3.36 ±0.57	2.42 ±0.29	4.36 ±0.14	0.94 ±0.10	0.50 ±0.05	0.12 ±0.01	0.24 ±0.03
pH 4.0	2.59 ±0.51	7.15 ±0.14	6.15 ±1.74	5.08 ±0.07	3.50 ±0.83	3.08 ±0.05	3.53 ±0.93	0.49 ±0.00	0.22 ±0.03	1.20 ±0.08
pH 3.0	3.06 ±0.24	4.29 ±1.07	7.62 ±0.67	4.08 ±0.31	4.99 ±0.60	5.83 ±1.70	2.49 ±0.22	0.66 ±0.05	0.24 ±0.04	0.92 ±0.31
KP										
pH 5.6	0.51 ±0.02	1.78 ±0.29	2.88 ±0.24	3.03 ±0.54	1.20 ±0.33	2.17 ±0.23	0.73 ±0.00	0.43 ±0.05	0.08 ±0.01	0.21 ±0.04
pH 5.0	1.65 ±0.67	3.04 ±0.62	3.67 ±0.85	3.12 ±0.69	2.46 ±0.41	3.05 ±0.66	1.02 ±0.13	0.48 ±0.09	0.12 ±0.03	0.19 ±0.03
pH 4.0	3.84 ±1.43	2.93 ±0.41	6.90 ±1.40	2.86 ±0.51	2.64 ±0.26	1.47 ±0.12	2.31 ±0.29	0.39 ±0.09	0.17 ±0.01	0.15 ±0.01
pH 3.0	2.43 ±0.31	2.09 ±0.23	6.51 ±0.92	3.82 ±0.24	2.26 ±0.28	0.71 ±0.08	1.68 ±0.29	0.48 ±0.05	0.12 ±0.02	0.19 ±0.04
KR										
pH 5.6	1.86 ±0.12	2.89 ±0.15	3.46 ±0.18	2.76 ±0.13	1.58 ±0.30	4.79 ±0.67	0.66 ±0.03	0.33 ±0.03	0.11 ±0.00	0.20 ±0.01
pH 5.0	1.56 ±0.09	2.37 ±0.36	4.14 ±0.28	3.35 ±0.46	2.11 ±0.22	3.78 ±0.24	0.71 ±0.03	0.45 ±0.03	0.07 ±0.01	0.15 ±0.02
pH 4.0	4.02 ±0.31	1.70 ±0.42	9.30 ±1.05	1.29 ±0.37	3.66 ±0.10	1.09 ±0.25	2.37 ±0.14	0.26 ±0.11	0.28 ±0.05	0.17 ±0.01
pH 3.0	1.74 ±0.26	3.49 ±0.30	12.27 ±2.97	3.16 ±0.27	5.97 ±0.61	2.75 ±0.50	2.76 ±0.43	0.47 ±0.06	0.42 ±0.08	1.01 ±0.17

treatments are shown in Figure 4.12. FM *Anthoxanthum* grew better in unlimed FM soil than either SMB or KP seedlings. Overall the addition of $Ca(OH)_2$ significantly influenced shoot and root dry weight yields. Total and shoot dry weights were also significantly different between soils, as was the interaction factor, soil*Ca*race (Table 4.8).

4.4.3.2 *Holcus lanatus*

Mean total, shoot, and root dry weight yields of *Holcus* originating from FM, SMB, SMM, KP, and KR after growth in all five soils with or without $Ca(OH)_2$ are graphed in Figure 4.13. Similar to *Anthoxanthum* both FM and SMB seedlings had significantly greater yields when the organic soils, FM and SMB, were limed (Table 4.8). Both FM and SMB seedlings also grew better in KP soil without any $Ca(OH)_2$ addition, and growth in SMM soil was either greater (SMB) or unaffected by $Ca(OH)_2$ (FM). Growth of *Holcus* originating from any of the five sites was very low in KR soil. Maximum growth in this soil was by KR races, and FM races did not grow at all in this soil. Addition of lime increased total yields of SMM *Holcus* in FM, SMB, and root yields in KP soil. Yields

however were greater in SMM soil without any addition of $Ca(OH)_2$. Both the root and shoot dry weight yields of KP *Holcus* improved when grown in the organic soils with added $Ca(OH)_2$. Yields of these same seedlings did not improve in either SMM or KP soil where $Ca(OH)_2$ had been added. Finally, yields of KR seedlings were improved with the addition of $Ca(OH)_2$ to FM, SMB, and SMM soil, but not to KP soil. The differences between liming treatments are shown in Figure 4.14. The dry weight yields of *Holcus* were significantly different among the races and soil origins (Table 4.8). The soil*Ca*race interaction factor was also significant (Table 4.8).

Table 4.8. Statistical analyses for total, root and shoot dry weight yields of *Holcus lanatus* and *Anthoxanthum odoratum* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR) and grown in limed and unlimed soils. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; n.s, not significant. Degrees of freedom for *Holcus* and *Anthoxanthum* (in parentheses) are : race 4(2), soil type 3(2), $Ca(OH)_2$ 1(1), and race*Ca*soil interaction 12 (4).

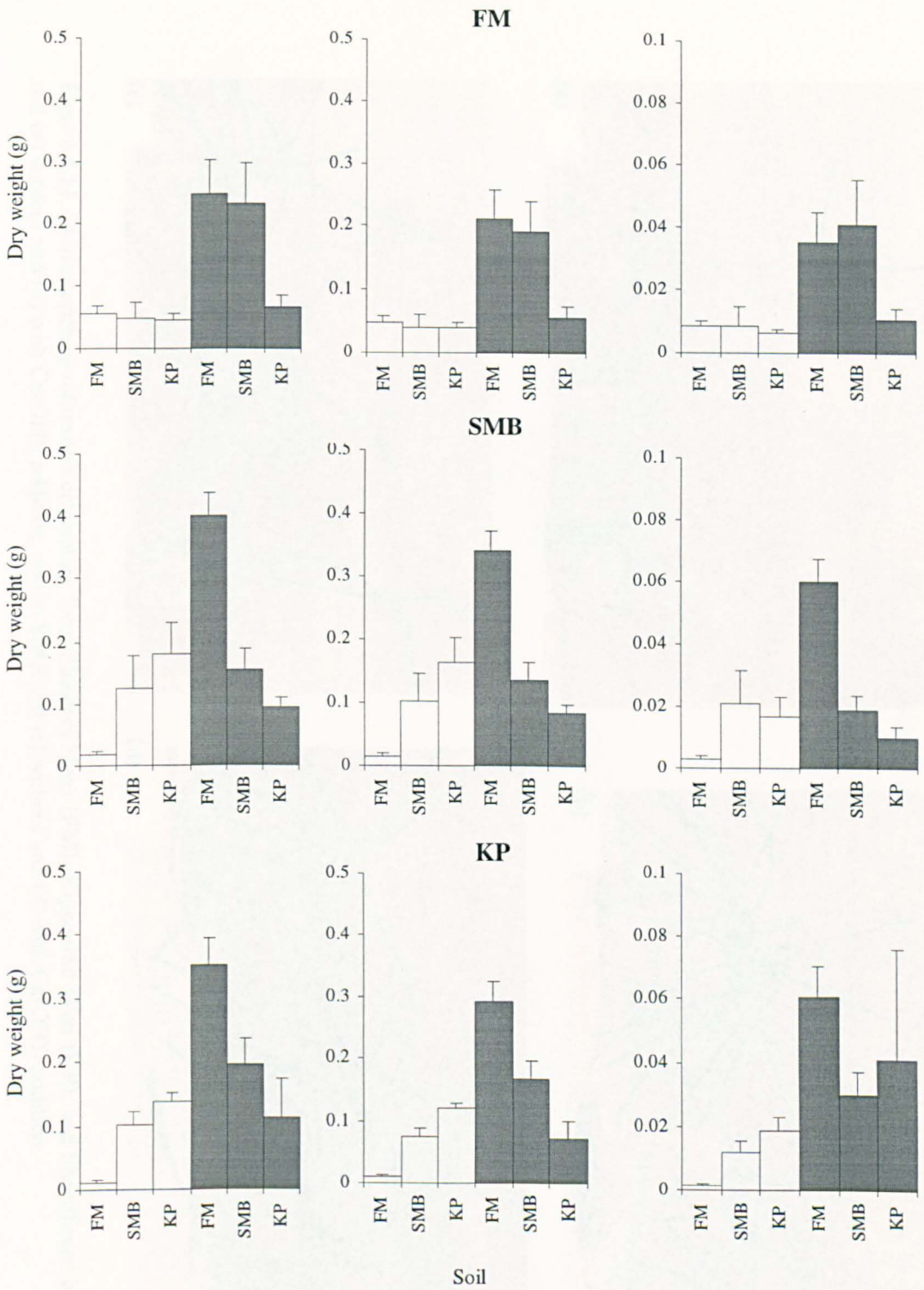| Measurement		Race		Soil		Ca		Race*Ca*Soil interaction factor	
		F	p	F	p	F	p	F	p
					Holcus lanatus				
	Dry weights								
Shoot		4.84	***	14.98	***	112.78	***	1.38	n.s
Root		8.34	***	8.90	***	20.20	***	2.94	**
Total		8.52	***	14.91	***	69.82	***	2.16	**
					Anthoxanthum odoratum				
	Dry weights								
Shoot		2.78	n.s	4.48	**	43.90	***	3.03	**
Root		1.06	n.s	1.62	n.s	20.56	***	1.39	n.s
Total		2.37	n.s	3.80	**	40.50	***	2.84	**

Figure 4.11. Mean total, shoot, and root dry weights (left to right) (± s.e) of *Anthoxanthum odoratum* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), and Kippenrait Glen (KP) after growth in FM, SMB, or KP soil with (■) or without (□) Ca(OH)$_2$ addition.
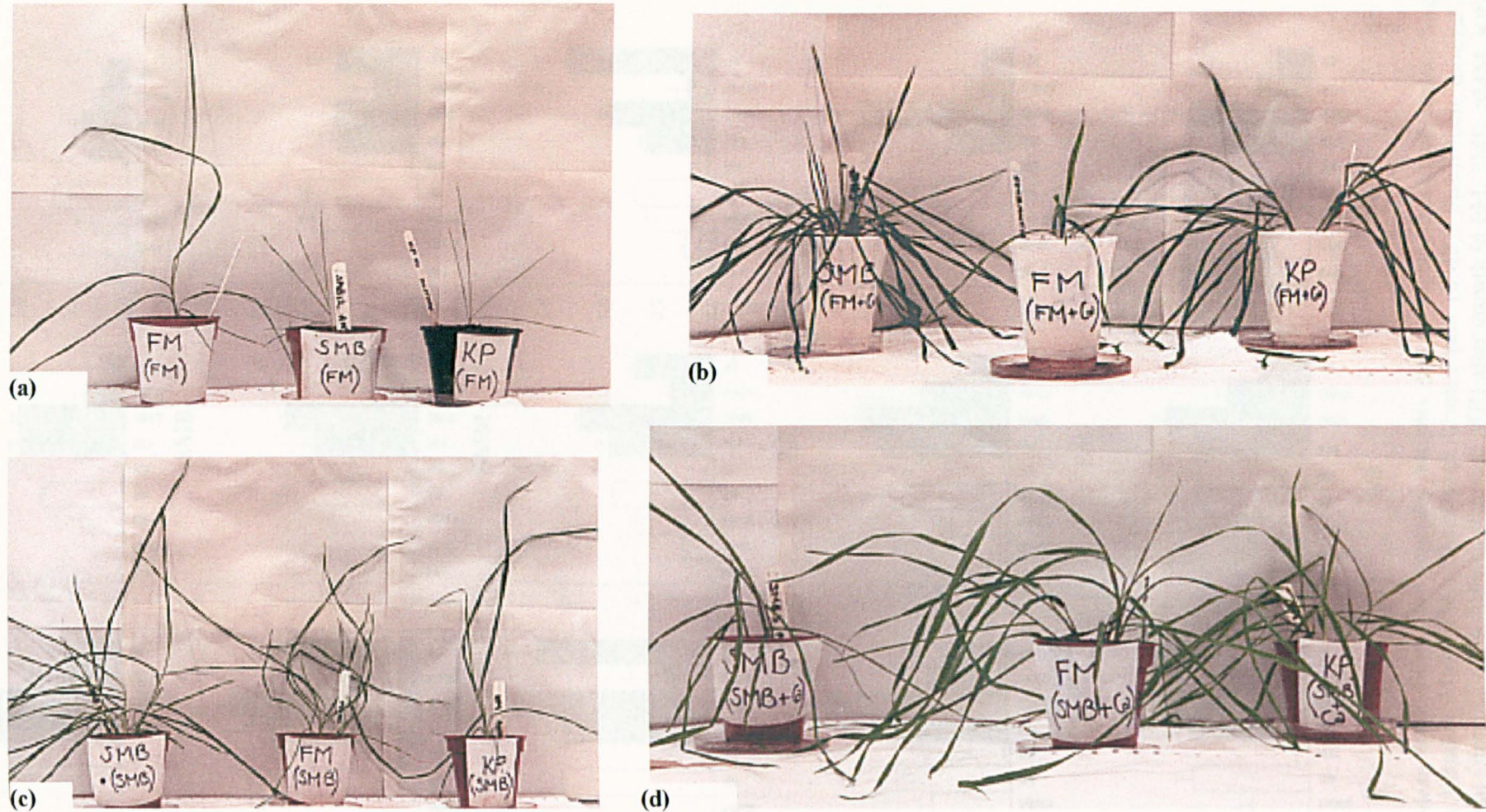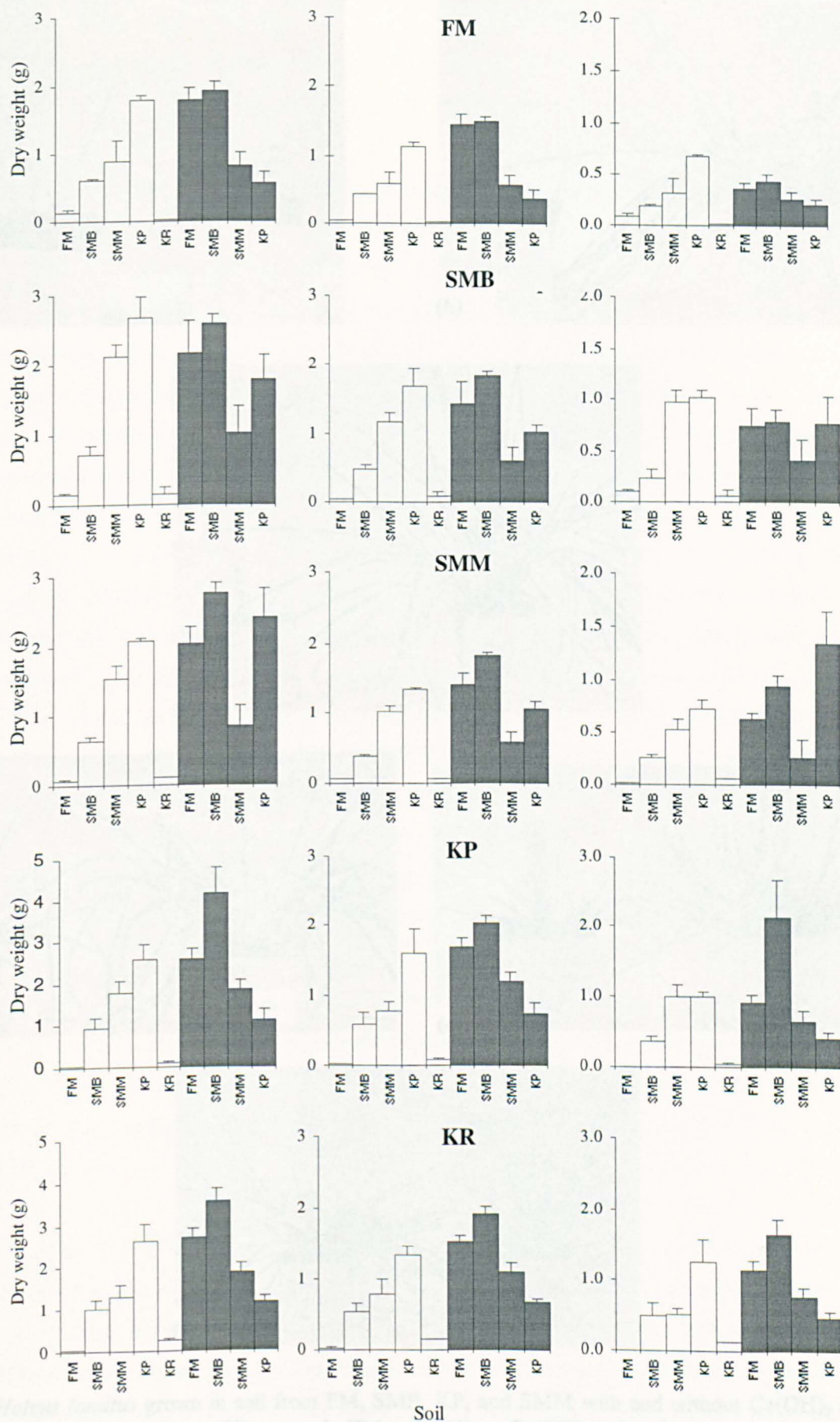
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Figure 4.12. *Anthoxanthum odoratum* originating from Flanders Moss (FM), Kippenrait Glen (KP), and Sheriffmuir blanket peat (SMB) grown in FM soil (a) without and (b) with Ca(OH)$_2$ addition, and in SMB soil (c) without and (d) with Ca(OH)$_2$ addition.

Figure 4.13. Mean total, shoot, and root dry weights (left to right) (± s.e) of *Holcus lanatus* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR) after growth in FM, SMB, SMM, KP soil with (■) or without (□) Ca(OH)$_2$ addition. The plants were also grown in the KR soil which was originally lime-rich and hence did not have a Ca(OH)$_2$ addition treatment.
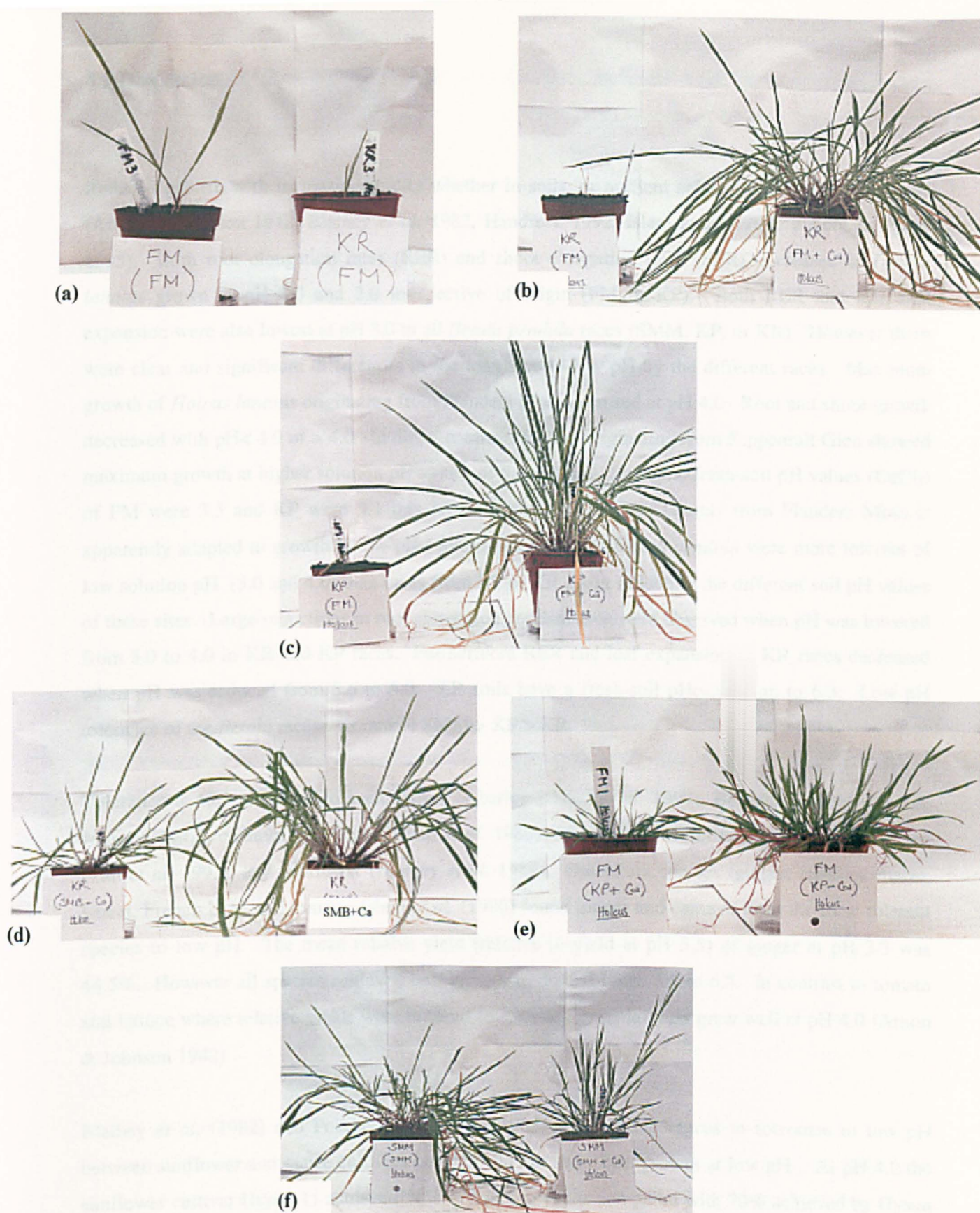
Figure 4.14. *Holcus lanatus* grown in soil from FM, SMB, KP, and SMM with and without Ca(OH)₂ addition. (a), *Holcus* originating from FM and KR grown in FM soil without Ca(OH)₂ ; (b), *Holcus* originating from KR grown in FM soil without and with Ca(OH)₂ (left to right); (c), *Holcus* originating from KP grown in FM soil without and with Ca(OH)₂ (left to right); (d), *Holcus* originating from KR grown in SMB soil without and with Ca(OH)₂ (left to right); (e), *Holcus* originating from FM grown in KP soil with and without Ca(OH)₂ (left to right); and (f), *Holcus* originating from SMM grown in SMM soil without and with Ca(OH)₂ (left to right).

4.5 Discussion

Reduced growth with increasing acidity whether in soils, or nutrient solutions, is well documented (Arnon & Johnson 1942, Blamey *et al.* 1982, Handreck 1992, Islam *et al.* 1980, Yokota & Ojima 1995). Both root elongation rates (RER) and shoot elongation rates (SER) decreased in *Holcus lanatus* grown at pH 3.0 and 2.0 irrespective of origin (FM or KP). Both RER and leaf area expansion were also lowest at pH 3.0 in all *Betula pendula* races (SMM, KP, or KR). However there were clear and significant differences in the tolerance to low pH by the different races. Maximum growth of *Holcus lanatus* originating from Flanders Moss occurred at pH 4.0. Root and shoot growth decreased with pH< 4.0 or > 4.0. In direct contrast, *Holcus* originating from Kippenrait Glen showed maximum growth at higher solution pH values of 5.0 and 5.6. Since the fresh-soil pH values ($CaCl_2$) of FM were 3.5 and KP were 5.1 this is not surprising. *Holcus lanatus* from Flanders Moss is apparently adapted to growth at low pH. Sheriffmuir races of *Betula pendula* were more tolerant of low solution pH (3.0 and 4.0) than races from KP or KR again reflecting the different soil pH values of these sites. Large reductions in root elongation and leaf area were observed when pH was lowered from 5.0 to 4.0 in KR and KP races. Furthermore RER and leaf expansion in KR races decreased when pH was reduced from 5.6 to 5.0. KR soils have a fresh-soil pH_{CaCl_2} of up to 6.3. Low pH tolerance of the *Betula* races was ranked SMM > KP > KR.

Tolerance to low pH has also been shown in barley (Osaki *et al.* 1997), Bermuda-grass (Arnon & Johnson 1942), cassava and ginger (Islam *et al.* 1980), maize (Poschenrieder *et al.* 1995), sorghum (Tan *et al.* 1993), and sunflower (Blamey *et al.* 1982). Out of six species (ginger, cassava, maize, wheat, French bean and tomato) Islam *et al.* (1980) found ginger and cassava were the most tolerant species to low pH. The mean relative yield (relative to yield at pH 5.5) of ginger at pH 3.3 was 44.5%. However all species achieved best growth in the pH range 5.5 to 6.5. In contrast to tomato and lettuce where relative yields were reduced to < 35%, Bermuda-grass grew well at pH 4.0 (Arnon & Johnson 1942).

Blamey *et al.* (1982) and Poschenrieder *et al.* (1995) showed differences in tolerance to low pH between sunflower and maize cultivars but they did not show best growth at low pH . At pH 4.0 the sunflower cultivar Hysun 11 achieved 90% of best top yields compared with 70% achieved by Hysun 32. Hysun 11 also showed the least increase in root yields when increasing solution pH from 4.0 to 6.5. The maize cultivar BR201F was tolerant of H^+ ions whereas the cultivars Ardour 250 and C525M were H^+-sensitive. Root and coleoptile elongation were greater in BR201F at low pH. Demonstrations of tolerance differences among races of naturally occurring species are rare. Handreck (1992) did show that the ferns *Asplenium trichomanes*, *Ceterach officinarum*, and *Phyllitis*

scolopendrium, which are reputed to require alkaline-growing media, were intolerant of pH values <5.0.

Tolerance differences between the *Betula* races were not seen in the root and top dry weights, which increased with increasing pH in all races at similar rates. However, dry weights were significantly greater at pH 4.0 in FM *Holcus* than at pH 5.0 or 3.0 or 2.0. Dry weights were highest at pH 5.6 but were not significantly different from those at pH 4.0. In contrast, the highest dry weights in KP races were at pH 5.6 and 5.0, and these were significantly different from, and about double those at pH < 5.0.

Root:shoot dry weight ratios in *Holcus lanatus* were lower at pH 3.0 and 4.0, increasing both as pH increased from 4.0 to 5.6, and as it decreased from 3.0 to 2.0. Raising the pH from 4.0 to 4.8 increased the root weight ratios of all six species studied by Islam *et al.* (1980), indicating a greater response in root growth than shoot growth to increasing pH.

A solution pH of 3.5 was lethal to 11 cultivars of subterranean clover (Kim *et al.* 1985), and to all four sunflower cultivars studied by Blamey *et al.* (1982). Both pH 2.0 and 3.0 were lethal to *Holcus* races from KP but only pH 2.0 was lethal to FM races over the 15 days. At the end of the treatment FM *Holcus* grown at pH 3.0 were still alive and appeared healthy although growth rates were low. The drop in root and shoot elongation rates was less than in KP seedlings. However the severe plasmolysis and disintegration of fine cell structure at pH 3.0 indicated that the FM races were dying. Yokota & Ojima (1995) also found root elongation was irreversibly curtailed by 20 h treatment at pH 4.0 in alfalfa.

Signs of H^+ injury in roots are well documented. Islam *et al.* (1980) investigated six species and found the roots of all grown at pH 3.3 were injured. Roots were thickened, discoloured brown or dull grey, and lateral root growth was inhibited. Root tips of French bean (cv. Redlands Pioneer) died in the solutions at pH 4.0. There were very distinct differences in the symptoms of H^+ injury between FM and KP races of *Holcus lanatus*. At pH 3.0 root tips of KP seedlings were black in colour and dead, suggesting no further root cell division and extension was possible. The roots were brownish in colour, the root tips severely swollen, and new roots were stunted and discoloured. In contrast, root tips of FM were only slightly discoloured or swollen. However lateral root growth in both was absent. Laterals only appeared at pH 5.0 and 5.6.

Many studies have reported reductions in ion transport at low pH, and after prolonged periods loss of previously absorbed solutes. Increasing solution pH increased concentrations of N, K, and Fe, and had no effect on Mg in sunflower tissues (Blamey *et al.* 1982). Guerrier (1982) found increasing

acidity decreased K absorption in sorghum. All mineral elements, except N, were lower in barley at low pH (Osaki *et al.* 1997). An increase in pH from 3.3 to 5.5 strongly increased the concentrations of P, K, Ca, and Mg in the tops of species studied by Islam *et al.* (1980). The close correlation between the H-induced inhibition of root elongation and H-induced reduction in specific absorption rates (SAR) of P, Ca, Mg, B, and Fe in maize, led Poschenrieder *et al.* (1995) to conclude alterations in nutrient uptake play an important role in H^+ ion toxicity. Moreover Schubert *et al.* (1990) proposed nutrient uptake in Broad Beans was altered by low pH as a result of a reduction in the plants ability to release H^+ ions by ATPase activity.

The nutrient analyses of *Holcus* corroborate the studies above. Root K concentrations, and both shoot and root Mg, decreased with increasing acidity. The root K concentrations only decreased significantly between pH 3.0 and pH 2.0 in the more tolerant FM seedlings. However, contrary to the studies above, both root Ca concentrations, shoot K concentrations, and shoot and root Fe concentrations, actually increased with increasing acidity. Shoot Ca concentrations were unaffected by pH. Islam *et al.* (1980) also found highest Fe concentrations at the lower pH (pH 4.0) and concentrations dropped with decreasing acidity. Kim *et al.* (1985) showed a depression in total P concentration of clover when the solution pH was increased from 4.0 to 4.5 or 5.0.

The mineral concentrations of *Betula* leaves and roots suggest H^+-induced reduction in growth was not a direct result of H^+-inhibition of nutrient uptake. All minerals translocated to leaves and absorbed by roots increased with an increase in H^+ concentration. That is with the exception of root Ca where concentrations decreased with pH in KP and KR seedlings. This suggests that there may be an ion-specific effect by H^+ at the root cell surface. The tolerant seedlings from SMM were able to continue absorbing Ca at the roots. The H^+-tolerant maize cultivar, BR201F, had higher root Ca concentrations at low pH values compared with the H^+-sensitive cultivar, HS7777 (Llugany *et al.* 1995). Llugany *et al.* (1995) proposed this ability to maintain higher Ca levels at low pH contributed to H^+ ion tolerance in this cultivar.

H^+ may specifically displace Ca^{2+} from apoplastic binding sites and this may be of primary importance in growth inhibition. Addition of Ca to low pH solutions is known to alleviate H^+ ion toxicity. Handreck (1992) found ferns requiring a 'basic' medium for growth were able to tolerate pH <5.0 provided an adequate supply of Ca was available. Yan *et al.* (1992) found additional Ca^{2+} enabled the roots of Broad Bean to continue extruding H^+ ions. Displacement of Ca^{2+}, from the external surface of the plasma membrane, and inhibition of Ca-uptake by roots, are likely the initial effect of H^+ ions progressing to a general inhibition of nutrient uptake. *Holcus* plants were beginning to show signs of a general reduction in nutrient uptake. They also appeared more damaged by high H^+ concentrations

than did *Betula*. *Betula*, treated for 4 weeks, were more tolerant of low pH than *Holcus*, treated for only 10 days.

Ciamporová *et al.* (1995) compared the structure of nodal roots of two wild acid-tolerant grasses: *Deschampsia flexuosa* and *Nardus stricta*. The grasses were grown in polluted acid soil (pH 3.4) and unpolluted soil (pH 5.2) in Central Slovakia. Heavy metals were not at toxic concentrations however the grasses were exposed to additional stress from toxic concentrations of Al and SO_4. The stress conditions resulted in a significant reduction of the whole-root area and central cylinder (stele). Whole-root area also decreased with increasing acidity in KP and also in FM with the exception of pH 3.0. The stress conditions which induced a reduction in stelar tissue in the two wild grasses were not observed in *Holcus*. Stele area was increased in FM at pH 3.0 and unaffected in KP seedlings. However *Deschampsia* was found to be more tolerant of soil acidity than *Nardus* and Ciamporová *et al.* (1995) attributed this to the preservation of the cortex by *Deschampsia*. The cortex is vital for absorption, transport and accumulation of water and solutes. The proportion of tissue occupied by cortex was increased with increasing acidity in FM races but fell by about 10% in KP races. This may reflect a structural adaptation by FM races.

Growth response to the addition of $Ca(OH)_2$ were obvious in both *Holcus lanatus* and *Anthoxanthum odoratum*. However best growth by acid-tolerant races such as FM and SMB did not occur as expected in unlimed soils. Yields of both SMB and FM *Holcus*, and FM *Anthoxanthum* increased with $Ca(OH)_2$ additions. However the growth of SMB *Anthoxanthum* in the organic SMB soil was unaffected by lime addition. It should also be noted that races of *Holcus* from FM were unable to grow in KR soil which has a pH_{CaCl_2} of up to 6.3. Both FM and SMB races of both species grew better in unlimed KP and SMM soil where pH values were < 5.0. *Holcus* races from KP required $Ca(OH)_2$ additions to improve yields in the acidic organic soils, but not in the acidic mineral soil from SMM. $Ca(OH)_2$ addition to SMM soil was necessary to improve KR yields. Races of both SMM and KP grew significantly better in their native soils without $Ca(OH)_2$ addition.

The lack of consistency between pH tolerance of races grown in nutrient solutions and those grown in pot experiments emphasises the difficulties in interpreting individual effects of low pH on plant growth. In the soil experiments factors other than high H^+ concentrations are simultaneously affecting plant growth and may influence growth to a greater extent than H^+ alone.

4.6 Conclusions

- Races of *Holcus lanatus* from Flanders Moss were tolerant to low pH (pH <4.0).

- Races of *Holcus lanatus* from Flanders Moss showed preferential root and shoot growth at pH 4.0.

- Tolerance in *Holcus lanatus* was partly achieved by maintaining root cortex area.

- Races of *Betula pendula* from Sheriffmuir were more tolerant to low pH (pH 3.0 and 4.0) than those from Kippenrait Glen and Kinloch Rannoch.

- Races of *Betula pendula* from Kinloch Rannoch were least tolerant of pH 3.0-5.6.

- Uptake of P, K, Mg, and Fe and translocation to shoots were unaffected by H^+ in *Betula pendula*.

- Uptake of Ca, by H^+-sensitive races, was significantly reduced by high H^+ concentrations.

- Tolerance of low pH in both *Holcus* and *Betula* among races reflected the soil pH of their provenance.

- Addition of $Ca(OH)_2$ sometimes improved dry weight yields in *Holcus lanatus* and *Anthoxanthum odoratum*.

- Apart from FM and SMB, dry weights of races were greater in the soils of their natural origin with no $Ca(OH)_2$ added.

Chapter 5

Response of *Betula pendula* Roth. to increasing concentrations of aluminium

5.1 Introduction

Aluminium toxicity is widely considered to be the most important growth-limiting factor for plants in most strongly acid soils (pH < 5.0), and primarily associated with the poor plant growth (especially root development) in these soils (Adams 1984, Blamey *et al.* 1986, Foy 1984, Horst 1995, Horst *et al.* 1983, Kamprath 1984, Pegtel 1987, Ryan *et al.* 1992, Ryan & Kochian 1993, Sasaki *et al.* 1995, Staß & Horst 1995, Taylor 1988). Aluminium is believed to be toxic to plant growth at micromolar concentrations (Ryan *et al.* 1994), and primarily as monomeric Al^{3+} (Andersson 1988, Alva *et al.* 1986, Barceló *et al.* 1996, Kinraide 1993). Early ecological work, using naturally occurring plant species, investigating Al toxicity was discussed in Chapter 1.

5.1.1 Al toxicity

The reduction in crop productivity in acid soils has provoked great interest in determining the physiological and biochemical mechanisms through which Al is toxic (Carr & Ritchie 1993, Tan *et al.* 1989). Consequently, extensive investigation into the effects of Al has primarily been carried out on crop species. Genetic variation between cultivars in Al tolerance has been found in most cultivated species. Ultimately the aim of this research is to breed Al-tolerant crop genotypes and increase yield in arable soils (Aniol 1996, Baligar *et al.* 1990, Barceló *et al.* 1996). Crop species which have been widely used include: barley (Sasaki *et al.* 1995), bean (Malavolta *et al.* 1981, Massot *et al.* 1992), cowpea (Horst *et al.* 1983), maize (Barceló *et al.* 1996), ryegrass (Nelson & Kiesling 1980, Rengel & Robinson 1990), sorghum (Blamey *et al.* 1986, Guerrier 1982, Malavolta *et al.* 1981, Tan *et al.* 1993), soybean (Kamprath 1984, Horst *et al.* 1992, Staß & Horst 1995, Spehar 1994) and finally, perhaps most frequently, wheat (Baligar *et al.* 1990, Huang *et al.* 1993, Kinraide 1988, Ryan & Kochian 1993, Ryan *et al.* 1994, Wheeler 1994, Wheeler & Edmeades 1995, Wright *et al.* 1989).

More recently however, the effects of acid rain and pollutants, suggested to account for the decreased vitality of forest ecosystems in Europe and North America, have led to the investigation of Al toxicity in naturally occurring species. Brunet (1994) investigated the interacting effects of pH, Al, and base

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cations on the growth of the woodland grasses *Bromus benekenii* and *Hordelymus europaeus*. Both Godbold & Kettner (1991) and Godbold *et al.* (1995) studied the effects of Al on *Picea abies*, and finally, De Graaf *et al.* (1997) attributed the decline in *Arnica montana*, and *Cirsium dissectum* to increased soil acidification and Al solubility.

5.1.2 Symptoms of Al toxicity

Foy (1984) described the foliar symptoms of Al toxicity to either resemble those of P deficiency (overall stunting, small, dark green leaves and late maturity, purpling of stems, leaves, and leaf veins, and yellowing and death of leaf tips), or those of Ca deficiency (curling of young leaves, and collapse of growing parts or petioles), or Fe deficiency (interveinal chlorosis). He described the roots as being stubby, brown and brittle with thickened root tips and laterals. There was no fine branching of the root system, and root hair length and number were reduced in *Trifolium repens* (Care 1995). The appearance of Al toxicity symptoms in plants does not correlate well with a 'threshold' concentration of Al in solution or soils.

Thickened, stunted roots, and chlorotic leaves, have frequently been shown. Examples include *Arnica* (40 and 80 mg Al l^{-1}, Pegtel 1987), sorghum and sunflower (0.17 and 0.38 mg Al l^{-1}, Blamey *et al.* 1986), and wheat (0.14 mg Al l^{-1}, Kinraide 1988). The yellowing of leaves with necrotic tips has been reported in *Arnica* (Pegtel 1987, De Graaf *et al.* 1997).

5.1.3 Effects of Al on root elongation

The mechanism(s) resulting in the inhibition of plant growth remain unclear and there are many proposed physiological and biochemical effects of Al. This chapter primarily investigated the effects of Al on root elongation and possible interference with nutrient uptake in *Betula pendula*.

The most easily recognised symptom, and rapid response of plants to Al toxicity, is the inhibition of root elongation (Barceló *et al.* 1996, Ryan *et al.* 1994, Staß & Horst 1995, Taylor 1988). Al inhibits root elongation within hours (Horst 1995), while reductions in shoot growth occur later (Barceló *et al.* 1996). From this it followed that the retardation of root growth was the principal and primary site of Al toxicity (Foy 1984). The root apex in particular was believed to be the critical site for Al injury, and also the site of preferential Al accumulation (Horst 1995, Ryan *et al.* 1993).

Numerous reports have shown Al-induced inhibition of root growth, using a wide range of Al concentrations. After 2 h of Al treatment (0.68 mg Al l^{-1}) in soybean, root elongation rate was

reduced to 50 % of controls (Horst *et al.* 1992). Exposure of *Picea abies* seedlings to 1.35, 5.40, or 10.79 mg Al l^{-1} inhibited root growth within one day (Godbold & Kettner 1991).

The inhibition of root elongation is thought to be the result of Al-induced inhibition of cell division or cell elongation in root tip meristems. Early studies by Clarkson (1965) suggested Al blocked the cell cycle during DNA synthesis thereby reducing mitotic activity in root apical meristems of onion. In agreement, later publications (Matsumoto *et al.* 1976b) showed Al-accumulation in nuclei as well as the inhibition of DNA synthesis by Al. Contrary to these early reports, recent studies have shown that Al-treated roots can recover and resume apical growth, suggesting that the effect of Al on the root meristem is not permanent. Horst *et al.* (1983) demonstrated the inhibition of cell division after short-term (6 h) Al treatment in cowpea was partially restored within 18 h. Furthermore the radial mobility of Al in roots is low, with little Al entering the symplast from the apoplast, and this does not corroborate with the observed time-scales of root growth inhibition (Corrales *et al.* 1997, Delhaize *et al.* 1993a, Godbold *et al.* 1988, Hodson & Wilkins 1991, Marienfeld & Stelzer 1993). Meristem cell nuclei appear structurally stable, and changes in meristematic cell ultrastructure occur very slowly after Al exposure (Bennet & Breen 1991).

Recent hypotheses of Al phytotoxicity are based on the rapid binding of Al to sensitive sites in the apoplast (Barceló *et al.* 1996, Blamey *et al.* 1990, Blamey *et al.* 1993a, Horst 1995). Horst (1995) suggested that the competition for these binding sites determined the Al-induced inhibition of root elongation. Binding sites include the pectic matrix of the cell wall; cell-wall constituents such as enzymes, extensin, and xyloglucan; and phospholipids and carboxyl chains of proteins on the plasma membrane surface. Al may cross-link the carboxyl groups of the pectin fraction of cell walls causing a reduction in cell wall extensibility and elasticity (Barceló *et al.* 1996, Barceló & Poschenrieder 1990). Blamey *et al.* (1993b) showed Al bound to an artificial Ca pectate membrane and reduced water permeability. Gunsé *et al.* (1997) found Al (1.35 mg l^{-1}) decreased maize root cell wall elasticity and root hydraulic conductivity.

5.1.4 Interference with nutrient uptake by Al

A frequent consequence of Al injury is the reduction in the uptake of essential nutrients (Foy 1984, Taylor 1988). Al has been suggested to bind to the polar regions of phospholipids or proteins on the plasma membrane (Barceló *et al.* 1996). The resultant structural and functional alterations in the plasma membrane affect membrane permeability and transport processes which in the long-term severely limit nutrient acquisition (Ryan & Kochian 1993). Sasaki *et al.* (1995) postulated that the Al-induced depression in the plasma membrane H^+-ATPase activity reduced K^+ efflux in barley roots resulting in a disturbance of the membrane potential and subsequent ion transport. K^+ net-efflux was

also reduced by increased Al concentrations (8.1 mg Al l^{-1}) in soybean cells in suspension culture (Staß & Horst 1995). Wagatsuma *et al.* (1995) also showed K leakage from the root tips of pea seedlings (cv. Kinusaya). A sensitive indication of Al-induced alteration of membrane properties is the induction of callose (1,3-β-glucan) synthesis (Horst 1995). Binding of Al to the negative charges of the plasma membrane surface reduce fluidity and open stretch-activated Ca^{2+} channels. The resultant increase in the cytoplasmic Ca^{2+} concentration activates callose synthesis. Al concentration in the root tips, and Al-induced inhibition of root elongation, have been correlated with callose concentration in maize root tips (Barceló *et al.* 1996).

Despite evidence of reduced K^+ efflux and induced callose synthesis, clear indicators of structural modifications to the plasma membrane, there is little evidence of major membrane injury at phytotoxic Al concentrations. Calba & Jaillard (1997), Kinraide (1988), and Horst *et al.* (1992) found no disruption in the membrane functional integrity of wheat and soybean by Al. The proton pump in wheat was intact, the membrane was not leaky, and the ATP biosynthesis adequate for vigorous proton extrusion. Results showing enhanced K^+ efflux were suggested to be the result of prolonged treatment and excessive Al concentrations (Horst *et al.* 1992). Kinraide (1993) found small hyperpolarisation of the root-cell-membrane electrical potential. No Al-induced reductions in root pressure and root cell turgor pressure were observed in a Al-sensitive maize variety indicating no general breakdown in membrane integrity, and ion pumping to the stele was maintained (Gunsé *et al.* 1997).

Symptoms of prolonged Al stress resemble those of Ca deficiency and imply Al is particularly disruptive to the uptake of Ca^{2+}. Numerous reports have shown Al-induced inhibition of Ca uptake and species adapted to soil acidity frequently show high Ca efficiency (Barceló *et al.* 1996). Al has been suggested to block the Ca^{2+} channels of the root plasma membrane (in a similar fashion to La^{3+}) which leads to a reduction in net Ca^{2+} uptake, cytoplasmic Ca^{2+} deficiency, and disturbance of Ca^{2+} homeostasis (Rengel *et al.* 1995, Ryan *et al.* 1994). Huang *et al.* (1993), Ryan & Kochian (1993), and Ryan *et al.* (1993) all showed evidence to support a reduction in Ca^{2+} uptake by Al but only in the localised area of the root apex. Ca was reduced to very low levels in the cortical cell walls of Al treated *Picea abies* roots (Hodson & Wilkins 1991). However as described above a prerequisite for callose formation, induced by Al, is an increase in cytosolic Ca^{2+} activity. When considering whole roots, Ryan *et al.* (1994), showed a severe inhibition of root growth in wheat at 0.07 mg Al l^{-1} with no concurrent reduction in Ca^{2+} uptake. Investigations with ion-selective microelectrodes or radioactive Ca tracers (Ca^{45}) have recently been used to show root elongation is not directly coupled with Ca^{2+} uptake into the root and translocation to the shoot (Barceló *et al.* 1996). Reduced Ca^{2+} influx as a primary mechanism of Al toxicity is considered unlikely (Horst 1995, Kinraide *et al.* 1994, Ryan *et al.*

1994) but the disruption in Ca homeostasis may play a role in the initial stages of Al-induced inhibition of root elongation.

Al has been associated with decreased plant concentrations of Ca and Mg in French bean (Massot *et al.* 1992), ryegrass (Rengel & Robinson 1990), and sorghum, and reduced concentrations of K in non-tolerant sorghum hybrids (Malavolta *et al.* 1981). K uptake increased with increasing Al concentration in Al-tolerant sorghum hybrids and similarly increased in sunflower at high Al (Blamey *et al.* 1986). However Al led to lower absorption of PO_4 in both hybrids. Wheeler (1994) found reductions in the shoot and root concentrations of N, P, K, Ca, Mg, and S with increasing Al in wheat. Tan *et al.* (1993) showed severe reductions in Mg uptake by Al in sorghum genotypes but little affect on Ca and P. In contrast the nutrient concentrations in the roots of *Arnica montana*, *Calluna vulgaris*, and *Cirsium dissectum* were not influenced by Al (De Graaf *et al.* 1997). Despite the reduction in growth in *Arnica* by Al, shoot Mg was not affected.

5.1.5 Tolerance to Al

Plant species and cultivars within species differ greatly in their tolerances to potentially toxic levels of Al (Foy 1984). Hypothesised mechanisms of Al tolerance basically stem from external mechanisms where Al entry across the membrane is limited and sensitive extracellular sites protected, and from internal mechanisms where Al is detoxified in the cytoplasm (Taylor 1995). The former include immobilisation of Al at the cell wall or low cell wall CEC; selective permeability of the plasma membrane; formation of plant-induced pH barriers in the rhizosphere or root apoplasm; exudation of chelator ligands; exudation of phosphate; and Al efflux. Internal resistance mechanisms include chelation in the cytosol (with Si or organic acids); compartmentation in vacuoles; evolution of Al-tolerant enzymes; and elevated enzyme activity (Bennet & Breen 1991, Foy 1984, Jones 1961, Malavolta *et al.* 1981, Taylor 1988, Taylor 1991, Taylor 1995). However detoxification of Al in the cytoplasm is not considered to play an important role in Al tolerance (Barceló *et al.* 1996). The interaction between Al toxicity and both Si and organic acids will be reviewed in Chapters 6 and 7.

An increase in the pH of the immediate solution surrounding plant roots or rhizosphere decreases the toxicity of Al through the formation of $Al(OH)_3.3H_2O$ a sparingly soluble monomeric species, or the precipitation of $Al(OH)_2.H_2PO_4$ (Foy 1984, see Chapter 1, Table 1.1). Both Miyasaka *et al.* (1989) and Taylor (1991) suggested plant-induced changes in rhizosphere pH were not major factors determining tolerance to Al.

Blamey *et al.* (1990) proposed differential Al tolerance resulted from differences in root CEC. An Al-tolerant cultivar of *Lotus pedunculatus* (cv. Grasslands Maku), with a low root CEC, absorbed less Al

from solution than did an Al-sensitive cultivar of *Lotus corniculatus* (cv. Maitland), with a high root CEC (Blamey *et al.* 1990). The differences in CEC were proposed to reflect differences in the degree of cell wall pectin methylation. Al-tolerant genotypes have roots with low CEC, and higher concentrations of Al are required to precipitate the relatively highly methylated pectins associated with low CEC.

Tolerance is not consistently achieved through the exclusion of Al uptake by roots. That is Al-tolerant cultivars do not always show lower root or shoot concentrations of Al. This is contrary to the evidence from Horst *et al.* (1983) who associated tolerant genotypes with lower Al uptake and Tan *et al.* (1989) who reported sensitive cultivars of rice accumulated more Al than resistant ones. Tolerance has often been shown in plants with high root or shoot Al concentrations. Plant tissues of *Calluna vulgaris* accumulated Al with increasing solution Al (up to 13.49 mg Al l^{-1}) despite no concurrent reduction in growth (De Graaf *et al.* 1997). Nevertheless Al does not generally accumulate in the tops of Al sensitive species (Foy 1984). The low mobility of Al in the plant cell would imply preferential retention of Al in plant roots (Bennet & Breen 1991). Al accumulating species such as tea or pine trees do have a high internal tolerance to Al. Matsumoto *et al.* (1976a) showed Al accumulated in the epidermal cells of old tea leaves which had thickened cell walls. Foliar concentrations of P, K, Ca, Mg, Fe, Mn, and Zn are no lower than concentrations in non-accumulating species (Foy 1984).

Al tolerance has been associated with a greater uptake of K and Mg in potato cultivars, and with a greater Mg uptake in maize by Foy (1984). Rengel *et al.* (1995) suggested differential tolerance principally arose through differential blockage of Ca^{2+} channels and maintenance of calcium uptake. Al-tolerant wheat cultivars were able to resist Al inhibition of Ca^{2+} uptake (Huang *et al.* 1995, Ryan & Kochian 1993). Cultivars of barley, soybean, and snap bean were shown to resist Al-induced changes in Ca deficiency (Foy *et al.* 1978).

Bennet & Breen (1991) suggested maintaining root growth in the presence of Al was dependent upon the activity of peripheral root cap cells. They found concomitant reductions in root growth with declines in root cap amyloplast cell numbers. Polysaccharide material produced in the Golgi bodies was distributed via the amyloplasts. This mucilage binds Al reducing any injury to root meristems.

5.1.6 Beneficial effects of aluminium

Despite not being an essential element for plant growth, Al in low concentrations occasionally stimulates plant growth (Foy 1984, Kinraide 1993, Taylor 1988). Al concentrations of 3 mg l^{-1} at pH 4.5 were detrimental to the Sonora 63 wheat cultivar from Mexico but markedly beneficial to the BH1146 cultivar (Foy 1984). Similar stimulation in plant growth were shown in *Arnica montana* and

Deschampsia flexuosa at <5 mg Al l^{-1} (Pegtel 1987), in nine sorghum hybrids at 3 mg Al l^{-1} (Malavolta *et al.* 1981), and in *Cirsium dissectum* at 1.35 mg Al l^{-1} (De Graaf *et al.* 1997). Konishi *et al.* (1985) provided evidence showing both a promotion in P absorption and stimulation in growth by Al in tea. Al and K content in the roots and shoots, and N in the shoots, increased with increasing Al supply. Tan *et al.* (1989) showed increased uptake of PO_4, K, Ca, and Mg by rice at low Al levels.

Foy *et al.* (1978) summarised proposed explanations for Al-enhanced growth at low Al concentrations. Explanations included: increased Fe solubility and availability in calcareous soils, prevention of internal Fe deficiency through displacement of Fe from inactive sites in calcicolous plants, prevention of P toxicity or promotion of P uptake, reduction in growth rate and prevention of Ca depletion, alteration of growth regulators, and finally, protection against Cu/Mn toxicity.

The most recent hypothesis to explain this stimulation in growth involves the alleviation of H^+ ion toxicity by Al^{3+} (Kinraide 1993). The magnitude of growth enhancement increases with decreasing pH. Root growth was reduced in *Picea abies* by 2.70 and 10.79 mg Al l^{-1} at pH 4 and 5, but at pH 3.2 only 10.79 mg Al l^{-1} significantly reduced growth (Godbold *et al.* 1995).

5.2 Aims

- To determine the effects of increasing Al concentrations (2-35 mg l^{-1}) on the growth of races of *Betula pendula*. (The reasons for the choice of study species were given in Chapter 2, Section 2.2.)

- To examine all aspects of growth: root elongation, root production, leaf expansion, and dry matter production in Al containing solutions.

- To relate race-specific responses to Al to their natural soil types.

- To determine the effects of Al on nutrient acquisition.

5.3 Methods

Seeds of *Betula pendula* were collected in August/September 1995 from FM, SMM, KP, and KR (Chapter 2, Table 2.1). The seeds were stored in dry and dark conditions at room temperature until the start of the experiment. Seeds were germinated in 1 % agar (DIFCO Laboratories 1995) in December 1995 following the methods in Chapter 4 (Section 4.3.1.2). At the first leaf stage they were transferred into an initial culture solution with no added Al and at pH 5.6 (Chapter 4, Section 4.3.1.2).

The composition of the culture solutions is given in Chapter 4 (Table 4.1). Stock solutions of 100-strength of NH_4OH, Na_2SO_4, $NH_4H_2PO_4$, KFeEDDHA, $Ca(NO_3)_2.4H_2O$, $CaCl_2.6H_2O$, $Mg(NO_3)_2.6H_2O$, H_3BO_3, KH_2PO_4, $Al(NO_3)_3.9H_2O$, and MES buffer, and 1000-strength of $MnSO_4.4H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, and $(NH_4)_6Mo_7O_{24}.4H_2O$ were made up and diluted appropriately. Beakers were covered in tinfoil to prevent algal growth. Solutions were stirred daily and the pH corrected where necessary to 5.6 using 1M NaOH or 1M HCl. Culture solutions were changed twice per week. After four weeks growth in culture solution with no added aluminium seedlings of similar size were separated into beakers which each held 2 seedlings. At this stage seedlings were on average 5.88-8.64 cm in height, with three to five roots in total 40.42-73.30 cm in length, and 10-17 leaves of 26.1-40.9 cm^2 total area. Aluminium was then added to the culture solutions in the form $Al(NO_3)_3.9H_2O$ and at the following concentrations : 0 mg l^{-1} (control), 2 mg l^{-1} (74 μM), 5 mg l^{-1} (185 μM), 10 mg l^{-1} (370 μM), 15 mg l^{-1} (555 μM), 25 mg l^{-1} (925 μM), and 35 mg l^{-1} (1295 μM) Al. The following abbreviations are used corresponding to the treatments 0 Al, 2 Al, 5 Al, 10 Al, 15 Al, 25 Al, and 35 Al. Culture solutions were adjusted to pH 4.2 and corrected when necessary each day. There were between five and eight replicate seedlings per treatment per site depending on the number of seedlings available. Subsamples of 5 ml from each of six beakers, from each of the seven treatments, were withdrawn from fresh culture solution, and from solutions one, two, three, and four days old, during the first two weeks of treatments. Solutions were analysed to monitor nominal element concentrations using the same analytical techniques as those in Chapter 3. Thereafter solutions were changed after the first 5-ml extractions on day 3.

The number of roots and their lengths, the number of leaves and their length and maximum breadth, the height of the seedlings, and the number of buds were recorded before seedlings were put into treatments and after treatments at harvesting. To determine the leaf area expansion over the treatment period a relationship between actual leaf area and measured values of length and breadth was established. One hundred leaves, collected from separate birch seedlings, which were grown alongside experimental seedlings, were scanned and their area were measured using NIH 5b Image. A regression equation between leaf area and leaf maximum breadth and length was then determined and

used to estimate the leaf area of experimental seedlings before treatments began and after harvesting (Area=(3.44(Length)+1.26(Breadth))-3.88, F=82.31, p<0.001). Both absolute growth (AGR=cm^2 7 days^{-1}) and relative growth (RGR=cm^2 cm^{-2} 7 days^{-1}) rates were then determined. Seedlings were harvested after 12 weeks growth in treatment (14 Jan-8 Apr 1996). Roots, stems, and shoots were separated, rinsed in deionised water, and dried in an oven at 60 °C for 48 h and the dry weights of leaves, stems, and roots recorded. Root:shoot ratios were determined. Prior to drying the lateral root growth of seedlings was observed under the binocular microscope. The number and length of lateral roots were estimated from 10 cm lengths of primary root.

Between 100 and 300 mg of oven-dried leaves and roots were digested in a sulphuric acid-hydrogen peroxide mixture (Allen 1989) in a block digester at 330 °C and filtered through No.44 Whatman filter paper and made up to 100 ml. Concentrations of calcium and magnesium were measured using a Varian AA-575 S atomic absorption spectrophotometer with a nitrous oxide-acetylene flame. An air-acetylene flame was used to determine sodium, potassium (flame emission) and iron concentrations. Total aluminium was measured with a Pye Unicam SP9 Atomic Absorption Spectrophotometer fitted with a Unicam GF90 furnace and FS90 furnace autosampler. Unicam 919 series atomic absorption software was used. P was measured on a Tecator FIAstar 5010 flow injection auto-analyser using the stannous chloride-ammonium molybdate method.

5.4 Results

5.4.1 Number and elongation of roots

5.4.1.1 Root elongation

There was no significant difference in the length of the longest roots between plants at the beginning of the experiment. The mean increase in the longest root length was also not significantly different between plants from either different origins or Al treatments (Table 5.1).

The mean rates of root elongation (RER, cm day^{-1}) are shown in Figure 5.1. RER were significantly affected by the origin of the plants and also by the Al treatment (Table 5.1). In plants originating from both FM and KP root elongation was greater at the lower Al concentrations, 2 and 5 Al (especially 5 Al), and lowest at 35 Al. 35 Al induced a 50 % and 80 % reduction in root elongation relative to control plants (RRE) and 5 Al stimulated RRE by 60 % and 40 % in plants from FM and KP (Figure 5.1 and Table 5.2). Root elongation was significantly reduced at all Al concentrations (compared with controls) in KR races (by up to 80 %). However, in the presence of Al, elongation was greatest at 35 Al. Root elongation of SMM races was only reduced at the lower Al concentrations (2 and 5 Al). RRE was greatest at 25 Al (>100 % increase compared to control plants). RRE was significantly correlated (negatively) with shoot Al. As shoot Al concentrations increased, RRE decreased. There was no significant correlation with root Al.

5.4.1.2 Number of root s

Figure 5.2 shows the mean increase in the number of roots between the start of treatment and harvesting. The effects of Al on the root number were similar to the effects on elongation. The increase in root number was not significantly different between the plant origins but root numbers were significantly affected by Al concentration (Table 5.1). At lower Al treatments, Al stimulated root production over the experimental period in plants from FM (2, 5, and 10 Al), and KP (2 and 5 Al). However in the same races increasing the Al concentration led to a significant decrease in the number of roots (about 60 % reduction, Table 5.2). Like root elongation, root numbers were reduced by all concentrations of Al in seedlings from KR. Al promoted root production in seedlings from SMM in all but the highest Al concentration where root numbers were reduced by about 50 % (Figure 5.2 and Table 5.2).

Table 5.1. General linear models for root, shoot, and dry weight growth measurements in races of *Betula pendula* from Flanders Moss (FM), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR), and grown in 0 Al, 2 Al, 5 Al, 10 Al, 15 Al, 25 Al, and 35 Al. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; n.s, not significant. Degrees of freedom are : race 3, Al 6, race*Al interaction 18.

| Measurement	Race F	p	Al F	p	Race*Al interaction F	p
Root growth						
Elongation of longest root	0.86	n.s	1.48	n.s	1.84	*
Root elongation rate	3.46	*	2.74	**	1.66	n.s
Relative RER	21.35	***	3.79	**	10.59	***
Increase in number of roots	1.29	n.s	3.49	**	2.03	**
Leaf expansion						
Total leaf area	5.86	***	5.91	***	2.13	***
% total area = <1 cm^2	9.07	***	12.15	***	3.59	***
% total area =1-2 cm^2	8.01	***	6.30	***	3.48	***
% total area =2-5 cm^2	4.71	**	4.00	**	2.58	**
% total area =>5 cm^2	11.69	***	9.22	***	2.24	**
Absolute growth rate, AGR	5.86	***	5.91	***	2.13	***
Relative growth rate, RGR	3.46	*	2.74	*	1.66	n.s
Leaf & bud number						
Total number of leaves	12.00	***	31.31	***	6.38	***
% total number=<1 cm^2	9.87	***	14.56	***	1.45	*
% total number=1-2 cm^2	15.33	***	9.10	***	5.36	***
% total number=2-5 cm^2	11.19	***	13.24	***	5.18	***
% total number=>5 cm^2	26.51	***	14.88	***	6.02	***
Bud production	6.13	***	12.88	***	5.99	***
Seedling height						
Height increase	15.92	***	10.39	***	4.75	***
Dry weights						
Shoot	9.21	***	10.54	***	3.60	***
Root	4.60	**	3.54	**	3.25	***
Stem	6.40	***	11.45	***	1.66	n.s
Total	2.82	*	10.04	***	1.98	*
Root:shoot ratio	4.86	**	3.71	**	2.93	***
Relative root yield	36.96	***	5.35	***	5.30	***
Relative shoot yield	13.28	***	13.39	***	4.44	***
Ionic composition						
Shoots						
P	1.81	n.s	2.03	n.s	2.74	**
K	29.71	***	10.55	***	12.26	***
Na	6.53	***	13.75	***	3.81	***
Ca	5.29	**	13.10	***	1.25	n.s
Mg	3.49	**	16.29	***	3.20	***
Al	5.46	**	11.57	***	3.96	***
Fe	0.84	n.s	4.23	**	1.73	n.s
Roots						
P	2.50	n.s	1.05	n.s	0.99	n.s
K	2.20	n.s	7.01	***	2.96	***
Na	9.69	***	18.31	***	2.99	***
Ca	1.74	n.s	3.44	**	2.58	**
Mg	0.33	n.s	51.79	***	0.37	n.s
Al	1.21	n.s	8.68	***	3.45	***
Fe	3.56	**	33.91	***	3.92	***
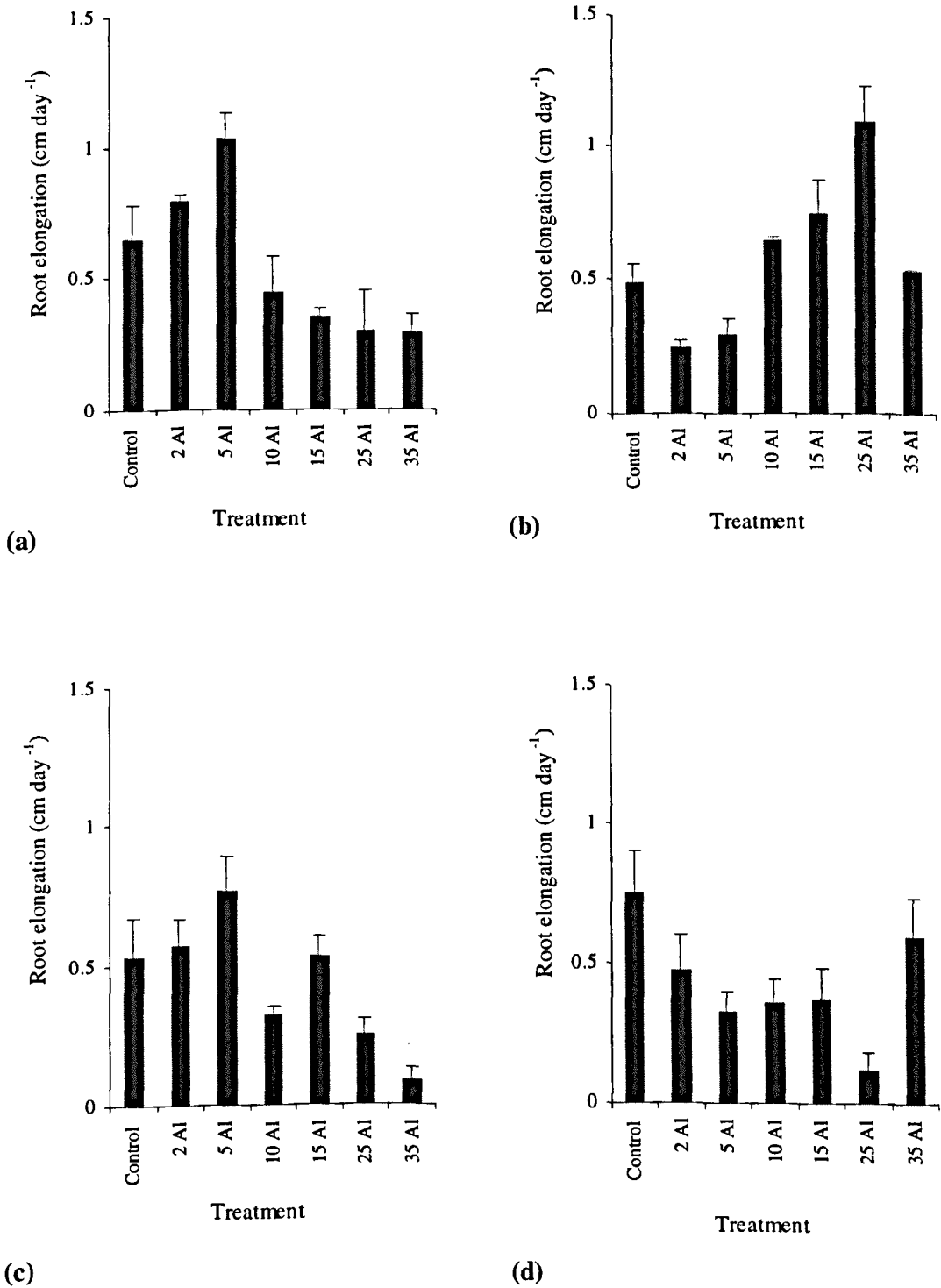
Figure 5.1. Mean rates of root elongation (cm day^{-1}, ± s.e.) of *Betula pendula* from (a) Flanders Moss (FM), (b) Sheriffmuir (SMM), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR), grown in 0 (control), 2, 5, 10, 15, 25, and 35 mg Al l^{-1} at pH 4.2.
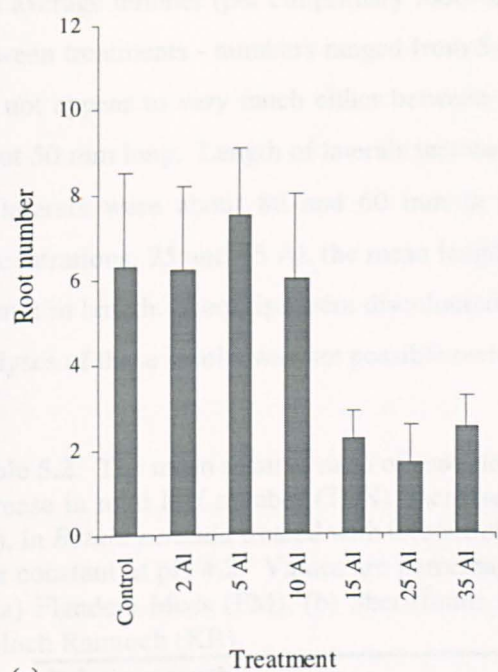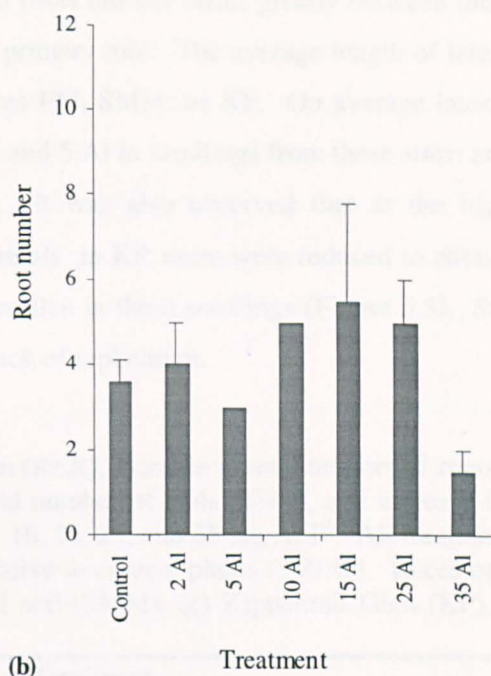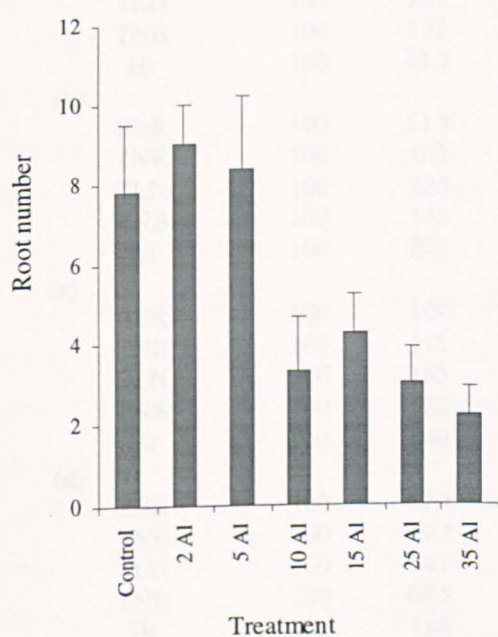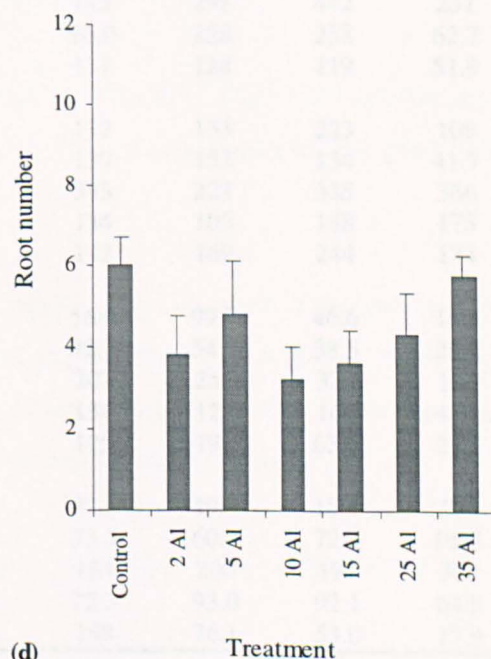
Figure 5.2. Increase in mean total number of roots (± s.e.) of *Betula pendula* from (a) Flanders Moss (FM), (b) Sheriffmuir (SMM), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR), grown in 0 (control), 2, 5, 10, 15, 25, and 35 mg Al l^{-1} at pH 4.2.

5.4.1.1 Lateral root growth

The average number (per cm primary root) of lateral roots did not differ greatly between the sites or between treatments - numbers ranged from 5-7 cm^{-1} primary root. The average length of lateral roots did not appear to vary much either between the races FM, SMM, or KP. On average laterals were about 50 mm long. Length of laterals increased at 2 and 5 Al in seedlings from these sites: at 2 and 5 Al laterals were about 80 and 60 mm in length. It was also observed that at the highest Al concentrations, 25 and 35 Al, the mean length of laterals in KR races were reduced to about 22 and 19 mm in length. Root tips were discoloured and swollen in these seedlings (Figure 5.3). Statistical analyses of these results was not possible owing to lack of replication.

Table 5.2. The mean relative rates of root elongation (RER), increase in total number of roots (TNR), increase in total leaf number (TLN), increase in total number of buds (TNB), and increase in height (Ht), in *Betula pendula* treated with 0 (control), 2, 5, 10, 15, 25, and 35 mg Al l^{-1}. All treatments were kept constant at pH 4.2. Values are percentages relative to control plants (100 %). Races originated in (a) Flanders Moss (FM), (b) Sheriffmuir mineral soil (SMM), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR).

Relative growth (%)	Control	2 Al	5 Al	Treatment 10 Al	15 Al	25 Al	35 Al
(a)							
RER	100	122	158	67.5	54.2	46.6	45.4
TNR	100	98.7	119	87.0	35.6	26.3	39.5
TLN	100	267	259	143	298	442	231
TNB	100	132	165	96.0	228	258	62.7
Ht	100	95.2	112	117	124	119	51.9
(b)							
RER	100	51.8	59.3	132	153	223	108
TNR	100	102	83.3	139	153	134	41.7
TLN	100	220	190	333	222	335	366
TNB	100	145	113	134	106	188	173
Ht	100	295	91.2	112	169	244	174
(c)							
RER	100	108	144	56.0	99.7	46.6	16.5
TNR	100	115	107	42.7	54.5	38.5	28.2
TLN	100	163	88.7	202	251	329	184
TNB	100	182	80.7	134	122	161	45.8
Ht	100	140	55.9	125	191	63.7	29.7
(d)							
RER	100	62.1	44.5	47.8	49.9	15.4	78.1
TNR	100	63.3	80.0	53.3	60.0	72.2	95.8
TLN	100	141	144	184	200	192	308
TNB	100	69.5	44.9	72.3	93.0	92.1	64.9
Ht	100	186	108	148	76.1	53.0	17.9

5.4.2 Leaf area and number

There were no signs of Al toxicity in the leaves of seedlings from FM, SMM, or KP. However the leaves of seedlings from KR were slightly chlorotic at the highest Al concentration, 35 Al (Figure 5.4).

5.4.2.1 Total leaf area

Before seedlings were treated with Al there were no significant differences in total leaf area between individuals or sites. However after treatments there were significant differences between both sites and Al concentrations (Table 5.1 and Figure 5.5). At all Al concentrations in FM, SMM, and KR races the mean total leaf area was significantly greater than the control plants. This increase in leaf area with Al was lowest in plants from KR. With the exception of SMM, leaf area was lowest at 35 Al (although still greater than controls). Leaf area was significantly increased at the lowest 2 Al, and greater 10, 15, and 25 Al concentrations in plants from KP.

5.4.2.2 Leaf area according to size categories

Figure 5.6 depicts the proportion of total leaf area which consisted of leaves in the following size categories : $<1 cm^2$, 1-2 cm^2, 2-5 cm^2, and >5 cm^2. The increase in leaf area in each category over the experimental period was significantly different between races and Al treatments (Table 5.1). An increase in Al led to a concurrent increase and decrease in the % of total leaf area comprising leaves <2 cm^2 and leaves >5 cm^2. This was particularly reflected in KR races. At 35 Al only 4 % of the total leaf area represented leaves >5 cm^2 in area compared with 39 % at 2 Al. Whereas at 35 Al, 31 % of total leaf area comprised leaves of <2 cm^2 compared with 12 % at 2 Al. In seedlings from FM, SMM, and especially KP, the % leaf area comprising larger leaves (>5 cm^2) was significantly increased at the lower Al treatment (2 Al) compared with controls (Figure 5.6).

5.4.2.3 Leaf expansion

Leaf expansion calculated as the absolute growth rate (AGR=cm^2 7 day^{-1}) and relative growth rate (RGR=cm^2 cm^{-2} 7 day^{-1}) are graphed in Figure 5.7. The Al concentration and plant origin both significantly affected AGR and RGR (Table 5.1).

5.4.2.4 Absolute growth rate

In FM, SMM, and KR races, absolute growth was significantly enhanced in the presence of Al. In both FM and KR AGR was lowest at 35 Al but still greater than control plants. In KP, AGR was greatest at 2 Al and lower than control plants at 35 Al but not significantly. There was a significant negative correlation between AGR and shoot Al but no correlation with root Al.

5.4.2.5 Relative growth rate

Relative growth rate (RGR) was unusually high at 2 Al (FM), 15 Al (KP), and 10 Al (KR) but standard deviations were very large at these points.

In FM, SMM, and KR, Al enhanced relative growth rates (RGR). Only in FM and KR did this growth decrease at 35 Al. In SMM, RGR increased with increasing Al concentration and was highest at the highest Al treatments, 25 and 35 mg l^{-1}. In KP, RGR was significantly lower than controls at the two highest concentrations, otherwise Al stimulated growth. There was no correlation between RGR and either shoot or root Al.
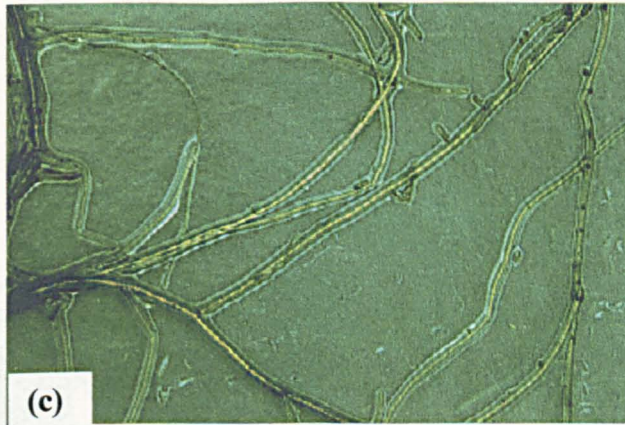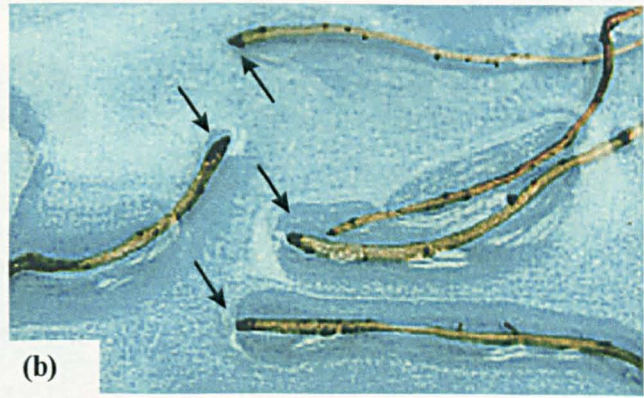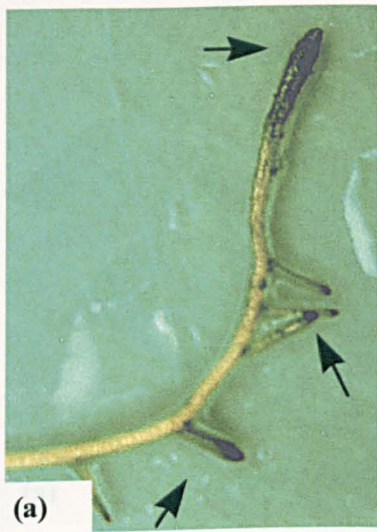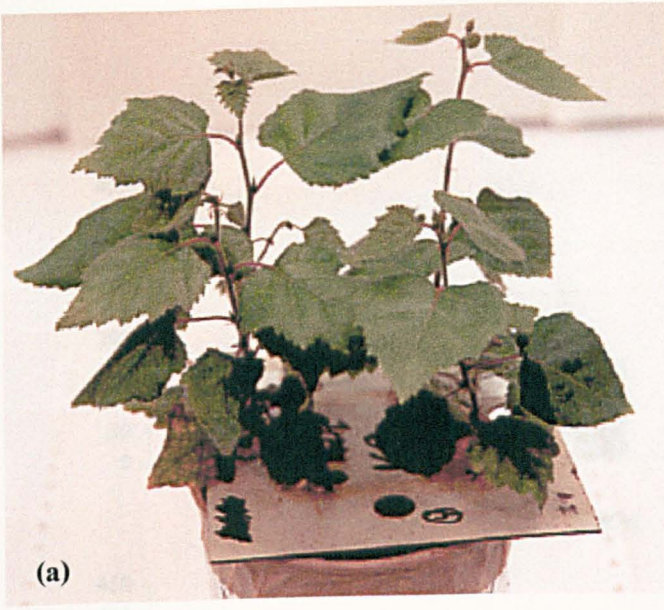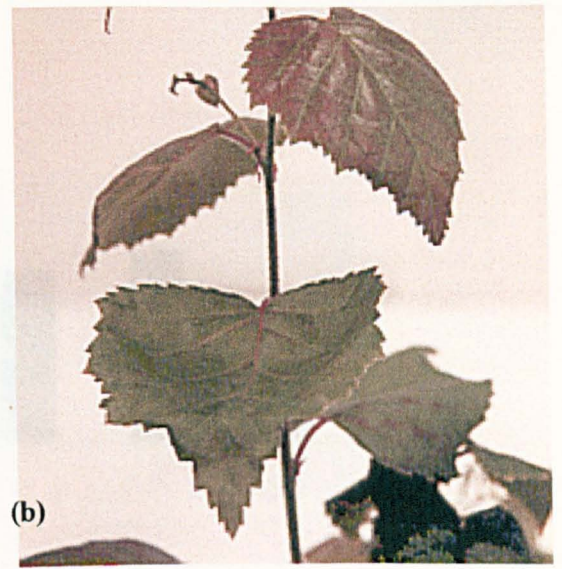
Figure 5.3. Roots of *Betula* seedlings originating from Kinloch Rannoch (KR) after treatment with (a)/(b) 35 mg Al l^{-1} and (c) without Al (controls). Arrows indicate thickened, discoloured root tips of Al-treated roots.
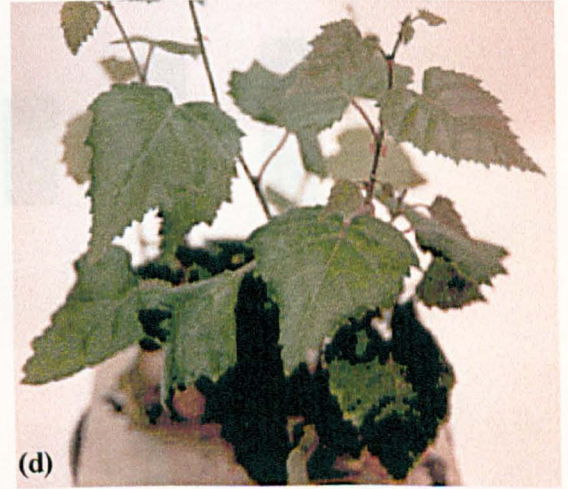
Figure 5.4. *Betula pendula* originating from Flanders Moss (FM) (a, c, d, and e), Sheriffmuir mineral soil (SMM) (b), and Kinloch Rannoch (KR) (f). Birch seedlings were grown in (a) control, (b) 2 mg l^{-1} Al, (c) 5 mg l^{-1} Al, (d) 10 mg l^{-1} Al, and (e,f) 25 mg l^{-1} Al. Leaf area was increased at low levels of Al and reduced at high levels.
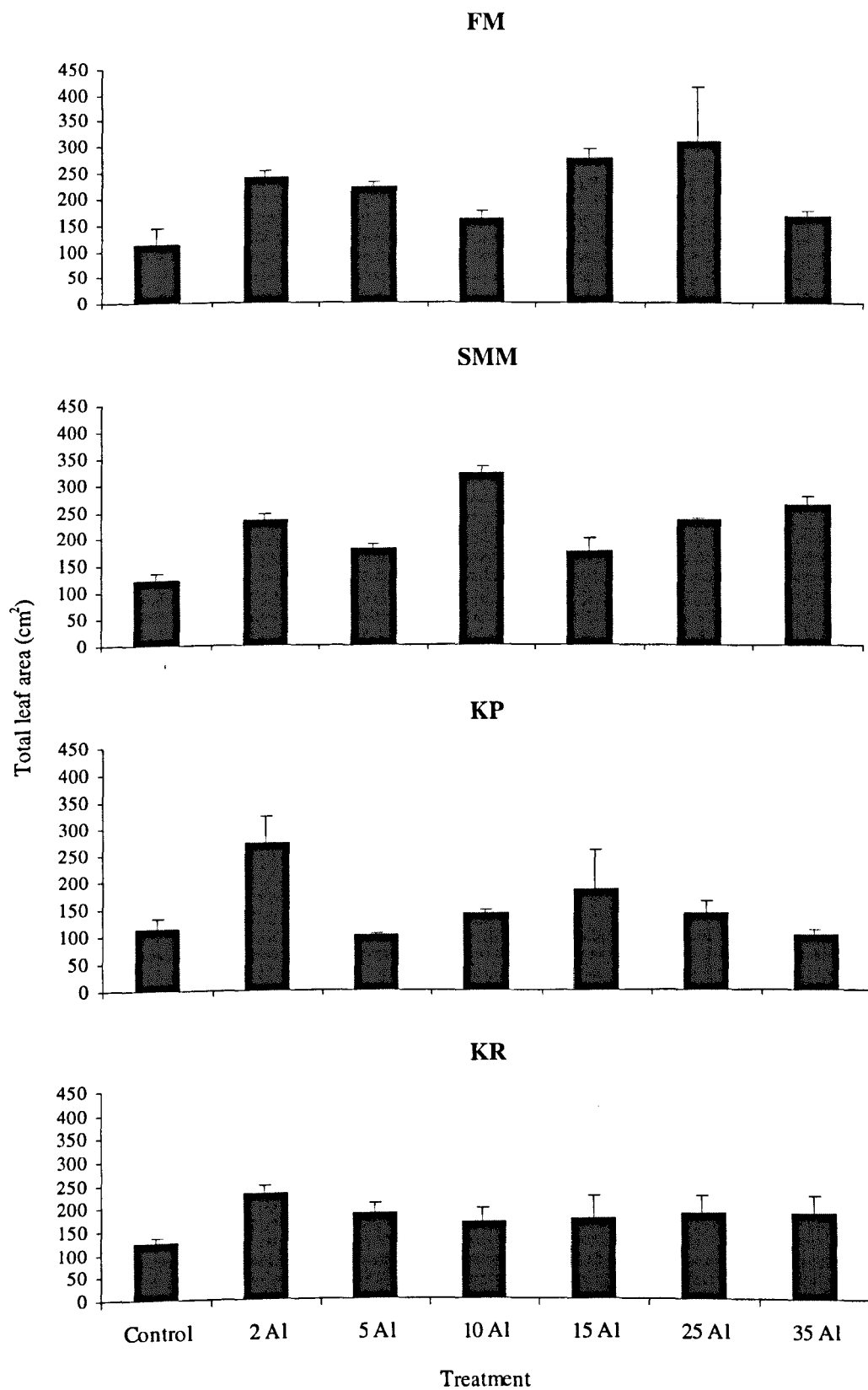
Figure 5.5. Mean total leaf area (cm^2, ± s.e) of *Betula pendula* from (a) Flanders Moss (FM), (b) Sheriffmuir (SMM), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR), grown in 0 (control), 2, 5, 10, 15, 25, and 35 mg Al l^{-1} at pH 4.2.
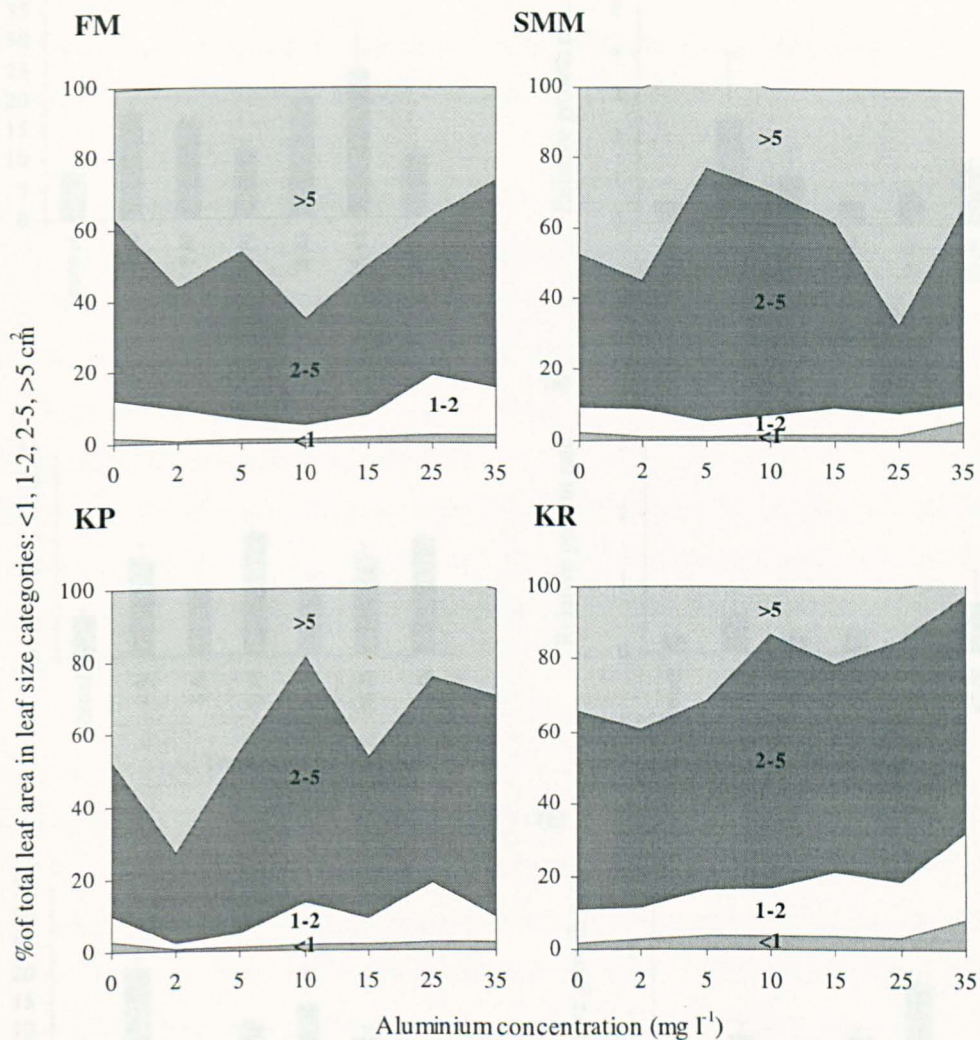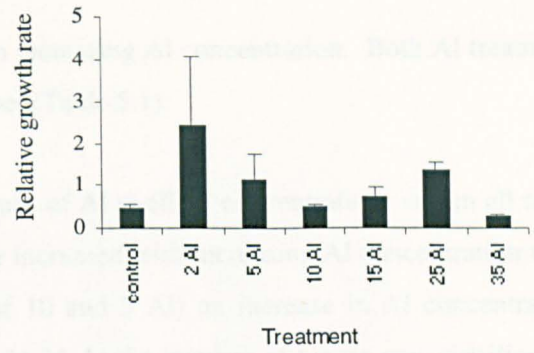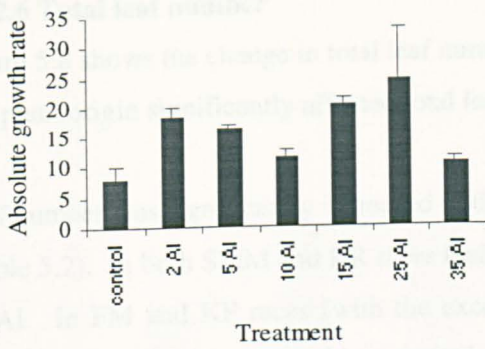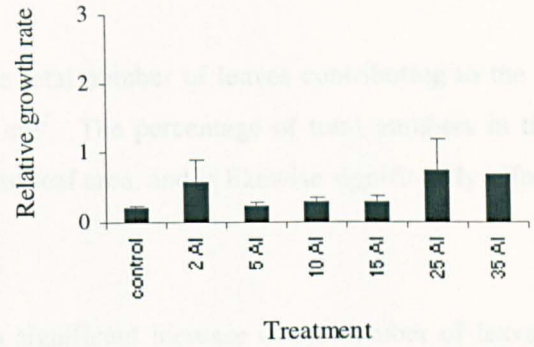
Figure 5.6. Percentage of total leaf area of *Betula pendula* comprising the leaf size categories: <1 cm^2, 1-2 cm^2, 2-5 cm^2, and >5 cm^2 after growth in 0, 2, 5, 10, 15, 25, and 35 mg Al l^{-1}. Seedlings originated from Flanders Moss (FM), Sheriffmuir (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR).

(a)

(a)
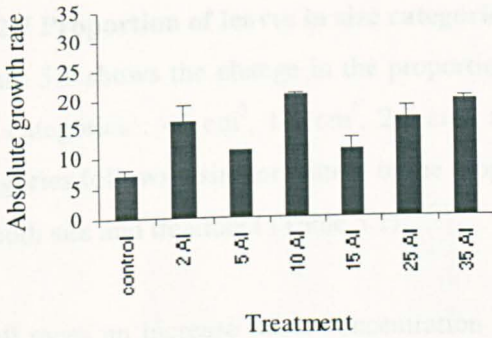
(b)

(b)
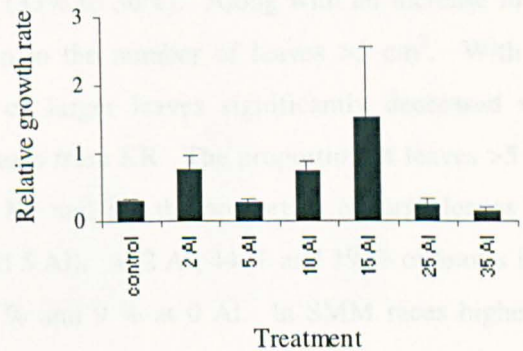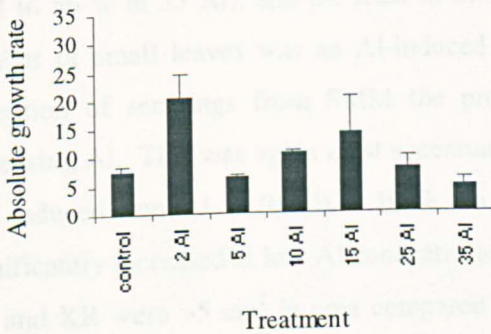
(c)

(c)
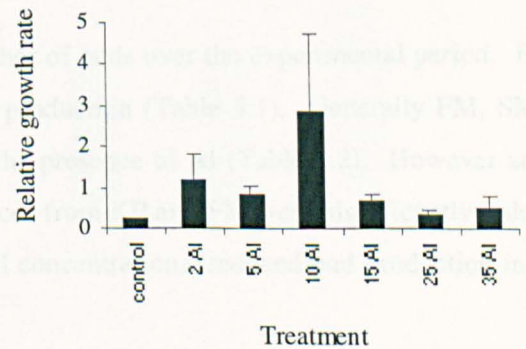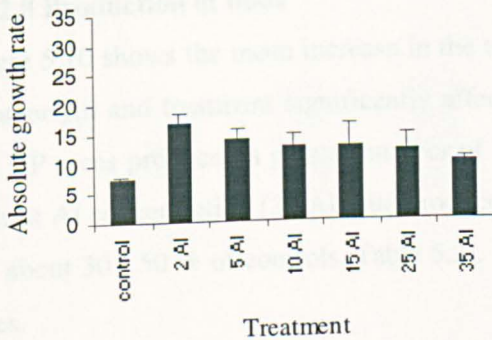
(d)

(d)

Figure 5.7. Mean absolute leaf growth rates (cm^2 7 day^{-1}, ± s.e.) and mean relative leaf growth rates (cm^2 cm^{-2} 7 day^{-1}, ± s.e.) of *Betula pendula* from (a) Flanders Moss (FM), (b) Sheriffmuir (SMM), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR), grown in 0 (control), 2, 5, 10, 15, 25, and 35 mg Al l^{-1}.

5.4.2.6 Total leaf number

Figure 5.8 shows the change in total leaf number with increasing Al concentration. Both Al treatment and plant origin significantly affected total leaf number (Table 5.1).

Leaf number was significantly increased in the presence of Al at all Al concentrations and in all races (Table 5.2). In both SMM and KR races leaf number increased with increasing Al concentration until 35 Al. In FM and KP races (with the exception of 10 and 5 Al) an increase in Al concentration resulted in a greater number of leaves until 25 Al. At 35 Al the number of leaves was significantly less but still greater than controls.

5.4.2.7 Proportion of leaves in size categories

Figure 5.9 shows the change in the proportion of the total number of leaves contributing to the leaf size categories : <1 cm^2, 1-2 cm^2, 2-5 cm^2, and >5 cm^2. The percentage of total numbers in these categories follows a similar pattern to the proportion of leaf area, and is likewise significantly affected by both site and treatment (Table 5.1).

In all races an increase in Al concentration led to a significant increase in the number of leaves of small area (<2 cm^2). The largest Al-induced production of small leaves occurred in KR races (37 % at 0 Al to 56 % at 35 Al), and the least in SMM races (33% to 36%). Along with an increase in the number of small leaves was an Al-induced reduction in the number of leaves >5 cm^2. With the exception of seedlings from SMM the proportion of larger leaves significantly decreased with increasing Al. This was again most accentuated in plants from KR. The proportion of leaves >5 cm^2 was reduced from 31 % (0 Al) to 10 % (35 Al). In KP and KR the proportion of large leaves was significantly increased at low Al concentrations (2 and 5 Al). At 2 Al, 44 % and 19 % of leaves from KP and KR were >5 cm^2 in area compared with 31 % and 9 % at 0 Al. In SMM races higher Al concentrations did not reduce the number of larger leaves. At 25 Al, 51 % of leaves were >5 cm^2 in area compared with 24 % at 0 Al.

5.4.2.8 Production of buds

Figure 5.10 shows the mean increase in the total number of buds over the experimental period. Both plant origin and treatment significantly affected bud production (Table 5.1). Generally FM, SMM, and KP races produced a greater number of buds in the presence of Al (Table 5.2). However at the highest Al concentration (35 Al) bud production in races from KP and FM were significantly reduced (by about 30 - 50 % of controls, Table 5.2). Al, at all concentrations, reduced bud production in KR races.

Figure 5.8. Mean total number of leaves (± s.e) of *Betula pendula* from (a) Flanders Moss (FM), (b) Sheriffmuir (SMM), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR), grown in 0 (control), 2, 5, 10, 15, 25, and 35 mg Al l^{-1} at pH 4.2.

Figure 5.9. Percentage of total leaf number of *Betula pendula* comprising the leaf size categories: <1 cm^2, 1-2 cm^2, 2-5 cm^2, and >5 cm^2 after growth in 0, 2, 5, 10, 15, 25, and 35 mg Al l^{-1}. Seedlings originated from Flanders Moss (FM), Sheriffmuir (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR).
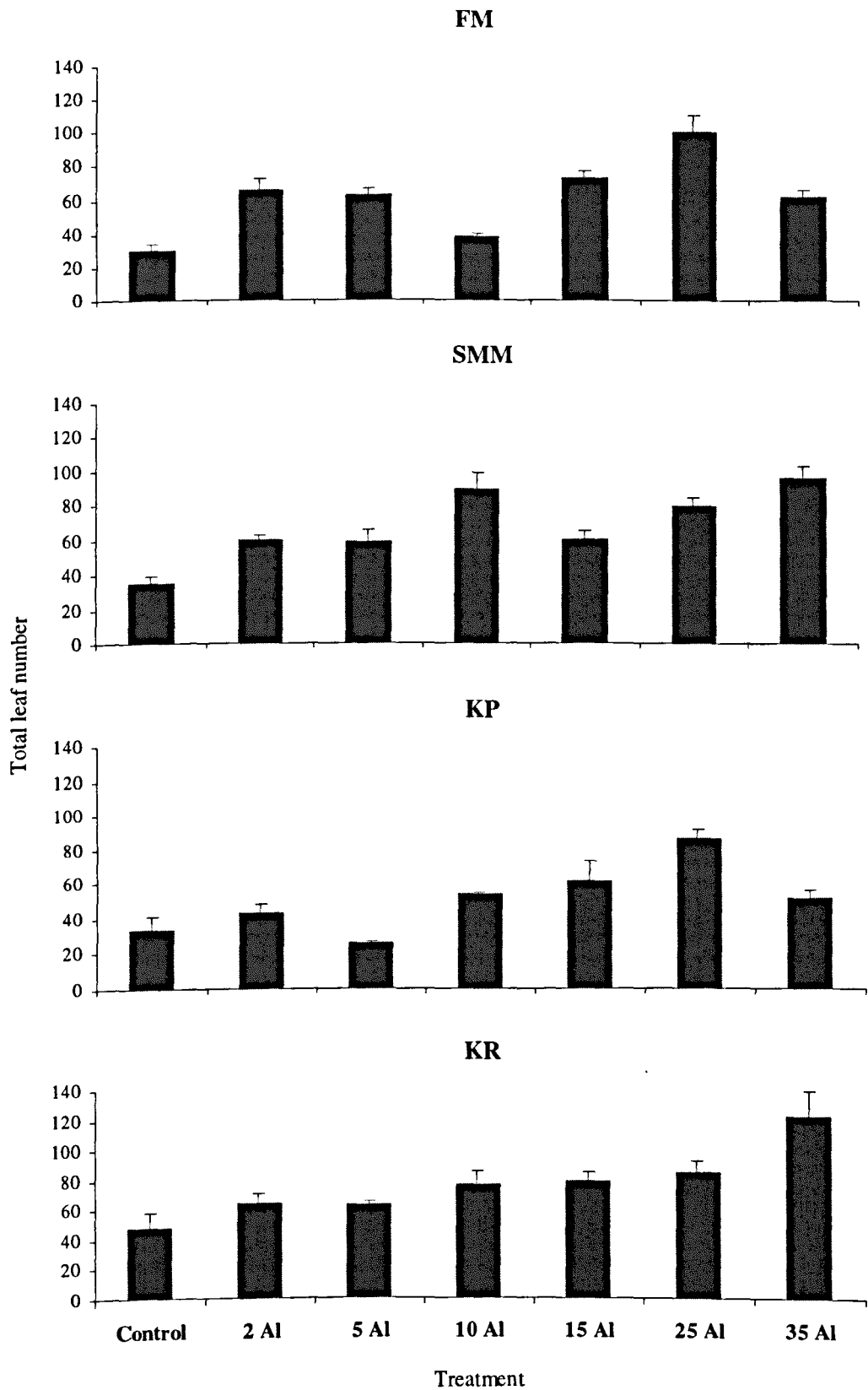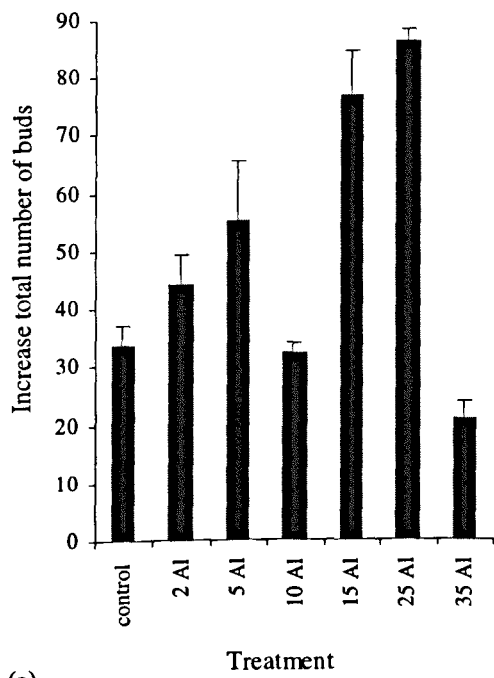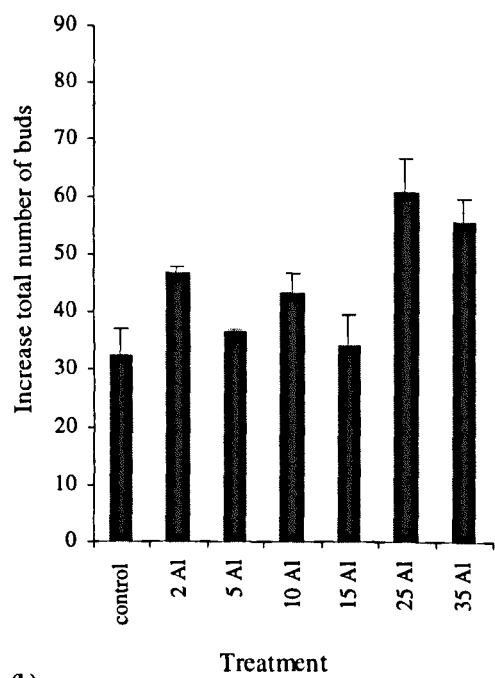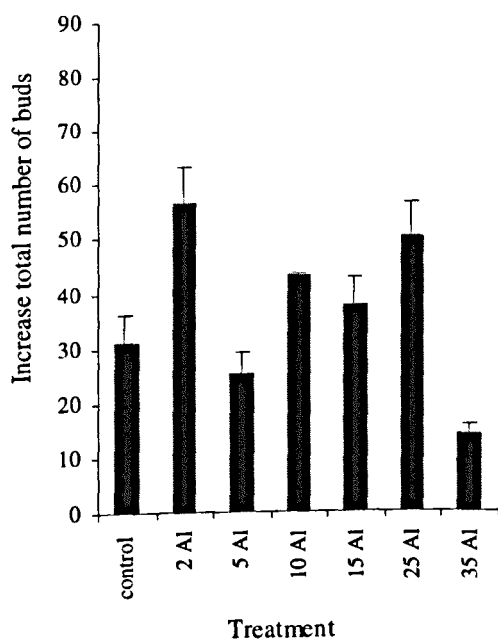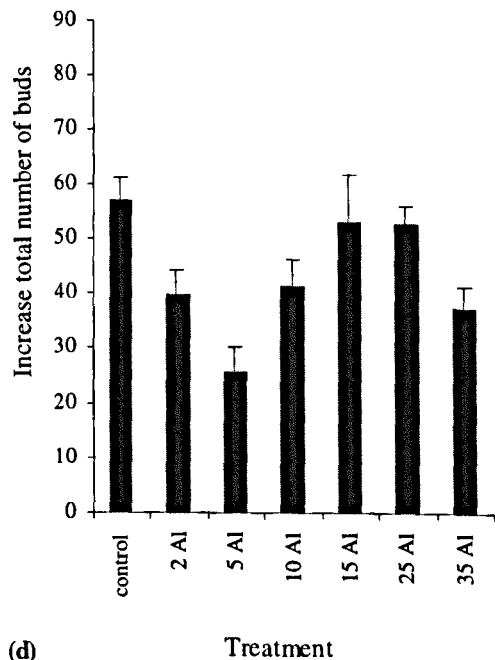
Figure 5.10. Mean (± s.e.) increase in the total number of buds in *Betula pendula* from (a) Flanders Moss (FM), (b) Sheriffmuir (SMM), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR), grown in 0 (control), 2, 5, 10, 15, 25, and 35 mg Al l^{-1} at pH 4.2.

5.4.3 Seedling height

The growth of seedlings in terms of incremental height over the experimental period was significantly affected by plant origin and treatment (Figure 5.11 and Table 5.1). Seedlings from FM (not significant) and SMM increased in height to a greater extent with Al than without Al. At 35 Al height was significantly reduced in FM seedlings (seedlings were about 4 cm shorter). Seedlings from SMM at 2 Al were significantly stimulated in growth by Al and about 3 times taller than controls. There was no reduction in height at 35 Al in SMM races: seedlings were 1.5-2 times taller than controls (Table 5.2). In both KP and KR races the heights of seedlings were greater than controls when grown at low Al concentrations (2-15 Al) and (2-10 Al). At higher concentrations heights were reduced by about 40 % (Table 5.2).

5.4.4 Dry weights

5.4.4.1 Total, shoot, stem, and root dry weights
Figure 5.12 shows the determined dry weights of seedlings at harvest. Both the plant origin and treatment affected all dry weights (Table 5.1).

At all Al concentrations seedlings from FM, SMM, and KR had greater mean shoot dry weights compared with controls. Mean shoot dry weights were overall greater in FM seedlings. The largest shoot dry weights in FM and SMM races were at 25 Al (significantly higher than controls). Shoot dry weights were significantly greater than controls in plants from KP only at 10, 15, and 25 Al.

Similar to shoot dry weight, root dry weights of seedlings from SMM, were greater in the presence of Al. In contrast root dry weights were significantly greater in seedlings from FM when exposed to higher Al concentrations (15-35 Al), and lower at 2 and 5 Al. Root dry weights were significantly greater after exposure to 25 and 35 Al in KP, and did not vary much in KR seedlings.

Stem dry weights increased significantly in FM races at the higher 15 and 25 Al concentrations, and in SMM at 10-25 Al.

In FM, SMM, and KR (slightly) total dry weights were greater in the presence of Al than in its absence. Dry weights were highest at 25 Al in FM, SMM, and KP. There was little difference in total dry weights between treatments in KR races.
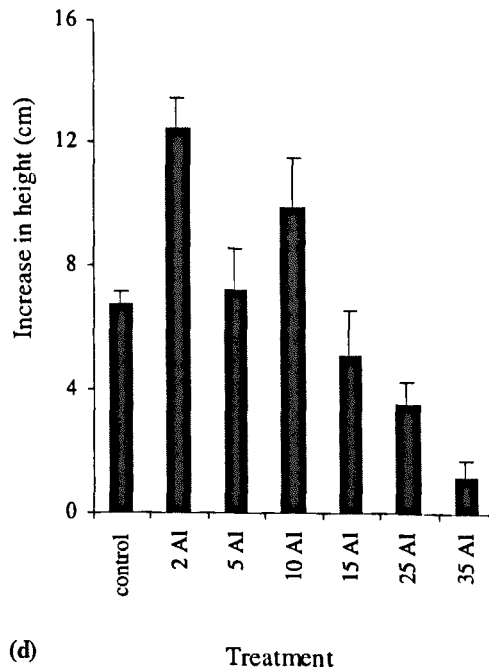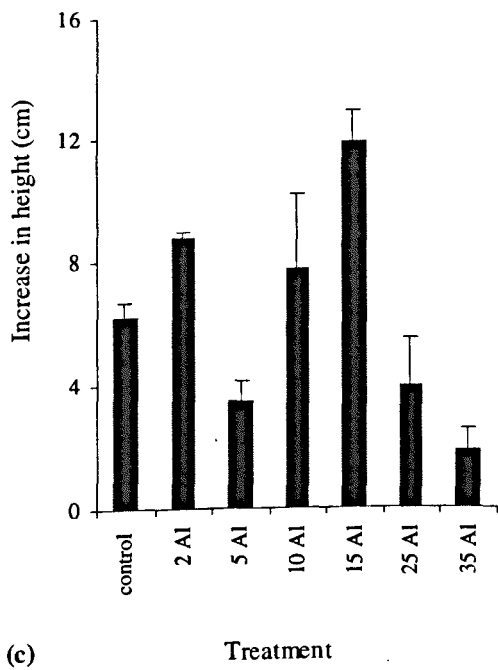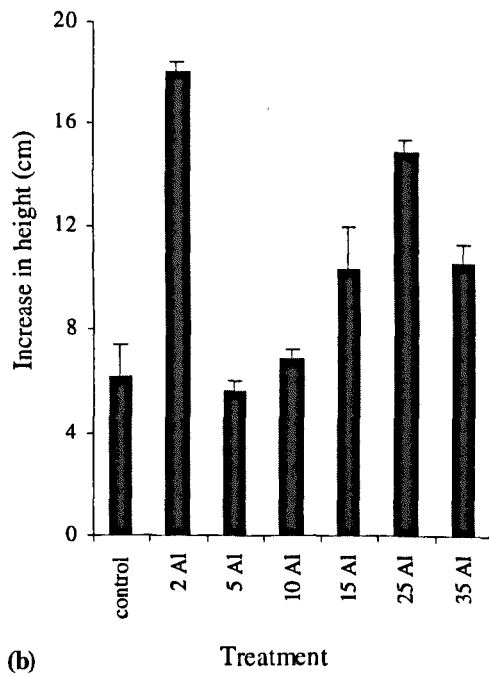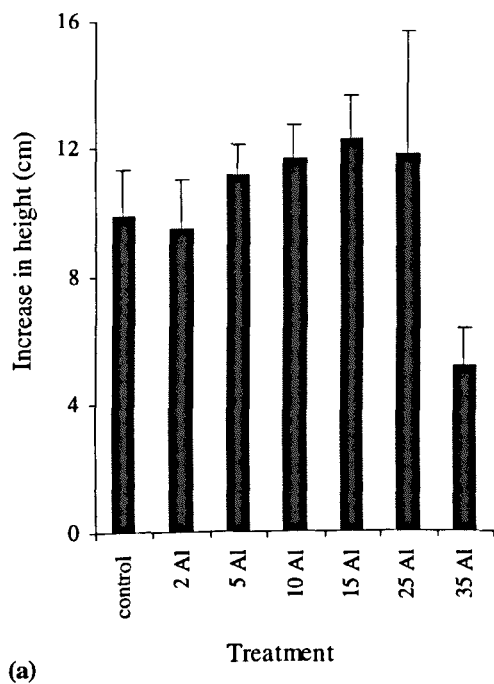
Figure 5.11. Mean (± s.e.) increase in height (cm) of *Betula pendula* from (a) Flanders Moss (FM), (b) Sheriffmuir (SMM), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR), grown in 0 (control), 2, 5, 10, 15, 25, and 35 mg Al l^{-1} at pH 4.2.
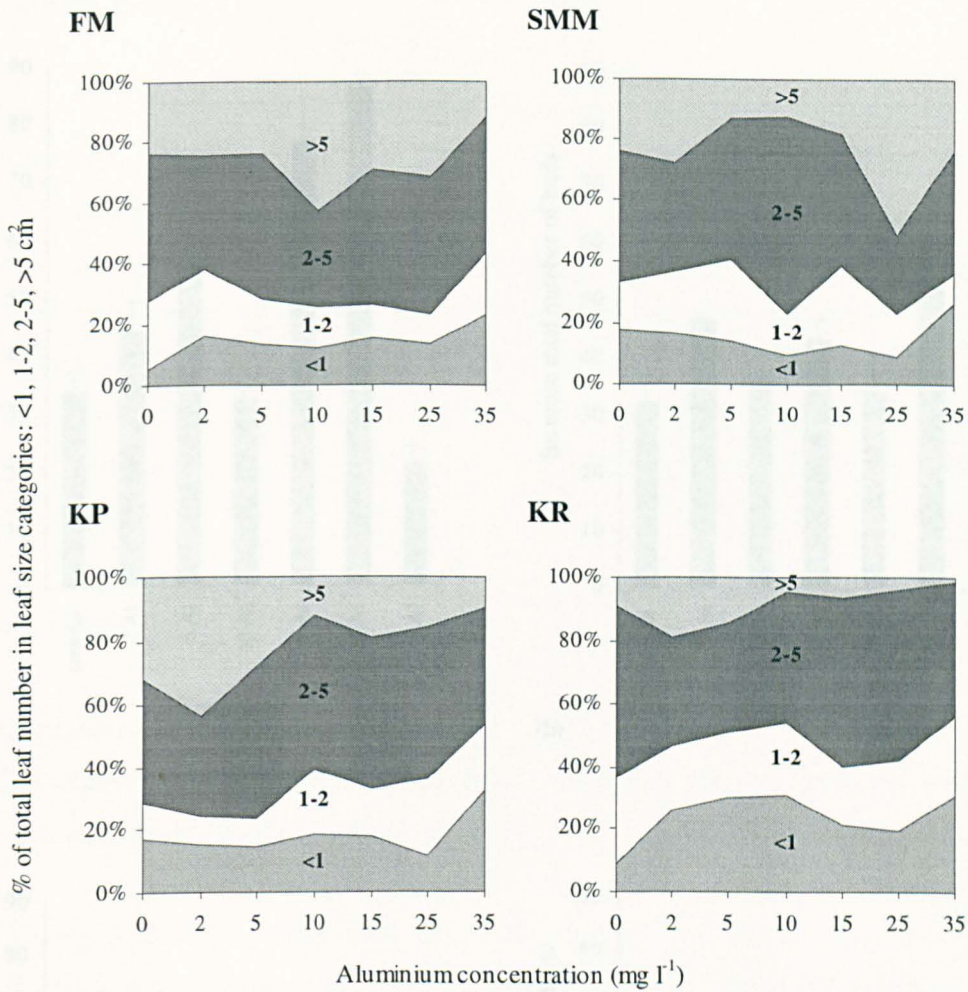
FM
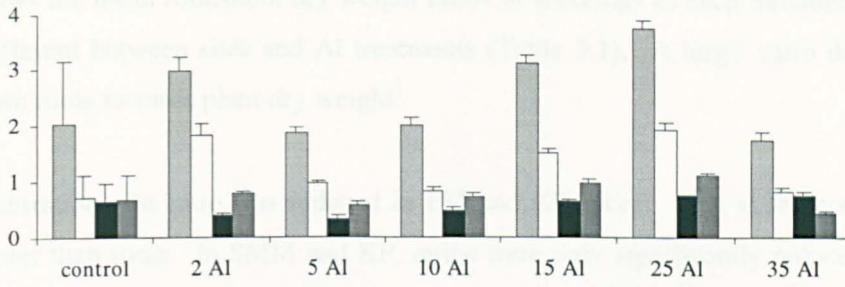
SMM
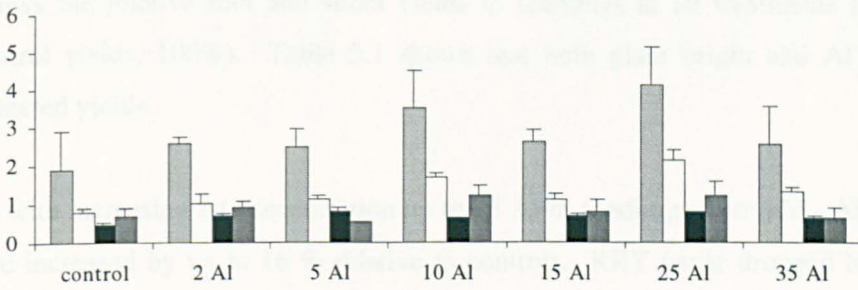
KP

KR

Treatment

Figure 5.12. Total (▨), shoot (☐), root (■), and stem (▨) dry weights of *Betula pendula* from Flanders Moss (FM), Sheriffmuir (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR) grown in 0 (control), 2, 5, 10, 15, 25, and 35 mg Al l^{-1} at pH 4.2.

5.4.4.2 Root:shoot ratio

Figure 5.13 shows the mean root:shoot dry weight ratios of seedlings in each treatment. Ratios were significantly different between sites and Al treatments (Table 5.1). A larger ratio depicts a greater contribution from roots towards plant dry weight.

At all Al concentrations the ratio was reduced in FM and KR races. That is the contribution from shoots was greater than roots. In SMM and KP, ratios were only significantly reduced at the higher Al treatments: 10, 25 and 35 Al in SMM, and 10, 15, and 25 Al in KP.

5.4.4.3 Relative root (RRY) and shoot (RSY) yields

Figure 5.14 shows the relative root and shoot yields in seedlings at all treatments from each site (relative to control yields, 100%). Table 5.1 shows that both plant origin and Al concentration significantly affected yields.

RRY increased with increasing Al concentration up to 25 Al in seedlings from FM. At 15 and 25 Al root yields were increased by up to 16 % relative to controls. RRY never dropped below 50 % of controls (51.6 % at 5 Al). All Al concentrations stimulated root production in SMM, RRY were always >100 % and greatest at 2 Al (173 %). In plants from KP and KR root yield was enhanced by Al (>100 %) at the lower (2 and 5 Al) and higher concentrations (25 and 35 Al). RRY never decreased below 50 % of controls at any site in any treatment.

Greatest RSY were in FM, SMM, and KR seedlings. At all Al concentrations shoot yields were greater than control plants. Up to 200 % enhanced shoot growth. The only reduction in RSY was in seedlings from KP at 2, 5, and 35 Al: RSY were about 50 % (at 2 and 5 Al) and 88 % at 35 Al. There was a significant negative correlation between RRY and shoot Al. There was no correlation between RRY and root Al, or between RSY and either root or shoot Al.

5.4.5 Chemical analyses of foliage

Table 5.3 shows the chemical analyses of the shoots and roots of seedlings. Solution Al concentration significantly affected the uptake of Al, K, Na, Ca, Mg, and Fe by the roots and their translocation to the shoots (Table 5.1). Plants from different origins reacted differently to the Al treatments. There were significant differences between sites in the uptake of K and Fe by the roots, and K, Na, Ca, Mg, and Al translocation to the shoots (Table 5.1). Uptake of P was not significantly influenced by either Al concentration or plant origin in either roots or shoots (Table 5.1).
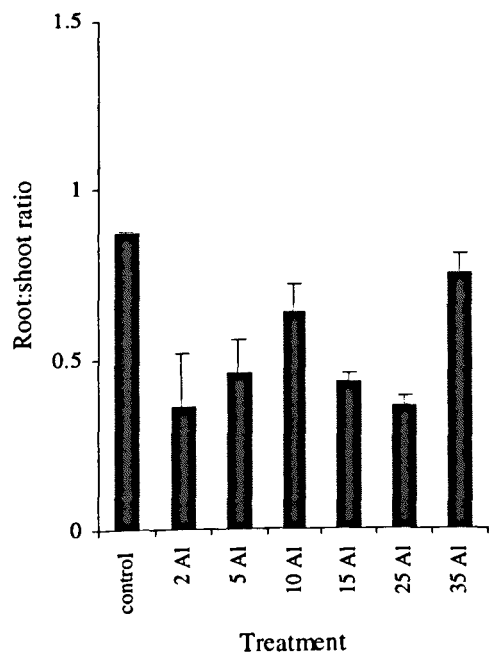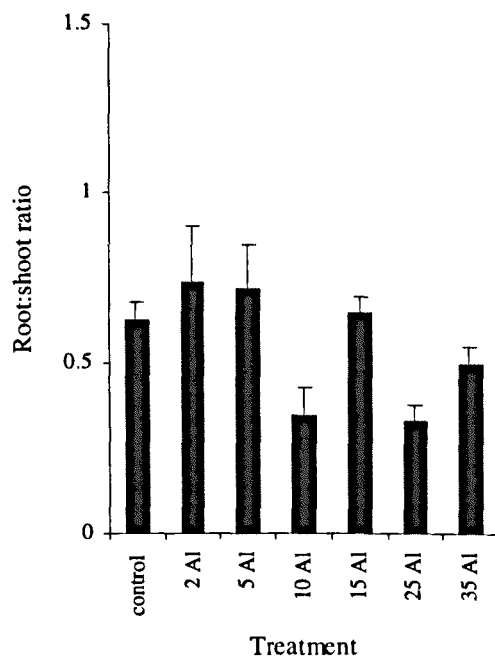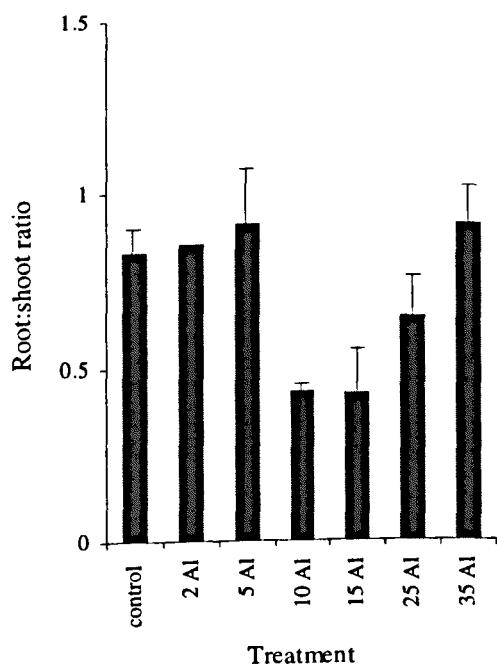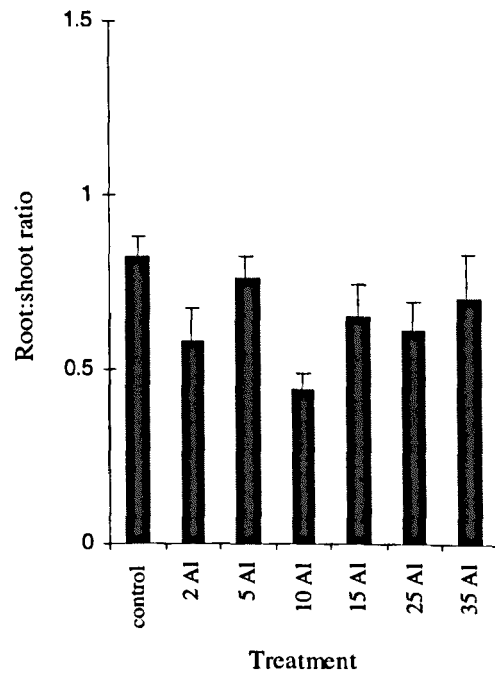
Figure 5.13. Mean root:shoot ratios (± s.e.) of *Betula pendula* from (a) Flanders Moss (FM), (b) Sheriffmuir (SMM), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR), grown in 0 (control), 2, 5, 10, 15, 25, and 35 mg Al l^{-1} at pH 4.2.

(a)

(b)

(c)

(d)
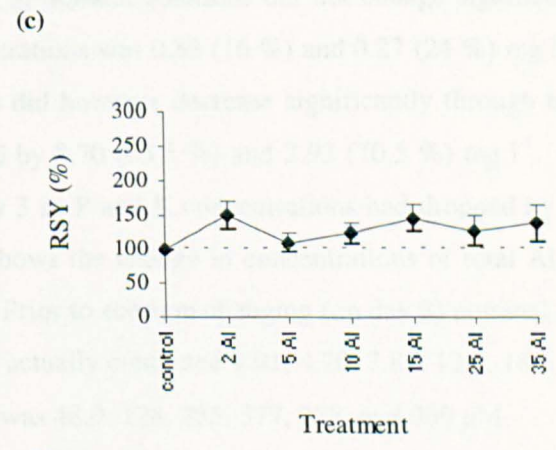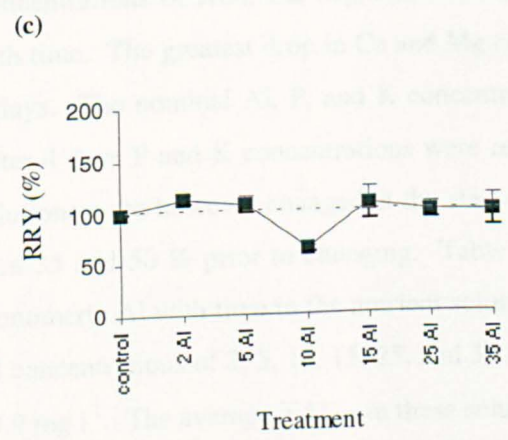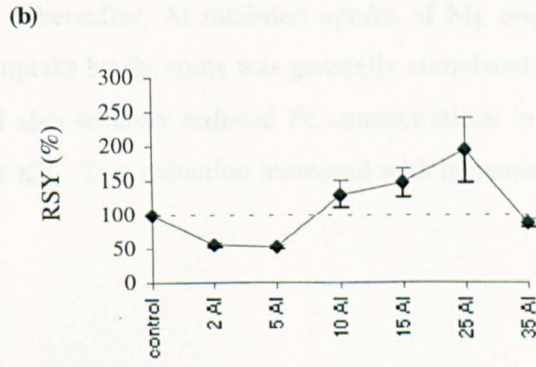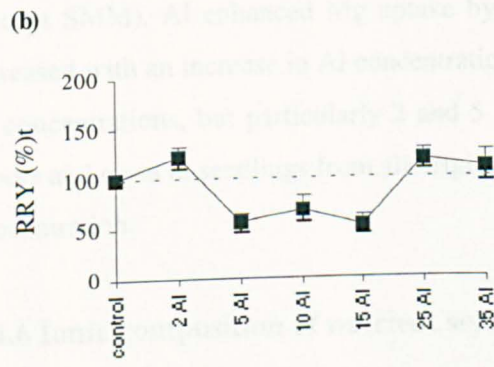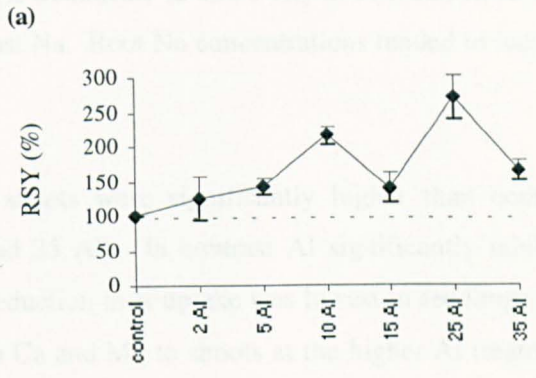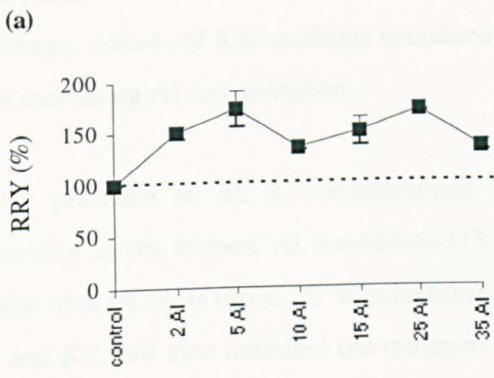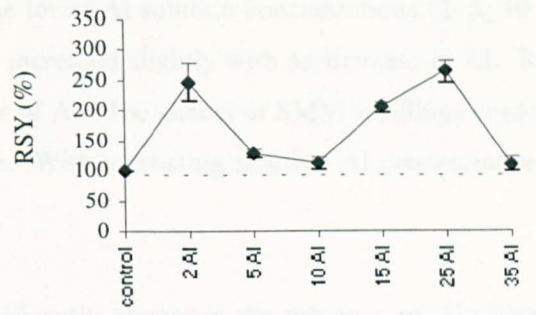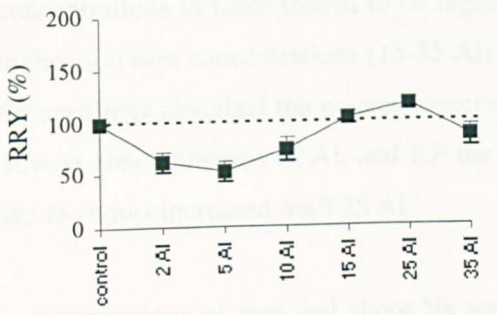
Figure 5.14. Mean (± s.e.) relative root (RRY) and shoot (RSY) yields (%) of *Betula pendula* from (a) Flanders Moss (FM), (b) Sheriffmuir (SMM), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR), grown in 0 (control), 2, 5, 1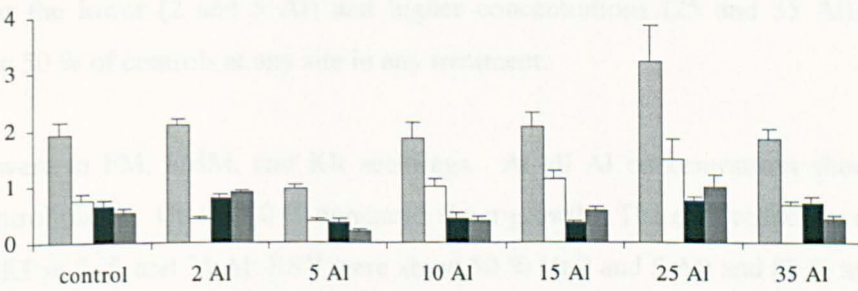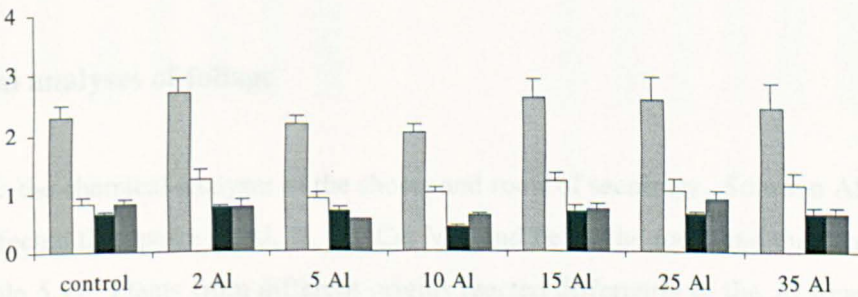0, 15, 25, and 35 mg Al l^{-1} at pH 4.2. 100 % relative root and shoot yields (control plants) are indicated by dashed lines.

Al concentrations in roots tended to be highest at the lower Al solution concentrations (2, 5, 10 Al). At higher solution concentrations (15-35 Al) uptake increased slightly with an increase in Al. Roots of KR seedlings absorbed the greatest concentrations of Al. The shoots of SMM seedlings contained the lowest concentrations of Al, and KP the highest. With increasing solution Al concentration, Al uptake to shoots increased until 25 Al.

The concentrations of root and shoot Na were significantly greater in the presence of Al compared with controls. Shoot Na was highest at the lowest Al treatments (2 and 5 Al) in FM, SMM, and KR seedlings. Shoots of KR seedlings contained the most Na. Root Na concentrations tended to increase with increasing Al concentration.

In the presence of Al, K concentrations in the shoots were significantly higher than controls, especially at the highest Al treatments (15, 25, and 35 Al). In contrast Al significantly inhibited uptake of K by roots (up to 25 % reduction). The reduction in K uptake was lowest in seedlings from KP and KR. Al also inhibited the transport of both Ca and Mg to shoots at the higher Al treatments (10-35 Al). The reduction in shoot Ca concentration was rarely more than 0.5 mg g^{-1}. At 2 and 5 Al (except SMM), Al enhanced Mg uptake by roots. Thereafter, Al inhibited uptake of Mg and this increased with an increase in Al concentration. Ca uptake by the roots was generally stimulated at all Al concentrations, but particularly 2 and 5 Al. Al also severely reduced Fe concentrations in both shoots and roots in seedlings from all origins except KR. This reduction increased with increasing Al concentration.

5.4.6 Ionic composition of nutrient solutions

Concentrations of NO_3, Ca, Mg, Na, Fe, and SO_4 in nutrient solutions did not change significantly with time. The greatest drop in Ca and Mg concentrations was 0.83 (16 %) and 0.27 (24 %) mg l^{-1} in 4 days. The nominal Al, P, and K concentrations did however decrease significantly through time. After 4 days P and K concentrations were reduced by 8.70 (63.5 %) and 2.93 (70.5 %) mg l^{-1}. The solutions were however changed at the start of day 3 so P and K concentrations had dropped by less than 35 and 50 % prior to changing. Table 5.4 shows the change in concentrations of total Al and monomeric Al with time in the nutrient solutions. Prior to solution changing (on day 3) nominal total Al concentrations of 2, 5, 10, 15, 25, and 35 mg l^{-1} actually contained 1.01, 4.20, 7.81, 12.0, 18.6, and 29.9 mg l^{-1}. The average ΣAl_{mono} in these solutions was 48.0, 128, 255, 377, 778, and 999 µM.

Table 5.3. Mean ionic composition (mg g^{-l} oven-dried plant material, ± s.e) of *Betula pendula* roots and shoots from *Flanders Moss* (FM), *Sheriffmuir mineral soil* (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR) treated with 2, 5, 10, 15, 25, and 35 mg Al l^{-1}. Control seedlings were grown at pH 4.2 with no added aluminium.

Treatment	P Shoot	P Root	K Shoot	K Root	Na Shoot	Na Root	Ca Shoot	Ca Root	Mg Shoot	Mg Root	Al Shoot	Al Root	Fe Shoot	Fe Root
FM							mg g^{-1}							
Control	1.55 ±0.53	5.94 ±0.65	6.01 ±1.09	12.39±2.4	0.13 ±0.06	3.33 ±0.82	2.02 ±0.21	1.80 ±0.02	0.80 ±0.15	0.64 ±0.13	0.00 ±0.00	0.00 ±0.00	0.20 ±0.05	1.85 ±0.72
2 Al	3.04 ±0.53	3.68 ±0.34	11.48±1.20	5.29 ±1.35	0.30 ±0.08	4.22 ±1.49	2.98 ±0.25	1.99 ±0.12	2.01 ±0.46	0.48 ±0.16	0.18 ±0.07	0.59 ±0.11	0.19 ±0.01	0.52 ±0.16
5 Al	1.31 ±0.11	4.07 ±0.95	6.69 ±1.41	3.53 ±0.89	0.61 ±0.23	4.89 ±1.12	2.31 ±0.28	2.55 ±0.12	1.03 ±0.17	0.20 ±0.05	0.04 ±0.01	0.15 ±0.02	0.13 ±0.02	0.04 ±0.01
10 Al	2.89 ±0.22	5.02 ±1.32	8.62 ±0.74	4.68 ±1.10	0.14 ±0.01	5.78 ±1.25	1.61 ±0.27	1.57 ±0.18	0.92 ±0.03	0.47 ±0.10	0.27 ±0.03	0.19 ±0.06	0.15 ±0.01	0.20 ±0.04
15 Al	1.28 ±0.22	4.67 ±0.50	6.10 ±0.56	3.65 ±0.59	0.20 ±0.02	4.18 ±0.32	1.33 ±0.26	1.88 ±0.24	0.53 ±0.11	0.06 ±0.02	0.11 ±0.06	0.19 ±0.03	0.16 ±0.04	0.10 ±0.03
25 Al	1.69 ±0.01	4.22 ±0.64	6.34 ±0.75	2.99 ±0.24	0.12 ±0.02	4.29 ±0.22	1.04 ±0.04	1.78 ±0.12	0.41 ±0.06	0.04 ±0.01	0.05 ±0.00	0.37 ±0.09	0.10 ±0.00	0.08 ±0.01
35 Al	1.37 ±0.08	4.67 ±0.27	9.23 ±0.98	3.95 ±0.47	0.19 ±0.04	5.05 ±1.69	1.78 ±0.10	2.16 ±0.26	0.58 ±0.11	0.05 ±0.01	0.26 ±0.05	0.35 ±0.03	0.12 ±0.02	0.10 ±0.00
SMM														
Control	1.58 ±0.10	5.18 ±0.93	6.51 ±0.91	10.65 ±0.30	0.10 ±0.03	2.73 ±0.66	2.31 ±0.36	1.95 ±0.29	1.51 ±0.15	0.62 ±0.10	0.00 ±0.00	0.00 ±0.00	0.33 ±0.12	1.23 ±0.26
2 Al	1.89 ±0.28	7.94 ±3.37	6.51 ±1.36	4.44 ±1.27	0.59 ±0.02	3.90 ±0.71	2.57 ±0.32	1.26 ±0.07	1.06 ±0.13	0.37 ±0.01	0.07 ±0.02	1.05 ±0.06	0.12 ±0.00	0.50 ±0.27
5 Al	1.16 ±0.02	5.00 ±0.01	5.25 ±0.93	6.46 ±1.84	0.16 ±0.01	3.55 ±0.80	1.79 ±0.18	2.61 ±0.51	0.64 ±0.15	0.19 ±0.05	0.16 ±0.02	0.27 ±0.11	0.11 ±0.03	0.11 ±0.00
10 Al	1.11 ±0.25	5.80 ±0.68	7.49 ±0.50	3.90 ±0.22	0.17 ±0.01	5.08 ±0.15	2.00 ±0.09	1.23 ±0.05	0.91 ±0.07	0.40 ±0.06	0.10 ±0.01	0.27 ±0.05	0.15 ±0.01	0.22 ±0.02
15 Al	1.14 ±0.25	3.25 ±0.21	5.95 ±0.11	4.57 ±0.46	0.50 ±0.13	7.67 ±1.02	1.94 ±0.35	1.63 ±0.13	0.75 ±0.09	0.08 ±0.01	0.12 ±0.01	0.26 ±0.06	0.12 ±0.00	0.13 ±0.03
25 Al	1.30 ±0.05	5.21 ±0.18	5.78 ±0.31	2.44 ±0.15	0.09 ±0.02	4.08 ±0.45	1.00 ±0.04	2.39 ±0.14	0.32 ±0.04	0.05 ±0.01	0.04 ±0.01	0.24 ±0.02	0.10 ±0.01	0.10 ±0.01
35 Al	2.11 ±0.01	5.23 ±1.47	10.72±0.40	5.76 ±0.28	0.15 ±0.01	7.07 ±0.31	1.46 ±0.05	3.41 ±0.10	0.55 ±0.05	0.09 ±0.02	0.13 ±0.01	0.31 ±0.03	0.11 ±0.01	0.12 ±0.00
KP														
Control	1.52 ±0.15	3.34 ±0.76	6.05 ±0.57	5.48 ±0.78	0.16 ±0.03	1.86 ±0.32	1.48 ±0.13	2.03 ±0.10	0.74 ±0.13	0.69 ±0.03	0.00 ±0.00	0.00 ±0.00	0.13 ±0.03	0.75 ±0.23
2 Al	1.52 ±0.02	4.85 ±0.10	5.60 ±0.06	4.31 ±1.48	0.20 ±0.01	6.20 ±0.74	2.20 ±0.08	3.29 ±0.17	0.89 ±0.05	0.54 ±0.04	0.08 ±0.01	0.29 ±0.05	0.07 ±0.03	0.24 ±0.12
5 Al	2.00 ±0.09	11.67±3.54	0.72 ±0.10	2.34 ±0.29	0.71 ±0.16	5.88 ±1.21	2.14 ±0.85	2.06 ±0.52	0.97 ±0.28	0.13 ±0.07	0.11 ±0.02	0.31 ±0.08	0.15 ±0.03	0.16 ±0.03
10 Al	1.39 ±0.14	5.71 ±2.07	5.58 ±1.02	5.16 ±1.26	0.16 ±0.05	3.67 ±0.74	0.93 ±0.03	2.20 ±0.38	0.73 ±0.03	0.40 ±0.06	0.26 ±0.07	1.40 ±0.63	0.16 ±0.03	0.49 ±0.18
15 Al	2.54 ±0.75	4.73 ±0.79	6.67 ±0.46	3.27 ±0.07	2.05 ±0.92	3.97 ±0.25	0.99 ±0.19	2.48 ±0.91	0.21 ±0.10	0.07 ±0.00	0.24 ±0.06	0.23 ±0.06	0.13 ±0.02	0.13 ±0.06
25 Al	1.60 ±0.32	4.47 ±0.73	7.09 ±1.10	3.54 ±0.54	1.62 ±0.18	1.97 ±0.20	1.33 ±0.09	1.78 ±0.37	0.63 ±0.02	0.06 ±0.02	0.38 ±0.10	0.33 ±0.05	0.17 ±0.04	0.14 ±0.03
35 Al	1.21 ±0.34	0.65 ±0.29	6.44 ±1.61	4.50 ±0.52	0.80 ±0.05	2.35 ±0.43	1.60 ±0.25	2.48 ±0.14	0.50 ±0.04	0.15 ±0.02	0.17 ±0.06	0.31 ±0.06	0.14 ±0.03	0.13 ±0.03
KR														
Control	1.82 ±0.13	3.49 ±0.03	6.45 ±0.89	5.15 ±1.10	0.09 ±0.01	1.57 ±0.36	1.98 ±0.18	1.33 ±0.26	0.98 ±0.11	0.66 ±0.12	0.00 ±0.00	0.00 ±0.00	0.27 ±0.02	1.05 ±0.19
2 Al	1.80 ±0.43	3.95 ±1.02	5.78 ±0.60	3.75 ±0.28	0.35 ±0.04	3.44 ±0.72	2.90 ±0.18	2.82 ±0.40	1.20 ±0.24	0.45 ±0.04	0.15 ±0.00	0.38 ±0.11	0.13 ±0.00	0.24 ±0.08
5 Al	1.52 ±0.07	3.66 ±1.02	0.37 ±0.06	3.13 ±0.03	8.39 ±0.34	3.45 ±1.40	2.40 ±0.17	2.31 ±0.20	1.00 ±0.11	0.16 ±0.04	0.11 ±0.01	0.66 ±0.02	0.17 ±0.03	0.45 ±0.05
10 Al	2.76 ±0.60	2.20 ±0.12	4.69 ±2.25	3.19 ±0.39	6.67 ±0.51	2.92 ±0.44	1.88 ±0.27	2.34 ±0.27	1.16 ±0.18	0.47 ±0.07	0.12 ±0.01	0.67 ±0.06	0.10 ±0.01	1.18 ±0.12
15 Al	1.61 ±0.26	4.88 ±0.54	8.14 ±1.13	3.16 ±0.31	1.60 ±0.48	8.78 ±0.09	1.73 ±0.13	2.26 ±0.40	0.63 ±0.09	0.11 ±0.04	0.18 ±0.04	0.23 ±0.02	0.22 ±0.03	0.11 ±0.03
25 Al	1.39 ±0.16	2.94 ±0.46	7.97 ±1.36	2.15 ±0.06	0.43 ±0.18	2.24 ±0.16	1.61 ±0.22	2.03 ±0.50	0.61 ±0.17	0.04 ±0.02	0.25 ±0.05	0.29 ±0.08	0.13 ±0.01	0.08 ±0.02
35 Al	1.60 ±0.14	5.56 ±0.83	11.44±0.78	3.66 ±0.56	1.97 ±0.59	5.61 ±0.84	1.79 ±0.08	2.04 ±0.15	0.55 ±0.03	0.09 ±0.06	0.14 ±0.02	0.33 ±0.06	0.14 ±0.01	0.15 ±0.01

Table 5.4. Changes in the concentration of total Al and monomeric Al (ΣAl_{mono}) with time in the nutrient culture solutions: 2 Al, 5 Al, 10 Al, 15 Al, 25 Al, and 35 Al. Solutions were analysed immediately after preparation (0), and one (1), two (2), three (3), and four (4) days later during the first two weeks of experimental treatments.

Solution age (days)	Nutrient Solution					
	2 Al	**5 Al**	**10 Al**	**15 Al**	**25 Al**	**35 Al**
Total Al						
0	1.14±0.04	4.57±0.24	8.37±0.15	12.2±0.17	18.8±0.21	30.9±0.31
1	1.01±0.03	4.13±0.16	7.77±0.11	11.9±0.17	18.7±0.04	30.4±0.19
2	0.89±0.03	3.89±0.05	7.29±0.07	11.7±0.18	18.3±0.09	28.3±0.23
3	0.82±0.03	3.82±0.03	7.13±0.01	11.2±0.07	17.5±0.10	26.2±0.20
4	0.80±0.01	3.82±0.03	7.00±0.01	10.8±0.14	15.4±0.06	24.8±0.13
ΣAl_{mono}						
0	60.3±1.07	157±4.51	302±4.28	469±5.19	993±6.55	1358±59.8
1	50.3±0.67	124±5.99	264±3.94	370±3.16	712±7.28	968±10.9
2	33.5±1.61	102±4.24	198±1.56	291±2.87	628±3.94	669±7.78
3	24.1±1.78	86.9±2.51	146±3.10	197±3.13	312±7.60	272±8.36
4	3.29±0.53	60.3±0.98	104±3.13	92.9±3.59	203±4.36	166±9.33

5.5 Discussion

Birch is regarded as an Al tolerant plant (Clegg & Gobran 1995). Seedlings of *Betula pendula* grown in Ingestad's solution culture with optimal P supply did not show any effect of Al on growth until Al concentrations were greater than 3 mM (80.9 mg l^{-1}). The birch seedlings in this study which originated from SMM, FM, and KP were also tolerant of Al (up to 35 mg Al l^{-1}). The highest concentrations of Al actually stimulated growth in seedlings from SMM but reduced growth in FM and KP. However seedlings from KR were Al-sensitive and growth was reduced at all the Al concentrations used (2-35 mg l^{-1}).

Low concentrations of Al (2 and 5 Al) enhanced growth in seedlings from FM and KP. Root elongation and root number were significantly increased relative to control plants at 2 and 5 Al. Uptake of Ca was stimulated by low Al concentrations, and translocation of both Ca and Mg to the shoots was enhanced at low Al treatments. Despite this increased growth at low Al concentrations, Al concentrations in the roots was also highest at these concentrations. Enhanced growth at low concentrations of Al has also been shown in wheat cultivars by Foy (1984), in *Arnica montana* and *Deschampsia flexuosa* by Pegtel (1987) and in tea by Konishi *et al.* (1985). Similar to this study, although different nutrients, Konishi *et al.* (1985) showed stimulation in P absorption.

Growth was reduced at the higher Al concentrations (15-35 Al) in FM and KP seedlings. Root elongation, root number, and relative growth rate were reduced. However root elongation, number, and relative growth rate increased with increasing Al concentration in SMM plants. Relative growth rate was highest at 35 Al. In contrast RER, root number, and RGR, were significantly reduced at all Al concentrations in KR plants.

The presence of Al stimulated absolute growth rate, leaf production and total leaf area. However the way in which leaf area was affected differed between the tolerant seedlings and the sensitive ones of KR. In FM, SMM, and KR seedlings, even at the highest concentration, total leaf area was increased in the presence of Al. Total leaf area was increased at most Al concentrations in KP seedlings. The lowest increase in total leaf area occurred in KR seedlings. In the sensitive seedlings of KR Al induced the production of many leaves of small area (<2 cm^2). Al also induced higher numbers of smaller leaves in seedlings from FM and KP but to a lesser extent than in those from KR. At lower Al concentrations, in FM and KP seedlings, Al induced the production of large leaves (>5 cm^2). This effect of Al was not apparent in SMM seedlings where there was no reduction in the number of leaves >5 cm^2 in area at the higher Al concentrations. Konishi *et al.* (1985) also observed the development of very large leaves in tea plants grown in nutrient solutions with up to 172.7 mg Al l^{-1}. However they did not quantify leaf area.

Seedlings were significantly taller in FM and SMM plants in the presence of Al. Whereas in KP and KR height only increased at low Al concentrations. Van Praag & Weissen (1985) also showed Al reduced the stem height of *Picea abies* and *Fagus sylvatica*.

Shoot dry weights and RSY increased in the presence of Al in all races. Root dry weights and RRY also increased with Al but only in SMM, FM, and KP races. There was no Al-induced increase in root dry weights in KR plants. The measurement of growth used in assessing the Al affect on growth determines the results obtained. KR seedlings were sensitive to Al but this was not obvious in all aspects of their growth. Shoot dry weight increased in the presence of Al in KR seedlings implying enhanced growth but this was due to an increase in the production of small leaves. Also root dry weights did not change with Al and this was due to the change in the root system from a fine-branching, thin diameter root system to one with stunted thickened laterals. Although KR seedlings were the most sensitive to Al, there was never greater than a 50 % reduction in either RRY or RSY at any Al concentration. Relative growth rates (g g^{-1} day^{-1}) were not significantly reduced at 1 mM (27 mg l^{-1}) Al in *Betula* seedlings grown in nutrient solution by Göransson & Eldhuset (1995), significant reductions were only seen at concentrations greater than 3 mM (81 mg l^{-1}). The races of birch used here were more sensitive to Al. These authors did not investigate the effects of Al on *Betula* at concentrations <1 mM (27 mg l^{-1}).

Root Al concentrations were highest at the lower Al treatments 2 and 5 Al despite the enhanced growth. Between 10 and 35 mg Al l^{-1}, Al uptake by the roots, and transport to the shoots, increased with increasing Al concentration. However the concentrations of Al in the shoots were, even at the highest Al treatment, very low (never above 0.38 mg l^{-1}). In fact they were lower than concentrations of Al in the shoots of field birch (Appendix 2). Malavolta *et al.* (1981) found Al toxicity in sorghum was associated with 0.64 mg g^{-1} of Al in lower leaves and 1.22 mg g^{-1} in upper leaves (Foy 1984). At the higher Al treatments, 10-25 Al, concentrations of shoot Al were greatest in SMM seedlings. This contradicts evidence of Massot *et al.* (1992) where sensitive bean cultivars accumulated greater concentrations of Al in the shoots. In contrast the roots of both SMM and KR plants had the highest concentrations of Al which implies SMM seedling tolerance to Al arises through internal detoxification mechanisms, and KR seedling sensitivity is related to a lack of these internal exclusion mechanisms. However the Al contents were measured in whole roots and Al uptake along the root axis is non-uniform. Godbold *et al.* (1995) found the Al content of roots was concentrated at the root tips. The use of x-ray microanalysis could also have given an indication as to where the Al was located in the roots. Godbold *et al.* (1995) found the Al was mostly in the root cortex cell walls of *Picea abies*. It would have been interesting to see if there was a difference in the distribution of any absorbed Al between the most tolerant seedlings from SMM and the least tolerant from KR. However X-ray microanalysis is at its least sensitive in the area of the x-ray spectrum containing the Al peak (Hodson & Wilkins 1991). Observations on the root cell ultrastructure would have shown if Al induced any disruption in the Golgi apparatus or amyloplast numbers and hence reduced production of excreted polysaccharide materials. There may have been differences between the races in mucilage production, suggested to have protective functions against Al toxicity, which correlated with their degree of Al tolerance.

With the exception of 2 and 5 Al, Al did significantly affect nutrient uptake : K, Mg, and Fe uptake by roots was reduced by Al. Ca, Mg, and Fe transport to the shoots was similarly reduced in the presence of Al. This corroborates with evidence from Massot *et al.* (1992), Malavolta *et al.* (1981), Rengel & Robinson (1990), and Wheeler (1994) where Al toxicity was manifested by a reduction in nutrient uptake. However Ca uptake by the roots was actually greater in the presence of Al than in its absence despite a reduced growth at the higher Al treatments in FM and KP, and at all treatments in KR. This contradicts hypotheses of Al blocking Ca channels, and thereby disrupting ion flow and membrane permeability. Na uptake was also stimulated in the presence of Al, at all concentrations, in both the roots and shoots, and K in the shoots alone. K uptake was also stimulated by Al in Al-tolerant hybrids of wheat and in sunflower (Blamey *et al.* 1986). Processes other than disturbances in ion uptake were causing the depression in growth. The work of Göransson & Eldhuset (1995), who showed that the mechanisms for Ca and Mg uptake by *Betula pendula* seedlings (grown in nutrient solution) still functioned in the presence of Al concentrations as high as 1 mM (27 mg l^{-1}) for several

weeks, corroborates with this study. They highlighted the importance of considering the uptake of nutrients in relation to the plant requirements. Ericsson & Ingestad (1988) demonstrated that the internal concentration of P required for a constant maximal relative growth rate (g g^{-1} day^{-1}) was 60 % of what was found when plants were grown in nutrient solutions with a free P supply (Göransson & Eldhuset 1995). Although a direct comparison is not possible owing to differences in plant ages, concentrations of shoot Na, K, and Fe were no different from concentrations in field plants (Appendix 2). Ca and Mg were significantly lower than field plants (about 20 %) but the actual difference between control plants and Al treated plants, although sometimes significant, was no more than 0.40 mg l^{-1}. To fully assess whether or not the diminished uptake of nutrients by Al was reducing growth, a knowledge of the seedlings ionic compositions at maximal growth rates would be necessary.

The concentrations of P, K, and Al in the culture solutions decreased significantly with time. Within 3 days P had dropped to about 8 - 9 mg l^{-1}. However P uptake by plants in the presence of Al was not significantly different from controls at any Al concentration or in any site, and also generally higher than field values (Appendix 2). Furthermore P concentrations never dropped below the range found within the soil solutions. Culture solutions were changed at the start of day 3 so that retained Al concentrations were never below about 70 % of nominal concentrations. The decline in nominal concentrations of P and Al is frequently attributed to the formation of hydroxy-phosphate precipitates (Wheeler 1994).

Göransson & Eldhuset (1995) only saw symptoms of Al toxicity in birch seedlings when solution Al concentrations were 3-6 mM (80.9-161.9 mg l^{-1}) and root tissue Al concentrations exceeded 4.5 mg g^{-1} d.w.$^{-1}$. At these concentrations root tips, and laterals, were swollen and discoloured. In this study symptoms of toxicity in both the roots and shoots of KR birch seedlings were visible at the lower Al concentration of 35 mg l^{-1}. Also the roots only contained 0.33 mg g^{-1} Al when these symptoms were apparent. In an earlier study, Göransson & Eldhuset (1991) found *Pinus sylvestris* was more sensitive to Al than *Picea abies*, and symptoms of toxicity were visible at 5.4 mg Al l^{-1}. *Betula* appear to be more Al-tolerant than *Pinus* but less tolerant than *Picea*.

The races of *Betula pendula* differed significantly in their tolerance to Al. The races were ranked in increasing order of Al tolerance as : KR<KP<FM<SMM. The results favoured the interpretation that growth damage occurred because of Al-induced changes in cell replication and root growth rather than disturbances in nutrient uptake. The differential tolerance of Al by the races also follows the pattern of Al concentrations in the soils from where the plants originated (Chapter 3). SMM soils have the highest concentrations of total Al and monomeric Al and KR the lowest.

5.6 Conclusions

- Races of *Betula pendula* were ranked in their tolerance to Al: SMM> FM> KP> KR.

- Differential tolerance of Al by birch races was correlated with Al concentrations of their natural soils.

- Low Al concentrations (nominal concentrations of 2 and 5 mg l^{-1}, and actual concentrations of 1.0 and 4.2 mg l^{-1}) enhanced growth in FM and KP races. Root elongation and number, and leaf expansion were greater at these Al concentrations than in control solutions with no Al. Ca uptake by roots, and translocation of Ca and Mg to shoots, were similarly increased.

- Higher Al concentrations (nominal concentrations of 15-35 mg l^{-1}, and actual concentrations of 12.0-29.9 mg l^{-1}) reduced growth in FM and KP seedlings.

- All treatment concentrations of Al reduced growth of KR seedlings and stimulated production of leaves <2 cm^2 in area. Symptoms of Al toxicity were visible in KR plants at 35 Al.

- Root elongation, root numbers, and relative growth rate increased with an increase in Al concentration in SMM plants.

- Root and shoot tissue Al concentrations were highest in SMM plants.

- Al, at higher concentrations (>5 mg l^{-1}) altered nutrient acquisition by plants in all races.

Chapter 6

Al/Si interactions in *Anthoxanthum odoratum* L. and *Holcus lanatus* L.

6.1 Introduction

6.1.1 Speciation of silicon and aluminium

Silicon and aluminium are the second and third most abundant elements after oxygen in the Earth's crust. Silicon exists as silica and silicates, especially in aluminosilicates of rocks and soil minerals. It is liberated very slowly during weathering as monosilicic acid, $Si(OH)_4$, which is available for plant uptake (Baylis *et al.* 1994, Birchall 1990, Birchall 1992, Hodson & Evans 1995). Aluminosilicate minerals weather at different rates: highly weathered soils such as oxisols and ultisols can be quite low in soluble Si, and organic soils (histosols) may be even lower (Hodson & Evans 1995). Jones & Handreck (1969) found concentrations of $Si(OH)_4$ varied from 116 µM to 1115 µM in soil solutions.

Monosilicic acid, $Si(OH)_4$, has a solubility in water about 2000 µM at 25 °C with a pH<7.5. The solubility is similar from pH 2-9 but rises at pH> 9 owing to silicate anion formation: $Si(OH)_3O^-$ (pK_{a1} 9.82/25 °C) and $Si(OH)_2O_2^{2-}$ (pK_{a2} 11.84/25 °C) (Hodson & Evans 1995, Raven 1983).

The speciation of Al is more complex than that of Si (Chapter 1, Section 1.2.3): the octahedral hexahydrate $[Al(H_2O)_6]^{3+}$, abbreviated as Al^{3+}, exists only in very acidic solutions. The aluminium species: $[Al(H_2O)_5(OH)]^{2+}$ or $AlOH^{2+}$ and $[Al(H_2O)_4(OH)_2]^+$ or $Al(OH)_2^+$ appear at the more basic pH's. Minimum solubility occurs at pH 6.5 with the precipitation of aluminium hydroxides ($Al(OH)_3$), and at more basic pH's (about pH 8) still the solubility again rises with the formation of the tetrahedral aluminate anion, $Al(OH)_4^-$ (Arp & Quimet 1986, Baylis *et al.* 1994, Birchall 1990, Martin 1986).

6.1.2 Silicon as a beneficial element

Silicon is regarded as a beneficial element for many plants, especially rice. Si deficiency is known as a major limiting factor for rice growth (Ma & Takahashi 1991, Miyake & Takahashi 1978, Wang *et al.* 1994) and rice shoots typically contain >5 % Si on a dry weight basis (Wallace 1993). Silicon applications, usually as Ca or Na silicates or basic slag, have been shown to increase P uptake by crops and to reduce P fixation in soils (Galvez *et al.* 1987). The beneficial effect of Si on rice was

suggested by Ma & Takahashi (1991) to be the result of a higher P/Mn ratio within the plant and not an increased P availability in the soil. Symptoms of Si deficiency have been reported in tomato plants: retarded growth, increased transpiration rate (> ~30 %), failure of pollination and fruit production, wilting of branches and leaves, and necrosis in leaves and branch tips (Aller *et al.* 1990). Silicon was shown to be beneficial to animals (Carlisle 1972, Schwartz & Milne 1972). Rats and chicks were fed silicon-deficient diets which reduced weight gains and induced changes in the formation and structure of collagenous connective tissue and bone.

Silicon has often been shown to improve the growth of plants under the stress of Mn toxicity (Barceló *et al.* 1993, Epstein 1994). Horst & Marschner (1978) showed Si improved the growth of cowpea when exposed to high Mn. Alleviation of Mn toxicity symptoms in maize were not due to a Si-induced decrease in Mn uptake, but to an increased tolerance of high Mn concentrations in the plant tissues (Barceló *et al.* 1993). In agreement, Williams & Vlamis (1957) found Si prevented the formation of necrotic spots of high Mn concentrations in barley leaves without reducing the overall Mn concentration in the tops. Silicon has similarly been shown to alleviate Mn toxicities in beans, lettuce, potato, rice, rye, ryegrass, sugarcane, and tomato (Galvez *et al.* 1987, Lewin & Reimann 1969, Okuda & Takahashi 1965, Peaslee & Frink 1969).

The essentiality of Si is still not accepted (Ma *et al.* 1997) except for growth in diatoms where Si-starvation studies imply that it is essential for DNA and chlorophyll synthesis (Exley *et al.* 1993). Epstein (1994) considers Si to be greatly undervalued in plant nutrition studies since plants are naturally exposed to Si in soil solutions and growing them without Si in hydroponic cultures produces plants which he feels are "in important aspects experimental artefacts".

6.1.3 Aluminium/silicon interactions

More recently, studies have shown a unique interaction between Si and Al. Birchall *et al.* (1989) when studying Al toxicity in Atlantic salmon fry (*Salmo salar* L.) showed a marked increase in the survival, and reduction in gill damage, of fry in the presence of Si. There was also a significant reduction in the uptake of Al: fish only absorbed 0.4 μM (0.01 mg) Al per gram dry mass in treatment with 7 μM (0.19 mg l^{-1}) Al and 93 μM Si, compared with >2 μM (>0.05 mg) Al in treatments with 7 μM Al (0.19 mg l^{-1}) and only 0.6 μM Si. Birchall *et al.* (1989) suggested that Al in the presence of Si was unavailable for binding at the gill epithelial sites or for systemic absorption due to the formation of Al-Si complexes known as hydroxyaluminosilcate (HAS) species and later work suggested that Si played a major role in the rejection of Al by biological systems (Birchall 1990, Hodson & Evans 1995). The reaction of silicic acid with Al to form HAS species reduced the biological availability, and hence toxicity, of Al. Since these first demonstrations in fish, this unique bioinorganic chemistry

between Al and Si, has also been confirmed in man. It is now established that gastro-intestinal absorption of Al is greatly reduced in the presence of $Si(OH)_4$, and that the intake of dietary $Si(OH)_4$ influences the excretion of Al via the kidneys implying an involvement in Al homeostasis (Bellia *et al.* 1994, Birchall *et al.* 1996). Most recently, silicic acid has been connected with the possible association between Al and the occurrence of Alzheimer's disease (Birchall 1992, Birchall *et al.* 1996).

Birchall (1992) has suggested that in non-plant systems "the role of silicon (as silicic acid) is to aid the exclusion of Al from organisms". The question of whether or not this is a general mechanism of Si, and is also true of plants, has been the basis of recent research.

6.1.4 Examples of Al/Si interactions in plants

The work which has been carried out on Al/Si interactions in higher plants has often shown both alleviative effects of Si, and no or little alleviation (Hammond *et al.* 1995). Silicon amelioration of Al toxicity has been shown in barley, corn, sorghum, soybean, and teosinte. In contrast Si did not have any ameliorative effects in cotton, pea, and rice (Hodson & Evans 1995). Contradictory results have been found in wheat when grown with Al and Si.

Hammond *et al.* (1995) grew barley (cv. Bronze) in nutrient solutions (at pH 4.5) with Al (0-1.35 mg l^{-1}) and Si (0-2800 μM). The presence of Si increased total dry weights in all Al treatments and prevented Al-induced inhibition of root elongation. Root length was equal to that of control plants when grown in 25 μM (0.68 mg l^{-1}) Al with 2000 μM Si. Si also restored Ca concentrations in roots and shoots to those approaching those in control plants, and Al uptake by roots was significantly reduced. At 2800 μM Si, Al was not detectable in plants of either 25 μM (0.68 mg l^{-1}) or 50 μM (1.35 mg l^{-1}) Al treatments. The inhibition of root elongation by 20 μM (0.54 mg l^{-1})Al (at pH 4.3) in corn (cv. golden cross bantam) was alleviated by the addition of silicic acid. Increasing the $Si(OH)_4$ concentration from 500 μM to 2000 μM increased the alleviative effect (Ma *et al.* 1997). Both Galvez *et al.* (1987) and Hodson & Sangster (1993) showed ameliorative effects of Si in sorghum grown in the presence of Al (in solutions at pH's 4.5 and 4.0). The latter authors found root growth inhibition by Al (0-2.70 mg l^{-1}) was reduced by adding Si (0-2800 μM), and the root:shoot dry weight ratios were equal to control plant ratios (grown with no Al). However total dry weights were not increased with Si. Like Hammond *et al.* 1995, they found Ca concentrations in cortical cell walls were higher in the presence of Si. Galvez *et al.* (1987) using a higher concentration of Al, up to 296 μM (8.10 mg l^{-1}), found a similar ameliorative effect by Si (0-3600 μM) on root elongation. Si restored the root growth of two cultivars to 50% and 30% and increased the root:shoot ratios to those of the controls. The ameliorative effect of Si on root elongation in soybean was dependent upon the

pH of the growth medium. Greater concentrations of Si were required for amelioration at lower pH values (pH 4.0) where Al was more toxic (Baylis *et al.* 1994). Finally, Barceló *et al.* (1993) showed Si concentrations as low as 4 μM were sufficient to ameliorate depressions in root and shoot length. Si prevented growth inhibition at [Al]$_{mm}$ concentrations as high as 35 μM (0.94 mg l^{-1}) at pH 4.0. In agreement with Hammond *et al.* (1995), they found plants grown in both Al and Si had lower tissue concentrations of Al. Root Si concentrations increased with increasing Al concentrations in solutions (0-3.24 mg l^{-1}). Likewise Si concentrations in root cortical cell walls of Al-tolerant *Picea abies* increased after treatment with Al (6000 μM) (Hodson & Wilkins 1991).

Okuda & Takahashi (1965), M.J. Hodson & A.G. Sangster (unpublished), and K.E. Hammond & M.J. Hodson (unpublished) were unable to detect any amelioration of Al toxicity (in solutions at pH's 5.5, 4.5, and 4.5) by Si in rice, wheat, or pea (Hodson & Evans 1995). Similarly, Li *et al.* (1989) showed no effect of Si, ranging from 0-2800 μM, on the toxicity of Al (2.70 mg l^{-1}) to cotton growth. However they did show a slight decrease in [Al] with the addition of Si. In contrast to the unpublished results of Hammond & Hodson, Cocker *et al.* (1998) found Si did ameliorate Al-induced reductions in root growth in both tolerant and sensitive-Al cultivars of wheat (cv. Atlas 66 and Scout 66). 2000 μM Si significantly ameliorated the toxic effects of 100 μM (2.70 mg l^{-1}) Al in Atlas 66 (at pH's < 5.0). Only 5 μM Si was required to prevent any toxic effects of 1.5 μM (0.04 mg l^{-1}) Al in Scout 66. However the presence of Si did not reduce Al uptake by roots.

6.1.5 Plant uptake of Si and Al

Silicon is taken up by plants as Si(OH)$_4$, suggesting Si(OH)$_4$ is the species which crosses the plant membrane. Once inside the plant, transport of Si(OH)$_4$ is almost entirely confined to the xylem. Xylem concentrations of Si are frequently very high suggesting polymeric forms of Si are present and/or chelation with organic complexes is occurring (Hodson & Evans 1995). The high values of pK_{a1} and pK_{a2} of Si(OH)$_4$ relative to the pH of the plant cell cytoplasm (usually 7.0-7.4), vacuoles (< pH 6), and of apoplastic compartments such as the xylem and cell walls (<pH 7), were suggested by Raven (1983) to make it highly unlikely that a significant fraction of Si(OH)$_4$ in plants is present as anions such as "SiO$_3^{2-}$".

Most of the Si(OH)$_4$ taken up by higher plants is eventually deposited as solid amorphous silica (SiO$_2$.xH$_2$O), called phytoliths or "plant stones". Once deposition has occurred no remobilization has been observed (Birchall *et al.* 1996, Hodson & Evans 1995, Raven 1983).

Hodson & Evans (1995) identified four main groups of higher plants with respect to Al and Si uptake and transport :

1) Al accumulators (mostly arborescent dicotyledons)
2) Si accumulators (including wetland and dryland grasses, and fern allies such as *Equisetum sp.*)
3) Moderate amounts of both Al and Si transported to shoots (including gymnosperms and some arborescent dicotyledons)
4) Herbaceous dicotyledons which largely exclude both Al and Si from the shoots.

The accumulation of very high amounts of both Si and Al in plant tissues appears to be mutually exclusive. Dryland grasses have between 1-3 % dry weight as silica which appears to be approximately the silica that would be expected on the basis of passive uptake (Hodson & Evans 1995).

6.1.6 Mechanisms of silicon alleviation of Al toxicity

Hodson & Evans (1995) proposed four possible mechanisms of amelioration of Al-damage by Si: solution effects, co-deposition of Al/Si in the plant, effects in cytoplasm and on enzyme activity, and finally, possible indirect effects of silicon. Wallace (1993) also suggested silicon-induced alteration of the plant cation-anion balance as an explanation for Si alleviation of Al toxicity in Gramineae.

6.1.6.1 Solution effects

It has frequently been suggested that $Si(OH)_4$ reduces the total concentration of soluble and available toxic Al ($[Al]_{mono}$). Ma *et al.* (1997) showed an alleviative effect by Si on Al toxicity in corn, and suggested this was the result of a decrease in toxic concentrations of Al^{3+} through the formation of Al-Si complexes: nominal concentrations of 20 μM (0.54 mg l^{-1}) Al were reduced to about 15 (0.42 mg l^{-1}), 10 (0.27 mg l^{-1}), and 5 μM (0.14 mg l^{-1}) Al with the addition of 500, 1000, and 2000 μM Si. However the studies of Barceló *et al.* (1993) and Cocker *et al.* (1998) found amelioration by Si was not only the result of these solution effects (described above) but also due to a Si-induced reduction in the internal toxicity of Al. Plants grown with or without Si, differed significantly in growth despite the similar $[Al]_{mono}$ concentrations in solution (Barceló *et al.* 1993). Li *et al.* (1989) found concentrations of reactive Al (measured using the Aluminon colorimetric method) did not decline measurably in solutions with 50 (1.35) or 100 μM (2.70 mg l^{-1}) Al, and only by 6-15 % in solutions with 200 μM (3.24 mg l^{-1}) Al.

The formation of HAS species as a mechanism of Si amelioration, involves the reaction of silicic acid with basic Al species from $[Al(H_2O)_5OH]^{2+}$ to $[Al(OH)_4]^-$. These are precursors which lead to the formation of amorphous solids such as protoimogolite, allophanes and crystalline, tubular solid imogolite, $(HO)_3Al_2O_3.SiOH$ (Birchall 1990, Birchall *et al.* 1996). The formation of solids is slow, but it is the early formation of sub-colloidal species which are believed to influence the bioavailability of Al. 100 μM Si has been shown to be required for the formation of stable HAS species which

mostly have a Si:Al ratio of 0.5. Exley & Birchall (1992) showed the formation of HAS species in solutions of low total Al concentration (0.27 mg l^{-1}) and formation increased with pH and higher Si concentrations (up to 500 μM). Both Baylis $et\ al.$ (1994) and Birchall & Chappell (1988) believed the formation of HAS species began from pH 4.0 and upwards. However at these lower pH's the species were unstable hence liberating Al into solution. Farmer (1986) also showed soluble Al-Si complexes with typical Al/Si ratios of 2.2 to 3.5 in synthetic solutions at pH 3.5 to 4.5. The stability of HAS species with respect to competition from other ligands such as carboxylate or phosphate is only significant at pH 6.5 and above (Exley & Birchall 1992).

In those studies of Al/Si interaction described above (Section 6.1.4) it is unlikely that HAS formation is playing an important role in Si amelioration of Al toxicity since they were mainly carried out at pH 4.5. However HAS formation could be important in the rhizosphere where a boundary layer of near neutral pH may exist.

Lumsdon & Farmer (1995) recently cast doubt on the role of HAS species in ameliorating Al toxicity (Exley $et\ al.$ 1997). They determined a solubility expression to describe the formation of a protoimogolite sol the precursors to which are similar, if not identical, to the HAS species described by Exley & Birchall (1992). This equilibrium expression precludes the presence of HAS species in acidic waters. Exley $et\ al.$ (1997), however, provided further support for the HAS hypothesis by showing that Al when present as HAS species was not toxic to fish. They did recognise that at pH's lower than 5.2 the HAS species formed would not be sufficiently stable towards dissolution.

These interactions of hydroxyl Al with silicic acid are unique. No such interactions occur between Si(OH)$_4$ and Ca^{2+} or Mg^{2+} at less than pH 9.0 and it seems likely that formation of HAS species, and hence reduction in bioavailable Al, plays an important role in the amelioration of Al toxicity by Si. However it is also likely that HAS formation is not the only mechanism through which Si achieves such alleviation.

6.1.6.2 Co-precipitation of Al and Si in plants

Chemical analyses of phytoliths and microanalysis have shown Al/Si deposition does occur in plants. Godde $et\ al.$ (1988) found both Si and Al colocalized in the needles of $Picea\ abies$. Hodson & Wilkins (1991) similarly found colocalization of Al and Si but in the root cortical cell walls of $Picea$. Al was not detectable inside the endodermis. Si deposition increased with increasing Al concentration in treatments. Hodson & Sangster (1993) suggested deposits of Al/Si in the outer tangential walls of sorghum roots, were a mechanism for lowering the amount of Al penetrating further into the root cortex. The principal locations of Al in the root do not appear to change greatly in the presence of Si, but the amounts of Al deposited at these sites can increase dramatically. In

agreement with Hodson & Sangster (1993), Barceló *et al.* (1993) believed the formation of aluminosilicate compounds in root cortex cell walls could inhibit uptake of Al into the protoplast.

6.1.6.3 Cation-anion balance

When large amounts of anionic Si (as silicates) participate in the cation-anion balance to add to an excess of anion uptake by plant roots, equivalent amounts of hydroxyl ions are expelled from roots, thereby increasing the rhizosphere pH, and hence decreasing uptake of Al and Fe (Wallace 1993).

6.1.6.4 Effects in the cytoplasm, on enzyme activity, and indirect effects

After entering the cell, aluminium becomes associated with nuclei and mitochondria as well as remaining in the cytosol. The majority of this Al is complexed, either with P-containing compounds or with proteins. Si cytoplasmic concentrations are expected to be much lower than Al (Hodson & Evans 1995).

Hodson & Evans (1995) suggested that Si may ameliorate Al toxicity indirectly through effects on Ca uptake and transport. As described in Chapter 5, Al has frequently been shown to inhibit Ca uptake and transport, and is implicated in Ca homeostasis. The presence of Si along with Al in the media has been found to increase Ca in the root cortical cell walls by Hodson & Sangster (1993), and enhance Ca uptake and transport to the shoots (Hammond *et al.* 1995).

The reports published to date on the Al/Si interactions in higher plants tended to use young seedlings (generally younger than 10 days), short growth periods (usually a maximum of 20-21 days), different plant ages and development stages, different nutrient media, and plants with different initial Si states. Hammond *et al.* (1995), Hodson & Sangster (1993), and Li *et al.* (1989) used nutrient media with background solutions of 0.5M $Ca(NO_3)_2$. Under these conditions experiments must be limited in time to prevent nutrient deficiency limiting growth, the interactions of Al/Si with other nutrients cannot be addressed, and Ca itself has frequently been shown to ameliorate Al toxicity. Soluble Si exists as monosilicic acid over the pH range 2-9, however most studies (Barceló *et al.* 1993, Baylis *et al.* 1994, Galvez *et al.* 1987, Hammond *et al.* 1995, Li *et al.* 1989) add Si as K or Na silicates which may also affect the interpretation of results. These factors may all contribute towards the inconsistency between reports.

6.2. Objectives

- To determine whether or not silicic acid ameliorates Al-induced growth reduction in *Anthoxanthum odoratum* L. or *Holcus lanatus* L. (The reasons for the choice of study species were given in Chapter 2, Section 2.2.)

- To assess any differences in amelioration between different races and relate these to their natural distribution.

- To investigate if, at a single Al concentration, ameliorative effects increase with greater concentrations of Si.

- To establish whether the concentration of Si required increases with increasing Al concentration.

- To investigate whether or not silicic acid prevents Al toxicity by production of hydroxyaluminosilicates which are not in a biologically available form for plant uptake.

- To investigate nutrient uptake by plants in the presence of Al with or without Si.

6.3 Methods

Seeds of *Anthoxanthum odoratum* and *Holcus lanatus* were collected in June 1995 and August 1996 from FM, SMM, KP, and KR (Chapter 2, Table 2.1). The seeds were stored in dry and dark conditions at room temperature until the start of the experiment. Seeds were germinated on acid-washed sand on filter paper in May 1996 (*A.odoratum*) and August 1996 (*H.lanatus*) in the Stirling University growth rooms. The Petri dishes were kept under a photoperiod of 16 h light and 8 h dark with a PAR of 200 μmol m^{-2} s^{-1}. Temperature was 20°C during the day and 15°C during the night. Seeds germinated after seven days (*Anthoxanthum*) and 3-4 days (*Holcus*) and were watered with dilute culture solution (ten times dilution) for about seven days before being transferred into full strength culture solution. At this stage they were removed from Petri dishes and carefully threaded through thin glass tubes with deionised water. The glass tubes were suspended from the lids of 600-ml beakers (Figure 6.1) in an initial culture solution with no added Al or Si and at pH 5.6 (Chapter 4, Section 4.3.1.1).

The composition of the culture solutions was the same as that used in Chapter 4 (Tables 4.1 and 4.2). Stock solutions of 100-strength of NH_4OH, Na_2SO_4, $NH_4H_2PO_4$, KFeEDDHA, $Ca(NO_3)_2.4H_2O$, $CaCl_2.6H_2O$, $Mg(NO_3)_2.6H_2O$, H_3BO_3, KH_2PO_4, $Al(NO_3)_3.9H_2O$, and MES buffer, and 1000-strength of $MnSO_4.4H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, and $(NH_4)_6Mo_7O_{24}.4H_2O$ were made up and diluted appropriately. Beakers were covered in tinfoil to prevent algal growth and arranged in a randomised block design (Figure 6.1). Solutions were stirred daily and their pH adjusted where necessary to pH 5.6 (initial solution) and pH 4.2 (treatment solutions) using 1M NaOH or 1M HCl. Culture solutions were changed twice per week.

6.3.1 Experiment 1

The effects of aluminium and silicic acid on the growth of *Anthoxanthum odoratum* originating from FM, SMB, and KR were determined. Aluminium was added to the culture solutions in the form $Al(NO_3)_3.9H_2O$ at the following concentrations: 1.3 mg l^{-1} (50 μM) and 2.7 mg l^{-1} (100 μM) Al. Silicic acid was added from a stock solution which was prepared by passing sodium silicate solution through a column packed with Amberjet 1200H (H$^+$ form) to give nominal silicic acid concentrations ([Si(OH)$_4$]$_n$) of 500 and 1000 μM in the nutrient solutions. Al and Si(OH)$_4$ were added in the following combinations : 0 mg l^{-1} Al + 0 μM Si(OH)$_4$ (control), 1.3 mg l^{-1} Al, 2.7 mg l^{-1} Al, 1.3 mg l^{-1} Al + 500 μM Si(OH)$_4$, 1.3 mg l^{-1} Al + 1000 μM Si(OH)$_4$, 2.7 mg l^{-1} Al + 500 μM Si(OH)$_4$, 2.7 mg l^{-1} Al + 1000 μM Si(OH)$_4$. The following abbreviations are used corresponding to the treatments: control, 1.3Al, 2.7Al, 1.3Al 500Si, 1.3Al 1000Si, 2.7Al 500Si, and 2.7Al 1000Si. Culture solutions

were kept at pH 4.2. There were 10 replicate seedlings per treatment per site. Solutions were changed every three days.

The number of roots and their lengths, and the number of blades and tillers and their lengths, were recorded before seedlings were put into treatments, on the day treatments began and thereafter at seven day intervals until harvesting.

Seedlings were harvested after 21 days growth in +Al/Si treatments (1 Jun-22 Jun 1996). Roots and shoots were separated, rinsed in deionised water, and dried in an oven at 60 °C for 48 h and the dry weights recorded. Root:shoot ratios were determined.

Between 100 and 300 mg of oven-dried leaves and roots were digested in a sulphuric acid-hydrogen peroxide mixture (Allen 1989) in a block digester at 330 °C. Digested plant material was filtered through No. 44 Whatman filter paper and made up to 100 ml. Concentrations of Ca and Mg were measured using a Varian AA-575 atomic absorption spectrophotometer with a nitrous oxide-acetylene flame. An air-acetylene flame was used to determine K (flame emission) and Fe concentrations. Total Al ($[Al]_T$) was measured with a Pye Unicam SP9 Atomic Absorption Spectrophotometer fitted with a Unicam GF90 furnace and FS90 furnace autosampler. Unicam 919 series atomic absorption software was used. Total Si was not analysed because of the unavailability of the equipment. P was measured on a Tecator FIAstar 5010 flow injection auto-analyser using the stannous chloride-ammonium molybdate method.

6.3.2 Experiment 2

The effects of aluminium and silicic acid on the growth of *Holcus lanatus* originating from FM, SMB, SMM, KP and KR were determined. Aluminium was added to the culture solutions in the form $Al(NO_3)_3.9H_2O$ and at the following concentrations: 25 mg l^{-1} and 35 mg l^{-1} Al. Silicic acid was prepared in the same way as Experiment 1 to give nominal silicic acid concentrations ($[Si(OH)_4]_n$) of 1500 and 2500 μM in the nutrient solutions. Al and Si(OH)$_4$ were added in the following combinations: 0 mg l^{-1} Al + 0 μM Si(OH)$_4$ (control), 25 mg l^{-1} Al, 35 mg l^{-1} Al, 25 mg l^{-1} Al + 1500 μM Si(OH)$_4$, 25 mg l^{-1} Al + 2500 μM Si(OH)$_4$, 35 mg l^{-1} Al + 1500 μM Si(OH)$_4$, 35 mg l^{-1} Al + 2500 μM Si(OH)$_4$, 1500 μM Si(OH)$_4$, and 2500 μM Si(OH)$_4$. The following abbreviations are used corresponding to the treatments: control, 25Al, 35Al, 25Al 1500Si, 25Al 2500Si, 35Al 1500Si, 35Al 2500Si, 1500Si, and 2500Si. Culture solutions were kept at pH 4.2. There were 5 replicate seedlings per treatment per site. Solutions were changed every three days.

Subsamples of 5 ml from each of six beakers, from each of the seven treatments, were withdrawn from fresh culture solution, and from solutions one, two, three, and four days old during the first two weeks of experimental treatments. Solutions were analysed to monitor nominal element concentrations using the same analytical techniques as those in Chapter 3. Thereafter solutions were changed after the first 5-ml extractions on day three. Separate subsamples, collected in the same manner, were filtered through a series of pore sizes: No.1 Whatman, 5 μM, and 0.2 μM. Viskin tubing (Medicell) was used as a dialysis membrane. This had a pore size of 2.4 nm and Molecular Weight Cut Off of 12-14000 which should only allow very small species of Al to pass from the nutrient solutions outside the tubing into the ultrapure water inside. Tubing lengths of 10 cm were cut and heated (not greater than 50 °C) in a dilute EDTA solution (0.01 M) for 1 h. Tubing was then washed in ultrapure water and immersed in 5% v/v HNO_3 which was agitated using magnetic stirrers for at least 24 h. The tubing was once more thoroughly washed and immersed in ultrapure water for a further 12 h. This step was repeated until the water surrounding the tubing had a conductivity <2 μS cm^{-1}. 2.5 ml of ultrapure water was added to each dialysis bag. The tubing was secured using clips and immersed in nutrient solution (extracted subsamples) for 24 h. Al and Si permeating the filter papers and dialysis tubing were analysed using a Pye Unicam SP9 Atomic Absorption Spectrophotometer fitted with a Unicam GF90 furnace and FS90 furnace autosampler, and expressed as the mole fraction of the total Al/Si concentration in the nutrient solution.

The concentrations of ($[Al^{3+}]$) and activities of ($\{Al^{3+}\}$) free Al^{3+} in the nutrient solutions (with or without Si) were calculated by GEOCHEM (Sposito & Mattigold 1980). The concentration of monomeric Al species in the nutrient solutions, $[Al]_{mono}$, was also determined by the 60 s Pyrocatechol violet method as described by Kerven et al. (1989).

Measurements of root and shoot growth were the same as those taken in Experiment 1 but at four week intervals. At harvest, prior to drying, the lateral root growth of seedlings was observed under a binocular microscope. The number and length of lateral roots were estimated from 10 cm lengths of primary root. Leaf area was determined, from scanned images of photocopied leaves, using NIH Image 5b.

Roots and shoots were harvested in the same manner as Experiment 1 after 16 weeks growth (14 Sep 1996-4 Jan 1997), and their dry weights recorded. The proportion of dead foliage was also determined. Digestion of plant material and analyses were the same as in Experiment 1. However total Si was also determined using a Pye Unicam SP9 AAS fitted with a Unicam GF90 furnace and FS90 furnace autosampler.

6.3.3 Experiment 3

Alongside experiment 2, the effects of 35 mg l^{-1} Al and 35 mg l^{-1} Al with 2500 μM Si in solution at pH 5.6 on the growth of *Holcus lanatus* originating from FM, SMB, KP, and KR were determined. These corresponded to the treatments 35Al$_{pH5.6}$ and 35Al 2500Si$_{pH5.6}$. The buffer MES (1000 μM) was used to maintain solution pH.

Subsamples of 5 ml were also withdrawn from these treatments and analysed in the same manner as Experiment 2. Measurements of roots and shoots were recorded as above, and lateral root growth and leaf area measured prior to harvesting and foliar analysis.

(a)

(b)
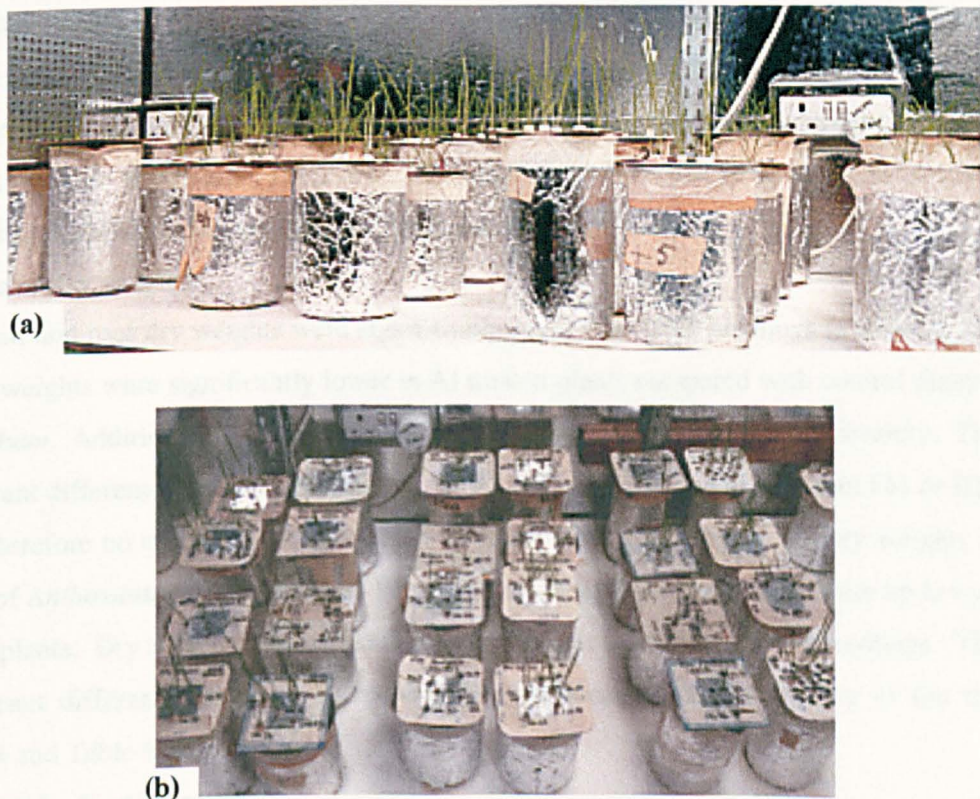
Figure 6.1. Seedlings of *Holcus lanatus* were threaded through glass tubes and suspended from the lids of beakers (a). Beakers were covered in tinfoil to prevent algal growth and arranged in a randomised block design (b).

6.4 Results

6.4.1 Al/Si interactions in *Anthoxanthum odoratum* (Experiment 1)

6.4.1.1 Root elongation and number

Al had a significant effect on total root lengths but not root numbers (Table 6.1). Root growth was inhibited in SMB seedlings but no amelioration was achieved with the addition of silicic acid (Figure 6.2). In FM and KR, 1.3 and 2.7 Al (FM) and 1.3 Al (KR), improved root growth and increased root numbers greater than that of control plants (0 mg Al l^{-1}). The addition of 500 and 1000 μM Si increased both root length and numbers still further (Figure 6.2). Root numbers and lengths were not significantly different between sites. However the Al*Si interaction factor was significant (Table 6.1).

6.4.1.2 Shoot growth and blade number

Neither total shoot length, tiller nor blade number were significantly adversely affected by Al (Table 6.1). Similar to root elongation, Al increased shoot growth and blade number in FM and KR (Figure 6.3). The addition of Si enhanced shoot growth in FM seedlings. There was a reduction in the total shoot length of SMB seedlings in the presence of Al (no reduction in blade number) but the overall effect of Al was not significant (Table 6.1). The addition of 1000 μM Si to solutions of 1.3 mg Al l^{-1} increased shoot lengths to those of control SMB plants (Figure 6.3).

6.4.1.3 Plant dry weights

Total, shoot, and root dry weights were significantly greater in SMB seedlings (Table 6.1 and Figure 6.4). Dry weights were significantly lower in Al treated plants compared with control plants in SMB *Anthoxanthum*. Addition of Si increased total and shoot dry weights but not significantly. There were no significant differences between dry weights of Al and Al+Si treated plants from FM or KR (Figure 6.4) and therefore no overall significant effect of either Al or Si (Table 6.1). Dry weights of shoots and roots of *Anthoxanthum* from FM and KR grown in Al and Al+Si solutions were up to twice those of control plants. Dry weights increased further with greater Si addition in FM seedlings. There were no significant differences between root:shoot ratios between treatments in any of the three sites (Figure 6.4 and Table 6.1).

6.4.1.4 Plant ionic composition

Al significantly reduced uptake of K, Mg, and Fe but not in all three races (Table 6.1 and Table 6.2). Transport of P and Ca to shoots was also significantly reduced in the presence of Al. The addition of Si increased shoot P concentrations of SMB races to concentrations of control plants. There were significant differences between sites in Al uptake by roots and transport to shoots (Table 6.1). SMB plants absorbed the most Al and KR races the least. Al uptake increased with an increase in solution

FM

SMB

KR
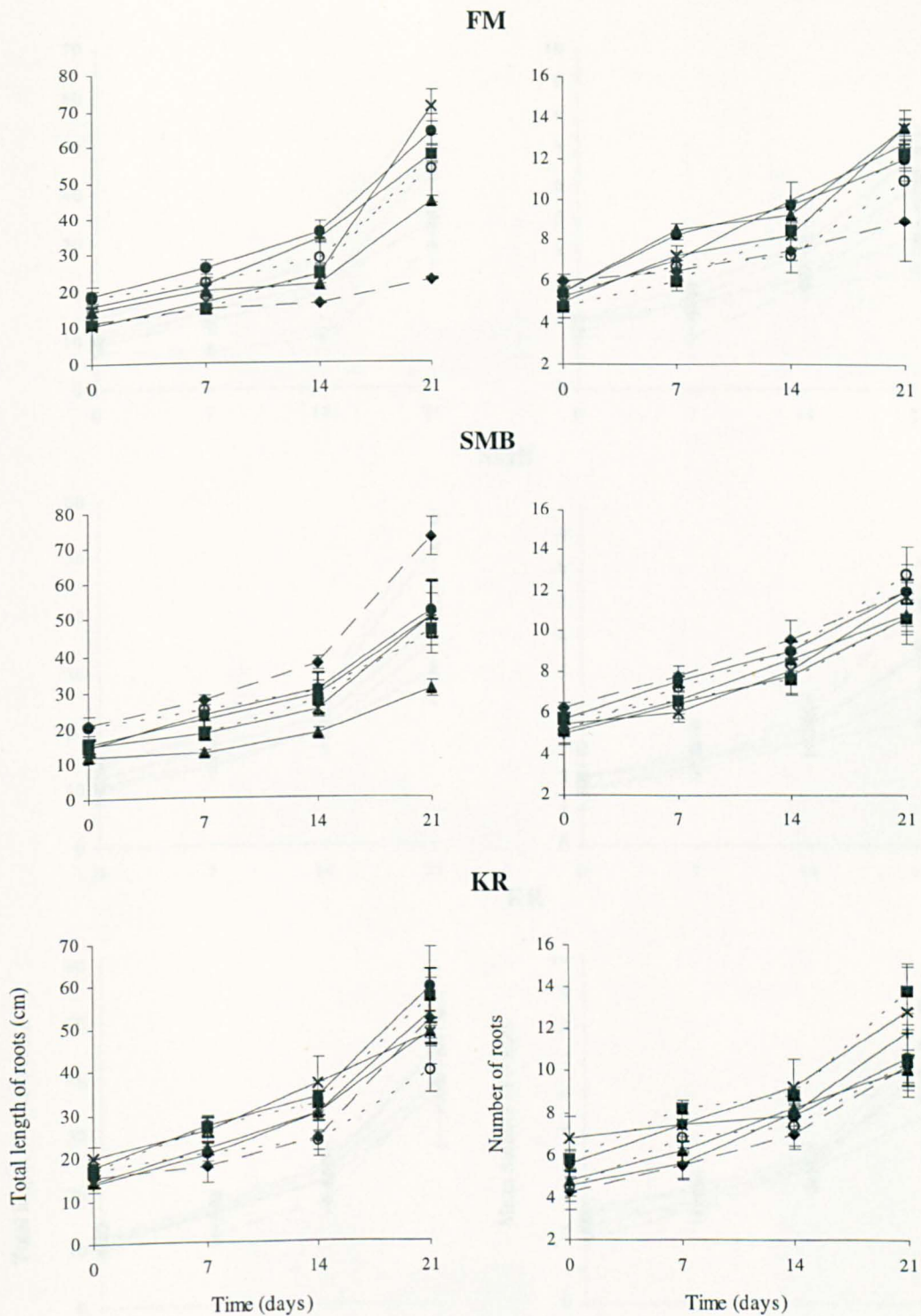
Figure 6.2. The increase in mean total length of roots (± s.e) and root number (± s.e) in *Anthoxanthum odoratum* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), and Kinloch Rannoch (KR) grown in control (◆), 1.3Al (■), 2.7Al (O), 1.3Al 500Si (▲), 1.3Al 1000Si (×), 2.7Al 500Si (●), and 2.7Al 1000Si (+) nutrient solutions. _ _ _ _ , control. -------, Al treatments. _____, Al+Si treatments. Treatments started at day 0.

FM

SMB

KR

Total length of shoots (cm)

Mean number of blades

Time (days)

Time (days)

Figure 6.3. The increase in mean total length of shoots (± s.e) and number of blades (± s.e) in *Anthoxanthum odoratum* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), and Kinloch Rannoch (KR) grown in control (◆), 1.3Al (■), 2.7Al (O), 1.3Al 500Si (▲), 1.3Al 1000Si (×), 2.7Al 500Si (●), and 2.7Al 1000Si (+) nutrient solutions. – – – –, control. -------, Al treatments . _____, Al+Si treatments.

Figure 6.4. Mean total (□), shoot (▨), and root dry weights ■) (± s.e), and root : shoot ratios (■) (± s.e) of *Anthoxanthum odoratum* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), and Kinloch Rannoch (KR) grown in (1) control, (2) 1.3Al, (3) 2.7Al, (4) 1.3Al 500Si, (5) 1.3Al 1000Si, (6) 2.7Al 500Si, and (7) 2.7Al 1000Si nutrient solutions.

Al concentration, particularly in SMB races. Al was transported to the shoots of both FM and SMB races but was not detected in the shoots of KR *Anthoxanthum*. Si reduced translocation of Al to the shoots in both FM and SMB races.

6.4.2 Al/Si interactions in *Holcus lanatus* at pH 4.2

6.4.2.1 Root elongation and number

Figure 6.5 shows the increase in total root lengths at four-week intervals. Between 0 and 4 weeks, before treatments started, there was little difference in root growth between plants. Root elongation was significantly affected by Al and Si between 4 and 12 weeks, but not significantly different between sites (Table 6.3). Al reduced root elongation, the reduction increasing with Al concentration. Total root lengths at harvest were between 30-50 % of control plants (Table 6.4). The addition of 1500 and 2500 μM Si(OH)$_4$ to nutrient solutions with Al improved root elongation. Total root lengths were significantly greater in all Al+Si solutions compared with Al-Si solutions. In general, the greater the concentration of Si the greater the amelioration of Al-inhibited root growth. This was especially pronounced in *Holcus* originating from KR. Relative total root lengths in Al+Si treated plants were generally > 80%. The Al*Si interaction factor was significant at p<0.001 (Table 6.3).

Al also caused a significant reduction in the number of roots in *Holcus* seedlings (Figure 6.6 and Table 6.3). Total root numbers were significantly different between sites (Table 6.3). Relative root numbers were 44.4 % (25 Al) and 51.7 % (35 Al) in KR seedlings which were most affected. Relative total root numbers were never <60 % in FM seedlings (Table 6.4). The addition of Si significantly increased root numbers. This was least apparent in KR seedlings where only the highest Si concentration, 2500 μM, in combination with the lowest Al concentration of 25 mg l^{-1}, prevented a reduction in root number.

6.4.2.2 Lateral root growth

Figure 6.7 shows the mean numbers of lateral roots per cm primary root, and the mean length of laterals (mm). Laterals were significantly reduced in length and number in the presence of Al (Table 6.3). They became stunted and swollen (Figure 6.8). With the addition of Si(OH)$_4$ length of laterals increased up to four-fold and numbers were significantly greater compared with Al-treated roots. This was least apparent in roots treated with 35Al and 1500Si.

Table 6.1. Statistical analyses for root and shoot growth measurements, dry weights, and plant ionic compositions in *Anthoxanthum odoratum* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), and Kinloch Rannoch (KR), and grown in 0 Al, 1.3 Al, 2.7 Al, 1.3 Al 500 Si, 1.3 Al 1000 Si, 2.7 Al 500 Si, and 2.7 Al 1000 Si at pH 4.2. *,$p<0.05$; **, $p<0.01$; ***, $p<0.001$; n.s, not significant. Degrees of freedom are : site 2, Al 2, Si 2, and Al*Si interaction 4.

Measurement		Site F	p	Al F	p	Si F	p	Al*Si interaction F	p
Root growth									
Increase in total root length		0.82	n.s	3.80	*	1.38	n.s	3.34	*
Increase in number of roots		0.55	n.s	2.52	n.s	2.31	n.s	3.70	*
Tops growth									
Increase in total shoot length		2.37	n.s	1.69	n.s	1.58	n.s	1.18	n.s
Increase in total tiller number		2.26	n.s	0.67	n.s	1.57	n.s	1.05	n.s
Increase in total blade number		2.36	n.s	0.56	n.s	1.05	n.s	0.17	n.s
Dry weights									
Shoot		8.52	***	0.58	n.s	0.74	n.s	1.27	n.s
Root		10.87	***	0.44	n.s	0.27	n.s	0.45	n.s
Total		9.49	***	0.54	n.s	0.62	n.s	1.02	n.s
Root:shoot ratio		2.19	n.s	4.87	**	0.43	n.s	0.77	n.s
Ionic composition									
Shoot	P	1.73	n.s	31.26	***	8.41	***	3.78	**
	K	2.03	n.s	26.13	n.s	0.35	n.s	1.26	n.s
	Ca	18.59	***	9.56	***	0.88	n.s	2.01	n.s
	Mg	13.56	n.s	18.71	n.s	0.82	n.s	3.86	**
	Al	126.8	***	215.8	***	4.91	**	15.53	***
	Fe	35.62	***	3.78	*	3.96	*	5.87	***
Root	P	0.70	n.s	14.56	***	0.97	n.s	1.07	n.s
	K	3.17	*	24.05	***	2.64	n.s	1.97	n.s
	Ca	15.55	***	2.13	n.s	0.91	n.s	2.43	n.s
	Mg	6.40	**	19.82	***	2.09	n.s	0.99	n.s
	Al	16.17	***	86.57	***	0.40	n.s	2.49	*
	Fe	14.83	***	7.91	***	7.10	***	6.78	***

Table 6.2. Mean ionic composition (mg g^{-1}, ± s.e) of shoots and roots of *Anthoxanthum odoratum* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), and Sheriffmuir mineral soil (SMM) grown in combinations of Al and Si. †, below detection level (4.2 µg ml^{-1}).

Treatment	P Shoot	P Root	K Shoot	K Root	Ca Shoot	Ca Root	Mg Shoot	Mg Root	Al Shoot	Al Root	Fe Shoot	Fe Root
FM												
Control	8.96	12.10	29.99	39.55	5.91	9.70	1.38	1.15	†	†	0.36	7.25
	±0.73	±0.97	±0.58	±2.45	±2.09	±1.63	±0.05	±0.18			±0.08	±2.09
1.3Al	8.39	13.49	43.08	42.00	2.41	6.68	1.15	1.13	3.45	9.76	0.52	6.86
	±0.26	±0.79	±2.45	±6.08	±0.43	±0.44	±0.04	±0.23	±0.96	±2.26	±0.01	±0.79
2.7Al	7.97	10.06	42.83	31.63	3.07	11.38	2.05	0.56	20.21	10.38	1.53	1.43
	±0.86	±1.25	±5.86	±2.67	±0.74	±2.74	±0.64	±0.25	±3.81	±0.91	±0.16	±0.08
1.3Al + 500Si	8.78	12.29	48.28	38.40	2.98	6.74	1.23	0.84	4.24	9.32	0.37	1.28
	±0.43	±1.19	±1.99	±3.09	±0.41	±1.68	±0.10	±0.07	±0.66	±2.62	±0.10	±0.32
1.3Al + 1000Si	5.83	9.94	38.64	41.78	3.81	6.99	0.77	1.16	2.89	13.50	0.39	3.46
	±0.23	±0.62	±1.69	±3.70	±1.36	±0.66	±0.10	±0.07	±0.69	±0.89	±0.08	±1.33
2.7Al + 500Si	4.56	5.74	39.98	25.40	2.00	5.87	0.77	0.66	2.33	4.31	0.33	0.41
	±0.26	±0.68	±3.89	±3.07	±0.13	±0.89	±0.08	±0.06	±0.54	±1.58	±0.06	±0.07
2.7Al + 1000Si	7.35	9.89	36.52	28.25	1.97	6.32	1.10	1.28	2.74	7.62	0.28	0.43
	±0.69	±1.76	±2.95	±5.68	±0.28	±1.20	±0.09	±0.18	±0.94	±1.22	±0.01	±0.11
SMB												
Control	8.11	12.65	40.53	49.63	2.13	4.29	1.08	1.82	†	†	0.39	4.61
	±0.42	±0.47	±1.88	±2.72	±0.04	±0.30	±0.13	±0.26			±0.01	±0.48
1.3Al	7.82	11.90	38.96	42.48	2.46	5.69	0.94	1.14	1.97	7.78	0.25	3.62
	±0.60	±0.99	±3.18	±4.19	±0.20	±0.41	±0.11	±0.35	±0.12	±1.50	±0.02	±1.07
2.7Al	4.92	17.25	31.20	35.22	2.90	8.28	0.87	1.41	5.75	20.32	0.29	6.44
	±0.65	±5.78	±3.02	±5.46	±0.40	±0.92	±0.11	±0.59	±1.58	±3.39	±0.01	±2.41
1.3Al + 500Si	8.26	13.86	38.05	43.88	2.38	7.47	0.99	1.85	2.81	15.26	0.29	1.27
	±0.33	±1.42	±2.36	±3.43	±0.17	±1.46	±0.07	±0.12	±0.74	±3.68	±0.01	±0.07
1.3Al + 1000Si	6.08	8.73	39.34	42.01	2.61	5.33	0.69	0.73	1.07	7.04	0.27	4.65
	±0.50	±0.49	±4.53	±1.97	±0.14	±0.63	±0.09	±0.13	±0.13	±1.03	±0.02	±0.30
2.7Al + 500Si	6.90	7.32	47.68	28.07	3.33	5.66	0.42	0.80	2.16	11.94	0.42	2.98
	±0.44	±0.99	±1.89	±3.43	±0.97	±1.12	±0.18	±0.15	±0.60	±1.16	±0.06	±0.79
2.7Al + 1000Si	8.47	10.09	40.51	31.88	2.38	8.24	1.02	1.23	2.87	12.88	0.62	6.21
	±0.56	±0.53	±4.88	±2.67	±0.21	±2.64	±0.19	±0.13	±0.44	±2.54	±0.22	±1.83
KR												
Control	9.21	12.30	41.73	44.52	2.51	5.48	1.24	1.58	†	†	0.48	7.16
	±0.92	±1.63	±2.72	±5.68	±0.27	±0.63	±0.05	±0.20			±0.04	±0.89
1.3Al	7.88	10.82	33.58	35.10	1.63	5.31	0.76	0.98	†	7.44	0.50	14.46
	±0.62	±0.72	±4.52	±3.66	±0.11	±0.98	±0.04	±0.14		±1.44	±0.05	±2.63
2.7Al	6.93	10.31	26.83	22.94	3.22	4.84	0.69	1.17	†	5.34	0.61	4.11
	±1.18	±2.62	±5.14	±4.61	±0.60	±0.79	±0.10	±0.26		±0.95	±0.02	±0.94
1.3Al + 500Si	7.79	9.96	29.65	29.54	2.16	3.38	0.61	0.76	1.30	3.81	0.50	2.66
	±0.34	±1.58	±2.14	±5.04	±0.31	±0.75	±0.05	±0.11	±0.15	±1.11	±0.03	±0.64
1.3Al + 1000Si	4.99	5.16	33.63	22.76	1.87	4.30	0.60	0.49	1.50	7.46	0.42	6.41
	±0.38	±0.88	±3.26	±4.09	±0.15	±0.91	±0.07	±0.14	±0.15	±1.15	±0.02	±0.64
2.7Al + 500Si	5.62	8.65	31.25	35.44	2.01	3.66	0.46	0.72	†	6.74	0.48	5.38
	±0.32	±0.37	±1.91	±4.15	±0.23	±0.18	±0.08	±0.10		±0.68	±0.01	±1.47
2.7Al + 1000Si	7.80	11.87	38.95	44.63	1.94	5.17	0.87	1.11	†	6.05	0.44	6.80
	±0.58	±0.27	±0.88	±4.16	±0.28	±0.62	±0.23	±0.11		±0.66	±0.02	±1.67

6.4.2.3 Shoot growth

Al significantly reduced total shoot lengths relative to control plants but the overall effect of Al, including Al+Si treatments, was not significant (Figure 6.9 and Table 6.3). The shoots became chlorotic and died. Figures 6.10(a) and (b) contrast *Holcus* grown in control and Al solutions.
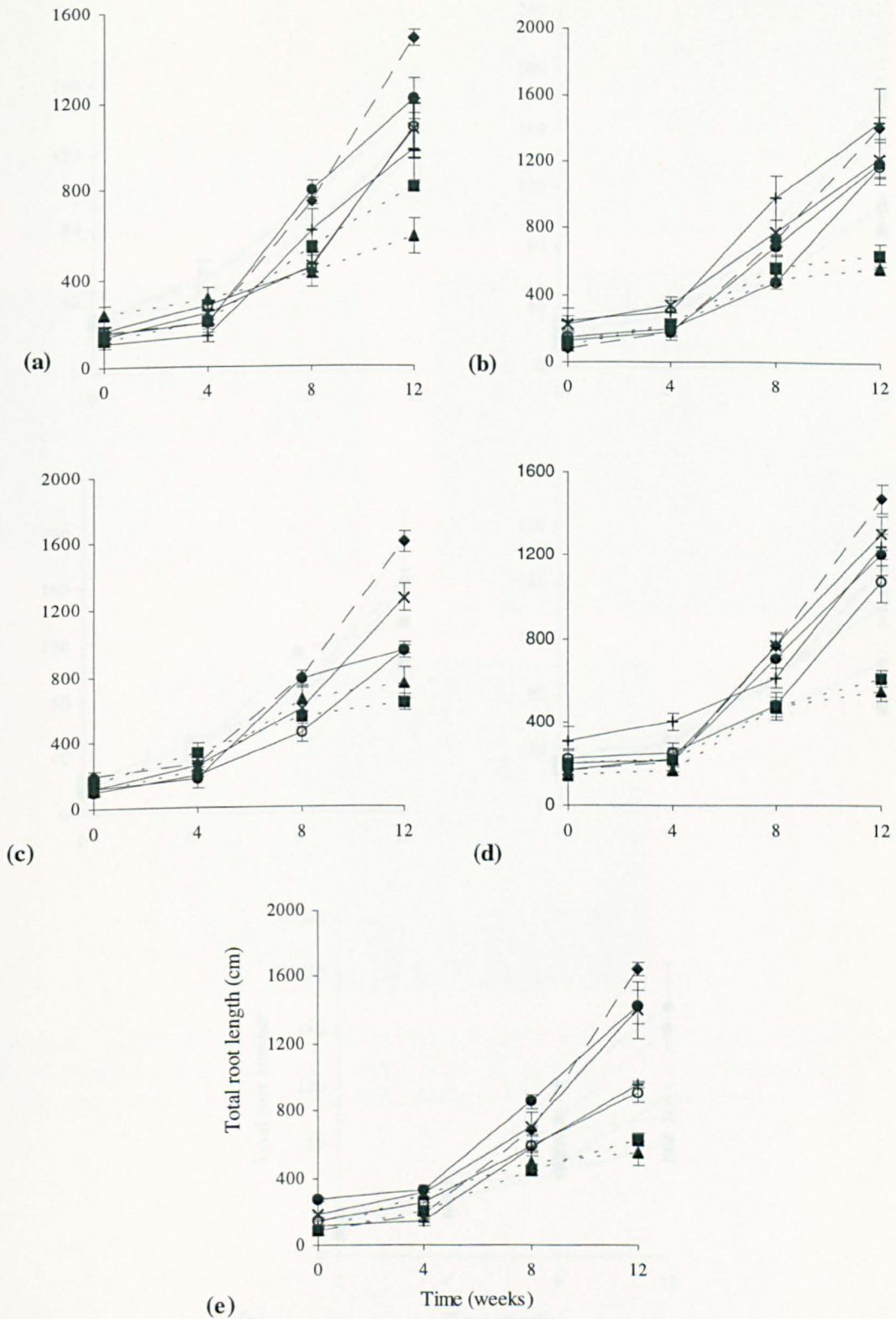
Figure 6.5. Increase in mean total root length (± s.e) in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR) grown in control (♦), 25Al (■), 35Al (▲), 25Al 1500Si (+), 25Al 2500Si (●), 35Al 1500Si (O), 35Al 2500Si (×) nutrient solutions at pH 4.2. ------, Al treatments. _____, Al+Si treatments. _ _ _ _ , controls. Treatments began after 4 weeks and lasted 8 weeks.
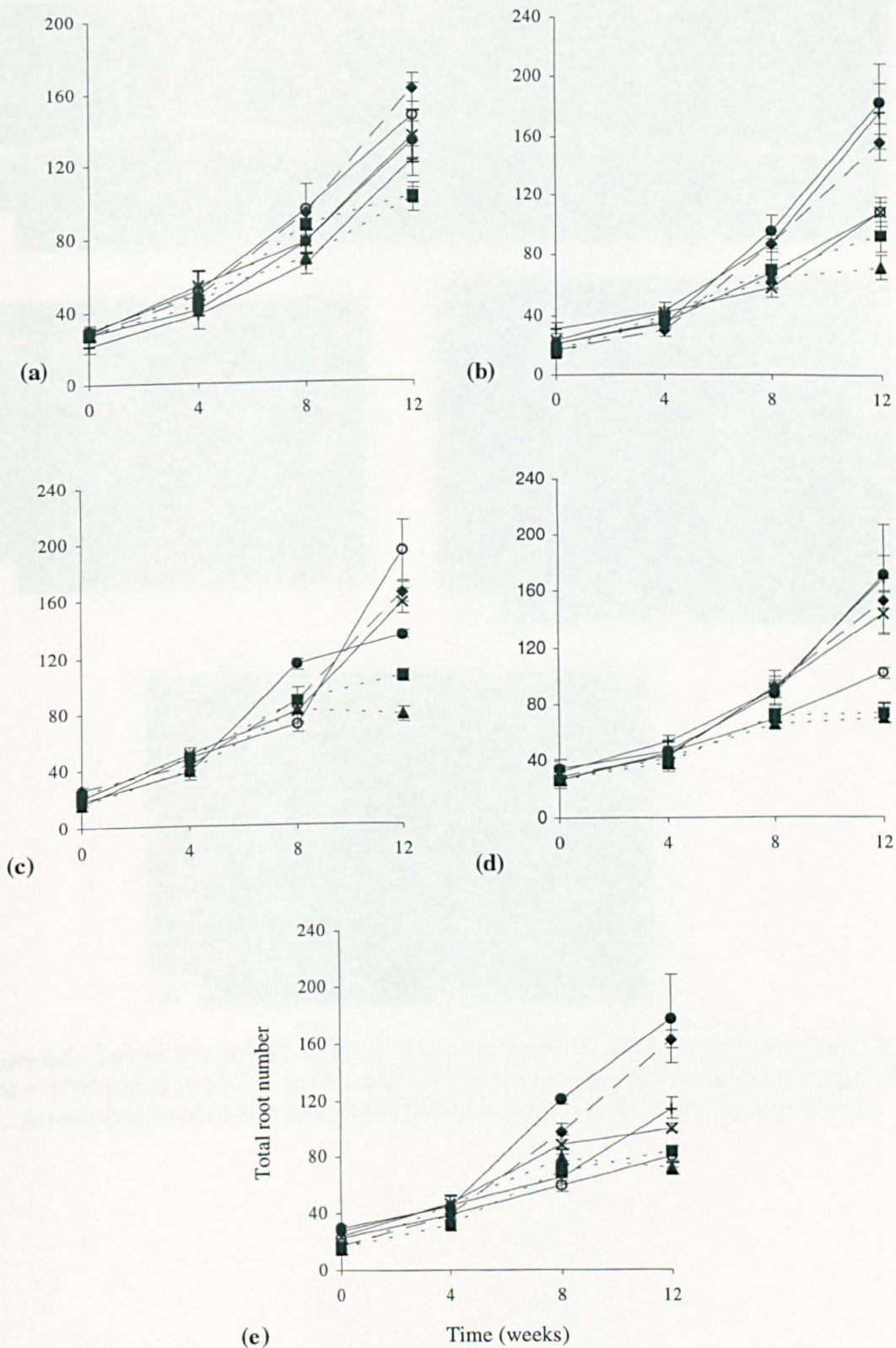
Figure 6.6. Increase in mean total number of roots (± s.e) in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR) grown in control (◆), 25Al (■), 35Al (▲), 25Al 1500Si (✚), 25Al 2500Si (●), 35Al 1500Si (O), 35Al 2500Si (×) nutrient solutions at pH 4.2. ...., Al treatments. _____, Al+Si treatments. – – – – , controls. Treatments began after 4 weeks and lasted 8 weeks.
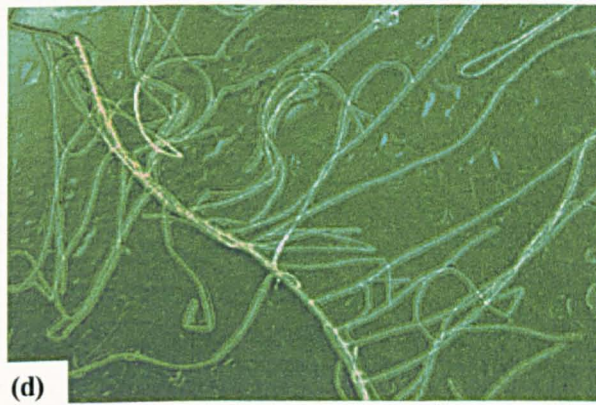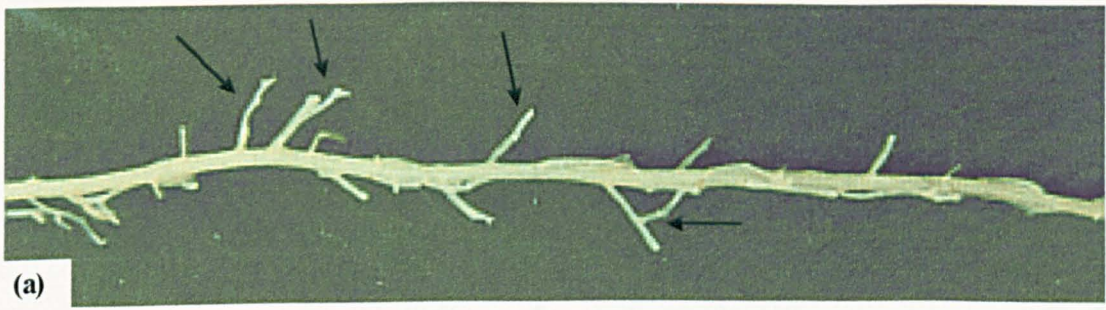
Figure 6.8. Lateral root growth in *Holcus lanatus* treated with (a) 35 mg Al l⁻¹ at pH 4.2, (b) 35 mg l⁻¹ Al + 1500 µM Si at pH 4.2, (c) 35 mg l⁻¹ Al + 2500 µM Si at pH 4.2, and (d) 35 mg l⁻¹ Al at pH 5.6. Arrows indicate stunted and thickened laterals in roots of plants grown in Al at pH 4.2.
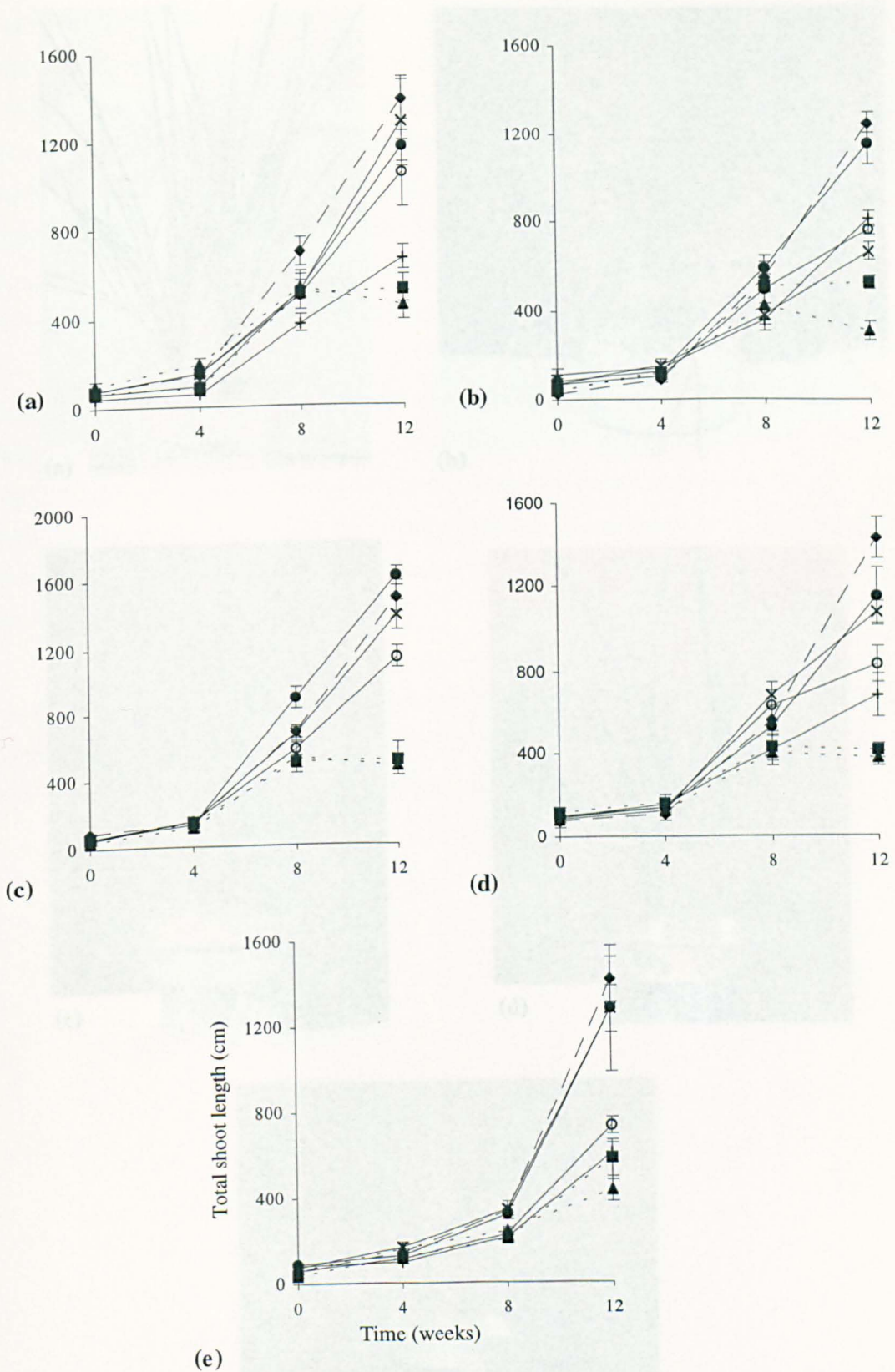
Figure 6.9. Increase in mean total shoot length (± s.e) in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR) grown in control (◆), 25Al (■), 35Al (▲), 25Al 1500Si (+), 25Al 2500Si (●), 35Al 1500Si (O), 35Al 2500Si (×) nutrient solutions at pH 4.2. ------, Al treatments. _____, Al+Si treatments. _ _ _ _, controls. Treatments began after 4 weeks and lasted 8 weeks.

Figure 6.10. *Holcus lanatus* grown in (a) control, (b) 35 mg Al l^{-1}, (c) 25 mg Al l^{-1} + 2500 μM Si, (d) 1500 μM Si, and (e) 2500 μM Si nutrient solutions. All nutrient solutions were kept at pH 4.2.

Although greater, total shoot lengths in plants grown in Al with the lower Si concentration (1500 μM) were not significantly different from shoot lengths in Al treatments alone. However the increased shoot lengths in plants of Al + 2500 μM Si treatments were significantly greater than in Al alone (Figure 6.9). Relative total shoot lengths were as high as 108 % (25 Al 2500 Si, SMM) (Table 6.4). The Al*Si interaction factor was significant at the $p<0.001$ level. Total shoot lengths were significantly different among sites (Table 6.3). Shoot length increased with Si to a greater extent in SMM and FM seedlings. The improvement in shoot growth with the addition of 2500 μM Si to Al-containing nutrient solutions, relative to that in 25 Al, is shown in Figures 6.10 (b) and (c).

Al also significantly reduced tiller number (Figure 6.11 and Table 6.3). Tiller production was not affected by Al in SMM seedlings until after eight weeks growth. However the addition of Si, whether 1500 or 2500 μM, did not significantly improve the vegetative growth of seedlings (Table 6.3). Tiller number was only increased to that of the controls in KR seedlings grown in 35Al 2500Si and 25Al 1500Si solutions. Relative tiller numbers (relative to controls, 100 %) in these plants were 76.7 % and 82.2 %.

Al reduced blade numbers but not always significantly (Figure 6.12 and Table 6.3). Si significantly increased blade numbers to control levels in FM (35Al 2500Si), SMB (25Al 1500Si and 25Al 2500Si), and SMM (25Al 2500Si, 35Al 1500Si, and 35Al 2500Si).

Table 6.3. Statistical analyses for root and shoot growth masurements, dry weights, and plant ionic compositions in *Holcus lanatus* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR), and grown in 0Al, 25Al, 35Al, 25Al 1500Si, 25Al 2500Si, 35Al 1500Si, 35Al 2500Si, 1500Si, and 2500Si at pH 4.2. *,p<0.05; **, p<0.01; ***, p<0.001; n.s, not significant. Degrees of freedom are : site 4, Al 2, Si 2, and Al*Si interaction 4.

Measurement		Site F	p	Al F	p	Si F	p	Al*Si interaction F	p
Root growth									
Increase in total root length		0.53	n.s	4.85	**	6.85	***	61.04	***
Increase in number of roots		3.61	**	12.38	***	9.07	***	33.23	***
Length lateral roots				6.33	**	1.28	n.s	3.57	*
Number lateral roots				11.49	***	23.76	***	8.62	***
Tops growth									
Increase in total shoot length		5.91	***	0.11	n.s	17.06	***	86.61	***
Increase in total tiller number		17.93	***	7.74	***	2.54	n.s	22.25	***
Increase in total blade number		0.12	n.s	0.28	n.s	4.54	*	19.61	***
Leaf Area (FM only)				9.34	***	20.35	***	1.51	n.s
Dry weights									
Shoot		0.25	n.s	0.92	n.s	11.07	***	13.36	***
Root		1.63	n.s	7.94	***	3.09	*	1.25	n.s
Total		0.38	n.s	1.73	n.s	9.80	***	11.41	***
Root:shoot ratio		1.31	n.s	10.35	***	10.76	***	10.79	***
Dead shoot		0.80	n.s	27.84	***	6.35	**	5.27	**
Ionic composition									
Shoot	P	2.10	n.s	276.3	***	0.40	n.s	11.30	***
	K	9.52	***	20.97	***	8.37	***	12.58	***
	Ca	1.91	n.s	20.34	***	13.29	***	8.93	***
	Mg	3.63	*	181.2	***	2.30	n.s	11.12	***
	Al	23.28	***	103.7	***	10.71	***	8.34	***
	Fe	4.59	**	3.13	*	9.89	***	6.57	***
Root	P	2.83	*	3.82	*	49.65	***	16.51	***
	K	14.11	***	12.98	***	39.38	***	8.88	***
	Ca	5.35	***	3.61	*	0.94	n.s	2.15	n.s
	Mg	11.08	***	169.0	***	5.21	**	6.51	***
	Al	14.15	***	76.00	***	1.44	n.s	10.86	*
	Fe	13.05	***	96.82	***	1.71	n.s	3.38	*
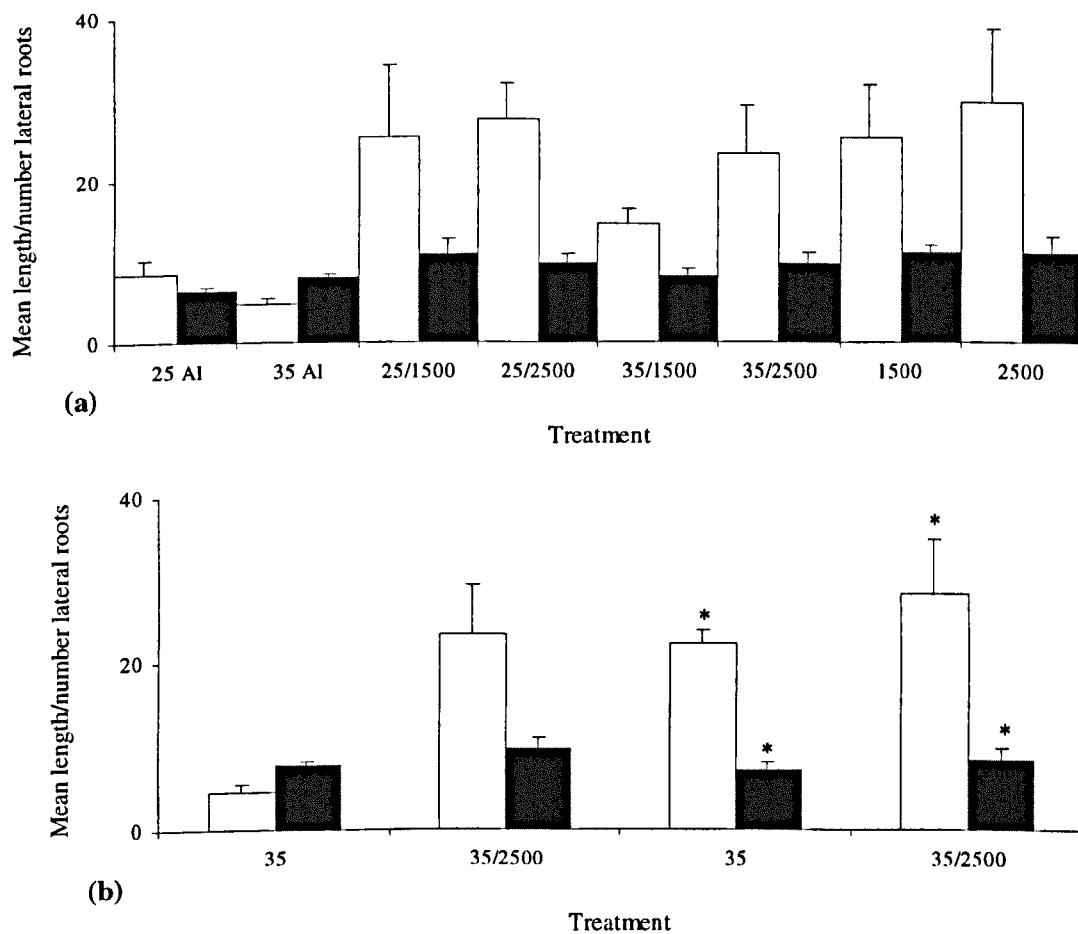
Figure 6.7. Mean length (mm) (□) and number (per cm primary root) (■) of lateral roots (± s.e) of *Holcus lanatus* grown in nutrient solutions containing combinations of Al and Si(OH)₄. (a) Nutrient solutions were adjusted to pH 4.2 (experiment 2), and (b) at pH 4.2 or pH 5.6 (experiment 3). Solutions adjusted to pH 5.6 are indicated by *.

Table 6.4. The mean relative total length of roots (TLR), total number of roots (TNR), total length of shoots (TSL), total number of blades (TNB), and total number of tillers (TNT) in *Holcus lanatus* treated with combinations of Al and Si. All treatments were adjusted to pH 4.2. Seedlings originated from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR). Values are percentages relative to control plants (100 %). n.d, no data.

Relative growth (%)	Control	25 Al	35 Al	25/1500	25/2500	35/1500	35/2500	1500	2500
(a)									
TRL	100	54.6	39.4	66.3	81.5	72.8	72.2	105	64.7
TNR	100	62.7	62.1	75.4	82.1	90.6	83.5	83.1	61.1
TSL	100	38.1	32.8	47.9	84.6	75.7	92.8	73.4	30.9
TNB	100	36.8	35.1	52.0	79.1	74.5	106	36.1	39.4
TNT	100	41.1	40.2	27.0	36.8	41.2	51.0	24.5	24.5
(b)									
TRL	100	43.4	38.4	98.0	81.1	79.7	83.0	103	39.4
TNR	100	59.1	45.7	112	117	69.6	69.6	101	44.8
TSL	100	41.9	24.7	64.6	92.1	60.8	52.9	61.2	30.3
TNB	100	46.6	35.6	91.7	104	72.0	54.6	71.6	40.7
TNT	100	35.0	28.2	53.9	57.3	45.2	29.6	45.6	34.0
(c)									
TRL	100	39.5	46.6	n.d	99.0	59.5	79.1	n.d	38.2
TNR	100	64.3	47.2	n.d	81.9	117	96.0	n.d	61.9
TSL	100	34.4	31.6	n.d	108	76.1	92.9	n.d	60.5
TNB	100	38.5	35.9	n.d	116	96.6	81.3	n.d	96.7
TNT	100	28.5	30.6	n.d	73.6	60.3	61.1	n.d	42.8
(d)									
TRL	100	41.6	37.5	84.1	81.7	73.1	88.7	82.1	46.7
TNR	100	48.4	46.0	110	112	66.8	93.9	78.2	74.8
TSL	100	28.6	26.1	54.9	80.4	57.7	74.8	50.1	49.0
TNB	100	52.6	39.4	57.5	112	75.6	78.2	36.8	57.5
TNT	100	37.5	22.5	82.2	46.1	28.3	76.7	55.8	86.8
(e)									
TRL	100	38.5	33.4	58.3	86.4	55.3	85.1	84.4	27.4
TNR	100	51.7	44.4	70.4	108	49.5	61.9	109	37.2
TSL	100	41.4	30.7	42.7	90.3	51.9	90.4	84.8	44.3
TNB	100	49.5	31.1	50.8	85.0	51.2	77.6	50.2	33.9
TNT	100	38.3	20.0	22.3	32.3	19.7	60.7	38.3	35.8
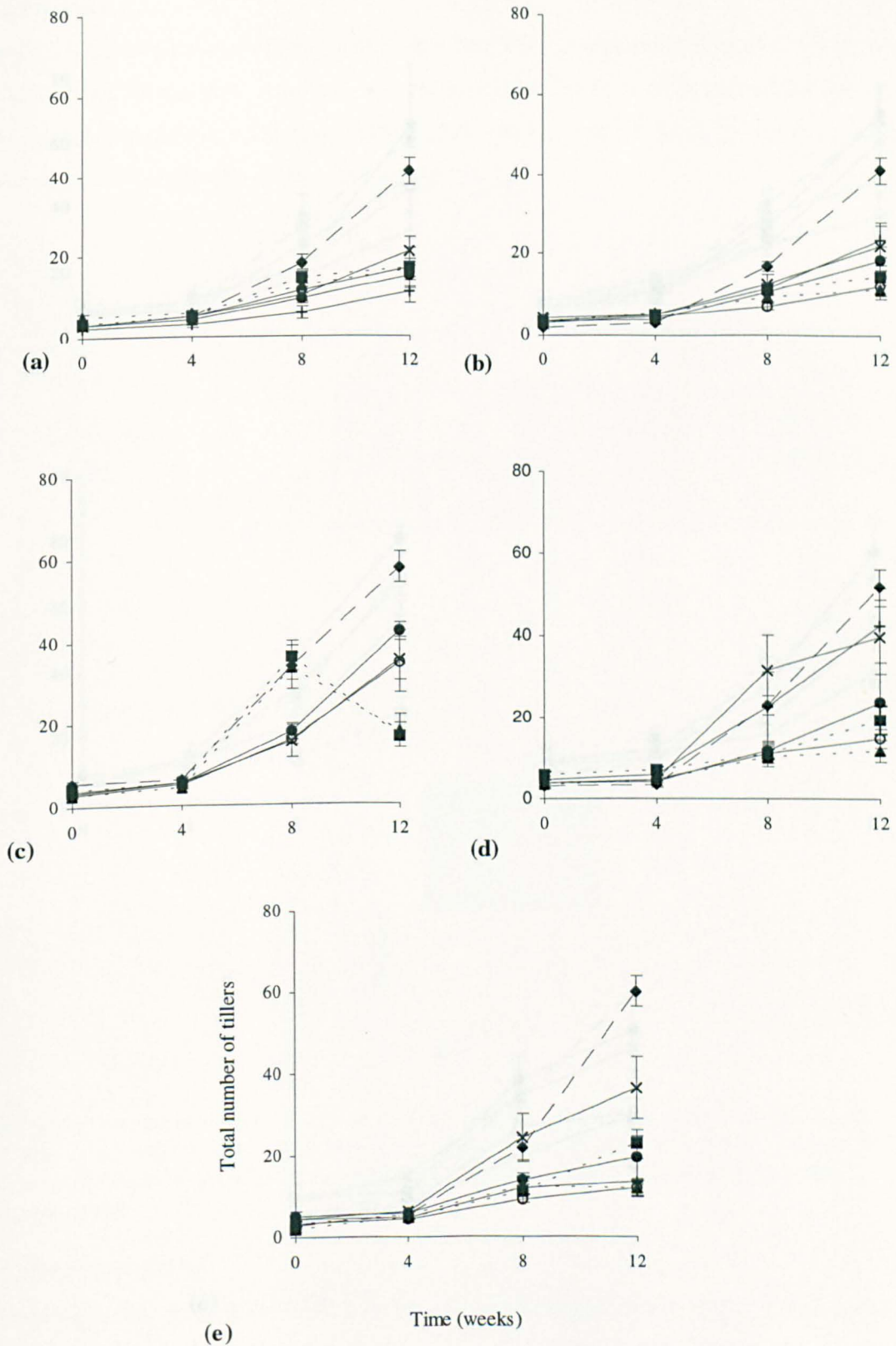
Figure 6.11. Increase in mean total number of tillers (± s.e) in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR) grown in control (◆), 25Al (■), 35Al (▲), 25Al 1500Si (+), 25Al 2500Si (●), 35Al 1500Si (○), 35Al 2500Si (×) nutrient solutions at pH 4.2. _____, Al treatments. _____, Al+Si treatments. _ _ _ _ , controls. Treatments began after 4 weeks and lasted 8 weeks.
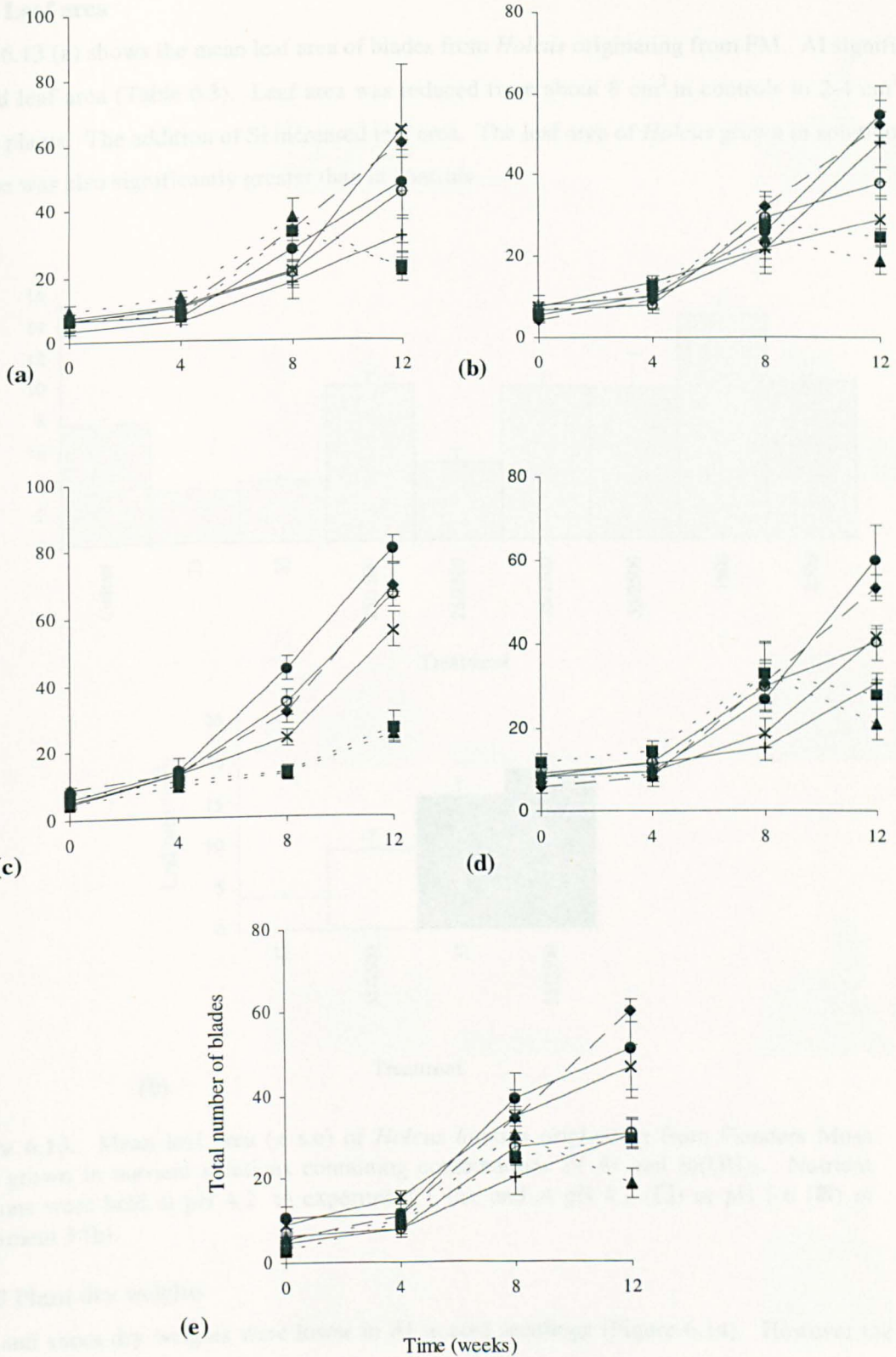
Figure 6.12. Increase in mean total number of blades (± s.e) in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR) grown in control (♦), 25Al (■), 35Al (▲), 25Al 1500Si (+), 25Al 2500Si (●), 35Al 1500Si (O), 35Al 2500Si (×) nutrient solutions at pH 4.2. _ _ _ _ _ , Al treatments. _____ , Al+Si treatments. _ _ _ _ , controls. Treatments began after 4 weeks and lasted 8 weeks.

6.4.2.4 Leaf area

Figure 6.13 (a) shows the mean leaf area of blades from *Holcus* originating from FM. Al significantly reduced leaf area (Table 6.3). Leaf area was reduced from about 8 cm^2 in controls to 2-4 cm^2 in Al treated plants. The addition of Si increased leaf area. The leaf area of *Holcus* grown in solutions with Si alone was also significantly greater than in controls.

(a)

(b)
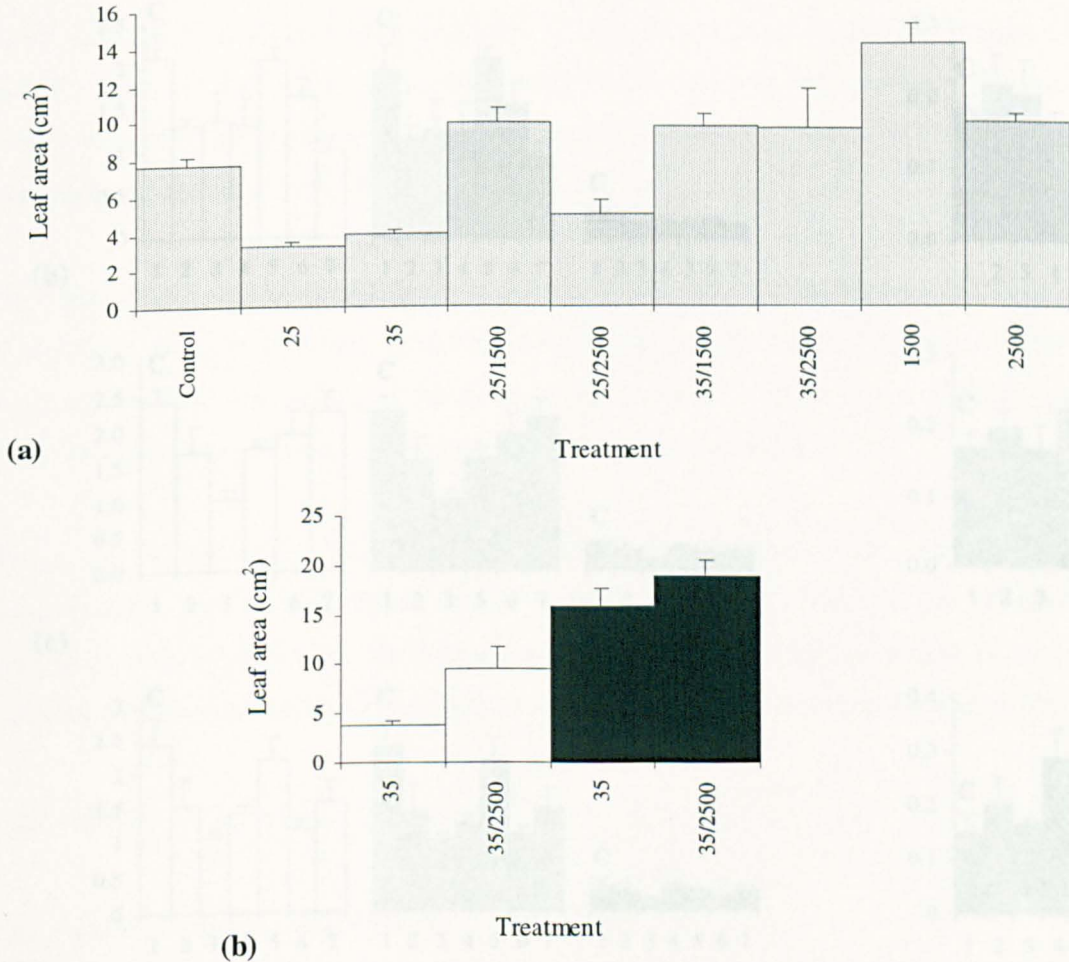
Figure 6.13. Mean leaf area (± s.e) of *Holcus lanatus* originating from Flanders Moss (FM) grown in nutrient solutions containing combinations of Al and Si(OH)$_4$. Nutrient solutions were held at pH 4.2 in experiment 1 (a), and at pH 4.2 (□) or pH 5.6 (■) in experiment 3 (b).

6.4.2.5 Plant dry weights

Total and shoot dry weights were lower in Al treated seedlings (Figure 6.14). However the overall effect of Al on total, shoot, and root dry weights was not significant (Table 6.3). Addition of Si generally increased total, shoot, and root dry weights but dry weights did not increase with an increase in Si concentration. There were significant differences in root:shoot ratios (Table 6.3). A small but consistent increase in ratios in *Holcus* treated with either Al or Al+Si was evident (Figure 6.14). The highest ratios were in plants grown in 25Al 2500Si.
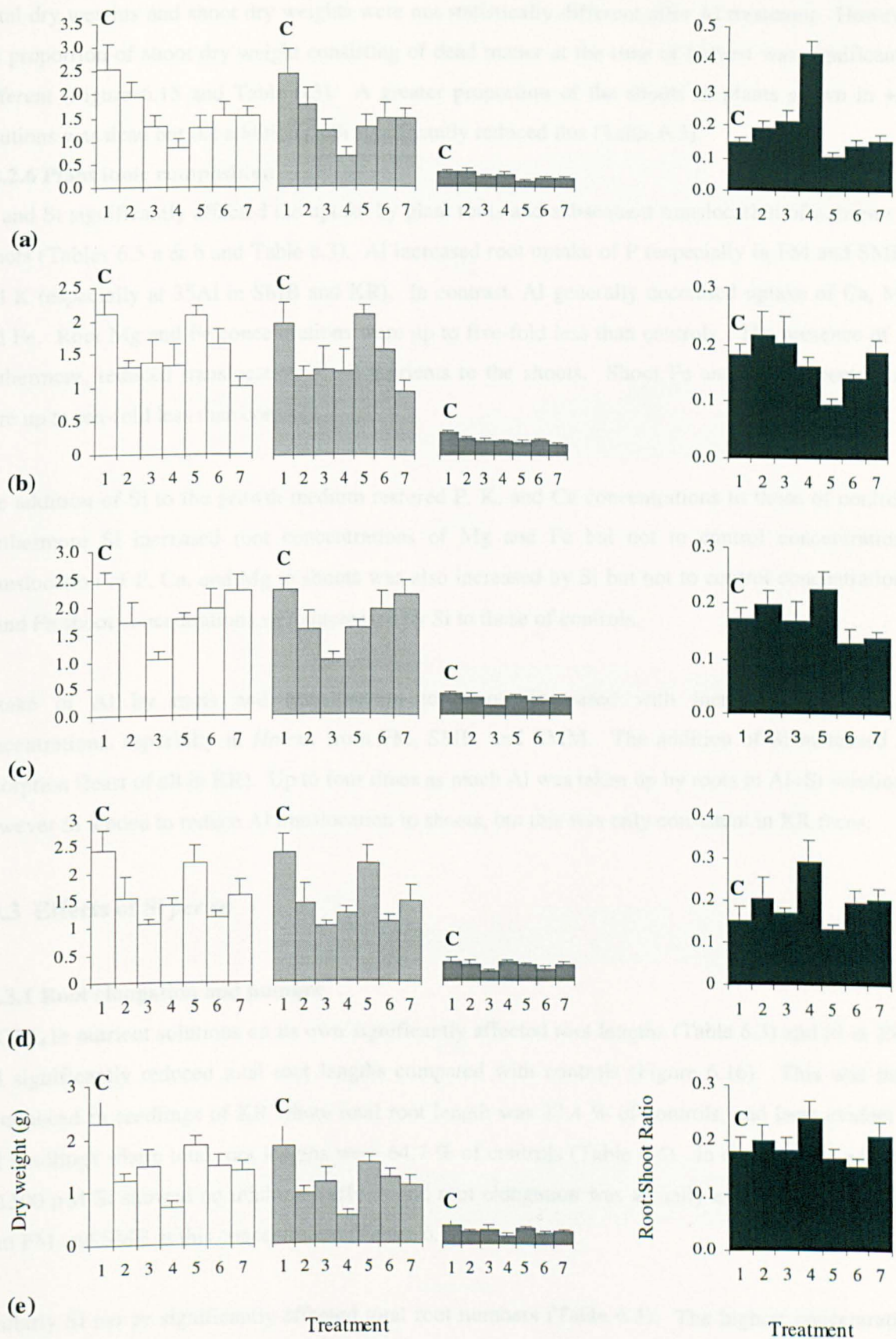
Figure 6.14. Mean total (□), shoot (▨), and root (▦) dry weights (± s.e), and root:shoot ratios (■, ± s.e) of *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR). Seedlings were grown in (1) control, (2) 25 Al, (3) 35 Al, (4) 25Al 1500Si, (5) 25Al 2500Si, (6) 35Al 1500Si, and (7) 35Al 2500Si nutrient solutions. The letter C indicates control plants.

Total dry weights and shoot dry weights were not statistically different after Al treatment. However the proportion of shoot dry weight consisting of dead matter at the time of harvest was significantly different (Figure 6.15 and Table 6.3). A greater proportion of the shoots of plants grown in +Al solutions was dead but the addition of Si significantly reduced this (Table 6.3).

6.4.2.6 Plant ionic composition

Al and Si significantly affected the uptake by plant roots and subsequent translocation of nutrients to shoots (Tables 6.5 a & b and Table 6.3). Al increased root uptake of P (especially in FM and SMB), and K (especially at 35Al in SMB and KR). In contrast, Al generally decreased uptake of Ca, Mg, and Fe. Root Mg and Fe concentrations were up to five-fold less than controls. The presence of Al furthermore, reduced translocation of all nutrients to the shoots. Shoot Fe and Mg concentrations were up to ten-fold less than controls.

The addition of Si to the growth medium restored P, K, and Ca concentrations to those of controls. Furthermore Si increased root concentrations of Mg and Fe but not to control concentrations. Translocation of P, Ca, and Mg to shoots was also increased by Si but not to control concentrations. K and Fe shoot concentrations were increased by Si to those of controls.

Uptake of Al by roots and translocation to shoots increased with increasing solution Al concentrations, especially in *Holcus* from FM, SMB, and SMM. The addition of Si increased Al absorption (least of all in KR). Up to four times as much Al was taken up by roots in Al+Si solutions. However Si tended to reduce Al translocation to shoots, but this was only consistent in KR races.

6.4.3 Effects of Si *per se*

6.4.3.1 Root elongation and number

$Si(OH)_4$ in nutrient solutions on its own significantly affected root lengths (Table 6.3) and Si at 2500 μM significantly reduced total root lengths compared with controls (Figure 6.16). This was most pronounced in seedlings of KR where total root length was 27.4 % of controls, and least evident in FM seedlings where total root lengths were 64.7 % of controls (Table 6.4). In contrast, the addition of 1500 μM Si showed no inhibitory effects and root elongation was actually enhanced in seedlings from FM and SMB at this concentration (Figure 6.16).

Similarly Si *per se* significantly affected total root numbers (Table 6.3). The highest concentration reduced root numbers in seedlings from SMB and KR to 44.8 % and 37.2 % of control plants (Figure 6.17 and Table 6.4).
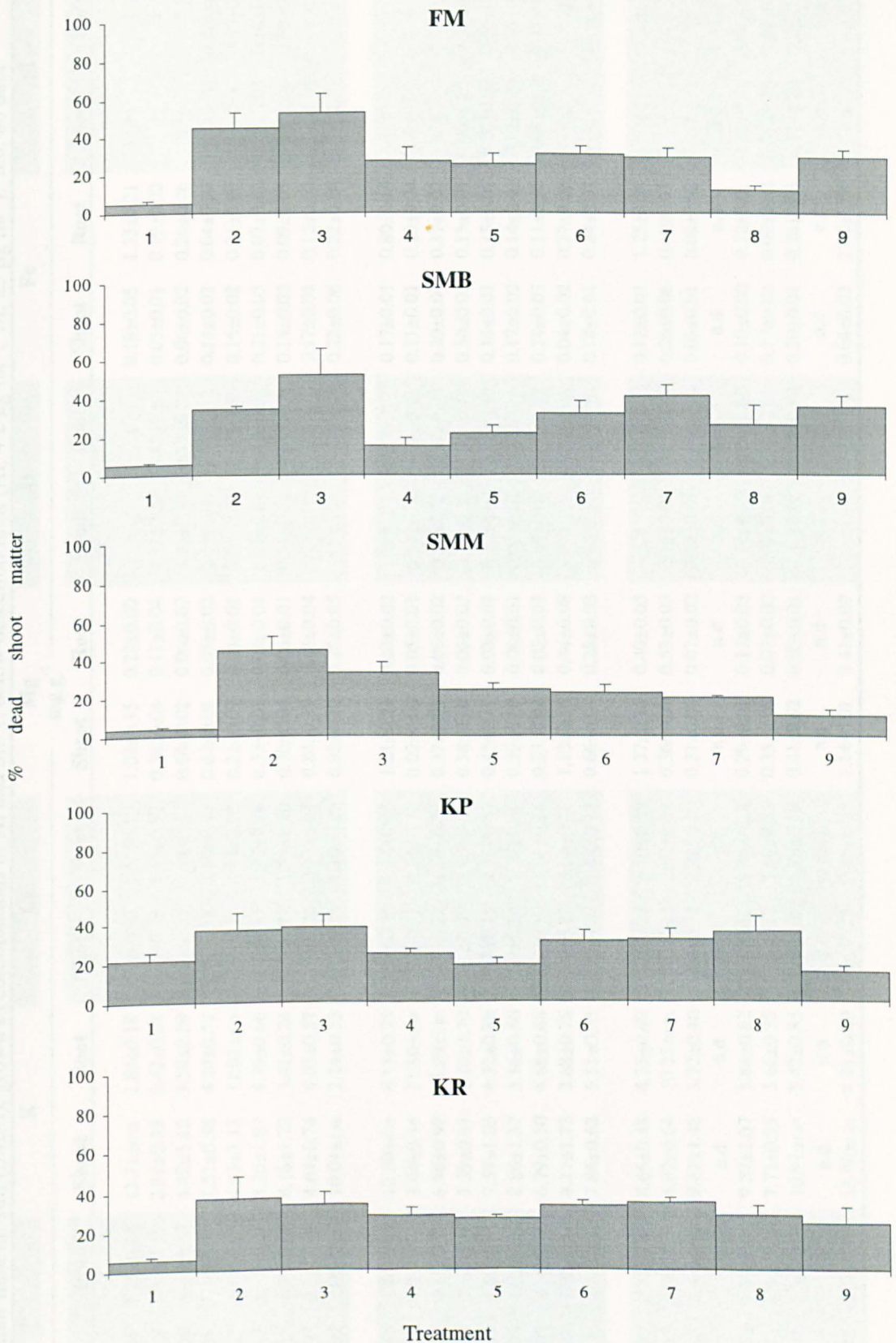
Figure 6.15. Mean percentage dead shoot matter (± s.e) in *Holcus lanatus* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR). Seedlings were grown in (1) control, (2) 25Al, (3) 35Al, (4) 25Al 1500Si, (5) 25Al 2500Si, (6) 35Al 1500Si, (7) 35Al 2500Si, (8) 1500Si, and (9) 2500Si nutrient solutions.

Table 6.5a. Mean ionic composition (mg g^{-1} dry weight, ± s.e) of shoots and roots of *Holcus lanatus*, originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), and Sheriffmuir mineral soil (SMM), grown in combinations of Al and Si. †, below detection limit (Al, 4.2 μg ml^{-1}; Si, 25 μg ml^{-1}). n.d, no data.

| Treatment	P		K		Ca		Mg mg g^{-1}		Al		Fe		Si	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
FM														
Control	6.85±0.26	1.25±0.24	13.71±0.22	2.88±0.18	3.00±0.22	1.51±0.19	1.07±0.15	0.22±0.00	†	†	0.18±0.05	1.33±0.21	†	†
25 Al	0.93±0.12	3.35±0.55	2.31±0.35	2.42±0.88	1.33±0.13	1.35±0.32	0.08±0.04	0.11±0.04	0.32±0.20	3.45±0.83	0.03±0.01	0.19±0.02	†	†
35 Al	0.80±0.06	3.88±0.40	4.42±1.12	3.59±0.29	1.60±0.15	2.18±0.57	0.04±0.02	0.06±0.02	0.35±0.04	0.42±0.07	0.06±0.02	0.26±0.06	†	†
25 Al + 1500 Si	0.97±0.05	1.98±0.38	7.51±0.38	4.59±0.57	1.48±0.18	1.50±0.14	0.62±0.08	0.05±0.02	0.13±0.04	0.39±0.12	0.14±0.03	0.04±0.01	0.17±0.03	0.23±0.05
25 Al + 2500 Si	1.36±0.23	1.57±0.35	4.63±1.13	11.03±2.52	0.91±0.15	1.74±0.26	0.22±0.07	0.09±0.01	0.32±0.06	0.97±0.25	0.16±0.02	0.21±0.07	0.31±0.04	0.65±0.13
35 Al + 1500 Si	1.30±0.12	1.52±0.21	8.26±1.07	4.39±0.66	1.32±0.15	1.37±0.34	0.39±0.07	0.04±0.01	0.03±0.01	0.12±0.02	0.21±0.05	0.07±0.02	0.08±0.03	0.18±0.04
35 Al + 2500 Si	1.37±0.18	1.92±0.38	8.16±1.72	3.41±0.24	1.21±0.21	1.59±0.20	0.30±0.04	0.06±0.01	0.57±0.11	0.64±0.03	0.14±0.02	0.09±0.01	0.18±0.06	0.66±0.02
1500	2.65±0.93	2.11±0.08	4.64±0.74	4.03±0.57	1.69±0.26	0.93±0.04	0.84±0.19	0.48±0.04	†	†	0.17±0.03	0.12±0.02	n.d	n.d
2500	2.95±0.92	2.09±0.30	10.04±1.56	2.24±0.53	2.39±0.23	1.10±0.06	0.95±0.12	0.25±0.05	†	†	0.22±0.06	0.22±0.04	n.d	n.d
SMB														
Control	5.22±0.75	2.44±0.19	12.50±0.75	8.13±0.75	2.04±0.26	2.14±0.06	1.21±0.21	0.28±0.02	†	†	0.17±0.01	0.80±0.16	†	†
25 Al	1.08±0.10	4.29±0.75	3.44±0.36	11.80±0.88	1.32±0.31	1.74±0.16	0.05±0.03	0.05±0.01	0.12±0.00	0.26±0.11	0.11±0.01	0.20±0.04	†	†
35 Al	1.11±0.15	4.16±0.36	6.96±0.98	13.09±2.87	1.14±0.12	1.63±0.12	0.37±0.03	0.06±0.02	0.31±0.02	0.37±0.05	0.20±0.04	0.31±0.07	†	†
25 Al + 1500 Si	1.93±0.29	2.12±0.14	7.39±0.81	7.13±1.70	2.11±0.49	2.30±0.06	0.38±0.10	0.06±0.02	0.10±0.03	1.20±0.19	0.30±0.05	0.13±0.01	0.06±0.01	0.99±0.08
25 Al + 2500 Si	1.35±0.19	1.59±0.12	7.57±1.05	4.32±0.89	1.65±0.11	2.56±0.52	0.45±0.11	0.03±0.01	0.12±0.03	0.72±0.10	0.16±0.01	0.15±0.01	0.20±0.02	0.56±0.07
35 Al + 1500 Si	1.38±0.09	1.87±0.03	8.86±1.37	5.16±0.60	1.39±0.34	1.77±0.05	0.39±0.10	0.08±0.01	0.05±0.01	0.42±0.16	0.17±0.02	0.14±0.03	0.12±0.04	0.35±0.08
35 Al + 2500 Si	1.09±0.07	2.18±0.19	6.79±0.50	4.68±0.68	1.11±0.17	1.86±0.16	0.33±0.04	0.02±0.01	0.05±0.02	0.70±0.17	0.24±0.03	0.11±0.02	0.08±0.02	0.37±0.14
1500	4.60±0.44	2.95±0.48	8.17±1.73	2.68±0.76	2.10±0.22	3.04±0.46	1.13±0.16	0.44±0.08	†	†	0.04±0.02	0.77±0.05	n.d	n.d
2500	2.62±0.90	2.27±0.38	7.86±0.62	5.51±0.73	1.29±0.27	1.41±0.17	0.66±0.04	0.28±0.03	†	†	0.18±0.01	0.24±0.08	n.d	n.d
SMM														
Control	4.53±0.49	2.75±0.12	8.66±0.48	4.33±0.49	3.16±0.27	1.50±0.19	1.37±0.14	0.40±0.05	†	†	0.12±0.01	1.25±0.04	†	†
25 Al	1.04±0.19	2.90±0.83	4.82±0.64	10.27±1.32	2.41±0.22	2.52±0.62	0.36±0.01	0.53±0.03	0.15±0.06	0.11±0.01	0.26±0.06	0.10±0.03	†	†
35 Al	1.01±0.15	5.48±1.09	9.47±1.40	5.92±0.46	1.10±0.31	2.43±0.29	0.31±0.05	0.07±0.02	0.22±0.05	0.50±0.14	0.06±0.01	0.08±0.02	†	†
25 Al + 1500 Si	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
25 Al + 2500 Si	1.85±0.22	1.78±0.01	9.57±1.07	3.84±0.82	1.11±0.12	1.88±0.24	0.29±0.01	0.13±0.01	0.89±0.22	0.25±0.03	0.10±0.00	0.12±0.01	0.35±0.01	0.44±0.03
35 Al + 1500 Si	1.27±0.12	1.95±0.03	7.71±0.95	3.66±0.35	1.20±0.17	1.69±0.13	0.35±0.08	0.07±0.02	0.07±0.01	0.28±0.07	0.17±0.02	0.09±0.02	0.31±0.04	0.59±0.01
35 Al + 2500 Si	1.28±0.04	1.32±0.09	10.91±1.67	5.42±0.85	1.29±0.05	1.76±0.19	0.41±0.02	0.05±0.01	0.34±0.02	0.62±0.10	0.20±0.01	0.28±0.04	0.07±0.02	0.36±0.02
1500	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
2500	4.78±0.25	4.38±0.98	13.90±1.19	4.21±0.09	1.54±0.26	2.15±0.75	1.14±0.01	0.41±0.09	†	†	0.04±0.03	1.82±0.64	n.d	n.d

Table 6.5b. Mean ionic composition (mg g^{-1} dry weight, ± s.e) of shoots and roots of *Holcus lanatus*, originating from Kippenrait Glen (KP) and Kinloch Rannoch (KR), grown in combinations of Al and Si. †, below detection limit (Al, 4.2 µg ml^{-1}; Si, 25 µg ml^{-1}). n.d, no data.

Treatment	P		K		Ca		Mg mg g^{-1}		Al		Fe		Si	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
KP														
Control	4.52±0.48	4.15±0.58	9.68±2.02	3.14±0.78	2.62±0.33	1.59±0.16	0.99±0.19	0.17±0.02	†	†	0.21±0.02	0.46±0.26	†	†
25 Al	0.81±0.24	3.42±0.54	5.27±0.91	2.97±0.24	1.38±0.10	1.00±0.03	0.30±0.03	0.01±0.00	0.16±0.06	0.20±0.07	0.05±0.03	0.03±0.00	†	†
35 Al	1.32±0.15	4.50±0.61	3.32±0.52	6.58±0.90	1.44±0.18	0.71±0.11	0.55±0.10	0.04±0.00	0.18±0.04	0.27±0.01	0.04±0.01	0.03±0.01	†	†
25 Al + 1500 Si	1.74±0.25	2.10±0.00	9.11±1.37	1.60±0.23	2.06±0.32	3.19±0.11	0.56±0.12	0.09±0.01	0.08±0.00	1.21±0.12	0.22±0.01	0.10±0.01	0.31±0.03	0.51±0.03
25 Al + 2500 Si	0.99±0.11	1.80±0.14	9.40±0.79	3.83±0.55	1.59±0.12	1.89±0.45	0.34±0.05	0.25±0.02	0.29±0.07	0.54±0.10	0.16±0.02	0.09±0.04	0.16±0.02	0.47±0.01
35 Al + 1500 Si	1.33±0.06	1.97±0.11	10.73±0.51	5.36±0.44	1.27±0.11	1.34±0.02	0.41±0.04	0.05±0.02	0.24±0.04	0.41±0.14	0.19±0.04	0.15±0.03	0.11±0.02	0.48±0.04
35 Al + 2500 Si	0.97±0.05	1.30±0.08	9.21±1.88	3.96±0.51	1.06±0.24	1.51±0.43	0.32±0.01	0.05±0.00	0.03±0.01	0.35±0.10	0.19±0.04	0.05±0.01	0.45±0.05	0.31±0.00
1500	3.61±0.13	2.91±0.23	6.42±0.57	4.00±0.59	1.01±0.19	1.15±0.03	0.67±0.07	0.19±0.01	†	†	0.09±0.03	0.15±0.03	n.d	n.d
2500	3.90±0.81	3.35±0.21	9.60±1.28	4.19±0.79	1.09±0.09	1.85±0.31	0.72±0.10	0.25±0.04	†	†	0.16±0.03	0.41±0.19	n.d	n.d
KR														
Control	3.27±0.14	3.30±0.46	6.18±0.44	3.51±0.03	1.35±0.12	1.65±0.06	0.68±0.09	0.46±0.01	†	†	0.21±0.02	1.94±0.23	†	†
25 Al	1.47±0.33	4.27±0.23	4.00±1.40	8.50±1.77	1.12±0.14	1.46±0.27	0.02±0.02	0.19±0.04	0.23±0.03	0.13±0.03	0.06±0.03	0.18±0.02	†	†
35 Al	1.39±0.18	4.41±0.70	7.74±1.10	14.5±0.92	1.35±0.25	2.02±0.36	0.45±0.08	0.25±0.03	0.39±0.10	0.51±0.05	0.19±0.03	0.09±0.05	†	†
25 Al + 1500 Si	1.39±0.15	2.40±0.35	9.76±1.30	5.89±0.98	1.65±0.11	2.10±0.26	0.53±0.08	0.06±0.02	0.06±0.01	0.42±0.11	0.14±0.02	0.06±0.02	0.05±0.02	0.80±0.22
25 Al + 2500 Si	1.41±0.10	1.83±0.25	5.04±1.38	1.47±0.36	1.74±0.26	2.03±0.41	0.60±0.17	0.06±0.02	0.04±0.01	0.35±0.07	0.27±0.03	0.28±0.02	0.16±0.04	0.54±0.03
35 Al + 1500 Si	1.14±0.04	1.90±0.13	8.73±1.42	2.24±0.50	1.67±0.16	1.91±0.26	0.39±0.08	0.04±0.01	0.07±0.01	0.14±0.01	0.20±0.05	0.14±0.01	0.16±0.04	0.34±0.10
35 Al + 2500 Si	1.86±0.03	1.47±0.23	7.03±0.26	1.88±0.53	1.21±0.04	1.47±0.21	0.39±0.05	0.03±0.01	0.03±0.01	0.08±0.03	0.18±0.03	0.17±0.04	0.07±0.03	0.22±0.02
1500	1.98±0.31	2.64±0.25	4.21±0.45	2.11±0.28	1.52±0.27	1.45±0.04	0.66±0.11	0.35±0.10	†	†	0.17±0.04	1.53±0.41	n.d	n.d
2500	2.85±0.20	2.71±0.37	5.84±0.68	5.19±0.70	0.84±0.09	1.20±0.08	0.43±0.07	0.32±0.06		†	0.14±0.06	1.66±0.21	n.d	n.d

Figure 6.16. Increase in mean total root length (± s.e) in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR) grown in control (◆), 1500 μM Si (▲), and 2500 μM Si (■) nutrient solutions at pH 4.2. Treatments began after 4 weeks and lasted 8 weeks. SMM seedlings were not treated with 1500 μM Si(OH)$_4$. _ _ _ _ , controls. _____ , Si treatments.

Time (weeks)

Total root number

Figure 6.17. Increase in mean total number of roots (± s.e) in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR) grown in control (◆), 1500 µM Si (▲), and 2500 µM Si (■) nutrient solutions at pH 4.2. Treatments began after 4 weeks and lasted 8 weeks. SMM seedlings were not treated with 1500 µM Si(OH)₄. _ _ _ _ , controls. _____ , Si treatments.

6.4.3.2 Shoot growth

Similar to root elongation, 2500 μM Si significantly inhibited shoot growth (Table 6.3). Total shoot lengths were greatly reduced compared with controls (Figure 6.18 and Table 6.4). Shoots became chlorotic and died (Figure 6.10(e)). The lower Si concentration of 1500 μM also reduced shoot lengths, especially in KP seedlings where relative shoot lengths were 50.1 % (Table 6.4). However no such reduction in shoot growth was seen in KR seedlings (relative shoot lengths of 84.8 %) and Figure 6.10(d) shows the healthy appearance of *Holcus* tops growing in 1500 μM Si.

With some exceptions, both concentrations of Si reduced vegetative reproduction in terms of tiller production and blade number. Relative tiller numbers were as low as 24.5 % (Table 6.4) (Figures 6.19 and 6.20).

6.4.3.3 Plant dry weights

There were no consistent patterns in dry weights between Si treated plants and controls (Figure 6.21). Differences between treatments were not significant. The lower Si concentration increased root dry weights and root:shoot ratios relative to control plants, in all plants except those of SMM

6.4.3.4 Plant ionic composition

The presence of silicic acid increased P uptake by roots beyond that in controls in FM, SMB and SMM races. K, Ca, and Mg uptake was inconsistent but generally greater or equal to controls. Fe uptake however was reduced by Si (Table 6.5 a and 6.5 b).

6.4.4 Al/Si interaction at pH 4.2 and pH 5.6 in *Holcus lanatus* (Experiment 3)

6.4.4.1 Root elongation and number

During the initial four weeks in treatment, neither 35Al alone nor 35Al with 2500Si at pH 5.6, inhibited root growth. Root lengths were no different from control plants. In contrast 35Al in solution at pH 4.2 had an immediate inhibitory effect on root elongation. After 4 weeks treatment Al at pH 5.6 did reduce root elongation but not greatly (Figure 6.22). In fact Al actually stimulated root growth in KR seedlings far beyond controls. Relative total root lengths at harvest were between 66.8 % and 138 % (Table 6.7). Al+Si did not significantly inhibit root growth at either pH 4.2 or pH 5.6. Root lengths tended to be larger in Al+Si at pH 4.2 compared with pH 5.6. The lowest relative root length in either of these two treatments was 66.0 %. Total root lengths were significantly affected by pH, Si, and the pH*Si interaction factor was significant p<0.001 (Table 6.6).
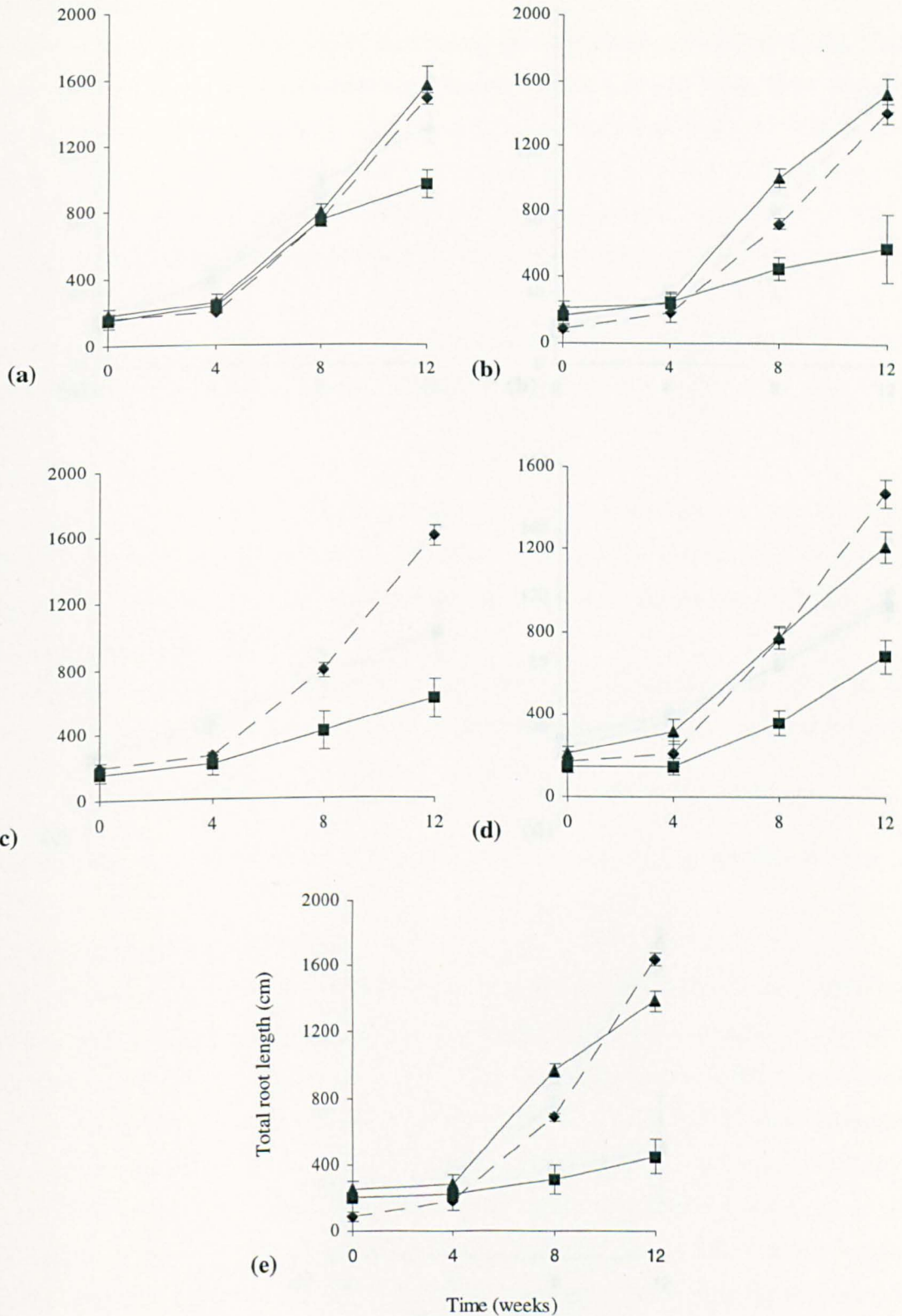
Figure 6.18. Increase in mean total shoot length (± s.e) in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR) grown in control (◆), 1500 μM Si (▲), and 2500 μM Si (■) nutrient solutions at pH 4.2. Treatments began after 4 weeks and lasted 8 weeks. _ _ _ _ , controls . _____, Si treatments. SMM seedlings were not treated with 1500 μM Si(OH)₄.
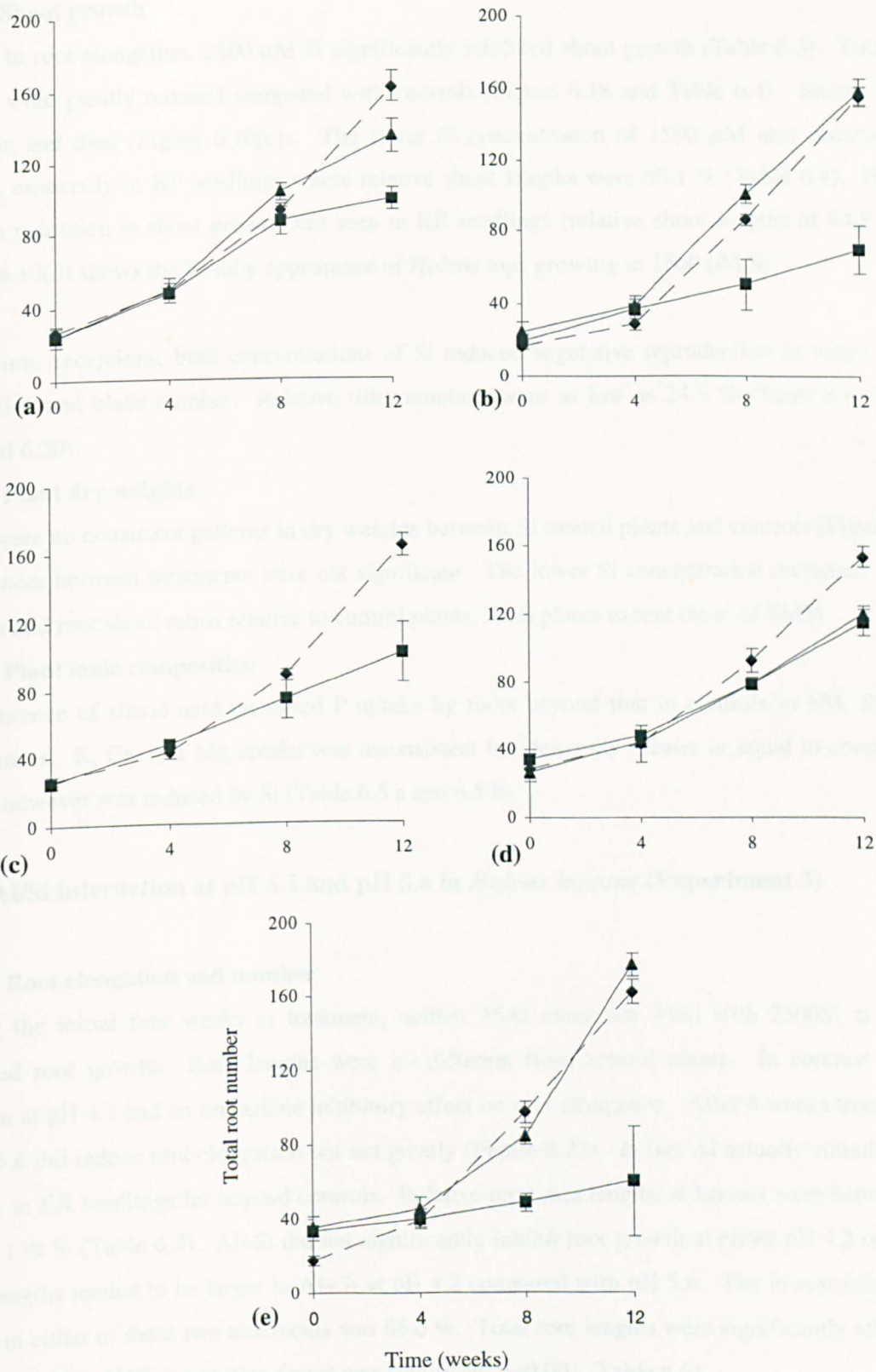
Figure 6.19. Increase in mean total number of tillers (± s.e) in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR) grown in control (◆), 1500 μM Si (▲), and 2500 μM Si (■) nutrient solutions at pH 4.2. Treatments began after 4 weeks and lasted 8 weeks. _ _ _ _ , controls. _____, Si treatments. SMM seedlings were not treated with 1500 μM Si(OH)$_4$.
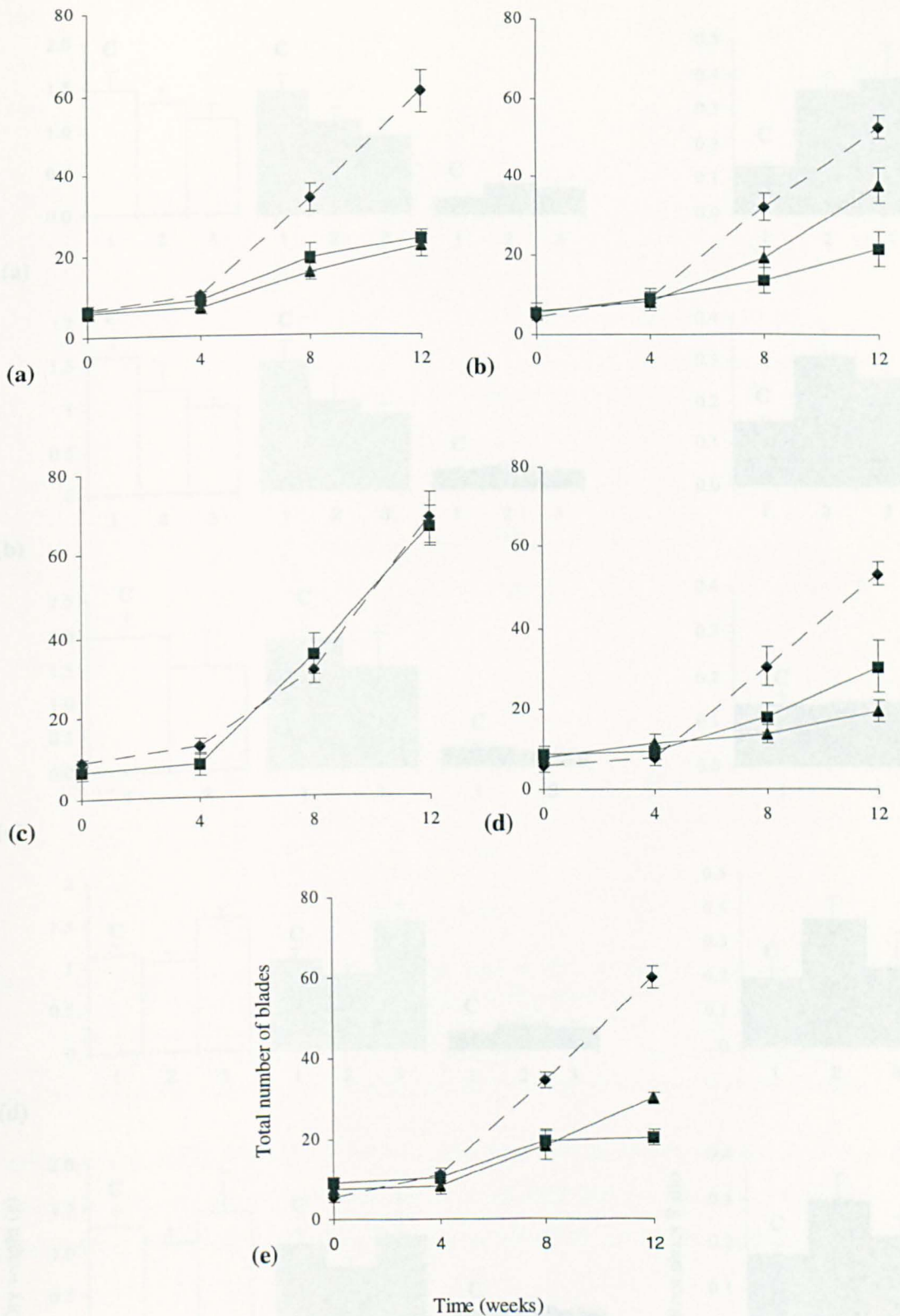
Figure 6.20. Increase in mean total number of blades (± s.e) in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR) grown in control (◆), 1500 μM Si (▲), and 2500 μM Si (■) nutrient solutions at pH 4.2. Treatments began after 4 weeks and lasted 8 weeks. _ _ _ _, controls. _____, Si treatments. SMM seedlings were not treated with 1500 μM Si(OH)$_4$.
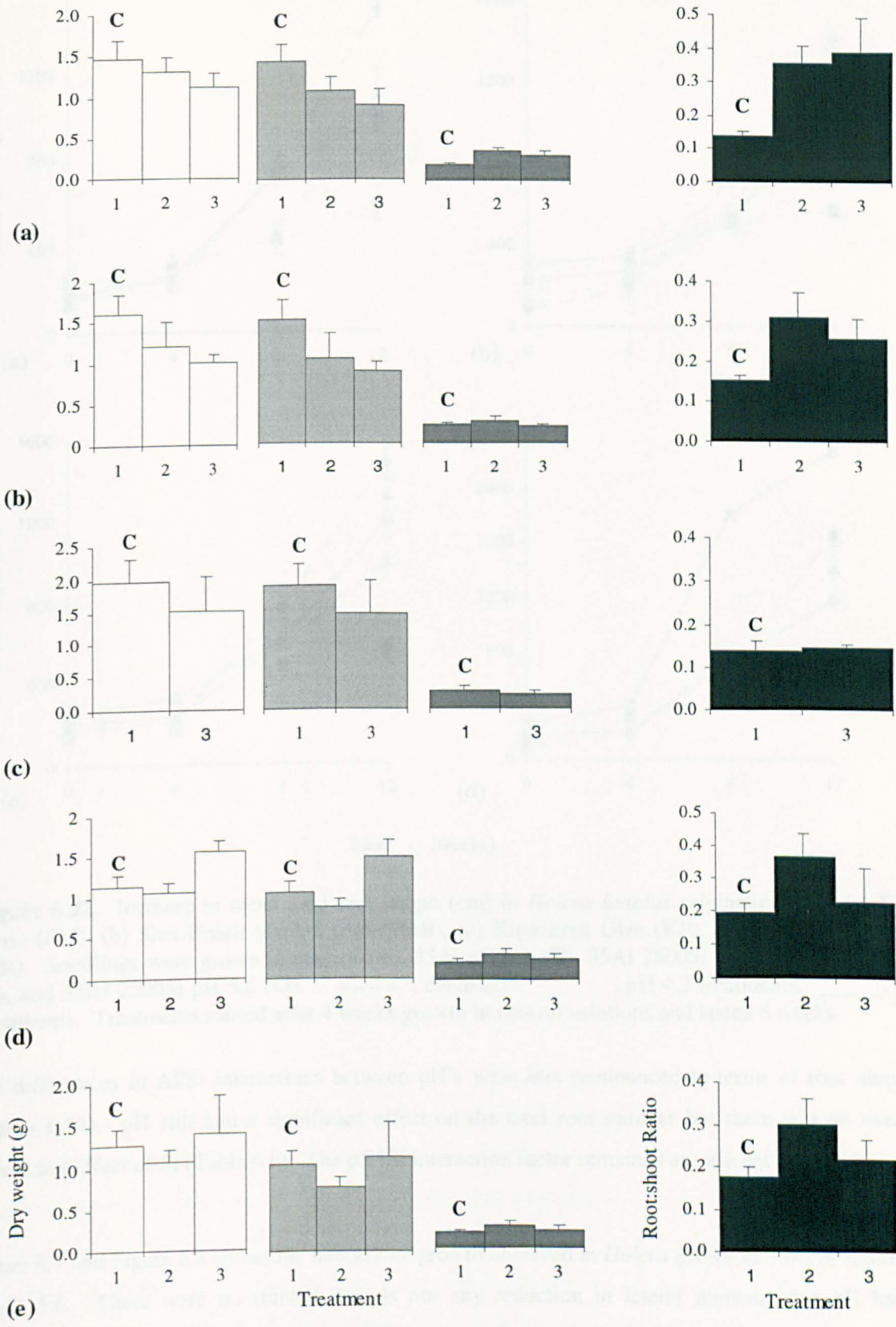
Figure 6.21. Mean total (□), shoot (▨), and root (■) dry weights (± s.e), and root:shoot ratios (■, ± s.e) of *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR). Seedlings were grown in (1) control, (2) 1500 μM Si, and (3) 2500 μM Si nutrient solutions. The letter C indicates control plants.
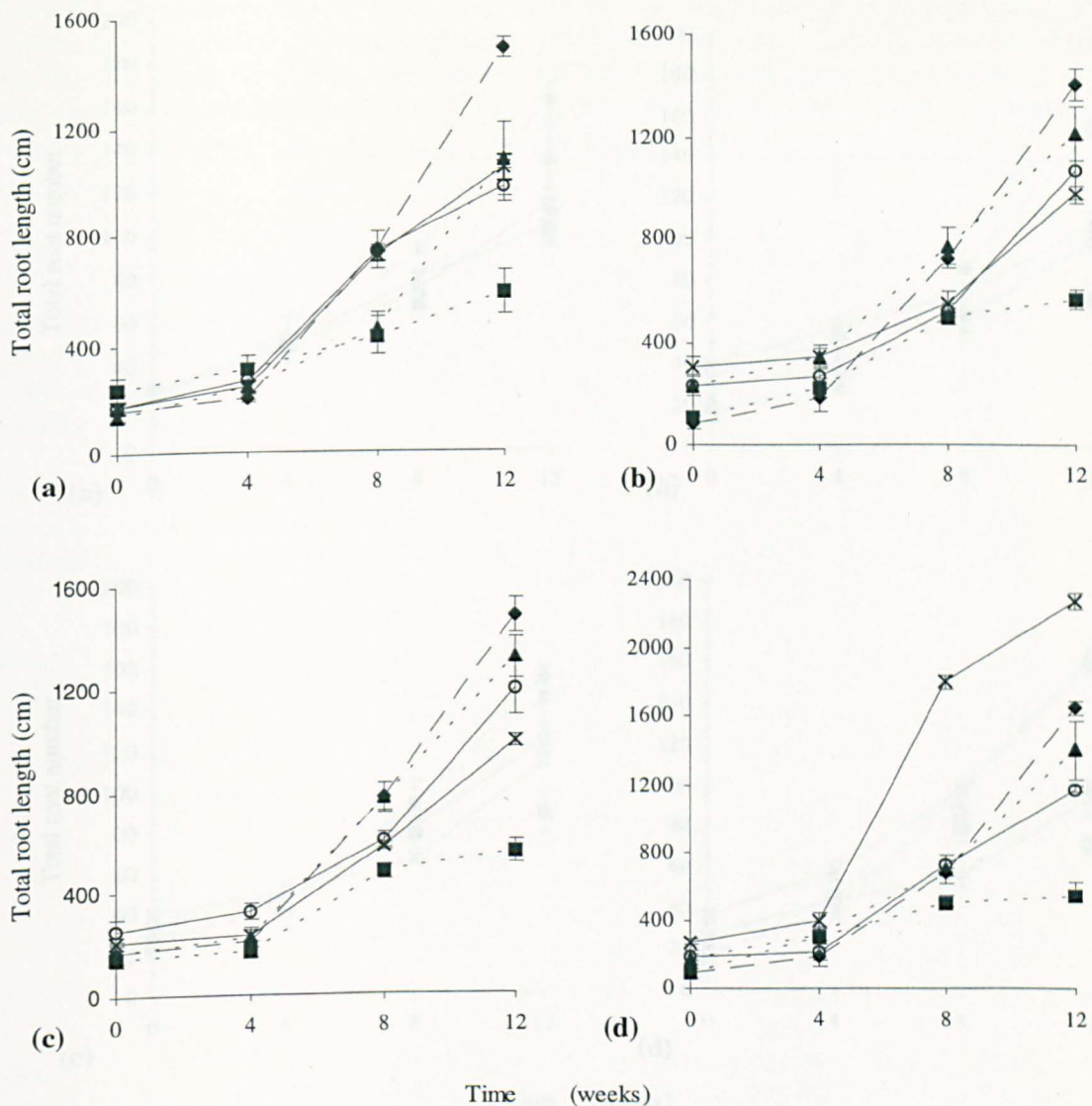
Figure 6.22. Increase in mean total root length (cm) in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR). Seedlings were grown in control (♦), 35Al pH 4.2 (■), 35Al 2500Si pH 4.2 (▲), 35Al pH 5.6 (×), and 35Al 2500Si pH 5.6 (O). ---- , controls. ...., pH 4.2 treatments. _____, pH 5.6 treatments. Treatments started after 4 weeks growth in control solutions and lasted 8 weeks.

The differences in Al/Si interactions between pH's were less pronounced in terms of root number (Figure 6.23). pH still had a significant effect on the total root number but there was no overall significant effect of Si (Table 6.6). The pH*Si interaction factor remained significant at $p<0.001$.

Figure 6.7 and Figure 6.8 shows the lateral root growth observed in *Holcus* grown in 35Al in solutions at pH 5.6. There were no stunted laterals nor any reduction in lateral numbers and pH had a significant effect on lateral length (Table 6.6).
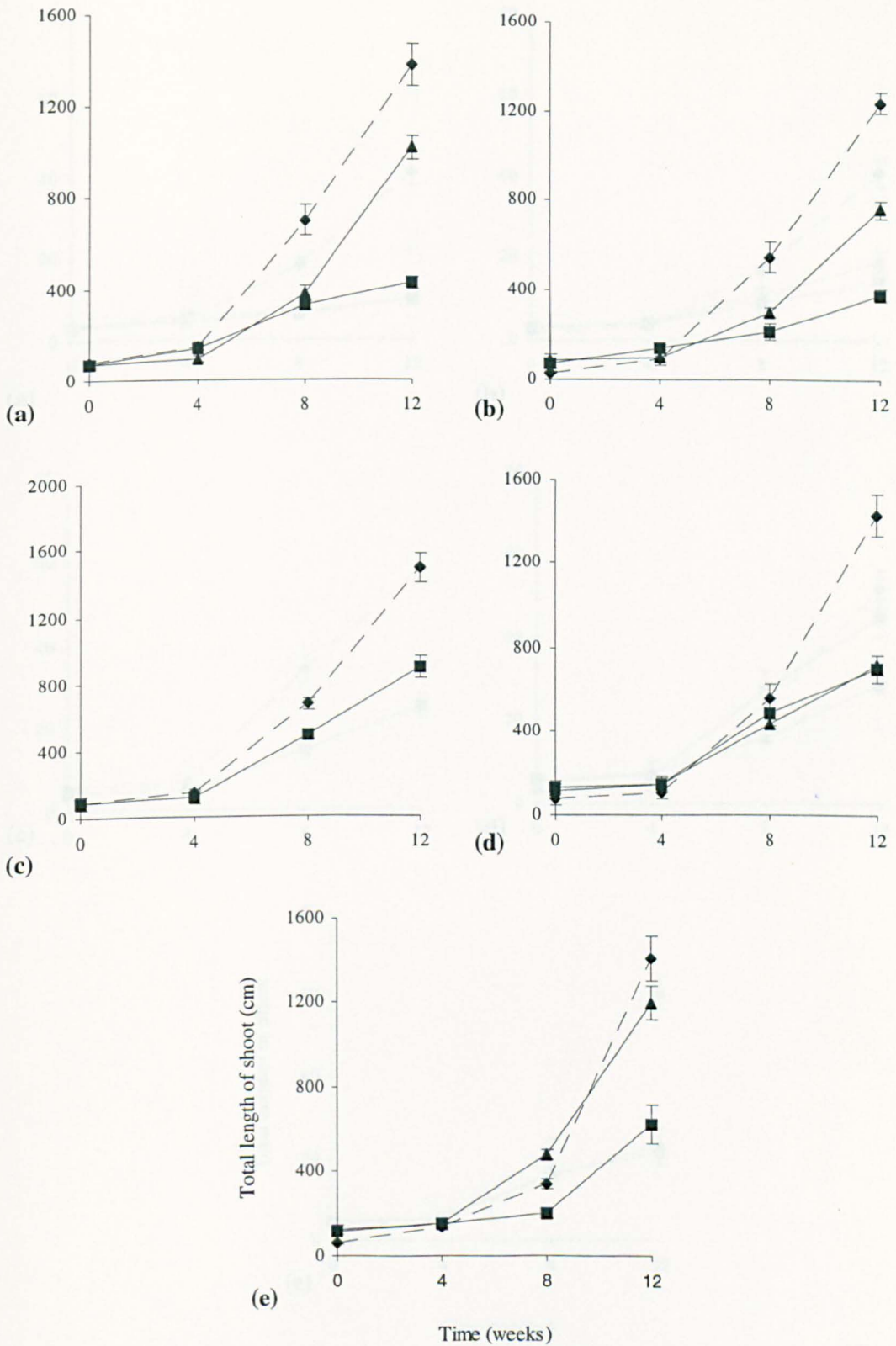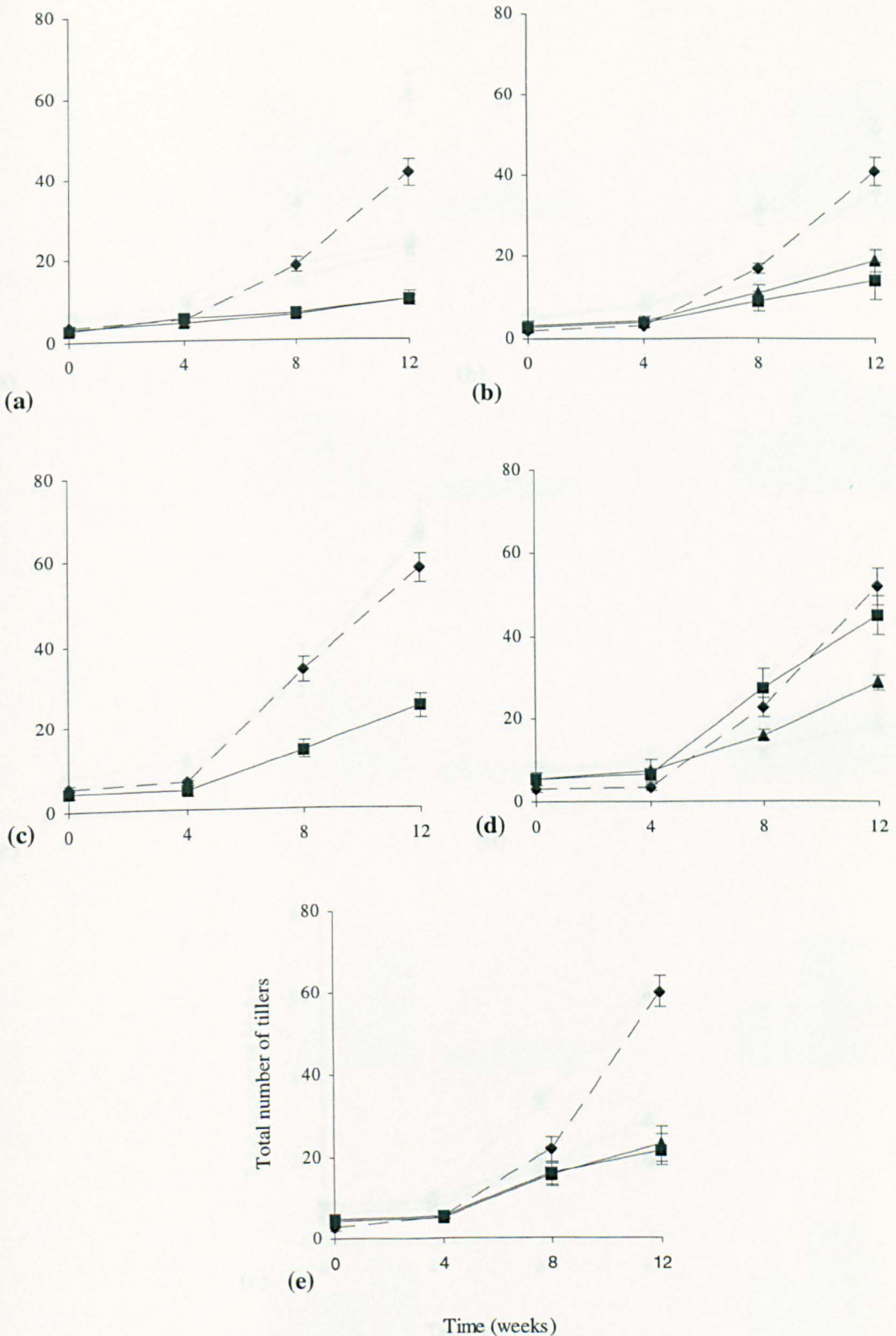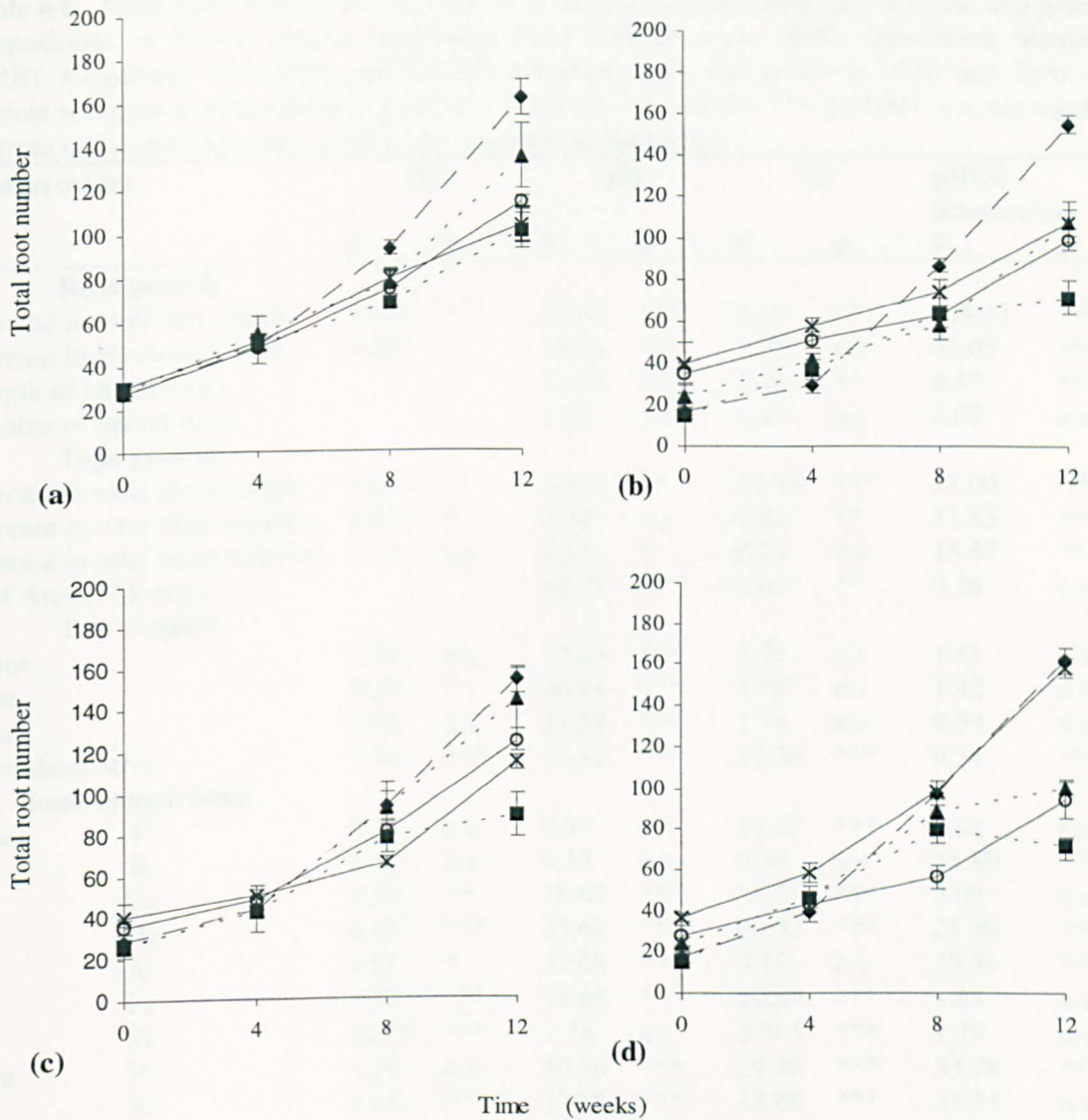
Figure 6.23. Increase in mean total number of roots in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR). Seedlings were grown in control (◆), 35Al pH 4.2 (■), 35Al 2500Si pH 4.2 (▲), 35Al pH 5.6 (×), and 35Al 2500Si pH 5.6 (O). ----- , controls. ...., pH 4.2 treatments. _____, pH 5.6 treatments. Treatments started after 4 weeks growth in control solutions and lasted 8 weeks.

6.4.4.2 Shoot growth

A similar pattern to root growth occurred in shoot growth (Figure 6.24). Total shoot lengths were substantially reduced by Al in solution at pH 4.2. At pH 5.6, Al was significantly less toxic and shoot lengths increased. Relative total root numbers in these solutions were never below 50.4 % and as much as 80.0 % (Table 6.7). Likewise Al+Si at either pH 4.2 or pH 5.6 did not inhibit shoot growth. This was most pronounced in *Holcus* from SMB. Shoot lengths in $Al_{pH5.6}$ and Al+Si treatments were no different. pH and Si both had a statistically significant effect on shoot growth and the pH*Si interaction factor was significant p<0.001 (Table 6.6).

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Table 6.6. Statistical analyses for root and shoot growth measurements, dry weights, and plant ionic compositions in *Holcus lanatus* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Kippenrait Glen (KP), and Kinloch Rannoch (KR), and grown in 35Al and 35Al 2500Si nutrient solutions at either pH 4.2 or pH 5.6. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; n.s, not significant. Degrees of freedom are : site 3, pH 1, Si 1, and pH*Si interaction 1.

Measurement		Site F	p	pH F	p	Si F	p	pH*Si interaction F	p
Root growth									
Increase in total root length		25.98	***	72.82	***	8.33	**	108.11	***
Increase in number of roots		3.80	*	14.94	***	1.91	n.s	43.05	***
Length of lateral roots				11.23	***	7.70	**	6.47	***
Number of lateral roots				1.01	n.s	1.67	n.s	0.07	n.s
Tops growth									
Increase in total shoot length		3.01	*	10.16	**	51.99	***	37.00	***
Increase in total tiller number		3.67	*	0.78	n.s	7.26	**	11.83	***
Increase in total blade number		2.71	n.s	5.53	*	0.35	n.s	15.57	***
Leaf Area (FM only)				46.79	***	8.62	**	3.28	n.s
Dry weights									
Shoot		1.24	n.s	15.23	***	3.21	n.s	1.41	n.s
Root		3.99	*	40.14	***	1.58	n.s	1.92	n.s
Total		0.53	n.s	21.38	***	1.74	n.s	0.59	n.s
Root:shoot ratio		7.74	***	14.88	***	12.38	***	9.34	**
Ionic composition									
Shoot	P	2.27	n.s	0.87	n.s	12.27	***	4.02	n.s
	K	1.36	n.s	0.19	n.s	0.33	n.s	14.80	***
	Ca	4.58	**	18.86	***	15.31	***	3.09	n.s
	Mg	6.40	***	33.61	***	24.93	***	21.90	***
	Al	3.51	*	31.88	***	0.15	n.s	25.81	***
	Fe	6.73	***	11.65	**	20.89	***	1.68	n.s
	Si	10.32	***	1.78	n.s	279.5	***	1.78	n.s
Root	P	1.26	n.s	59.96	***	29.49	***	33.78	***
	K	4.81	**	179.8	***	18.88	***	23.23	n.s
	Ca	2.20	n.s	19.73	***	0.01	n.s	0.10	n.s
	Mg	15.60	***	10.24	**	0.07	n.s	39.94	***
	Al	6.82	***	0.29	n.s	0.03	n.s	0.91	n.s
	Fe	2.76	n.s	89.37	***	0.02	n.s	6.15	*
	Si	18.87	***	5.04	*	337.8	***	5.04	*

Table 6.7. The mean relative total length or roots (TLR), total number of roots (TNR), total length of shoots (TSL), total number of blades (TNB), and total number of tillers (TNT) in *Holcus lanatus* treated with 35 mgl^{-1} Al and 35 mgl^{-1} Al with 2500 μM Si in solutions at pH 4.2 or pH 5.6. Holcus originated from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR). Values are percentages relative to control plants (100 %). n.d, no data.

| Relative growth (%)	Control$_{pH4.2}$	35 Al $_{pH 4.2}$	Treatment 35/2500$_{pH4.2}$	35 Al $_{pH 5.6}$	35/2500$_{pH5.6}$
(a)					
TRL	100	39.4	72.2	70.2	65.6
TNR	100	62.1	83.5	63.6	70.1
TSL	100	32.8	92.8	65.9	60.2
TNB	100	35.1	106	68.1	57.0
TNT	100	40.2	51.0	52.9	35.3
(b)					
TRL	100	38.4	83.0	67.1	72.9
TNR	100	45.7	69.6	69.2	64.2
TSL	100	24.7	52.9	80.0	72.4
TNB	100	35.6	54.6	94.3	95.7
TNT	100	28.2	29.6	61.2	79.1
(c)					
TRL	100	37.5	88.7	66.8	80.7
TNR	100	46.0	93.9	74.3	81.1
TSL	100	26.1	74.8	52.7	50.5
TNB	100	39.4	78.2	71.1	69.6
TNT	100	22.5	76.7	33.7	26.7
(d)					
TRL	100	33.4	85.1	139	71.3
TNR	100	44.4	61.9	97.7	58.6
TSL	100	30.7	90.4	50.4	71.3
TNB	100	31.1	77.6	92.3	67.6
TNT	100	20.0	60.7	43.0	43.7
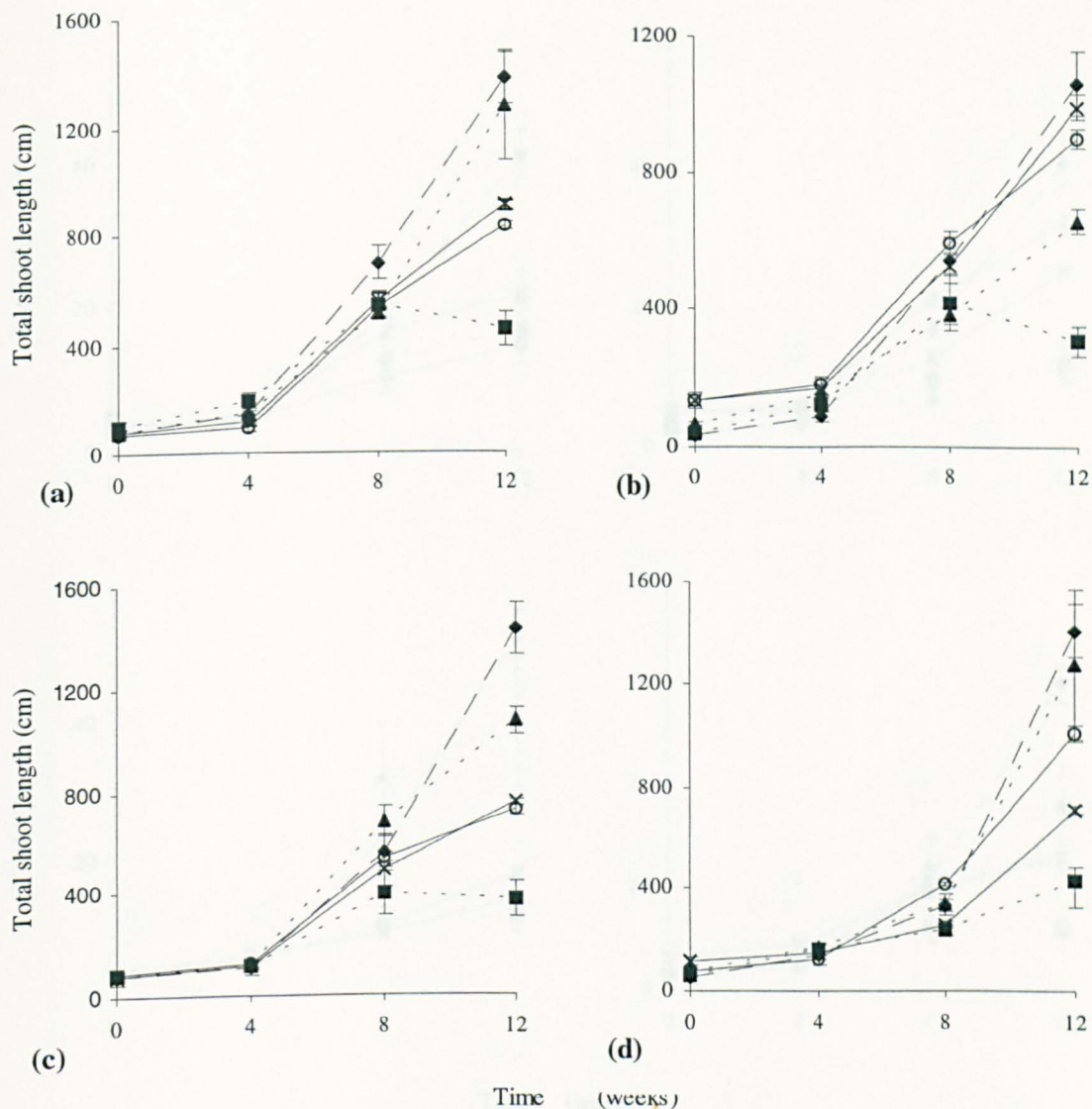
Figure 6.24. Increase in mean total length of shoots in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR). Seedlings were grown in control (◆), 35Al pH 4.2 (■), 35Al 2500Si pH 4.2 (▲), 35Al pH 5.6 (×), and 35Al 2500Si pH 5.6 (O). _ _ _ _ , controls. ...., pH 4.2 treatments. _____, pH 5.6 treatments. Treatments started after 4 weeks growth in control solutions and lasted 8 weeks.

Si addition did not significantly improve vegetative growth of seedlings. Figure 6.25 shows very little difference in tiller numbers between Al and Al+Si treatments at either pH level, and pH did not significantly effect tiller numbers (Table 6.6). Al in solution at pH 5.6 was only less toxic to seedlings from SMB and, to a lesser extent, KR. 35Al 2500Si in solution at pH 4.2 reduced tiller production to a lesser extent than at pH 5.6.
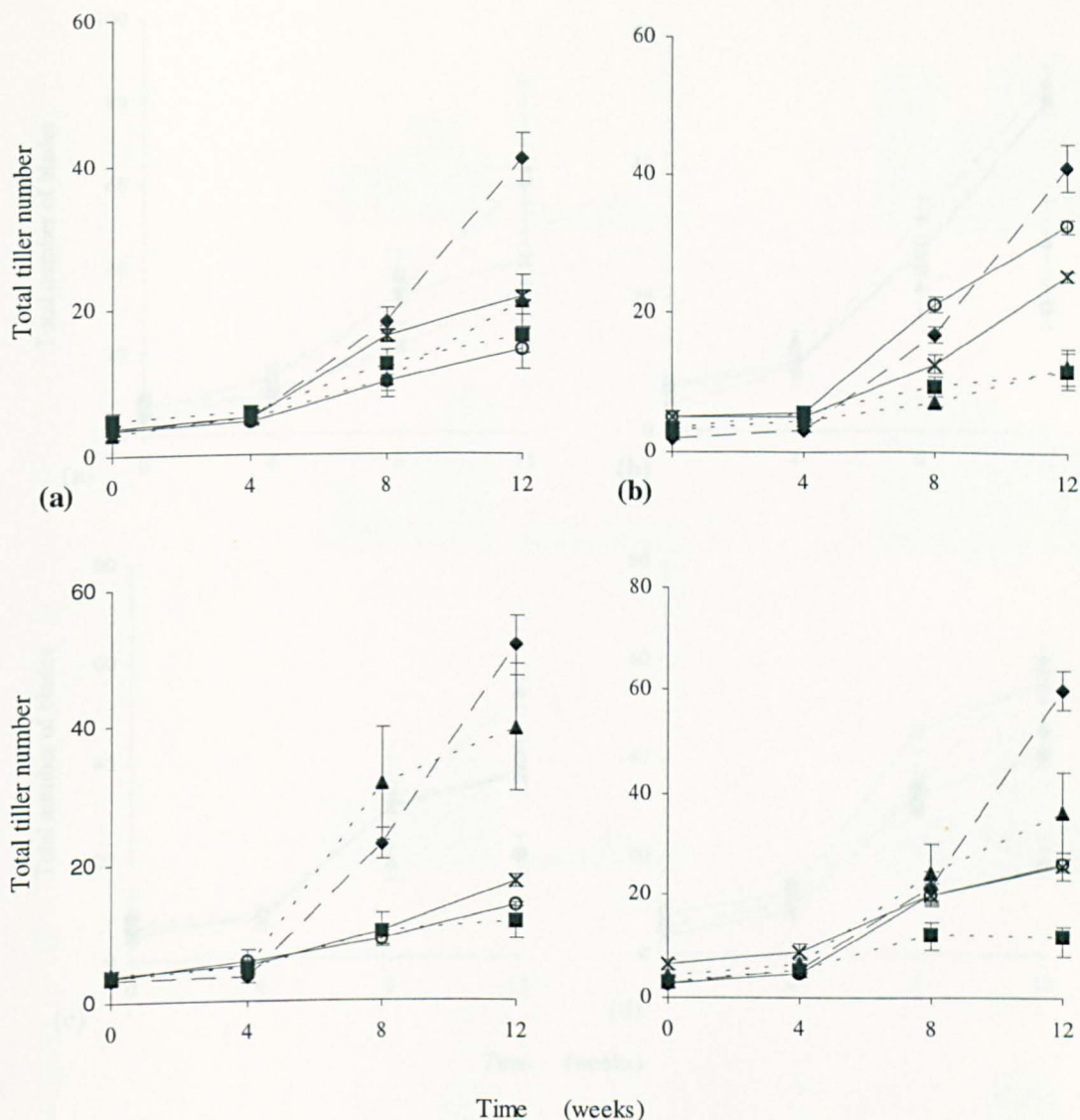
Figure 6.25. Increase in mean total number of tillers in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR). Seedlings were grown in control (◆), 35Al pH 4.2 (■), 35Al 2500Si pH 4.2 (▲), 35Al pH 5.6 (×), and 35Al 2500Si pH 5.6 (○). – – – – , controls. ...., pH 4.2 treatments. _____, pH 5.6 treatments. Treatments started after 4 weeks growth in control solutions and lasted 8 weeks.

The previous pattern of no Al toxicity in solutions at pH 5.6 was again evident in blade numbers (Figure 6.26). There were also no differences between Al in solution at pH 5.6 and Al in solution with 2500 μM Si at either pH 4.2 or pH 5.6. Hence Si did not significantly affect blade numbers but pH had a significant affect (Table 6.6). Like root elongation, blade numbers were greater than those of controls in KR plants at $35Al_{pH\,5.6}$.

Leaf area was significantly greater in *Holcus* grown in either 35Al or 35Al 2500Si at pH 5.6 compared with pH 4.2. Figure 6.13 shows the mean leaf area in *Holcus* originating from FM.
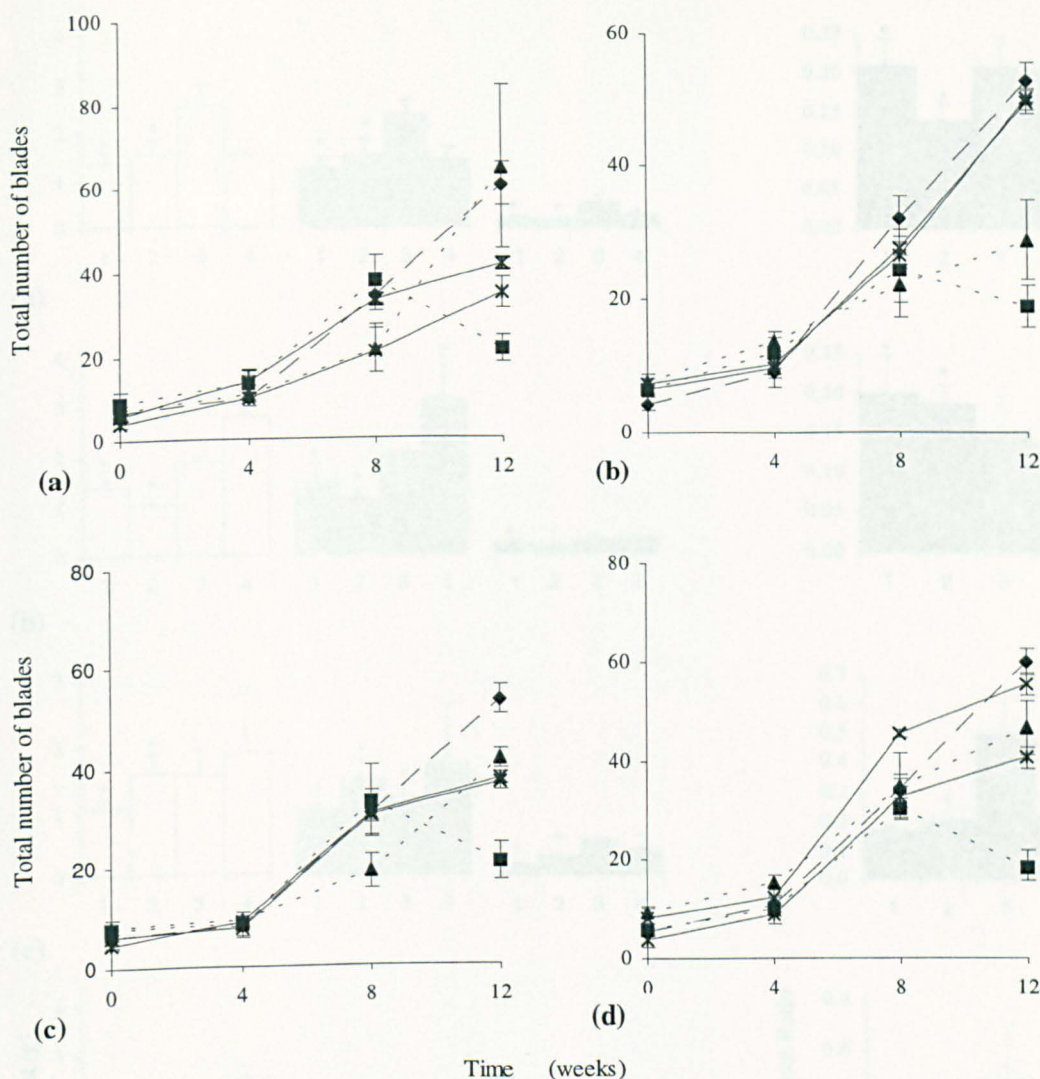
Figure 6.26. Increase in mean total number of blades in *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR). Seedlings were grown in control (◆), 35Al pH 4.2 (■), 35Al 2500Si pH 4.2 (▲), 35Al pH 5.6 (×), and 35Al 2500Si pH 5.6 (O). − − − − , controls. ...., pH 4.2 treatments. _____ , pH 5.6 treatments.

6.4.4.3 Plant dry weights

Total, shoot, and root dry weights were generally higher in either 35Al or 35Al 2500Si at pH 5.6 (particularly the latter) but this was not consistent among sites (Figure 6.27 and Table 6.6). Root:shoot ratios of KP and KR seedlings were significantly greater in Al solutions at pH 5.6 compared with pH 4.2.

6.4.4.4 Plant ionic composition

P, K, Ca, Mg, and Fe uptake by roots in 35Al solutions was significantly lower at pH 5.6 than at pH 4.2 (Tables 6.5, 6.6 and 6.8). Likewise K and Ca uptake in 35Al 2500Si were lower at pH 5.6. Nutrient uptake by roots in solutions of $35Al_{pH\ 5.6}$, $35Al\ 2500Si_{pH\ 4.2}$, and $35Al\ 2500Si_{pH\ 5.6}$ did not differ greatly. Translocation of P to shoots increased in 35Al solutions at pH 5.6 in FM and SMB races, but
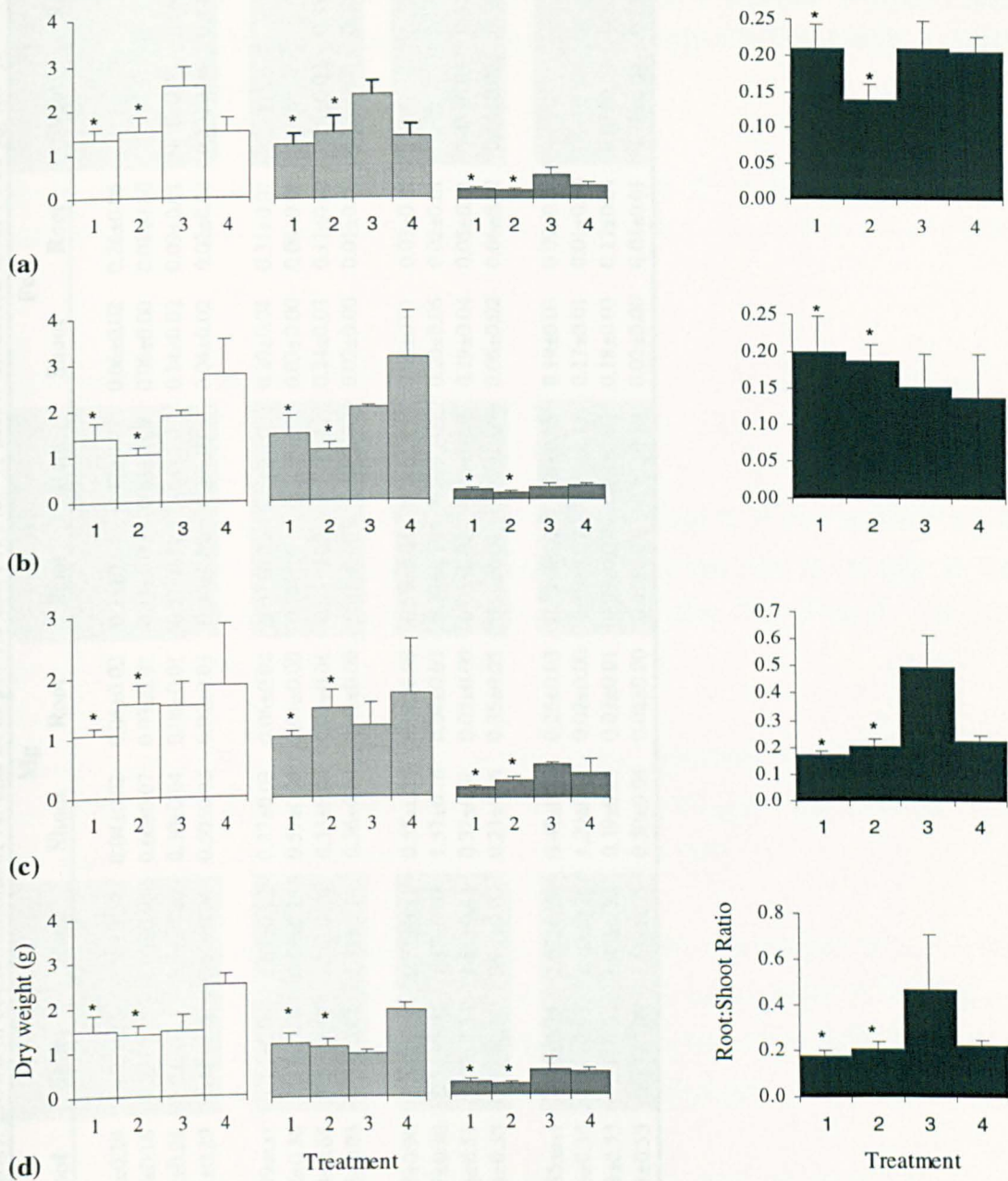
Figure 6.27. Mean total (□), shoot (▨), and root (▨) dry weights (± s.e), and root:shoot ratios (■, ± s.e) of *Holcus lanatus* originating from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Kippenrait Glen (KP), and (d) Kinloch Rannoch (KR). Seedlings were grown in (1) 35 Al, (2) 35Al 2500Si, (3) 35Al, and (4) 35Al 2500Si nutrient solutions at pH 4.2 or pH 5.6. *, indicates nutrient solutions at pH 4.2.

Table 6.8. Mean ionic composition (mg g^{-1} dry weight, ± s.e) of shoots and roots of *Holcus lanatus*, originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Kippenrait Glen (KP), and Kinloch Rannoch (KR), grown in combinations of Al and Si at pH 4.2 and pH 5.6. †, below detection level (< 25 µg ml^{-1}).

Treatment	P Shoot	P Root	K Shoot	K Root	Ca Shoot	Ca Root	Mg Shoot	Mg Root	Al Shoot	Al Root	Fe Shoot	Fe Root	Si Shoot	Si Root
FM														
35Al $_{pH\,4.2}$	0.80±0.06	3.88±0.40	4.42±1.12	3.59±0.29	1.60±0.15	2.18±0.57	0.04±0.02	0.06±0.02	0.35±0.04	0.42±0.07	0.06±0.02	0.26±0.06	†	†
35Al $_{pH\,5.6}$	1.14±0.12	1.83±0.09	5.62±0.69	1.12±0.16	1.80±0.05	1.05±0.08	0.60±0.07	0.03±0.01	0.11±0.00	0.83±0.09	0.06±0.00	0.01±0.00	†	†
35Al 2500Si $_{pH\,4.2}$	1.37±0.18	1.92±0.38	8.16±1.72	3.41±0.24	1.21±0.21	1.59±0.20	0.30±0.04	0.06±0.01	0.57±0.11	0.64±0.03	0.14±0.02	0.09±0.01	0.18±0.06	0.66±0.02
35Al 2500Si $_{pH\,5.6}$	1.24±0.10	1.56±0.15	5.70±0.17	1.04±0.29	1.48±0.26	0.67±0.04	0.50±0.02	0.02±0.01	0.34±0.02	0.39±0.09	0.04±0.02	0.02±0.00	0.33±0.04	0.49±0.05
SMB														
35Al $_{pH\,4.2}$	1.11±0.15	4.16±0.36	6.96±0.98	13.09±2.87	1.14±0.06	1.63±0.12	0.37±0.03	0.06±0.02	0.31±0.02	0.36±0.05	0.20±0.04	0.31±0.07	†	†
35Al $_{pH\,5.6}$	1.46±0.21	2.43±0.37	6.81±0.97	1.42±0.36	1.17±0.09	0.68±0.13	0.57±0.05	0.01±0.00	0.24±0.01	0.33±0.02	0.03±0.00	0.00±0.00	†	†
35Al 2500Si $_{pH\,4.2}$	1.09±0.07	2.18±0.19	6.79±0.51	4.68±0.68	1.11±0.17	1.86±0.16	0.33±0.04	0.02±0.01	0.05±0.02	0.70±0.17	0.24±0.03	0.11±0.02	0.08±0.02	0.33±0.14
35Al 2500Si $_{pH\,5.6}$	1.00±0.11	2.29±0.64	5.19±0.52	1.05±0.03	1.39±0.01	1.31±0.15	0.36±0.04	0.02±0.00	0.07±0.00	0.32±0.00	0.03±0.00	0.01±0.00	0.23±0.03	0.20±0.03
KP														
35Al $_{pH\,4.2}$	1.32±0.15	4.50±0.61	3.32±0.52	6.58±0.90	1.44±0.18	0.71±0.11	0.55±0.10	0.04±0.00	0.18±0.04	0.26±0.01	0.04±0.01	0.03±0.01	†	†
35Al $_{pH\,5.6}$	0.54±0.05	2.32±0.11	8.30±0.80	1.57±0.30	2.74±0.04	1.47±0.03	1.37±0.16	0.04±0.03	0.73±0.18	0.22±0.02	0.20±0.06	0.02±0.01	†	†
35Al 2500Si $_{pH\,4.2}$	0.97±0.05	1.30±0.08	9.21±1.88	3.96±0.51	1.06±0.24	1.51±0.43	0.32±0.01	0.05±0.00	0.03±0.01	0.35±0.10	0.19±0.04	0.05±0.01	0.45±0.05	0.31±0.00
35Al 2500Si $_{pH\,5.6}$	2.56±0.87	1.29±0.17	5.45±0.17	2.24±0.35	1.73±0.31	0.84±0.08	0.23±0.06	0.35±0.05	0.54±0.01	0.32±0.09	0.06±0.02	0.06±0.02	0.16±0.02	0.38±0.01
KR														
35Al $_{pH\,4.2}$	1.39±0.18	4.41±0.70	7.74±1.10	14.55±0.92	1.35±0.25	2.02±0.36	0.45±0.08	0.25±0.03	0.39±0.10	0.55±0.05	0.19±0.03	0.09±0.05	†	†
35Al $_{pH\,5.6}$	0.98±0.03	2.02±0.46	8.46±1.09	1.36±0.35	2.47±0.55	1.10±0.26	1.20±0.41	0.02±0.00	0.73±0.09	0.44±0.11	0.17±0.01	0.01±0.00	†	†
35Al 2500Si $_{pH\,4.2}$	1.86±0.03	1.47±0.23	7.03±0.26	1.88±0.53	1.21±0.04	1.47±0.21	0.39±0.05	0.03±0.01	0.03±0.01	0.08±0.03	0.18±0.03	0.17±0.04	0.07±0.03	0.22±0.02
35Al 2500Si $_{pH\,5.6}$	1.81±0.06	2.01±0.05	6.38±0.63	1.73±0.55	1.13±0.08	1.65±0.05	0.50±0.04	0.02±0.00	0.07±0.01	0.65±0.19	0.02±0.00	0.04±0.01	0.21±0.04	0.15±0.00

decreased in KP and KR races. K, Ca, and Mg shoot concentrations were greater in $35Al_{pH\,5.6}$ solutions compared with $35Al_{pH\,4.2}$. Shoot Fe concentrations were greatly reduced in $35Al\,2500Si_{pH\,5.6}$ compared with $35Al\,2500Si_{pH\,4.2}$ treatments.

Al uptake by roots in Al+Si solutions at pH 5.6 was generally lower than at pH 4.2, particularly in FM and SMB. Al translocation to shoots in 35Al solutions was greatly increased at pH 5.6 in KP and KR races.

6.4.5 Al/Si speciation in nutrient solutions

6.4.5.1 Nutrient solutions at pH 4.2.

Tables 6.9 and 6.10 gives the total concentrations of Al ($[Al]_{TOT}$) and Si ($[Si]_{TOT}$) analysed in nutrient solutions filtered through 5 µM and 0.45 µM filter paper, and that passing through the dialysis membrane. The solutions were analysed immediately after preparation (0), and one (1), two (2), three (3), and four (4) days thereafter.

The measured concentration of monomeric Al species ($[Al]_{mono}$) decreased with time in all solutions irrelevant of Si addition. Solutions were changed on day 3 when $[Al]_{mono}$ was about 50 % of that in the original solution. $[Al]_{mono}$ was reduced a little more in the presence of Si.

$[Al]_{TOT}$ (5 µM) also decreased with time. There was less of a difference between $[Al]_{TOT}$ in +Al and Al+Si solutions, with the exception of 35Al 2500Si where $[Al]_{TOT}$ was reduced to 25 % of the original solution. Most of the Al passed through a 0.45 µM filter, ranging from 56 to 100 % (but more often 80-100 %). However only 7-36 % of this Al passed through a dialysis membrane.

$[Si]_{TOT}$ (5 µM) like Al decreased with time. More Si passed the 0.45 µM filter in 25Al solutions compared with 35Al solutions. Between 26 and 96 % of Si passed through 0.45 µM pore in Si solutions with no added Al. The majority of Si, 70-94 %, was small enough to permeate the dialysis membrane.

6.4.5.2 Nutrient solutions at pH 5.6

There was a large reduction in $[Al]_{mono}$ at pH 5.6 compared with pH 4.2 (about a 20-fold difference) but little change with time. $[Al]_{TOT}$ (5 µM) was about 60 % of those at pH 4.2, and the majority passed through 0.45 µM. Between 81-94 % of Al species were also small enough to pass through the dialysis membrane.

At pH 5.6 the reduction in $[Si]_{TOT}$ (5 μM) was less pronounced than at pH 4.2. However with time the proportion passing through 0.45 μM was greatly reduced (from 79 to 26 %). 83.0 % of Si passed through the dialysis tubing.

Table 6.9. Total Al concentration ($[Al]_{TOT}$) in nutrient solutions (up to four days old) passing through filters of 5 μM, 0.45 μM, and a dialysis membrane. Total monomeric Al ($[Al]_{mono}$) was measured using the Pyrocatechol Violet colorimetric method. Predicted Al activity ({Al}) after GEOCHEM-PC. The mole fraction of the $[Al]_{TOT}$ concentration passing through 0.45 μM or dialysis membranes is given in parentheses.

Nutrient Solution	Solution Age (days)	{Al}GEOCHEM (μM)	$[Al]_{mono}$ (μM)	$[Al]_{TOT}$ 5 μM (mg l^{-1})	$[Al]_{TOT}$ 0.45 μM (mg l^{-1})	$[Al]_{TOT}$ Dialysis membrane (mg l^{-1})
25Al	0	300	482±6.88	18.8±0.38	17.7±0.38(0.94)	1.25±0.72(0.07)
	1		345±3.20	16.8±1.02	14.9±0.98(0.89)	
	2		307±4.23	11.0±0.32	8.78±0.32(0.80)	
	3		107±3.81	10.3±0.15	7.76±0.15(0.76)	
	4		98.4±2.09	7.57±0.47	6.88±0.47(0.91)	
35Al	0	408	719±11.27	30.6±0.28	25.4±1.54(0.83)	6.59±0.05(0.22)
	1		491±4.96	28.8±0.13	25.1±0.32(0.87)	
	2		357±9.50	27.8±0.18	24.5±0.18(0.88)	
	3		131±6.48	26.2±0.20	23.9±0.20(0.91)	
	4		85.6±3.23	24.6±0.37	20.2±0.44(0.82)	
25Al 1500Si	0	300	406±17.33	20.2±1.28	19.8±1.14(0.98)	5.43±0.04(0.27)
	1		329±2.11	20.2±0.10	19.5±0.28(0.97)	
	2		292±6.78	20.0±0.23	19.8±0.25(0.99)	
	3		131±6.48	4.08±0.47	3.98±0.46(0.98)	
	4		79.6±2.57	2.96±0.24	3.03±0.03(1.03)	
25Al 2500Si	0	300	462±7.03	26.4±1.02	26.0±1.09(0.99)	3.51±0.04(0.13)
	1		299±4.95	24.0±0.03	23.8±0.02(0.99)	
	2		296±4.23	17.7±1.34	17.6±1.30(1.00)	
	3		117±4.09	14.9±0.20	14.8±0.19(0.99)	
	4		76.9±4.01	14.0±0.24	13.8±0.17(0.98)	
35Al 1500Si	0	408	720±11.27	36.1±1.02	34.4±1.07(0.95)	13.0±1.48(0.36)
	1		424±25.36	27.8±0.15	26.7±0.38(0.96)	
	2		304±4.23	21.8±0.14	20.5±0.29(0.94)	
	3		266±9.11	18.3±0.18	16.7±0.10(0.91)	
	4		217±9.80	14.4±0.20	11.9±0.34(0.83)	
35Al 2500Si	0	408	696±25.77	33.9±0.49	31.0±0.48(0.92)	5.20±0.35(0.15)
	1		395±23.25	18.6±0.30	18.9±0.15(1.01)	
	2		313±40.73	10.7±0.29	10.5±0.28(0.99)	
	3		329±19.45	8.04±0.51	5.23±0.15(0.65)	
	4		219±10.86	8.70±0.51	4.90±0.21(0.56)	
35Al pH5.6	0	41	24.5±0.29	16.2±0.39	15.2±0.18(0.94)	15.3±0.14(0.94)
	1		23.2±1.67	15.8±0.14	15.3±0.12(0.97)	
	2		18.8±1.59	16.7±0.10	14.1±0.25(0.85)	
	3		21.1±0.63	15.0±0.31	13.2±0.16(0.88)	
	4		19.7±0.17	11.5±1.42	10.8±0.33(0.94)	
35Al 2500Si pH5.6	0	41	36.2±3.31	33.9±0.06	32.5±0.58(0.96)	27.6±0.28(0.81)
	1		26.0±1.31	22.2±0.17	20.8±0.23(0.94)	
	2		20.5±0.61	21.9±0.65	18.1±0.38(0.83)	
	3		19.8±0.79	21.8±0.52	17.3±035(0.80)	
	4		21.9±1.41	19.9±0.20	16.6±0.09(0.83)	

Table 6.10. Total Si concentration ([Si]$_{TOT}$) in nutrient solutions (up to four days old) passing through filters of 5 μM, 0.45 μM, and a dialysis membrane. The mole fraction of the [Si]$_{TOT}$ concentration passing through 0.45 μM or dialysis membrane is given in parentheses.

Nutrient Solution	Solution Age (days)	[Si]$_{TOT}$ 5 μM (μM)	[Si]$_{TOT}$ 0.45 μM (μM)	[Si]$_{TOT}$ Dialysis membrane (μM)
25Al 1500Si	0	801±25.6	905±29.5(1.13)	542±26.5(0.68)
	1	757±51.1	757±51.1(1.00)	
	2	728±78.1	713±25.6(0.98)	
	3	702±27.5	657±6.64(0.94)	
	4	684±36.4	675±45.4(0.99)	
25Al 2500Si	0	2222±97.9	2089±42.9(0.94)	2077±107(0.94)
	1	1730±289	1328±116(0.77)	
	2	1384±71.1	1132±69.3(0.82)	
	3	812±55.6	750±70.8(0.92)	
	4	880±70.9	812±57.9(0.92)	
35Al 1500Si	0	1571±35.9	1524±106(0.97)	1200±135(0.76)
	1	1483±36.3	905±193(0.61)	
	2	1624±192	188±26.7(0.12)	
	3	1515±215.3	112±31.8(0.07)	
	4	1731±153	79.2±15.6(0.05)	
35Al 2500Si	0	2751±165	1790±118(0.65)	1111±102(0.40)
	1	2498±129	1052±78.1(0.42)	
	2	1477±11.7	993±128(0.68)	
	3	1067±108	713±25.6(0.67)	
	4	1495±29.5	1052±78.1(0.70)	
1500Si	0	2195±7.66	1876±61.9(0.79)	1686±27.0(0.77)
	1	1660±10.2	1645±32.0(0.51)	
	2	1253±15.3	1193±4.19(0.36)	
	3	1058±5.11	975±14.4(0.34)	
	4	971±19.4	898±1.47(0.26)	
2500Si	0	3648±106	2297±10.1(0.63)	2556±312(0.70)
	1	2182±15.3	1979±12.1(0.91)	
	2	1928±12.9	1664±78.1(0.86)	
	3	1748±7.22	1683±60.7(0.96)	
	4	1531±54.2	1297±10.2(0.85)	
35Al 2500Si pH5.6	0	3102±76.6	2439±51.1(0.79)	2565±172(0.83)
	1	3118±67.8	1600±231(0.51)	
	2	2704±51.1	967±19.1(0.36)	
	3	2262±135	779±12.4(0.34)	
	4	2321±106	607±35.4(0.26)	

6.5 Discussion

Aluminium only inhibited the growth, and reduced the dry weights, of *Anthoxanthum* originating from Sheriffmuir blanket peat (SMB). The Al-induced inhibition of root elongation in these seedlings was not reversed with the addition of Si. However the reduction in shoot growth by Al was ameliorated with the addition of 1000 μM Si(OH)$_4$. Addition of Si also increased both the total and shoot dry weights and reduced Al uptake and transport to shoots. In contrast, these low concentrations of Al (1.3 and 2.7 mg l^{-1}) were actually beneficial to the growth of *Anthoxanthum* originating from Flanders Moss (FM) and Kinloch Rannoch (KR). Root and shoot growth were both increased in the presence of Al, and dry weights were up to two-fold higher than control plants. The addition of 500 and 1000 μM Si stimulated growth further. With the exception of root uptake of Fe by KR races, Al did not have any effects on nutrition. P, K, Mg, and Fe uptake in these plants (KR) was reduced and translocation to the shoots sometimes impaired. Fe uptake at 1.3 mg l^{-1} Al, in KR races, was double that of control plants. This result supports the hypothesis of Grime & Hodgson (1969) who proposed low concentrations of Al competed with Fe for root binding sites, thereby releasing more Fe for uptake.

The findings of this study contradicted those of Davies & Snaydon (1972) who found a high correlation among races of *Anthoxanthum odoratum*, Al-tolerance and their natural soils. They found that high concentrations of Al in culture solutions had less effect upon root growth in races from acid soils compared with those from calcareous soils. The SMB races, of this study, originating from acid soils, were more affected than KR races from calcareous soils. It should be noted that Davies & Snaydon (1972) used tillers collected from their natural sites and therefore already adapted to the soil conditions. Furthermore, FM and SMB races originate from acid soils but these are peats and naturally low in Al (Chapter 3).

The higher concentrations of Al used in Experiment 2 (25 and 35 mg l^{-1} Al) significantly inhibited all aspects of growth in *Holcus lanatus*. Silicon addition to the nutrient solutions, as 1500 and 2500 μM Si(OH)$_4$, was shown to unequivocally alleviate the effects of Al toxicity. Amelioration tended to increase with increasing Si concentration but this was less consistently observed. These results are in agreement with those of Barceló et al. (1993), Cocker et al. (1998), Galvez & Clark (1991b), Galvez et al. (1987), Hammond et al. (1995), Hodson & Sangster (1993), and Ma et al. (1997) who also showed amelioration of Al toxicity using Si. Furthermore Ma et al. (1997) found the alleviative effect of Si increased with increasing Si concentrations.

The symptoms of Al toxicity observed were thickening of root tips, reduced lateral root growth (in terms of number), and thickened stunted lateral roots. The shoots were chlorotic and the majority died. These symptoms have been consistently reported in the literature. The lateral roots of Al-treated coffee were also thicker, shorter, and fewer in number compared with control plants (Pavan & Bingham 1982). With the addition of Si visual symptoms of Al stress were absent.

The most frequent symptom of Al-damage, also observed here, is the inhibition of root elongation. Root elongation was restored to a maximum of 99.0 % and a minimum of 55.3 % with Si addition. Si restored root growth of two sorghum cultivars to 50 % and 30 % in studies by Galvez et $al.$ (1987), the least tolerant cultivar showing the lowest relative root growth. Relative root and shoot growth (in terms of length and numbers of roots, shoots, blades and tillers) were generally lowest in $Holcus$ originating from KR. Measured concentrations of soil solution Al ($[Al]_{TOT}$) were lowest at this site where soil pH's were up to pH_{H_2O} 6.2. At these pH's the solubility of Al is at its lowest and aluminate is predominant (pH> 6.2).

Shoot growth in Al+Si treatments was frequently greater than control plants but tiller production was not always significantly enhanced in the presence of Si. This was again especially evident in KR seedlings but not characteristic of SMM seedlings which responded best to Si. The soil pH of SMM suggests the dominant Al form present is Al^{3+}. This race is therefore naturally exposed to the toxic forms of monomeric Al. Furthermore these results are compatible with studies such as Galvez et $al.$ (1987) where the least Al-tolerant crop cultivars showed the least response to Al+Si solutions. In this study the race, KR, which experiences the lowest Al concentrations (in non-toxic forms) also showed the least amelioration by Si.

Dry weights of barley seedlings were increased when grown in solutions containing both Al and Si (Hammond et $al.$ 1995). Si did not have a significant effect on the dry weights of $Holcus$ $lanatus$. However there were trends of increasing total and shoot dry weights in Al+Si treatments. This was particularly evident in SMM $Holcus$. Root dry weights were not significantly different between control, or Al, or Al+Si treatments. Hodson & Sangster (1993) also found Si did not increase dry weights. However both Hodson & Sangster (1993), and Galvez et $al.$ (1987) found lower root:shoot dry weight ratios in Al-treated sorghum. Ratios were increased in the presence of Si. The root:shoot ratios of $Holcus$ $lanatus$ were not reduced in the presence of Al and were often higher than control plants. The addition of Si did not significantly change ratios.

The addition of Si to Al nutrient solutions was found by both Hammond et $al.$ (1995) and Hodson & Sangster (1993) to increase Ca concentrations in roots and shoots to control concentrations. Ca uptake by roots of $Holcus$ was reduced by Al in all races except SMM. Absorption and subsequent

translocation to the shoots was increased in Al+Si treatments to control concentrations. SMM races experience toxic concentrations of Al in their natural distribution and are therefore likely to be more tolerant of Al-induced inhibition of Ca uptake. Ryan *et al.* (1994) provided evidence contrary to the common hypothesis that Al^{3+} inhibits root growth by reducing Ca^{2+} uptake. They found treatment of wheat (cv. Scout 66) with 2.64 μM (0.07 mg l^{-1}) Al at pH 4.5 severely inhibited root growth without affecting Ca^{2+} uptake. P and K concentrations in *Holcus* roots were actually greater in Al-treated plants. Marienfeld & Stelzer (1993) and Rengel & Robinson (1990) also showed K-uptake and transport into the stele (Marienfeld & Stelzer 1993) of oat and ryegrass roots was not affected by Al-stress. However the concentrations of both these nutrients translocated to *Holcus* shoots was greater in Al+Si treated plants. Pavan & Bingham (1982) suggested the Al-reduced transport of P to coffee tops arose through precipitation of P on the root surface or in the root cells rendering it immobile. It is likely that P was precipitated in the root (surface or cell walls) of *Holcus*, especially since actual root P concentrations were greater than those of Al+Si-treated and control plant roots. Both Barceló *et al.* (1993) and Cocker *et al.* (1998) showed reduced Al uptake in plant tissue after growth in Al+Si solutions. Root Si concentrations increased with increasing Al concentrations in the solutions. Si concentrations in the root and shoot tissues of *Holcus lanatus* from FM increased with an increase in either Si or Al concentrations in nutrient solutions. There were no consistent differences in the other four races. Al uptake and translocation to shoots increased with an increase in solution Al concentration in all five races. Concentrations of Al in the roots of Al+Si treated *Holcus lanatus* were surprisingly higher than in Al-treated plants. However translocation to the shoots was lower (although not consistently) implying Al is detoxified at the roots and prevented from reaching the shoots. This was particularly evident in SMB and KR races. The Al/Si analyses of roots suggest Al is precipitated as aluminosilicates, probably in the root cortical cell walls.

Hodson & Sangster (1993), and Hodson & Wilkins (1991) provided evidence, using X-ray microanalysis, to show Al-Si co-deposition in outer cell walls of the root epidermis of sorghum and *Picea abies*. Only one study to date, Godde *et al.* (1988), has investigated similar co-deposition in plant tops. Al has been shown to be deposited in leaf cell walls of tropical tree Al-accumulators (Cuenca *et al.* 1991). Furthermore, Galvez & Clark (1991) showed Al transported to sorghum shoots increased in the presence of Si but growth was not inhibited. Co-deposition as a means of detoxification in roots seems likely, and this, together with the potential deposition in plant shoots, warrants further study.

The alleviation of Al toxicity did not appear to be due to a Si-induced reduction in Al uptake by *Holcus* roots but to a greater tolerance of Al in the plant tissues. Barceló *et al.* (1993) also suggested the alleviation of Mn toxicity through Si addition was not achieved by lower Mn uptake, but by greater tolerance of high Mn concentrations in the plant tissues. The $[Al]_{mmm}$ measured in Al+Si

solutions did not decline significantly. Nominal concentrations of 25 and 35 mg l^{-1} Al ([Al]$_{TOT}$ 5 μM) were only reduced to 60-90 % of original solutions. GEOCHEM did not predict a reduction in the free activity of Al in Al+Si solutions. Between 74 and 76 % of Al was predicted to be present as a free metal. There was no evidence of hydroxyaluminosilicate production in Al+Si solutions from the dialysis analysis. Molar proportions of Al, in Al+Si solutions, passing through the dialysis membrane ranged from 0.13-0.36 and were no different from proportions in solutions of Al alone (0.07-0.22). It should be noted however that the solutions were not stable and dialysis analysis was only carried out in initial solutions (0 days). HAS formation may have increased with time. The results emphasise the necessity for constant monitoring of solutions.

These results contradict those of Barceló et al. (1993) who found Si reduced [Al]$_{mono}$ by about 50 %. The [Al]$_{mono}$ in 25Al (averaged over three days before solution change), 378 μM, was only reduced to 342 μM and 285 μM in 25Al and 1500Si and 25Al and 2500Si. [Al]$_{mono}$ in 35Al, 522 μM, was reduced to 482 μM and 468 μM. These corresponded to reductions of 10 %, 25 %, 8 %, and 10 %. Al was therefore still in an available and toxic form for plant uptake, and the formation of HAS species was insignificant. At pH 4.2 HAS formation would not be expected to contribute significantly to the availability of Al.

A recent study, Corrales et al. (1997), was able to distinguish the ameliorative effects of Si on Al-induced inhibition of maize growth from Al/Si interactions in nutrient solutions by pretreating plants with 1000 μM Si for 72 h (+Si) prior to Al exposure for 24 h in nutrient solutions with no added Si. These plants, +Si, showed greater RER compared with plants not pretreated with Si (-Si). The ameliorative effect was a result of reduced Al uptake in +Si plants and not a consequence of decreased Al availability in the solutions.

Silicon at lower concentrations was beneficial to the growth of Holcus lanatus. Relative root elongation and root numbers were frequently greater than controls. Hammond et al. (1995) also found Si alone, at 2000 μM, increased both root length and dry weight in barley. 700 μM Si improved root growth by 69-87 % in Coker 315, DPL90, and McNair cotton cultivars (Li et al. 1989). Contrary to Marschner et al. (1990), Si supply increased root and shoot maize dry weights and increased both root and shoot P concentrations (Corrales et al. 1997). P uptake, and translocation to shoots, was increased in FM, SMB, and SMM races of Holcus by Si. However high Si concentrations of 2500 μM significantly reduced growth in Holcus. These Si-induced reductions in growth were often more extreme than Al-induced reductions (25 Al). Seedlings originating from KP and SMM were least affected by 2500 μM Si(OH)$_4$. Seedlings from the organic soils, FM and SMB, and from KR were most affected. Analysed Si concentrations were least in the soil solutions from these sites (Chapter 3). Hammond et al. (1995) found reduced root concentrations of Ca in barley seedlings

grown with 2800 μM Si *per se*. Similar reductions in Ca were not evident in *Holcus lanatus* at high Si concentrations (2500 μM). Dry matter yields were lower than control plants in seedlings from two of the five sites (FM and SMB). Analysed Si concentrations in soil solutions from these two sites were also at their lowest. Similarly, Galvez *et al.* (1987) found Si concentrations of 2670-3560 μM reduced shoot yields.

Aluminium (35Al) was not toxic to *Holcus* growth when in solution at pH 5.6. At this pH the dominant species of monomeric Al expected are $Al(OH)^{2+}$ and $Al(OH)_2^+$ and the actual measured $[Al]_{mono}$ were greatly reduced compared with solutions at pH 4.2. GEOCHEM also predicted that at pH 5.6 70.2 % of Al was complexed by OH compared to only 8 % at pH 4.2. Al, in solution at pH 5.6, greatly stimulated root elongation in *Holcus* whose provenance was KR. However there were reductions in shoot elongation although these were less severe than with Al in solution at pH 4.2. These monomeric species are therefore not as toxic as Al^{3+} but do show some toxicity to *Holcus lanatus*. There were no reductions in dry weights of plants grown in solution with Al at pH 5.6 compared with control plants. Pavan & Bingham (1982) showed Al-induced reductions in the growth of coffee seedlings. The regression equation they obtained for shoot growth plotted against GEOCHEM-calculated activity for Al^{3+} had a greater correlation coefficient than regressions of shoot growth against $AlSO_4^+$ or $AlOH^{2+}$ activity. Brenes & Pearson (1973) also found Al^{3+} activity to be the best index of Al toxicity for corn and sorghum. Kinraide (1991) however, believed Al^{3+} on its own has only been shown to be toxic to wheat cultivars in an earlier experiment by Kinraide & Parker (1989). Red clover, lettuce, and turnip were not sensitive to Al^{3+} but were sensitive to mononuclear hydroxy-Al. Amelioration of Al^{3+} toxicity by protons, was suggested by Kinraide (1991) to possibly account for the apparent toxicity of mononuclear hydroxy Al species.

The Al uptake by roots increased significantly at the higher pH in *Holcus*. Godbold *et al.* (1995) also showed Al uptake by *Picea abies* increased with pH from 4.0 to 5.0 and this was mainly due to greater concentrations in root cortex cell walls.

The results of the dialysis analyses of 35Al 2500Si solutions at pH 5.6 show hydroxyaluminosilicates were formed in these solutions and were also not toxic to the growth of *Holcus lanatus*. Exley *et al.* (1997) showed HAS species were not toxic to *Salmo salar* at pH 5.5.

6.6 Conclusions

- Al inhibited the growth of SMB races of *Anthoxanthum*, but stimulated growth in FM and KR races.

- Despite enhanced growth, nutrient acquisition was reduced in Al-treated *Anthoxanthum*.

- Fe uptake alone was stimulated by Al in KR races of *Anthoxanthum*.

- Silicic acid, at both concentrations, ameliorated Al toxicity in *Holcus lanatus*. Root lengths, shoot lengths, laterals, leaf area, and dry weights were increased in Al+Si-treated plants compared with Al-treated plants.

- Amelioration by Si increased with an increase in silicic acid concentration.

- Si restored nutrient uptake and translocation to shoots to that in control plants.

- Alleviation of Al toxicity was not the result of a reduction in Al uptake, nor was it the result of the formation of HAS species in the nutrient solutions.

- At 1500 μM, Si enhanced growth in *Holcus*, particularly in races which also originated from the most Si-rich soils.

- At 2500 μM, Si inhibited growth in *Holcus*, particularly in FM and SMB races which also originated from those soils with the least Si.

- Al was not toxic to *Holcus* in solutions at pH 5.6. Root elongation was stimulated by Al in KR races at this pH.

- Hydroxyaluminosilicate species, formed at pH 5.6, were not toxic to *Holcus*.

Chapter 7

Changes in the root cell anatomy and ultrastructure of *Holcus lanatus* L. grown with Al and Si

7.1 Introduction

The majority of proposals regarding the initial site of action by Al implicate changes in the root elongation rate. Clarkson (1965, 1969) believed a reduction in root elongation was the most obvious consequence of Al treatment. Furthermore he suggested Al action involved a blockage of the cell cycle during DNA synthesis and reduced mitotic activity in root apical meristems. Later publications, such as Matsumoto *et al.* (1976b), showed Al-accumulation in nuclei as well as inhibition of DNA synthesis, and furthermore, they suggested Al bound to nucleic acids.

However recent studies have shown that Al-treated roots can recover and resume apical growth, suggesting that the effect of Al on the root meristem is not permanent, and more likely to be a result of apoplastic rather than symplastic Al (Horst 1995). Observed meristem cell nuclei appear structurally stable, and changes in meristematic cell ultrastructure occur very slowly after Al exposure (Bennet & Breen 1991).

Al uptake was shown by Bennet *et al.* (1985) to initially occur in peripheral root cap cells and only very slowly reached the remaining cells of the root apex. Cap mucilages have been found to have a very high affinity for binding Al (Horst *et al.* 1982). The slow movement of Al within plant cells is generally agreed, and substantiated by chemical analyses of roots and shoots implying that the primary site of Al action must coincide with the site of Al uptake (root cap).

Peripheral cap cells secrete mucilaginous, polysaccharide materials to the cell exterior. Their secretory function is reflected in their ultrastructure (e.g. Golgi body, secretory vesicles, amyloplasts). Recent publications have shown ultrastructural changes in these cells after Al exposure. Exposing Al-sensitive *Danthonia linkii* to Al, resulted in a reduction in secretory vesicle size (in peripheral cap cells), Golgi body number, and amyloplast size (in central cap cells) of root cap cells. These changes were reflected in the lower volume of mucilage produced by the root tips (Crawford & Wilkens 1997). A similar reduction in mucilage production was observed by Puthota *et al.* (1991) in the Al-sensitive wheat cultivar Victory. Mucilage polysaccharide accumulated between the plasmalemma

and cell wall, and its transport to the cell exterior was inhibited. After only 4 h exposure to Al, Golgi body volume was less than 20% of plants grown without Al. Al inhibited the development of the Golgi apparatus function in cells of the quiescent centre in decapped maize roots (Bennet *et al.* 1985). Within 6 h of exposure the numbers of secretory vesicles, Golgi bodies and cisternae per dictyosomal stack were reduced. Al-treated roots also failed to regenerate a root cap, unlike maize treated with no Al.

The evidence above implies Al is inhibiting cell division and root elongation rates indirectly via a primary effect on root cap function. Bennet & Breen (1991) described a new theory implicating a stimulus-response coupling with Al toxicity. Common environmental signals, such as gravity and light, induce changes in plant root growth rates. These adaptive responses can include both growth stimulation or growth retardation. All such stimuli investigated to date have apparently shown signal perception in the root cap. The signal is then translated into a growth response after its transduction between interacting cell populations (Bennet & Breen 1991). They proposed that Al could equally act as one such environmental stimulus. Furthermore they suggested a possible connection in stimulus-response mechanisms between the activity of the root cap (signal strength) and possible tolerance mechanisms. Al tolerance perhaps reflects a plant's ability to maintain the activity of the root cap and cap secretion in the presence of Al. Johnson & Bennet (1990) found Al tolerance coincided with an unusually high level of root cap activity in *Aristida junciformis* (Bennet & Breen 1991).

7.2 Aims

- To determine the location of Al within the root cells of *Holcus* using hematoxylin staining and compare Al localization in Al-treated and Al+Si-treated roots.

- To investigate ultrastructural changes in the root tips of *Holcus lanatus* after exposure to Al.

- To investigate any differences in cell ultrastructure between Al-treated and Al+Si-treated roots of *Holcus* and discuss possible mechanisms of Si alleviation of Al toxicity regarding cell ultrastructure and root caps.

7.3 Methods

Root anatomy and ultrastructure were investigated in *Holcus lanatus*, originating from FM, treated with solutions of Al and Si as described in Chapter 6.

7.3.1 Light microscopy

For light microscopy the end 15-mm portions of five main roots from one plant in each treatment were cut. After removing the terminal 5 mm, the root sections were fixed in 2 % gluteraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) and kept at room temperature for 2 h. The root segments were vacuum infiltrated and placed in a fridge overnight. They were then drained and dehydrated with graded ethanol and embedded in JB4 Resin. Cross-sections (7 nm thick) were cut using an ultramicrotome and stained with hematoxylin.

Also for light microscopy terminal 10-mm root sections were fixed in FAA (13 ml formaldehyde: 5 ml glacial acetic acid: 200 ml 50 % aqueous ethanol) and embedded in paraffin wax (Paraplast Embedding Media) following dehydration with graded ethanol. Thin cross-sections (7 nm) were cut using a ultramicrotome and stained with Safranin and Light Green. Several measurements were recorded from each prepared slide: diameter of root, number of cortical cells, diameter of central stele, width of epidermis.

7.3.2 Electron microscopy

For electron microscopy terminal 10 mm root portions were embedded in Spurr's resin. Roots were excised and fixed in 2.5 % gluteraldehyde in 100 mM sodium cacodylate buffer at pH 7.2 for 2 h at room temperature. Root tips were then washed in three changes of the buffer for 10 min each, and post-fixed in 1 % osmium tetroxide in 100 mM buffer for 2 h at room temperature (in a fume cupboard). Root tips were washed in buffer, en-bloc stained in 2 % uranyl acetate (in 30 % acetone) for 1 h, and dehydrated in a graded acetone series consisting of 40 min in each of 60, 90, and 100 % acetone (diluted with deionised water). This was followed by 1 h in 100 % anhydrous acetone. The samples were infiltrated overnight in a mixture of 100% acetone and Spurr's low-viscosity resin (1:1), followed by 100 % Spurr's resin overnight on a rotary mixer. Root tips were trimmed back to the terminal 2-mm portion and these tips embedded in flat Beem capsules with fresh Spurr's resin and polymerised for 48 h at 60 °C. Ultra-thin longitudinal sections of root tips were cut using a Reichert Ultracut E Ultramicrotome and diamond knife (about 90 nm thick), stretched using xylene vapours, collected on 200 mesh copper grids, and stained with 8 % uranyl acetate in distilled water for 20 min

and Reynolds lead citrate for 7 min. They were observed under a Philips 301 transmission electron microscope (TEM) and micrographs taken.

7.3.3 Hematoxylin staining

Five plants per treatment were randomly chosen for hematoxylin staining of root tips after Polle *et al.* (1978). The staining solution consisted of 2 g hematoxylin and 0.2 g of $NaIO_3$ dissolved in one litre of deionised water. The plants were rinsed and transferred from nutrient solutions into deionised water for 30-60 min. The roots were subsequently dipped in staining solution for 15 min. After staining, the seedlings were washed in flowing deionised water for 1 min and photographed.

The distribution of Al in the root tissue zones (approximately 0.5-1 cm from the tip) was also observed in cross-sections cut from the JB4 embedded root portions. Sections were stained with hematoxylin, mounted with DPX mountant and observed under a optical Zeiss microscope.

7.4 Results

7.4.1 Hematoxylin staining

Root tips of *Holcus* seedlings treated with 25 and 35 mg l^{-1} Al stained a dark purple after immersion in hematoxylin. Al acts as a binding agent of hematein, an oxidised component of the hematoxylin solution. The stainable region of the root tip coincides with the region of root elongation. Where no staining occurs root cells are expected to elongate normally (Polle *et al.* 1990). With increasing Si concentration in the nutrient solution +Al (25 or 35Al), the extent of staining diminished (Figure 7.1). Staining of root cross-sections showed similar patterns. There was no staining in control sections (Figure 7.2 d). Staining was evident within the outer cortical cells and on the outer root surface in roots treated with Al (Figures 7.2 a and b). There was some staining on the root outer surface of roots grown in 35Al 2500Si (Figure 7.2 c) revealing the presence of Al deposits on the root surface of both Al and Al+Si treated plants.

7.4.2 Root anatomy

Table 7.1 gives the mean root diameter, stele diameter and number of cortical cells in *Holcus* grown in Al and Al+Si solutions. Both root diameters and stele diameters were greater in Al-treated roots
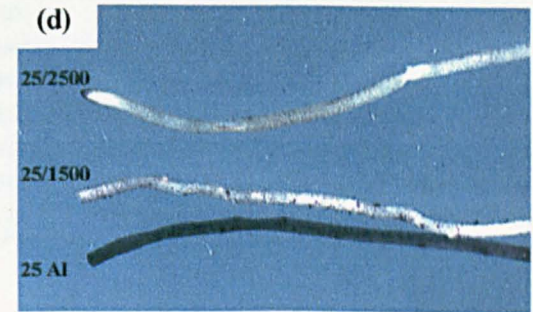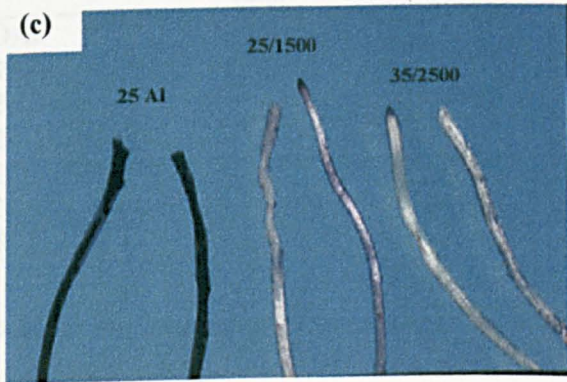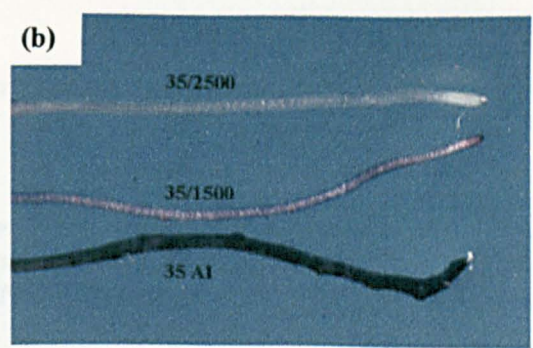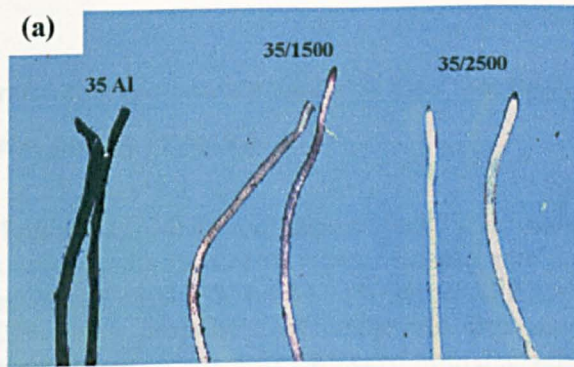
Figure 7.1. Hematoxylin staining of roots of *Holcus lanatus* treated with (a)/(b) 35 mg Al l^{-1}, 35 mg Al l^{-1} + 1500 µM Si, 35 mg Al l^{-1} + 2500 µM Si, and with (c)/(d) 25 mg Al l^{-1}, 25 mg Al l^{-1} + 1500 µM Si, and 25 mg Al l^{-1} + 2500 µM Si.
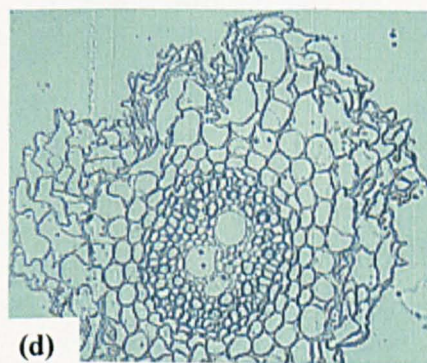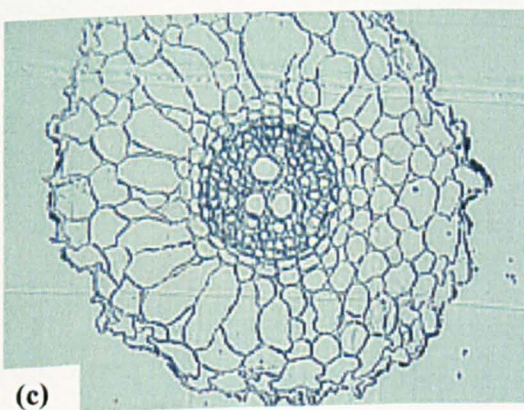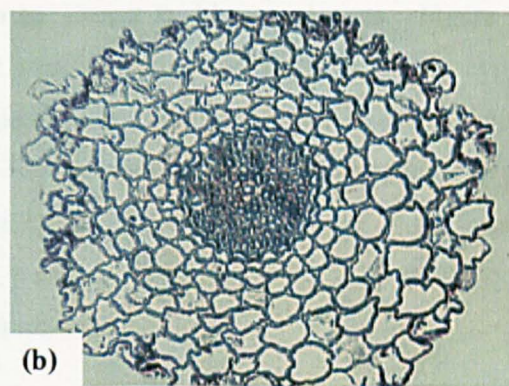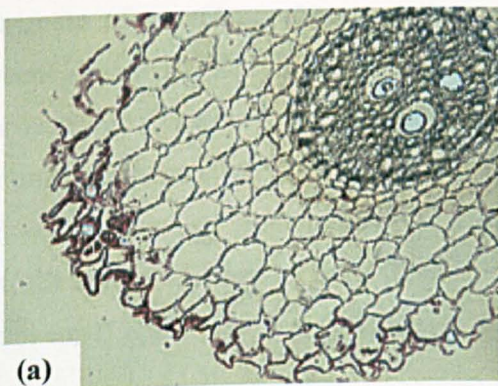
Figure 7.2. Hematoxylin staining of root cross-sections from *Holcus lanatus* after treatment in (a)/(b) 35 mg Al l^{-1}, (c) 35 mg Al l^{-1} + 2500 µM Si, and (d) control nutrient solutions.

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reflecting the swollen nature of their root tips. The number of cortical cells in Al-treated roots was also greatly increased.

Table 7.1. Mean root diameter and area, stele diameter and area, cortex area and number of root cortical cells (± s.e) in *Holcus lanatus* root cross-sections embedded in paraffin. Seedlings were grown in 25Al, 35Al, 25Al 1500Si, 25Al 2500Si, 35Al 1500Si, and 35Al 2500Si nutrient solutions. The mean proportion of whole-root area occupied by vascular tissues and cortex are given in parentheses. Areas were estimated from diameter measurements.

Treatment	Root diameter (mm)	Root area (mm^2)	Stele diameter (mm)	Stele area (mm^2)	Cortex area (mm^2)	Cortical cell number
0Al	0.22 ±0.01	0.70 ±0.04	0.09 ±0.01	0.25±0.02(36.6)	0.45±0.02(63.4)	80 ±2.45
25Al	0.31 ±0.01	0.98 ±0.04	0.11 ±0.01	0.35±0.02(35.6)	0.64±0.03(64.4)	108 ±3.64
35Al	0.31 ±0.02	0.98 ±0.05	0.12 ±0.01	0.37±0.02(39.1)	0.61±0.04(60.9)	98 ±5.57
25Al 1500Si	0.27 ±0.02	0.86 ±0.07	0.10 ±0.01	0.32±0.02(39.6)	0.53±0.05(60.4)	87 ±1.53
25Al 2500Si	0.21 ±0.01	0.65 ±0.05	0.08 ±0.01	0.24±0.02(37.8)	0.41±0.04(62.2)	65 ±5.19
35Al 1500Si	0.22 ±0.01	0.70 ±0.03	0.09 ±0.01	0.28±0.01(40.1)	0.42±0.02(59.9)	78 ±2.00
35Al 2500Si	0.29 ±0.02	0.91 ±0.08	0.10 ±0.01	0.30±0.03(33.5)	0.60±0.05(66.6)	96 ±10.2

7.4.3 Root cap cell ultrastructure

Figures 7.3 and 7.4 show several Transmission Electron Microscope (TEM) micrographs of longitudinal sections through the root tips of *Holcus lanatus* grown in control, 35Al, and 25Al 2500Si nutrient solutions. Immature cells were highly vacuolated irrespective of treatment (Figures 7.3 and 7.4). Vacuolation increased towards the outer cortical cells (Figures 7.3 d and e). The extent and increase of vacuolation however appeared to be greater in Al treated roots. Figure 7.3 c shows vacuoles almost enveloping the cell nucleus and occupying the majority of the cytoplasm. No obvious reductions in E.R., Golgi bodies, or mitochondria were visible in Al treated roots (Figures 7.3 b and f, compared with Figures 6.4 f and g). Neither nuclei nor cell walls appeared to be disrupted. However very few amyloplasts were observed in Al treated roots but these were abundant in both Al+Si treated and control roots (Figure 7.4 b, c and e). What appeared to be secretory cells on the root cap (Figure 7.4 h) were also absent in Al treated roots.
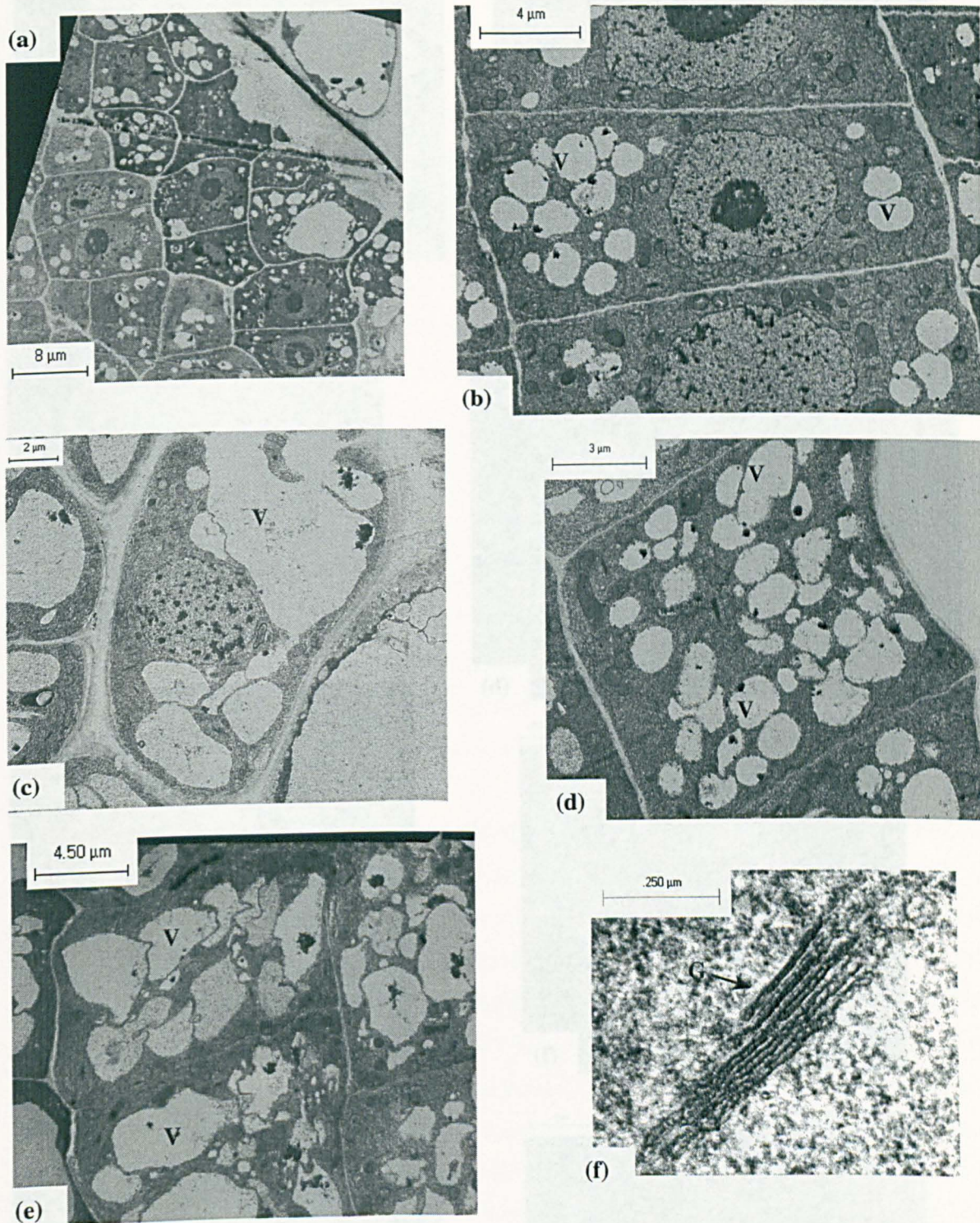
Figure 7.3. TEM micrographs showing cortex cells on a longitudinal root tip section of *Holcus lanatus* treated with 35 mg l^{-1} Al. The immature cortex cells, (a)-(e) were heavily vacuolated with vacuoles often enveloping the nucleus (c). Vacuolation increased in the outer cortex cells, (d) and (e), to a greater extent in Al treated roots compared with controls. Mitochondria and Golgi bodies (f) were still abundant in Al treated roots. G = Golgi body, V = vesicle.
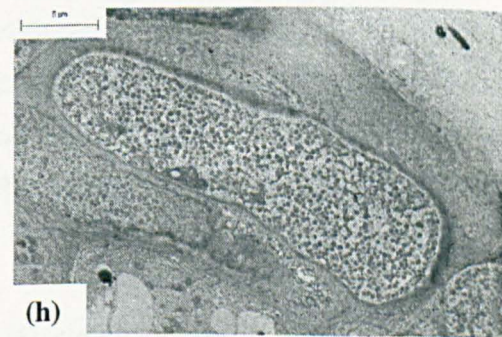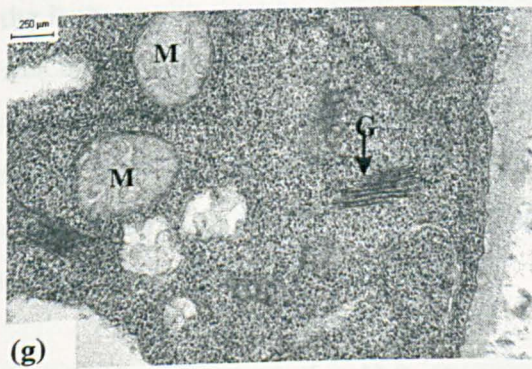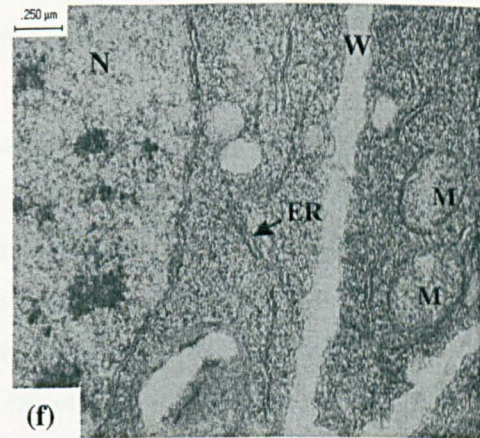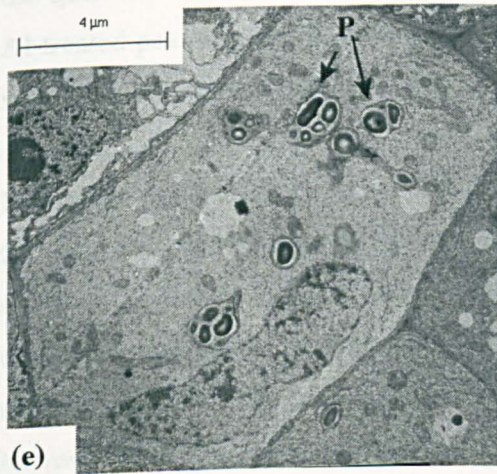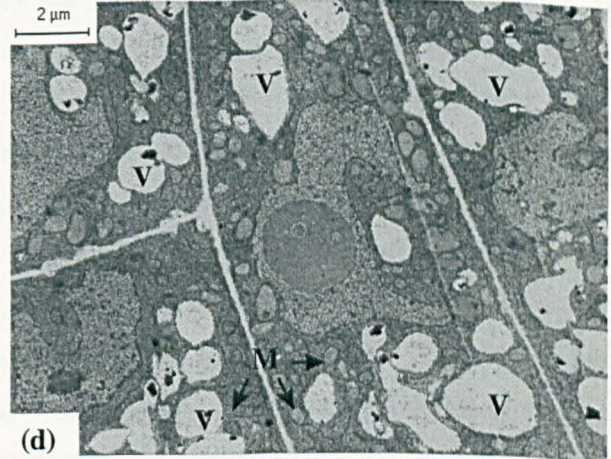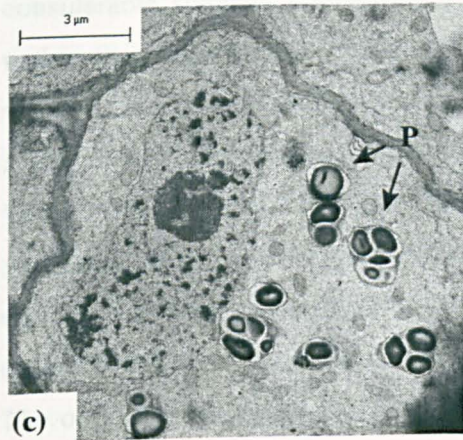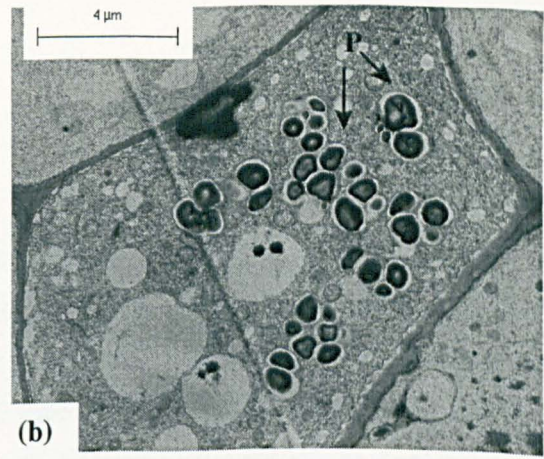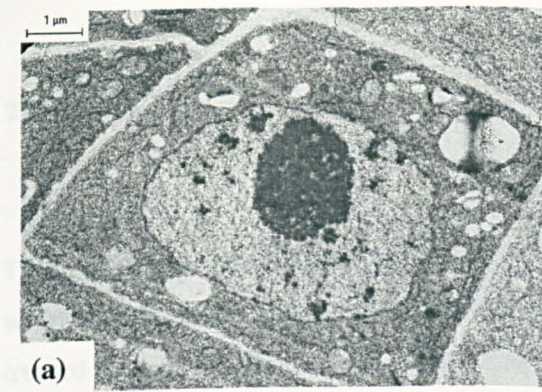
Figure 7.4. TEM micrographs showing cortex cells of *Holcus lanatus* grown in control and 25 mg Al l^{-1} + 2500 μM Si nutrient solutions. Both (a) control and (d) Al+Si-treated roots were very vacuolated. Plastids were abundant in control cells, (b) and (c), and frequent in the cells of Al+Si treated roots (e). ER, Golgi bodies and mitochondria were abundant in both (f) control and (g) Al/Si treated root cells. Secretory cells were present in the outer root cap of cells of both treatments (h). ER = Endoplasmic reticulum, G = Golgi body, M = mitochondrion, N = nucleus, P = plastid, V = vesicle, W = cell wall.

7.5 Discussion

The association between hematoxylin and Al has frequently been used to identify the location of Al within plant tissues. Root tips of Al-treated *Holcus* stained a dark purple, while those of Al+Si-treated *Holcus* barely stained at all. Likewise root cross-sections of Al-treated plants exhibited considerable staining, predominantly associated with the epidermal cell wall, with little staining within the cortical cells. Intracellular staining was however not apparent in Al+Si-treated roots, where Al was mainly found on the outer surface of the root. The patterns of hematoxylin staining in *Holcus* were in agreement with patterns found in maize (Corrales *et al.* 1997) and in wheat (Kinraide 1988). Corrales *et al.* (1997) also found hematoxylin staining in epidermal and outer cortex cells of Al-treated maize, and Al deposits on the root surface of maize roots pretreated with Si. Further staining was also visible in the cell walls of root endodermal cells of Al-treated maize. Kinraide (1988) found no intracellular staining in root cap and epidermal cells of Al-treated wheat (cv. Tyler). The only cells which stained intracellularly were those of the quiescent centre. The significance of the quiescent centre will be discussed later. In contrast, Wagatsuma *et al.* (1995) found staining of Al-treated pea roots was only localised to the epidermis when plants were grown in high salt nutrient solutions. If grown in low salt solution, almost all the cortical cells stained.

In a recent publication, Bennet (1997) warned scientists of the use of hematoxylin as a universal stain for Al. He showed staining patterns between Al-tolerant and Al-sensitive plants did not always correlate with differences in Al uptake, and the roots of Al-stressed pea plants did not consistently respond to hematoxylin despite Al being present at the time of staining. He also found that some roots not treated with Al also responded positively to hematoxylin. Control *Holcus* (0 Al) did not however show any positive staining in this study. However the whole root Al contents contradicted the hematoxylin staining pattern of Al+Si-treated plants. The Al concentration measured implied Al was taken up by the roots but the hematoxylin staining implied Al was mainly present as deposits on the root surface. The Al concentrations may only reflect Al deposits attached to the root surface which were digested along with the roots. Combining the use of hematoxylin staining, ionic composition analysis, and x-ray microanalysis (EDXA) analysis may be useful in confirming the exact location of Al within root cells.

Despite the limitations of EDXA (discussed in Chapter 5) in identifying Al deposits, the technique may be more sensitive than hematoxylin at lower concentrations of Al. EDXA has been used to show the Al distribution in the roots of *Pinus strobus*, oat, *Picea rubens*, and wheat.

Al preferentially accumulated in the cell walls of root cortical tissue of an Al-sensitive wheat cultivar (Carazinho x Egret) (Delhaize *et al.* 1993a) and electron dense globular deposits were observed between the cell wall and cell membrane of the epidermal cells of the Al-sensitive Wagrigal cultivar (Wheeler *et al.* 1992). Only after prolonged exposure was Al detected within the cells (Delhaize *et al.* 1993a). Similarly Al was predominantly localised in the walls of peripheral cortex cells of oat (Marienfeld & Stelzer 1993, Marienfeld *et al.* 1995). A steep decreasing [Al]-gradient was observed from the rhizodermis towards the stele. Intracellular Al concentrations were always low and no enhanced Al concentrations were ever detected in the cell nuclei.

The results of both hematoxylin staining and EDXA generally imply the plasma membrane acts as an effective barrier against Al-influx into cells. Influx only seems to occur after prolonged exposure. The inhibition of root elongation and reduction in mitotic activity appear more likely to be indirect effects of Al, contrary to the suggestion that they were the direct result of intracellular Al binding to DNA and inhibition of DNA-synthesis (Matsumoto *et al.* 1976b). Minocha *et al.* (1992) actually found increased DNA-synthesis during the first few hours of Al treatment (Marienfeld & Stelzer 1993). Furthermore the reduction in root elongation and mitotic activity observed in oat seedlings exposed to Al did not coincide with any detection of intracellular [Al]. However the minimum detection limits of EDXA are themselves high (2-3 mM) and the presence of intracellular Al can therefore not be entirely excluded (Horst 1995).

Ultrastructural investigations showed that the meristematic tissues of the Al-treated *Holcus* roots showed increased vacuolation compared with control and Al+Si treated roots. The cells however remained intact and there were no obvious alterations in the ultrastructure. There was no visible alteration of nuclear fine structure. Nuclei contained large nucleoli in control, Al, and Al+Si treated roots. The fine structure of the mitochondria and presence of the E.R. and Golgi bodies also appeared unaltered in Al-treated roots. Similar results were found in ultrastructural investigations in Al-treated oat roots by Marienfeld *et al.* (1995), and in *Pinus strobus* seedlings by Schier & McQuattie (1995). Cortical cells were vacuolated and vacuolation proceeded from the inner cortex to the rhizodermis in both control and Al-treated oats but vacuolation was more advanced in Al-treated oats, as it was in Al-treated *Holcus*. Increasing Al concentration from 12.5 to 100 mg l^{-1} resulted in more and larger vacuoles in *Picea abies* meristem cells (Hecht-Buchholz *et al.* 1987). Marienfeld *et al.* (1995) also found no alteration in the nuclear fine structure, or in numbers of mitochondria, E.R , or plastids. In this study the numbers of plastids in *Holcus lanatus* were greatly reduced in Al-treated plants but not in control or Al+Si treated plants.

If Al were binding to DNA and inhibiting its synthesis Marienfeld *et al.* (1995) suggested that this binding would not specifically inhibit DNA synthesis but should block general nuclear functions.

However both their TEM observations, and the TEM observations in this study, provided no evidence for this. There did not appear to be any ultrastructural deviations in nuclei from Al-treated roots from control roots.

The increased vacuolation, observed by Marienfeld et al. (1995) and in this study, also did not appear to be a means of detoxification. Vacuoles tended to be located close to nuclei suggesting no pinocytotic function and no precipitations or fibrillar material within the vacuoles were visible. In contrast element distribution studies of Schlegel et al. (1992) in Picea rubens showed high concentrations of Al (109 mM) in vacuoles of root cortical cells. Wheeler et al. (1992) showed electron dense deposits in vacuoles of wheat roots exposed to Al which they suggested may contain Al. No deposits however were observed in vacuoles of Holcus treated with Al.

Marienfeld et al. (1995) suggested that the increased vacuolation was unlikely to be a direct effect of Al but rather an effect of growth retardation. Similar increased vacuolation was described by Barlow & Adam (1989) for cold-stressed plants (Marienfeld et al. 1995).

Crawford & Wilkens (1997) investigated ultrastructural changes in the root cap cells of two Al-tolerant native Australian grasses : Danthonia linkii and Microlaena stipoides. This study was more advanced in that they used image analysis in order to quantify ultrastructural changes. Lower Al concentrations (1-2 mg l^{-1}) produced larger root cap cells and high Al concentrations (5-10 mg l^{-1}) produced smaller cells. No change in root cap cell size was observed in Holcus but no quantitative measurements were made. The more Al-tolerant grass, Microlaena, contained 90 % more Golgi bodies and had 50 % larger amyloplasts than Danthonia. Although Golgi bodies were observed in Al-treated Holcus again no quantitative assessment was made. However practically no amyloplasts were observed in Al-treated roots whereas amyloplasts were abundant in both control and Al+Si treated roots. The size of amyloplasts in Al treated roots that were present was not determined. The number of mitochondria in Danthonia was not affected by Al. There did not appear to be any reduction in mitochondria number in Holcus.

Crawford & Wilkens (1997) also found that the size of secretory vesicles in peripheral cap cells of Danthonia were significantly smaller after exposure to 5 and 10 mg l^{-1} Al. Secretory vesicles in the more Al-tolerant Microlaena were on the other hand unaffected. Secretory vesicles are produced by Golgi bodies. This suggested that production and export of mucilage was dramatically reduced in Danthonia. Microlaena produced mucilage in considerably greater quantities than Danthonia. It was also observed in Holcus lanatus that peripheral cap cells did not appear to have any secretory vesicles in Al-treated roots. This was not the case in either control or Al+Si treated roots.

The ultrastructural observations and hematoxylin staining of root cortical cells imply the entry of Al into the symplast and subsequent impairment of cellular functions were not the primary effects of Al toxicity. However it appears that greater concentrations of Al were able to enter the symplast and be translocated to shoots compared with Al+Si treated roots. This contradicts the findings of other researchers such as Wagatsuma *et al.* (1995) who found Al accumulated in large amounts in the younger and outer cells of pea roots and cell destruction was extensive in these regions. Wagatsuma *et al.* (1995) also observed that the plasma membrane was destroyed, a feature which was not observed in the TEM micrographs of *Holcus lanatus*.

Ultrastructural studies have frequently and successfully been used to investigate Al phytotoxicity but not apparently to investigate silicon's amelioration of Al toxicity. The investigations here suggest that the production of mucilage could be involved in the mechanisms of overcoming Al toxicity by Si. Similar suggestions were made to explain increased Al tolerance by Crawford & Wilkens (1997). Unfortunately mucilage production by *Holcus* roots was not quantified. However the only significant differences in cell ultrastructure between Al- and Al+Si-treated *Holcus* were an increase in vacuolation, a reduction in amyloplasts, and an absence of secretory vesicles in the peripheral cap cells of Al-treated roots. Secretory vesicles are produced by Golgi bodies and quantification of Golgi numbers may have provided further evidence implicating mucilage production in Si protection. Bennet & Breen (1991) have suggested that maintenance of root growth in the presence of Al is dependent upon the activity of peripheral root cap cells. They found reductions in root growth alongside reductions in amyloplast number which also distribute polysaccharide material produced by Golgi bodies. Further investigations with quantitative analysis are required to fully elucidate the mechanisms through which Si alleviates Al toxicity.

Ryan *et al.* (1993) showed Al-induced inhibition of root elongation was neither increased nor decreased after the removal of the root cap, contradicting theories involving root cap cell activity and Al tolerance. The cells of the quiescent centre respond to the removal of the root cap by entering mitosis and regenerating a new cap, and were shown by Kinraide (1988) and Galsomies *et al.* (1992) to stain intracellularly with hematoxylin. Ryan *et al.* (1993) made no reference to whether or not root caps regenerated. Bennet *et al.* (1985) did however show that Al-treated roots failed to regenerate a new root cap. The cells of the quiescent centre and their role in Al-toxicity deserve further attention. Whether or not root caps regenerate in the presence of Al+Si also requires further investigation, as does the potential role of Si in protecting the quiescent centre cells and allowing root cap regeneration.

7.6 Conclusions

- Root tips of Al-treated *Holcus* stained a dark purple in hematoxylin, and the staining decreased in Al+Si-treated plants.

- Al was primarily located in the peripheral cortical cell walls of roots. Al precipitates on root outer surfaces were found in Al+Si-treated *Holcus*.

- Al-treated roots were swollen and contained a larger number of cortical cells. The proportion of whole-root area occupied by stele and cortex did not change among treatments.

- The fine structure of nuclei, nucleoli, plasma membranes, and cellular constituents were not affected by Al.

- Vacuolation was greater in Al-treated *Holcus*.

- Root cap cells of Al-treated roots had fewer amyloplasts and no visible secretory vesicles.

- Root cap cells of Al+Si-treated roots did not lack amyloplasts or secretory vesicles.

Chapter 8

Organic acids reduce aluminium toxicity in *Holcus lanatus* L. and stimulate growth in *Deschampsia flexuosa* (L.) Trin.

8.1 Introduction

Organic acids have been shown by several authors to detoxify aluminium (Foy *et al.* 1990, Gerke 1994, Harper *et al.* 1995, Kerven *et al.* 1991, Ostatek-Boczynski *et al.* 1995, Ownby & Popham 1989, Slattery & Morrison 1995, Suhayda & Haug 1986, Suthipradit *et al.* 1990). As early as 1933, Mattson recognised that plants growing in soils high in organic matter did not exhibit symptoms of Al toxicity at the same pH that soils low in organic matter did (Hargrove & Thomas 1981). Later both Evans & Kamprath (1970) and Thomas (1975) found less exchangeable Al in organic soils compared with mineral soils despite the low pH of organic soils (Hargrove & Thomas 1981). The effect increased with decreasing soil pH (Thomas 1975).

In a similar manner to the suggested detoxification of Al by hydroxyaluminosilicates, organic acids are thought to chelate soluble, phytotoxic Al (Al^{3+} and hydroxy-Al ions) and render it biologically unavailable. Low molecular weight, short chain aliphatic and aromatic (discussed in Chapter 1) organic acids are common components of soil solutions and released into the solution through microbial decomposition of organic matter or exudation from plant roots (Hue & Amien 1989, Ostatek-Boczynski *et al.* 1995). Complexes between organic ligands and Al, formed at low pH, vary in strength (Ostatek-Boczynski *et al.* 1995). The high molecular weight fulvic and humic acids form far more stable complexes than the low molecular weight carboxylic acids (Harper *et al.* 1995). Fulvic and humic acids are less susceptible to microbial degradation than the low molecular weight acids and can therefore provide a more permanent amelioration of Al toxicity (Harper *et al.* 1995).

The ameliorative effect of organic acids has been shown by investigators both in acid soils and in nutrient solutions. Hargrove & Thomas (1981) grew barley (cv. Kearney) in an acid fragipan soil from western Kentucky with added peat. Plant growth was better, and exchangeable Al lower, at any given pH with increasing amounts of organic matter. Hue & Amien (1989) added ground shoot material of cowpea, Leucaena, or guinea grass at 0, 5, 10 & 20 g kg^{-1} to an acid Ultisol (pH_{H_2O} 4.0, 50 % Al saturation). They grew an Al-sensitive tree legume, *Sesbania cochinchinensis*, for four weeks in each treatment. Organic matter additions increased biomass production. Cowpea and Leucaena were

more effective than guinea grass in ameliorating Al toxicity. They suggested that the detoxification of Al occurred via two paths: first the reduction in soluble Al resulting from an increase in soil pH after manure additions, and secondly, the complexation of the remaining soluble Al by organic ligands, particularly low molecular weight organic acids. The same reasoning was proposed by Bessho & Bell (1992) who found applications of organic matter (ground barley and tree legume) prevented Al toxicity in mungbean.

There was no reduction in dry matter production of ryegrass grown in nutrient solutions with 20 or 200 mg Al l^{-1} as Al citrate or oxalate (Muchovej et al.1988). Similarly Kerven et al. (1991) showed no inhibition in root elongation of mungbean (cv. Berken) when seedlings were grown with citrate, L-malate, and oxalate in Al solutions.

Both Harper et al. (1995) and Tan & Binger (1986) investigated Al detoxification in maize using the high molecular weight humic and fulvic acids. Harper et al. (1995) added both fulvic and humic acids (extracted from Eucalyptus leaves) at three nominal concentrations: 40, 120, and 360 mg C l^{-1} to solutions with 0.8 mg Al l^{-1} (30 μM). In all treatments with both organic acid and Al, relative root length was significantly greater than that in treatments with Al alone. Monomeric Al was totally complexed by organic acids. Dry weights increased and plants appeared green and healthy in appearance when grown with Al and humic acid (Tan & Binger 1986). Addition of humic acid also reduced Al uptake by shoots and suppressed reductions in leaf P concentrations.

Differences in the complexing ability of organic ligands have been frequently shown. Bruckert (1970) classified short-chain carboxylic acids into three groups of Al complexers: strong complexers (such as citric, oxalic, and tartaric acids), moderate complexers (such as malic, malonic, and salicylic acids), and finally, weak complexers (such as succinic, lactic, formic, acetic, and phthallic acids).

Hue et al. (1986) showed a positive correlation between the ability of these acids to detoxify Al and the relative position of OH/COOH groups on their main C chain. Positions which favoured the formation of stable 5-or 6- bond ring structures with Al were common to the strong Al complexers. These acids had either 2 pairs of OH/COOH attached to two adjacent carbons (citric and tartaric) or two COOHs directly connected (oxalic). The formation of complexes of Al^{3+} with hydroxy carboxylic acids, and their stability constants, was reviewed by Motekaitis & Martell (1984). Hue et al. (1986) found relative root growth of cotton seedlings, grown in solutions with both Al and organic chelates, followed Bruckert's classification. Within the strong complexers, the alleviative effect of the acids increased in the order : citric > oxalic > tartaric. Cotton roots grew normally in solutions with 0.5 mg Al l^{-1} (18.5 μM Al) provided 50 μM citric acid was also added. Slightly less effective were oxalic and tartaric acid: cotton grown in solutions with 0.25 mg Al l^{-1} (9.25 μM Al) with 50 μM

of either oxalic or tartaric acid had relative root lengths of 101 and 93% (relative to plants grown with no Al or organic acids). In contrast, the weak Al complexer, succinic acid, had virtually no protective effect in solutions with concentrations of Al > 0.10 mg l^{-1} (3.7 μM). The results of Ostatek-Boczynski et al. (1995) agreed with those of Hue et al. (1986). Root growth of mung-bean was unaffected by Al in solutions with nominal Al concentrations of 0.14 and 0.54 mg l^{-1} (5 and 20 μM) as Al citrate (molar ratio 1:1), and was only marginally reduced by Al oxalate at the same concentration. Both citrate, and to a lesser extent succinate, were able to stimulate regrowth of wheat after 5 h treatment in Al solutions (Ownby & Popham 1989). However malate was found to be ineffective despite being considered a moderate Al detoxifier.

Several investigators have also shown a correlation between organic acids and differential Al tolerance (Foy et al. 1990, Galvez et al. 1991, Klimashevskii & Chernysheva 1980, Pellet et al. 1995). One possible mechanism of Al tolerance may be the chelation of Al, either in the cytoplasm by internal organic ligands, or in the rhizosphere by root exuded organic ligands. Foy et al. (1987) found Al stress induced by 1.5 and 3.0 mg Al l^{-1} (55 and 110 μM at pH 4.5) reduced concentrations of citric, succinic, and total organic acids in the roots of Al-sensitive "Kearney" barley but not the Al-tolerant "Dayton". Al tolerance was associated with an ability to maintain normal concentrations of organic acids in the presence of Al (Foy et al. 1990). Similar results were found in Al-tolerant sorghum cultivars by Cambraia et al. (1983) and Galvez et al. (1991), and in maize hybrids by Suhayda & Haug (1986). The tolerant cultivars of both crops accumulated significantly higher concentrations of t-aconitic and malic acids than Al-sensitive cultivars.

More recently however Foy et al. (1990) grew five wheat cultivars, representing a range in Al tolerance, in nutrient solutions containing 0 or 2 mg l^{-1} Al. The cultivar roots and shoots were analysed for organic acids. Al treatment reduced concentrations of c-aconitic but increased fumaric and malic acid concentrations in the plant tops. Similarly c-aconitic concentrations were reduced in wheat roots, while concentrations of fumaric, malic, succinic, and total organic acids were increased. However differential Al tolerances were neither consistently correlated with differences in foliar acid concentrations, nor with changes in these concentrations under Al stress. Suthipradit et al. (1990) also found contradictory results to the authors above. Oxalic and malic acids were compared with fulvic acid as Al detoxifiers in soybean, cowpea, and green gram. Concentrations were within the mid-range of reported values for acid soil solutions. There was no inhibition of growth in any of the three crops when grown in Al with fulvic acid. However neither oxalic or malic acid (both known as strong Al complexers) had any ameliorative effect of Al toxicity at 50 μM. A far greater proportion of Al remained in the monomeric form in solutions with these two acids.

Few investigations have looked at the effects of organic ligands *per se* on plant growth. Concentrations of fulvic and humic acids in the range 25-250 mg C l^{-1} have been shown to stimulate root elongation (Harper *et al.* 1995). Root elongation of maize was increased by 30 % by humic acid at 40 mg C l^{-1}, and by 36 % by fulvic acid at 120 mg C l^{-1} (Harper *et al.* 1995). However there are few records of the effects of individual carboxylic acids on plant growth.

8.2 Aims

- To analyse soil solutions (from five soil types) for organic acid content and prepare nutrient solutions based on these measurements.

- To investigate the amelioration of Al toxicity in the presence of organic acids in *Holcus lanatus*. (The reasons for the choice of study species were given in Chapter 2, Section 2.2).

- To determine the effectiveness of different organic acids in ameliorating Al toxicity.

- To determine whether or not amelioration is concentration dependent.

- To investigate the effects of organic acids, with no added Al, on the growth of *Holcus lanatus* and *Deschampsia flexuosa*.

8.3 Methods

8.3.1 Soil solution analysis of carboxylic acids

Fresh soils, collected from all five sites, were centrifuged according to the methods in Chapter 3. The collected solution was immediately filtered through a 0.45 μM membrane filter prior to HPLC analysis. To concentrate samples for HPLC analysis subsamples of 2 ml of soil solutions were freeze-dried and redissolved in 0.5 ml of sulphuric acid.

Fourteen commonly occurring organic acids were determined using high performance liquid chromatography (HPLC). Detection was by a ACS Model 750/14 Mass Detector at 214 nm. Solvent delivery was via a Waters M-45 HPLC pump, controlled by a Waters 680 Automated Gradient Controller. All solvents were filtered through an 0.45 μM membrane filter and degassed prior to use. Quantification of peaks was achieved using a Shimadzu C-6RA recording integrator. The analytical column was a OA-2000 Aromatic Acids Column, with the dimensions 0.65 x 10 cm. The mobile phase used was 0.025 M H_2SO_4 at a constant flow rate of 0.3 ml min^{-1}. Organic acids were

quantitatively identified by comparing the retention times and peak areas of soil-solution chromatograms with those of HPLC-grade chemical standards. The organic acids isolated, in order of elution, were: oxalic, citric, tartaric, malonic, lactic, formic, acetic, propionic, malic, fumaric, glutaric, glycolic, phthalic, and succinic acid.

8.3.2 Al/organic acid interaction in nutrient solutions

Seeds of *Holcus lanatus* were collected in August 1996 from FM, SMB, SMM, KP, and KR (Chapter 2, Table 2.1). The seeds were stored in dry and dark conditions at room temperature until the start of the experiment. Seeds were germinated on acid-washed sand on filter paper in January 1997 in the Stirling University growth rooms. The Petri dishes were kept under a photoperiod of 16 h light and 8 h dark with a PAR of 200 μmol m^{-2} s^{-1}. Temperature was 20 °C during the day and 15 °C during the night. Seeds germinated after three to four days and were watered with dilute culture solution (diluted 10 times) for about seven days before being transferred into full strength culture solution. At this stage they were removed from Petri dishes and carefully threaded through thin glass tubes with deionised water. The glass tubes were suspended from the lids of 600-ml beakers in an initial culture solution with no added Al or organic acids and at pH 5.6 (Chapter 4, Section 4.3.1.1).

The composition of the culture solutions was the same as that used in Chapter 4 (Tables 4.1 and 4.2). Stock solutions of 100-strength of NH$_4$OH, Na$_2$SO$_4$, NH$_4$H$_2$PO$_4$, KFeEDDHA, Ca(NO$_3$)$_2$.4H$_2$O, CaCl$_2$.6H$_2$O, Mg(NO$_3$)$_2$.6H$_2$O, H$_3$BO$_3$, KH$_2$PO$_4$, Al(NO$_3$)$_3$.9H$_2$O, and MES buffer, and 1000-strength of MnSO$_4$.4H$_2$O, ZnSO$_4$.7H$_2$O, CuSO$_4$.5H$_2$O, and (NH$_4$)$_6$Mo$_7$O$_{24}$.4H$_2$O were made up and diluted appropriately. Beakers were covered in tinfoil to prevent algal growth and arranged in a randomised block design. Solutions were stirred daily and pH's corrected where necessary to pH 5.6 (initial solution) and pH 4.2 (treatment solutions) using 1M NaOH or 1M HCl. Culture solutions were changed twice per week in initial solutions.

8.3.2.1 Experiment 1

The effects of one strong Al complexer, tartaric acid, and one weak complexer, formic acid (Hue *et al.* 1986) on the growth of *Holcus lanatus* were determined. Aluminium was added to the culture solutions at 35 mg l^{-1} Al in the form Al(NO$_3$)$_3$.9H$_2$O and organic acids were added at 100 and 1000 μM in the following combinations: 0 mg l^{-1} Al + 0 μM organic acids (control), 35 mg l^{-1} Al, 35 mg l^{-1} Al + 100 μM formic acid, 35 mg l^{-1} Al + 1000 μM formic acid, 35 mg l^{-1} Al + 100 μM tartaric acid, and 35 mg l^{-1} Al + 1000 μM tartaric acid. The following abbreviations are used corresponding to the treatments: control, 35Al, 35Al 100F, 35Al 1000F, 35Al 100T, and 35Al 1000T. Culture solutions were kept at pH 4.2. There were five replicate seedlings per treatment per site. Solutions were changed every 2 days to minimise microbial breakdown (Kerven *et al.* 1991).

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The number of roots and their lengths, and the number of blades and tillers and their lengths, were recorded on the day treatments began and at harvest. Seedlings were harvested after 28 days growth in +Al/organic acid treatments (1 Feb-28 Feb). Roots and shoots were separated, rinsed in deionised water, and dried in an oven at 60 °C for 48 h and the dry weights of shoots and roots recorded. Root:shoot ratios were determined.

Prior to drying, leaf area of ten blades per treatment, per site, were determined. Leaves were photocopied, and the photocopy scanned. Area was determined from the scanned image using NIH Image 5b.

Between 100 and 300 mg of oven-dried leaves and roots were digested in a sulphuric acid-hydrogen peroxide mixture (Allen 1989) in a block digester at 330 °C. Digested plant material was filtered through No. 44 Whatman filter paper and made up to 100 ml. Concentrations of Ca and Mg were measured using a Varian AA-575 atomic absorption spectrophotometer with a nitrous oxide-acetylene flame. An air-acetylene flame was used to determine K (flame emission) and Fe concentrations. Total Al ($[Al]_T$) was measured with a Pye Unicam SP9 Atomic Absorption Spectrophotometer fitted with a Unicam GF90 furnace and FS90 furnace autosampler. Unicam 919 series atomic absorption software was used. P was measured on a Tecator FIAstar 5010 flow injection auto-analyser using the stannous chloride-ammonium molybdate method.

Solutions were analysed to monitor nominal element concentrations using the same analytical techniques as those in Chapter 3. Subsamples of 5 ml from each of six beakers, from each of the six treatments, were withdrawn from fresh culture solutions, and from solutions one, two, three, and four days old. Thereafter solutions were changed every two days.

The concentrations of ($[Al^{3+}]$) and activities of ($\{Al^{3+}\}$) free Al^{3+} in the nutrient solutions with and without organic acids were calculated by GEOCHEM (Sposito & Mattigold 1980). The concentration of monomeric Al species in the nutrient solutions, $[Al]_{mono}$, was also determined by the 60 s Pyrocatechol violet method as described by Kerven et al. (1989).

8.3.2.2 Experiment 2

The effects of organic acids alone on the growth of Deschampsia flexuosa originating from SMM, and Holcus lanatus originating from FM, SMB, SMM, KP and KR were determined. Deschampsia was grown in control (no added acids), 100 μM succinic acid, 1000 μM succinic acid, 100 μM formic acid, and 1000 μM formic acid nutrient solutions. The following abbreviations are used corresponding to the treatments : 100S, 1000S, 100F, and 1000F. Holcus were grown in control, 1000 F, and 1000 μM tartaric acid (1000 T). Culture solutions were adjusted to pH 4.2. There were five replicate seedlings per treatment per site. Solutions were changed every 3 days.

Measurements of root and shoot growth were the same as those taken in Experiment 1. Roots and shoots were harvested in the same manner as Experiment 1 after 28 days growth, and their dry weights recorded. Prior to drying, the leaf area of ten blades per treatment, per site of origin, were determined from scanned images using NIH Image 5b on leaf photocopies.

8.4 Results

8.4.1 Organic acids in soil solutions

Table 8.1 lists the organic acids present in the soil solutions and their estimated concentrations. Soil solutions of calcareous soils, KR, predominantly consisted of glycolic acid which was also present in acid soils but did not dominate the acid spectrum in these soils. The organic soils, and brown forest soils of KP, comprised a greater number of organic acids than either KR or SMM soils. Acetic, formic, glycolic, and succinic acids dominated FM and SMB soil solutions. SMM soils were high in acetic and glycolic acids. Total organic acid concentrations decreased in the following order: FM> KP> SMB> KR> SMM. Concentrations increased about 20-fold between FM and SMM soils.

8.4.2 Al detoxification by organic acids

8.4.2.1 Root elongation and number

The addition of organic acids significantly improved the growth of *Holcus* compared with Al-treated plants. Root elongation rates (RER) and numbers were significantly greater in these treatments than in Al-treated plants (Figures 8.1 and 8.2). This was particularly pronounced in FM races where RER in Al+organic acid-treated plants was about twice that of Al-treated plants. Organic acids were least effective in SMM races. The type of acid, formic or tartaric, did not significantly affect RER (Table 8.2), both were equally effective in ameliorating Al toxicity. Amelioration was however, significantly greater at higher concentrations of acid (Table 8.2). Relative RER were generally equal to control plants at these higher concentrations (Table 8.3). Root numbers were also increased in the presence of organic acids but, with the exception of FM, they were not increased to control numbers (Figure 8.2). Relative root numbers ranged from 27.9 % in SMM races to 107 % in FM races (Table 8.3). Tartaric acid was more effective in preventing Al-induced reduction in root numbers, as was the higher concentration of both acids (Table 8.2).

Table 8.1. Organic acid composition and concentration (µM) of soil solutions extracted from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR). ND, below detection limit. Percentage of total organic acids (listed in order of elution) is given in parentheses.

Organic Acid	FM	SMB	SMM	KP	KR
Oxalic acid	1694.9 (2.2%)	48.99 (0.2%)	ND	ND	ND
Citric acid	ND	664.6 (2.5%)	ND	ND	ND
Tartaric acid	263.0 (0.4%)	872.0 (3.3%)	ND	ND	ND
Malonic acid	6449.4 (8.5%)	1420 (5.3%)	687.8 (18.1%)	82.5 (0.1%)	1244.7 (17.1%
Lactic acid	2244.2 (3.0%)	ND	ND	ND	ND
Formic acid	3496.1 (4.6%)	5003.7 (18.8%)	ND	5088.3 (8.7%)	93.4 (1.3%)
Acetic acid	19135.8 (25.1%)	ND	919.0 (24.2%)	52044.9 (89.2%)	ND
Propionic acid	75.6 (0.1%)	159.0 (0.6%)	ND	6.0 (0.1%)	ND
Malic acid	271.0 (0.4%)	ND	ND	607.4 (1.0%)	759 (10.4%)
Fumaric acid	13.0 (0.1%)	ND	150.7 (4.0%)	ND	3.3 (0.1%)
Glutaric acid	119.0 (0.2%)	ND	ND	ND	ND
Glycolic acid	14446.2 (19.0%)	4706.3 (17.7%)	2000.0 (52.7%)	496.9 (0.9%)	5143.0 (70.7%
Phthalic acid	14.2 (0.1%)	21.9 (0.1%)	41.1 (1.1%)	3.5 (0.1%)	ND
Succinic acid	27934.2 (36.7%)	13700 (51.5%)	ND	ND	34.0 (0.5%)
TOTAL	**76156.7**	**26596.5**	**3798.7**	**58329.5**	**7277.4**

8.4.2.2 Shoot elongation, tiller and blade number

Shoot elongation rates (SER), like RER, were significantly increased in Al+organic acid solutions (Figure 8.3). The extent of amelioration was less than in the case of RER, relative SER were at most 88.8 % (Table 8.3). Tartaric acid was more effective than formic acid but amelioration was independent of acid concentration (Table 8.2). Figure 8.4 shows the improved shoot growth in the presence of Al.

Vegetative growth of seedlings, in terms of tiller and blade number, was significantly better in Al+organic acid-treated plants than in Al-treated plants (Figure 8.5). Tiller numbers of FM and SMB races grown with organic acids were equal to control plants in all sites. Relative numbers in these two races ranged from 75.7 to 164 % (Table 8.3). Only 1000 µM tartaric acid prevented an Al-induced reduction in tiller production in SMM races (Table 8.3). Blade numbers were most increased in SMB, SMM, and KR races. There were no significant differences between the type or concentration of acid (Table 8.2).

Leaf area was significantly increased in the presence of tartaric acid at any concentration and formic acid at 1000 µM (Figure 8.6 and Table 8.2).

8.4.2.3 Plant dry weights

The addition of organic acids to nutrient solutions significantly increased dry weights, particularly in FM, KP, and KR races (Figure 8.7). Tartaric acid increased total and root dry weights significantly more than formic acid (Table 8.2) as did higher acid concentrations. Al increased root:shoot ratios compared with control plants. Organic acids reduced ratios, particularly in SMM, KP, and KR.

Formic acid, and lower acid concentrations of 100 μM, reduced ratios significantly more than either tartaric acid or 1000 μM solutions (Table 8.2).

Table 8.2. Statistical analyses for root and shoot growth measurements, dry weights, and plant ionic compositions in *Holcus lanatus* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR), and grown in 0 Al, 35 Al, 35 Al 100F, 35 Al 1000 F, 35 Al 100 T, 35 Al 1000 T. *, p<0.05; **, p<0.01; ***, p<0.001; n.s, not significant. Degrees of freedom are : site 4, organic acid (formic or tartaric) 1, acid concentration (100 μM or 1000 μM) 1, and acid*concentration interaction 1.

Measurement		Site		Organic Acid		Concentration		Acid*Concentration interaction	
		F	p	F	p	F	p	F	p
Root growth									
Root elongation rate		2.79	*	1.46	n.s	63.38	***	0.55	n.s
Increase in number of roots		15.60	***	4.43	*	9.24	**	0.22	n.s
Tops growth									
Shoot elongation rate		8.42	***	9.34	**	0.09	n.s	0.35	n.s
Increase in total tiller number		15.78	***	0.10	n.s	0.29	n.s	4.39	**
Increase in total blade number		22.06	***	0.50	n.s	0.22	n.s	5.27	*
Leaf Area (FM only)				9.36	**	9.10	**	14.75	***
Dry weights									
Shoot		3.37	*	0.64	n.s	8.30	**	13.55	***
Root		6.31	***	61.97	***	142.2	***	0.04	n.s
Total		5.41	***	8.85	**	36.13	***	10.61	**
Root:shoot ratio		0.87	n.s	27.99	***	38.22	***	4.81	*
Ionic composition									
Shoot	P	6.87	***	7.87	**	27.50	***	27.28	***
	K	11.44	***	1.09	n.s	0.91	n.s	1.45	n.s
	Ca	1.70	n.s	0.26	n.s	27.70	***	12.94	***
	Mg	1.49	n.s	22.64	***	89.07	***	48.79	***
	Al	20.54	***	0.13	n.s	1.04	n.s	29.08	***
	Fe	4.80	**	0.32	n.s	5.31	*	2.27	n.s
Root	P	5.78	***	0.61	n.s	0.62	n.s	0.21	n.s
	K	3.46	*	1.31	n.s	0.25	n.s	5.33	*
	Ca	4.74	**	9.81	**	4.12	***	4.85	*
	Mg	6.15	***	0.58	n.s	0.01	n.s	3.53	n.s
	Al	3.41	*	4.39	*	14.77	***	7.31	**
	Fe	15.94	***	0.30	n.s	3.25	n.s	23.36	***
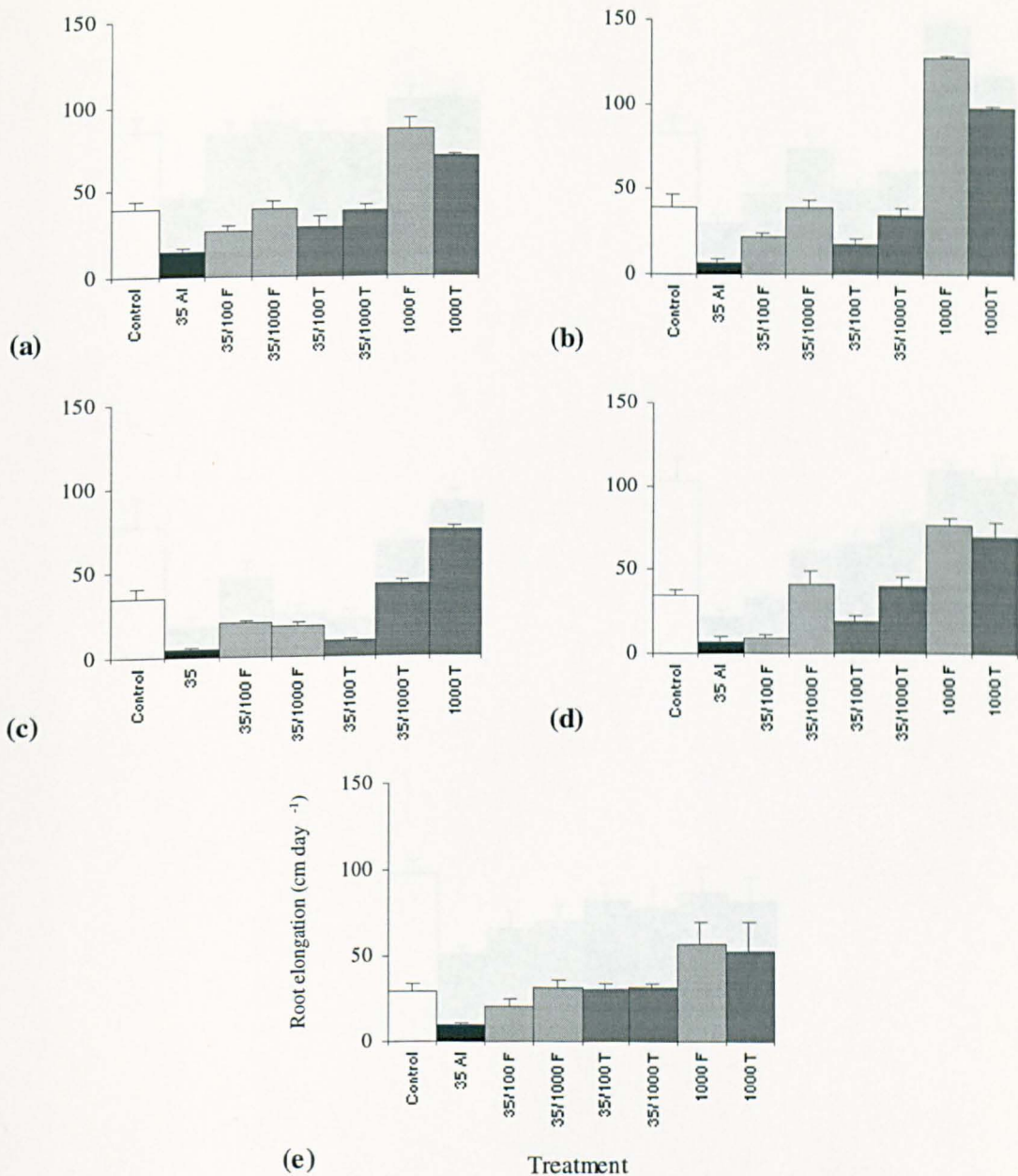
Figure 8.1. Mean rates of root elongation (cm day^{-1},± s.e) in *Holcus lanatus* L grown in control (□), 35 mg l^{-1} Al (■), 100 μM formic acid + 35 mg l^{-1} Al, 1000 μM formic acid + 35 mg l^{-1} Al, 100 μM tartaric acid + 35 mg l^{-1} Al, 1000 μM tartaric acid + 35 mg l^{-1} Al, 1000 μM formic acid, and 1000 μM tartaric acid nutrient solutions. *Holcus* originated from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR). ▨ , treatments including formic acid. ▨, treatments including tartaric acid.

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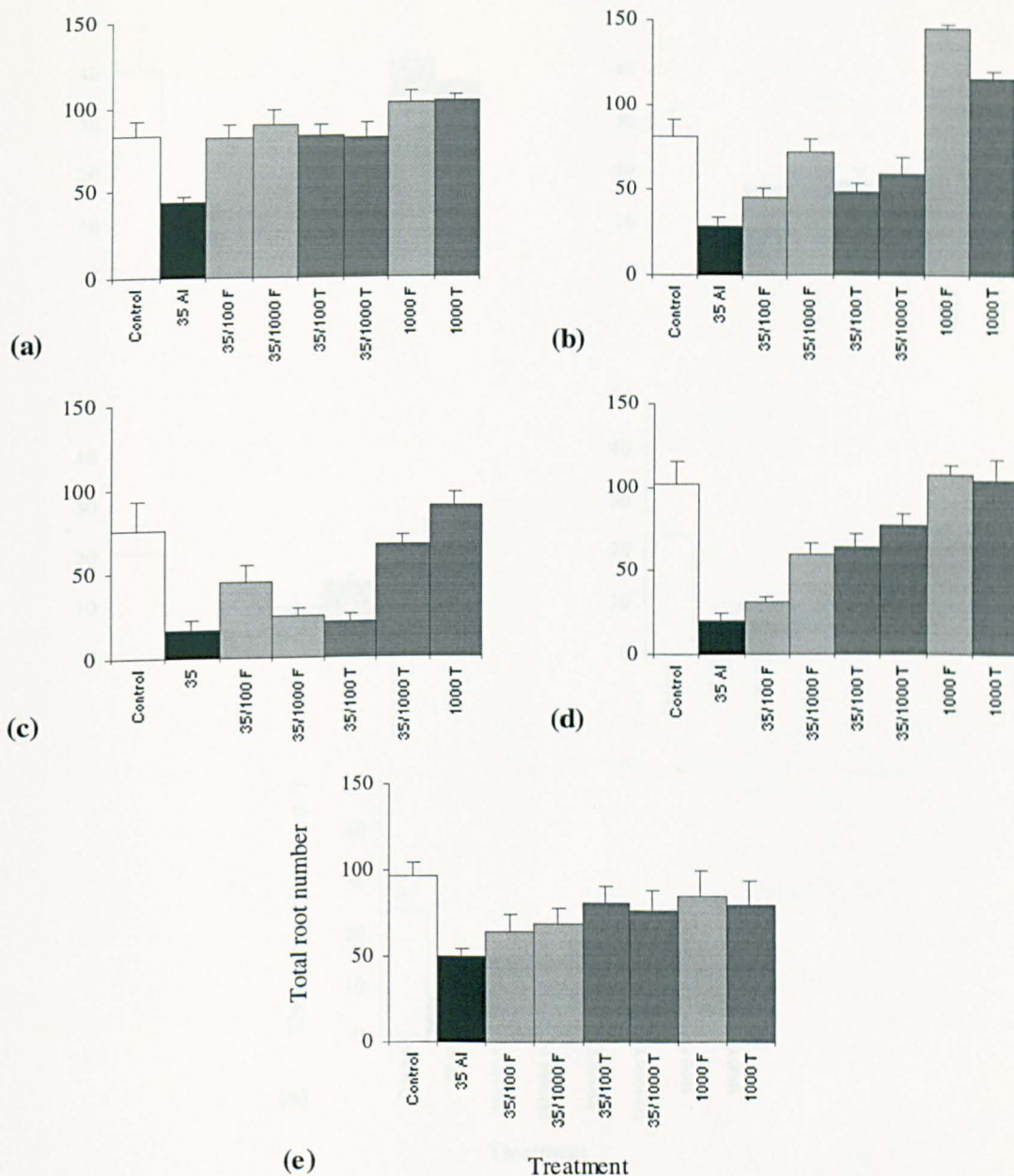
Figure 8.2. Mean increase in total root number (± s.e) in *Holcus lanatus* L grown in control (□), 35 mg l^{-1} Al (■), 100 µM formic acid + 35 mg l^{-1} Al, 1000 µM formic acid + 35 mg l^{-1} Al, 100 µM tartaric acid + 35 mg l^{-1} Al, 1000 µM tartaric acid + 35 mg l^{-1} Al, 1000 µM formic acid, and 1000 µM tartaric acid nutrient solutions. *Holcus* originated from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR). ▨ , treatments including formic acid. ▤ , treatments including tartaric acid.

Figure 8.3. Mean shoot elongation (cm day^{-1},± s.e) in *Holcus lanatus* L grown in control (☐) , 35 mg l^{-1} Al (■), 100 μM formic acid + 35 mg l^{-1} Al, 1000 μM formic acid + 35 mg l^{-1} Al, 100 μM tartaric acid + 35 mg l^{-1} Al, 1000 μM tartaric acid + 35 mg l^{-1} Al, 1000 μM formic acid, and 1000 μM tartaric acid nutrient solutions. *Holcus* originated from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR). ☐ , treatments including formic acid. ■ , treatments including tartaric acid.
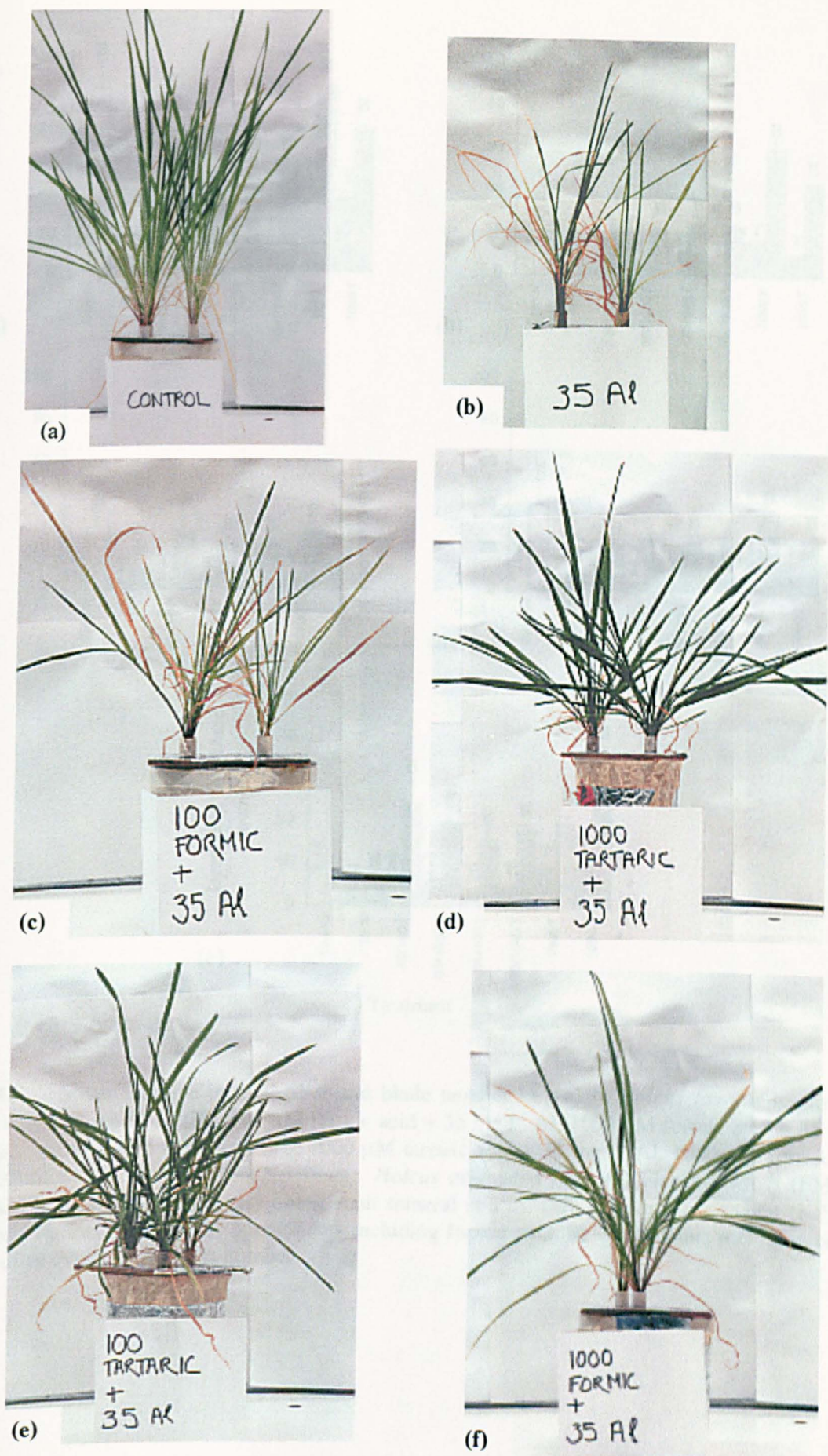
Figure 8.4. *Holcus lanatus* grown in (a) control, (b) 35Al, (c) 35Al 100F, (d) 35Al 1000T, (e) 35Al 100T, and (f) 35Al 1000F nutrient solutions.
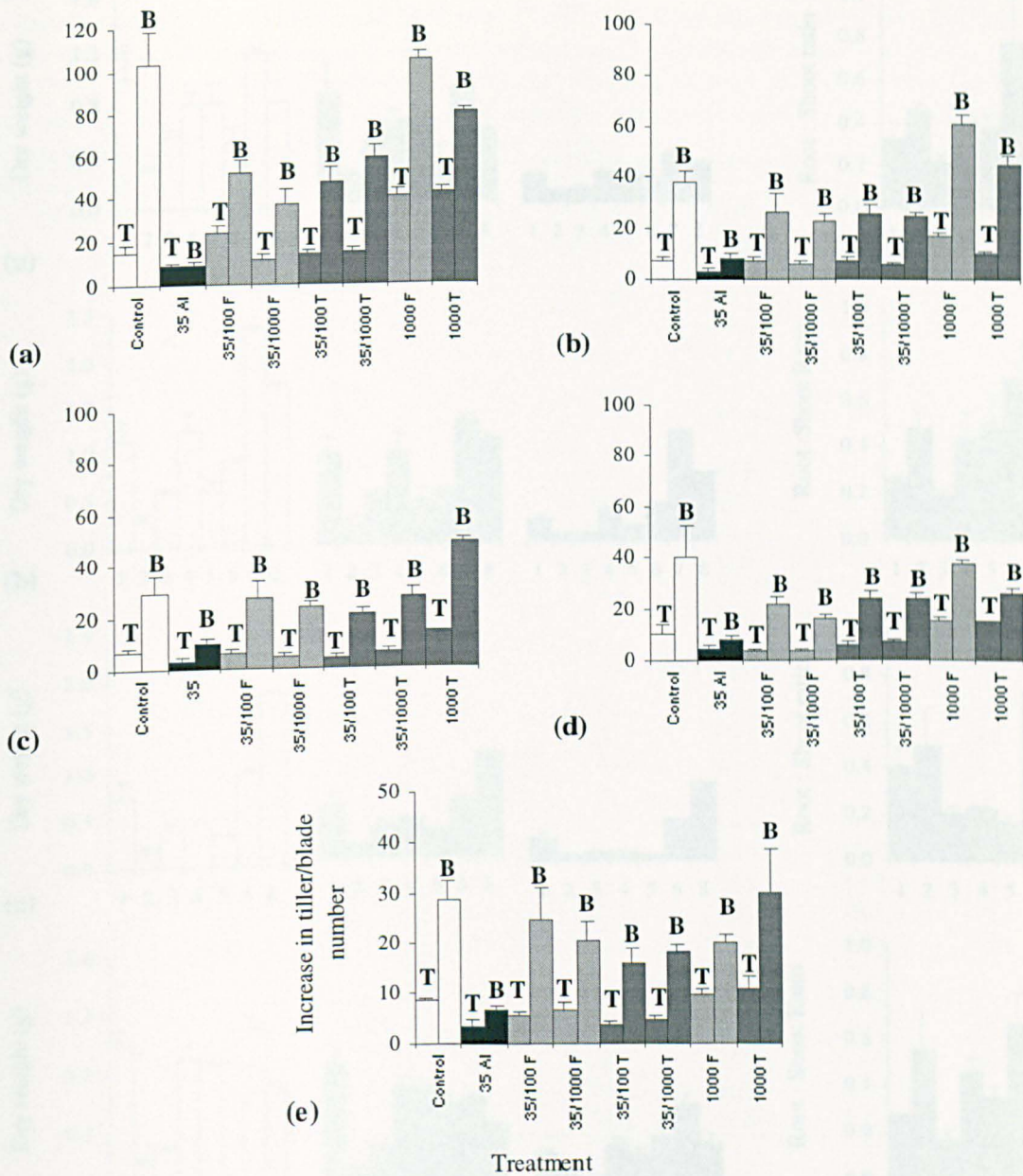
Figure 8.5. Mean increase in total tiller and blade number (± s.e) in *Holcus lanatus* L grown in control (☐) , 35 mg l^{-1} Al (■), 100 µM formic acid + 35 mg l^{-1} Al, 1000 µM formic acid + 35 mg l^{-1} Al, 100 µM tartaric acid + 35 mg l^{-1} Al, 1000 µM tartaric acid + 35 mg l^{-1} Al, 1000 µM formic acid, and 1000 µM tartaric acid nutrient solutions. *Holcus* originated from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch Rannoch (KR). ▨ , treatments including formic acid. ▨ , treatments including tartaric acid. **T**, tiller number. **B**, blade number.
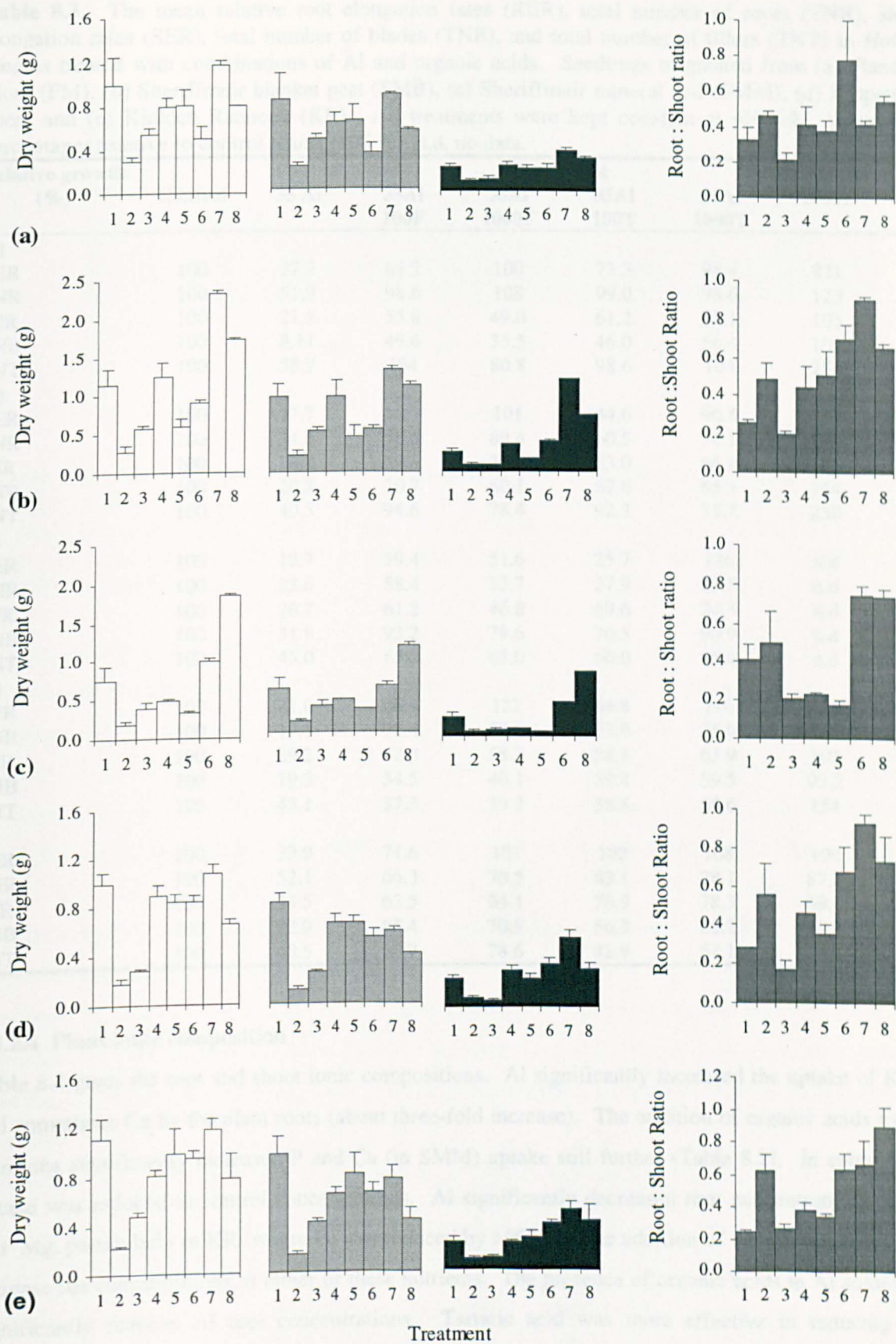
Figure 8.7. Mean total (□), shoot (▨), and root (■) dry weight (g, ± s.e), and root:shoot ratio (▨, ± s.e) in *Holcus lanatus* L grown in control (1), 35 mg l⁻¹ Al (2), 100 μM formic acid + 35 mg l⁻¹ Al (3), 1000 μM formic acid + 35 mg l⁻¹ Al (4), 100 μM tartaric acid + 35 mg l⁻¹ Al (5), 1000 μM tartaric acid + 35 mg l⁻¹ Al (6), 1000 μM formic acid (7), and 1000 μM tartaric acid (8) nutrient solutions. *Holcus* originated from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen (KP), and (e) Kinloch rannoch (KR).

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Table 8.3. The mean relative root elongation rates (RER), total number of roots (TNR), shoot elongation rates (SER), total number of blades (TNB), and total number of tillers (TNT) in *Holcus lanatus* treated with combinations of Al and organic acids. Seedlings originated from (a) Flanders Moss (FM), (b) Sheriffmuir blanket peat (SMB), (c) Sheriffmuir mineral soil (SMM), (d) Kippenrait Glen, and (e) Kinloch Rannoch (KR). All treatments were kept constant at pH 4.2. Values are percentages relative to control plants (100 %). n.d, no data.

Relative growth (%)	Control	35 Al	35Al 100F	35Al 1000F	35Al 100T	35Al 1000T	1000F	1000T
(a)								
RER	100	37.2	68.2	100	73.3	98.4	221	180
TNR	100	51.9	98.6	108	99.0	98.6	123	124
SER	100	21.2	55.8	49.0	61.2	53.1	105	93.6
TNB	100	8.11	49.6	35.5	46.0	56.4	101	77.5
TNT	100	58.9	164	80.8	98.6	103	276	288
(b)								
RER	100	17.7	56.7	101	44.6	90.8	332	254
TNR	100	34.6	56.5	89.4	60.5	74.1	180	143
SER	100	20.8	59.8	71.8	63.0	66.9	126	110
TNB	100	20.8	70.7	60.1	67.6	65.4	164	120
TNT	100	40.5	94.6	78.4	92.3	75.7	230	140
(c)								
RER	100	12.7	59.4	51.6	25.7	126	n.d	220
TNR	100	22.6	58.4	32.7	27.9	88.5	n.d	120
SER	100	26.7	61.2	46.8	69.6	84.3	n.d	131
TNB	100	31.8	93.2	79.6	70.5	90.9	n.d	164
TNT	100	45.0	85.0	65.0	60.0	85.0	n.d	205
(d)								
RER	100	21.0	26.4	122	54.8	116	226	206
TNR	100	19.1	30.4	58.1	63.0	76.0	105	102
SER	100	29.2	42.8	58.3	88.8	63.9	103	82.6
TNB	100	19.3	54.5	40.1	59.4	59.5	93.2	64.4
TNT	100	43.1	37.3	39.2	58.8	68.6	154	150
(e)								
RER	100	33.9	71.6	107	102	108	190	178
TNR	100	52.1	66.3	70.5	83.1	78.1	87.8	82.3
SER	100	34.5	63.5	64.1	76.9	78.3	69.8	59.0
TNB	100	22.9	85.4	70.8	56.3	63.2	69.4	104
TNT	100	40.5	66.7	78.6	42.9	57.1	115	131

8.4.2.4 Plant ionic composition

Table 8.4 gives the root and shoot ionic compositions. Al significantly increased the uptake of K, P and sometimes Ca by the plant roots (about three-fold increase). The addition of organic acids to Al solutions significantly increased P and Ca (in SMM) uptake still further (Table 8.2). In contrast K uptake was reduced to control concentrations. Al significantly decreased root concentrations of Fe and Mg, particularly in KR (where Fe was reduced by >90 %). The addition of organic acids did not increase the concentrations of either of these nutrients. The presence of organic acids in Al solutions significantly reduced Al root concentrations. Tartaric acid was more effective in reducing Al concentrations in FM and SMB, conversely formic acid was more effective in SMM, KP, and KR (Table 8.2). An increase in formic acid concentration reduced Al concentrations further still.
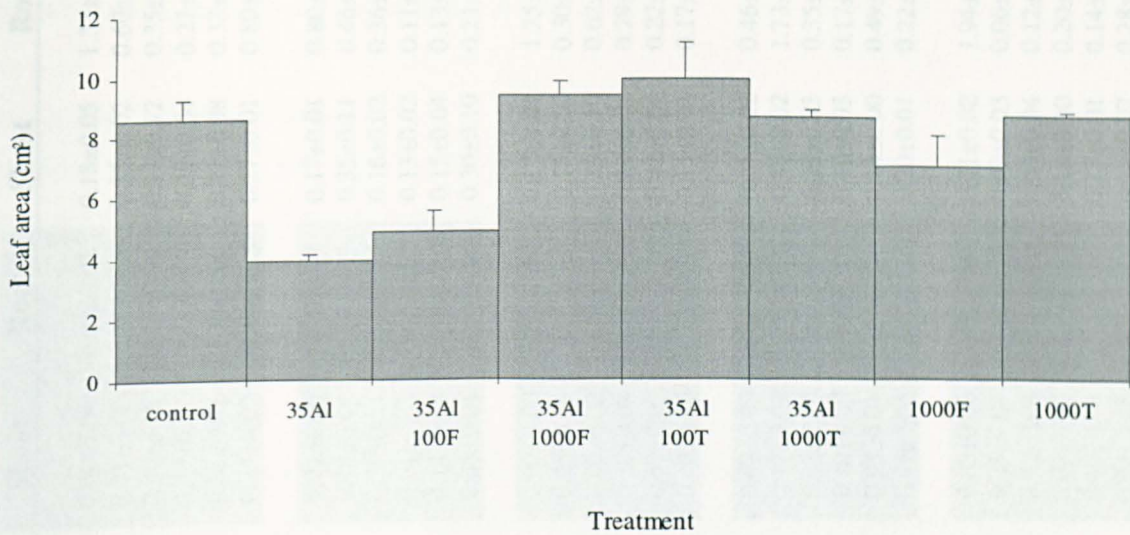
Figure 8.6. Mean leaf area (cm^2, ± s.e) in *Holcus lanatus* originating from Flanders Moss (FM) grown in control, 35 mg l^{-1} Al (35Al), 35 mg l^{-1} Al + 100 µM formic acid (35Al 100F), 35 mg l^{-1} Al + 1000 µM formic acid (35Al 1000F), 35 mg l^{-1} Al + 100 µM tartaric acid (35Al 100T), 35 mg l^{-1} Al + 1000 µM tartaric acid (35Al 1000T), 1000 µM formic acid (1000F), and 1000 µM tartaric acid (1000T) nutrient solutions.

Al significantly reduced the transport of P, K (not SMM, KP, or KR), Mg (not in FM), and Ca (not in FM and KR) to plant shoots. In SMM, KP, and KR races, Al increased K transport to shoots, and in all races increased Fe translocation. The addition of organic acids increased shoot Fe concentrations still further, particularly in KR (up to four-fold increase). The addition of organic acids tended to increase or decrease the translocation of other nutrients to match control concentrations. Contrary to root uptake of Al, organic acids increased Al translocation to shoots but only in FM races. In SMB, SMM, KP, and KR, organic acids, with the exception of 35Al 1000T, decreased shoot Al concentrations. Translocation of Al to shoots was not significantly different between acids.

8.4.2.5 Al speciation in nutrient solutions

The analysed [Al]$_{mono}$ in Al and Al+organic acid solutions of increasing age are listed in Table 8.5. Solutions, with the exception of 35Al 1000T, were relatively stable. There was a dramatic reduction in [Al]$_{mono}$ in the presence of organic acids. Tartaric acid reduced Al concentrations to a greater extent than formic acid.

Table 8.5. Concentrations of [Al]$_{mono}$ (µM) in Al (35Al) and Al+organic acid (35Al 100F, 35Al 1000F, 35Al 100T, 35Al 1000T) nutrient solutions, measured using the Pyrocatechol Violet colorimetric method. Concentrations are given for initial solutions (0), and solutions sampled one (1), two (2), three (3), and four (4) days later during the first two weeks of experimental treatments.

Age of nutrient solution (days)	[Al]$_{mono}$ (µM)				
	35Al	35Al 100F	35Al 1000F	35Al 100T	35Al 1000T
0	609±12.5	47.8±0.00	38.3±0.73	38.7±0.12	42.6±0.12
1	606±20.1	37.7±2.30	41.5±2.44	38.6±0.29	43.9±0.49
2	568±17.9	44.8±0.17	44.2±3.66	40.8±0.24	23.0±1.46
3	511±60.6	40.9±0.12	47.8±0.00	40.3±0.26	9.65±0.49
4	562±9.15	39.5±0.50	47.8±0.00	39.7±0.37	11.0±0.73

Table 8.4. Mean ionic composition (mg g⁻¹dry weight, ± s.e) of shoots and roots of *Holcus lanatus*, originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR), grown in combinations of Al and formic (100 and 1000 µM) or tartaric acid (100 and 1000 µM). The ionic composition of control plants was taken from Experiment 2, Chapter 6, Section 6.3.2.

Treatment	P		K		Ca		Mg		Al		Fe	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
FM												
Control	6.85±0.26	1.25±0.24	13.71±0.22	2.88±0.18	3.00±0.22	1.51±0.19	1.07±0.15	0.22±0.01	0.01±0.00	0.02±0.01	0.18±0.05	1.33±0.21
35 Al	1.40±0.03	3.59±0.07	12.64±0.64	4.41±0.50	5.76±0.13	1.57±0.19	1.33±0.14	0.10±0.06	0.21±0.08	2.13±0.25	0.25±0.02	0.60±0.19
35 Al + 100 F	1.80±0.47	3.59±0.73	11.26±0.65	2.75±0.41	3.49±0.11	1.73±0.09	0.75±0.03	0.10±0.02	0.80±0.18	1.35±0.07	0.37±0.12	0.35±0.01
35 Al + 1000 F	1.92±0.10	5.87±0.19	17.07±3.13	2.26±0.03	5.12±0.19	1.12±0.03	1.05±0.11	0.03±0.01	0.39±0.12	0.66±0.07	0.29±0.04	0.27±0.09
35 Al + 100 T	4.63±0.18	7.28±0.16	12.66±0.63	2.51±0.21	2.94±0.46	1.55±0.06	0.62±0.13	0.03±0.01	0.05±0.03	0.35±0.00	0.41±0.08	0.33±0.09
35 Al + 1000 T	1.24±0.13	5.74±0.01	17.77±0.35	3.26±0.06	4.92±0.02	2.11±0.01	2.10±0.04	0.06±0.01	0.73±0.07	0.40±0.02	0.27±0.01	0.80±0.05
SMB												
Control	5.22±0.75	2.44±0.19	12.50±0.75	8.13±0.75	2.04±0.26	2.14±0.06	1.21±0.21	0.28±0.02	0.01±0.00	0.01±0.01	0.17±0.01	0.80±0.16
35 Al	1.54±0.16	2.82±0.93	6.93±1.91	5.92±0.58	1.91±0.17	2.15±0.03	0.17±0.02	0.22±0.07	0.44±0.04	1.64±0.23	0.35±0.11	0.46±0.18
35 Al + 100 F	1.13±0.06	6.39±0.01	10.40±1.56	3.97±0.90	1.85±0.09	2.26±0.18	0.30±0.00	0.08±0.03	0.25±0.01	1.05±0.47	0.16±0.02	0.36±0.13
35 Al + 1000 F	0.44±0.11	3.49±0.04	8.75±0.91	3.26±0.01	2.88±0.34	2.95±0.26	0.62±0.00	0.01±0.00	0.01±0.00	0.05±0.03	0.13±0.02	0.11±0.02
35 Al + 100 T	1.90±0.40	3.99±0.10	9.66±1.83	3.34±0.24	1.36±0.17	2.76±0.07	0.30±0.01	0.01±0.00	0.14±0.04	0.30±0.10	0.15±0.04	0.13±0.02
35 Al + 1000 T	0.74±0.11	5.23±0.92	7.17±1.38	4.26±1.09	6.79±1.65	8.49±3.14	1.88±0.33	0.21±0.04	0.06±0.01	0.46±0.09	0.30±0.10	0.21±0.06
SMM												
Control	4.53±0.49	2.75±0.12	8.66±0.48	4.33±0.49	3.16±0.27	1.50±0.09	1.37±0.14	0.40±0.05	0.00±0.00	0.01±0.00	0.12±0.01	1.25±0.04
35 Al	1.85±0.38	3.52±0.19	14.15±0.47	7.67±1.42	3.90±0.59	2.55±0.09	0.66±0.15	0.05±0.02	0.25±0.04	0.96±0.15	0.18±0.00	0.30±0.11
35 Al + 100 F	1.68±0.10	6.38±0.06	11.28±0.32	4.36±1.52	5.80±1.81	2.65±0.05	1.00±0.34	0.08±0.02	0.06±0.02	0.32±0.10	0.29±0.10	0.62±0.08
35 Al + 1000 F	1.83±0.12	9.00±0.25	13.33±0.39	4.06±0.32	2.61±0.22	1.96±0.06	0.68±0.07	0.10±0.03	0.28±0.04	0.28±0.01	0.22±0.02	0.29±0.01
35 Al + 100 T	3.34±0.10	7.16±0.08	16.45±0.94	4.61±0.06	2.60±0.72	5.42±0.02	0.32±0.01	0.06±0.01	0.62±0.07	0.48±0.10	0.42±0.04	0.22±0.03
35 Al + 1000 T	0.76±0.12	4.02±0.08	7.54±1.03	3.66±0.24	3.64±0.32	3.12±0.15	1.47±0.17	0.03±0.01	0.10±0.05	0.63±0.07	0.15±0.02	0.17±0.01
KP												
Control	4.52±0.48	4.15±0.58	9.68±2.02	3.14±0.78	2.62±0.33	1.59±0.16	0.99±0.19	0.17±0.02	0.00±0.00	0.01±0.00	0.21±0.02	0.46±0.26
35 Al	3.97±0.23	4.76±0.12	21.75±0.15	10.01±0.26	2.18±0.13	1.71±0.15	0.38±0.04	0.24±0.02	0.17±0.02	1.37±0.06	0.33±0.02	1.73±0.11
35 Al + 100 F	1.25±0.07	4.42±0.40	13.30±0.22	5.00±0.65	2.19±0.23	2.35±0.15	0.42±0.01	0.11±0.05	0.22±0.08	0.33±0.03	0.29±0.03	0.35±0.02
35 Al + 1000 F	0.51±0.02	2.38±0.19	8.52±0.26	5.02±0.04	4.05±0.48	3.10±0.10	0.92±0.06	0.10±0.01	0.10±0.00	0.03±0.01	0.10±0.03	0.17±0.03
35 Al + 100 T	2.12±0.10	4.15±0.48	11.84±0.73	2.00±0.43	1.09±0.05	1.37±0.00	0.26±0.02	0.15±0.02	0.03±0.01	1.53±0.20	0.14±0.00	0.49±0.04
35 Al + 1000 T	0.90±0.12	6.58±1.10	10.27±1.22	4.22±0.41	4.56±0.40	4.45±0.58	1.86±0.12	0.02±0.01	0.45±0.06	0.57±0.15	0.20±0.01	0.22±0.03
KR												
Control	3.27±0.14	3.30±0.46	6.18±0.44	3.51±0.03	1.35±0.12	1.65±0.06	0.68±0.09	0.46±0.01	0.00±0.00	0.01±0.01	0.21±0.02	1.94±0.23
35 Al	2.58±0.36	4.23±0.14	9.41±3.38	3.70±1.09	3.25±0.55	2.40±0.19	0.56±0.07	0.01±0.00	0.25±0.04	0.75±0.13	0.09±0.03	0.06±0.01
35 Al + 100 F	1.62±0.19	5.48±0.47	11.77±1.03	5.00±0.04	1.72±0.14	2.20±0.02	0.37±0.03	0.03±0.01	0.32±0.04	0.22±0.05	0.30±0.04	0.12±0.02
35 Al + 1000 F	2.77±1.81	5.00±0.78	11.06±1.64	2.64±0.43	3.24±0.35	1.73±0.51	0.74±0.13	0.06±0.03	0.05±0.02	0.29±0.09	0.19±0.00	0.20±0.04
35 Al + 100 T	5.48±0.79	5.65±0.74	7.86±0.94	3.91±0.29	1.80±0.35	1.91±0.07	0.28±0.08	0.01±0.00	0.10±0.05	0.44±0.05	0.17±0.01	0.14±0.01
35 Al + 1000 T	0.81±0.04	4.70±1.26	9.51±1.04	3.44±0.81	4.90±0.32	3.10±0.78	2.30±0.40	0.02±0.01	0.22±0.08	0.70±0.12	0.28±0.07	0.28±0.05

8.4.3 Growth stimulation by organic acids

8.4.3.1 *Deschampsia flexuosa*

Succinic and formic acid significantly increased RER, and this increase was greater at higher concentrations (Figure 8.8). SER was also significantly improved in the presence of organic acids (particular by formic acid). Formic acid, but not succinic acid, increased tiller and blade number compared with control plants. Figure 8.9 shows the improved growth of plants in the presence of these organic acids.

Both organic acids significantly increased total, shoot, and root dry weights, and root:shoot ratios compared with control dry weights and ratios. Both acids were equally effective and at both 100 µM and 1000 µM (Figure 8.10).

Concentrations of Ca in *Deschampsia* roots and translocation to shoots was significantly greater in solutions with organic acids than in control solutions. The presence of organic acids did not significantly change the concentrations of any other nutrients (Table 8.6).

Table 8.6. Mean shoot and root ionic compositions (mg g^{-1} ± s.e) of *Deschampsia flexuosa* originating from Sheriffmuir mineral soil (SMM) grown in control, 100 µM succinic acid (100 S), 1000 µM succinic acid (1000 S), 100 µM formic acid (100 F), and 1000 µM formic acid (1000 F) nutrient solutions.

Treatment	P		K		Ca mg g^{-1}		Mg		Fe	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	4.25	5.16	16.09	9.27	2.55	1.32	1.14	0.68	0.41	1.46
	±0.93	±0.44	±4.46	±2.53	±0.73	±0.22	±0.30	±0.10	±0.13	±0.52
100 S	3.87	4.47	11.31	5.82	2.96	2.82	1.34	0.98	0.23	1.30
	±0.00	±0.52	±1.56	±0.54	±0.29	±0.42	±0.17	±0.37	±0.05	±0.09
1000 S	3.82	4.13	12.31	7.03	4.88	2.24	1.54	0.80	0.21	0.98
	±0.10	±0.36	±0.45	±1.30	±0.37	±0.06	±0.08	±0.04	±0.07	±0.19
100 F	3.25	4.56	10.67	5.98	5.99	1.52	1.15	0.57	0.19	0.91
	±0.08	±0.10	±0.29	±0.36	±0.02	±0.29	±0.03	±0.02	±0.04	±0.03
1000 F	3.45	4.38	8.30	6.73	6.96	1.09	1.26	0.47	0.33	0.65
	±0.18	±0.70	±0.57	±1.20	±1.03	±0.15	±0.06	±0.10	±0.11	±0.19

8.4.3.1 *Holcus lanatus*

With the exception of KR, RER and root numbers were significantly greater in the presence of formic and tartaric acid (Figure 8.1 and Table 8.7). This was particularly pronounced in FM and SMB and least in KP. A similar pattern was seen in SER and blade numbers (Figures 8.3 and 8.5). Formic acid increased blade numbers to a greater extent in FM and SMB, while tartaric acid was more effective in SMM and KR. Total and root dry weights were significantly greater after organic acid addition, particularly in FM, SMB, and SMM. Formic acid increased dry weights more than tartaric acid.

Tartaric and formic acid significantly increased root uptake of K (up to 2.5-fold), Fe (up to two-fold), Ca (up to five-fold), Mg (up to five-fold), and P (up to three-fold) (Tables 8.7 and 8.8). Organic acids also significantly increased translocation of Fe (up to two to three-fold greater than control plants), Ca (up to four-fold), and Mg (up to two-fold). This improvement in plant nutrition was not consistent among the sites, increased nutrient uptake was in general least evident in KR races and most pronounced in FM and SMB races.

Table 8.7. Statistical analyses for root and shoot growth measurements, dry weights, and plant ionic compositions in *Holcus lanatus* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR), and grown in three treatments: control, 1000T, and 1000F. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; n.s, not significant. Degrees of freedom are : site 4, treatment 2, and site*treatment interaction 8.

Measurement		Site		Treatment		site*treatment interaction	
		F	p	F	p	F	p
Root growth							
Root elongation rate		13.74	***	101.07	***	6.69	***
Increase in number of roots		1.62	n.s	12.17	***	4.91	***
Tops growth							
Shoot elongation rate		25.01	***	5.51	**	3.07	*
Increase in total tiller number		16.26	***	23.39	***	1.52	n.s
Increase in total blade number		20.30	***	1.97	n.s	2.55	*
Dry weights							
Shoot		6.76	***	2.50	n.s	3.00	**
Root		43.50	***	117.21	***	14.24	***
Total		15.37	***	14.09	***	5.35	***
Root:shoot ratio		5.95	***	83.47	***	5.49	***
Ionic composition							
Shoot	P	9.03	***	0.76	n.s	5.52	***
	K	171.84	***	10.44	***	2.93	*
	Ca	3.12	*	37.31	***	4.36	***
	Mg	12.91	***	15.43	***	16.99	***
	Fe	39.13	***	6.71	**	9.15	***
Root	P	22.15	***	91.16	***	26.64	***
	K	11.44	***	30.75	***	4.79	***
	Ca	7.18	***	152.68	***	43.83	***
	Mg	29.35	***	137.69	***	97.82	***
	Fe	4.21	**	5.91	**	9.32	***

Table 8.8. Mean ionic composition (mg g^{-1}, ± s.e) of shoots and roots of *Holcus lanatus* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR) grown in control, 1000 µM formic acid or 1000 µM tartaric acid.

Treatment	P		K		Ca		Mg		Fe	
	mg g^{-1}									
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
FM										
Control	6.45	1.25	15.25	2.88	3.17	1.51	1.23	0.22	0.16	1.34
	±0.45	±0.24	±1.55	±0.18	±0.23	±0.19	±0.19	±0.01	±0.04	±0.21
1000 F	7.84	4.32	13.74	4.07	4.21	2.89	3.88	0.24	0.40	0.39
	±0.38	±0.11	±0.47	±0.04	±0.20	±0.04	±0.07	±0.02	±0.03	±0.04
1000 T	4.19	5.40	11.66	7.17	4.24	9.93	1.65	0.99	0.40	2.31
	±0.47	±0.06	±0.49	±0.10	±0.15	±0.25	±0.20	±0.01	±0.01	±0.18
SMB										
Control	4.21	2.62	9.56	5.85	2.13	2.07	0.29	0.32	0.14	1.17
	±0.74	±0.26	±1.90	±1.98	±0.33	±0.10	±0.01	±0.04	±0.02	±0.45
1000 F	2.13	9.88	3.06	9.70	8.04	10.86	0.07	1.46	0.08	1.46
	±0.07	±0.07	±0.11	±0.34	±0.30	±0.17	±0.00	±0.06	±0.01	±0.12
1000 T	3.75	5.40	5.07	9.03	6.56	3.23	0.07	0.21	0.11	0.25
	±0.12	±0.64	±0.08	±0.27	±0.29	±0.16	±0.00	±0.02	±0.02	±0.06
SMM										
Control	4.53	2.75	8.66	4.33	3.16	1.50	1.37	0.40	0.12	1.25
	±0.49	±0.12	±0.48	±0.48	±0.27	±0.09	±0.14	±0.05	±0.01	±0.04
1000 F	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
1000 T	3.79	2.76	9.39	4.95	5.84	2.83	1.72	0.38	0.15	0.55
	±0.12	±0.05	±0.35	±0.03	±0.09	±0.11	±0.05	±0.01	±0.00	±0.06
KP										
Control	3.87	3.62	8.72	3.75	2.38	1.40	0.86	0.20	0.19	0.79
	±0.74	±0.77	±1.72	±0.95	±0.33	±0.25	±0.18	±0.03	±0.03	±0.43
1000 F	3.44	4.59	4.79	8.09	6.44	4.35	1.36	0.37	0.10	0.37
	±0.37	±0.61	±0.87	±1.25	±1.28	±0.31	±0.16	±0.07	±0.01	±0.05
1000 T	4.66	5.85	9.74	10.67	7.55	6.88	1.90	0.78	0.14	0.76
	±0.09	±0.74	±0.15	±0.25	±0.26	±1.66	±0.06	±0.02	±0.01	±0.16
KR										
Control	3.27	3.31	6.18	3.51	1.35	1.65	0.68	0.46	0.08	1.94
	±0.14	±0.45	±0.44	±0.03	±0.12	±0.06	±0.09	±0.01	±0.01	±0.23
1000 F	5.33	3.02	6.36	5.74	6.81	5.32	1.28	0.47	0.14	0.64
	±1.77	±0.66	±1.85	±0.94	±1.37	±0.90	±0.21	±0.11	±0.04	±0.10
1000 T	6.44	2.80	8.65	5.31	11.58	3.39	1.97	0.31	0.18	0.75
	±1.23	±0.05	±0.87	±0.52	±3.02	±0.20	±0.33	±0.03	±0.03	±0.13

Figure 8.8. Mean root elongation (cm day^{-1}, ± s.e), increase in root number (± s.e), shoot elongation (cm day^{-1}, ± s.e), and increase in tiller and blade number (± s.e) in *Deschampsia flexuosa* from Sheriffmuir (SMM) grown in control (☐), 100 µM succinic acid, 1000 µM succinic acid ☐), 100µM formic acid, and 1000 µM formic (■) acid nutrient solutions. **T**, tiller number. **B**, blade number.
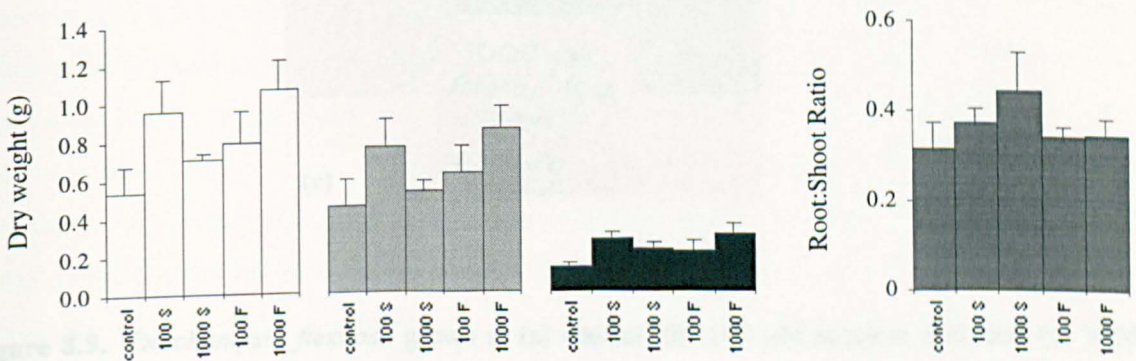
Figure 8.10. Total (☐), shoot (☐), and root (■) dry weights (g, ± s.e), and root:shoot ratios ☐ , ± s.e) in *Deschampsia flexuosa* from Sheriffmuir (SMM) grown in control, 100 µM succinic acid, 1000 µM succinic acid, 100µM formic acid, and 1000 µM formic acid nutrient solutions.
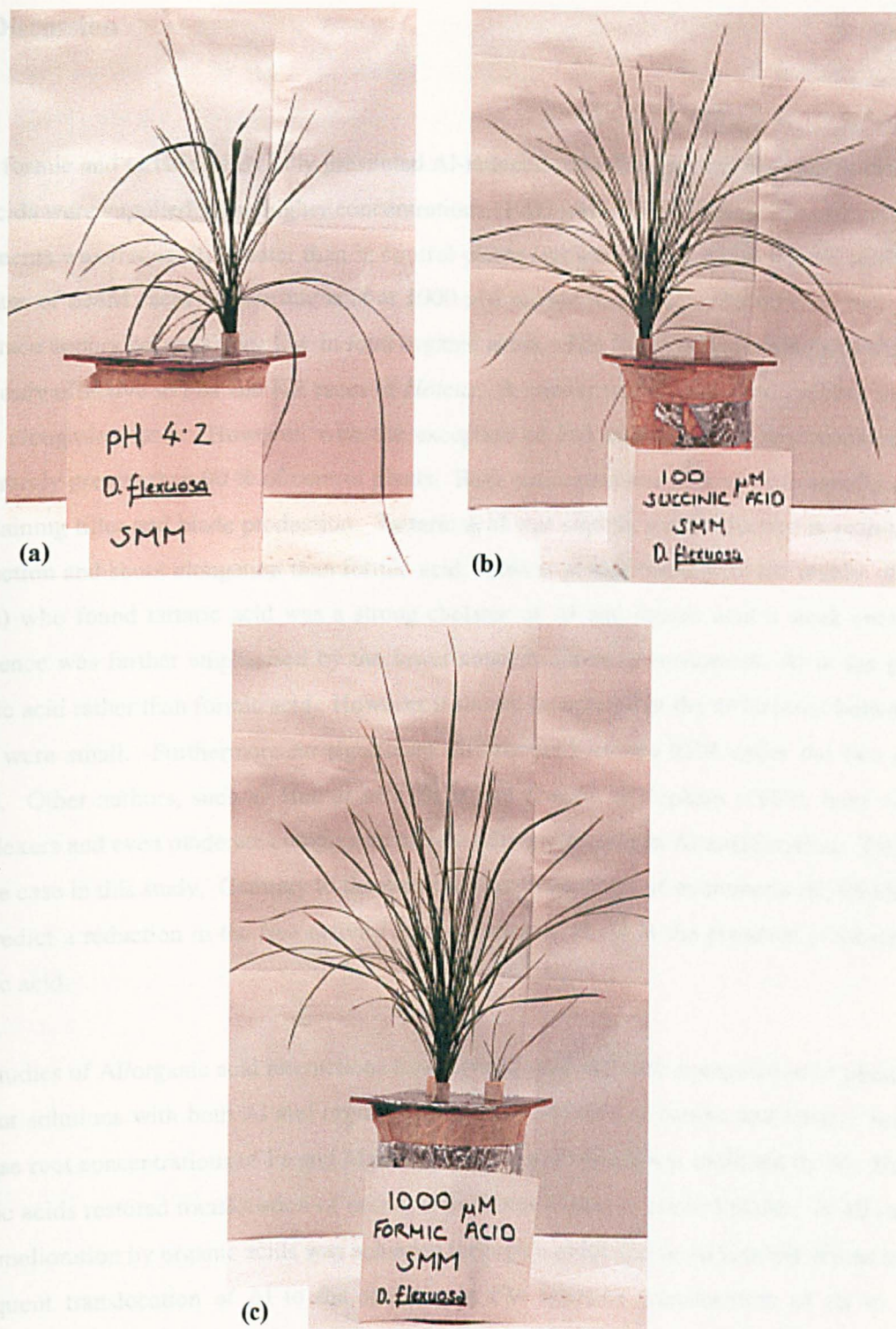
Figure 8.9. *Deschampsia flexuosa* grown in (a) control, (b) 100 μM succinic acid, and (c) 1000 μM formic acid nutrient solutions.

8.5 Discussion

Both formic and tartaric acids fully prevented Al-induced reductions in root elongation rates provided the acids were supplied at the higher concentrations (1000 μM). Furthermore root elongation in these treatments was frequently greater than in control plants (grown without Al or organic acids). Only in the case of SMM races was formic acid at 1000 μM unable to prevent inhibition of root elongation. This race occurs on soils very low in total organic acids. The lower concentrations of organic acids were only effective in FM and KR races of *Holcus*. A similar pattern was found in root numbers and shoot elongation rates. However, with the exception of FM races, relative root numbers and SER were rarely greater than 90 % of control plants. Both concentrations of acid were equally effective in maintaining tiller and blade production. Tartaric acid was slightly more effective in maintaining root production and shoot elongation than formic acid. This is in agreement with the results of Hue *et al.* (1986) who found tartaric acid was a strong chelator of Al and formic acid a weak chelator. This difference was further emphasised by the lower concentrations of monomeric Al in the presence of tartaric acid rather than formic acid. However it should be noted that the differences between the two acids were small. Furthermore no significant differences between RER under the two acids were found. Other authors, such as Hue *et al.* (1986) and Ownby & Popham (1989), have found weak complexers and even moderate complexers to be totally ineffective in Al amelioration. The same was not the case in this study. Contrary to the measured concentrations of monomeric Al, GEOCHEM did not predict a reduction in the free activity or concentration of Al in the presence of either formic or tartaric acid.

Few studies of Al/organic acid interactions have investigated the ionic composition of plants grown in nutrient solutions with both Al and organic acids. The addition of formic and tartaric acids did not increase root concentrations of Fe and Mg, the absorption of which was inhibited by Al. However the organic acids restored translocation of nutrients to shoots to that in control plants. In all races except FM, amelioration by organic acids was achieved through a reduction in Al concentrations in roots and subsequent translocation of Al to the shoots. In FM however translocation of Al to plant tops increased in the presence of organic acids. In this race amelioration was most likely achieved through internal complexation between Al and organic acids. Muchovej *et al.* (1988) also found ryegrass accumulated Al as Al-citrate complexes.

Few studies have investigated the effects on plant growth of organic acids in nutrient solutions without Al. Both succinic and formic acids enhanced root and shoot elongation in *Deschampsia* originating from SMM. Root numbers, and tiller and blade numbers were also increased beyond numbers in control plants (no organic acids). Formic acid was particularly effective. Furthermore

these organic acids enhanced Ca uptake and translocation to the shoots. Both the organic acids, formic and tartaric acid, at 1000 μM stimulated RER and root production in *Holcus* races from FM, SMB, SMM, and KP. Growth was no different from control plants in KR races. Formic acid is only present at concentrations less than 100 μM, and tartaric acid is absent, in KR soils. In contrast both acids were isolated in high concentrations from the organic soils FM and SMB: about 200-900 μM tartaric acid and 3000-5000 μM formic acid. The organic acids also stimulated nutrient absorption by roots and translocation to shoots. Higher tissue nutrient concentrations were also found in KR races. Contradictory results were found by Lynch (1980): root extension in barley was reduced by up to 26 % in the presence of acetic, citric, formic, lactic, propionic, and succinic acids in nutrient solution at pH 5.5. However Lynch (1980) used high concentrations of 5 M organic acids which are not representative of soil concentrations.

The results indicate that the organic acids facilitate nutrient uptake, and thereby increase plant growth. This may allow plants growing in acidic organic soils to maintain growth under adverse conditions. The organic soils, FM and SMB, contained the highest concentrations of organic acids, and in accordance growth stimulation was highest in races originating from these sites. Furthermore the amelioration of Al toxicity by organic acids was most effective in these races.

Unfortunately no quantification of root organic exudates in *Holcus* was made. Several researchers have shown Al-stimulated release of organic acids from plant roots and furthermore have associated this with Al tolerance (Delhaize *et al.* 1993b, Klimashevskii & Chernysheva 1980, Suhayda & Haug 1986). These studies have not included naturally occurring species, an area which deserves further investigation.

8.6 Conclusions

- The organic acids, formic and tartaric acid, effectively prevented Al-damage in *Holcus lanatus*. RER, SER, root, tiller, and blade numbers were almost equal to those of control plants.

- The stronger Al-chelator, tartaric acid, was slightly more effective than the weak-chelator, formic acid.

- Amelioration was achieved through substantial reductions in toxic monomeric Al, and likely formation of organo-Al complexes.

- Al uptake by roots and transport to shoots was inhibited in the presence of organic acids.

- Organic acids increased nutrient transport to shoots to that in control plants.

- Succinic and formic acids, and tartaric and formic acids *per se*, enhanced growth in *Deschampsia flexuosa* and *Holcus lanatus*.

- Organic acids *per se* enhanced nutrient absorption by roots and increased transport to shoots.

Chapter 9

Phenolic compounds and their effect on the growth of *Holcus lanatus* L.

9.1 Introduction

"Phenolic" or "polyphenol" is defined chemically as a substance possessing an aromatic ring which bears a hydroxyl substituent, including functional derivatives such as esters, methyl esters, and glycosides (Harborne 1989, Kuiters 1990). Phenolic acids occur widely in soils (Whitehead *et al.* 1983) at concentrations of 10 to 1000 μM (Kuiters & Sarink 1987). They arise mainly via decomposition of plant materials, although some may be synthesised by soil microorganisms, and are mainly confined to the organic A horizons (Kuiters & Sarink 1986, Whitehead *et al.* 1981). Once in the soil, phenolics are subject to further decomposition as part of the process of humus formation (humification). The identification of phenolic compounds, particularly monomeric phenolic acids, in both soils and plant residues implicated their involvement in allelopathic effects between competing plant species (Whitehead *et al.* 1982). Indeed phenolic substances, in particular benzoic and cinnamic acid derivatives, are the most frequently alleged substances (allelochemicals) involved in allelopathy (Blum 1996, Vaughan & Ord 1990) with soil concentrations and concentrations in the dominant plant species often correlating (Kuiters & Sarink 1986, Vaughan & Ord 1991b, Whitehead *et al.* 1982). Benzoic acid derivatives include vanillic, syringic, p-hydroxybenzoic, and protocatechuic acids. Cinnamic acid derivatives include ferulic, sinapic, p-coumaric, and caffeic acids.

Rice (1984) defined the term allelopathy, first coined by Molisch (1937), as the mechanism by which one plant or microbial species influences the germination, growth and development of a different species through the production of chemical compounds (secondary metabolites) released into the environment. Besides phenolic acids, other potential allelochemicals include organic acids, hydroxamic acids and volatiles (Blum *et al.* 1992).

Although phenolic acids polymerise into the more complex humus compounds such as humic and fulvic acids they are continually present in the soil solution with some seasonal fluctuation (Kuiters 1989) and can significantly inhibit germination and subsequent seedling growth (Baziramakenga *et al.* 1995). At concentrations between 100 and 1000 μM many phenolics are toxic to plants (Whitehead *et al.* 1981) since their relatively simple molecular structure allows them to be easily taken up by plant roots. More recent studies have shown their adverse influence on plant growth at lower

concentrations (1 to 50 µM) particularly where culture media were dilute and hence more similar to soil solutions (Vaughan & Ord 1991a, Vaughan et al. 1993).

Concentrations of 1 and 100 µM gallic and protocatechuic acids reduced root and shoot lengths of the legumes *Lens esculenta* and *Rhynchosia minima* but their germination was not inhibited until the concentrations reached 10000 µM (Nandakumar & Rangaswamy 1985). Kuiters & Sarink (1987) grew several herbaceous woodland species in nutrient solutions with mixtures of seven commonly occurring phenolic acids at concentrations of 100, 1000, and 10000 µM at pH 5.5. There were significant reductions in the growth and chlorophyll concentrations of *Silene dioica* at the highest phenolic acid concentration. Subsequently Kuiters (1989) also showed high concentrations of phenolic acid mixtures (10000 µM) inhibited primary root length, number and length of secondary roots, and dry weights in *Deschampsia flexuosa* and *Senecio sylvaticus*. Root growth was also inhibited in pea, again at high concentrations (1000 µM) of monomeric phenolic acid mixtures (Vaughan & Ord 1990). Concentrations as low as 1 µM also suppressed root growth as long as the N content of solutions was limiting. The root and shoot biomass of *Ipomoea lacunosa* were reduced by 52 and 26 % when grown with 5000 µM ferulic acid (Liebl & Worsham 1983). Ferulic, along with vanillic and p-coumaric, also inhibited leaf expansion in cucumber (Blum et al. 1989, Lehman et al. 1994). In agreement with Nandakumar & Rangaswamy (1985), Blum et al. (1992) found no germination inhibition in clover by phenolic acids in mixtures or individually at 0 to 2000 µM. However mixtures of phenolic acids did reduce radicle and hypocotyl length. The shoot lengths, fresh weight, and transpiration rates of Broad bean (cv. Alfred) were only affected by very high salicylic acid concentrations (3500 µM) which would rarely be extracted from field soils (Manthe et al. 1992). However low concentrations (1 µM) did result in stomatal closure.

Inhibition of germination requires unrealistic high concentrations of phenolic acids, rarely present in soils (Rüdiger & Lohaus 1987). Benzoic and salicylic acids decreased seed germination of barley to 60% but only at a concentration of 5 M (Lynch 1980). Moreover phenolic acids tend to affect germination with respect to the time taken rather than the number of seeds germinating (Kuiters 1990).

Rice (1984) outlined the possible mechanisms through which allelochemicals, such as phenolics, inhibited plant growth. These included effects on the following: mineral uptake, cell division and elongation, gibberellin- or indoleacetic acid (IAA)-induced growth, photosynthesis, respiration, stomatal opening, protein synthesis, lipid and organic acid metabolism, and enzyme activity. Glass (1974) found p-hydroxybenzoic acid at concentrations of 25 mM inhibited K uptake in excised barley roots. Similarly, the phenolic acids, salicylic and ferulic acid (5000 µM at pH 4.0), both inhibited $K^+(^{86}Rb^+)$ absorption in excised oat root tissue (Harper & Balke 1981). Salicylic acid, at 500 µM and

pH 4.5, induced leakage of organic compounds and electrolytes from the cell (Harper & Balke 1981). Similarly Kuiters & Sarink (1987) showed significant reductions. K and P uptake by roots, and Na, Ca and Mg translocation to the shoots, were reduced by phenolic acids (10000 μM at pH 5.5) on herbaceous woodland species. P uptake by cucumber was inhibited by ferulic, p-coumaric, and vanillic acids at concentrations of 2270, 3000, and 6730 μM at pH 5.5 (Lyu et $al.$ 1990). Baziramakenga et $al.$ (1995) suggested that a reduction in soybean nutrient absorption by intact soybeans with benzoic and cinnamic acids was a consequence of reduced membrane integrity, which was in turn a result of decreased sulfhydryl groups essential to plasma-membrane transport proteins and lipid peroxidation. Depletion of sulfhydryl groups could therefore result in the inactivation of such transport enzymes. The leakage of electrolytes from treated soybean increased with time. The ions, PO_4^- and SO_4^{2-}, were lost at a tenth of the rate of K^+ and Cl^-.

Contrasting results were found by Vaughan & Ord (1991a) on the uptake of radioactively-labelled ions by excised pea root tips grown in solutions with p-coumaric, vanillic, and hydroxybenzoic acids. The lowest phenolic concentrations (100 μM) had no effect on the Ca and Na root concentrations and the highest concentrations (1000 μM) actually enhanced the uptake of both cations. Uptake of Ca, Mg, and Na, was also stimulated in the roots of $Silene$ $dioica$ at 100 μM (Kuiters & Sarink 1987).

The effects of phenolic acids on plant growth are dependent upon the specific acid(s), and their concentration. Cinnamic acids are generally more inhibitory than their corresponding benzoic acid derivatives, and monohydroxy phenols more inhibitory than dihydroxy compounds. Blum (1996) found the relative toxicities of phenolic acids to cucumber seedlings were : ferulic and sinapic acid> p-coumaric acid> vanillic and syringic acid> caffeic acid and p-hydroxybenzoic acid> protocatechuic acid. p-coumaric, ferulic, and salicylic acids were the most effective inhibitors of $Deschampsia$ $flexuosa$ and $Chamerion$ $angustifolium$ growth (Kuiters 1989), and vanillic, caffeic, and syringic acids the least toxic. Ferulic, sinapic, and p-coumaric acids are cinnamic acid derivatives. At concentrations of 500 μM, the order of decreasing toxicity to pea seedlings was ferulic> p-coumaric> vanillic> p-hydroxybenzoic acid (Vaughan & Ord 1990).

Recent evidence has however shown that the concentrations of individual phenolic acids extracted from field soils are more often than not below concentrations reported to have phytotoxic effects (Blum 1996, Blum et $al.$ 1992). Laboratory bioassays suggest the action of single phenolic acids is not representative of mixtures of acids, which occur in field soils. Results show field concentrations of acid mixtures cause inhibitory effects despite individual concentrations being lower than their inhibitory concentrations (Blum et $al.$ 1989, Blum 1996). A 30% reduction in absolute leaf expansion of cucumber required 0.23 μmol g^{-1} of ferulic acid but required only 0.05 μmol g^{-1} when 0.06, 0.17, and 0.04 μmol g^{-1} p-coumaric, p-hydroxybenzoic, and vanillic acids were also present (Blum 1996).

The effects of mixtures of phenolic acids on plant growth can be antagonistic, synergistic, or additive when compared to the combined effects of the individual compounds (Blum 1996, Lyu *et al.* 1990).

Recently Northup *et al.* (1995) introduced an alternative theory suggesting a potential role for phenolics in plants as an adaptation to soil acidity. They suggested that phenolic-rich foliage may benefit the producers. To date polyphenol-rich vegetation in acidic soils has been assumed to act as a herbivore deterrent whereby less foliage is lost to herbivory (Lege *et al.* 1995, Siqueira *et al.* 1991). Nicolai (1988) found higher polyphenol concentrations in beech growing in acidic soils compared with less acidic soils, and Muller *et al.* (1987) showed tannins increased in both dogwood and red maple along a gradient of increasing acidity (Northup *et al.* 1995). Northup *et al.*(1995) investigated the interaction between the phenolic content of Pygmy forests near Mendocino, California, and soil acidity. They found significant differences in foliar condensed tannin and phenolic concentrations within different species, and these concentrations were inversely related to soil pH. Vegetation, such as the Pygmy forest, with a high phenolic content leads to an accumulation of leaf-litter and a mor humus layer. Northup *et al.* (1995) postulated that this humus layer could provide an alternative medium for root growth and nutrient cycling when mineral soil conditions were unfavourable. Litterfall N could be retained in a form (complexed with litterfall proteins) that was difficult for other organisms to utilise providing a competitive advantage for polyphenol-rich vegetation. Phenolic acids could also potentially compete with PO_4 for sorption sites on clay surfaces or even release "fixed" P thereby increasing its availability for plant uptake. Hydroxybenzene acids can form strong complexes with Al, Fe, and Mn, which usually precipitate PO_4 in acid soils. Chelating phenolics would therefore simultaneously improve PO_4 availability and detoxify Al. Kuiters & Sarink (1987) showed that low concentrations of phenolics (1 µM) stimulated production of shoot and root biomass in *Deschampsia flexuosa*, *Milium effusum*, and *Silene dioica*. These studies all point towards a potential positive role of phenolic acids in ameliorating acid soil infertility and minimising nutrient loss within these soils. The capacity to produce polyphenol-rich foliage would allow species to sustain their productivity, whereas the ability to produce lower concentrations in less acidic soils would allow a greater proportion of their photosynthate to go towards growth (Northup *et al.* 1995).

9.2 Aims

- To quantify foliar concentrations of 11 commonly occurring phenolic acids using high performance liquid chromatography in races of *Betula pendula*, *Calluna vulgaris*, *Erica tetralix*, and *Holcus lanatus*.

- To quantify concentrations of the same phenolic acids in five soil types from FM, SMB, SMM, KP, and KR.

- To establish any correlation between phenolic acid contents of dominant plant species and soils.

- To identify the most common phenolic acids present in the soils of all five study sites, prepare culture solutions reflecting any differences in phenolic acid composition between soils, and measure the growth of *Holcus lanatus* in these solutions. (The reasons for the choice of study species were given in Chapter 2, Section 2.2).

- To assess the potential role of phenolics in plants as adaptations to soil acidity.

- To investigate the effects on growth of *Holcus lanatus* in soils incubated with dried plant material of *Betula*, *Calluna*, *Erica*, and *Holcus*.

9.3 Methods

9.3.1 Total phenolics

Foliage was collected from *Betula pendula*, *Calluna vulgaria*, *Erica tetralix*, and *Holcus lanatus*, when present, from the five study sites: FM, KP, KR, SMB, and SMM in August 1996. Foliage was air-dried for 2 weeks before grinding. Sub-samples (100 mg) of ground foliage were extracted for 24 h in 50 % aqueous methanol following Northup *et al.* (1995). Extracts were filtered through No. 41 Whatman filter paper and made up to 100 ml for analysis.

Subsamples of 25 g of soil from each of the five sites were extracted with 0.25 M citrate following Blum (1997). 25 g soil was added to 250 ml conical flasks and extracted for 2.5 h with 100 ml citrate at pH 7.0. EDTA extracts were not used to determine total phenolics since citrate, unlike EDTA, does not interfere with the Folin & Ciocalteau's reagent (Blum 1997).

Total phenolics were measured using Folin-Ciocalteau Phenol Reagent following recommendations by Box (1983). Values obtained using this reagent represent relative available total phenolic acids rather than absolute total phenolic acids because the reagent reacts with other substances besides phenolic acids such as cyclic amino acids (Blum 1997). The reagent was stored in a dark bottle at 4 °C. 1.5 ml Na_2CO_3 (200 g l^{-1}) and 0.5 ml Folin-Ciocalteau phenol reagent were added to 10 ml sample (soil or plant extract) in that order. After 60 min at room temperature, the absorbance was measured at 750 nm against deionised water (blank). Absorbance data were obtained using a LKB Novaspec Spectrophotometer. The use of Na_2CO_3 instead of 1 M NaOH prevented the formation of a haze which was also found by Box (1983). Tannic acid was used as a standard and the concentration of total phenolics expressed as mg tannic acid equivalents (TAE) per g dry weight soil/plant material.

9.3.2 Monomeric phenolic acids

Soil and plant samples (as above) were extracted using 0.25 M EDTA (pH 7) after Blum *et al.* 1994. The extracts were concentrated in a rotating vacuum evaporator at 30 °C to 2-5 ml. Subsamples of 50 μl were then injected into the HPLC.

Phenolic acids were separated using a LDC Analytical HPLC system consisting of a ConstaMetric 4100 solvent delivery system with LDC Membrane Degasser, SpectraSYSTEM SN4000, and a SpectraSYSTEM UV3000 absorbance detector set at 254 nm. A 5-μM particle Bondapak C_{18} column (3.9 mm x 150 mm) was used to isolate the acids which were identified and quantified, based on retention times of standards, using ThermoSeparation Products SpectraSystem software PC1000. Solvent A consisted of 97.25 % water: 2 % methanol: 0.5 % acetic acid: 0.25 % ethyl acetate. Solvent B consisted of 80 % methanol: 17 % water: 2 % acetic acid: 1 % ethyl acetate. The phenolic acids isolated were: gallic, protocatechuic, p-hydroxybenzoic, vanillic, caffeic, syringic, p-coumaric, salicylic, benzoic, ferulic, and sinapic acids. The 11 acids (listed in elution order) were separated within a 70-minute period using the solvent gradient described in Table 9.1.

Table 9.1. Solvent gradient system program used to separate phenolic acids from soil and plant extracts.

Minutes	Flow (ml min^{-1})	Solvents (%)	
		A	B
initial	1.3	94.0	6.0
4.2	1.3	100.0	0
11.5	0.5	92.5	7.5
13.0	1.5	91.0	9.0
17.0	1.0	85.5	14.5
40.0	1.0	66.0	34.0
50.0	1.0	0	100.0
60.0	1.0	0	100.0
70.0	1.0	92.0	8.0

9.3.3 Addition of ground foliage to soils

A mixture of ground shoot material of *Betula pendula*, *Calluna vulgaris*, and *Erica tetralix* (7:2:1) was added to soil from FM, SMM, and KR at the following rates : 0, 0.5, and 1.0 g organic mixture 100 g^{-1} soil. After air-drying the organic material was milled to powder using a stainless-steel grinder and incubated with soil in plastic bags at 90 % field capacity for two weeks. Soil was brought to field capacity (90 %) with deionised water and stored in open ended plastic bags which allowed adequate aeration (Bessho & Bell 1992).

Holcus lanatus seeds were germinated in wet filter paper with acid-washed sand and transferred into treatment soils seven days after germination. *Holcus* originating from FM, SMM, and KR, were grown in 78-mm pots with soil from each of the three sites, and each of the three treatments. There were five replicates per treatment.

9.3.4 Growth of *Holcus lanatus* in nutrient solutions

Seeds of *Holcus lanatus* from FM and KR, collected in August 1996, were germinated on acid-washed sand on filter paper in October 1997 in the Stirling University growth rooms. The conditions were the same as those in Chapter 8. Seedlings were transferred after about 10 days into an initial culture solution with no added phenolics and at pH 5.6 (Chapter 4, Section 4.3.1.1). Seedlings were grown in this initial culture solution for two weeks and then transferred into experimental solutions (described below).

Phenolic acids were added to nutrient solutions in three equimolar mixtures and at each of two pH values. The mixtures were based on the monomeric phenolic acids analysed in the extracted soils from all five sites (Section 9.2.2) and pH values were chosen to reflect average pH of the soil solutions in FM and KR (Chapter 3) : pH 4.0 and pH 6.5. Mixture 1 comprised benzoic, ferulic and sinapic acids at 50 µM; mixture 2 comprised gallic, protocatechuic, p-hydroxybenzoic, and vanillic acids at 50 µM; and mixture 3 comprised all seven phenolics at 50 µM. The three phenolics in mixture 1 were absent from KR and KP soils, but present in the acidic soils of FM, SMB, and SMM. Acids in mixture 2 were generally present in both non-acidic (KR) and acidic (FM, SMB, SMM) soils. Culture solutions were adjusted to pH 4.0 and 6.5 using 1M NaOH or 1M HCl. There were four replicate seedlings per treatment. Solutions were changed every other day to minimise microbial degradation of the phenolic acids (Kuiters *et al.* 1987).

The number of roots and their lengths, the number of blades and tillers and their lengths, were recorded on the day treatments began, 1 Nov 1997, and at harvest, 21 days later on 22 Nov 1997.

9.4 Results

9.4.1 Soil total phenols and water-soluble phenolic acids

Tables 9.2 and 9.3 list the total phenols measured using Folin Ciocalteau's reagent (FC), and monomeric phenolic acids identified and quantified using HPLC. Total phenols (FC) and total monomeric phenolics (HPLC) were not correlated. With the exception of FM, total phenols were highest in the acid soils, particularly SMB, and lowest in the calcareous soil, KR (Table 9.2). The same trend was seen in total amounts of phenolic acids (HPLC) (Table 9.3), FM, SMB, and SMM soils had the highest total concentrations, and KR the lowest. The distribution of individual phenolic acids in the soils varied with soil type (Table 9.3). Acid soils contained benzoic, ferulic, and sinapic acids which were completely absent from KP and KR. These acids comprised about 50 and 90 % of FM and SMM soils. Syringic acid represented 68 % of KR phenolics and was absent from all other soils.

9.4.2 Plant total phenols and monomeric phenolic acids

Tables 9.4 and 9.5 list the total phenol contents of plant material from Folin Ciocalteau's reagent (FC), and monomeric phenolic acids from HPLC. *Calluna vulgaris* was the most phenolic rich (FC), particularly in FM races. *Erica tetralix* from different provenances contained similar total phenol concentrations, and *Betula pendula* was most phenol-rich in KR and then FM. The grass, *Holcus lanatus*, contained very low amounts of total phenol, the highest concentrations were found in KR races (Table 9.4). With the exception of *Holcus* the total monomeric phenolic acids, determined using HPLC, were highest in the tissues of acidic races (Table 9.5). This was particularly pronounced in *Erica tetralix*: FM races contained eight times as much total phenolics as KR races (Table 9.5). In other species the difference was about two fold between the acidic and calcareous races. Syringic and ferulic acids dominated the phenolic spectrum of *Betula* from FM, SMM, and KP. These acids represented far lower proportions in KR races, which conversely had a substantially greater proportion of salicylic acid. Both FM and KR races of *Erica* were high in syringic acid but FM races were equally high in ferulic acid. The phenolic acids, vanillic and ferulic, comprised a greater proportion of total phenolics in FM *Calluna* than in KR *Calluna*, which was dominated by syringic acid. The most notable difference in *Holcus* phenolic composition was a substantially larger proportion of p-coumaric acid in acidic races compared with KP and KR.

Table 9.2. Concentrations of total phenols (Tannic acid equivalents mg g⁻¹ dry wt) in soils collected from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR).

Site	Total phenols (TAE mg g⁻¹)
FM	0.07
SMB	0.31
SMM	0.19
KP	0.15
KR	0.05

Table 9.3. Water-soluble phenolic acids (ng g⁻¹ dry wt) in soils from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR). The percentages of each compound in the total spectrum are given in parentheses. Phenolic acids are listed in order of increasing retention time. ND= below detection level.

Phenolic compound	FM	SMB	SMM	KP	KR
Gallic acid	0.12(18.8)	0.54(70.1)	0.09(12.2)	0.13(74.0)	0.01(9.4)
Protocatechuic acid	0.06(9.4)	ND	ND	ND	ND
p-hydroxybenzoic acid	0.13(20.3)	0.04(5.2)	ND	ND	0.01(7.5)
vanillic acid	ND	0.10(13.0)	ND	0.05(25.1)	0.02(12.2)
caffeic acid	ND	ND	ND	ND	ND
syringic acid	ND	ND	ND	ND	0.09(68.3)
p-coumaric acid	ND	ND	ND	ND	ND
salicylic acid	ND	ND	ND	ND	ND
benzoic acid	0.12(18.8)	ND	0.04(5.4)	ND	ND
ferulic acid	ND	0.07(9.1)	0.54(73.0)	ND	ND
sinapic acid	0.21(32.8)	0.02(2.6)	0.07(9.5)	ND	ND
TOTAL	**0.64**	**0.77**	**0.74**	**0.18**	**0.13**

Table 9.4. Concentrations of total phenols (mg tannic acid equivalents g⁻¹ dry weight) in leaf matter of *Betula pendula* L., *Calluna vulgaris* L., *Erica tetralix* L., and *Holcus lanatus* L. originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR). The gaps in the data are because three of the species do not occur at all the sites.

Site	Total phenols (TAE mg g⁻¹)			
	Holcus lanatus L.	*Erica tetralix* L.	*Calluna vulgaris* L.	*Betula pendula* L.
FM	0.13	3.26	7.19	3.37
SMB	0.19	-----	4.31	-----
SMM	0.16	-----	-----	2.74
KP	0.08	-----	-----	1.94
KR	0.31	3.83	6.64	4.14

Table 9.5. Water-soluble phenolic acids (µg g^{-1} dry wt) in leaf matter of *Betula pendula* (a), *Calluna vulgaris*, *Erica tetralix*, and *Holcus lanatus* originating from Flanders Moss (FM), Sheriffmuir blanket peat (SMB), Sheriffmuir mineral soil (SMM), Kippenrait Glen (KP), and Kinloch Rannoch (KR). The percentages of each compound in the total spectrum of water-soluble phenolics are given in parentheses. Phenolic acids are listed in order of increasing retention time. ND= below detection level.

Phenolic compound	*Betula pendula* L.			
	FM	SMM	KP	KR
Gallic acid	ND	ND	ND	ND
Protocatechuic acid	0.16(2.0)	0.02(0.2)	0.32(6.3)	0.01(0.2)
p-hydroxybenzoic acid	0.02(0.2)	0.01(0.1)	0.05(0.9)	0.24(3.7)
vanillic acid	0.10(1.3)	1.36(10.5)	0.08(1.6)	0.09(1.4)
caffeic acid	0.26(3.3)	0.47(3.7)	0.14(2.7)	0.61(9.4)
syringic acid	5.51(68.7)	5.36(41.3)	2.62(50.9)	1.23(19.0)
p-coumaric acid	ND	0.65(5.0)	ND	0.17(2.6)
salicylic acid	0.42(5.2)	1.13(8.7)	ND	1.59(24.6)
benzoic acid	0.39(4.9)	1.13(8.7)	0.26(5.0)	1.16(17.9)
ferulic acid	1.15(14.3)	2.74(21.1)	1.67(32.5)	1.37(21.2)
sinapic acid	0.01(0.1)	0.10(0.8)	0.01(0.2)	ND
TOTAL	8.02	12.97	5.14	6.47

Phenolic compound	*Calluna vulgaris* L.			*Erica tetralix* L.	
	FM	SMB	KR	FM	KR
Gallic acid	ND	ND	ND	0.05(0.1)	0.07(1.6)
Protocatechuic acid	0.30(1.4)	0.31(1.5)	0.05(0.3)	0.01(<0.1)	0.09(1.9)
p-hydroxybenzoic acid	0.23(1.1)	0.02(0.1)	0.02(0.1)	0.01(<0.1)	ND
vanillic acid	7.68(35.4)	0.48(2.4)	0.16(1.1)	0.27(0.8)	0.22(5.0)
caffeic acid	0.30(1.4)	1.44(7.1)	0.18(1.2)	0.55(1.6)	0.16(3.7)
syringic acid	5.70(26.3)	13.76(67.3)	10.06(70.3)	12.67(37.0)	3.09(69.9)
p-coumaric acid	1.05(4.8)	1.22(6.0)	0.86(6.0)	1.24(3.6)	ND
salicylic acid	ND	1.92(9.4)	1.93(13.5)	3.98(11.6)	0.20(4.4)
benzoic acid	0.88(4.0)	1.29(6.3)	0.70(4.9)	3.64(10.6)	0.28(6.3)
ferulic acid	4.96(22.8)	ND	ND	10.90(31.8)	0.30(6.8)
sinapic acid	0.62(2.8)	ND	0.38(2.6)	0.93(2.7)	0.02(0.4)
TOTAL	21.71	20.45	14.32	34.26	4.42

Phenolic compound	*Holcus lanatus* L.				
	FM	SMB	SMM	KP	KR
Gallic acid	ND	ND	ND	0.06(0.4)	0.33(2.9)
Protocatechuic acid	0.18(3.9)	0.24(2.4)	0.40(2.4)	0.26(1.7)	0.22(1.9)
p-hydroxybenzoic acid	0.01(0.2)	0.05(0.5)	0.22(1.3)	0.01(<0.1)	0.01(0.1)
vanillic acid	0.07(1.5)	0.71(7.1)	0.06(0.3)	0.08(0.5)	0.30(2.6)
caffeic acid	0.05(1.0)	0.14(1.4)	0.06(0.4)	ND	0.03(0.2)
syringic acid	0.68(15.1)	2.13(21.3)	0.01(0.1)	8.01(52.2)	3.10(27.1)
p-coumaric acid	0.38(8.4)	1.50(15.0)	8.82(52.9)	0.12(0.8)	0.03(0.2)
salicylic acid	0.47(10.5)	0.84(8.4)	0.37(2.2)	0.65(4.2)	1.23(10.7)
benzoic acid	1.86(41.1)	1.73(17.2)	6.25(37.5)	0.31(2.0)	2.26(19.7)
ferulic acid	0.79(17.6)	2.65(26.4)	0.23(1.4)	5.83(37.9)	3.93(34.4)
sinapic acid	0.04(0.8)	0.04(0.4)	0.24(1.5)	0.03(0.2)	0.02(0.2)
TOTAL	4.52	10.03	16.67	15.36	11.44

Table 9.6. Water-soluble phenolic acids (μg g^{-1} dry wt) in mixture of ground dried plant material added to FM, SMM, and KR soil. The percentages of each compound in the total spectrum are also given. Phenolic acids are listed in order of increasing retention time. ND= below detection level.

Phenolic compound	Concentration (μg g^{-1})	%
Gallic acid	ND	ND
Protocatechuic acid	0.26	1.4
p-hydroxybenzoic acid	0.01	0.1
vanillic acid	0.45	2.4
caffeic acid	1.74	9.4
syringic acid	11.50	62.3
p-coumaric acid	0.65	3.5
salicylic acid	1.92	10.4
benzoic acid	0.89	4.8
ferulic acid	0.78	4.2
sinapic acid	0.26	1.4
TOTAL	18.46	100

9.4.3 Addition of dried plant material

The phenolic acid composition of the ground material added to soils is listed in Table 9.6. The majority (60 %) was represented by syringic acid. The addition of plant material (litter addition) to the soils significantly affected both the dry weights, and root:shoot dry weight ratios, of *Holcus*. Furthermore the response of plants differed significantly between soil types, plant races, and litter additions (Table 9.7). The race*soil*litter addition interaction factor was significant at $p < 0.001$. With the exception of one treatment (discussed below) there was an approximate ten-fold reduction in dry weight (d.w) with addition of 0.5 g plant material 100 g^{-1} soil, and 100-fold reduction with addition of 1.0 g plant material 100 g^{-1} soil (Table 9.8 and Figure 9.1). The percentage reduction in dry weights with the addition of plant material are given in parentheses. The roots of plants became stunted and discoloured, with many branched and stunted lateral roots, after litter additions (Figure 9.2).

Growth of KR and SMM races was stimulated with the lower rate of addition when grown in FM soil, and only in FM soil. Shoot and root dry weights of KR races were more than two fold greater than control plants (no litter addition). Root d.w of SMM plants were about 50 % greater than controls. D.w of FM races did not change greatly between control plants and plants grown in FM soil with 0.5 g litter addition 100 g^{-1} soil.

There was no stimulation in growth at the higher litter addition rate (1.0 g 100 g^{-1} soil), or in either KR or SMM soil with any litter addition. The reduction in growth, after 1.0 g litter addition 100 g^{-1} soil, was consistently lowest in FM soil in all three races. Moreover the largest reduction in d.w after plant addition occurred in all three races when grown in KR soil.

After litter addition, irrelevant of soil type, the root:shoot ratios of all races were significantly increased, and generally increased with an increase in litter addition (Table 9.7). The lowest increase in ratios occurred in all races when grown in FM soil. FM races had significantly lower root:shoot ratios in all soil types and at all rates of litter addition.

Table 9.6. Statistical analyses of plant dry weights, and root:shoot ratios, of *Holcus* originating from FM, SMM, and KR (race), grown in soil from FM, SMM, and KR (soil type) with additions of plant material at 0, 0.5, and 1.0 g 100g⁻¹ soil (plant addition). Degrees of freedom are: race 2, soil type 2, plant addition 2, and interaction factor 8. ***, p<0.001; **, p<0.01; *, p<0.05; n.s, not significant.

Dry weight	Race		Soil type		Plant addition		Race*Soil*Addition interaction	
	F	p	F	p	F	p	F	p
Total dry weight	3.21	*	192.80	***	629.26	***	7.91	***
Shoot dry weight	4.29	*	159.23	***	404.46	***	10.11	***
Root dry weight	3.19	*	264.96	***	535.93	***	7.84	***
Root:shoot ratio	3.43	*	119.28	***	33.18	***	2.34	*

9.4.4 Growth of *Holcus* in hydroponic solutions

Root elongation rate (RER) was not significantly affected by phenolic acid additions or nutrient solution pH. However the pH*phenolic mixture interaction factor was significant at p<0.01, and the response of the two races to phenolic acids differed significantly (Table 9.9). In FM races RER's were greater at pH 4.0, with or without phenolic acids, compared with pH 6.5. Conversely RER's were greater in all treatments at pH 6.5 than at pH 4.0 in KR races. Furthermore at pH 4.0, RER's were greatest, in both races, in mixture 3 (Figure 9.3).

The mean increase in total root numbers were significantly greater in FM than KR irrelevant of treatment (Table 9.9 and Figure 9.3). The pH*phenolic mixture interaction factor was significant at p< 0.001. Total root numbers significantly decreased in KR seedlings, at pH 4.0, in the presence of phenolic acids. Root numbers were unaffected in KR races at pH 6.5, and in FM plants at either pH.

Shoot elongation rate (SER) was significantly greater in FM races, particularly at pH 4.0 (Table 9.9 and Figure 9.4). Shoot growth was enhanced in mixtures 2 and 3 in this race, and unaffected by treatment at pH 6.5. There was no similar stimulation in shoot growth in KR plants where SER was depressed in mixtures 2 & 3 at pH 6.5.

Tiller and blade numbers increased in FM plants, at pH 4.0, with phenolic acids (mixture 2 and 3) (Figure 9.4). In contrast numbers decreased in KR races, especially in mixture 3. Growth was unaffected at pH 6.5 in either race.
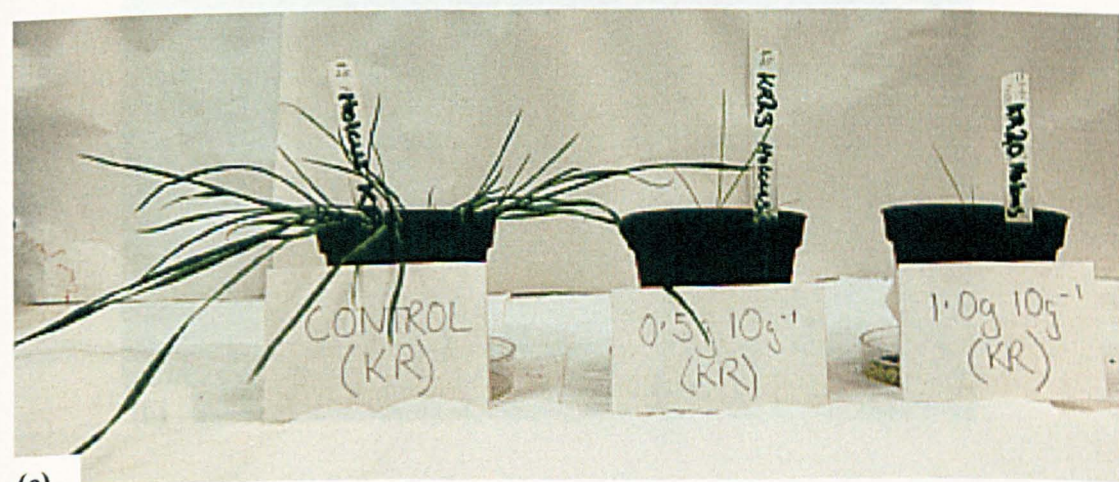
Figure 9.1. Reduced growth of (a) Flanders Moss (FM), (b) Sheriffmuir mineral soil (SMM), and (c) Kinloch Rannoch (KR) races of *Holcus lanatus* grown with increasing amounts of litter addition: 0 g 100 g⁻¹, 0.5 g 100 g⁻¹, and 1.0 g organic mixture 100 g⁻¹ soil.

Table 9.9. Statistical analyses of root elongation rate (RER), shoot elongation rate (SER), and increase in total root number (TRN), tiller number (TNTill), and blade number (TNB), of *Holcus* originating from FM and KR (race), grown in nutrient solution with mixtures of phenolic acids (mixture) at pH 4.0 and 6.5 (pH). Degrees of freedom are: race 1, mixture 3, pH 1, and interaction factor 3. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s, not significant.

	Race		Mixture		pH		pH*mixture interaction	
	F	p	F	p	F	p	F	p
RER	4.04	*	1.03	n.s	0.16	n.s	5.32	**
SER	14.60	***	2.32	n.s	8.81	**	6.98	***
TRN	123.74	***	2.30	n.s	12.32	***	2.38	n.s
TNTill	122.21	***	0.39	n.s	4.12	*	0.89	n.s
TNB	31.22	***	0.15	n.s	9.28	**	4.50	**

(a)

(b)

Figure 9.2. Roots of Kinloch Rannoch (KR) races of *Holcus lanatus* after growth in soil with 1.0 g 100 g^{-1} litter addition. Roots were stunted and thickened with thickened laterals, also stunted in length.
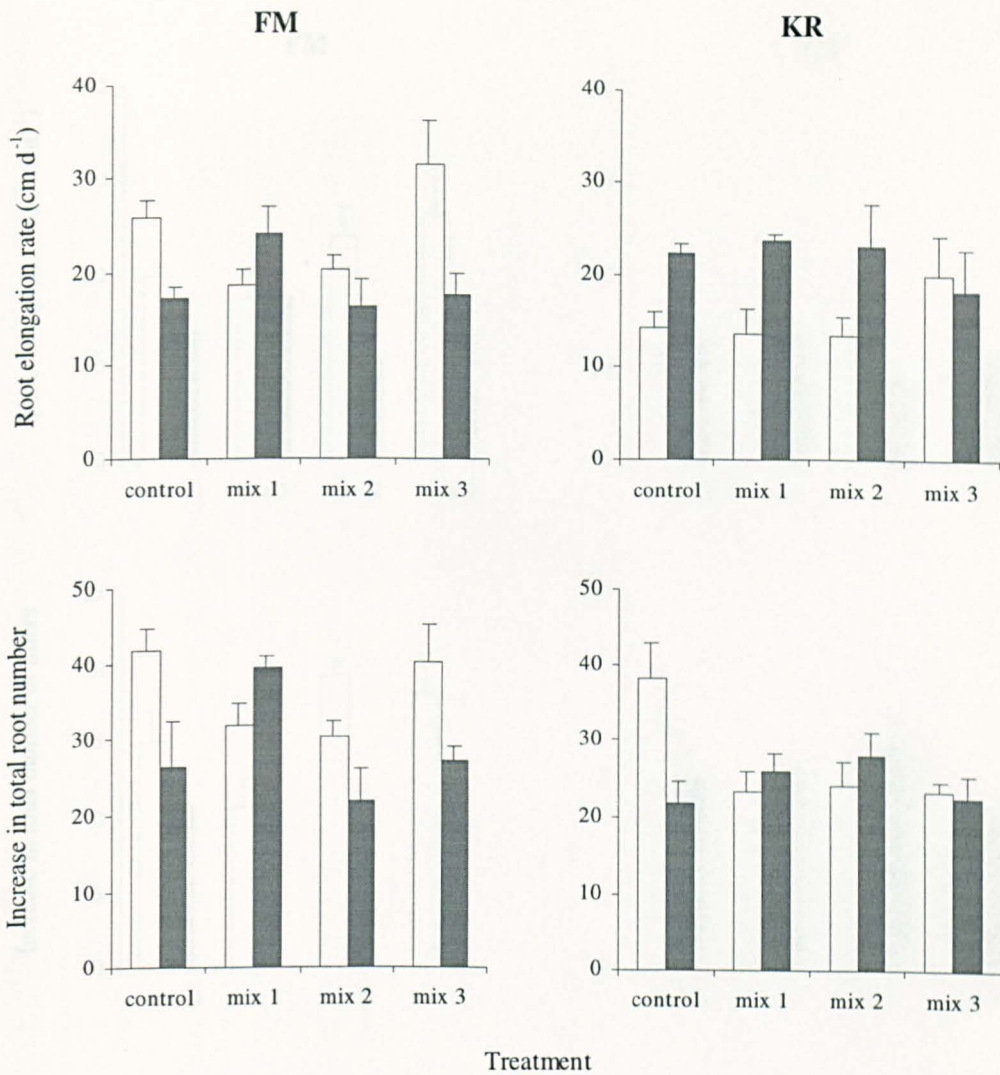
Figure 9.3. Mean root elongation rates (cm day^{-1}, ± s.e) and increase in total root number (± s.e) in *Holcus lanatus* originating from Flanders Moss (FM) and Kinloch Rannoch (KR). Seedlings were grown in nutrient solutions with and without phenolic acid mixtures at pH 4.0 (□) and pH 6.5 (▨).

Figure 9.4. Mean shoot elongation rates (cm day^{-1}, ± s.e), increase in total tiller number (± s.e), and increase in total number of blades (±s.e) in *Holcus lanatus* originating from Flanders Moss (FM) and Kinloch Rannoch (KR). Seedlings were grown in nutrient solutions with and without phenolic acid mixtures at pH 4.0 (□) and pH 6.5 (■).
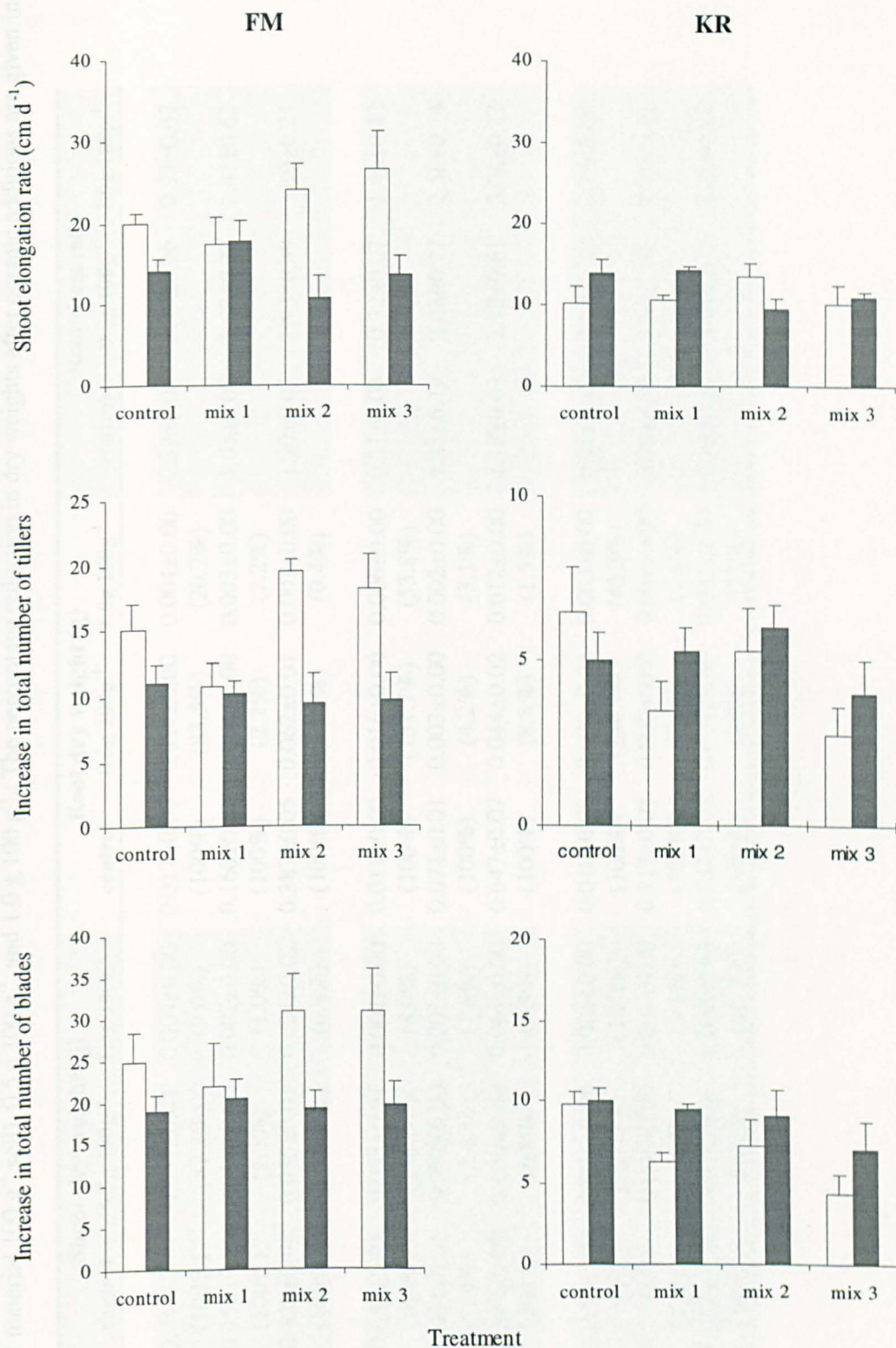
Table 9.8. Mean shoot and root dry weights (± s.e), and root:shoot ratios (± s.e), of *Holcus lanatus* originating from Flanders Moss (FM), Sheriffmuir (SMM), and Kinloch Rannoch (KR) grown in soil with added plant material. Plants were grown in each of three soils collected from FM, SMM, and KR. Additions were at the following rates: control (0 g plant material 100 g^{-1} soil), 0.5 g 100 g^{-1}, and 1.0 g 100 g^{-1}. The percentage reduction in dry weights after organic additions are given in parentheses.

Species Origin (Soil Type)	Shoot dry weight (g)			Root dry weight (g)			Root:shoot ratio		
	control	0.5g 100g^{-1}	1.0g 100g^{-1}	control	0.5g 100g^{-1}	1.0g 100g^{-1}	control	0.5g 100g^{-1}	1.0g 100g^{-1}
Flanders Moss									
FM	0.043±0.01 (100%)	0.037±0.01 (87.3%)	0.004±0.00 (9.0%)	0.017±0.00 (100%)	0.014±0.00 (83.5%)	0.004±0.00 (20.7%)	0.39±0.06	0.19±0.06	0.93±0.07
SMM	0.150±0.02 (100%)	0.002±0.00 (1.3%)	0.002±0.00 (1.0%)	0.160±0.03 (100%)	0.004±0.00 (2.2%)	0.003±0.00 (2.2%)	1.05±0.08	1.88±0.32	2.43±0.63
KR	0.323±0.04 (100%)	0.035±0.01 (10.8%)	0.001±0.02 (0.4%)	0.387±0.05 (100%)	0.062±0.01 (16.0%)	0.001±0.00 (0.4%)	1.20±0.07	1.93±0.39	1.10±0.21
Sheriffmuir									
FM	0.052±0.01 (100%)	0.055±0.01 (106.0%)	0.004±0.00 (8.0%)	0.011±0.00 (100%)	0.016±0.00 (151.7%)	0.006±0.00 (53.8%)	0.21±0.03	0.32±0.07	1.40±0.13
SMM	0.064±0.01 (100%)	0.002±0.00 (2.4%)	0.001±0.00 (1.6%)	0.074±0.01 (100%)	0.003±0.00 (4.2%)	0.002±0.00 (3.1%)	1.23±0.19	2.03±0.12	2.58±0.74
KR	0.647±0.07 (100%)	0.044±0.02 (6.8%)	0.004±0.00 (1.5%)	0.647±0.07 (100%)	0.044±0.02 (8.8%)	0.012±0.00 (1.5%)	1.18±0.03	2.12±0.61	3.04±0.13
Kinloch Rannoch									
FM	0.030±0.01 (100%)	0.073±0.04 (247.5%)	0.005±0.00 (18.0%)	0.010±0.00 (100%)	0.030±0.01 (284.7%)	0.005±0.00 (45.3%)	0.51±0.25	0.54±0.15	0.89±0.09
SMM	0.133±0.01 (100%)	0.002±0.00 (1.7%)	0.001±0.00 (1.1%)	0.113±0.02 (100%)	0.006±0.00 (5.3%)	0.004±0.00 (3.4%)	0.86±0.18	2.54±0.48	2.75±0.42
KR	0.466±0.04 (100%)	0.004±0.00 (0.8%)	0.002±0.00 (0.4%)	0.554±0.05 (100%)	0.011±0.00 (2.0%)	0.003±0.00 (0.5%)	1.19±0.05	3.18±0.62	1.43±0.25

9.5 Discussion

The phenolic acids of acidic soil races of *Betula pendula* were dominated by syringic and ferulic acids. In contrast in KR races the phenolic acids were represented by a more even mixture of syringic, salicylic, benzoic, and ferulic acids. The phenolic composition in *Calluna vulgaris* and *Erica tetralix* was dominated by syringic acid in both acidic and non-acidic races. However ferulic acid was notably higher in acidic races.

The soils of FM, SMB, SMM, KP, and KR, were represented by eight phenolic acids: gallic, protocatechuic, p-hydroxybenzoic, syringic, benzoic, ferulic, and sinapic acids. The latter three were confined to the acidic soils, both the organic (FM and SMB) and the mineral (SMM). Conversely, syringic acid dominated the calcareous KR soil and was absent from the acidic soils and brown forest soils of KP.

The species: *Betula pendula*, *Calluna vulgaris*, *Erica tetralix* influenced the total amounts of phenolic acids extracted from the soils by HPLC. Plant and soil concentrations were positively correlated at $p< 0.05$ (*Betula* and *Calluna*), and $p< 0.01$ (*Erica*). Races naturally occurring on the organic soils, FM or SMB, and on the acidic mineral soil (SMM), contained greater amounts of phenolic acids (HPLC), as did their respective soils. Concentrations in KR races were the lowest measured, in accordance with the low concentrations extracted from KR soils, and were about five-fold lower than acidic soils. In agreement with these findings, Whitehead *et al.* (1982) showed four graminaceous species and eight dicotyledenous species markedly influenced the amounts of phenolic compounds extracted from the supporting soils. Similarly Kuiters & Denneman (1987) found differences in water-soluble phenol concentrations in soils were partly explained by differences in the phenolic litter properties of the dominant tree species.

There was less consistency between individual acids identified and quantified in plants and soils. The phenolic spectrum of *Erica tetralix*, originating in KR, was dominated by syringic acid (69.9 %). Likewise the phenolic acid extracted from KR soil were mainly syringic acid (68.3 %). Ferulic acid (73.0 %) made up the majority of phenolics extracted from SMM soil, and was the second highest acid in *Betula pendula* from this site.

The total amount of phenolics, measured using FC, and the total concentration of monomeric phenolics, measured using HPLC, were not correlated. Values based on FC were about 10^6 fold greater than total concentrations obtained from HPLC. Contrary to these results Blum *et al.* (1991) found positive correlations between HPLC and FC measurements. However a number of organic

substances, not necessarily possessing a phenolic hydroxyl group, together with inorganic substances are known to reduce the FC reagent (Box 1993). This is likely to account for differences in values obtained between methods. Furthermore Blum *et al.* (1991) did not recommend the use of the FC method to estimate absolute concentrations of phenolic acids in soil extracts.

The most widely accepted theory to explain predominance of polyphenol-rich vegetation in forests on acidic soils is based on the assumption that greater phenolic concentrations reduce herbivory (Northup *et al.* 1995). Recent evidence has cast doubt on this theory: within individual tree species on a range of soils, higher concentrations of phenolics did not reduce herbivory in *Quercus* or *Fagus sylvatica* (Glyphis & Puttick 1989, Balsberg Pahlson 1989). Moreover the results of these studies provide further evidence towards the hypothesis, suggested by Northup *et al.* (1995), which suggests polyphenol-rich vegetation is an adaptive response to soil acidity. Siqueira *et al.* 1991 also showed phenolic acids facilitated nutrient uptake, particularly Fe, under acidic conditions.

Addition of organic mixtures to soil from KR and SMM severely reduced the growth of *Holcus lanatus* originating from FM, SMM, and most of all KR. In agreement with Vaughan & Ord (1991b) the root morphology and architecture were modified, and increases in fresh weight inhibited, by the phenolics. However growth was not reduced in FM races, and stimulated in KR and SMM races, when grown in FM soil amended with the lower rate of organic addition. The organic mixture was predominantly represented by syringic acid (62.4 %), a benzoic acid derivative. Benzoic acids have usually been shown to be less inhibitory than cinnamic acids (Kuiters & Sarink 1986). Einhellig *et al.* (1985) also showed negative effects on the growth of sorghum after amending soil with three weed residues. The degree of growth reduction was correlated with the quantity of dried residue added. The same was found in *Holcus lanatus*.

Although differences were not significant, root elongation rates of both KR and FM races of *Holcus* were higher than control plants in nutrient solutions with a mixture of 7 phenolic acids at pH 4.0. This was not the case at pH 6.5. Shoot elongation rates, tiller and blade numbers were also increased in FM races. Moreover root numbers, tiller and blade numbers, were reduced in KR races in nutrient solutions with phenolic acids at pH 4.0. The absence of growth inhibition in FM races at pH 4.0 or 6.5 is in accordance with the higher concentration of phenolic acids present in the organic FM soil. The life-time of phenolics in this soils will be more prolonged as a result of lower microbial activity. In contrast most phenolics will be rapidly metabolised by microorganisms in KR soil leading to a rapid removal of these acids from the soil. Moreover in KR soil phenolics are likely to be present in the dissociated, less toxic state.

The pKa values for simple phenolic acids are about 4.5. Therefore phenolic acids in the nutrient solutions at pH 4.0 are predicted to be in a undissociated form which is more lipid soluble and easily taken up at the root membrane (Kuiters 1990). This explains why growth inhibition in KR races only occurred in solutions at pH 4.0.

9.6 Conclusions

- The total amount of monomeric phenolic acids extracted from the races of dominant species in FM, SMB, SMM, and KR influenced the concentrations in respective soils.

- Individual monomeric phenolic acids extracted from either plants or soils were not consistently correlated.

- Total phenolics measured using Folin Ciocalteau's reagent and HPLC were not correlated.

- Organic additions to FM, SMM, and KR soils inhibited growth of all three races of *Holcus lanatus* except at the lower rate of addition in FM soil. In this soil, organic residues stimulated the dry matter production of KR and SMM races.

- Phenolic acids, in hydroponic solutions, did not inhibit growth of FM races of *Holcus lanatus*, at either pH 4.0 or pH 6.5.

- Growth was sometimes increased in both FM and KR races in acidic solutions with added phenolic acids.

- Growth of KR races was sometimes reduced in the presence of phenolic acids but only at pH 4.0.

Chapter 10

General Discussion

10.1 Tolerance to low pH in *Holcus lanatus* and *Betula pendula*

10.1.1 *Holcus lanatus*

Increasing acidity, with pH < 4.0, inhibited root elongation, root production, shoot elongation, and tiller production in races of *Holcus* from both FM and KP. Inhibition of growth was most pronounced in KP races which did not tolerate pH < 5.0. KP races showed symptoms of toxicity which were absent or far less severe in FM races (stunted, swollen and discoloured roots, and rapid wilting and death of shoots).

According to Rorison (1986) races most tolerant of soil acidity maintain a stable root:shoot ratio much better than potentially faster growing plants which are intolerant of acidic conditions. There was less variation in root:shoot dry weight quotients between pH values in FM races compared with KP races.

In KP races, increasing the H^+ concentration led to a sudden wilting of shoots and their subsequent death. Gunsé *et al.* (1997) found a high H^+ concentration significantly reduced the hydraulic conductivity of root cells of H^+-sensitive maize varieties (Ardour 250) but not of H^+-tolerant varieties (BR 201F). Plasmolysis of the cortical cells presumably contributed to reduced water uptake. Wilting and death of shoots in FM *Holcus* was far less pronounced than in KP races. Furthermore the proportion of root tissue occupied by cortex increased with increasing acidity in FM races but decreased in KP races. Ciamporová *et al.* (1995) proposed that the preservation of cortex tissue, essential for absorption and transport of water and nutrients, reflected structural adaptations to acid-stress.

10.1.2 *Betula pendula*

Rates of root elongation, root production, and leaf expansion in both KP and particularly KR races of *Betula* were severely inhibited by pH< 5.0. This reflects the slightly acidic and calcareous nature of

their natural soils. Growth in SMM races was not adversely affected by decreasing solution pH from 5.6 to 3.0.

Rorison (1986) suggested that plants most capable of surviving acidic environments tended to have inherently slow rates of growth and low nutrient requirements. SMM races of *Betula* maintained a steady low rate of root elongation, 2.5-3.5 cm day^{-1}, which did not change markedly with a change in acidity. In contrast RER of KP and KR races varied from 2.0-5.0 and 3.0-6.0 cm day^{-1} at pH > 5.0, and then dropped substantially to about 0.5 cm day^{-1} as the pH decreased.

10.2 Effects of Al on *Anthoxanthum odoratum*, *Betula pendula*, and *Holcus lanatus*

10.2.1 pH 4.2 culture solutions

10.2.1.1 *Anthoxanthum odoratum*

Aluminium at low concentrations, 1.3 mg l^{-1}, stimulated growth in races of *Anthoxanthum odoratum* from FM and KR but was inhibitory to SMB races. The higher concentration of 2.7 mg l^{-1} also stimulated growth in FM but not KR races. Similar positive growth responses to Al in nutrient solutions have frequently been shown in crops (Foy *et al.* 1978) including: rice (3 mg l^{-1}), tropical legumes (0.5 mg l^{-1}), eucalyptus (1 mg l^{-1}), tea (27 mg l^{-1}), sugar beet (1 mg l^{-1}), corn (3.5 mg l^{-1}), and wheat (3 mg l^{-1}).

Al has been proposed to displace Fe from metabolically inactive sites increasing Fe uptake, and to promote P uptake by blocking negative charges on cell wall sites (Foy *et al.* 1978). Fe uptake was not increased in FM races but was increased in KR at the lower concentration of 1.3 mg l^{-1}. At this concentration roots contained twice as much iron as plants which received no Al. In both FM and KR races Fe translocation to the shoots was increased in the presence of Al - this was not the case in SMB plants.

Al uptake by roots was lowest in KR races, and translocation to the shoots was inhibited.

10.2.1.1 *Holcus lanatus*

Aluminium (at nominal concentrations of 25 and 35 mg l^{-1} and [Al]$_{mm}$ concentrations of 378 and 522 μM) significantly reduced root elongation, primary and lateral root numbers, shoot elongation, tiller production, and leaf area of all races of *Holcus lanatus*. Inhibition of growth increased with an increase in nominal Al concentration. This Al-induced reduction in growth was to a certain extent correlated with the natural distribution of races of *Holcus*. Relative root and shoot growth was

generally lowest in KR races. A more detailed measurement of growth at daily (or hourly) intervals would most likely have revealed bigger differences in growth among races.

Root uptake of P and K increased in Al-treated plants. In contrast Al inhibited root uptake of Ca, Mg, and Fe, and translocation of all nutrients to plant tops. The effect of Al differed between *Holcus* races. The increases in root P concentrations were greatest in races from the acid peats: FM and SMB, and least in that from KP. The increase in root K concentrations were most pronounced in SMB and KR races, and least pronounced in those from FM. SMM races were generally least effected by Al-induced reductions in nutrient absorption and translocation to the shoots.

Tolerance to Al has been related to a plant's ability to use P and Ca efficiently in the presence of Al (Andersson 1988, Barceló *et al.* 1996, Foy & Brown 1964, Foy *et al.* 1978, Jones 1961). The two races SMM and KR were most effective in transporting P and Ca from roots to shoots in the presence of Al, and the races from FM, SMB, and KP were least effective. To a certain extent this is explained by their natural distribution. SMM races are found in acidic soils with the highest concentrations of Al. FM and SMB races are from acidic soils but these are low in Al and the Al present is more than likely complexed by organic matter and therefore non-toxic. KP soils are slightly acidic and at this pH should contain optimum levels of plant nutrients. That these three races should not be tolerant of Al is therefore expected.

The concentrations of Ca in the shoots of *Holcus lanatus* collected from the field were highest in the KP, and particularly, KR races. Similarly the highest P concentrations were found in KP races but the lowest in KR races. As expected concentrations of these two nutrients did not vary greatly between the three races from acidic (FM, SMB, SMM), either organic or mineral, soils.

Quellette & Dessureaux (1958) found Al-tolerant races of alfalfa contained more Ca and less Al in their shoots than those that were Al-sensitive (Davies & Snaydon 1972). This was also true of SMM races of *Holcus lanatus*.

At the lower Al concentration (25 mg l^{-1} Al) FM and SMB races accumulated the most Al in their roots. This is in agreement with Delhaize *et al.* (1993a) who showed sensitive root apices of wheat accumulated more Al. FM roots contained up to 30 times more Al. However at the higher solution Al concentration, 35 mg l^{-1} Al, the races KR and SMM contained the highest Al concentrations. Greater Al precipitation in roots, in SMM races, may reflect greater Al tolerance. Differences in Al accumulation between different parts of the root system (apices, mature zones) were not quantified in this study.

Foy (1996) found shoot Al concentrations in barley were greater in Al-sensitive cultivars than Al-tolerant cultivars. Generally the shoots of FM, SMB, and KR races accumulated the most Al in their shoots. This would be expected from their native soils.

10.2.1.2 *Betula pendula*

Al, at low concentrations (nominal concentrations of 2 and 5 mg l^{-1} and [Al]$_{mono}$ concentrations of 48 and 127.5 μM), sometimes stimulated root elongation, root numbers, lateral root lengths and numbers, leaf expansion, relative and absolute growth rates and seedling height. At high concentrations (nominal concentrations of 15-35 mg l^{-1} and [Al] $_{mono}$ concentrations of 376.6 and 998.5 μM) Al often inhibited growth. The effects of Al were dependent upon the race and reflected their natural origin. The tolerance to Al between races was of the order SMM>FM>KP>KR.

Al was beneficial to, and stimulated growth, at lower concentrations, in races from FM and KP. Root elongation was stimulated by up to 58 % and total leaf area was up to three-fold greater. Furthermore this increase in leaf area was a result of an increase in the number of larger leaves (> 5 cm^2). At the highest concentration Al reduced root elongation, root production, relative growth rates, and seedling height. Like the lower Al concentrations total leaf area was also increased. However this was a result of an increase in the number of small leaves (< 2 cm^2).

Al, at all concentrations, damaged the growth of KR races, reflecting the calcareous, near neutral, soils of its natural origin. Root elongation, root number, lateral root lengths and numbers, and seedling height were all reduced in the presence of Al. However total leaf area was increased at all Al concentrations but this was primarily a result of an increase in the production of small leaves, < 2 cm^2 in area. Janhunen *et al.* (1995) similarly showed a reduction in the size, and increase in the density of needles of *Pinus sylvestris* and *Picea abies* after treatment with high concentrations of Al (150 mg l^{-1}).

At the highest Al concentration there were visible symptoms of Al toxicity in KR plants. This was not the case in any of the other three races. Root tips were swollen, discoloured and necrotic. Lateral roots were stunted, swollen and discoloured. The leaves of these birch were slightly chlorotic.

The growth of SMM races was not adversely affected by Al at any of the experimental concentrations used. Root elongation was greater at the higher concentrations, root production was increased by all but the highest concentration, leaf area was increased at all concentrations with no reduction in leaf expansion (i.e. no reduction in leaves >5 cm^2 in area), and relative growth rates and seedling height increased with increasing Al concentration.

Contrary to *Holcus lanatus,* the uptake and subsequent translocation of P to plant tops was unaffected by Al (at any concentration) in all races, even KR. This P-efficiency in the presence of Al reflects the

tolerant nature of *Betula* to Al. Root absorption of Ca was also not reduced by Al in any race, and was generally higher in the KP and KR races reflecting their greater requirement for Ca. Subsequent translocation to the shoots was slightly reduced by Al concentrations >10 mg l^{-1} but not severely. The plants retained their ability to translocate Ca in the presence of Al. Al did however reduce the uptake by roots of K, Mg, and Fe, and translocation of Mg and Fe to shoots, in all races.

Root Al concentrations were up to 15 times higher than shoot concentrations. Therefore, in agreement with Corrales *et al.* (1997), Kinraide (1988), Delhaize *et al.* (1993a), and Marienfeld *et al.* (1995), Al was preferentially located in the roots, and although no histological techniques were used to determine the location within the roots, the literature would suggest it accumulates in cortical cell walls. This suggests *Betula* tolerates Al by excluding it from the symplast. Other proposed mechanisms of Al tolerance include complexation of Al by root organic exudates or precipitation of Al through an increase in rhizosphere pH. Al was not chelated around the roots of *Betula* grown in rhizoboxes with agar containing aluminon (data not given). Aluminon complexes Al and the agar turns from pink to clear (Dinkelaker *et al.* 1993). Also there was no significant increase in nutrient solution pH, however only the pH of the bulk solution was measured, and not that of the rhizosphere in particular.

The Al concentrations in shoots of SMM races were lower than the other three races. This implies the most tolerant race is also the most efficient at preventing Al uptake into the symplast. Root elongation was significantly correlated with shoot Al concentration. However Al concentrations were lower than those found in field plants (Chapter 2, Table 2.2).

In agreement with Rorison (1986), the number and lengths of lateral roots in the Al-tolerant *Betula* race, SMM, were no different from seedlings grown in 0 mg Al l^{-1} reflecting the effective distribution of dry matter between laterals and root hairs in the presence of Al. In contrast the lateral roots of KR seedlings were swollen, stunted and fewer in number. Rates of root elongation of the SMM race in nutrient solution with no added Al were about half that of KR races.

Howeler & Cadavid (1976) found the stimulus in growth by low Al concentrations in rice was greater in Al-tolerant cultivars than in Al-sensitive cultivars. The beneficial effect of 2 and 5 mg l^{-1} Al on root elongation in FM and KP races was not seen in SMM races which were the most Al-tolerant. However the stimulus was greater in FM races which were more Al-tolerant than KP races. Fe uptake was not increased in these races at low Al concentrations, and P uptake was only increased in KP races at 5 mg Al l^{-1}.

Kinraide (1988) proposed Al^{3+} amelioration of H^+ toxicity explained the beneficial effect seen at low concentrations of Al. He suggested Al^{3+} reduced cell-surface activity of the toxic cation, and, simultaneously, the toxic cation reduced the activity of the ameliorative cation which, at lower concentrations than the toxic cation, could itself be toxic. At pH 4.2 root elongation of KP races of *Betula* would not be optimum. RER increased substantially by increasing solution pH from 4.0 to 5.0. In contrast RER in SMM races did not change significantly over the pH range 3.0-5.6. Al, at 2 and 5 mg l^{-1}, could therefore have ameliorated the adverse effects of H^+ ions on the KP races in the same manner as that described by Kinraide (1988). This would also explain the lack of stimulation seen in SMM races which were not inhibited by H^+ ions.

10.2.2 pH 5.6 nutrient solutions

Al in solution was significantly less toxic at pH 5.6 compared with pH 4.2. Monomeric Al was greatly reduced at pH 5.6 explaining the reduced toxicity. The monomeric Al species present at this pH would also be different from those dominant at pH 4.2 ($Al(OH)^{2+}$ and $Al(OH)_2^+$ compared with Al^{3+}). This implies the hydroxy-monomers are far less toxic, and actually stimulated growth in KR races. This race accumulates the most Al in the field, and the same Al species are likely to be present from the pH of KR soil solutions (pH range 5.1-6.5). Foy *et al.* (1978) suggested that Al increases the Fe availability in calcareous soils or in slightly acid nutrient solutions, and prevents internal Fe deficiency. However uptake of Fe was not significantly improved at pH 5.6. Mg, Ca, and K translocation to shoots was improved at pH 5.6, particularly in KP and KR races which naturally grow in soils of higher pH than either FM or SMB. Furthermore, P did not appear to be precipitated (with Al) in the plant roots, of any race, at pH 5.6. Al was translocated to the shoots in far greater quantities than at pH 5.6, particularly in KP and KR races.

10.3 Amelioration of Al toxicity by Si and organic acids

10.3.1 Al/Si interactions

Si, as silicic acid, effectively ameliorated Al toxicity: root and shoot lengths, lateral root and blade numbers, and leaf area were significantly improved provided Al was present with Si. Plant performance was dependent upon Si concentration, increasing the concentration from 1500 to 2500 μM increased amelioration of Al-toxicity. In general, races of *Holcus* from FM and SMB showed the least improvement and KP and SMM races showed the greatest improvement in growth. This reflects the Si concentrations measured in the soil solutions: concentrations in SMM and KP soil were far

greater than in FM and SMB soil. Si, although not completely, restored nutrient uptake and translocation to shoots to that of control plants. In the presence of Si, Al uptake by roots was significantly increased but translocation to shoots was reduced. This implies Si can effectively prevent Al from entering the symplast and probably forms alumino-silicate precipitates. The cross-sections stained with hematoxylin showed Al precipitates on the outer surface of the root which were not present in Al-treated roots.

Si did not ameliorate Al toxicity through HAS formation (as proposed by Birchall *et al.* 1996). The results of dialysis did not show formation of HAS at pH 4.2. Furthermore monomeric Al was reduced but not sufficiently to explain the amelioration. It is therefore likely that Si increased the internal tolerance of Al and this may have been a result of Al/Si co-precipitation or maintenance of Golgi activity (discussed below).

HAS species were formed in nutrient solutions at pH 5.6 and, in agreement with Exley *et al.* 1997, they were not found to be toxic.

At the lower concentration, 1500 μM, Si without Al promoted growth in *Holcus*. Root elongation, root production, and plant dry weights were increased beyond that in plants grown with no added Si. Si increased root uptake of P, and sometimes K, Ca, and Mg. In contrast, Si at the higher concentration of 2500 μM inhibited growth, and this was particularly pronounced in the races from the acid peats, FM and SMB. Again reflecting the lower Si concentrations measured in soil solutions extracted from these soils. The toxicity of Si at higher concentrations is of importance in soils where Al concentrations are low.

10.3.2 Al/Organic acid interactions

The addition of organic acids to solutions containing Al prevented Al-induced reductions in the growth of *Holcus*. Root elongation, root numbers, shoot elongation, tiller and blade numbers, dry weights and leaf area were almost equal to those of plants grown without both Al and organic acids (controls). This was particularly pronounced in FM races, and least pronounced in SMM races. These differences among the races reflect the organic acid contents of their native soils: total concentrations of measured organic acids were highest in FM and lowest in SMM. The stronger Al complexer, tartaric acid, was more effective in preventing Al damage than formic acid which is reported to form weak complexes with Al (Hue *et al.* 1986). The organic acids improved translocation of nutrients to shoots which was inhibited by Al. Contrary to Si, organic acids were effective in reducing both root uptake and subsequent transport to shoots of Al. This was achieved through a dramatic reduction in the concentrations of monomeric Al, and likely formation of organic-

Al complexes. Monomeric Al was reduced below 50 μM which at pH 4.2 was not toxic to all the *Holcus lanatus* races (data not given).

10.4 Effects of organic and phenolic acids on *Deschampsia flexuosa* and *Holcus lanatus*

10.4.1 Organic acids

Organic acids alone stimulated the growth of *Deschampsia flexuosa* and *Holcus lanatus*. Rates of root and shoot elongation and root numbers were increased beyond rates in control plants (grown with no added organic acids). Vegetative reproduction was also stimulated via an increase in tiller production. This stimulation in growth, in *Holcus lanatus*, was particularly pronounced in FM and SMB races, reflecting the high concentrations of organic acids which these races experience in their native soils. Enhanced growth was achieved through an increase in nutrient uptake and subsequent transport to shoots. Similarly, reflecting their ecological distribution, nutrient uptake was most enhanced in FM and SMB races and least in KR races. The soil solutions of KR contained the lowest total concentration of organic acids.

10.4.2 Phenolic acids

Phenolic acids in the acidic peaty soils appear to contribute to the plants adaptation to low pH. In agreement with the recent hypothesis of Northup *et al.* (1995), phenolic acids in nutrient solutions enhanced growth of FM races of *Holcus* under acidic conditions (pH 4.0), but reduced the growth of KR races. The same phenolic acids did not stimulate growth at pH 6.5. In their native soils, phenolic acids of KR are likely to be dissociated and not readily available for plant uptake and therefore not toxic. In contrast in the acidic organic soils of FM these phenolics are undissociated and their life-time enhanced due a lower microbial activity. Despite their available form for root absorption they were not toxic to the FM race. Addition of organic matter to soil inhibited *Holcus* growth except in the organic FM soil where the organic residues stimulated the growth of SMM and KR races allowing them to survive the sub-optimal conditions of this soil.

10.5 Future research

Both Al and H^+ ions not only affected root lengths and rates of elongation but also changed root morphology and root system architecture. The roots became stubby, stunted, swollen, and brittle with bent, brown tips. As a result of the injury to the plant roots the ability of the plant to absorb water (and nutrients) diminishes. The shoots then become chlorotic, wilt and die. Barceló & Poschenrieder (1990) highlighted the frequent correlation between drought tolerance and metal resistance in plants adapted to metalliferous soils. Despite this coincidence in plant adaptations very little attention has been given to water relations in either Al or H^+-stressed plants.

This reduction in the root system volume may not be the only reason for these induced water deficiencies in plants. A reduction in cell wall elasticity and plasma membrane permeability could account for the reduction in water stress tolerance. Zhao *et al.* (1987) and Gunsé *et al.* (1997) found reductions in root cortical cell membrane permeability in Red Oak and maize, and reductions in cell wall elasticity and extensibility (maize), in the presence of Al. Si prevented shoot wilting and presumably maintained water relations in *Holcus lanatus*. However cell wall or plasma membrane hydraulic properties in Al+Si-treated plants have not to date been investigated. The ultrastructural investigations in *Holcus lanatus* showed secretory vesicles and amyloplasts were absent or greatly reduced in Al-treated plants implying the Golgi activity was affected. Cell wall synthesis would therefore also be affected. The Golgi activity in Al+Si-treated plants did not appear to be adversely affected by Al. This area also requires further investigation along with the accurate quantification of changes occurring in the cell ultrastructure and subsequent implications in cell wall mechanical properties (elasticity and extensibility).

The shoots of *Holcus* grown in pH 2.0 and 3.0 wilted within 1 day of treatment, and this was far more pronounced in the H^+-sensitive KP race which did not recover than in the H^+-tolerant FM race. This loss in turgor, together with plasmolysis of cortical cells, indicates severe water stress. Wilting was more pronounced and faster in *Holcus* grown at low pH than in *Holcus* grown in +Al solutions. Furthermore there was no observed plasmolysis of root cortical cells in Al-treated plants despite the longer treatment period. The effects of Al and H^+ ions on plant water relations appear to differ. Gunsé *et al.* (1997) using pressure-probe techniques showed H^+ toxicity affected the whole-root conductivity (Lp_r) to a greater extent than the hydraulic conductivity of root cells (Lp_c) in a H^+-sensitive cultivar of maize (Ardour 250). In contrast, Al reduced Lp_c more than Lp_r in an Al-sensitive cultivar (BR201F). This new technique will allow further research into the race-specific responses of whole root and root cell water relations to low pH. The FM races were tolerant of low pH and

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maintained turgor and presumably water uptake and transport. Determining whether or not this was related to differences in FM and KP root and root cell hydraulic conductivity would be of interest.

10.6 Extrapolation of studies using nutrient solutions to the field

Nutrient solutions allow the researcher to present a known concentration of nutrients and/or toxins to plants. Furthermore the pH can be easily maintained, the nutrient concentrations at the actual root surface can be controlled, the solution can easily be sampled and pH or nutrients adjusted, sterile conditions can be used, and the plants can be removed and measured without injury (Rorison 1969). However it is difficult to extrapolate the results of measurements on plant individuals in a greenhouse/growth room to the behaviour of the same species growing under natural conditions. Nutrient solutions do not reproduce the seasonal changes of the environment, spatial variation in nutrients/toxins, competition between individuals of the same or other species, soil compaction, mycorrhizal infection, or soil organic matter and decomposing leaf litter. These factors probably account for the discrepancies between results of solution culture and plant performance in soil pot experiments or in the field. The growth response of *Holcus lanatus* in nutrient solutions of decreasing pH did not correlate well with responses to limed and unlimed soil. In an attempt to minimise these effects it is necessary to study the effects of soil acidity in culture solutions which reflect the ionic environment of plants under field conditions. Investigations of Al-toxicity, such as Davies & Snaydon 1972, Hammond *et al.* 1995, Hodson & Sangster 1993, Li *et al.* 1989, Kinraide 1993, Kinraide *et al.* 1994, Ryan *et al.* 1993, 1994, have frequently used background solutions of $Ca(NO_3)_2$ which does not reflect the natural soil solution.

A comparison of foliar element concentrations of the study species in nutrient solutions with those collected from the field (Appendix 2) showed minor differences. Concentrations of K, Mg, and Fe in *Holcus* races grown in solution were very close to concentrations found in field races. Ca concentrations of KP and KR races were about 30 % of field concentrations. However this was expected as Ca concentrations in nutrient solutions were based on FM soil concentrations which were substantially lower than in these two sites. P concentrations in all *Holcus* races were usually about two-fold greater in solution than in field plants. There was a high correlation in shoot ionic composition between plants from the field and those grown in solution in both *Deschampsia* and *Anthoxanthum*. There was more variation between the element concentrations in field and solution grown *Betula*. In all races P, K and Fe were similar between the field and solution plants. Ca and Mg were however up to 60 % lower in solution grown plants than field plants. It should be noted that field specimens were collected from mature birch. The close correlation achieved between solution

grown plants and field specimens adds to the advantages of using nutrient solutions when determining the effects of ion toxicity and reinforces faith in their relevance.

Although the same 35Al treatment reduced the growth of *Holcus* in different experiments involving nutrient solutions (with Al and silicic acid (Chapter 6), with Al and organic acids (Chapter 8) there were some significant differences in the ionic composition of the plants and in race-specific growth response in the two experiments. The P and Mg concentrations of both roots and shoots, and the Ca, Al, and Fe concentrations of shoots, were consistent between experiments but root concentrations of Al and Fe were significantly greater in the second experiment. Moreover 35Al inhibited root elongation to a greater extent in SMB, SMM, and KP races in the second experiment compared with the first. It is important to consider the large variation in plant response when using nutrient solutions despite maintaining constant conditions.

The responses to low pH, Al, silicic acid, organic acids, and phenolic acids, by the different races could be accurately predicted from their field ionic compositions, and from the ionic composition of their natural soil environments. However under natural conditions these races will periodically be exposed to extreme conditions which tend to be ignored in solution cultures. That is the chemical composition of soil solutions varies greatly with time and this may be a result of season, temperature, rainfall and soil water content, decomposition and nitrification rates, or element uptake by plants and microorganisms. Soil solution concentrations of H and Al ions may during one year differ tenfold (Falkengren-Grerup 1994), and may be between two and six-fold greater during dry periods than under average moisture conditions (Joslin & Wolfe 1992). In a recent study, Quist (1995) highlighted the importance of such episodic events on plant growth. He determined the effect of episodes of different length (one or two weeks) and concentrations of H^+ (pH 3.8-4.5) and Al^{3+} (0-70 μM) on the growth of three forest-floor species: *Galium odoratum*, *Lamium galeobdolon*, and *Poa nemoralis*. Species occurring in less acidic soils, *Galium* and *Lamium*, were more sensitive to acid episodes, and did not always recover from them, compared with *Poa* which was found at a lower soil pH. The plasticity of the response to these toxicities by the species should be considered alongside its tolerance. Plants which are adapted to low pH or high Al concentrations should also be able to recover from episodes where these toxicities become even greater.

The probability of plants being exposed to episodes of elevated concentrations of H^+ and Al^{3+} ions has recently increased as a result of acid rain which is discussed below.

10.7 Implications of increasing soil acidification

Recent research has shown an increase in soil acidification through anthropogenic effects (Barak *et al.* 1997, Bouman *et al.* 1995, Hahn & Marschner 1998, Hartemink 1998, Kiss 1993, Kreutzer & Weiss 1998, Ingerslev 1997, Nouri & Reddy 1995, Raubauch *et al.* 1998, Ukrainetz *et al.* 1996). Chemical changes in the soil, associated with acid precipitation and the nitrification of ammoniacal fertilizers, include increases in the soil solution concentration of Al^{3+}, Mn^{2+}, Pb^{2+}, and Cd^{2+}, reductions in soil solution pH, and an enhanced loss of base cations through leaching (Matzner & Murach 1995).

Recent European initiatives have been taken to reduce emissions of SO_2, NO_x, and NH_3 (Ahokas 1997). Nevertheless acid deposition continues to increase although the main component is now considered to be nitrogen rather than sulphur (Goulding & Blake 1998, Matschonat & Vogt 1997, Matzner & Murach 1995). Alewell *et al.* (1997) investigated the soil solution response to experimentally reduced acid deposition in a *Picea abies* stand in Solling, Germany. They found the reversibility of soil acidification would be delayed for decades owing to desorption of previously stored SO_4, accompanied by cation leaching and Al release. In contrast experimental reduction of NO_3 deposition resulted in an immediate increase in soil pH.

As discussed in Chapter 1, soils can buffer H^+ ion input through ion exchange of clays, sesquioxides, and organic matter. Kiss (1993) estimated the total exogenous input of H^+ ions to soils (from atmospheric deposition, N-fertilizers, and superphosphate) as 6.18-7.30 kg H^+ ha^{-1} $year^{-1}$, which accounts for 35.6-29.0 % of the total H^+ input in soils (from both exogenous and endogenous sources). The soil buffer capacity is only effective for as long as the potential acidity can release exchangeable Al and H. Therefore with continued and amplified acidification the buffering capacity of the soils may be reduced or lost and the toxic effects of an increased Al^{3+} concentration can be expected.

The extent to which soils can be acidified by atmospheric input is highly variable and will essentially depend on the soil type, geology, and the quantity of drainage water. The soils of FM and SMB are rich in organic matter and primarily buffered through pH-dependent ion exchange with weak organic acids. The weathering of minerals is unimportant in these soils. An increase in acidity will reduce the cation exchange capacity, as a result of a decline in exchange sites (organic acids become undissociated as the pH drops), and therefore reduce the buffer capacity. The total exchangeable acidity of these soils is dominated by H^+ with little exchangeable Al^{3+} and therefore unable to assimilate incoming H^+ ions. Microbially-mediated reduction of NO_3, Fe_2O_3, MnO_2, and SO_4 will remove some of these protons. The mineral, SMM, soils are effectively buffered by Al-hydrolysis

reactions and exchange of Al and hydroxy-Al from the clay fraction (permanent charge component of the cation exchange capacity). However increasing amounts of Al and free H^+ ions will be exported from the soil to the drainage water. The pH of a mineral soil rarely falls below pH 4.0. The buffer capacity of a strongly acid mineral soil with 1 % clay is about 100-150 kg H^+ ha^{-1} $year^{-1}$. The calcareous, KR, and base-rich, KP, soils will be buffered so long as the exchangeable cations last, and Ca is released through dissolution of the limestone ($CaCO_3$) bedrock. The buffer capacity of a near-neutral soil with 1 % $CaCO_3$ is 150 kg H^+ ha^{-1} $year^{-1}$, substantially greater than the current exogenous H^+ input. The prevention of Al toxicity by Si will be an important factor in siliceous rich soils such as SMM and KP.

The impact of a pH-reduction in these soils depends on the initial pH of the soil. In the case of KR, a reduction in pH of 0.5 units will only reduce the soil pH to about 6.0 with almost no damaging effect on plant growth. While an equivalent pH reduction in FM soil will reduce the soil pH to 3.0 with likely serious effects. *Holcus* from FM grown at pH 3.0 survived much better than KP seedlings but the observed plasmolysis of the cortical cells did indicate serious damage.

Recent studies, including the present study, have shown naturally occurring species, and particularly trees, have a substantially higher Al-tolerance than agricultural crop species (Clegg & Gobran 1995, Godbold & Kettner 1991, Godbold *et al.* 1988, Göransson & Eldhuset 1995, Hecht-Buchholz *et al.* 1987, Janhunen *et al.* 1995). Crops tend to be sensitive at Al concentrations < 50 μM (1.35 mg l^{-1}). In contrast, at these concentrations, Al stimulated growth in *Anthoxanthum, Holcus* (data not given), and *Betula*. The effects of soil acidification, through anthropogenic means, will have a more significant influence on nutrient acquisition (rather than root elongation) and could lead to nutrient imbalances in these plants.

In conclusion, acid soils can be divided into two main groups: organic and mineral soils, and the division occurs at a soil pH of about 4.0 (measured using H_2O or a weak $CaCl_2$ solution such as 0.002 M). Plants growing in acid peats are adapted to high H^+ concentrations but not Al and growth is enhanced by both organic and phenolic acids. In contrast acid mineral soils are dominated by exchangeable Al^{3+} and plants are therefore tolerant of Al and but not necessarily low pH. The response to these factors by different races of *Holcus* and *Betula* in this study was in accordance with their natural distribution and soil environments. FM races of *Holcus* were able to continue growing in pH values less than 4.0 but were intolerant of high Al concentrations. SMM races of *Betula* and *Holcus* were least inhibited by Al. Si, in the form of silicic acid ($Si(OH)_4$), ameliorated Al toxicity, and this was most pronounced in those races who were naturally exposed to high concentrations of Si (SMM and KP). At high concentrations, Si *per se*, inhibited plant growth and this was most pronounced in those races who were found in soils of low Si concentration (FM and SMB). Finally,

phenolic acids in solutions at low pH (despite being in a undissociated and potentially toxic form) enhanced the growth of races originating from acidic organic soils (naturally phenolic-rich) but inhibited growth of non-acidic races such as KR.

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Appendix 1. Common and Latin names for species referred to throughout thesis.

Common name	Latin name
Alfalfa	*Medicago sativa* L.
Amaranth	*Amaranthus retroflexus* L.
Arnica	*Arnica montana* L.
Azalea	*Rhododendron spp.* L.
Barley	*Hordeum vulgare* L.
Beech	*Fagus sylvatica* L.
Bermuda-grass	*Cynodon dactylon* L. Pers.
Bloody Crane's-bill	*Geranium sanguineum* L.
Bracken	*Pteridium aquilinum* L. Kuhn
Bristle Bent	*Agrostis curtisii* Kerguelen
Broad Bean	*Vicia faba* L.
Buckwheat	*Fagopyrum esculentum* Moench
Canola	*Brassica campestris* L.
Cassava	*Manihot esculenta* Crantz
Coffee	*Coffea arabica* L.
Common Bent	*Agrostis capillaris* L.
Common Bird's-foot-trefoil	*Lotus corniculatus* L.
Common Figwort	*Scrophularia nodosa* L.
Common Knapweed	*Centaurea nigra* L.
Cotton	*Gossypium hirsutum* L.
Cowpea	*Vigna unguiculata* L.
Cranberry	*Vaccinium oxycoccos* L.
Creeping soft-grass	*Holcus mollis* L.
Creeping Bent	*Agrostis stolonifera* L.
Crimson Clover	*Trifolium incarnatum* L.
Cross-leaved Heath	*Erica tetralix* L.
Cucumber	*Cucumis sativus* L.
Dog's Mercury	*Mercurialis perennis* L.
Dogwood	*Cornus florida* L.
Downy Birch	*Betula pubescens* Ehrh.
Fodder Burnet	*Sanguisorba minor* Scop.
French Bean	*Phaseolus vulgaris* L.
Ginger	*Zingiber officinale* Roscoe
Greater Bird's-foot-trefoil	*Lotus pedunculatus* Car.
Groundnut	*Arachis hypogaea* L.
Guinea Grass	*Panicum maximum* L.
Hare's-tail Cottongrass	*Eriophorum vaginatum* L.
Hart's-tongue	*Phyllitis scolopendrium* L. Newman
Heath-grass	*Danthonia linkii* Kunth
Heath Bedstraw	*Galium saxatile* L.
Heath Groundsel	*Senecio sylvaticus* L.
Heather	*Calluna vulgaris* L. Hull
Kermes oak	*Quercus coccifera* L.
Lesser Hairy-brome	*Bromus benekenii* Lange Trimen
Lettuce	*Lactuca sativa* L.
Leucaena	*Leucaena leucocephala* L.
Lucerne	*Medicago sativa* L.
Loblolly pine	*Pinus taeda* L.
Maidenhair Spleenwort	*Asplenium trichomanes* L.
Maize	*Zea mays* L.

Appendix 1 (continued). Common and Latin names for species referred to throughout thesis.

Common name	Latin name
Mat-grass	*Nardus stricta* L.
Meadow Thistle	*Cirsium dissectum* L. Hill
Morning glory	*Ipomoea lacunosa* L.
Mung-bean	*Vigna radiata* L. Wilczek
Norway Spruce	*Picea abies* L. Karst
Oat	*Avena sativa* L.
Onion	*Allium cepa* L.
Pea	*Pisum sativum* L.
Pedunculate Oak	*Quercus robur* L.
Potato	*Solanum tuberosum* L.
Quaking-grass	*Briza media* L.
Red Campion	*Silene dioica* L. Clairv
Red Maple	*Acer rubrum* L.
Red Oak	*Quercus rubra* L.
Red Spruce	*Picea rubens* Sarg
Rice	*Oryza sativa* L.
Rosebay Willowherb	*Chamerion angustifolium* L. Scop
Rough Hawkbit	*Leontodon hispidus* L.
Rustyback	*Ceterach officinarum* Willd.
Rye	*Secale cereale* L.
Ryegrass	*Lolium multiflorum* Lam
Sainfoin	*Onobrychis viciifolia* Scop.
Scots Pine	*Pinus sylvestris* L.
Serradella	*Ornithopus sativus* Brot.
Sheep's-fescue	*Festuca ovina* L.
Silver Birch	*Betula pendula* Roth.
Small scabious	*Scabiosa columbaria* L.
Sorghum	*Sorghum bicolor* L. Moench
Soybean	*Glycine max* L. Merill
Squinancywort	*Asperula cynanchica* L.
Subterranean Clover	*Trifolium subterraneum* L.
Sugarcane	*Saccharum officinarum* L.
Sunflower	*Helianthus annuus* L.
Sweet Vernal Grass	*Anthoxanthum odoratum* L.
Sycamore	*Acer pseudoplatanus* L.
Tea	*Camellia sinensis* L.
Teosinte	*Zea mays* L. ssp. *mexicana*
Tomato	*Lycopersicon esculentum* Miller
Upright Brome	*Bromopsis erecta* Hudson Fourr.
Wavy Hair-grass	*Deschampsia flexuosa* L. Trin
Weeping-grass	*Microlaena stipoides* Labill R. Br.
Weymouth Pine	*Pinus strobus* L.
Wheat	*Triticum aestivum* L.
White Clover	*Trifolium repens* L.
Wood Barley	*Hordelymus europaeus* All.
Wood Meadow-grass	*Poa nemoralis* L.
Wood Millet	*Milium effusum* L.
Woodruff	*Galium odoratum* L. Scop
Wood-sorrel	*Oxalis acetosella* L.
Yellow Archangel	*Lamium galeobdolon* (L.) L.
Yorkshire-fog	*Holcus lanatus* L.

Appendix 2. Element composition of leaves and blades (mg g^{-1} dry weight) collected from the study species in the field. Bulked samples were collected in July-September 1995, air-dried and ground prior to analysis. Samples were collected from the sites: Flanders Moss (FM), Sheriffmuir (SMM and SMB), Kippenrait Glen (KP) and Kinloch Rannoch (KR).

Site	P	K	Ca	Mg mg g^{-1}	Na	Al	Fe
Betula pendula Roth.							
FM	2.46	7.65	8.02	2.63	0.47	0.40	0.27
SMM	2.06	6.26	9.48	2.03	0.30	0.19	0.07
KP	2.58	9.20	9.53	1.84	0.23	0.24	0.10
KR	1.73	6.68	9.43	2.27	0.15	0.47	0.22
Anthoxanthum odoratum L.							
FM	3.43	22.11	1.50	1.32	0.56	0.14	0.09
SMB	3.59	24.96	1.42	0.83	0.17	0.34	0.15
SMM	2.33	20.63	2.34	0.88	0.57	0.26	0.04
KP	3.31	23.18	2.99	1.20	0.87	0.23	0.08
KR	1.21	17.38	3.56	0.58	0.29	0.49	0.28
Deschampsia flexuosa (L.) Trin							
FM	1.76	14.23	1.84	1.15	0.10	0.10	0.05
SMB	1.42	13.49	1.55	0.61	0.14	0.18	0.12
SMM	1.43	10.07	2.47	0.95	0.08	0.21	0.34
KP	1.76	12.80	1.97	1.00	0.11	0.22	0.15
KR	1.57	15.59	6.43	0.68	0.17	0.23	0.12
Holcus lanatus L.							
FM	1.76	12.16	2.46	1.23	0.12	0.12	0.13
SMB	1.46	14.11	2.23	0.71	1.58	0.23	0.28
SMM	1.23	12.22	2.94	0.82	1.48	0.28	0.25
KP	2.09	13.39	3.47	1.14	0.29	0.32	0.15
KR	0.89	14.55	4.31	0.45	1.85	0.30	0.15