Uptake of Fluorides by the Plant Root

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Abstract

Fluoride (F) is one of the most common airborne pollutants and its phytotoxicity is well known. Major sources of airborne F pollution are brickworks, aluminium smelters and phosphate fertiliser factories. Fluoride is also an impurity in phosphatic fertilisers (2-3%) and this is the major source of F contamination in agricultural soils. Until recently F added to the soil was considered to adsorb strongly to the soil and therefore was unavailable to the plant. However, some recent studies in agricultural and industrial situations have shown increases in water extractable F in soils, which could be potentially available to the plant. In solution, F can exist as a number of complexes with aluminium, boron, hydrogen, silicon and iron. There are limited data available on how the ionic speciation of F in solution affect F uptake by the plant. One of the reasons for the small number of studies examining F uptake by plants is the analytical difficulty of analysing F in plant material.

The main objectives of this thesis were:

1. to verify a sealed chamber acid digestion technique for dissolution of plant material for total F analysis by a F ion selective electrode,

2. to improve this technique for routine, rapid F analysis of plant material,

3. to identify, through the literature and computer modelling, the inorganic ionic species of F which could be present in the soil solution, and

4. to determine which of these ionic species of F are taken up by the plant root and those which are toxic to the plant.

A sealed chamber acid digestion technique was used for dissolution of plant materials for analysis of F by an ion selective electrode. Verification of this method was by analysis of standard reference materials. However, when the certified F value of the standard reference material was not obtained, digestion acids, ratio of acid:sample, times of digestion and energy sources (convection or microwave) were modified in an attempt to improve this method.

To determine which ionic species of F are taken up by the plant root and those which are toxic to the plant, oats and tomatoes were grown in solution cultures. Solution culture parameters were modified to give a variety of F complexes in solution (F^-, AlF^{2+}, AlF_2^+, AlF_3^0, AlF_4^-, HF and BF_4^-). Speciation of solution were modelled using the GEOCHEM-PC and MINTEQA2 computer modelling programs. Laboratory studies highlighted some limitations of these modelling programs.

A variety of analytical techniques were employed to compare the measured concentrations of ionic species of F in solution with the calculated concentrations. Fluoride ion selective electrode procedures were compared with an anionic capillary ion analyser (CIA) procedure. Using the CIA, a method to measure simultaneously the free F ion and the BF_4^- complex in solution was verified.

The major findings of the thesis were as follows:

1. Acid digestion of plant material for the determination of F is not suitable for determination of total F in plant material. This method is limited to 60-75% recovery due to the occlusion of F in silicate minerals within the plant. However, it may be suitable for determination of bioavailable F in plant material: further studies are required to confirm this.

2. Some ionic species of aluminium-F (AlF^{2+} or AlF_2^+) are phytotoxic at concentrations which can be found in polluted soils.

3. The uptake and toxicity of F is affected by its speciation, and the rate of F uptake at equivalent activities in solution is in the order: $BF_4^+ > HF > AlF^{2+} = AlF_2^+ > AlF_3 = AlF_4^- = F^-$. However, toxicity is in the order $HF > BF_4^+ > AlF^{2+} = AlF_2^+ > AlF_3 = AlF_4^- = F^-$.

4. Changes in the ionic species of F, particularly F^-, AlF and HF, in solution could increase uptake of F by plant roots to phytotoxic levels, or to levels that could cause fluorosis in cattle and sheep grazing on the plant.

Declaration

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

I consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying.

January, 1996.

Signed:

Daryl Philip Stevens.

Publications from the Thesis

The following papers have arisen from the work done in this thesis.

Journal Articles:

Stevens D P, McLaughlin M J and Alston A M 1995 Limitations of acid digestion techniques for the determination of fluoride in plant material. Commun. Soil Sci. Plant Anal. 26,1823-1842.

McLaughlin M J, Tiller K G, Naidu R and Stevens D P 1996 The behaviour and environmental impact of contaminants in fertilisers: A review. Aust. J. Soil. Res.34, 1-54.

Stevens D P, McLaughlin M J and Alston A M 1995 Plant root uptake of aluminium fluorides from solution culture. Plant Soil. (In Review)

Conference Articles:

Stevens D P, McLaughlin M J and Alston A M 1994 Are aluminium fluoride complexes phytotoxic? 15th World Congress of Soil Sci, Acapulco, Mexico. 3b:254-255 (Proceedings)

Stevens D P, McLaughlin M J and Alston A M May 1995 Speciation of fluoride and uptake of fluoride by plants. Third International Conference on the Biogeochemistry of Trace Elements, Paris, France. Theme B3. (Proceedings)

Stevens D P, McLaughlin M J and Alston A M October 1995 Differences in uptake and phytotoxicity of the free fluoride ion and fluoride complexed with aluminum, boron and hydrogen. October 1995 ASA CSSA, SSA Annual Meeting, St. Louis, Missouri. (Abstract, Oral presentation by McLaughlin).

Stevens D P, McLaughlin M J and Alston A M February 1996 Uptake of fluoride by plants from fluoride-polluted soils. First International Conference on Contaminants in the Soil Environment in the Australasia-Pacific Region. Adelaide. pp. 63-64 (Proceedings).

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To my Grand Parents

Abbreviations

AEC	Anion exchange capacity.
AFS	Apparent free space (DFS + WFS).
AlF	AlF_x^{3-x}, where x ranges form 1 - 4 (x can range from 1 - 6. However, the complexes where x ranges from 1- 4 are most predominant and only these will be considered in this thesis).
CE	Convectional energy.
CEC	Cation exchange capacity.
DFS	Donnan free space.
F-ISE	Fluoride ion selective electrode.
ICP-AES	Inductively coupled plasma atomic emission spectrometry.
lsd	least significant difference.
ME	Microwave energy.
NCB	Non complexing buffer.
P-UCE	Plant uptake co-efficients (mmol F kg^{-1} plant/mmol F-ionic-species dm^{-3} of growth solution). Describes total plant uptake of F-ionic-species relative to the activity of an ionic species of F in solution.
rsd	Relative standard deviation.
S-UCE	Shoot uptake co-efficients (mmol F kg^{-1} plant shoot/mmol F-ionic-species dm^{-3} of growth solution). Describes uptake and translocation of F-ionic-species to the shoot relative to the activity of a ionic species of F in solution..
SRM	Standard reference material.
Teflon	Polytetrafluorethylene.
TISAB	Total ionic strength adjusting buffer.
WFS	Water free space.

1.0 Introduction

1.1 Background

Fluoride (F) is considered one of the most toxic inorganic pollutants. The biochemical and physiological mechanisms involved in the toxicity of F are not clear. It is thought that F is an effective inhibitor of enzymes and its calcium-precipitating powers affect membrane permeability. There are many airborne industrial sources of F pollution. Aluminum smelters, brickworks and phosphatic fertiliser factories are the major polluters. Non-airborne sources of F contamination in soils include fertilisers and irrigation water.

Fluoride is not considered an essential element for plants, but is an essential micronutrient for animals. However, excessive intake of F by animals can lead to fluorosis. Cattle are one of the more F-sensitive domestic animals, and a constant diet of dried plant material containing greater than 30 mg F kg^{-1} is considered to be above the toxic threshold (Davis, 1980).

Not only can F be toxic to animals, but the phytotoxicity of atmospheric F has been well documented and the phytotoxic species of F have been identified. Atmospheric F can cause immediate phytotoxicity, and has therefore been of primary concern. Until recently, much research has indicated that uptake of F from soils by plant roots is of little concern, as F added to the soil is rapidly removed from solution and becomes unavailable to the plant. However, recent research has shown high concentrations of water soluble and potentially plant available F in certain soil types near point sources of F pollution. Increased concentration of F in plant shoots can be toxic to the plant or toxic to the animals grazing on the plant.

Total F concentrations in soil do not correlate well with uptake of F by plant roots. Various extraction techniques used to measure readily soluble soil F (F presumed to be plant available) have been shown not to adequately measure plant available F, suggesting that it is not only the concentration of soluble F in the soil which affects uptake of F and phytotoxicity. Other research has indicated that when F is complexed with other ions in solution uptake of F by plant roots and its translocation to the shoots, are enhanced. Similarly, there are limited data to show that absorption of F by bacteria and some animals is affected by the speciation of F.

Further work is required to identify the species of F present in soil solution which are taken up by plant roots and are toxic to plants. These findings should be considered in developing an appropriate soil test for identification of F available for uptake by plant roots, and in identifying soil conditions which may lead to higher than normal concentrations of F in plants, which could lead to toxic effects on plants and/or animals.

To aid further research, a quick accurate method for determination of total F in plant material is required. The development of the F ion selective electrode (F-ISE) has improved the analysis of F in solution. However, because of the volatility and strong complexing ability of F, procedures for bringing plant F into solution for total F analysis have been very tedious. Newly developed techniques which use sealed chamber and acids for digestion promise to simplify this task.

1.2 Objectives

The four objectives of this study were:

1. to verify a sealed chamber acid digestion technique for dissolution of plant material for total F analyses by a F-ISE,

2. to improve the technique for dissolution of F in plant material so it can be used for routine, rapid analysis of F,
3. to identify, through a literature search, the ionic species of F which could be present in the soil solution, and
4. to determine which ionic species of F are taken up by the plant root and those which are toxic to the plant.

1.3 Outline of approach

To develop a quick accurate method for the determination of F in plant material, a sealed chamber acid digestion technique was tested against a plant standard reference material which was not previously available. This method was then modified to use sealed chamber microwave digestion vessels and strong acids to bring F and all other elements into solution for analysis by F-ISE and Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES), respectively.

Solution culture experiments determined the dependency of uptake of F by plants on the ionic form of F in solution. The major species of F which may be exposed to the plant roots were determined through a review of the literature and by modelling inorganic species in solution with GEOCHEM-PC. Solution culture parameters were adjusted to expose a variety of inorganic species of F to plant roots (oats and tomatoes). Dry weights and concentrations of all elements in plants shoots were monitored to aid in assessing the ionic species of F which are taken up and/or toxic to these plants, and to confirm that plant nutrients are not limited.

No attempt was made to assess the interaction of the free F ion with dissolved organic, colloidal or organo-mineral compounds which could be present in the soil solution. This was considered beyond the scope of this thesis.

2.0 Review of the literature

2.1 Introduction

The element fluorine is a unique halide chemically and is the most common halide in igneous rocks (Bohn *et al*, 1985). In the combined state, fluorine constitutes 0.078 % of the earth's crust. Fluorine is virtually absent in the free state as the diatomic molecular gas F_2, but is widely distributed in chemical combination, and as such is commonly referred to in literature under the general term 'fluoride' (F) (Shortland, 1988). This general term will be adopted in this thesis. The best known F-containing minerals are fluorite (CaF_2), fluorapatite ($Ca_5(PO_4)_3F$), and cryolite (Na_3AlF_6) (Brewer, 1965; Simons, 1954; Kumpulainen and Koivistoinen, 1977). The main sources of F in non-polluted soils are the weathered products of rocks.

Fluoride concentrations within soil depend on atmospheric input, anthropogenic additions, variation in soil parent material, the rate of F translocation through the soil profile and the rate of biological cycling of F. While total F in normal mineral soils averages 150 to 360 mg F kg^{-1}, higher concentrations up to 7070 mg F kg^{-1} have been reported.

Fluoride pollution originates from many sources: aluminium (Al) smelters; manufacturers of iron (Fe), bricks, fertilisers and glass; coal-fired power stations; irrigation water; and phosphorus fertilisers (Hocking *et al.*, 1980; Kremlenkova and Gaponyuk, 1984; Pickering, 1985; Kremlenkova and Gaponyuk, 1984; Stewart *et al.*, 1974). Any industry which uses raw materials containing even small amounts of F can release enough gaseous (*e.g.* HF, SiF_4) and particulate F (*e.g.* AlF_3, Na_3AlF_6, CaF_2) to enhance concentrations in soils and plants of surrounding areas (Pickering, 1985). Gaseous atmospheric F is considered to be far more toxic than particulate atmospheric F, as this F can be readily taken into the plant through the stomata (Muramoto *et al.*,

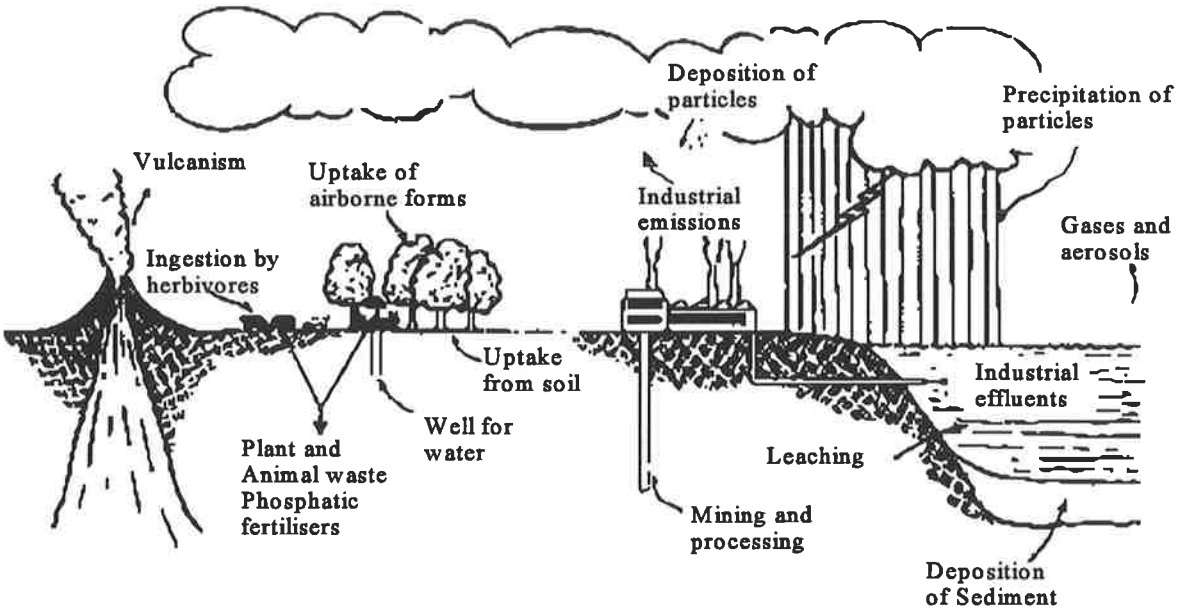


Figure 2.1 Sources and movement of fluoride in the environment (from Weinstein, 1977)

1991). Many authors have studied the toxic affects of atmospheric F (Leone *et al.*, 1956; Applegate and Adam, 1966; McCune *et al.*, 1964; Treshow *et al.*, 1967). The immediate visible symptom of atmospheric F toxicity is leaf necrosis, and a need to address this problem has contributed to a neglect of the study of effects of soil F on plants.

Atmospheric F may cause immediate phytotoxicity and is therefore of primary concern. However, through various channels the airborne F eventually enters the soil (Figure 2.1). Continual heavy application of F-containing fertilisers and disposal of F-contaminated wastes, could also increase and maintain high concentrations of soluble F in soil solutions, thereby increasing uptake by the plant. Recent research has indicated high concentrations (up to 192 mg F kg^{-1} soil) of water soluble F in soils surrounding Al reduction plants (Walton, 1987; Wenzel and Blum, 1991). There are limited data relating soil solution F directly to soil-extracted F. However, the data of Haidouti (1991) and Polomski *et al.* (1982a) suggest that the ratios between F in soil solutions:soil extracts ranges between 1:10 to 1:20 (*i.e.* between 10 to 20 mg F dm^{-3} of

soil solution) . These concentrations of F are similar or higher than those determined by Bar-Yosef and Lindsay (1986) to cause a significant restriction in growth rate of tomato and maize plants in solution culture (approximately 10 mg F dm^{-3}). Such concentrations could therefore contribute to elevated F concentrations and F toxicity in F-sensitive vegetation. However, the knowledge of plant-soil-F interactions is limited, such that not only the contribution of F from the soil to the plant is unknown, but little is known on the ionic species of F which are taken up by the plant, and those which are toxic to the plant.

2.2 The chemistry of fluoride

Fluorine is the first member of the halogens and the lightest element in Group VII, with an atomic weight of 18.998. The valence of F is 1- and its isotope with the greatest half life (109.8 minutes) is ^{18}F (Weast, 1988). It has a high electron affinity (electronegativity = 4.10 electron volts) and consequently, unique physical and chemical properties (Cotton *et al.*, 1987). Its properties often display different characteristics from those of other halogens. For example, alkaline earth F compounds (CaF_2, SrF_2) have low solubilities in water in contrast to $CaCl_2$ and $CaBr_2$. The radius of the F ion (1.36 Å) is similar to the hydroxyl ion (1.40 Å). The F ion therefore substitutes isomorphously with the hydroxyl ion in many silicate and phosphate minerals (Fleischer, 1974). Fluoride forms complexes with many cations in the soil solution with varying degrees of solubility (Table 2.1). In a complex system, with a single nominal concentration of F, the activities of complexes in solution are pH dependent.

2.3 Background concentrations and regulations of fluoride concentration in soils

Background concentrations of F in soils vary greatly depending on the parent rock of the soil and the dominant minerals in the soil (Table 2.2). Generally, high total F concentrations correspond with high mica and clay contents (Fleischer and Robinson, 1963).

Table 2.1 Solubility and dissociation constants of fluoride complexes.

Fluoride complex	Log K, 25°C, ionic strength = 0 (Smith and Martell, 1976)	Solubility (Weast, 1988) g dm^{-3}	°C
NaF	not reported	42.200	18
CaF_2	-10.40	0.016	18
AlF	7.00		Not reported
AlF_2	12.60		Not reported
AlF_3	16.70	5.590	25
AlF_4	19.10		Not reported
MgF_2	-8.18	0.076	18
KF	not reported	923.000	18
Na_2SiF_6	not reported	0.653	17
HBF_4	not reported	Soluble in all proportions	
HF	3.17	very soluble	
FeF_2	0.80	slightly soluble	
FeF_3	12.10	slightly soluble	

In the majority of soils, F concentrations are below the Dutch reference value for background concentrations, 200 mg F kg^{-1} (Moen *et al.*, 1986). Total soil F concentrations below this value are refered to as being in Category A. Concentrations greater than 400 mg F kg^{-1} (Category B) would generally be considered above background concentrations and would therefore be a value require further investigation. For concentrations above 2000 mg F kg^{-1} soil (Catergory C) the Dutch criteria state that soils require remediation. The reference values proposed by Moen *et al.* (1986) were further refined in 1988 by Moen to take into account soil clay content, where the reference values for F = 175 + (13 × % clay). The range of values listed in Tables 2.2 and 2.3 extend into the Category B and C criteria set down by the Dutch for assessment of F contaminated soils.

Table 2.2 Fluoride concentration in a range of rocks and soils.

Material	F concentration (mg kg^{-1})		Reference
	Range	Mean	
Soil type/location			
Maury silt loam/Tennessee		3650	MacIntire *et al.* (1955)
Harsell fine sandy loam/Tennessee		170	MacIntire *et al.* (1955)
137 soils/USA	trace-7070	292	Robinson and Edgington (1946)
6 soils/Austria	61-314	235	Wenzel and Blum (1992)
28 soils/New Zealand	68-540	200	Gemmell (1946)
Rock			
Basalt	20-1060	360	Fleischer and Robinson (1963)
Granite and granodiorite	20-2700	810	Fleischer and Robinson (1963)
Alkalic rocks	200-2250	1000	Fleischer and Robinson (1963)
Limestone	0-1210	220	Fleischer and Robinson (1963)
Andesite	0-780	210	Fleischer and Robinson (1963)

As the range of background concentrations of F in soil depends on soil type and parent rock, no one value could possibly be used for all soils to indicate Category A soil types. However, as Moen *et al.* (1986) suggest this system is an aid for examination, planning and implementing investigations and remedial actions. Each site must still be examined individually as no two sites are the same.

Table 2.3 Background concentrations of fluoride in soils from several countries.

mg F kg^{-1}	Soil	Location\Country	Reference
95 - 108	Calcareous	Beotia region, Greece.	Haidouti (1991)
166 (mean)	Silt clay	Liebefeld, Switzerland	Polomski *et al.* (1982a)
228 (mean)	Calcareous	Schitterwald, Switzerland	Polomski *et al.* (1982a)
70 - 618	Various	Illinois, United States	Omueti and Jones (1977)
22 - 220	Various	Bluff area, New Zealand	Manley *et al.* (1975)
19 - 26	Soil humus	Newfoundland, Canada	Sidhu (1979)

2.4 Accumulation of fluoride in soils

In the majority of cases, a high percentage of F input to soils is firmly retained (MacIntire *et al.*, 1955; Murray, 1984; MacIntire *et al.*, 1948; Gilpin and Johnson, 1980; Peek and Volk, 1985; Morshina and Fanaskova, 1987). Due to the reactivity of F species, F remains in the upper portion of the soil and there is little movement through the profile, although this is dependent on the soil type (Seth and Pandey, 1983).

Fluoride retention by soil varies with the F concentration in the aqueous phase, the soil type and soil pH (Bower and Hatcher, 1967). In acidic soils, retention is favoured in soils containing clays and poorly ordered hydrous oxides of Al (Pickering, 1985), and is not favoured in soils which possess low concentrations of amorphous Al species, clay and organic matter (Omueti and Jones, 1977a and b). In alkaline soils, Hani (1978) suggested that the fixation of F is essentially governed by the presence of calcium (Ca) phosphates and Ca carbonate. However, the data of

Wenzel and Blum (1992) led them to conclude that F in solution increases as pH increases above 6.5 due to a more negatively charged soil surface causing desorption of F.

In slightly acid soils (pH 5.5-6.5) F is strongly adsorbed to the soil and F accumulates in such soils. However, as the soil becomes more acidic or alkaline F solubility increases (Wenzel and Blum, 1992: Larsen and Widdowson, 1971) and F losses from the profile by either leaching for plant uptake would be greater.

2.5 Sources of fluoride contamination in soil

There are several sources of F which pollute the soil. These sources enter the soil through three major pathways: the atmosphere, fertilisers and soil amendments or water sources. These pathways are discussed below.

2.5.1 *Atmospheric input of fluoride to soil*

In the United States, F has been ranked as one of the most important phytotoxic air pollutants along with ozone, other oxidants, sulphur dioxide and pesticides (Heck *et al.*, 1973). However, F is considered to be the most phytotoxic of these pollutants and susceptible plant species can be injured at ambient concentrations of about 0.8 $\mu g\ F\ m^{-3}$ (Weinstein and Alcsher-Herman, 1982). The Australian and New Zealand Environment Council (1990) states that the maximum acceptable average ambient F concentration in the atmosphere over a 90 day period should be less than 0.5 $\mu g\ HF\ m^{-3}$. Background concentrations of F in uncontaminated air are usually below the detection limit of about 0.05 $\mu g\ F\ m^{-3}$ (Thompson *et al.*, 1971). However, there are many industries which release F into the atmosphere causing localised increases in atmospheric F concentrations (Table 2.4). As outlined above (Section 2.1), atmospheric F pollution originates

from many sources. Many of the gaseous by-products of these industries are potentially toxic when directly absorbed by plants and animals and many authors have studied the toxic affects of airborne F (Sidhu, 1979; Mitchell *et al.*, 1981; Jacobson *et al.*, 1966).

Airborne F which is not directly adsorbed by the soil, will eventually be transported to the soil through vegetation, chiefly by means of the plant litterfall and by the removal of external and internal foliar F by precipitation as rainfall, snow, dews, fogs and mists (Murray, 1982).

Table 2.4 Ambient fluoride concentrations in uncontaminated and contaminated air.

Background $\mu g\ F\ m^{-3}$	Contaminated $\mu g\ F\ m^{-3}$	Source of Contamination	Reference
0.15	4.34 - 5.14	Phosphorus plant	Sidhu, 1979
-	0.32 - 2.36	Al smelter	Hocking *et al.*(1980)
-	60 - 100	Al smelter	Macuch *et al.*(1969)
<0.1	0.1 - 1.0	Al smelter	O'Connor and Horsman, (1982)
<0.05	-	-	Thompson *et al.*(1971)

2.5.2 *Input of fluoride to soil in fertilisers and soil amendments*

Rock phosphates generally contain around 3.5 % F (Hart *et al.*, 1934), but concentrations can range from about 1 to 4 % (Becker, 1989). In the major process used in the manufacture of phosphatic fertilisers, phosphate rock (composed mainly of apatite) is treated with concentrated sulfuric acid and water to produce gypsum, phosphoric acid and hydrogen fluoride (Equation 2.1, taken from Rutherford *et al.*, 1994). During this process, part of the F is lost into the atmosphere as gaseous SiF_4 and HF. The concentration of F in the final fertiliser is also lowered further through dilution with S (superphosphates) or NH_4^+ (ammoniated phosphates). After processing

of the phosphate rock, phosphatic fertilisers contain between 1.3 to 3.0 % F (Table 2.5).

$$Ca_{10}(PO_4)_6F_2 + 10H_2SO_4 + 20H_2O \rightarrow 10CaSO_4.2H_2O + 6H_3PO_4 + 2HF \qquad \text{(Equation 2.1)}$$

A large proportion of the phosphorus applied to Australian soils since the 1920s has been in the form of single superphosphate (Gargett 1983; McLaughlin *et al.*, 1992). Assuming 1.5 % F in single superphosphate (Kumpulainen and Koivistoinen, 1977; Evans *et al.*, 1971), McLaughlin *et al.* (1996) estimated from data on imports of rock phosphate (Donald, 1964; Cook, 1982; ABARE, 1993) that approximately 2.5 million tonnes of F have been added to fertilised agricultural soils in Australia since the turn of the century. Assuming the fertilised area has been 25 million ha (an overestimate for earlier years), the average annual loading rate through fertilisation is 1.4 kg F ha^{-1} y^{-1}. If the bulk density of the soils is taken as 1.6 g cm^{-3} and F is incorporated into the top 10 cm of soil, the F concentration in soil would increase by 61 mg kg^{-1} over a 70 y period. This figure does not consider losses of F from the system by erosion, leaching or plant uptake. The rate of F increase in soil will be more rapid where higher rates of fertilisers are used. For example, up to 200 kg P ha^{-1} is added to soils when potatoes are grown (McLaughlin *et al.*, 1995). Assuming single superphosphate is used (9% P, 1.5% F), approximately 33 kg F ha^{-1} (20 mg F kg^{-1} soil, calculated using the above assumptions) will be added in a single application. Mortvedt and Sikora (1992) suggested that the concentration of F in soil would increase by 60 mg kg^{-1} if monoammonium phosphate containing 2 % F were applied at 60 kg ha^{-1} of P for 25 years. The assumed depth of incorporation and soil bulk density were not stated.

Phosphogypsum (which contains between 0.2 - 1.2 % F) is an acid by-product from the production of phosphatic fertilisers (Shainberg *et al.*, 1989; Rutherford *et al.*, 1994; Oates and

Caldwell, 1985). Phosphogypsum is commonly used as a soil amendment for sodic soils, and increasing world stockpiles of this by-product have led to research into its possible use for alleviation of soil acidity (Keerthisinghe *et al.*, 1991a; Oates and Caldwell, 1985). The work has been encouraging and could lead to wide spread use of phosphogypsum as a soil amendment, although this will contribute significantly to F concentrations in soils.

Table 2.5 Fluoride concentration in phosphate fertilisers.

Fertiliser	mg F kg^{-1}	Reference
Diammonium phosphate	15000 - 30000[A]	Mortvedt and Sikora, 1992
Monoammonium phosphate	16000 - 22000[A]	"
Triple superphosphate	13000 - 24000[A]	"
Superphosphate	15800[A]	Evans *et al.*, 1971
Phosphate rock	21500 - 27300[A]	"
Phospho-Potassium Borate (PKB)	17830[A]	Bovay, 1969
Superphosphate	2600[B]	Conover and Poole, 1981
Zinc oxide	733[B]	"
Dolomite	31.6[B]	"
Ammonium nitrate	6.9[B]	"

[A] Total fluoride
[B] Water soluble fluoride

Concentrations of F in fertilisers other than phosphatic fertilisers are much lower (Table 2.5) due to the raw materials used in their manufacture being low in F. However, there is an increasing pressure to use sewage sludge as a soil amendment as marine disposal of sewage sludge is being decreased and land disposal is now favoured. With the rapid increase in demand and production of hydrofluoric acid by industry, some of this F will be discharged to sewerage systems and eventually contribute to increased F concentrations in sewage sludges (Rea, 1979). Sewage sludges have been shown to contain up to 34,000 mg F kg^{-1}, but F concentrations generally range between

80-1950 mg kg^{-1} dried solids (Rea, 1979; Davis, 1980). Disposal of sewage sludges with high concentrations of F to soil will eventually increase the F concentration in the soil significantly.

2.5.3 *Water sources of fluoride input to soil*

The F concentration of surface and underground waters depends on the availability and solubility of F in rocks and soils, the porosity of the rocks and soils through which the water flows, the water flow velocity, the pH of the water and the concentration of Ca and other ions present. Fresh water usually contains less than 2 mg F dm^{-3} (World Health Organisation, 1970). Most irrigation water also contains less than 2 mg F dm^{-3}. However, in some areas F concentrations in water may reach up to 28 mg F dm^{-3} due to the source of water, *e.g.* deep aquifers or geothermal wells (Tracy *et al.*, 1984; Rowe *et al.*, 1973; Kubota *et al.*, 1982; Bower and Hatcher, 1967)

The F concentration of sea water ranges between 0.8 and 1.5 mg F dm^{-3} (Hemens *et al.*, 1975; Whitford, 1989; Kappanna *et al.*, 1962). Even though this is not generally used for irrigation, sea mist settling on coastal soils would be expected to add F to these soils.

2.6 Behaviour of fluoride in soils

The behaviour of F in soils is predominantly controlled by the clay, organic matter, Al, Fe, and Ca concentrations, pH and forms of F added to the soil. It is generally accepted that, up to approximately 0.6 mmol F dm^{-3} in soil solution, the relationship between solution and adsorbed F can be adequately described by the Freundlich or Langmuir equations (Peek and Volk, 1985; Tracy *et al.*, 1984: Robbins, 1986; Bower and Hatcher, 1967; Chhabra *et al.*, 1979). Above this concentration, it is likely that F is immobilized through precipitation. The proposed mechanisms involved in retaining F species in soils include (Morshina, 1980; Barrow, 1986; Ares, 1978):

1. chemical combination within the clay lattice,
2. adsorption from solution onto colloid surfaces,
3. mechanical retention in the soil solution (within soil micropores), and
4. precipitation.

The ways in which these variables affect the behaviour of F in soils are discussed in the following sections.

2.6.1 *Form of fluoride added to the soil*

The amount of atmospheric F which is adsorbed by the soil can vary greatly depending on the source of pollution. Many industries (Section 2.1) release gaseous HF, SiF_4 and/or particulate AlF_3, Na_3AlF_6, CaF_2 into the atmosphere (Pickering, 1985; Hocking *et al.*, 1980) which eventually enter the soil. The type and concentrations of F species in fertilisers vary greatly depending on product formulaton. For example, in boron-enriched PK fertiliser (PKB), 57% of the F is present as BF_4 (Bovay, 1969). The major form of F in rock phosphate is apatite ($Ca_5(PO_4)_3F$), with fluorite (CaF_2) present as a minor component (Rutherford *et al.*, 1994). Similarly, these forms (apatite and fluorite) would be found in phosphatic fertilisers. With suggestions that fluorosilicic acid (H_2SiF_6) could be added to suspension fertilisers produced from monoammonium phosphate (MAP) to prevent gelling of the suspension (Sikora *et al.*, 1992), H_2SiF_6 could also be added to soils through application of fertilisers. Fluoride added to the soil in irrigation or bore water is predominantly in the F^- form, although the speciation in solution is dependent on the pH and concentrations of other ions in the irrigation water.

2.6.2 *Chemistry of fluoride in soil*

In acid soils, F is complexed with Al, Fe, silica and the hydrogen ion, and in alkaline soils, with Ca. The dissociation constants for many F complexes found in soil solution were listed in Table 2.1. Generally at a pH less than 6, F begins to form complexes with Al and at pH < 4.0 F begins to form complexes with H^+. At neutral pH, F^- is the predominant ionic form in solution, which encourages adsorption to soil surfaces (see Section 2.6.4). Under alkaline conditions, Ca usually dominates F chemistry in the soil and F precipitates as CaF_2. However, under alkaline conditions the negative surface charge of the soil is increased, repelling the F ion from the soils surface (Wenzel and Blum, 1992; Larsen and Widdowson, 1971; Barrow and Ellis, 1986). These reactions and their effects on F reacting with the soil are discussed in more detail in the following sections.

2.6.3 *Reaction of fluoride with the soil surface*

There are two types of reactions of F with the soil surface: fast (adsorption) and slow (absorption). The theory and mechanisms involved in each are discussed below.

2.6.3.1 Fast reactions of fluoride with the soil surface

When F is equilibrated with soil or kaolinite, hydroxyl release has been attributed to the exchange of OH on the clay mineral lattice by F^- (Romo, 1954, cited Bower and Hatcher, 1967; Parfitt and Russell, 1977), a relatively fast reaction. However, there are much data showing that the replacement of lattice OH by F^- is of minor significance and that F adsorption at low F concentrations occurs primarily by exchange with OH of $Al(OH)_3$ and from other basic Al polymers adsorbed on mineral surfaces, rather than with the crystal lattice OH of clay minerals

(Samson, 1952; Huang and Jackson, 1965; Bower and Hatcher, 1967; Omueti and Jones, 1977b). This is probably due to the much greater surface area for OH exchange and OH groups on such polymers.

Desorption of F has been found to be largely by exchange of adsorbed F ions for hydroxide ions rather than escape of F ions and counter ions (Barrow and Shaw, 1982). Fluoride sorption has been described using the Freundlich model (Peek and Volk, 1985) and sorption is almost totally reversible (Hingston *et al.*, 1974).

2.6.3.2 Slow reactions of fluoride with the soil surface

A model developed by Barrow (1983) for describing the adsorption and desorption of phosphate by soil, was considered a general model which should apply to other specifically adsorbed anions and cations. In 1986, Barrow found that the model which closely described the effects on sorption of phosphate closely modelled the sorption and desorption of F.

One of the assumptions of the model is that the initial adsorption induces a diffusion gradient towards the interior of the particle which begins a solid state diffusion process. As the model uses similar mechanisms to predict desorption and adsorption for both F and phosphate, F must behave analogously to phosphate. This suggests that phosphate or any anion that has reacted with soil for a long period is not irreversibly fixed but has penetrated into the soil particles (Barrow, 1983). That observed desorption of F was adequately decribed by the model, added support to the argument that slow desorption is largely controlled by diffusion from within the solid phase.

It should be noted that Barrow's model questions the suggestion that slow desorption of phosphate occurs because phosphate forms a binuclear structures with oxide surfaces through a

second oxygen atom, giving a stable ring structure (Hingston *et al.*, 1974). The single charge of the F is unable to form binuclear structures.

2.6.4 *Factors affecting fluoride adsorption to soil*

Fluoride adsorption by soil varies with concentration of F in the aqueous phase, the soil type and soil pH (Bower and Hatcher 1967; Pickering, 1985; Omueti and Jones, 1977a and b). In the majority of soils, a high proportion of added F is firmly retained (MacIntire *et al.*, 1955; Murray, 1984; Gilpin and Johnson, 1980; Peek and Volk, 1985). In general, slightly acid soils (pH = 5.5-6.5) have the greatest affinity for F. Adsorption of the free F ion results from adsorption on the minerals of the kaolinite group, Al and Fe hydroxides, Al oxide, Ca carbonate, and cation-saturated organic matter (Morshina and Fanaskova, 1987). In alkaline soils, fixation is thought to be governed by the presence of Ca phosphates and Ca carbonate (Hani, 1978; Peek and Volk, 1986; Elrashidi and Lindsay, 1986b) and the increase in negative charge of soil surfaces (Wenzel and Blum, 1992; Larsen and Widdowson, 1971; Barrow and Ellis, 1986). These factors are discussed in detail below.

2.6.4.1 The effects of soil pH on fluoride adsorption to soil

The capacity of clays, which readily hydrolyse, to scavenge F effectively from the soil solution decreases with increasing pH, and with increasing F concentration (Slavek *et al.*, 1984; Morshina and Fanaskova, 1985 and 1987). Morshina and Fanaskova (1985 and 1987) found that at low concentrations (2-100 mg dm^{-3}) F adsorption to the soil did not depend on the type of compound (sodium, potassium or ammonium F) added but on the soil pH. As the concentration of F increases, other factors such as mobilisation of organic matter and metals and the nature of the F complex begin to affect F solubility. Alkaline calcareous sediments may deviate from this

generalisation, due to the probability of formation of CaF_2 (Slavek *et al.*, 1984). Slavek *et al.* (1984) postulated precipitation of F as AlF_3 at pH values less than 5 as the basic retention process at these pH values. However, at this pH, high concentrations of AlF_3 would be expected to remain in solution as a soluble complex (Farrah *et al.*, 1987; Barrow and Ellis, 1986).

Farrah *et al.* (1987) showed that the maximum sorption of F in soil occurred at pH 5.5-6.5. Several researchers have found the solubility of F in soil is at a minimum at pH 5.5-6.5 (Wenzel and Blum, 1992; Omueti and Jones, 1977b and 1980; Larsen and Widdowson, 1971). At lower pH, sorption of F declined due to preferential formation of AlF_x (x = 1-3) species lowering the activity of the F^- ion (Barrow and Ellis, 1986). It would be expected that the positive or zero charge on the AlF complexes would decrease the adsorption of this F complex and in very acid soils (pH less than 4.0) the formation of HF and SiF would affect adsorption similarly. However as Anderson *et al.* (1991) suggest, some AlF complexes may reside on the soil exchange phase and be involved in adsorption of F as this complex. Maximum solubility of F in soil at pH values less than 5.5 and greater than 6.5 is thought to be due to formation of soluble AlF complexes at low pH, and by desorption of free F due to repulsion by more negatively charged surfaces at higher pH (Wenzel and Blum, 1992; Larsen and Widdowson, 1971; Barrow and Ellis, 1986). Yet, if Ca is present, F solubility at pH values greater than 6.5 is thought to be controlled by precipitation of insoluble (0.016 g dm^{-3} at 18°C) CaF_2 (Weast, 1988; Slavek *et al.*, 1984; Hani, 1978). However, CaF_2 would only control F solubility when F concentrations in soil solution are greater than approximately 410 μM. This figure will vary with temperature and ionic strength.

The work of Slavek *et al.* (1984) and Hani (1978) was laboratory based (using pure minerals and limed soils) and the apparent contradiction between results of studies of F solubility at pH greater than 6.5 in soils could be explained by Ca replacing the H on clay exchange sites decreasing its

availability to complex with F, or by dissociation of H from the surface of clay minerals at high pH leaving a negative charge on the surface thus preventing adsorption of F.

Work by Barrow and Ellis (1986) suggests that the effects of pH on F retention by soil can be explained by the same principles used to explain the effects of pH on phosphate retention. These are changes with pH in the ion species present, and changes with pH in the electrostatic potential of the variable charge surfaces with which the species react. At neutral pH (6.5 ± 1.0), F in soil solution would be present predominantly as the negatively charged free F ion (F^-), which would be rapidly adsorbed by positively charged surfaces on edge faces of clays or oxide surfaces.

2.6.4.2 The effects of clay on adsorption of fluoride to soil

It is clear that the adsorption of F present in the aqueous phase can be influenced by the nature, amounts and form of any clays present (Slavek *et al.*, 1984; Bower and Hatcher, 1967). After leaching of F through the soil, Omueti and Jones (1980) found total concentration of F in soil increased with clay content. Previously Omueti and Jones (1977b) showed that the variable most highly associated ($r = 0.92$) with F sorption to soil was the clay content. Other significant variables were the pH ($r = 0.36$) and organic matter content ($r = 0.60$).

Bower and Hatcher (1967) measured adsorption by shaking soils and standard solutions of F. Fluoride adsorbed was recorded as F removed from solution after shaking for 16 h. Gibbsite ($Al_2O_3.3H_2O$), halloysite and kaolinite adsorbed more F than goethite, montmorillonite, and vermiculite, the latter adsorbing only traces of F (Bower and Hatcher, 1967). Kaolinite (1:1 layers of Al and silicate, as is halloysite) has a low cation exchange capacity (CEC) because of little isomorphic substitution (low negative charge) and low surface area. Gibbsite (a hydrous oxide clay) has a lower CEC (low negative charge) than kaolinite (Brady, 1974). Montmorillonite

and vermiculite are 2:1 silicate clays (characterised by an alumina sheet sandwiched between two silica sheets), with substantial isomorphic substitution of Mg with Al, and Al with Si (resulting in high negative charge) and therefore high CEC. The lower negative charges of the gibbsite, halloysite and kaolinite may allow F to become sufficiently close to the mineral surface to enhance exchange with hydroxyl groups or adsorb to the positive charges exposed at crystal edge faces, where this is not possible with montmorillonite and vermiculite due to the greater negative charge of the surface. This could explain the greater adsorption of F by the former clays compared with the latter. However, goethite ($Fe_2O_3.H_2O$) which has a low negative charge at pH values 4 - 8 (low CEC), adsorbs little F, suggesting the presence of Al enhances anion exchange with hydroxyl groups. In soils, Al is more soluble than Fe and thus Al would more active in forming bridges between the negatively charged clays and F.

However, the CEC of hydrous oxides, allophane and to a degree the kaolinite group is pH dependent. At high pH values, these clay surfaces tend to be negatively charged (the H ion dissociates from O leaving a negatively charged site) and at low pH positively charged due to protonation of OH groups. At low pH this would give these clays a high affinity for adsorption of anions, highlighting the importance of the type of clay present in the soil and the soil pH on adsorption of F.

2.6.4.3 The effects of aluminium and iron on adsorption of fluoride to soil

The binding of F in the soil was found to have a close relationship with the Al content of the soil (MacIntire *et al.*, 1955). Lysimeter studies by MacIntire *et al.* (1955) indicated that under certain conditions, the formation of relatively insoluble Al fluorosilicate ($Al_2(SiF_6)$) could account for the observed retention of F in soils. Later studies by Sikora *et al.* (1992) concluded that formation

of SiF_6^{2-} in soil is only of importance at pH less than 4.0. The high retention of F by soil has been attributed to not only Al, but also to Fe (Murray, 1984). Morshina and Fanaskova (1985) suggest that the preferred retention mechanism for F on the soils which they tested was precipitation of F on Al oxides. Aluminium may also act as a bridge between organic matter and F (see Section 2.6.4.4).

The results of Anderson *et al.* (1991) suggest that part of the Al on the soil exchange phase may in fact exist as fluoro-Al complexes, which implies a role for exchangeable Al in regulating apparent F solubility in acid soils. Elrashidi and Lindsay (1986a) found it unlikely that AlF_3 controls the F ion activity in strongly acid soils. Elrashidi and Lindsay (1987) found that increases in pH, due to application of F to the soil, were larger in acid soils compared with alkaline soils. The fact that acid soils contain more amorphous Al and Fe hydroxides than alkaline soils may help to explain these differences (Perrott *et al.*, 1976), and suggests that a large portion of F added to these soil adsorbs to the Al and Fe hydroxides, releasing hydroxyl ions into solution.

Batch experiments of Wenzel and Blum (1992) showed that the concentrations of F and Al in water extracts of acid soils were highly correlated, and that Al solubility is increased by F through the formation of soluble AlF complexes. However, the discussion in the above paragraphs suggests that soils containing Al minerals increase the adsorption of F to the soil. This contradiction could be explained by the fact that the number of adsorption sites for F would be much greater than the amount of desorbed soluble Al (which would desorb to form soluble AlF complexes) so that, the net effect is adsorption of F, or by the fact that batch experiments have limited application when predicting AlF chemistry at realistic soil water contents (Bond *et al.*, 1995).

2.6.4.4 The effects of organic matter on the adsorption of fluoride to soil

The F concentration of soil has been positively correlated (r = 0.60) with the concentration of soil organic matter (Omeuti and Jones, 1977a). However, more recent research (Omeuti and Jones, 1980) indicated that the total F content of the organic matter removed from soil was, on average, 9 mg F kg^{-1} soil, which is a small percentage of total F in the soil. This suggests that organic matter may not be an important factor in F adsorption. Positive correlations between total soil F and organic matter could be explained by F decreasing microbial activity, which decreases organic matter breakdown, leading to increased concentrations of organic carbon in the soil (Wilke, 1987; Rao and Pal, 1978). However, decreased microbial activity could decrease plant growth, decreasing carbon input into this system, having the opposite affect. The continual uptake of F by the plant from the lower strata and the deposition of this F upon the soil surface, could also contribute to a relationship between F and organic matter. Many of these effects are long-term and there are insufficient data to draw conclusions on the mechanisms involved.

Addition of F to acid and calcareous soils significantly increases extracted soluble organic carbon (Hani, 1978; Morshina, 1980; Gaponyuk *et al.*, 1982; Peek and Volk, 1986; Elrashidi and Lindsay, 1987). Kremlenkova and Gaponyuk (1984) showed that NaF increased the mobility of humic acids, yet humic and fulvic acids separated from soil do not adsorb F (Morshina and Fanaskova, 1987). These observation could be explained by F having a greater affinity for adsorption sites on the soil than negatively charged organic matter (fulvic or humic acids). Therefore, while organic matter may not be a primary factor in F adsorption, it could aid adsorption of F to soil by providing exchange sites for the F ion. Humic and fulvic acids, which generally possess some aromatic character and a range of acidic functional groups, strongly retain cations, particularly Fe^{3+} and Al^{3+} (Pickering, 1985). Therefore, F adsorption to soil probably

involves the formation of coordination bonds between these cations which are themselves adsorbed to organic matter. Peek and Volk (1986) suggested that organic C in soil extracts could be present as Al-OH-organic material. Theoretically the F could substitute for OH. The work of Kremlenkova and Gaponyuk (1984) could also be explained by colloidal movement of clay due to dispersion by increased concentrations of Na.

Increased organic matter concentrations in soil increase F concentrations and the mechanism of F retention is probably a combination of the processes discussed above. Further work is required to confirm this, but is beyond the scope of this thesis.

2.6.4.5 Adsorption of fluoride in calcareous, sodic and saline soils

Tracy *et al.* (1984) speculated that there were possibly two mechanisms responsible for the retention of F in sodic and saline sodic soils. Firstly, F could be adsorbed or precipitated by a mechanism that kept the F activity below that required for fluorite precipitation, and once this mechanism was saturated, the F activity increased until fluorite precipitation started to take place. Robbins (1986) found an additional adsorption mechanism to that of Tracy *et al.* (1984) on similar soils. Data (Langmuir isotherm) obtained by these authors indicated that one kind of surface or site removes F from solution over the range 0.0 - 1.2 mM F. Once these surfaces or sites are saturated, a second kind of site removes F from solution, and at some point before or after this second set of sites or surfaces is saturated, the fluorite ion activity product was exceeded and F was then removed from solution by fluorite precipitation. The first two adsorption mechanisms may be those outlined in Section 2.6.3.

The results of Chhabra *et al.* (1979) indicated that the amount of added F retained by soil decreases with increasing exchangeable sodium percentage (ESP), and increases in pH (*i.e.*

adsorption decreases with increase in pH from 8.4 - 9.8). The decrease in F adsorption in soils with high ESP may also be due to the greater anionic repulsion associated with the increased negative double-layer repulsion operative under such conditions (Chhabra *et al.*, 1979). Decreased adsorption is also favoured by the presence of excess Na which forms NaF, one of the more soluble F compounds (Table 2.1). Excess Na would also coincide with a decrease in the presence of Ca, decreasing precipitation of the relatively insoluble CaF_2 compound (Table 2.1).

2.6.5 *Fluoride mobility in soil*

Fluoride mobility in soil is governed by the factors which affect the adsorption of F to soil (Section 2.6.4). Wenzel and Blum (1992) found the contamination risk for ground water was low in slightly acid soils, and it was increased under strongly acid as well as under alkaline conditions, conditions where retention of F by soil was lowest. Pickering (1985) suggested that the main deleterious effect of mobile F species was contamination of ground water. Contrary to Pickering (1985), MacIntire *et al.* (1948) concluded from studies on silt and sandy loams, that no harmful concentration of F would develop in the ground waters from incorporation of the F-containing Ca silicate slag or from the incorporation of phosphatic fertilisers. However, MacIntire *et al.* (1948) experimented on soils of pH 5.5 - 6.2 (pH values at which maximum F adsorption occurs, see Section 2.6.4.1) and much of the F was added in poorly soluble form (*e.g.* CaF_2, see Table 2.1).

The work of Tracy *et al.* (1984), carried out over a 600 d period, showed that application of water containing 7 mg F dm^{-3} at 0.15 and 0.3 leaching fraction leads to no more than 2% of the added F being leached from 1 m deep soil (pH = 8.5-9.3) profiles. However, as more water and F were applied, the high F concentration in the soil moved to lower points in the profile, leading these

authors to speculate that these high concentrations could, with time, have an effect on the F concentrations of shallow ground waters.

Murray (1984) showed, over a 12 month period, that only 2.6 to 4.6% of F applied to column 0.1 m wide and 2 m long was leached (65 dm^3) as water-soluble F. The soil used for this experiment was a reconstructed profile of a sandy podzolic soil (pH = 5.7 to 6.0), a highly leached soil containing low quantities of Ca and humus and significant quantities of Al and Fe. The findings suggest that if these results can be applied to the same soils under field conditions, airborne F emissions from nearby industries, even under the most extreme conditions, would be unlikely to result in the F pollution of ground waters.

Therefore, it can be concluded that the mobility of F in the majority of soil types is too slow to pose a threat to ground waters.

2.7 Plant uptake of fluoride from soil

2.7.1 *Soil factors affecting fluoride uptake by plants*

Fluoride must be in a soluble or potentially soluble form to be available to the plant. The factors which control the adsorption and solubility of F in soil are discussed in this section in relation to F uptake by the plant.

2.7.1.1 The effects of adsorption of fluoride to soil on plant uptake of fluoride

As F must be present in the soil solution to be taken up by the plant roots, any conditions which encourage F adsorption to the soil will decrease F availability to the plant. As soluble F added to soil becomes more strongly bound to the soil over time (slow adsorption reaction, Section

2.6.3.2), it desorbs more slowly and becomes less available to the plant. The studies of Barrow and Shaw (1977) found that increasing the temperature and duration of the incubation period of soils with NaF, resulted in a lower concentration of F in the soil solution and less F desorbed, which would be expected to decrease the amount of F available for plant uptake.

2.7.1.2 The effects of soil pH on fluoride uptake by plants

Soil pH affects plant uptake of F by altering the adsorption of F to the soil (see Section 2.6.4.1) and changing the ionic species of F in the soil solution (see Sections 2.2 and 2.7.1.5). Changes in F adsorption, as a result of changes in pH, would be expected to affect F mobility in the soil and its uptake by plants. Decreases in soil pH can also increase the toxicity of other elements present in the soil, such as Al (see Section 2.10.4) and hydrogen ions (Johnson and Wilkinson, 1992). Such ions may affect the root membrane transport systems or cause damage to the root which will allow leakage of F past sites which regulate F uptake by the plant.

2.7.1.3 The fraction of fluoride in the soil taken up by plants

Total F in soil generally does not correlate well with plant uptake of F (Cooke *et al.*, 1976a; Gisiger, 1968) as it is only the soluble or potentially rapidly soluble F which is assumed to have the potential to be taken up by the plant (Brewer, 1965). Therefore, there have been several attempts to correlate readily extractable F in soil with F taken up by plants. Three main fractions of F in soil have been identified: readily soluble, potentially rapidly soluble or labile (the amount of fluoride that is able to replenish the soil solution when the latter is depleted), and insoluble fluoride (fluoride bound tightly within and on the crystalline structure of the soil particles, not rapidly soluble). Procedures used to measure these fractions are:

1. water extraction (readily soluble F),
2. 0.01 M $CaCl_2$ extraction (readily soluble F),
3. resin and mild acid extraction (readily and potentially soluble F), and
4. NaOH fusion (total F).

Readily soluble F is commonly measured by extraction of the soil using water or 0.01 M $CaCl_2$. However, extraction with 0.01 M $CaCl_2$ may not be a good measure of readily soluble F. Extracting with 0.01 M $CaCl_2$ could cause the precipitation of insoluble CaF_2 (Table 2.1).

Soil:water extraction ratios of up to 1:50 have been used (Haidouti, 1991; Polomski *et al.*,1982a; Wilke, 1987; Desaules *et al.*, 1992). However, as the response time of the F-ion selective electrode is positively related to the F concentration (see Section 2.7.3), the less diluted the sample the quicker, and more accurate, the analysis. Furthermore, there is evidence that excessively large water-to-soil water ratios should be avoided so that appreciable amounts of relatively insoluble F compounds like CaF_2 are not dissolved (Brewer, 1965). Larsen and Widdowson (1971) and Supharungsun and Wainwright (1982) found that soil:solution ratios of (water or 0.01 M $CaCl_2$) less than 1:6 resulted in increased extractable F g^{-1} soil.

An extraction ratio of 1:5 soil:water which provides a high detection limit per g soil, facilitates rapid, accurate analysis, and several authors have used such extraction ratios (Brewer, 1965; Larsen and Widdowson, 1971; Thompson *et al.*, 1979; Seth and Pandey, 1983; Tracy *et al.*, 1984). Good relationships between readily soluble F in soil and F concentrations in plants have been demonstrated by some authors, where low ratios of soil:water or soil:0.01M $CaCl_2$ have been used (Singh *et al.*, 1979a and 1980; Keerthisinghe *et al.*, 1991a). This suggests that readily soluble F is a good indicator of plant available F. However, data correlating readily soluble F in

soil with uptake of F by plants roots are limited, and F concentrations in plants have been shown by other authors (Cooke *et al.*, 1976a; Braen and Weinstein, 1985) not to correlate with readily soluble F in soil.

The results of Cooke *et al.* (1976a) were obtained from plants which were grown on fluorspar mine waste in an unmonitored atmosphere and which could have been subjected to varying levels of airborne F (gaseous or particulate). Cooke *et al.* (1976a) suggested that plant uptake may also have been affected by some other factors in this unusual environment. *e.g.* the high concentrations of lead and zinc in the mine waste might have had some effect. These results should therefore be considered with caution.

Soil solution F could also be considered a measure of readily soluble F. In four acid soils (pH = 3.7 - 4.9), Elkhatib *et al.* (1987) found soil solution concentrations of F ranging between approximately 3 to 35 mg F dm^{-3}. However, little data exist on the relationship between the concentration of F in the soil solution and the uptake of F by plants. The concentration of F in the soil solution can be lower than that of water extractable F, which could indicate that these two indices measure two pools of F within the soil system (Haidouti, 1991).

Larsen and Widdowson (1971) speculated that the uptake of F by plants would not be controlled by the concentration of readily soluble F alone. Fluoride, like phosphate and some other anions, is to a large extent adsorbed by soil material and its uptake must be governed by the amount of F that is able to rapidly replenish the soil solution when the latter is depleted (labile or potentially readily soluble F). A number of methods to determined labile F have been employed (Hall, 1968; Larsen and Widdowson, 1971; Braen and Weinstein, 1985). Braen and Weinstein (1985) related soil ready soluble F (0.01 M $CaCl_2$) and labile F (resin-extractable as described by Larsen and

Widdowson, 1971) to F content of orchard grass and red maple and found readily soluble F a better predictor of foliar F values than was labile F. Yet, Davison *et al.* (1985) found evidence of correlations (r not given) between resin-extractable F and F concentrations of ryegrass.

Differences in correlations for both soil soluble and labile F with foliar F suggest that there are other factors which affect F uptake that these extraction methods fail to take into account. There is little evidence relating the above measures (readily soluble, labile (potentially readily soluble) and soil solution F) to uptake of F by plants. The extraction of F by any given solvent is, at best, a method of estimating available F (Murray, 1981b; Ares, 1978). At present these measures appear to be unsubstantiated estimates of plant available F and further studies in this area are required to determine the best method for estimating plant available F in soil. Further work is needed to characterise the chemical nature of both fractions (labile and readily soluble) of F in the soil and how they relate to the absorption of F by plant roots (Braen and Weinstein, 1985; Wenzel and Blum, 1992; Barrow and Ellis, 1986).

2.7.1.4 The effects of soil nutrient status on fluoride uptake by plants

Concentrations of other anions and cations in the soil can affect the sorption and solubility of F. Soils high in Ca encourage precipitation of CaF_2, thus decreasing the availability of F to the plant (Chhabra *et al.* , 1979; Morshina and Fanaskova, 1985; Pickering, 1985). The effect would be similar for soils high in magnesium. The results of Street and Elwali (1983) revealed that liming F-contaminated acid sandy soils (pH 5.5 - 7.0) may decrease F in the soil solution by precipitation of CaF_2. Yet, Elrashidi and Lindsay (1986a) concluded that it is unlikely that CaF_2 controls F uptake in either slightly acid or neutral soils as other factors such as clay, organic matter and Al oxides exert the major influence on adsorption of F in these soils (Section 2.5.4).

Soils with high concentrations of SO_4^{-2} can affect F solubility and plant availability. Bar-Yosef and Lindsay (1986) explained the differences in F concentrations in leachates from columns, when F was added as CaF_2 or phosphogypsum, as being due to the presence of SO_4^{-2} in the phosphogypsum. The SO_4^{-2} led to formation of $CaSO_4^0$, which decreased the Ca activity and allowed a higher F concentration in the leachate. Therefore, high concentrations of SO_4^{-2} in calcareous soils may decrease F retention, increasing F leaching or availability of F to the plant.

Other anions which compete with F for adsorption sites may also affect the solubility and plant availability of F. Larsen and Widdowson (1971) suggested that phosphate could displace F from the solid phase. In agreement with Larsen and Widdowson (1971), Singh *et al.* (1979a) showed that with increased P (KH_2PO_4) application, the water extractable F in soils increased. Bar-Yosef and Lindsay (1986) considered that the higher concentrations of F in soil solutions where P had been applied as superphosphate, compared with no application of P, was due to the P competing with F for adsorption sites on the soil. Although the work of Bar-Yosef and Lindsay (1986) did not account for the addition of F as a contaminant in the superphosphate, they suggested that higher concentrations of P added to the soil increased F in soil solution due to P-F competition on common adsorption sites of the soil. Phosphorus, F and Si have been shown to compete for adsorption sites (Chien, 1980; Fey and Jenkins, 1980).

It is clear from the above that, by complexation or competition for absorption sites, plant nutrients can affect the solubility of F in soils and hence the plant availability of F.

2.7.1.5 The effects of ionic species of fluoride exposed to the root on plant uptake of fluoride

Much research has investigated the phytotoxic affects of total F concentrations in soil, solution culture and air (Section 2.9.1.1). Although the airborne ionic forms of F which are absorbed and phytotoxic are known (Section 2.4.1), there is little information on the forms of soil F which are taken up by the plant root and those which are toxic to the plant. In the soil solution, F can be present as the free F ion (F^-) or form soluble complexes with Al, Si, B and H. Complexes with H would exist in significant concentrations only in solutions from acidic soils, at solution pH values less than 4.0 (dissociation constant of HF = 3.17, Table 2.1) and where Al concentrations in solution are low as F has a strong affinity for Al (Lindsay, 1979).

Solution culture experiments, conducted under conditions where F would be present as F^- (pH = 5.5), have shown good correlations ($r^2 = 0.99$) with total F in solution and F taken up by plants (Bar-Yosef and Rosenberg, 1988). However, solution culture studies by Takmaz-Nisancioglu and Davison (1988) led them to hypothesis that the F ion itself is not readily taken up due to exclusion from uptake sites. Their research found that F present as AlF complexes was more readily taken up and translocated to the shoots than the F ion, due to more favourable charge of these AlF species. This observation is contrary to the findings of MacLean *et al.* (1992) who found that the concentration of F in shoots of plants was lower where Al was present with F in solution cultures, compared with plants grown with F in the absence of Al.

The presence of fluorosilicates in the soil solution would be uncommon, as simple computer modelling by Sikora *et al.* (1992) found that SiF_6^{2-} completely dissociates above pH 4 to H_4SiO_4 and F^- at total F concentrations < 1.0 mM. The model that Sikora *et al.* (1992) used contained Si and F only. If Al (which has a strong affinity for F) was also present in solution, lower pH and

high F concentrations would still not form SiF_6^{2-} (Allison *et al.*, 1991). However, addition of fluoroborates or fluorosilicates to solution culture has been found to increase the F concentration of plant shoots (Collet, 1969). The work of Collet (1969) did not indicate if the pH of the solution cultures was monitored. The addition of boric acid, ammonium fluorosilicate or potassium fluoroborate would decrease the pH of the solution. Decreases in pH could lead to the formation of HF. The cell permeability for HF is six orders of magnitude higher than for F^- (Gutknecht and Walter, 1981). Therefore, the formation of HF would be expected to affect the uptake and phytotoxicity of F.

There are limited and inconclusive data on the species of F which are taken up by the plant root and more research is required to identify these species and the mechanisms involved. This topic will be addressed in this thesis.

2.7.2 *Plant factors affecting fluoride uptake from soil*

The plant root has many important beneficial functions; anchoring the plant, synthesis of growth regulators, water absorption, metabolising photosynthate for root growth, and absorption of nutrients. Nutrients can also be absorbed in small amounts through the leaves. The leaves and roots are also efficient at absorbing non-essential elements (*e.g.* F), some of which can be detrimental to plant growth.

Gaseous or particulate F can be absorbed by the plant through the stomata of the leaf and to a much lesser extent throught the cuticle of the leaf (Chamel and Garrec, 1977; Weinstein and Alscher-Herman, 1982). However, uptake of F by plants from the soil is predominantly by absorption of F in the soil solution through the root. This section will discuss F uptake by the plant root with respect to the morphology of the root, related mechanisms of F absorption (using

current nutrient absorption models) and root kinetics. It is not intended to be definitive review of ion uptake by plants (the topic has been covered extensively by others, *e.g.* Hewitt and Smith, 1974; Kolek and Kozinka, 1992; Walker and Pitman, 1976), but to highlight briefly the theories and mechanisms which could explain uptake of F by plants.

Three types of ion-influx kinetics have been recognised:

1. passive ion movement of nutrients into the plant, which is independent on respiration energy,
2. passive ion uptake along an electrochemical gradient dependent on respiration energy, and
3. active ion uptake against an electrochemical gradient, requiring respiration energy (Barber, 1984).

Active uptake of ions is against a concentration gradient, requires energy and is selective. Uptake generally increases as solution concentration increases, however, a maximum rate is reached at higher concentrations. Carrier-mediated uptake is hypothesised as the mechanism for active uptake. Current theory suggests that ATP or molecules connected to ATP act as carriers and are located in the plasma membrane. These carriers combine with an ion on the outside of the membrane, transport the ion through the plasma membrane, then release the ion into the cytoplasm. Respiration energy is required for operating the carrier.

According to Hodges (1973) anions are actively transported across the plasma membrane into the cytoplasm. Rao (1977) speculated that uptake of F was an active process. However, in 1965 Venkateswarlu *et al.* had shown that processes requiring the expenditure of energy are required for absorption of chloride but not F, suggesting that F is not taken up actively. Data of Cooke *et*

al. (1978) and Garrec and Letuorneur (1981) have also shown that F uptake by the root is a passive diffusion process. Bosaormenyi and Cseh (1961) found F uptake was less than other halogens (I, B and Cl) at equal concentrations, suggesting that F is taken up by different mechanisms from other anions.

The free F ion is the most eletronegative of the halogens (Cotton *et al.,* 1987) and uptake may be influenced by the charge on the apoplasm. A portion of solution in the apoplasm, the Donnan free space (DFS) is affected by negatively charged sites (probably due to the carboxyl groups on the pectic cell wall matrix and another portion, the water-free space (WFS) is unaffected by negatively charged sites (Barber, 1984). The AFS (apparent free space = DFS and WFS) of the root represents 10 to 15% of the root volume. The negative charge of the DFS could lead to the exclusion of F^- from potential sites of F uptake.

Restrictions of the DFS on passive uptake of anions could be overcome by decreasing the negative charge in the apoplasm or by changing the charge of F^-. There are two mechanisms which could accomplish this.

1. The presence of divalent and trivalent cations would decrease the effective negative charge in the apoplasm because they dissociate much less than from exchange sites within the apoplasmic pathway (Barber, 1984). This effect would be greater for dicotyledons, the roots of which tend to have a greater cation exchange capacity than those of monocotyledons (Bowling, 1976).

2. Complexation of F^- with cations in solution could form neutral or positively charged complexes.

Overcoming exclusion of anions due to the repulsive charges of the DFS would result in a greater concentrations of the F ions being present in the apoplasm, which would in turn increase the concentrations of the F ion at the plasma membrane of the epidermis and cortex, which should favour F ion influx through passive ion movement. However, it is still uncertain if the cation exchange capacity has any role to play in the process of ion uptake, or if it is just an unimportant consequence of cell wall structure (Bowling 1976; Barber, 1984).

Membranes may contain pores with sizes similar to those of ions and therefore the movement inorganic ions would be related note only to their charge, but to their ionic radius and state of hydration (Hewitt and Smith, 1974). The F^- ion has a small ionic radii (1.36 Å), and complexation of F^- with other ions would affect the charge and size of the ion and may alter the uptake of F by plants. However, there are no data available on ionic radii of hydrated F complexes.

2.7.3 *Normal fluoride concentration in plants*

Concentrations of F in plants grown in soil free from anthropogenic F contamination (background concentrations) normally range from 0.1 to 15 mg F kg^{-1} (Cholak, 1959; Cooke *et al.*, 1976a; Hara *et al.*, 1977; Whitford, 1989). However, some species (*e.g. Dichapetalum, Thea, Gastrolobium, Camellia, Oxylobium, Acacia* and *Palicoure*) accumulate high concentrations of F (Vickery and Vickery, 1976; Jacobson *et al.* 1966; Weinstein and Alscher-Herman, 1982). Table 2.5 gives a summary of the range of F concentrations found in plants grown in areas considered to be free from F contamination.

2.8 Measurement of fluoride in plant material

Great care must be taken in preparing plant material for analysis of F to distinguish between internal and external F. The plants must be washed to remove external F, dried, and the F in the plant must then be brought into solution. Because of the volatility of some F compounds and strong complexation of F by metallic cations, bringing plant F into solution for total F analysis is still a tedious task, which the recent development of acid digestion techniques with sealed chambers promises to overcome. Once the F is in solution, it can be measured using the F ion selective electrode (F-ISE) in conjunction with a total ionic strength adjusting buffer (TISAB), an accepted and relatively interference-free method. Developments in analysis of F have been extensively reviewed by Cooke *et al.*, (1976b), Wang and Xu (1993) and Wang and Zhou (1994).

Table 2.5 Concentrations of fluoride in plant materials grown in soils unpolluted by fluoride.

Plant species	Plant part	F concentration in plant (mg F kg^{-1} dry weight)	Reference
Avena sativa	grain	6.8	von Gericke and von
	straw	14.8	Kurmies, 1955 (cited by
	root	36.8	Kumpulainen and
Triticum aestivum	grain	1.4	Koivistoinen, 1977)
	straw	1.8	"
Solanum tuberosum	tuber	0.25	"
	sprout	10.9	"
Daucus carota	root	0.21	"
	sprout	7.5	"
Avena sativa	shoot	40	Garber, 1968
Medicago sativa	shoot	10 - 20	Hansen *et al.*, 1958
Acer pseudoplatanus	shoot	18	Cooke *et al.*, 1976a
Lotus corniculatus	shoot	15	"
Dactylis glomerata	shoot	4.6	"
Camellia sinensis	shoot	67 - 3062	Zimmerman *et al.*, 1957

2.8.1 *Preparation of plant material for fluoride analysis.*

Because of the need to distinguish between internal and external F when analysing plant tissue, a washing procedure is required to remove all F external to the plant without removing internal F. Cooke *et al.* (1976a) evaluated several washing techniques for plants grown in soil, examining material after washing for surface particles by light or scanning electron microscopy to confirm the removal of surface particles. These authors found that use of either 0.2 % (v/v) 'Teepol' or 0.1 % (v/v) 'Teepol'/0.1 % (w/v) ethylene diaminetetra-acetic acid (EDTA) followed by four rinses in deionised water proved satisfactory in removing all surface particles. Samples were agitated and brushed for one minute in each solution. It was found necessary to wash root samples 2-3 times. Washing with de-ionised water alone or with 0.05-0.1 % (v/v) 'Teepol' proved unsatisfactory due to incomplete removal of surface particulates, while 0.3 M HCl caused leaching of internal F. Ares (1978) washed samples of airborne contaminated foliage by rinsing three times in 1 dm^3 of distilled water during 1 min. Murray (1981a) washed leaves in 0.5 % tetra sodium EDTA and 'Alconox' for 30 s, then rinsed them in distilled deionised water for a further 30 s. Both authors found these washing procedures satisfactory for their requirements. However, neither give justification for use of these washing procedure. Both procedures would be expected to remove a large proportion of the external F on plants.

Drying temperature is a potential source of error when analysing plant material for F. If drying temperatures are too high (>100°C) there may be loss of volatile F from samples (Hall, 1968). Although undried samples have been used to determine F concentrations (results expressed on a dry weight basis), samples are usually dried between 60-80 °C before being analysed (Cooke *et al.*, 1976b; Davison *et al.*, 1973; Ares, 1978; Murray, 1981a).

2.8.2 *Digestion of plant material for fluoride analysis*

There are a several methods commonly used to digest plant samples to release inorganically and organically bound F into solution in a form (F^-) that can be measured easily. Many of these methods have been reviewed (Cooke *et al.*, 1976b) and found to be subject to incomplete recovery of inorganic F due to losses of volatile F and overestimation of F due to contamination during the digestion procedure (Hall, 1968). Keerthisinghe *et al.* (1991b) compared several of these methods and found they gave incomplete recovery due to the use of fusion mixtures or acids inefficient at releasing F from silicates. Two methods which are commonly used at present are NaOH fusion and acid digestion.

2.8.2.1 Dissolution of plant fluoride by alkaline fusion.

Alkaline fusion of samples with sodium carbonate was used by Willard and Winter (1933) for releasing F from materials when perchloric acid would not decompose the samples. Variations of this fusion technique have been developed over the decades and sodium hydroxide is now commonly used to fuse soil and plant materials, releasing all bound F for analysis (Baker, 1972; McQuaker and Gurney, 1977).

2.8.2.2 Dissolution of plant fluoride by acid digestion

A number of acid extractants have been utilised by various researchers to release F from plant material. Cooke *et al.* (1976a) compared a nitric/perchloric mix (4:1 v/v) and a 0.5 M sulphuric acid extraction procedure with the ashing and fusion methods used by Hall (1968). Both methods were found to give satisfactory recovery of F from most species when compared with those of Hall (1968). However, Van Den Heede *et al.* (1975) tested four methods and established that the

two methods which used HNO_3 for digestion either did not completely free all F from the organic material (room temperature digest), or lost F due to the elevated temperatures used in the digestion (heated digest).

Keerthisinghe *et al.* (1991b) compared several digestion techniques (five ashing and fusion methods, an acid digestion method, and a oxygen flask combustion method) and the AOAC (1978) extraction method (975.04) with an acid digestion method proposed by themselves for the determination of low concentrations of F in plant material. Their method, which involved digesting 0.05 g of plant sample with 1.0 cm^3 of concentrated nitric within a sealed teflon chamber enclosed in stainless steel, proved superior to the others tested. The superiority of the method was attributed to the complete dissolution of F in the sample and the elimination of losses of F through volatilisation. The speed and relative ease of the method, compared with fusion methods, makes it an attractive technique for determination of total F in plant material.

However, due to the lack of a reference material with certified F concentration, this method is still to be verified. Verification of this procedure will be addressed in this thesis.

2.8.3 *Measurement of fluoride in solution*

Before the introduction of the F-ISE, analysis of alkaline-fused plant solutions for F required distillation of the fusion solution to remove interfering radicals before the F concentration in the final solution was determined titrimeterically or spectrophotometrically.

2.8.3.1 Colorimetric and titrimetric methods for measurement of fluoride in solution

Once F is in solution and separated from other interfering radicals by steam distillation from acid, F can be determined by titrating with standard thorium nitrate, using a zirconium-alizarin mixture

as an indicator (Willard and Winter, 1933). This method is still used to compare the effectiveness of new methods and is approved by the Association of Official Analytical Chemists as a Final Action Method (Jacobson and Weinstein, 1977; Helrich, 1990). However, this procedure is time-consuming and requires a great deal of apparatus.

2.8.3.2 Ion selective electrode methods for measurement of fluoride in solution

Fluoride must be in the F^- form to be detected by the electrode. The response of the F ion selective electrode (F-ISE) conforms to the Nernst equation (Equation 2.2, where E = measured electrode potential, E_0 = reference potential which is dependent on ionic background (constant), A = fluoride ion activity in solution, and S = Nernst slope (-58.5 ± 2.0 mV at 22°C))

$$E = E_0 + S \log(A) \qquad \text{(Equation 2.2)}$$

The F-ISE (Orion, 1991), used in conjunction with a buffer to adjust total ionic strength (TISAB) and to complex interfering radicals, allows selective measurement of F^- in a multi-element solution with few known interferences. Therefore, sample preparation can be simplified by removing the time consuming distillation steps involved in removing known interfering radicals from the solution prior to F analysis. The electrode is reported to have an approximate tenfold selectivity for F^- over OH^- and at least a thousand-fold selectivity for F^- over Cl^-, Br^-, I^-, HCO_3^-, NO_3^-, SO_4^{2-} and HPO_4^{2-} (Frant and Ross, 1966; Rechnitz, 1967).

2.8.3.3 Total ionic strength adjusting buffers (TISAB)

The TISAB performs three functions.

1. It fixes the ionic strength of the analyte: the ionic strength between samples and

standards must be equal so that measurements of activity by the electrode can be converted to concentrations.

2. It buffers the solution in a range which avoids OH^- interference and the complexation of F^- with H^+.
3. It complexes cations which may complex F^-, for example Al and Fe.

As outlined by Moore and Ritchie (1988), there are three TISABs in common use, TISAB I, III and IV. These TISABs contain citrate (0.001 M), CDTA (cyclohexylene dinitrilo tetra-acetic acid) and tartrate respectively as their decomplexing agents. Moore and Ritchie (1988) found TISAB IV gave the best recoveries of F when Al was present in solution. However, Davey *et al.* (1992) showed citrate (0.1 M) released more F bound to Al than did tartrate. Yet, 1.0 M sodium citrate gave poorer recovery than 0.1 M. This highlights the need to test the reagents and the concentrations used in TISABs thoroughly under the conditions in which they will be used.

2.9 Toxic concentrations of fluoride

The biochemical and physiological mechanisms involved in the toxicity of F are not clear. It is thought that F is an effective inhibitor of enzymes and its Ca precipitating powers interfere with membrane permeability (Suttie, 1977). Weinstein and Alscher-Herman (1982) suggest that once inside the plant cell, the toxic action of F^- is thought to be inactivation of metal ions (Ca, Mg, Mn and Zn) at their sites of physiological activity. Symptoms of F toxicity in vegetation include marginal or leaf tip chlorosis (yellowing) and/or necrosis (burning) and necrosis of the foliage (Woltz, 1964a and b; Rauch, 1983; Haidouti *et al.*, 1993). Symptoms of toxicity in cattle and sheep include dental and skeletal lesions (fluorosis), which lead to lameness and general stiffness, and appetite impairment (reviewed by Suttie, 1977; Samal and Naik, 1992; Hubb *et al.*, 1993).

2.9.1 *Phytotoxic concentrations of fluoride*

Vegetation can accumulate F from airborne gases and particles, water or soil. The sensitivity of plant species to F toxicity varies greatly from species to species. Some genera, *e.g. Dichapetalum, Thea, Gastrolobium, Camellia, Oxylobium, Acacia* and *Palicoure* accumulate F (Vickery and Vickery, 1976) and show none of the symptoms of F toxicity with F concentrations up to 4000 mg F kg^{-1} dry weight (Jacobson *et al.*, 1966; Weinstein and Alscher-Herman, 1982). Other genera are non-accumulators of F, and have shown signs of F toxicity at much lower F concentrations in their tissues. For example, plants of *Gladiolus* spp. may become necrotic with 20 mg F kg^{-1} shoot dry weight (Jacobson *et al.* 1966). Normal concentrations of F in leaves of non-accumulating F plants range from 0.1 to 15 mg F kg^{-1} (Section 2.7.3). There are similar differences between species of animal with respect to F toxicity, both plant and animal toxicity to F are discussed below.

2.9.1.1 Phytotoxic concentrations of airborne fluoride

Uptake of F by plants and phytotoxicity of airborne F has been well documented and will be considered here only briefly. (See Smith and Hodges (1979) for a comprehensive review.) The phytotoxicity of airborne F is usually attributed to HF, SiF_4, and soluble particulate materials such as NaF and AlF complexes (National Research Council, 1971). The major mode of entry of airborne F is through stomatal pores and there is limited uptake through the cuticle (Chamel and Garrec, 1977; Weinstein and Alscher-Herman, 1982). Susceptible species can be injured at atmospheric F concentrations 10 to 1000 times lower than those of the other major pollutants, *viz.* less than 0.8 µg F m^{-3} (Weinstein and Alscher-Herman, 1982). Mitchell *et al.* (1981) suggested that the 3 month ambient average atmospheric concentrations should be below 0.54 µg F m^{-3} to

prevent injury to eucalyptus leaves. The phytotoxic affects of airborne F can be decreased when F forms relatively insoluble F salts in the leaf (MacLean *et al.*, 1976).

2.9.1.2 Phytotoxic concentrations of fluoride in solution and in plants

Restriction in plant growth as a result of increasing F in the soil can result from either its accumulation in toxic quantities or the effect of added F on the absorption or balance of other nutrient elements in the plant (Singh *et al.*, 1979b). Cabbage plants grown in solution culture have shown significant restrictions in dry weights when the F concentration in solution was greater than 2632 µM (Hara *et al.*, 1977). However, these researchers did not consider the effect of increased Na in their treatments or the precipitation of CaF_2 and MgF_2 from the high concentrations of F employed, and therefore these results must be considered with caution. Bar-Yosef and Lindsay (1986) found the threshold for total F in solution culture which caused a significant restriction in growth rate was between 50 and 260 µM for tomato and between 260 and 530 µM in maize plants. Singh *et al.* (1979a) found significant restrictions in the yield of wheat when water extractable F (Brewer, 1965) in the soil was 1160 µM. At this concentration in soil, the F concentration of mature straw was 35 mg kg^{-1}. The work of Hansen *et al.* (1958) with turnips and lucerne led them to the general conclusion that plant growth was restricted when the F concentration of the tissues exceeded 60 mg F kg^{-1} on a dry weight basis. However, this is dependent on the plant species (Vickery and Vickery, 1976). In polluted soils, where the concentrations of readily soluble F are high, plant growth is generally restricted and plant F concentrations (Table 2.6) are higher than in plants grown in soils which are not contaminated (Table 2.5).

In contrast to heavily polluted soils, the concentrations of F in agricultural soils are generally low. Fluoride is added to agricultural soils through two main sources, fertilisers and irrigation water. Fluoride concentrations in plants have been increased through F contamination in fertilisers and irrigation water (Woltz, 1964a; Kudzin and Pashova, 1970). However, there do not appear to be any data indicating phytotoxic effects.

Table 2.6 Concentrations of fluoride in plants grown in substrates containing enhanced fluoride concentrations.

Plant species	F concentration of medium	F concentration (mg F kg^{-1} dried tissue)		Fraction of control weights [A]	Reference
	Soils (mg F kg^{-1})				
Lolium perenne	153700 (fluorspar mine waste)	1705 3327	shoot root	NR [B]	Cooke *et al.*, (1976a)
Medicago sativa	1600 (added to the soil as NaF)	130	shoot	0	Hansen *et al.*, (1958)
	Solution cultures (µM)				
Prunus persica	21052	1442 1286	leaf stem	0 0	Leone *et al.*, (1948)
Lycopersicon esculentum		2179	leaf	0	
Fagopyrum spp		1910	shoot	0	
Helianthus annus	5263	59 507	shoot root	1.0	Cooke *et al.*, (1978)
Zea mays	2631	54 680	shoot root	0.36 0.63	Bar-Yosef and Rosenberg (1988)
L ycopersicon esculentum		126 1310	shoot root	0.32 0.52	
Brassica oleracea	13158	490 39500	shoot root	0.71	Hara *et al.*, (1977)

[A] 0 = dead [B] NR= not recorded

2.9.2 *Plant fluoride concentrations toxic to animals*

Estimations made by Suttie (1977) of the amount of air inhaled per day by a cow of average size, indicate that the amount of F that can be absorbed by this route is very small. This lead Suttie (1977) to postulate that the major source of F in the diet of livestock in areas of F pollution is F-contaminated vegetation. Suttie did not consider that many grazing animals ingest soil which could also contribute to dietary F.

Among domesticated animals, cattle are the most sensitive to fluorosis (a skeletal disorder resulting from ingestion of high amounts of F over a prolonged period) followed by sheep and pigs, and then poultry, which are comparatively tolerant (Rose and Marier, 1977). Davis (1980) and Suttie (1977) reviewed F tolerance levels for domestic animals and suggested the threshold was 30 mg F kg^{-1} for cattle (20 mg F kg^{-1} higher than the maximum concentration likely to occur in uncontaminated ryegrass herbage) and 70 mg F kg^{-1} for sheep.

It is likely that toxicity of F to animals depends not only the concentration of F in the diet, but also the ionic species of F which are present. Tsunoda *et al.*, (1985) found that NaF was absorbed more readily than CaF_2 by human males, and complexing of F with Al decreases the osteo-dental symptoms of F toxicity in sheep (Kessabi *et al.*, 1986). Beyer *et al.* (1987) also found differences in the toxicity of F fed to caterpillars depending on the source of F supplied.

Fluoride can be transformed by a number of plant species into the highly toxic compound monofluoroacetate. Sodium monofluoroacetate is well known as the rabbit poison, 1080 (Aplin, 1968). Approximately 75 mg sodium monofluoroacetate is sufficient to kill a 50 kg sheep (Aplin, 1968). Two well-known Australian species which synthesis monofluoroacetates are *Gastrolobium* ssp. (Aplin, 1968) and *Acacia georginae* F. M. Bailey (Oelrichs and McEwan,

1961), and other species include *Oxylobium Dichapetalum* and *Palicourea marcgravii* (see review by Weinstein, 1977). The function of monofluoroacetate in the plant is unknown. The assumption that its synthesis by the plant is a response to high concentrations of available inorganic F in soil or water (Preuss *et al.*, 1970) is not supported by the finding that some plants containing monofluoroacetate grow in soils with low F concentrations (Hall, 1972). It could be hypothesised that the synthesis of monofluoroacetate may be a mechanism of the plants for protection against grazing animals.

2.10 Non-phytotoxic effects of fluoride on soil

2.10.1 *The effects of fluoride on soil microorganisms*

Increased concentrations of F in soil can have adverse effects on soil microflora and fauna. Fluoride and fluorosilicates have been shown to possess valuable properties as agricultural poisons, particularly as insecticides and fungicides (Jacob and Reynolds, 1928). One of the most notable characteristics of F as a pollutant is its tendency to accumulate in organisms, making serious adverse effects possible even at low concentrations if the organisms are exposed over time (Groth III, 1974; Garrec and Plebin, 1984; Breimer *et al.*, 1989). At high concentrations (1500 - 3000 mg F kg^{-1}), there is evidence that F may modify microbial processes in the soil (*e.g.* inhibition of enzymes responsible for the fermentation of carbohydrate-rich organic matter) which could slow carbon cycling, and thus decrease the fertility of the soil (Ares, 1978; Saha *et al.*, 1981; Gaponyuk *et al.*, 1982).

Links between F concentrations in micro-organisms and changes in the soil processes for which these organisms are responsible have been discussed by others (*e.g.* Kremlenkova and Gaponyuk, 1984). Murray (1981b) found high F concentrations in slaters in F polluted soils and

hypothesised that this could point to possible alterations to the processes of litter breakdown in soils by the inhibition of the activities of some organisms responsible for decomposition of litter. The findings of Beyer *et al.* (1987) support the hypothesis of Murray (1981b), suggesting that the probable explanation for a higher fraction of fine particles in the litter (O horizon) near an Al reduction plant was that F was toxic to organisms responsible for completing the final stages of litter decomposition. Rao and Pal (1978) found a positive correlation between concentrations of F in soil and soil organic matter content at eight sites near an Al factory in India and inferred that F decreased the activity of soil microorganisms responsible for litter decomposition. Significant increases in the organic matter of soils were not found until total soil F concentrations were greater than approximately 1000 mg F kg^{-1}.

Some attention has been given to the toxic affects of F on soil fauna (Wilke, 1987 and 1989; Becker and Ottow, 1985). However, the concentrations of F applied by these authors were high (up to 3605 mg F kg^{-1}, water extractable) relative to reported concentrations of water soluble F (up to 192 mg F kg^{-1}, water extractable) at contaminated sites (Wenzel and Blum, 1992). Therefore these data would not represent conditions in most contaminated soils. The effect of F on decomposer organisms has largely been ignored in the work on air pollution and, to date, little is known of the effect of F on soil flora or fauna (Murray, 1981a; Vogel and Ottow, 1991).

2.10.2 *The effects of fluoride on soil structure*

Addition of F to soil has been shown to alter chemical and biological properties of soils (Sections 2.6 and 2.10.1) by altering solubility of cations and organic matter, and possibly by decreasing the activity of some organisms responsible for the decomposition of litter. Such changes in soils would be expected to be reflected in changes in soil structure.

The studies of Gaponyuk *et al.* (1982) on sod-podzolic and sierozem soils demonstrated that 3000 mg F kg^{-1}, added as NaF, increased 2 - 3 fold the content of microaggregates (< 0.01 mm) found in these soils. In many soils F may affect soil structure. However, as Gaponyuk *et al.* (1982) acknowledged, the increase in content of < 0.01 mm microaggregates was probably due to the accumulation of sodium in the adsorbing complex of the soil. The effect of Na was not evaluated.

Morshina (1980) concluded that the accumulation of F compounds may have a significant effect on soil productivity not only because of their toxic action but also because of the changes that may occur in the physiochemical properties of soil during adsorption, such as dissolution and increased leaching of organic matter and decreases in certain exchangeable cations (precipitation of CaF_2 and MgF_2).

Although there are little data available on the effects of F on soil structure, the combination of biological and physical changes outlined above (Section 2.10.1 and 2.10.2) could contribute to changes in soil structure which may be beneficial (increased organic matter) or detrimental (increased microaggregates) to soil structure. However, many of the effects outlined above have been observed after addition of high amounts of F and would relate only to soils heavily contaminated with F, and the impacts of the cation accompanying F additions have seldom been isolated from the effects of F.

2.10.3 *The effects of fluoride on plant nutrient availability*

Fluoride may influence plant nutrition either by encouraging leaching of nutrients, removing them from the rhizosphere, or by altering the strength of their retention making them either more or less soluble and therefore altering their availability to the plant. Little is known about the effect of F on the solubility of chemical species in F contaminated soils (Elrashidi and Lindsay, 1987).

Bar-Yosef and Lindsay (1986) found that batch extraction (1 week) of a variety of soils with 0, 0.002, 0.02 and 0.1 M NaF increased, by variable extents, the mobility of all elements investigated. The order of increased mobility of elements was (Al, Fe, Ca) > (Mg, K, Mn, P) > (Cu, Zn, B, Mo, Ba) > (Cd, Cr, Ni). The increases in element mobility were far more pronounced at the highest F concentration. Similar effects on the mobility of K, Ca and Mg were observed from both NaCl and NaF addition, indicating that the increase in the solubilities of these elements is not related to the F ion alone. The Na ion was probably also implicated, acting as a cation exchanger for K, Ca and Mg on soil adsorption sites, increasing their mobility.

In contrast to Bar-Yosef and Lindsay (1986), Peek and Volk (1986) found concentrations Ca and Mg in solution decreased with increased F additions to three soil types (batch extraction for 24 h, with 0.0025 M NaF). These authors suggest that this was possibly due to the removal of polymeric Al coatings from clay surfaces by F, exposing additional adsorption sites on the clay for the adsorption of Ca and Mg. The decrease could also be explained by precipitation of CaF_2 and MgF_2 complexes, both of which have low solubilities (Table 2.1). Obviously, the effects of F on solubility of other elements in dependent on the soil type and F concentration.

Fluoride application to soil has also been shown to increase the solubility of organic matter (Morshina, 1980; Peek and Volk, 1986; Polomski *et al.*, 1982b) (Section 2.6.4). Elements such as Cu, B, Zn, and Ni are known to form stable organic complexes (Bar-Yosef and Lindsay, 1986), which could explain the increase in the mobility of these elements due to F application. Subsequent leaching of elements may remove them from the rooting zone of the soil, making them inaccessible to plants.

The work of Dickman and Bray (1941) and Swenson *et al.* (1949) considered the role of F ion

in the release of adsorbed phosphate and found F will replace phosphate chemically bound to Al or Fe. However, phosphate was much more effective at replacing F than F replacing phosphate. Fluoride could increase the rate of phosphate immobilisation when hydroxyapatite reacts with F ions to form the more stable fluorapatite (Larsen and Widdowson, 1969). However, as Larsen and Widdowson suggest, it is unlikely that the small quantity of F in superphosphate would decrease phosphate reactivity to an agronomically important extent. The concentrations of F used by these authors were in the range 2 to 8% F in the soil. Such concentrations of F are rarely experienced in soils (Table 2.2 and 2.3).

Plant availability of F could also be affected by the strong negative F ligand creating a large positive nuclear charge on B (boron) atoms. Singh and Randhawa (1979) suggested that F shows an affinity for B and helps in its transportation in water, which could increase leaching of B and its availability to plants.

2.10.4 *Alleviation of aluminium toxicity with fluoride*

The use of F to ameliorate Al toxicity in plants has been considered by several researchers (Keerthisinghe *et al.*, 1991a; Alva *et al.*, 1988; Alva and Sumner, 1988; Moore and Ritchie, 1988; Oates and Caldwell, 1985). The amelioration of Al toxicity could result from a combination of two possible mechanisms. Firstly, F adsorbed to hydroxides of Al and Al polymers on mineral surfaces could exchange with and release OH groups (Bower and Hatcher, 1967) which could precipitate insoluble Al as Al hydroxides (Figure 2.2). Secondly, F forms soluble AlF complexes which are considered less toxic than Al^{3+}, $Al(OH)^{2+}$ and $Al(OH)_2^{+}$ (Takmaz-Nisancioglu and Davison, 1988; MacLean *et al.*, 1992; Keerthisinghe *et al.*, 1991a; Hue *et al.*, 1986; Alva *et al.*, 1986).

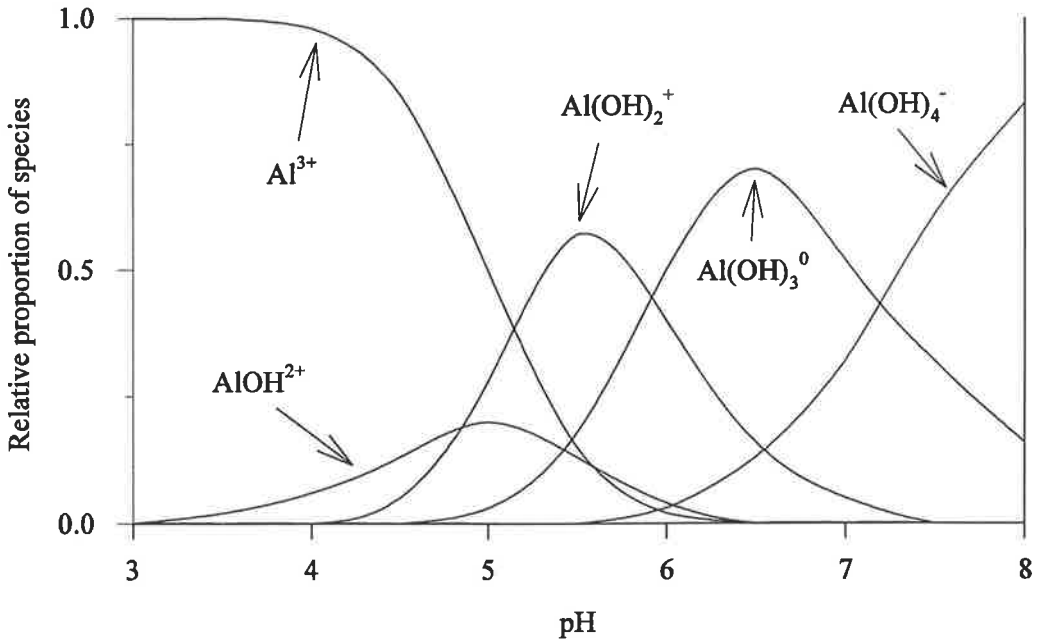


Figure 2.2 Hydroxylated species of aluminium in equilibrium with kaolinite (taken from Evans, 1988)

The use of F to alleviate Al toxicity has been encouraging. Several papers have assessed the use of phosphogypsum, an acidic by-product from the phosphate fertiliser industry (Alva et al., 1988; Alva and Sumner, 1989; Smith et al., 1994; Keerthisinghe et al., 1991a). Phosphogypsum contains high concentrations of F and SO_4, both of which can complex with Al thus decrease Al toxicity (Cameron et al., 1986). However, phosphogypsum may also contain other impurities (e.g. radionuclides) considered to be of environmental concern which may limit its application to agricultural soils (Rutherford et al., 1994). In contrast, Sikora et al. (1992) have suggested a possible role of F in promoting Al toxicity.

Other limitations to the use of F for alleviating Al toxicity in the soil have been highlighted by Shainberg *et al.* (1989), Keerthisinghe *et al.* (1991a) and Sikora *et al.* (1992). Keerthisinghe *et al.* (1991a) point out that the application of F to soils can increase F concentrations in plant material which may cause health problems for grazing livestock.

The effects on uptake of F by plants from application of F to acidic soils and of F complexing with Al are complex and at present not well defined. Further studies to determine the extent of any detrimental effects on plants or animals are required. The affects of Al and F on plants will be addressed in this thesis.

2.11 Summary

Some F compounds are readily soluble in water while others have sparing solubility. In the majority of soils a high percentage of F input is firmly retained by the soil. Retention of F by soils is favoured in acidic conditions, in soils of high clay content and having high concentrations of amorphous Al species.

Soil pH is the major variable controlling F sorption by soil and therefore F availability to plants. Sorption is greatest at pH 5.5-6.5. At lower pH, F adsorption declines due to preferential formation of AlF soluble species. At high pH, F adsorption declines due to the positive electrostatic potential of variable charge materials diminishing, raising the negative surface charge and resulting in a repulsion of negatively charged F. However, with high F concentrations in alkaline soils, retention of F is essentially governed by the presence of Ca, which precipitates soluble F out of solution as CaF_2.

The main non-anthropogenic sources of F in soils are the weathered products of rocks. Fluoride concentrations within soil will depend on atmospheric input, variation in soil parent material, the rate of F translocation through the soil profile and the rate of biological cycling of F. The total F in normal mineral soils averages 150 to 360 mg F kg^{-1}, but it can reach up to 7070 mg F kg^{-1}.

Fluoride has been found (Groth III, 1974) to fit most of the criteria for potentially important pollutants set down by the Nation Academy of Sciences, Washington. For example:

1. fluoride is a widespread pollutant,
2. fluoride is non-biodegradable,
3. fluoride is accumulated by a great many organisms,
4. fluoride is an element of high biological activity, with well-established toxic effects on a great many organisms,
5. information on potential effects on populations in the field and on ecological balances is virtually non-existent, and
6. it is possible that F may be transformed by some organisms in the natural environment into far more toxic organic F.

Fluoride pollution originates from many sources: Al smelters; manufactures of Fe, bricks, fertilisers and glass; coal-fired power stations; irrigation water; and phosphorus fertilisers. Any industry which uses raw materials containing even small amounts of F can release enough gaseous (*e.g.* HF, SiF_4) and particulate fluorides (AlF_3, Na_3AlF_6, CaF_2) to enhance elemental concentrations in surrounding areas. Gaseous atmospheric F is considered to be far more toxic than particulate atmospheric F, as this F can be readily taken into the plant through the stomata. Many authors have studied the toxic affects of atmospheric F.

Atmospheric F may cause immediate phytotoxicity and is therefore of primary concern. However, through various channels the airborne F eventually enters the soil. Continual heavy application of F-containing fertilisers and disposal of F-contaminated wastes could also increase and maintain high concentrations of soluble F in soil solutions, thereby increasing uptake by the plant. Recent research, has indicated high concentrations of water soluble F (up to 192 mg F kg^{-1} soil) in soils surrounding Al reduction plants and in soil solutions (10 to 20 mg F dm^{-3}) from several other soils. These concentrations of F are equal or higher than that determined by Bar-Yosef and Rosenberg (1988) to cause a significant reduction in growth rate of tomato and maize plants in solution culture. Such concentrations could therefore contribute to elevated F concentrations and F toxicity in F sensitive vegetation. However, the knowledge of plant-soil-F interactions is limited, such that not only the contribution of F from the soil to the plant is unknown, but little is known on the ionic species of F which are taken up by the plant, and those which are toxic to the plant.

This review has identified a number of topics which require further study:

1. verification of sealed chamber acid digestion techniques for the release of F from plant material for analysis,
2. identification of the ionic species of F which are taken plant roots and translocated to shoots, and
3. the mechanisms which the plant uses to take up F.

Topics one and two will be addressed in this thesis, and topic three discussed in relation to the findings from topics one and two.

Chapter 3

3.0 Material and methods

3.1 Introduction

The previous chapter has identified that the knowledge of plant soil interactions involving F is limited. This Chapter describes the general methods used for the ensuing solution culture studies (Chapters 5 - 8). Solution cultures were designed to determine the effects of the speciation of F in solution on uptake of F by the plant root and its toxicity to the plant.

3.2 Solution cultures

3.2.1 *Selection of plant species*

A range of plant species were selected for preliminary experiments. The plants selected included species known to be F-sensitive (toxic response when less than 500 μM F exposed to roots) and F-tolerant (toxic response when greater than 500 μM F exposed to roots) (Table 3.1). A toxic response is considered to be a significant decrease in plant dry weight compared with control plants.

Oat and tomato plants were selected for use in solution culture studies to allow comparisons between:

1. this study and previous studies,
2. plants which are monocotelydons and dicotyledons, and
3. a sensitive and tolerant plant species.

Preliminary experiments showed that oats and tomatoes were easy to establish and grow in solution cultures.

Table 3.1 Sensitivity of plant species to fluoride

Plant Species	Sensitivity [A]	Reference
Avena sativa (oats)	Greater uptake of F than in canola (probably tolerant)	Singh (1990)
Brassica napus (canola)	Less uptake of F than oats (probably tolerant)	Singh (1990)
Phaseolus vulgaris (bean)	Resistant to F and Al	Takmaz-Nisancioglu and Davison (1988)
Lolium multiflorum, Lam cv. S22 (italian ryegrass)	F tolerant	Davis (1980)
Lolium perenne L. cv. S23 (perennial ryegrass)	F tolerant	Cooke *et al.* (1976a)
Fagopyrum spp. (buckwheat)	F tolerant	Leone *et al.* (1948)
Lycopersicon esculentum cv. Grosse lisse (tomato)	F sensitive	Bar-Yosef and Rosenberg (1988)
Zea mays cv. Sweet corn	F sensitive	Bar-Yosef and Rosenberg (1988)
Eucalyptus punctata (grey gum)	F sensitive	Ivinskis and Murray (1984)
Eucalyptus fibrosa (iron bark)	F tolerant	Ivinskis and Murray (1984)

[A] In some cases there were insufficient data to determine if the plants were sensitive (toxic response < 500 μM F) or tolerant (toxic response >500 μM F) to F in solution.

3.2.2 *Nutrient solutions*

Initial nutrient concentrations (μM) in aerated solution cultures were similar to those of Blamey *et al.* (1990): 500 Ca, 512 S, 587 N (533 NO_3^-, 54 NH_4^+), 100 Mg, 117 Na, 350 K, 112 Cl, 6 B, 5 Fe, 1.0 Zn, 0.2 Cu, 1.0 Mn, 0.05 Mo, 25 P. Stock solutions (4.350 cm^3) were added to 8 dm^3 of deionised water before the solution was made to 8.7 dm^3 with deionised water. The B, Zn, Cu, Mn and Mo were mixed together as a micronutient stock solution. All other compounds were

added to pots as separate stock solutions (Table 3.2). Micronutrients (mixed micro-nutrients and Fe-EDTA) were added again on day 8 and solutions renewed on day 12. Plants were harvested on day 15 (oats) and day 16 (tomatoes).

3.2.3 *Plant germination and growth*

All experiments were conducted in growth cabinets at 25 ± 1°C with 12 h day and night periods. Light irradiance was 230 ± 35 μmol s^{-1} m^{-2}. Tomato and oat plants were germinated in the dark at 20°C on paper cloth dampened with deionised water. When the radicle length of tomato and oat plants were between 2 and 3 cm, 6 and 10 plants respectively were planted in plastic support baskets, made by lodging common woven shade cloth between two disposable plastic cups (7 cm diameter), from which the bases had been removed.

The design of the pots and support baskets was such that when the basket were placed in the 10 dm^3 pots containing 8.7 dm^3 of nutrient, the top of the nutrient solution was within 2 mm of the mesh bottoms of the pots. All support baskets contained approximately 25 cm^3 of white polyethylene beads to hold the stems of the plants upright (Plate 3.1). For 3 days after planting, clear plastic petri dishes were placed over baskets to prevent desiccation of the seedlings.

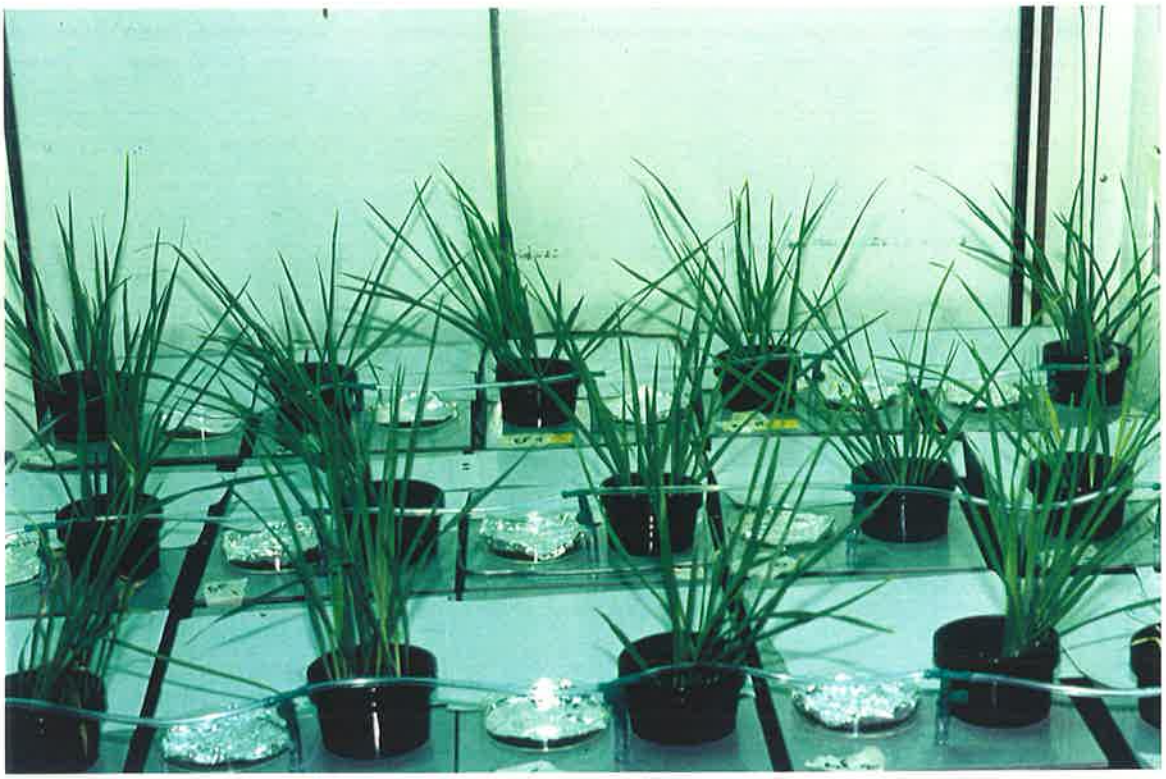


Plate 3.1 Solution culture system in a growth cabinet

Table 3.2 Salts required to make 500 cm^3 stock nutrient solutions.

Macro Nutrients		Micro Nutrients	
Salt	Weight (g) for 500 cm^3 stock solution	Salt	Weight (g) for 500 cm^3 stock solution
$CaSO_4.2H_2O$	0.389g/pot[A]	H_3BO_3	0.371
$Ca(NO_3)_2.4H_2O$	56.650	$ZnSO_4.7H_2O$	0.286
NH_4NO_3	4.800	$CuSO_4.5H_2O$	0.050
$MgSO_4.7H_2O$	24.650	$MnSO_4.H_2O$	0.168
NaCl	6.530	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.009
K_2SO_4	26.150	$C_{10}H_{12}O_8N_2FeNa.H_2O$	1.835
$K_2HPO_4.3H_2O$	5.706		

[A] - added directly to pot as salt.

3.3 Monitoring of solution cultures

3.3.1 *Monitoring of pH, micronutrients and macronutrients*

Samples (20 cm^3) were taken from each solution culture pot after addition of the treatments (day 4), before and after renewal of solutions (day 12) and at harvest (oats, day 15; tomatoes, day 16). Samples were stored in capped plastic tubes at room temperature, before analysis. The elemental composition of the samples was determined using inductively coupled plasma atomic emission spectrometry (ICP-AES) for B, Ca, Fe, K, Mg, Na, S, Zn and P, and a Waters capillary ion analyser (CIA) with a dichromate electrolyte for Cl, NO_3 and SO_4 (Millipore, 1993), unless stated otherwise.

The pH was monitored daily using a Hani Piccolo stick pH electrode (accuracy ± 0.02) or an Ingold electode attached to an Activon pH/mV meter (Model 101). No significant difference was found between results obtained from these two instruments.

3.3.2 *Measurement of total fluoride in solution culture*

The F concentrations in all solution cultures were determined by adding 0.50 cm^3 of TISAB III to 5.00 cm^3 of sample or standand, unless otherwise stated. The mV potential of these stirred samples or standards were determined using an Orion 720a pH/ISE meter and a double junction F-ISE (Orion model 96-09), unless otherwise stated. All measurement were conducted in a constant temperature room (20°C) and samples were insulated from the stirring plate with 1 cm thick polystyrene foam sheet to prevent heat transfer from stirrer to sample. The electrode was rinsed with deionised water, soaked in deionised water for 60 s, rinsed again, and blotted dry before the next measurement. The pH/ISE meter was linked to a computer which allowed a reading to be taken when the change in mV was less than 0.2 mV min^{-1}.

Standard curves were constructed from a 1000 mg F dm^{-3} stock solution made from NaF (AR grade, dried at 105^0C for 2 h). All F concentrations were calculated by direct calibration from the mV potentials of the standard curve (Orion, 1991). The standard curve was calibrated down to 0.1 mg F dm^{-3}. The 5 mg F dm^{-3} standard was checked for drift every 10 samples and standards were recalibrated if mV potential drift was greater than 1.4 mV, representing a 5 % change in F concentration.

3.4 Ionic species of fluoride in solution

Modelling of ionic activites in solution was accomplished using either GEOCHEM-PC (Parker *et al.*, 1987) or MINTEQA2 (Allison *et al.*, 1991). All calculations were completed using the standard data bases issued with these programs. Thermodynamic equilibrium constants considered important for each program, when calculating the speciation of F in solution, were compared with other sources of thermodynamic constants (Table 3.3). For all calculations the computed net charge of the solution at equilibrium represented an error or less than 3 % of the total charge of cationic species in solution, unless stated otherwise.

3.5 Harvesting of plants

During harvest, plants were photographed before roots were rinsed 3 times for 1 min each in 8.7 dm^3 of deionised water. Roots and shoots were separated, dried at 70 ℃ for 2 days and dry weights recorded. Samples were ground (< 1.0 mm) in a stainless steel mill and stored in sealed plastic containers at room temperature prior to analyses.

Table 3.3 Comparison of thermodynamic stability constants

Species	GEOCHEM-PC version 2	MINTEQ2A	Lindsay (1979)	Smith and Martell (1976)	Ball *et al.* (1980) Hem *et al.* (1973) [A]
	---------- Stability constant $\log_{10} K^0$, 25 C ----------				
HF	3.0	3.2	nr	3.2	3.2
AlF^{2+}	7.0	7.0	7.0	7.0	7.0
AlF_2^+	12.7	12.7	12.6	12.6	12.8
AlF_3	16.8	17.0	16.7	16.7	17.0
AlF_4^-	19.4	19.7	19.0	19.1	19.7
$Al(OH)^{2+}$	-5.0	-5.0	-5.0		-5.0
$Al(OH)_2^+$	-10.1	-10.1	-9.3		-10.0
$Al(OH)_3$	-16.8	-16.0	-15.0		
$Al(OH)_4^-$	-22.7	-23.0	-23.3		-23.0
$AlSO_4^+$	3.5	3.0	3.2		3.0
$AlPO_4$ (s)	19.1	nr	19.5		
$AlHPO_4^+$	19.8	nr	19.8		
$AlH_2PO_4^{2+}$	22.7	nr	22.7		
$Al(H_2PO_4)_2^+$	nr	nr	46.0		
$Al(H_2PO_4)_3$	nr	nr	68.0		
CaF_2 (s)	9.1	11.0		-10.41	
MgF_2 (s)	10.1	nr		-8.18	

[A] cited by Schecher and Driscoll (1987), s = solid, nr = not recorded

3.6 Analyses of plant material

Chapter 2 highlighted the current lack of a simple method for the rapid determination of F in plant material. In an attempt to develop such a method, several modifications to a method proposed by Keerthisinghe *et al.* (1991b) were investigated. A modification of this method allowed multi-element analyses of the same digest.

3.6.1 *Determination of F in plant material*

Verification and modification of the method proposed by Keerthisinghe *et al.* (1991b) for rapid deterimination of F in plant material will be discussed in Chapter 4. The method described in Section 4.2.1.5 was used to release F, from plant materials grown in solution cultures described in Chapters 5 - 8, into solution for analysis. Fluoride concentrations in these solutions were analysed as described in Section 4.2.

3.6.2 *Multi-element analyses of plant material*

Concentrations of Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn in the plant digests, obtained using the procedure described in Section 4.2.1.5 (Method ME_2), were measured with ICP-AES Zarcinas *et al.*, 1987).

Chapter 4

4.0 Determination of fluoride in plant material

4.1 Introduction

Before the introduction of the fluoride ion-selective electrode (F-ISE), the most widely used method for sample preparation (before analysis of the solutions for F) was alkali fusion followed by distillation to remove interfering radicals. Fluoride concentration in the final solution was determined by a spectrophotometric or a titrimetric method. These methods and others using the F-ISE have been reviewed (Cooke *et al.*, 1976b) and found to be subject to incomplete recovery of inorganic F due to losses of volatile F and overestimation of F due to contamination from furnace linings (Hall, 1968). Keerthisinghe *et al.* (1991b) compared several of these methods and found they gave incomplete recovery due to the use of fusion mixtures or acids inefficient at releasing F from silicates (Section 2.8.2.2).

The F-ISE (Orion, 1991), used in conjunction with a buffer to adjust total ionic strength (TISAB) and to complex interfering radicals, allows selective measurement of F in a multi-element solution with few known interferences. Therefore, sample preparation can be simplified by removing the time consuming distillation steps involved in removing known interfering radicals from the solution prior to F analysis. The work of Keerthisinghe *et al.* (1991b), with sealed chamber digestion vessels combined with nitric acid rather than alkaline fusion (McQuaker and Gurney, 1977) to release F from organic and inorganic compounds, promised to simplify sample preparation further and to overcome problems of contamination and incomplete recovery of F. Standard reference material (SRM) with a certified concentration of F has only recently become available and allows verification of the acid digestion procedure proposed by Keerthisinghe *et*

al. (1991b).

The objectives of the work reported in this Chapter were:

1. to validate the acid digestion procedure of Keerthisinghe *et al.* (1991b) using the SRM,
2. to compare this method with a similar method which uses microwave energy rather than convection energy in the digestion procedure, and
3. to develop and further simplify one of these methods so that it can be used for routine analysis of F and several other elements in plant material.

4.2 Materials and Methods

4.2.1 *Digestion of plant material*

Three major methods and two modifications of these methods were used to release F from the plant material prior to analysis:

1. the method described by Keerthisinghe *et al.* (1991b) and a modification of this method,
2. a microwave digestion procedure and a modification of the microwave digestion method, to allow F and multi-element analyses of the single digest, and
3. a NaOH fusion procedure.

4.2.1.1 Plant materials

Nine types of plant material were analysed.

1. Timothy (*Phleum pratense* L.): NIST SRM 2695h (National Institute of Standards and Technology Standard Reference Material, No. 2695, high), contaminated by atmospheric fallout from a nearby aluminium smelter.
2. A mixture of pasture species, supplied for an inter-laboratory comparison (Turner K

and Anderson G, pers. comm.).

3. Tea leaves (*Camellia sinensis* L.).
4. Subterranean clover (*Trifolium subterraneum* L.) grown in NaF contaminated soil, as used by Keerthisinghe *et al.* (1991b).
5. Maize (*Zea mays* L.)
6. Italian ryegrass (*Lolium multiforum* L.)
7. Tomatoes (*Lycopersicum esuculentum* cv. Floridade)
8. Peach leaves (*Prunus persica*): NIST SRM 1547
9. Potato tubers (*Solanum tuberosum*): a composite sample of mixed cultivars

The maize, Italian ryegrass and tomatoes were grown in solution cultures containing high concentrations of F.

All plant material, except for timothy, peach and the mixed pasture species (sampled as recommended by certifying organisations), were dried at 75°C for at least 48 h before analysis. All plant F concentrations are expressed on a dry weight basis. All analyses were carried out in triplicate, unless otherwise stated.

4.2.1.2 Release of fluoride from plant material by alkaline fusion (AF release)

The NaOH fusion was carried out as described by McQuaker and Gurney (1977), with the following modifications. Approximately 200 mg of plant sample was weighed accurately into a platinum crucible. Samples were fused with 5 cm^3 of 16 M NaOH over a bunsen burner for 10 min, and the fusion cake was dissolved and transferred with deionised water to a 50 cm^3 volumetric flask.

4.2.1.3 Release of fluoride from plant material by acid digestion and convection energy (CE release)

Keerthisinghe *et al.* (1991b): standard method (Method CE_1)

The digestion vessel consisted of a teflon (polytetrafluorethylene) bucket (21.0 cm^3) with a teflon lid tightly secured inside a stainless steel outer vessel with screw cap, as decribed by Keerthisinghe *et al.* (1991b). Plant material (0.050 g) was weighed into the vessel and 1.00 cm^3 16 M HNO_3 was added. The vessel was sealed and placed in an oven at 120°C for 6 h. The vessel was cooled before it was opened and the digest quantitatively transferred to a 5 cm^3 volumetric flask with deionised water and a 2.00 cm^3 aliqot was taken for analysis (Section 4.2.2.2).

Keerthisinghe *et al.* (1991b): modified method (Method CE_2)

A teflon vessel of similar design and volume (31.0 cm^3) to that of Keerthisinghe *et al.* (1991b) was used for sample preparation. It was sealed with a teflon lid. An aluminium sleeve and lid encompassed the teflon vessel and lid, which was clamped together with a stainless steel holder capable of sealing 6 vessels. Preliminary experiments found repeatability was improved using this sealing technique. Samples (0.100 g) were weighed directly into the teflon vessels and 1.00 cm^3 16 M HNO_3 was added to each. The vessels were sealed and placed in a convection oven at 120°C for 6 h. Modifications of the method consisted of variation in acid type, time, temperature and weight of material digested (See Section 4.2.7). The digestion vessels were cooled, opened and 20.00 cm^3 of 1.31 M trisodium citrate and 1.00 cm^3 of deionised water were dispensed directly into each vessel. Activity of F in the solution was either determined directly in the teflon

digestion vessel, or the solution was transferred to 80 cm^3 plastic vials which were sealed for storage at room temperature prior to analyses. There were no significant differences between F concentrations in samples measured directly or after storage. The ionic strength of the final mixture was designed to match that of the final mixture analysed by Method CE_1.

4.2.1.4 Release of fluoride from plant material by acid digestion and microwave energy (Method ME_1)

A laboratory microwave digestion system (Milestone mls 1200 Mega) with tetrafluormethaxil (TFM) vessels was used for sample preparation. Samples (0.100 g) were weighed directly into the 88.0 cm^3 TFM vessels to which was added either:

1. 1.00 cm^3 of 16 M HNO_3, or
2. 0.5 cm^3 16 M HNO_3 and 0.5 cm^3 18.7 M H_2SO_4, or
3. 0.86 cm^3 16 M HNO_3 and 0.22 cm^3 10.2 M HCl.

To prevent charring, 1.00 cm^3 deionised water was added to all samples and vessels were sealed with TFM lids. After a 15 min standing time, the samples were digested using the following program, 3 min at 250 watts, 30 s at 0 watts, 5 min at 300 watts, 30 s at 0 watts, 5 min at 600 watts and 1 min ventilation time. The digests were cooled under running water for 15 min before the chambers were opened and 20.00 cm^3 of 1.31 M trisodium citrate added. The final solutions were transferred to 80 cm^3 vials which were sealed for storage at room temperature prior to analyses.

4.2.1.5 Release of fluoride and nutrients from plant material by acid digestion and microwave energy (Method ME_2)

This method was a modification of the ME_1 method described above (Section 4.2.1.4), which allowed sufficient plant sample to be digested for analysis of F and multi-element analyses of plant material by ICP-AES (Section 3.6.2).

Samples (0.200 g) were weighed directly into the 88.0 cm^3 TFM vessels after which 1.50 cm^3 of 16 M HNO_3 was added. To prevent charring, 1.00 cm^3 deionised water was added to all samples and vessels were sealed with TFM lids. Digestion times were as described in Section 4.2.1.4. The digests were cooled under running water for 15 min before the chambers were opened. Condensation on the lids was washed into the digestion vessels and the digest was quantitatively tranferred with deionised water into 10 cm^3 marked glass mini digest tubes. Tubes were made to the mark with deionised water and mixed. A 5.00 cm^3 aliquot was added to 15 cm^3 of 1.31 M tri-sodium citrate solution.

The remaining digest solution was tranferred to plastic ICP sample-tubes, sealed and stored (4 °C) for analyses by ICP-AES. The verification of this method for multi-element analysis of plant material is described in Section 4.2.3.

For all acid digestion methods, digestion vessels were cleaned between digests by either washing them with deionised water before soaking them in 6 M HCl overnight or washing for 15 min in a 2% 'Decon' solution. No difference was found between these washing methods. Digestion methods were then rinsed four times with deionised water before drying.

4.2.2 *Measurement of fluoride in plant digests*

Fluoride concentrations in stirred solutions were determined with an Orion 720a pH/ISE meter and a double junction F-ISE (Orion model 96-09) as described in Section 3.3.2. The electrode was rinsed with deionised water, soaked in deionised water for 60 s, or 3 min where concentrations were below 0.05 mg F dm^{-3}, rinsed again, and blotted dry before the next measurement. Trisodium citrate was used to match the ionic strengths of the standards to those of the samples, as it is essential that both standards and samples have equivalent ionic strengths if the F-ISE measurement are to conform to the Nernst equation (Equation 4.1, where E = measured electrode potential, E_0 = reference potential which is dependent on ionic background (constant), A = fluoride ion activity in solution, and S = Nernst slope (-58.5 ± 2.0 mV at 22°C)). All F concentrations were calculated by direct calibration from a standard curve (Orion, 1991).

$$E=E_0+Slog(A) \qquad \text{(Equation 4.1)}$$

4.2.2.1 Measurement of plant fluoride released by alkaline fusion digestion

Analysis of F was as described by McQuaker and Gurney (1977) except that a five point standard curve was constructed, and the ionic strengths of the standards and samples matched.

4.2.2.2 Measurement of plant fluoride released by acid and convection or microwave energy

Standard solutions for Method CE_1 were prepared by diluting 1.00 cm^3 16 M HNO_3 and 1.00 cm^3 of standard with deionised water in a 5 cm^3 volumetric flask. Electrode potential was determined on 2.00 cm^3 of the sample or standard mixture with 8.00 cm^3 of 1.5 M tri-sodium citrate

(Keerthisinghe *et al.*, 1991b). Vickery and Vickery (1976) suggested that tri-sodium citrate meets the criteria for use as a TISAB (Section 2.8.3.3).

Standard solutions for Methods CE_2 and ME_1 were treated as follows to account for the effect of pH change on F-ISE potential (See Section 4.3.2). Solutions contained 1.00 cm^3 standard solution, 20.0 cm^3 tri-sodium citrate (1.31 M) and sufficient concentrated HNO_3 to match acidity in plant digests (0.615 cm^3, final solution pH = 5.40 ± 0.05). Where acids other than concentrated HNO_3 were used, equal volumes of these acids (H_2SO_4, $HClO_4$ or HCl) were added to standards and samples, and the pH of the standards were adjusted with HNO_3 to equal (± 0.05) those of the samples.

To account for the effect of pH change on F-ISE potential and the different volumes used to allow simultaneous multi-element analysis of plant material (Section 4.3.2), the standards for Method ME_2 were treated as follows. Solutions contained 2.50 cm^3 of standard, 1.94 cm^3 of deionised water, 0.56 cm^3 of 16 M HNO_3 and 15 cm^3 of 1.31 M tri-sodium citrate.

The F-ISE potentials of the above standard solutions were determined and plotted against log [F]. The F-ISE potentials of the samples (Sections 4.2.1.3 and 4.2.1.4) were measured and the concentrations of F calculated directly from the appropriate standard curve. The slopes of the standard curves were always within the specified range of 58.5 ± 2 mV. Calibration curves for methods CE_1, CE_2 and ME_1 were linear down to 0.10 mg F dm^{-3}. For method ME_2, which had a lower ionic strength, calibration curves were linear down to concentrations of 0.06 mg F dm^{-3}. Although standards were reproducible below these concentrations, a time greater than 30 minutes was required for:

1. washing the electrode (to ensure samples were not poisoned from previous samples),

and

2. obtaining a stable electrode potential.

Standard concentrations below 0.06 mg F dm^{-3} were therefore not used, thus setting the detection limit for F in plant material at 10 mg F kg^{-1}.

4.2.3 *Comparison of methods*

Samples of tea, timothy and clover were digested and analysed with Methods CE_1, CE_2, ME_1 and NaOH fusion to compare plant F concentrations. The tea standard and tomatoes grown in solution culture were digested and analysed with Methods ME_1 and ME_2 to compare F concentrations determined by these methods. To determine the accuracy and repeatability of the multi-element analyses of plant material with Method ME_2, peach leaves and potato tubers were digested with this method and the results compared with means obtained with a method used routinely in the laboratories CSIRO Division of Soils (McLaughlin *et al.*, 1994; McLaughlin, 1995).

4.2.4 *The effect of pH and and electical conductivity on F-ISE potential*

The pH of tea samples digested and analysed for F with Method A_1 were measured to determine the consumption of acid during the digestion process. The effect of pH on mV potential was determined by adding five volumes of concentrated HNO_3 (0.6, 0.7, 0.8, 0.9, 1.0 cm^3) and 1.00 cm^3 of the 1 or 10 mg F dm^{-3} standards to 5 cm^3 volumetric flasks. The solutions were diluted to 5 cm^3 with deionised water and 2.00 cm^3 of these solutions were added to 8.00 cm^3 of 1.5 M tri-sodium citrate. The final F concentrations in solutions were 0.4 mg dm^{-3} and 0.04 mg dm^{-3}. The mV potential (double junction F selective electrode (Orion 96-09)), pH (Orion 720a meter with ingold electrode) and electrical conductivity (EC) (Radiometer CDM 83 conductivity meter and

glass electrode) of the solutions were determined.

The effects of varying pH at constant EC, and varying EC at constant pH, on mV potential of the F-ISE were examined at two concentrations of F in solution (0.43 and 4.26 mg F dm^{-3}), under conditions similiar to method CE_2. Volumes of NaCl, HNO_3, trisodium citrate and deionised water used are listed in Tables 4.1 and 4.2. All mV potentals were determined with a double junction F selective electrode (Orion 96-09). To determine if changes in electrode potential at varying pH were artifacts of the double junction electrode, separate solutions with constant ionic strengths were prepared at one F concentration (4.26 mg F dm^{-3}). The mV potentials of these solutions were determined with a single junction F selective electrode (Orion 94-09) and reference electrode (Orion 90-01). The pH and EC of these solutions were measured as described for the double junction electrode.

Table 4.1 Volumes of NaCl, HNO_3, trisodium citrate and water added to determine effects of altered pH on F-ISE potential. Electrical conductivity was constant at 71.5 dS m^{-1}.

HNO_3 16 M (cm^3)	NaCl 4.8 M (cm^3)	Na-Citrate 1.31 M (cm^3)	Deionised H_2O (cm^3)	NaF Standard (cm^3)
0.90	0.00	20.0	1.57	1.00
0.50	0.82	20.0	1.14	1.00
0.30	1.25	20.0	0.91	1.00
0.10	1.56	20.0	0.80	1.00

4.2.5 *Recovery of added fluoride*

Recovery of inorganic F was measured following digestion (Methods CE_2 and ME_1) of 0.010 or 0.050 cm^3 aliquots of 1000 mg F dm^{-3} stock solutions of NaF. A separate set of solutions remained sealed at room temperature as controls.

Table 4.2. Volumes of NH_3, HNO_3, trisodium citrate and water added to determine effects of altered electrical conductivity on F-ISE potential. The pH was constant at 5.36.

HNO_3 16 M (cm^3)	NH_3 13.4 M (cm^3)	Na-Citrate 1.31 M (cm^3)	Deionised H_2O (cm^3)	NaF Standard (cm^3)
0.60	0.00	20.00	2.10	1.00
1.00	0.80	20.00	0.90	1.00
1.40	1.30	20.00	0.00	1.00

4.2.6 *The effect of boron on the solubility of fluorides in plants*

Under high temperatures and high concentrations of F and B, insoluble fluoroborate complexes may form in the acid digestion of plant material (Topchiev, 1959). The following experiment was conducted to determine if, under the conditions of acid digestion, concentrations of F and B in plant material were sufficient to form the very stable fluoroborate (BF_4^-) complex.

Using digestion method ME_1, 2.0 cm^{-3} of 8 M HNO_3 with 0, 230 or 4630 µM B were digested with 263 or 2630 µM F. The solutions were analysed to determine concentrations of F and results were expressed as a percentage of the total F added.

4.2.7 *Method modifications*

Different digestion parameters were altered to monitor their effects on F recovery with Method CE_2. Treatments involved testing acid mixtures, (0.8 cm^3 16 M HNO_3 and 0.2 cm^3 11.6 M $HClO_4$, 0.5 cm^3 16 M HNO_3 and 0.5 cm^3 18.7 M H_2SO_4, or 0.86 cm^3 16 M HNO_3 and 0.22 cm^3 10.2 M HCl), and a range of temperatures (20, 50, 75, 100, 120, 140, and 170°C), digestion times (1, 3, and 6 h) and sample weights (0.050, 0.100, 0.200 and 0.400 g).

4.2.8 *Mineralogy, and silica and fluoride concentration of plant materials and residues of plant digests.*

Plant material was analysed for total silica (Si) with X-ray fluorescence spectroscopy (XRF) (Norrish and Hutton, 1977). The mineralogy of the residue remaining after acid digestion was determined by X-ray diffraction (XRD). XRD patterns were recorded with a Philips PW 1800 microprocessor-controlled diffractometer using CoK$\propto$ radiation, variable divergences slit and graphite monochromator. Total F concentrations in the digest residues were determined by NaOH fusion (Sections 4.2.1.2 and 4.2.2.1).

4.3 Results

4.3.1 *Comparison of methods for analysis of fluoride in plant materials*

Fluoride concentrations in plant materials determined by the NaOH fusion method were generally significantly greater than those obtained by acid digestion procedures (Table 4.3). Fluoride concentrations determined by the NaOH fusion method for the SRM (timothy) matched the certified value, but F concentrations determined by all acid digestion procedures were two thirds or less of the certified value. Methods CE_2 and ME_1 gave similar results, but the F concentrations of timothy, clover and tea determined by these two methods were, on average, 20% lower than those obtained with Method CE_1.

A comparsion between Methods ME_1 and ME_2 for the determination of F concentrations in tea and tomatoes grown in solution cultures showed there were no significant differences between these two methods (Table 4.4).

Table 4.3 Fluoride concentrations of three plant materials determined by Methods CE_1, CE_2, ME_1 and NaOH fusion. Standard deviations are shown in parentheses, n = 3.

Method	Timothy (mg F kg^{-1})	Clover (mg F kg^{-1})	Tea (mg F kg^{-1})
CE_1	228 (8)	72.1 [A]	285 (4)
CE_2	173 (7)	60.0 (2)	229 (6)
ME_1	170 (9)	nd	227 (3)
NaOH fusion	284 (9)	83.8 (3)	274 (11)
Certified value[B]	277 ± 27	none	none

nd = not determined as insufficient sample,
[A] reported by Keerthisinghe (pers comm.),
[B] fusion and microdistillation from sulfuric acid followed by colorimetric alizarin measurement of F in solution

Table 4.4 Mean F concentration in tea and tomato material determined with Methods ME_1 and ME_2. Standard deviations shown in parenthesis, n = 3 (unless stated otherwise).

Plant material	Digestion method	
	ME_1 (mg F kg^{-1})	ME_2 (mg F kg^{-1})
Tea	227 (3)	225 (14)[A]
Tomato shoots	33 (4)	37 (9)
Tomato roots	260 (16)	284 (27)

[A] n = 48

4.3.2 *The effect of pH and and electical conductivity on F-ISE potential*

Theoretically, a change in electrode potential could arise from a change in the ionic strength of the solution (due to the pH change) affecting E_0 (Equation 4.1, Section 4.2.2; Section 4.4.4). However, the change in potential when pH was maintained constant and electrical conductivity varied from 65.4 to 85.1 dS m^{-1} was small; 2.17 and 0.03 mV for concentrations of 0.43 and 4.26

mg F dm^{-3} respectively (Figure 4.1).

This indicates that large changes in ionic strength at these molarities have negligible effects on the potential of the F-ISE. When ionic strength (measured as electrical conductivity) was kept constant and pH altered from 5.0 to 6.3, the change in potential was 7.9 and 6.8 mV for concentrations of 0.43 and 4.26 mg F dm^{-3} respectively (Figure 4.2).

Similar results to the double junction F-ISE were obtained using a F-ISE and single junction reference electrode (Figure 4.2). Blanks were always below the F-ISE detection limit (0.02mg F dm^{-3}) and the changes in electrode potential with pH were similar at both F concentrations. Samples were digested with 1.00 cm^3 of 16 M HNO_3 and standards were made with 1.00 cm^3 16 M HNO_3. When 20.0 cm^3 of 1.31 M trisodium citrate was dispensed into the digest or the standard, the pH of the final sample solutions were 5.41, yet the pH values of the standard solutions were 5.13. From the examination of pH effects on ISE potential for method CE_1, it is evident the decrease in acidity caused an increase in potential of up to 4.7 mV (Figure 4.3).

4.3.3 *Recovery of added fluoride*

Recoveries of inorganic F were approximately 100% for Methods CE_2 and ME_1 (Table 4.5).

Table 4.5 Recovery of inorganic F. Standard deviations shown in parenthesis, n = 3.

Compound	Amount	Method CE_2 (%)	Method ME_1 (%)
NaF	10 mg F dm^{-3}	106 (7)	101 (2)
NaF	50 mg F dm^{-3}	100 (7)	103 (1)

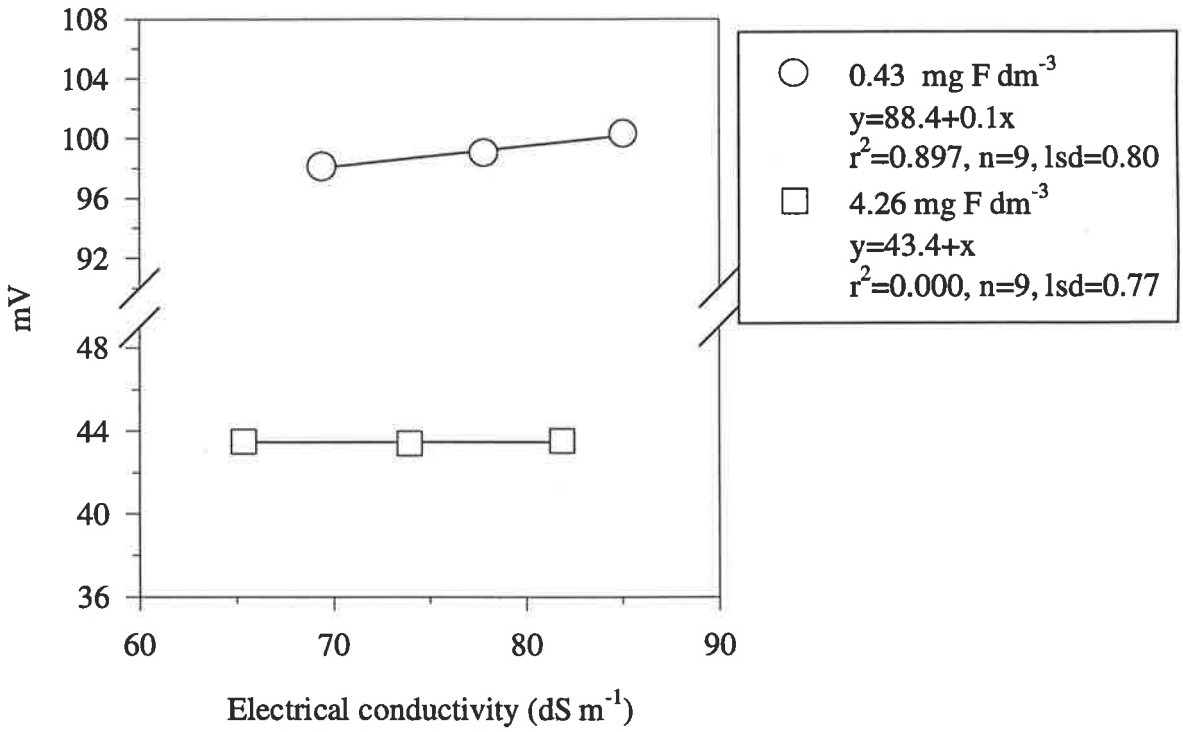


Figure 4.1 Effect of ionic strength (electrical conductivity) on F-ISE (ion selective electrode) potential with Method CE_2, pH constant (5.36). Points represent means, n = 3.

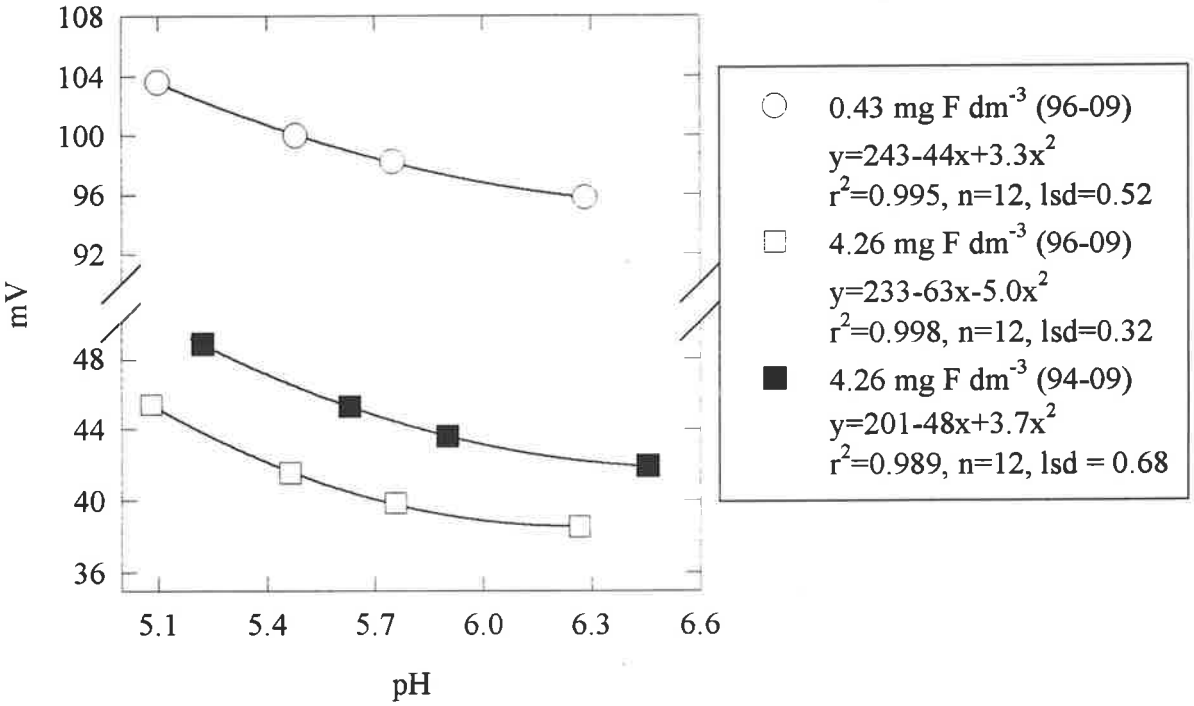


Figure 4.2 Effect of pH on F-ISE (ion selective electrode) potential with Method CE_2, ionic strength (electrical conductivity) constant (71 or 64 mS m^{-1}). 96-09 = double junction F-ISE. 94-09 = single junction F-ISE (solid symbols). Points represent means, n = 3.

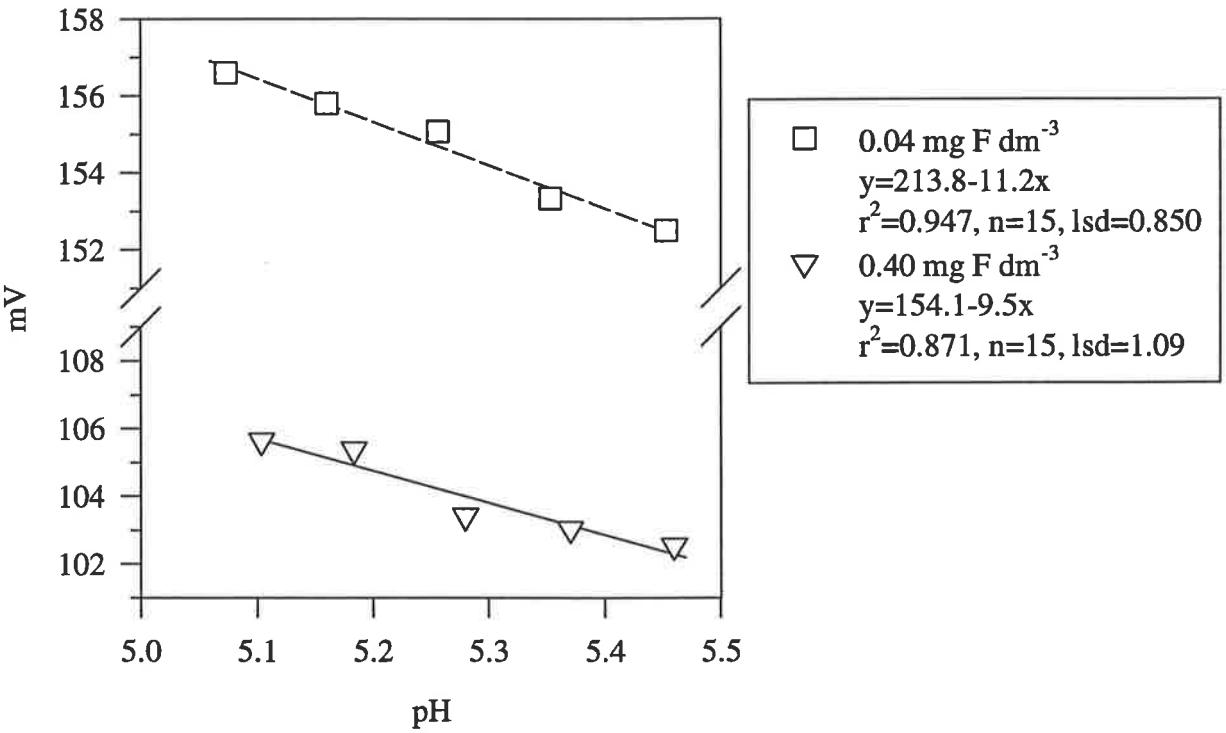


Figure 4.3 Effect of pH change on F-ISE potential with Method CE_1. Points represent means, n = 3.

4.3.4 *Method modifications for improving fluoride release from plant material*

Method CE_1 has been shown to overestimate F concentration of plant digests (see Section 4.4.2, Table 4.3). Altering digestion temperatures for Method CE_2 indicated that convection heat at 120°C releases the greatest proportion of F from the tea sample. Temperatures below 100°C decreased F release from plant material and temperatures greater than 120°C increased F recovery, far exceeding 100% of F concentrations of tea determined with AF release (data not shown). Digestion by microwave energy, rather than with convection energy, did not significantly improve release of F from any of the materials, except for ryegrass (Table 4.6).

Table 4.6 Fluoride concentration of plant materials digested with Method CE_2 and ME_1. Standard deviations are shown in parenthesis, n = 3.

Sample	Method CE_2 (Convection energy) mg F kg^{-1}	Method ME_1 (Microwave energy) mg F kg^{-1}
timothy	174 (7)	170 (9)
mixed species	47 (1)	45 (1)
maize root	116 (11)	128 (1)
maize shoot	11 (2)	13 (2)
ryegrass root	1100 (27)	1321 (26)
ryegrass shoot	293 (12)	364 (8)

Changes in the acid used for digestion (Method CE_2 and ME_1) had no significant effect ($p<0.05$) on the efficiency of F release from the timothy or tea material (Table 4.7).

Table 4.7 The effects of acid used for digestion on F content of timothy and Tea (Methods CE_2 and ME_1)

Method	Acid	Timothy (mg F kg^{-1})	Tea (mg F kg^{-1})
CE_2	HNO_3	174 (7)	230 (7)
CE_2	$HNO_3/HClO_4$	174 (6)	234 (4)
ME_1	HNO_3/H_2SO_4	183 (17)	nd
ME_1	HNO_3/HCl	175 (5)	248 (10)

nd = not determined

When digestion times were increased from 3 to 6 h (Method CE_2) there was no significant improvement in F release from plant material (data not shown). Changing sample weights did not significantly affect the determined F concentration of tea (Table 4.8). However, there was a significant decrease in measured F concentrations in timothy as the sample weight increased up to 400 mg. This decrease was not related to different final acidities of the tea and timothy digests as the pH of digests, for a particular sample weight, were identical and the pH of standards were matched for each sample weight.

Table 4.8 Effect of sample size on F concentrations obtained for timothy and tea with Method ME_2, digestion time = 6 h. Standard deviations shown in parenthesis, n = 3.

Sample	Timothy		Tea	
(g)	(mg F kg^{-1})	Analyte pH	(mg F kg^{-1})	Analyte pH
0.050	212 (7)	5.18 (0.01)	243 (12)	5.16 (0.02)
0.100	174 (6)	5.36 (0.05)	233 (5)	5.28 (0.02)
0.200	178 (3)	5.57 (0.03)	213 (3)	5.54 (0.02)
0.400	153 (5)	6.20 (0.07)	234 (6)	6.14 (0.02)

4.3.5 *The effect of boron concentrations in plants on the solubility of plant fluorides*

High concentrations of B in solutions had no significant effect on recovery of F added to digests (Figure 4.4).

4.3.6 *Mineralology, and silica and fluoride concentrations of plant material and residiues of plant digests*

Timothy and the mixed species (analysed by XRF) were found to be highly siliceous (8.4 and 9.6 g total Si kg^{-1}). Analysis by XRD of the solid residue of left after digestion with Method CE_2 indicated the residue of mixed species was predominantly quartz (intensity = 2500 cps), the residue after digestion of timothy was predominantly a mixture of quartz (intensity = 1300 cps) and feldspar (intensity = 960 cps). Timothy also contained a sharp peak corresponding to amphiboles (Figure 4.5). The residue left after digestion of timothy contained 83 mg F kg^{-1} (determined with the AF method).

4.3.7 *Comparison of methods for mult-element analyses of plant material*

Multi-element analyses of plant material (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn) were generally within 5 % of certified values or values obtained using the method of McLaughlin *et al.* (1994). Several of the elements considered of importance for solution culture studies are shown in Table 4.9. In the cases where differences between the results and the certified values were greater than 5 %, relative standard deviations from the means were usually less than 5 %.

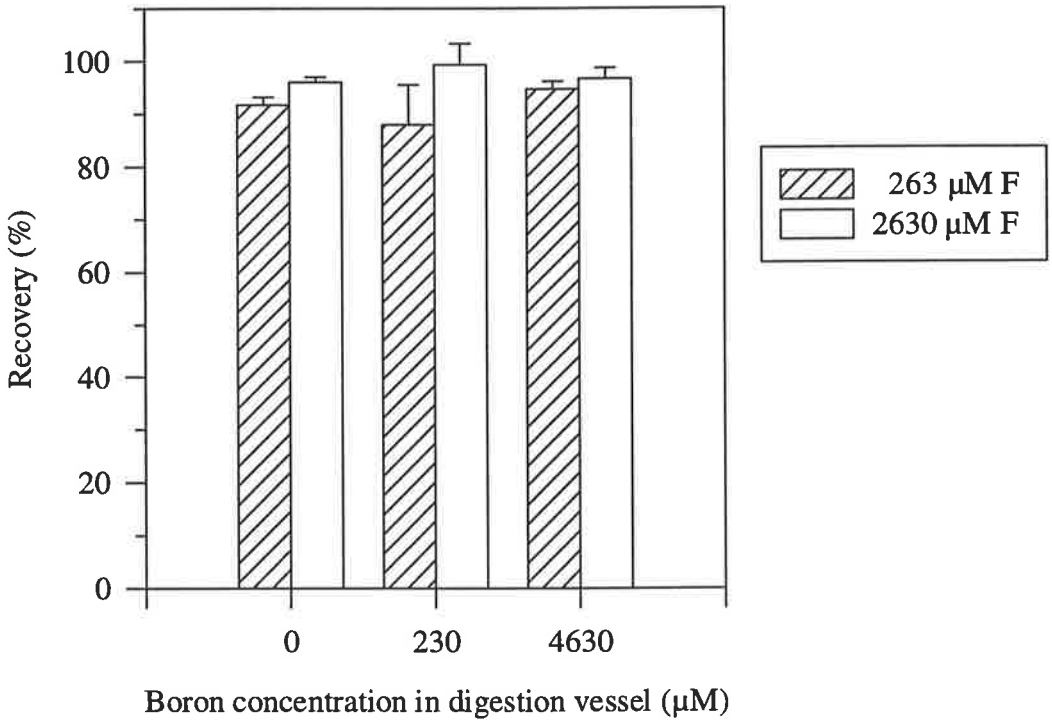


Figure 4.4 Effect of boron concentrations in digests (Method ME_1) on recovery of added F. Error bars represent one standard deviation from the mean, n = 3.

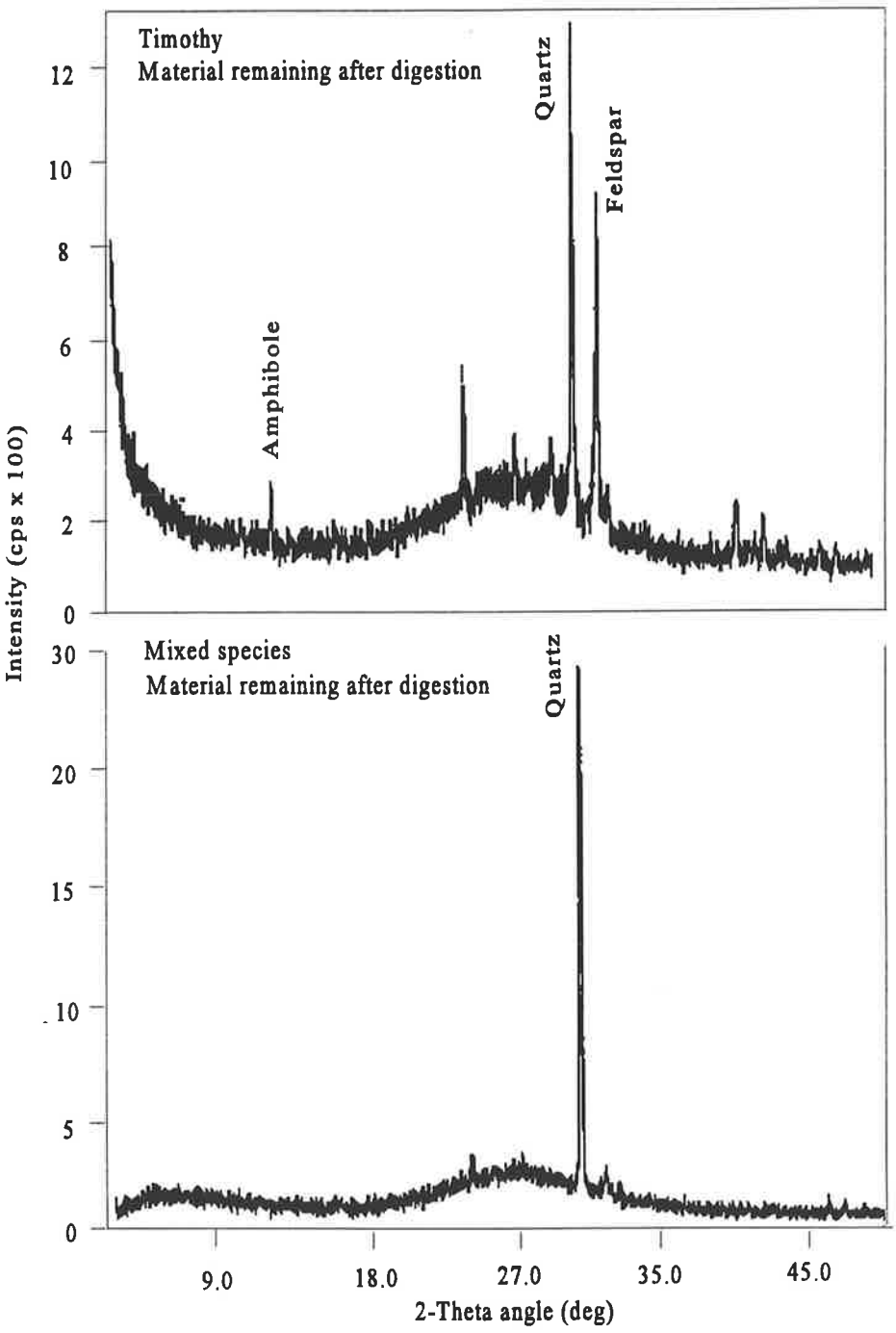


Figure 4.5 Results from XRD analysis of digest (Method CE_2) residues from samples of timothy and mixed species.

Table 4.9 Comparison of multi-element analyses of plant materials (Method ME_2) with certified values and a procedure used by McLaughlin *et al.* (1994). Standard deviation in parenthises, n=3.

Element	Peach leaf		Potato tuber	
	Method ME_2 (mg kg^{-1})	Certified value (mg kg^{-1})	Method ME_2 (mg kg^{-1})	McLaughlin *et al*, (1994) (mg kg^{-1})
Al	208 (9)	249	4.1 (0.5)	3
B	29 (1)	29	3.1 (0.4)	6
Ca	15705 (273)	15600	849 (35)	713
K	24522 (357)	24300	6533 (158)	6918
Mg	4174 (57)	4320	411 (2)	406

4.4 Discussion

4.4.1 *Comparison of methods for determination of fluoride in plant material*

The data in Table 4.3 show that either (a) there are F losses during all acid digestion procedures, or (b) acid digestion is not sufficient to release all of the F from plant material for analysis with the F-ISE. Furthermore, as Method CE_1 gave significantly higher F concentrations than Methods CE_2 and ME_1, then either method CE_1 was a more efficient procedure for releasing F from plant materials (unlikely given the similarity to Method CE_2), or there was some systematic error in F analyses.

Slight changes in the digestion procedure of ME_1 were made to allow multi-element analysis of the same digest used for F analysis (Method ME_2). Comparisons of the two methods (Table 4.4) showed no signicant difference between methods for analysis of F concentrations in tea and tomatoes. Higher variations were found with method ME_2, which was probably due to the extra steps involved in making the digests up to volume. However, relative standard deviation were still usually less than 10 %, indicating good repeatability.

4.4.2 *The effect of pH and and electical conductivity on F-ISE potential*

Similar changes in electrode potential with pH were found with single and double junction eletrodes (Figure 4.2), discounting the possibility of these results being an artifact of the double junction electrode. Fluoride contamination in the HNO_3 can be discounted as a source of error, as blanks were always below the F-ISE detection limit (0.02mg F dm^{-3}) and the changes in electrode potential were similar at both F concentrations in test solutions (0.43 and 4.26 mg F dm^{-3}). Effects of pH on F-ISE performance are usually related to a change in the relationship between free F^- ion activity and the total concentration of F in solution with decreasing solution pH, due to the formation of HF complexes. This effect becomes significant within one pH unit of the pK for HF (3.17, Table 3.3), so that it is recommended that total F concentrations in solution be determined with the F-ISE only above pH 5.0. However, the data indicate that pH affected electrode performance in conditions where HF complexes were unlikely to form. A possible reason for this effect is that the F-ISE relies on the potential of the reference electrode being the same in standard and sample solutions. Differences in activity may change the liquid junction potential of the reference electrode and contribute to the measured specific ion electrode potential.

The difference in F concentrations of plant materials obtained by Method CE_1, compared with Methods CE_2 and ME_1, can be explained by the consumption of acid through oxidation of organic matter during the digestion procedure. Samples were digested with 1.00 cm^3 of 16 M HNO_3 and standards were made with 1.00 cm^3 16 M HNO_3. When 20.0 cm^3 of 1.31 M trisodium citrate was dispensed into the digest or the standard, the pH of the final sample solutions were 5.41, yet the standards were pH 5.13. From the examination of pH effects on ISE potential for Method CE_1, it is evident the decrease in acidity can cause an increase in potential of up to 4.7 mV (Figure 4.3).

From Equation 4.1, such an increase in electrode potential equates to a 20% overestimation of the F concentration determined by Method CE_1 compared with Methods CE_2 and ME. The results obtained by the method suggested by Keerthisinghe *et al.* (1991b) would therefore be comparable to the results obtained by the other methods investigated by these authors, not significantly greater as suggested. A comparison of F concentrations in three plant materials (Table 4.3) shows that the average F concentration determined by Method CE_1 was 20% greater than those obtained with Methods CE_2 or ME, suggesting that the difference between these methods was due to incorrect measurement of F concentration in the digest solution.

Munns *et al.*(1992) found that activities F ion in soil extracts (soil pH ranging from 3.3 - 5.8) determined directly with a F-ISE were less than those calculated with GEOCHEM-PC. The brief description of the method for determination of F activity presented by Munns *et al.* (1992) does not provide sufficient information to determine if these differences are due to different sample and standard pH. However, the differences Munns *et al.* (1992) found between calculated and measured F ion activites could be explained if the pH of the standards were not adjusted. The differences in the pH of standards and samples would be the reverse of that found when using Method CE_1 . Standard pH would be much greater than sample pH, leading to an under-estimation of F activity in sample solutions, as found by Munns *et al.*(1992).

4.4.3 *Recovery of added fluoride*

Total recovery of inorganic F showed that there is no loss of volatile F using Methods CE_2 and ME_1, and hence the incomplete recovery from timothy (Standard reference material) was not due to loses of volatile F during the digestion procedure. Addition of organic material to inorganic F before digestion does not effect these recoveries (Tann, C., pers. comm.).

4.4.4 *Method modifications*

Method CE_1 has been shown to overestimate F concentration of plant materials (Table 4.3, Section 4.4.2). All attempts to improve the acid digestion procedures were unsuccessful. Altering digestion temperatures for Method CE_2 indicated that convection heat at 120°C (the temperature used by Keerthisinghe *et al.*, 1991b) releases the greatest proportion of F from the tea sample. Temperatures below 100°C decreased F recovery, probably due to incomplete release of F bound withing the plant material. At temperatures greater than 120°C recovery was greater than 100% due to released of F from the teflon (polytetrafluorethylene) digestion vessels. Release of F from the teflon at 140-170°C was unexpected, as teflon has excellent chemical resistance to oxidising acids and a working temperature up to 250°C (Windholz, 1983).

Digestion by ME, rather than with CE energy, did not significantly improve release of F from any of the materials, except for ryegrass (Table 4.6). However, the use of microwave energy is a quicker method for analyses, offering faster analyses.

Changes in the acid used for digestion (Table 4.7) did not improve the efficiency of F release from the timothy or tea material. Of the acids used, $HClO_4$ is considered a more powerful oxidising agent than HNO_3 (Zarcinas *et al.* 1987), H_2SO_4 has been reported to decompose resistant silicates in a sealed tube digestion method (Dolezal *et al.* 1968), and HCl is known to decompose a number of silicates (Bock, 1979). These finding suggests that a proportion of the F in these materials is bond strongly in some form which these acids cannot degrade.

Increasing digestion times from 3 to 6 h (Method CE_2) did not improve F release from plant material, indicating that longer digestion times will not increase the efficiency of this digestion

procedure to release F from plant material.

The significant decrease in measured F concentrations in timothy, as the sample weight increased up to 400 mg, was not found for tea (Table 4.8). The decrease in F released from timothy was not related to different final digest acidities between tea and timothy, suggesting that the way in which the F is bound within the plant material may differ between these two species. These data suggest that sample weights should be standardised for all materials to ensure sufficient acid is available to obtain optimal release of F from the range of plant materials being studied.

4.4.5 *The effect of boron concentrations in plants on the solubility of plant fluorides*

Assuming a 200 mg plant sample was digested, concentrations up to the equivalent of 250 mg kg^{-1} in dried plant material of F and B did not form stable fluoroborate complexes (BF_4^-) when digested using Method ME_1. Fluoroborate salts are formed under high (> 200 °C) temperatures with high concentrations of F and B and with loss of H_2O (Topchiev *et al.*, 1959). The temperature in the digestion vessel could form fluoroborate salts. However, fluoroborate salts are most unlikely to form under the conditions in the the sealed chamber digestion vessels because concentrations F and B are not excessive and there are no losses of H_2O from the sealed vessel.

4.4.6 *Mineralogy, and silica and fluoride concentration of plant material and residues of plant digests*

Fluoride release by acid digestion of the timothy could have been incomplete because F is bound tightly within silicates and cannot be released by these mineral acids. Both timothy and the mixed species were highly siliceous. However, for the mixed species, acid digestion gave comparable

results to fusion procedures (Anaqual report, unpublished data). This suggests that total Si concentration alone cannot explain incomplete recovery.

Analysis by XRD of the solid left after digestion from Method CE_2 found the residue of mixed species and timothy was predominantly quartz and/or feldspar both which are considered not to contain F or have F substituted in the lattice. Both samples would have been exposed to ambient contaminants such as dust. Timothy also contained a sharp peak corresponding to amphiboles. These common rock forming minerals have the general formula $X_2Y_5(SiAl)_8O_{22}(OH)_2$ (where X = Mg, Fe, Ca or Na and Y = Mg, Al, Fe^{2+}, and Fe^{3+}) and the OH group is highly substituted by F (Lapidus, 1990; Fleischer and Robinson, 1963). Confirmation that the residue contained F within silicate minerals was obtained by determining the F concentration in the residue by NaOH fusion. The residue left after digestion of timothy was found to contain the equivalent of 83 mg $F\ kg^{-1}$ plant material. This corresponds to the difference between the certified value and the F recovered by acid digestion.

It could be argued that mineral-bound F in plant material would not be biologically active in the short-term as it is extremely resistant to dissolution by mineral acids. Acid digestion procedures may therefore be acceptable for monitoring F concentrations in plants to indicate phytotoxicity or for assessing risks for herbivores ingesting F contaminated plant material. The sealed vessel acid digestion method tested here offers a simple, rapid method for F analyses and ensures complete recovery of acid-labile F without losses through volatilization.

4.4.7 *Comparison of methods for mult-element analysis of plant material.*

The digestion Method ME_2, designed to allow F and multi-element (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn) analyses of the same digest, showed good agreement with certified values for most elements. Results were similar to those obtained with a method used by McLaughlin *et al.* (1995) (Table 4.9). Both these findings, and the low relative standard deviation of concentrations of elements, showed that this method is accurate and repeatable, and is therefore suitable for use as a digestion procedure for F and multi-element analyses.

4.5 Conclusions

Sealed-vessel acid digestion methods which used microwave or convection heat (CE_2, ME_1 and ME_2) did not release all plant F for analysis by the F-ISE. The results of this investigation suggest that F bound strongly in some minerals found in plants cannot be released by acid digestion, leading to underestimation of the total F concentration in plants.

Differences in pH of samples and standards, due to acid consumption during the digestion process, results in an overestimation of F^- concentrations in digested plant material. Although trisodium citrate is a good complexing agent and maintains a high constant ionic strength, it is not sufficient to buffer pH when concentrated HNO_3 is added. The pH of the samples and standards must therefore be matched to within 0.05 pH units when acid digestion procedures are used to measure acid-labile F in plants.

Recognising the limitation to Method ME_2 as a measure of total F in plant material, this method was considered satisfactory for comparison of acid-labile F and concentrations of several other elements (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn) in plants grown in solution cultures (Chapters 5 - 8).

Chapter 5

5.0 Uptake of the free fluoride ion by plants

5.1 Introduction

In soil solutions of neutral to alkaline pH, F exists as the free F ion (F^-). At slightly acid pH (5.5 - 6.5) much of the F^- is adsorbed to the soil and at pH < 5.5 F^- is complexed with Al (Section 2.6.4.1). However, if high concentrations of F are added or solution pH is more alkaline, activities of F^- could increase in soil solutions and more F^- would be potentially available for uptake by the plant root. Physio-chemical properties of F^- which may influence F uptake by roots are its strong electronegativity (Section 2.2) and small ionic radius (Cotton *et al.*, 1987).

Solution culture experiments, conducted where F would be present as F^- (pH = 5.5), have provided good correlations ($r^2 = 0.99$) between total F concentrations in solution and F taken up by plants (Bar-Yosef and Rosenberg, 1988). Concentrations of total F in solution cultures above which plant growth is adversely affected by toxicity have been shown to vary considerable between plant species, ranging from between 50 - 260 µM for tomatoes to between 2532 - 13157 µM for cabbages (Hara *et al.*, 1977; Leone *et al.*, 1948; Bar-Yosef and Rosenberg, 1988). However, plant uptake and toxicity of F should be more dependent on the activity of F^- in solution rather than the total F present if F^- is the toxic agent.

The aims of this chapter were: a) to determine the effects of F^- activity in solution on uptake of F by the plant root and F toxicity to the plant, and b) to us GEOCHEM-PC to calculated F^- activities from published data to compare the uptake and toxicity of F^- from published data with the data in this Thesis.

5.2 Materials and methods

5.2.1 *Solution culture parameters*

To determine the effects of F^- activity in solution on uptake of F, tomato and oat plants were grown in full nutrient solutions (unless otherwise stated) containing the treatments described in Table 5.1. There were three replicates of all treatments except for the 53 - 841 µM NaF (inclusive) treatments in Experiment 5.1 which were single treatments.

Experiment 5.1 was designed to determine the effects of F^- activity in solution on plant uptake and toxicity of F. Experiment 5.2 was a control for Experiment 5.1, to determine if there were any effects of variable Na concentrations on plant dry weights. Experiment 5.3 repeated the work of Bar-Yosef and Rosenberg (1988), but with a different variety of tomato. Experiments 5.4 and 5.5 were designed to determine if other anions ($H_2PO_4^-$ and NO_3^-) competed with F^- for uptake. Solution culture parameters for all experiments (except Experiment 5.3) were as described in Section 3.2.2, with the following modifications.

In Experiments 5.4 (low P) and 5.5 (low P and N), no P was added to nutrient solutions on day 1 or 12. Phosphate stock solutions were added to solution cultures on days 5 and 8 to make final P concentrations in solutions up to 2.5 µM. In Experiment 5.5, no N was added to nutrient solutions on day 1 or new nutrient solution on day 12.

In Experiment 5.3, parameters were similar to those described by Bar-Yosef and Rosenberg (1988). Briefly, seeds were germinated in distilled water and 7 seedlings were grown to the second leaf stage in 1.0 dm^3 of aerated F-free quarter-strength Hoagland solution, with no changes of the solution. Seedlings were grown for a further 14 days in F-free solution with daily changes

of the solution. The plants were then thinned to 5 per pot, the F treatments were added and the plants grown for another 15 days. During the last 15 days, solutions were changed every 48 hours and pH, F, K, Ca, NO_3, P, S were monitored. Unlike Bar-Yosef and Rosenberg (1988), two plants from each container could not be sampled at 5 and 10 days from the addition of treatments because the root systems of the plants could not be separated. Therefore, five plants were grown for the full 15 days of the F treatments.

Plants were grown in growth cabinets under conditions as described in Section 3.2.3. The size of the growth cabinets limited this experiment to a control and F treatment, triplicated. The 530 μM KF treatment was chosen for two reasons: a) Bar-Yosef and Rosenberg (1988) found significant restrictions in growth rates compared with controls for this treatment, and b) effects from the formation of solid CaF_2 and MgF_2 (Section 5.3.2) on F uptake would be minimal in comparison with the highest F concentration (2630 μM) used by Bar-Yosef and Rosenberg (1988). Fluoride was added to the solutions as KF. Potassium chloride (KCl) was added as described by Bar-Yosef and Rosenberg (1988) to obtain equal K concentrations and osmotic potentials with all treatments.

Table 5.1 Treatments imposed on tomato and oat plants grown in solution cultures.

Experiment		Concentrations of F in nutrient solutions, and calculated ion activities (μM) and ionic strengths								
5.1	NaF	0	53	105	210	421	841	1684	3368	6736
	F activity	0	50	98	196	392	782	1476	2412	5130
	Ionic strength	2.74	2.79	2.85	2.95	3.16	3.58	4.29	5.04	7.94
5.2	NaCl	0						1684	3368	6736
	Ionic strength	2.74						4.43	6.11	9.45
5.3	KF	0				530	(repeat of Bar-Yosef and Rosenberg, 1988)			
	F activity	0				314				
	Ionic strength	16.97				16.72				
5.4	NaF (low P)	0			210		841	1684		
	F activity	0			196		782	1470		
	Ionic strength	2.67			2.88		3.50	4.21		
5.5	NaF (low P and N)	0			210		841	1684		
	F activity	0			198			1483		
	Ionic strength	1.94			2.17		2.80	3.50		

Ionic strengths and activities were calculated with GEOCHEM-PC and only oats were grown in Experiments 5.4 and 5.5.

5.2.2 *Monitoring of solution culture conditions*

In Experiments 5.4 and 5.5, P concentrations in solution were monitored daily using the modification by Blamey *et al.* (Pers. comm.) of the malachite green method published by Motomizu *et al.* (1983). The lower detection limit of the method was 1 µM. In Experiment 5.5, NO_3^- was monitored daily with high performance ion chromatography (HPIC), using the HPIC-AS4A separator (Dionex, 1987). This method had lower detection limit of approximately 160 µM NO_3^-.

5.2.3 *Modelling of the ionic species of fluoride in solution cultures*

Speciation in the solution cultures were calculated with GEOCHEM-PC as described in Section 3.1. The data described by authors of previous studies (Bar-Yosef and Rosenberg, 1988; Leone *et al.,* 1948; Hara *et al.,*1977; MacLean *et al.,* 1992) were used to calculate the speciation of ions in solution in these studies. If nutrient concentrations were described, but the salts added were not detailed (*e.g.* Leone *et al.*, 1948), the salts described in Table 3.2 were used for the calculations.

5.2.4 *Analysis of plant material*

Plant material was digested for F and multi-element analysis as described in Section 4.2.1.5. Digests were analysed for F as described in Section 4.2.2.2, Method ME_2. Plant nutrients were analysed in these digests as described in Section 3.6.2. Plants from each replication were analysed separately, except for treatments where shoot dry weights were decreased by 50 %. To provide enough sample, plants from all replications of these treatments were bulked for analysis.

5.2.5 *Statistical analyses*

For Experiment 5.1, plant dry weights were linearly regressed against F activities in solution and F concentrations in shoots were fitted to a sigmoidal curve (Equation 5.1, Section 5.3.4) with F ion activity in solution as the x axis. Least significant differences (lsd) were calculated for the line of best fit. Sigmoidal curves were found to describe best the effects of F^- activity in solution culture on F concentrations in plant material in this chapter and also for similar data from the literature. The implications of modelling uptake of F^- by a sigmoidal response are discussed in Section 5.4.3.

Except for Experiment 5.3, a one-way analysis of variance was used in conjunction with a Tukey test to determine significant differences between means of dry weights and F concentrations (if replicate samples had not been bulked to obtain enough material for chemical analysis, see Section 5.2.4). A student t-test was used to determine significant differences between means for Experiment 5.3. Unless otherwise stated, all errors are expressed as one standard deviation from the mean and $n = 3$.

5.3 Results

5.3.1 *Solution culture parameters*

Except for Experiments 5.4 and 5.5, analysis of nutrient solutions (before and after plant growth) indicated that at no time were any nutrients limiting. In Experiments 5.4 (low P) and 5.5 (low P and N), all detectable P had been removed from solution within 24 hours after addition. In Experiment 5.5, no NO_3 was detected in solutions.

5.3.2 *Modelling of ionic species of fluoride in solution*

In Experiment 5.1, where increasing concentrations of NaF were added to the nutrient solutions, F^- activities predicted by GEOCHEM-PC were directly proportional (activity:concentration = 0.94) to the concentration of F up to concentrations of 841 μM. GEOCHEM-PC predicted that higher concentrations of F led to precipitation of F with Mg and Ca, and therefore a decrease in the F activity:concentration ratio. With treatments greater than 841 μM NaF, where more than 40 % of Mg and Ca was predicted to be in the solid form, concentrations of Mg and Ca predicted by GEOCHEM-PC were generally significantly less than measured concentrations (Figure 5.1). Concentrations of F which caused precipitation were used in these studies to allow comparisons with earlier studies which were carried out under conditions where precipitation of F would have occurred (Hara *et al.*, 1977; Bar-Yosef and Rosenberg, 1988). For example, using the nominal concentrations of nutrients in the solutions of by Bar-Yosef and Rosenberg (1988), GEOCHEM-PC calculated that addition of 530 μM NaF to the nutrient solutions caused 28% of the F to be precipitated as MgF_2.

5.3.3 *The effect of the free fluoride ion activity in solution culture on plant dry weights*

Activities of F^- greater than 1473 μM in solution cultures (nominal F concentration = 3368 μM, Experiment 5.2) significantly ($p < 0.05$) decreased the dry weights of tomato shoots and roots (Figures 5.2 and 5.3). The highest F treatment (F^- activities = 5130 μM) had no effect on the dry weights of oat shoots or roots.

There were no significant changes in the dry weights of roots or shoot of oats or tomatoes when concentrations of NaCl equal to those of NaF were added to solution cultures (Figures 5.2 and 5.3).

In Experiment 5.3, there was no significant difference between shoot and root dry weights of plants grown in the control and 530 μM F treatments (Figure 5.4). Similarly, in Experiments 5.4 and 5.5 there were no significant differences between shoot and root dry weights of plants grown in control and F treatments (Figure 5.5).

5.3.4 *The effect of the free fluoride ion activity in solution culture on fluoride concentrations in plants*

At low F activities in solution (< 1476 μM), ratios of the F concentrations in roots:shoots were approximately 6:1 (Figure 5.6). When the lower range of F activities in solution (0 - 782 μM) were linearly regressed against F concentrations in the plants, there were significant ($p < 0.05$) linear relationships between F activities in solutions and F concentrations in tomato and oat shoots (Figure 5.6).

Throughout this thesis, shoot uptake co-efficient (S-UCE = mmol F kg^{-1} plant shoot/mmol F-ionic-species dm^{-3} of growth solution) will be used to describe uptake of ionic species through the roots and their translocation to the plants shoots relative to the activity of ionic species solutions. Concentrations of F in the shoots of plant is important because it is these concentrations which can be zootoxic. Shoot-UCEs also allow direct comparison with previous published data and between different ionic species. Fluoride ion S-UCE of tomatoes and oats grown at low F activities (≤ 1476 μM) were 2.3 ± 1.2 and 2.3 ± 1.9 dm^3 kg^{-1}, respectively (n = 9).

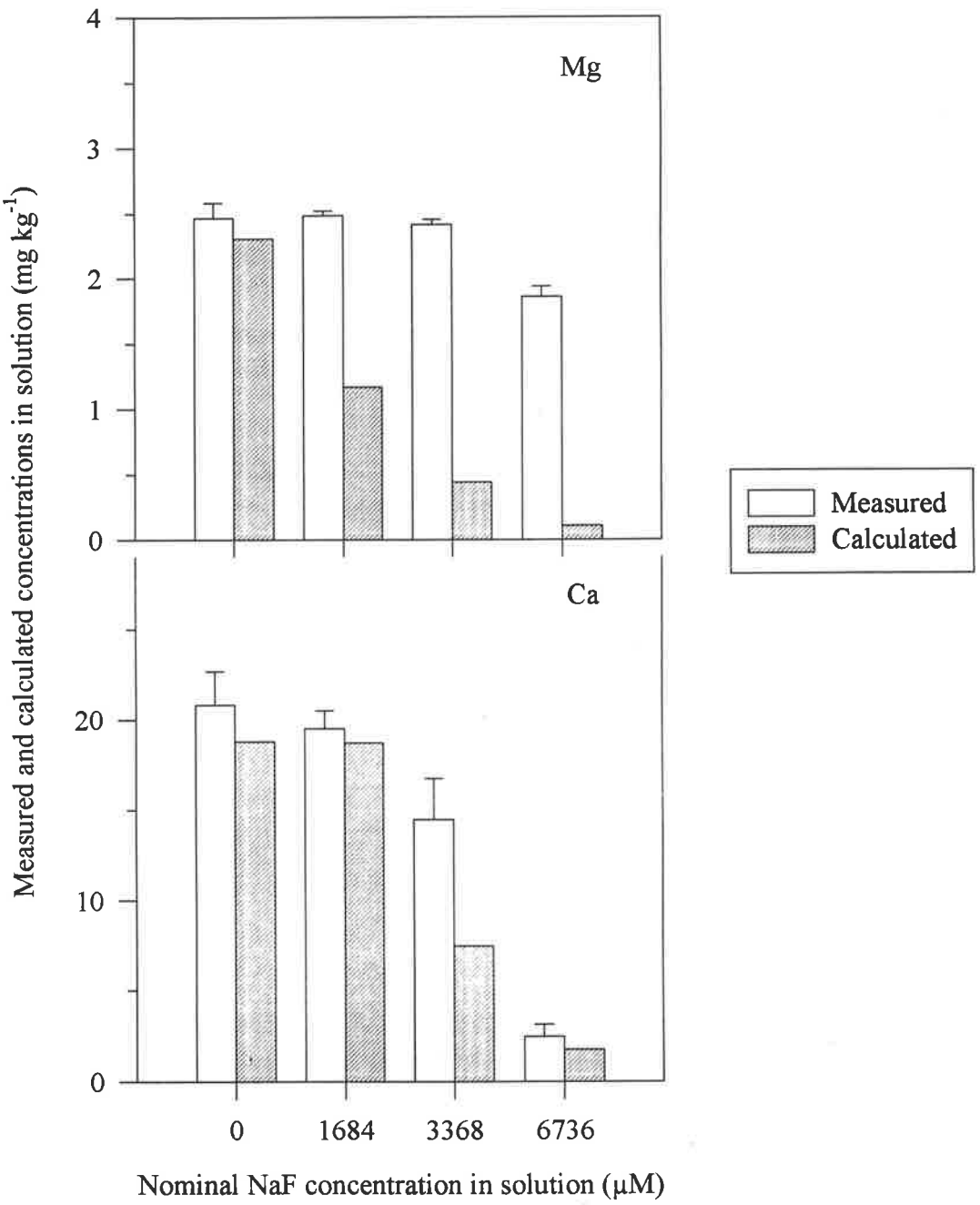
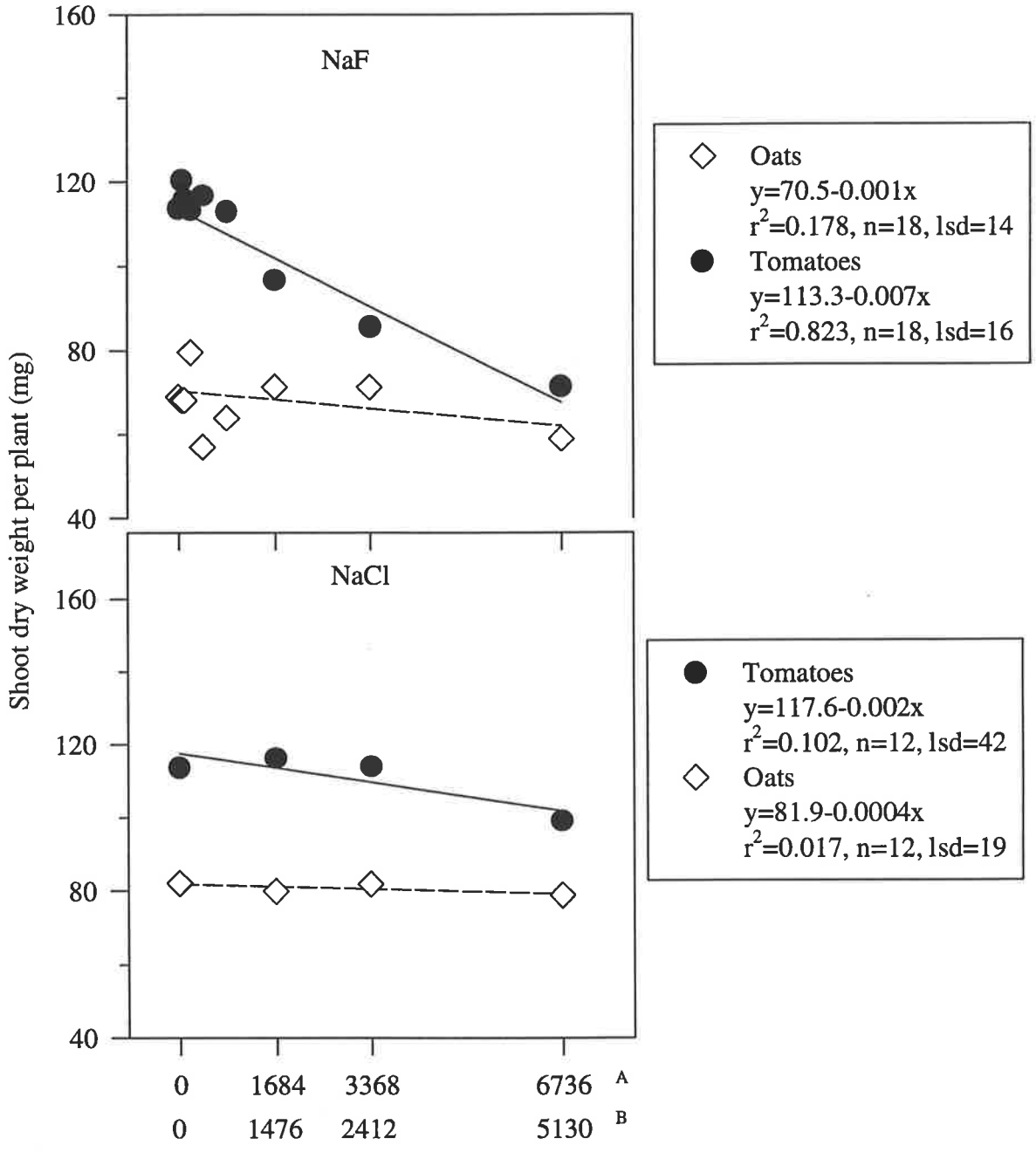


Figure 5.1 Calculated (GEOCHEM-PC) and measured (ICP-AES) concentrations of Ca and Mg in solution. Error bars represent one standard deviation from the mean, n = 3.



[A] Nominal concentrations of NaCl or NaF in solution (μM)
[B] Calculated F^- activity

Figure 5.2 Effect of NaF (F^- activity calculated with GEOCHEM-PC) or NaCl treatment in solution on dry weights of oats and tomatoes shoots. Points represent means, n = 3, except for NaF between 0 - 1476 μM F which are not replicated.

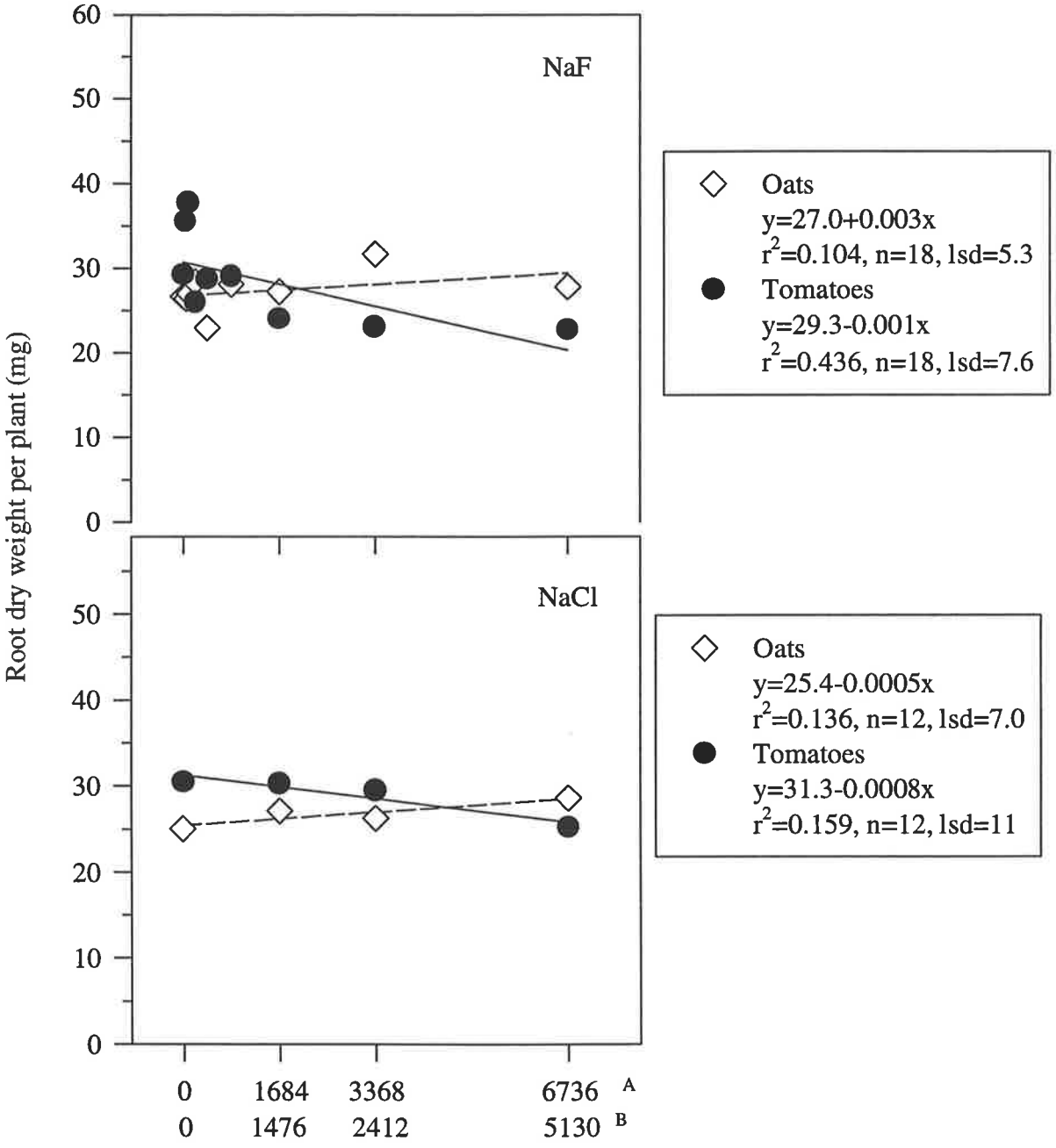


Figure 5.3 Effect of NaF (F^- activity calculated with GEOCHEM-PC) or NaCl treatment in solution on dry weights of oats and tomatoes roots. Points represent means, n = 3, except for NaF between 0 - 1476 μM F which are not replicated.

There was also a significant positive relationship between F^- in solution and F concentrations in oat roots. However, there was no significant relationship between F activity in solutions and F concentrations in tomato roots (Figure 5.6).

Fluoride uptake by the roots and shoots of tomato and oat plants increased rapidly when the F^- activity in solution was greater than 1476 μM. At the highest F treatments, maximum F concentrations in the shoots of oats (approximately 1000 mg F kg^{-1}) were approximately twice that found in tomato shoots (Figure 5.7). At high F activities in solution, ratios of the F concentrations in roots:shoots were within the 50 to 120:1 ratio (Figure 5.7). Mean S-UCEs of tomatoes and oats at high F activities (> 1476 μM) were 7.0 ± 1.8 and 12.4 ± 2.4 dm^3 kg^{-1}, respectively (n = 6).

At low F^- activities (< 1476 μM) in solutions, concentrations of F in roots of tomatoes and oats were approximately 6 - 8 times greater than those found in the shoots (Figure 5.6). At higher activities concentrations in roots were approximately 75 times greater than concentrations in shoots (Figure 5.7). The relationship between F in plant and F activities in solution (Figure 5.7) was best described by a sigmoidal model (Equation 5.1, where a = the first asymptote, b = the slope parameter, c = the value at the inflection point and d = the second asymptote).

$$y = \frac{(a-d)}{[1+(\frac{x}{c})^b]} + d \qquad \text{(Equation 5.1)}$$

Using the limited data available from previous published studies, element speciation analysis (with GEOCHEM-PC) indicated that F in previous studies would be present predominantly as F^-. In several of these studies F, Mg and Ca concentrations in solution were high enough to form solids (MgF_2 and CaF_2), which were significant percentages of the total concentrations of these elements.

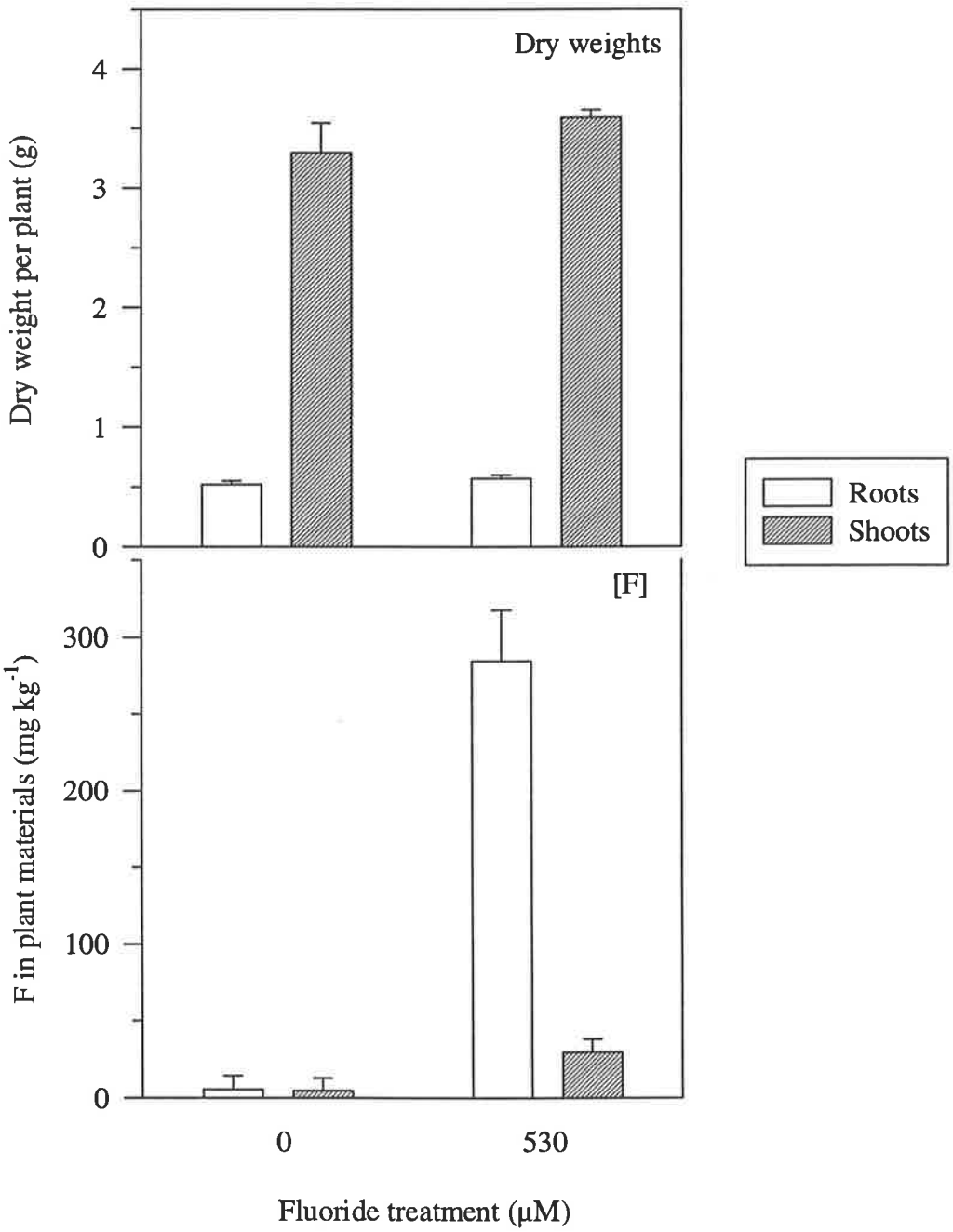


Figure 5.4 Repeat of Bar-Yosef and Rosenberg (1988) solution cultures. Effect of F concentrations in solution on dry weights and F concentration on roots and shoots of tomato. Error bars represent on standard deviation from the mean, n = 3.

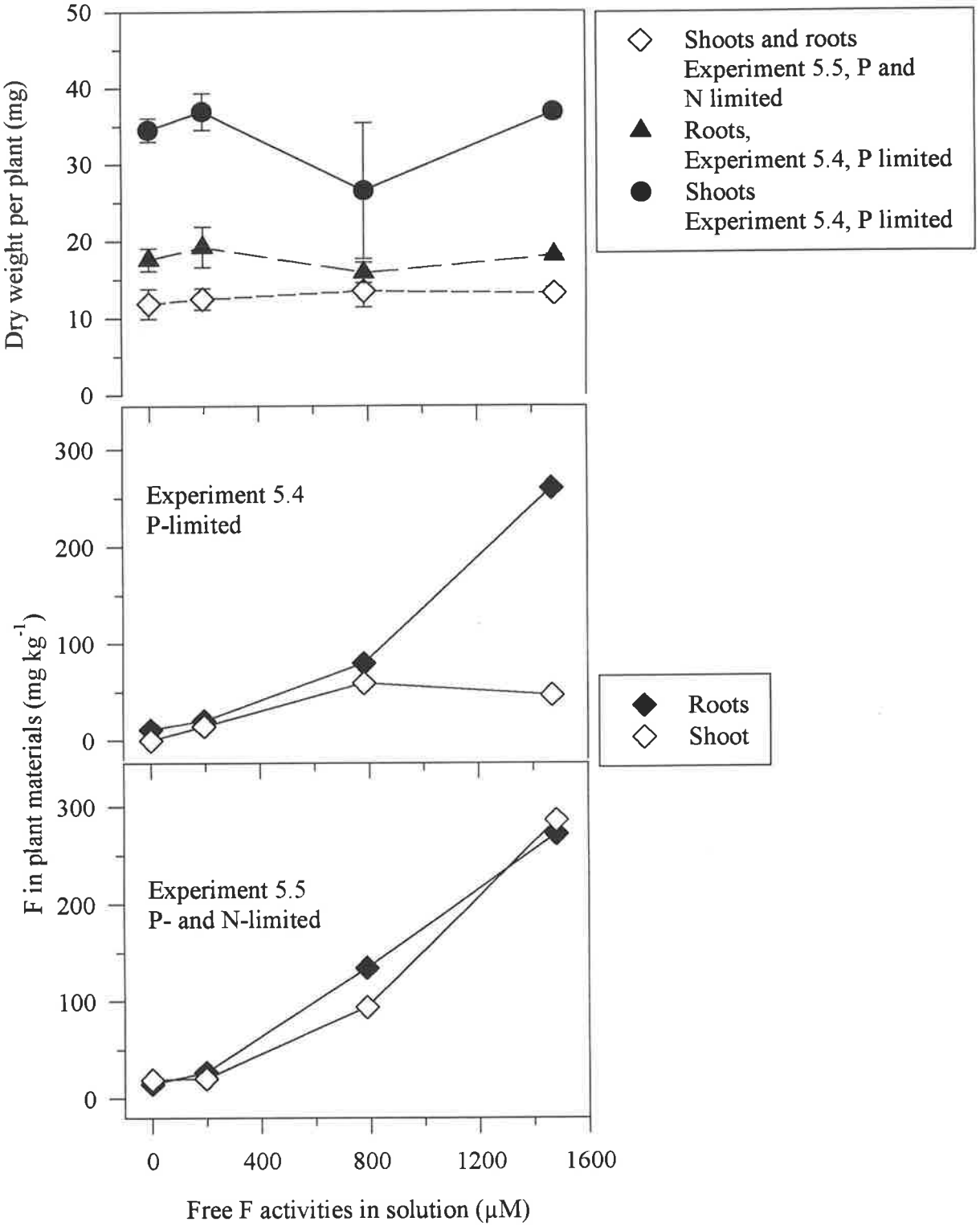


Figure 5.5 Effect of F^- activity in solution on dry weights and F concentrations of oats grown in solution with limited P or limited P and N. Error bars represent one standard deviation from the mean, n = 3.
Due to poor growth, F concentrations are single points determined from bulked samples.

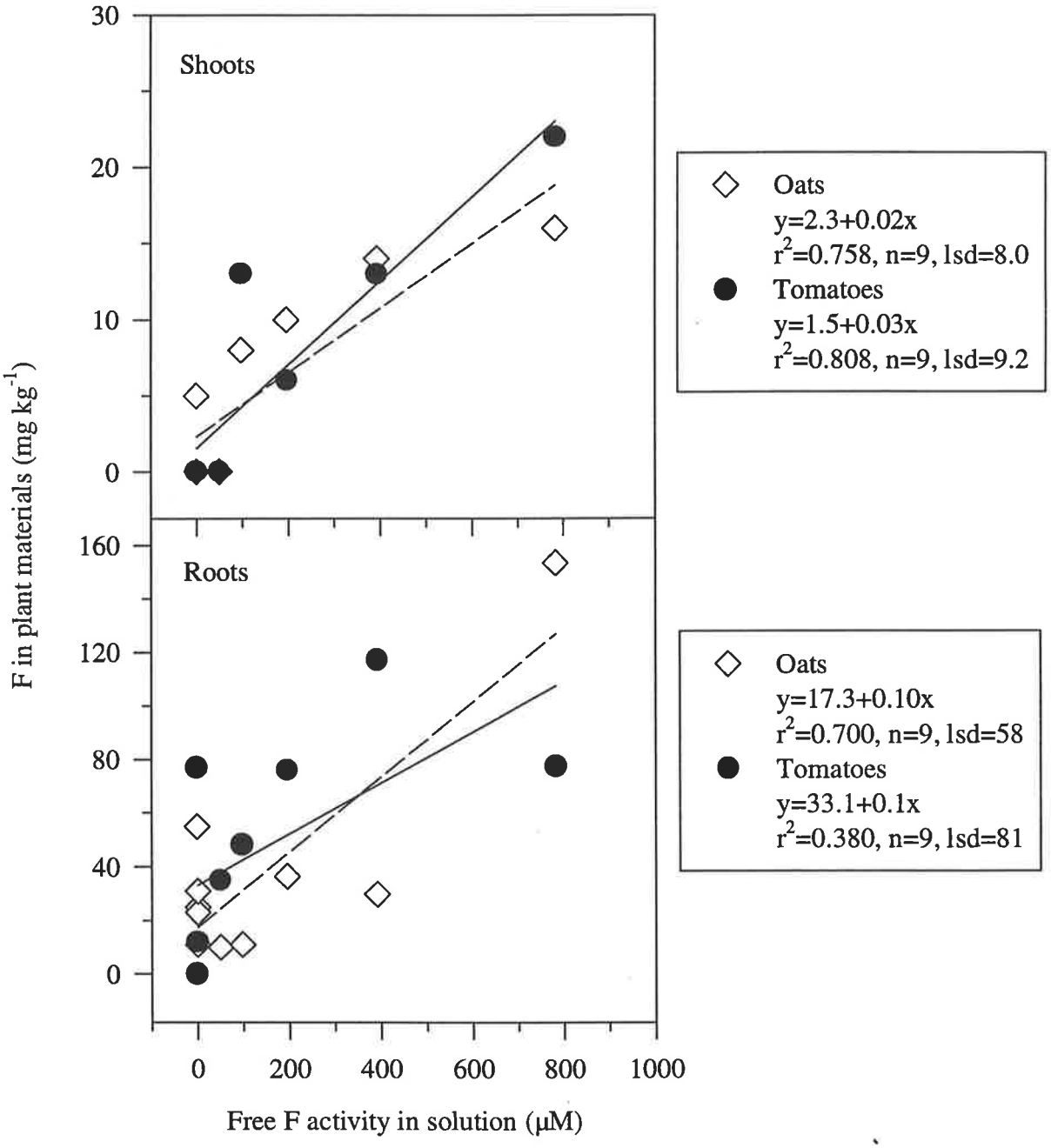


Figure 5.6 Effect of low activities of F^- (< 800 μM) in solution on F uptake by roots and shoot of tomatoes and oats. Points represent single measurements.

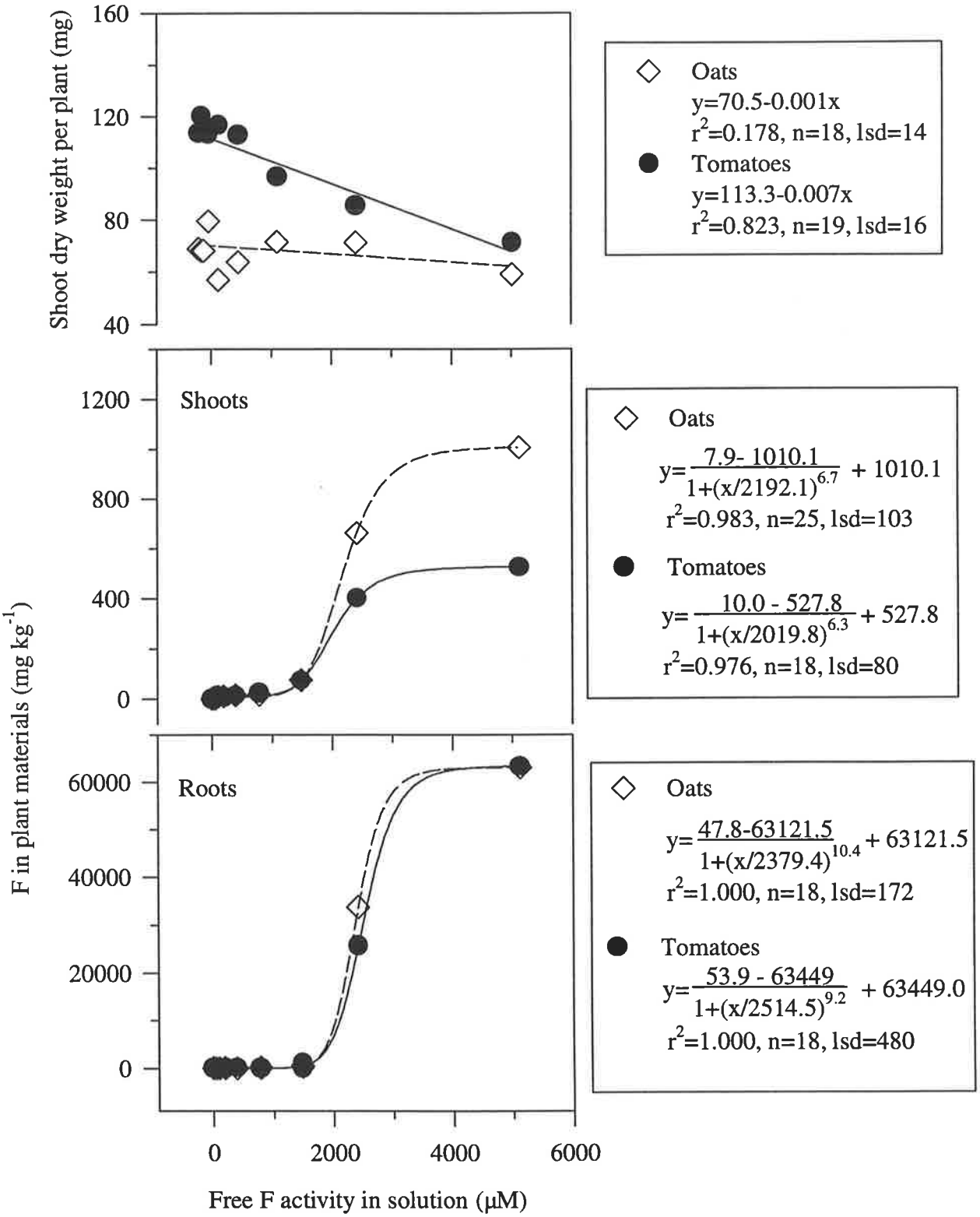


Figure 5.7 Effect of F^- activity in solution on shoot dry weights and F uptake by roots and shoots of tomatoes and oats. Points represent means, n = 3, except between 0 - 1476 μM F which are not replicated.

Using the speciation data calcualted from previous studies (McLean, *et al.*, 1992; Hara *et al.*, 1977; Leone *et al.*, 1948; Bar-Yosef and Rosenberg, 1988), F^- activity in solution and F concentrations in plant shoots were fitted to the sigmoidal model used above (Equation 5.1). Fluoride concentrations in shoots of a range of plant species fitted a sigmoidal model (Figure 5.8), similar to that obtained with data from Experiment 5.1. There were insufficient data points at high F activities in solutions used by MacLean *et al.* (1992) to fit the sigmoidal model and the range of F activities used by Bar-Yosef and Rosenberg (1988) was insufficient to determine the upper asymptote of the sigmoidal model (the line drawn represents an approximation assuming symmetry).

The mean ionic strengths of solutions in each study listed in Figure 5.8 (calculated from their data with GEOCHEM-PC) varied from approximately 2 to 16 mM. As ionic strength of the solutions increased, the inflection point of the curve occurred at lower F activities (Equation 5.1 - parameter c) (Figure 5.8). Appearance of symptoms of F toxicity or decreases in plant dry weights (indicated by the arrows on Figure 5.8) corresponded with or occurred immediately after rapid uptake of F, except for the data of Bar-Yosef and Rosenberg (1988). Bar-Yosef and Rosenberg (1988) found growth decreases before rapid increases in F uptake were observed.

As points of inflection of each F uptake curve increased, mean S-UCE of all plants species from each study increased and ionic strengths of solution cultures increased (Figure 5.9). Although the mean S-UCE calculated from the data of Bar-Yosef and Rosenberg (1988, Figure 5.9) decreases at the highest ionic strength, the mean was not significantly less than the mean UCEs calculated from the other studies. The mean S-UCEs were calculated using data from points before rapid uptake of F, assuming that at this point membrane function had been altered.

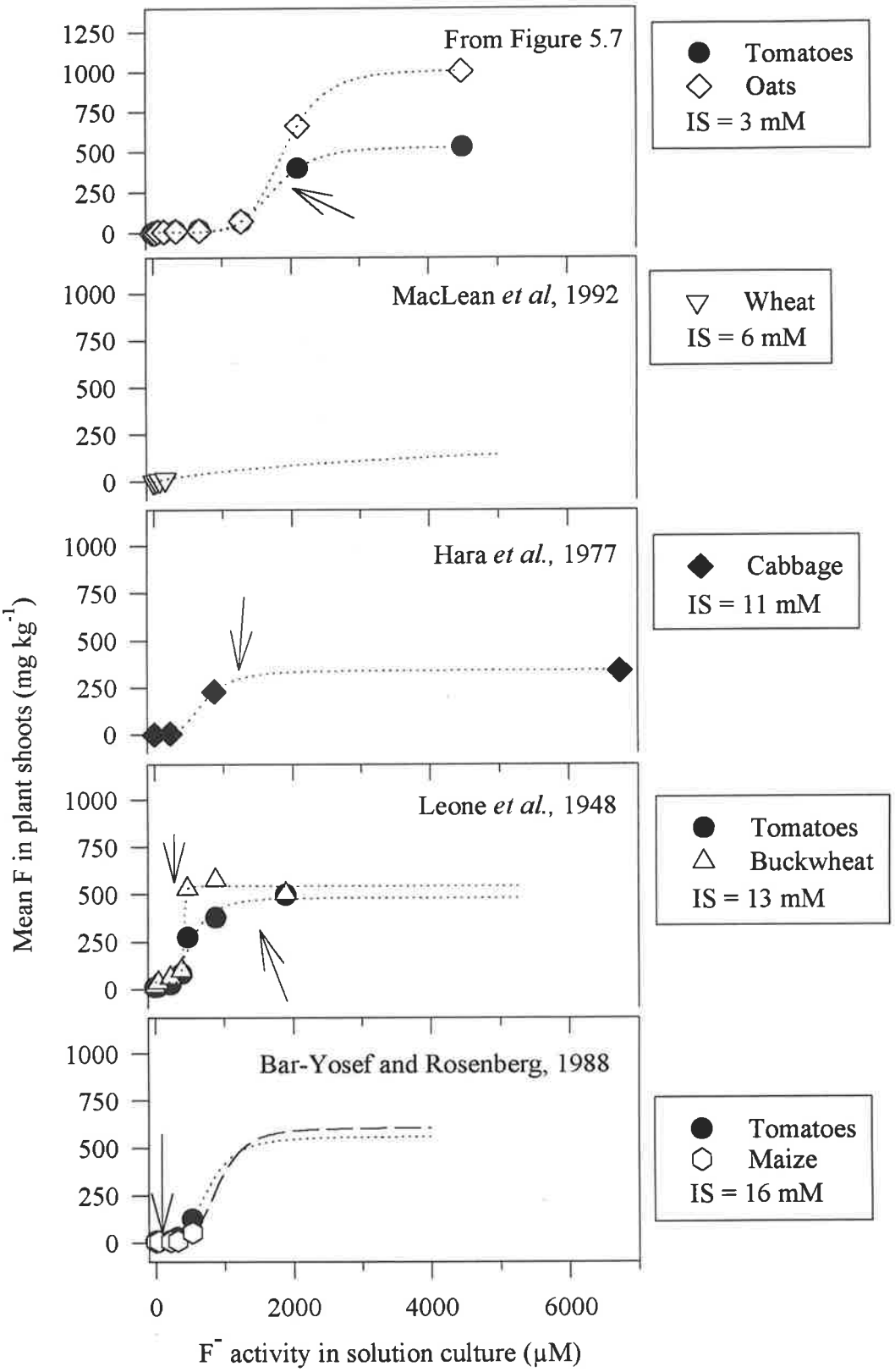


Figure 5.8 Effect of F^- activity (calculated with GEOCHEM-PC) on published F concentrations and toxicity in shoots. Observed toxic responses are indicated by arrows. No arrow indicates no toxic response observed. IS = mean ionic strength of solutions.

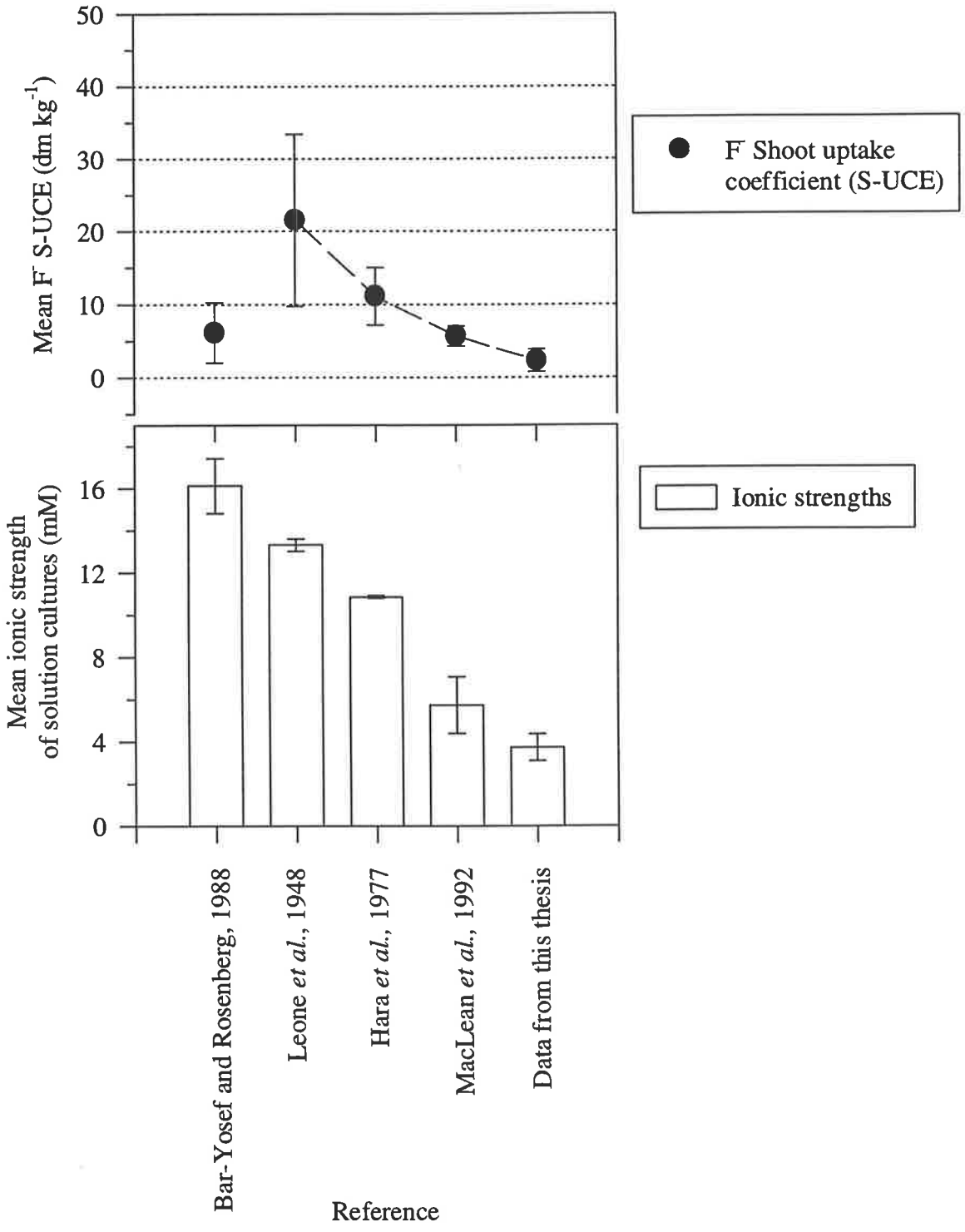


Figure 5.9 Effect of solution ionic strength on F^- uptake. Data from the current study and data from the literature. See Section 5.3.4 for explanation of uptake coefficients. Error bars represent one standard deviation from the mean, n ranged from 2 - 17.

In Experiment 5.1, concentrations of F in tomato shoots grown in solution with F^- activities of 314 μM were 10 mg F kg^{-1} (taken from the regression line in Figure 5.6) and the S-UCE was 1.7 ± 1.9 dm^3 kg^{-1} (error = one standard deviation of the linear model, Figure 5.6). In Experiment 5.3, a repeat of Bar-Yosef and Rosenberg (1988) with higher ionic strength solutions (Figure 5.9), mean F concentrations in shoots of tomatoes grown in solutions with F^- activities of 314 μM were 29.7 ± 8.5 mg F kg^{-1}. The mean S-UCE for these plants was 4.9 ± 1.1 dm^3 kg^{-1}, similar to that calculated from the data of Bar-Yosef and Rosenberg (1988) (4.7 dm^3 kg^{-1}).

In Experiment 5.4, concentrations of F in roots and shoots of oats were similar to those grown in experiments where P in solution was not limited (Figures 5.5 and 5.7). However, in Experiment 5.5 concentrations of F in oat shoots increased at the highest F activity in solution. For oats grown in solutions containing similar activities of F^-, there were no differences between concentrations of F in the roots of plants grown in Experiments 5.1, 5.4 and 5.5 (Figures 5.5, 5.6 and 5.7).

Plant uptake co-efficient (P-UCE = mmol F kg^{-1} plant shoots and roots/mmol F dm^{-3} of growth solution) will be used to describe the ionic species of F taken up by the plant relative to the activity of the ionic species in solution. This measure is important with respect to mechanisms of uptake and differences between P-UCE and S-UCE values suggest differences between plant uptake of ions and plant uptake and translocation of ions to the shoots. However, P-UCEs are also complicated by adsorption of ions to the root. It is not known how much of the ionic species of interest is internal and how much is external to the root. P-UCEs showed similar trends to S-UCEs. At low F activities in solution (0 - 1476 μM) P-UCEs for F^- were 6.8 ± 1.7 dm^3 kg^{-1} (n = 9) and 6.2 ± 2.7 dm^3 kg^{-1} (n = 9) for oats and tomatoes, respectively. At high F activities in solution (> 1476 μM) P-UCE's increased by at least 2 orders of magnitude . Plant-UCE's were 225.4 ± 12.0 (n = 6) and 144.8 ± 19.1 dm^3 kg^{-1} (n = 6) for oats and tomatoes, respectively.

5.3.5 *The effects of fluoride and nutrient limitations on Ca, Mg and P concentrations in plant shoots*

In Experiment 5.1, where high F treatments (6736 μM) affected dry weight of tomatoes but not oats (Figure 5.2), the calcium concentrations in the shoots of F^--treated oats were less than the controls, but still within the ranges specified as adequate by Reuter and Robinson (1986) (Table 5.2). However, the concentrations of Ca in tomato shoots were below that considered by Reuter and Robinson (1986) to be adequate and the plants could be described as Ca deficient (< 70% maximum yield, as defined by Reuter and Robinson, 1986). Mean dry weights of tomato shoots at the highest F treatment were 60 % of controls, *i.e.* those grown without F (Figure 5.2). There were no significant differences in Mg concentrations in shoot of oats or tomatoes for any treatments (Table 5.2).

In Experiments 5.4 and 5.5, phosphorus concentrations in the shoots of oats (Table 5.2) were less than in plants grown with adequate P (Experiment 5.1), but not in the range considered deficient by Reuter and Robinson (1986). However, plant dry weights in both these experiments were < 50 % of the maximum yield of plants grown with adequate P (Figures 5.2 and 5.5). Phosphorus concentrations in shoots of control plants were higher than considered adequate for shoots of plants grown in soils (Reuter and Robinson, 1986).

Table 5.2 Effects of fluoride and nutrient limitations on concentrations of Ca, Mg and P in plant shoots.

Experiment No.	Treatment (μM F)	Element concentration in plant (%)					
		Oats			Tomatoes		
		Ca	Mg	P	Ca	Mg	P
5.1 (high F)	0	0.59	0.23	1.50	2.2	0.44	1.2
	6736	0.31	0.22	1.10	1.0	0.53	1.5
5.4 (low P)	0	0.49	0.22	0.37			
	1684	0.41	0.20	0.33			
5.5 (low P and N)	0	0.53	0.26	0.65			
	1684	0.48	0.26	0.83			
Mean rsd[A] for element analysis, n=5		0.04	0.07	0.07	0.03	0.05	0.04
Adequate[B]		0.2-0.5	0.15-0.5	0.2-0.8[C]	1.4-4.0	0.4-0.8	0.3-1.2
Deficient[B]		<0.2	<0.12	<0.15-0.45	<1.0	<0.25	<0.4

[A] Relative standard deviation.

[B] From Reuter and Robinson (1986). These ranges are generally from plants older than those grown in the experiments in this chapter and from plants grown in soils, predominately under field conditions.

[C] Toxic range > 3.0 %

5.4 Discussion

5.4.1 *Modelling of ionic species of fluoride*

Although changes in concentrations of Ca and Mg were measured by ICP-AES in solutions containing high F, measured concentrations differed from those calculated with GEOCHEM-PC (Figure 5.1). Differences in measured and calculated values could be due to the use of incorrect thermodynamic constants in calculated values (Table 3.3), or due to the ICP-AES measuring suspended colloidal solids of either CaF_2 or MgF_2 in these solutions. The formation of solid CaF_2

or MgF_2 could alter the phytoavailability of Ca and Mg in these situations thereby affecting plant growth (see Section 5.4.4).

5.4.2 *The effect of the free fluoride ion activity in solution on dry weights of plants*

That high activities of F in solution did not affect dry weights of oat shoots or roots suggests that this plant species is able to tolerate high concentrations of F in solution, either by excluding F at the root or by detoxifying F at the cellular level in the plant. The activities of F^- in solution which significantly decreased dry weights of tomato shoots (between 1476 - 2412 μM) were similar to activities which Leone *et al.* (1948) found to cause visual necrosis of tomato leaves (between 884 - 1898 μM) which had been grown for a similar period to experiments reported in this thesis. However, these activities are significantly greater than those which Bar-Yosef and Rosenberg (1988) found to limit the growth rate of maize (314 μM) and tomatoes (213 μM) grown in solution cultures. The activities of F^- were calculated with GEOCHEM-PC from the data of Bar-Yosef and Rosenberg (1988) and Leone *et al.* (1948).

Difference between sensitivity of tomatoes and oats to F toxicity (measured as decreases in dry weight) could be due to: a) greater negative charge on the roots of the oat plants, giving their roots a greater capacity to repel the negatively charged F^- ions and prevent them from nearing sites of uptake (Section 2.7.1.5), or b) tomatoes being more sensitive to the low Ca concentrations in solution or in the plant, induced by precipitation of CaF_2. It is unlikely that difference in sensitivity of oats and tomatoes to F toxicity is due to greater negatvie charge on the roots of oat plants as monocotyledons generally have a less CEC than dicotyledons (Section 2.7.2). The data presented in Sections 5.4.3 and 5.4.4 (below) support the latter theory.

When the work of Bar-Yosef and Rosenberg (1988) was repeated (Experiment 5.3) no significant differences were found between the dry weights of control plants and those treated with 530 µM F (Figure 5.4). Bar-Yosef and Rosenberg (1988) found that 530 µM F decreased plant growth by 64 - 75 %. It is possible that the findings of Bar-Yosef and Rosenberg (1988), which contradict the findings of Leone *et al.* (1948) and the findings of this thesis, could be due to variations in sensitivity of tomato cultivars to F, although the differences in critical F concentrations is much greater than would normally be expected between cultivars. Variability between plant species in sensitivity to gaseous F is well documented (Ivinskis and Murray, 1984; Weinstein, 1977).

Dry weights within Experiments 5.4 and 5.5 did not differ significantly with increasing F concentrations up to 1476 µM F in solution, suggesting that F uptake was not increased to a toxic level by removal of anions ($H_2PO_4^-$ and NO_3^-) that could compete with F^- at uptake sites. These data suggest that F^- is not competitively excluded from sites of active uptake by other anions such as $H_2PO_4^-$ and NO_3^-.

5.4.3 *The effect of the free fluoride ion activity in solution on fluoride concentrations in plants*

At low activities of F^- in solution (< 1476 µM, Experiment 5.1), F concentrations in shoots of tomatoes and oat increased linearly (Figure 5.6). MacLean *et al.* (1992) found that F concentrations in shoots of wheat were linearly related to F activities in solution up to 169 µM (F activities were calculated with GEOCHEM-PC from the data of MacLean *et al.*, 1992). If uptake of F^- was regulated by the plant at low concentrations in solution, the concentration of F in shoots would be expected to increase to an upper asymptote, as described by Equation 6.1 (Section 6.4.4). However, linear uptake suggests that at low activities of F^- in solution, F uptake

is a passive process where F enters the plant through an extra-cellular pathway, probably leaking past the endodermal barrier at the root tips, where it is not fully formed, or where lateral roots penetrate the endodermis (Perry and Greenway, 1973; Pitman, 1982; Davison *et al.*, 1985). The ratio of F in roots:shoots of oats and tomatoes ranged from approximately 6:1 - 75:1 (Figures 5.6 and 5.7), suggesting that translocation of F^- with in the plant is restricted through some mechanism in the root.

Fluoride concentrations in oat shoots and roots, and tomato shoots showed significant linear correlations with F^- activity in solution. However, F concentrations in tomato roots were not linearly related to solution F^- activities. This could be explained by: a) variable desorption or entrapment of F from the AFS of the tomatoes roots during the washing procedure, or b) variability in separation of roots from shoots. When harvesting tomatoes, the definition between roots and shoots is not as distinct as oats. With oats the seed defines the boundary between roots and shoots. This is not the case with tomatoes. If the boundary between roots and shoots is weighted towards the shoot, dilution of the small amount of root material produced by tomatoes would affect measured F concentrations in the roots.

At low F^- activities in solution, if F uptake is via an extra-cellular pathway, F concentrations in plants should be equivalent to 2-3% of the plants water flow (Perry and Greenway, 1973). Approximately 500 cm^{-3} of nutrient solution were depleted over the growth period of tomatoes and oats (not accounting for evaporation losses). Total plant dry mass per container was approximately 0.66 g. If F activity in solution is 782 μM, F concentrations in the plant would be 0.5 mg F kg^{-1}, much lower than the F concentrations found for this treatment (approximately 6 mg F kg^{-1}). These calculation suggest that even at low F activities plant uptake of F^- is not only due to flow via an extra-cellular pathway.

The increase in both P-UCEs and S-UCEs, reflecting the rapid uptake of F by the plant at F^- activities greater than 1476 μM, suggested that uptake of F by plants at these activities would not follow a linear response. When the higher activities of F^- in solution were included in the F uptake curve (Figure 5.7), the data presented here and the reinterpretation of data found in the literature (Figure 5.8) presents strong evidence that uptake of F^-, by a range of plant species, follows a sigmoidal pattern. Hewitt and Smith (1974) attributed sigmoidal uptake patterns to substrate co-operation (the affinity of an enzyme for its substrate increasing with the substrate concentration). Substrate co-operation is thought to be due to structural changes in the protein induced by the substrate as the substrate activity increases. In the case of F, a non-essential element, it is unlikely that F activity at the site of uptake causes substrate co-operation. However, it could be postulated that at high F concentrations in the plant, F affects Ca in the membrane to an extent that membrane permeability is altered (see Section 5.4.4). The change in permeability would have to be selective to F, as general membrane damage would be reflected in increased concentrations of other elements, which was not the case.

Hewitt and Smith (1974) suggested that, in investigations of ion uptake by roots, it is important to include Ca ions or results would be meaningless due to permeability changes which take place in cell membranes in the absence of Ca. The toxic mechanisms of F are thought to be effective inhibition of enzymes and/or precipitation of Ca interfering with membrane permeability (Suttie, 1977), supporting the postulate above that F activity at the cell membrane must be high enough to affect Ca concentration before membrane permeability is altered. Changes in membrane permeability would overcome the barriers of the roots cortex to F uptake and increase F concentrations in plant to phytotoxic levels.

In previous solution culture studies summarised in Figure 5.8, rapid uptake of F generally coincided with growth restrictions (Hara *et al.*, 1977; Leone *et al.*, 1948; tomatoes, this thesis). However, rapid uptake of F does not always coincide with growth restrictions: MacLean *et al.* (1992); Bar-Yosef and Rosenberg (1988); Oats, this thesis. Oats grown over a large range of F activities (0 - 5130 μM), which produced concentrations up to approximately 1000 mg F kg^{-1}, showed no growth reductions: it is a plant species tolerant to F, but not an accumulator. Other genera (*e.g. Dichapetalum, Thea, Gastrolobium, Camellia, Oxylobium, Acacia* and *Palicoure*) accumulate fluoride (Vickery and Vickery, 1976) and show none of the symptoms of F toxicity with F concentrations up to 4000 mg F kg^{-1} dry weight (Jacobson *et al.* 1966; Weinstein and Alscher-Herman, 1982). No growth restrictions were found by MacLean *et al.* (1992) because the concentrations of F used by these authors were too low to affect membrane permeability.

However, it is difficult to explain the toxic responses reported by Bar-Yosef and Rosenberg (1988). The concentrations of F in the shoots of tomatoes and maize studied by Bar-Yosef and Rosenberg (1988), where significant restrictions in growth rates were recorded, were no greater than 12.4 mg F kg^{-1}. The concentrations of F in these plants were unlikely to be significantly different from those of control plants, although insufficient data were presented to confirm this. Tomato shoots grown under the same conditions as Bar-Yosef and Rosenberg (1988) in Experiment 5.3 contained 29.7 ± 8.5 mg F kg^{-1}, no different from the mean concentration of F in shoots (28 mg F kg^{-1}) found by Bar-Yosef and Rosenberg (1988) for the same treatment. However, growth reductions in other studies were associated with much higher concentrations of F in plant shoots (> 100 mg F kg^{-1}).

The work of Hansen *et al.* (1958), involving turnips and lucerne, led them to the general conclusion that plant dry weights were restricted when the F concentration of the tissues exceeded 60 mg F kg^{-1} on a dry weight basis. However, this figure is very dependent on the plant species (Vickery and Vickery, 1976). For example, plants of *Gladiolus* spp. (one of the most sensitive to F toxicity) may become necrotic with 20 mg F kg^{-1} dry weight in their shoots (Jacobson *et al.*, 1966). Yet, Bar-Yosef and Rosenberg (1988) recorded significant growth rate restrictions in maize and tomatoes at approximately half this concentration. One reason may be differences in F sensitivity between cultivars of tomatoes, as discussed above (Section 5.4.2). However, such a large difference between cultivars of a single species is unlikely.

Differences between S-UCE for tomatoes grown in low ionic strength solution cultures (1.7 dm^3 kg^{-1}) in Experiment 5.1 and those found by Bar-Yosef and Rosenberg (1988) which was also repeated in Experiment 5.3 (4.7 and 4.9 dm^3 kg^{-1}, respectively), could be explained by differences in ionic strength of solutions (Figure 5.9). Similarly, differences in activities of F in solution where F concentrations in shoots increased rapidly (point of inflection of sigmoidal curve) determined from published data and data obtained in Experiment 5.1 (Figure 5.8), could also be explained by differences in ionic strengths of solutions (Figure 5.9). Increases in ionic strength of a solution indicate an increase in the activities of cation and anions in solution. Increasing cation activity in solution, especially divalent cations, and the suppression of the negative charge of the DFS was demonstrated by Walker and Pitman (1976). It is likely that the F^- must overcome the negative charge of the DFS of the root to allow movement to sites where it can be taken up. Increasing the ionic strength of the solution would aid in suppression of the DFS charge and allow higher activities of F at uptake sites.

In Experiments 5.4 and 5.5, plant dry weights were restricted, requiring replicated shoot and root samples to be bulked to obtain sufficient sample for analysis: this made it impossible to determine whether the differences recorded were significant. Interpretation of the data was also complicated by the likely effect of low NO_3^- on membrane function and permability. There is also strong evidence in the literature indicating that uptake of F by roots is a passive, diffusive process (Venkateswarlu *et al.*, 1965; Cooke *et al.*, 1978; Garrec and Letuorneur, 1981) and therefore research into the competitive inhibition of F^- by NO_3^- and $H_2PO_4^-$ was not pursued. However, from the limited data obtained where solution P was limiting, oats shoots showed no increase in F concentrations, suggesting that F uptake is not competitively inhibited by $H_2PO_4^-$. In Experiment 5.5, where NO_3^- was also limited, F concentrations in oat shoots increased, possibly due to limited N supplies affecting membrane function.

5.4.4 The *effect of fluoride on Ca, Mg and P concentrations in shoots*

Calcium concentrations in the shoots of tomato plants grown with the highest F treatment were found to be significantly lower than controls and not within the range considered adequate (Table 5.2). However, Ca concentrations in oat shoots were within the range considered adequate, suggesting that growth reduction due to high F^- activities in solution may be due to complexation of Ca in the tomato root or due to lower activities of Ca in solution through precipitation with F. Comparison of the critical concentrations for Ca in oats and tomatoes shoots (Table 5.2) indicate that tomatoes have a greater demand for Ca, supporting this conclusion. The mechanisms through which F is toxic are thought to involve, in part, interference with membrane permeability through precipitation with Ca (Suttie, 1977). If Ca concentrations in plants are already low (*e.g.* the tomatoes grown here), plants would be more sensitive to F exposure. The toxic action of F is also thought to involve the inactivation of Mg at its sites of physiological activity (Weinstein and

Alscher-Herman, 1982). Concentrations of Mg in plant shoots showed no changes with F treatment suggesting that F concentrations in solutions had no effect on Mg nutrition in the plant (Table 5.2).

In experiments where P was limited (Experiment 5.4 and 5.5), P concentrations in shoots of oats were within the range considered adequate by Reuter and Robinson (1986) (Table 5.2). However, dry weights of plants were less than 70 % of oats grown without a limited P supply. By the definition of Reuter and Robinson (1986), an element is considered deficient if yield is decreased to less than 70 % of maximum yield. These results suggest that for oats grown in solution culture, the adequate range set by Reuter and Robinson (1986) (mostly taken from field or pot studies), is not applicable. Phosphorus concentrations in shoots of plants grown in control solutions were higher than consider adequate by Reuter and Robinson (1986), but were not considered toxic.

5.5 Conclusions

The data from this chapter suggest that, at low F activities (< 1476 μM), uptake of F^- by the plant roots is a passive, diffusive intra-cellular process and there is probably also uptake via an extra-cellular pathway where the endodermis of the root acts as a leaky barrier to this ion. The low uptake of F uptake by the plant root at low activities is thought to be due to exclusion of F^- from sites of uptake by the negative charge of the DFS. This mechanism is influenced by the ionic strength of the external growth solution, which suppresses the negative charge of the DFS as ionic strength increases.

At high F activities, F is readily taken up to high concentrations in shoots, concentrations that vary between plant species. It is suggested that at high activities, F selectively alters the permeability to Fof cell membranes in the cortex and endodermis, thus allowing rapid influx of

F until F concentrations in shoots become toxic. The complexation of Ca with F has been proposed as the mechanism of selectively altering membrane permeability.

Chapter 6

6.0 Uptake of fluoride complexed with aluminium by plants grown in solution culture

6.1 Introduction

The previous chapter described plant uptake of the free F^- ion. However, in acid soils (pH < 5.0 - 5.5) growth limitations from Al toxicity become severe due to release of Al into solution. Rhizotoxic species of aluminium (Al_r) are thought to be Al^{3+} $AlOH^{2+}$ and $Al(OH)_2^+$ (Wright *et al.*, 1987). Once in solution, Al can also form several soluble complexes with F (*e.g.* AlF^{2+}, AlF_2^+, AlF_3^0, AlF_4^-). Complexation of Al with F has been shown to ameliorate the toxic affects of Al_r, suggesting that AlF_x^{3-x} complexes are less toxic than Al_r (Cameron *et al.*, 1986; MacLean *et al.*, 1992; Takmaz-Nisancioglu and Davison, 1988). In the formula AlF_x^{3-x}, x is commonly equal to 1- 4, but can be as high as 6. These complexes are referred to as AlF in this thesis.

Current data relating to increased uptake of F due to the complexation with Al are contradictory (Section 2.7.1.5). It is not possible to determine from the data whether F is more readily taken up as F^- or as certain AlF complexes, and which of the latter, if any, are toxic to the plant. The aims of this chapter were to determine: a) the toxicity of AlF complexes compared with F^- and Al_r, and b) if the speciation of AlF in solution exposed to the root affects F uptake by the plant.

6.2 Material and methods

6.2.1 *Solution culture parameters*

Solution culture parameters were as described in Section 3.2.2.

6.2.2 *Fluoride and aluminium treatments added to solution culture*

Treatments of Al and F added to basal nutrient solutions are shown in Table 6.1. All treatments in Experiments 6.1 - 6.3 (Table 6.1) were replicated three times, except for oats grown in Experiment 6.2 with 1684 μM F which were replicated six times. Solution pH was 4.2 ± 0.05. Due to poor growth of plants in some treatments, extra pots were prepared to obtain sufficient sample for analyses.

6.2.3 *Modelling of the ionic species of fluoride in solution cultures: GEOCHEM-PC v MINTEQA2*

The most predominant species of F, Al or AlF were modelled with GEOCHEM-PC and MINTEQA2 as described in Section 3.4.

6.2.4 *Measurement of total fluoride concentrations in solution*

Moore and Ritchie (1988) have shown that TISAB IV is much more effective than TISAB III in releasing F complexed with Al. Therefore, in solutions containing Al, F concentrations were determined as described in Section 3.3.2, with the following modifications. TISAB IV was used in place of TISAB III and 5 cm^3 volumes of TISAB were mixed with samples or standards (Moore and Ritchie, 1988).

Table 6.1 Treatments imposed on tomatoes and oats grown in solutions and the activities of Al_r, F^- and AlF species calculated with GEOCHEM-PC.

Exp. no.	Salts and ions	Concentrations and activities in nutrient solutions (μM)						
6.1	$AlCl_3$	0	18.5	37	74	185	370	741
	Al_r activity[B]	0	5.7	11.7	23.1	37[A]	37[A]	38[A]
6.2	$AlCl_3$:NaF	0:1684	185:1684	370:1684	555:1684	741:1684	741:3368	1482:336
	F^- activity	1476	947	489	158	33	861	37
	Al_r activity[B]	0	2.7×10^{-6}	4.3×10^{-5}	1.6×10^{-3}	0.1	1.5×10^{-5}	0.16
	AlF^{2+} activity	0	2.15×10^{-2}	0.18	2.2	28	0.15	49
	AlF_2^+ activity	0	10	44	172	468	46	903
	AlF_3^0 activity	0	123	267	345	195	502	417
	AlF_4^- activity	0	47	52	22	2.6	172	6.1
6.3[C]	$AlCl_3$:NaF	0:0	46:105	185:421	370:841	555:1263	741:1684	
	F^- activity	0	13	23	29	32	33	
	Al_r activity[B]	0	4.8×10^{-2}	5.3×10^{-2}	6.9×10^{-2}	8.3×10^{-2}	9.9×10^{-2}	
	AlF^{2+} activity	0	5	11	17	22	28	
	AlF_2^+ activity	0	32	126	243	357	468	
	AlF_3^0 activity	0	5	37	88	140	195	
	AlF_4^- activity	0	2.6×10^{-2}	3.4×10^{-1}	1	2	3	

[A] Al predicted to precipitate as $Al_2(SO_4)_3$ or $Al(OH)_3$

[B] Al_r = potentially rhizotoxic aluminium (the sum of Al^{3+}, $AlOH^{2+}$ and $Al(OH)_2^+$ activities)

[C] Tomatoes were not grown in this Experiment

6.2.5 *Measurement of ionic fluoride (F^-) in solution*

The ionic species of F in solution were measured and the measured values compared with those calculated with GEOCHEM-PC (Version 2) (Parker *et al.*, 1987). To measure the activity of F^- in the solutions with Al, a non-complexing buffer (NCB), similar to that of Takmaz-Nisancioglu and Davison (1988), was used in conjunction with a F-ISE (Section 3.3.2). The NCB was made by dissolving 8.203 g of CH_3COONa in 80 cm^3 of deionised water and adjusting the pH to 6.5 (NCB 6.5) or 4.2 (NCB 4.2) with concentrated H_2SO_4. To make the ionic strength of the two buffers equal, 4.22 cm^3 of 4.8 M NaCl was added to NCB 6.5 before diluting both NCBs to 100 cm^3 with deionised water. The pH of the NCB used for standard solutions was 6.5, to prevent any formation of HF in these solutions. The pH of the NCB used for the samples was equal to that of the samples (4.2) to minimise any changes in speciation of ions in solution by addition of the NCB.

6.2.6 *Measurement* of *reactive Al in solution*

As a measure of Al_r, Al concentrations in the solutions of Experiments 6.2 and 6.3 were quantified with the labile Al 8-hydroxyquinoline method of James *et al.* (1983) using the modifications of Alva *et al.* (1989). Sample and standard volumes of 4.00 cm^3 were used. Measured activities were compared with those predicted by GEOCHEM-PC.

6.2.7 *Solution ageing effects*

The following experiment was designed to determine if there were changes with time in speciation in nutrient solutions in the absence of plants. Complete nutrient solutions (Section 3.2.1), with and without 370 µM $AlCl_3$ added were maintained at 20°C with no plants. Duplicate

samples (10 cm^3) were taken at 0.5, 1, 2, 4, 24 and 48 h intervals (t = 0, when nutrient stock added to deionised water). The concentration of B, Ca, Fe, K, Mg, Na, S, Zn and P in samples were determined with ICP-AES.

6.2.8 *Analysis of plant material*

Plant material was digested as described in Section 4.2.1.5. Digests were analysed for F as described in Section 4.2.2.2, Method ME_2. Plant nutrients were analysed in these digests as described in Section 3.6.2. Plants from each treatment were analysed separately.

6.2.9 *Statistical Analyses*

In all experiments, a one-way analysis of variance was used in conjunction with a Tukey test to determine significant differences between means. For Experiment 6.1, Al concentrations in plants were linearly regressed against F activities in solution. In Experiment 6.2, F and Al concentrations in shoots were fitted to Equation 6.1 (Section 6.3.8) with F ion activity in solution as the independent variable. Equation 6.2 was found to describe best the effects of AlF activity in solution on F concentrations in plant material. The implications of uptake of AlF by this model are discussed in Section 6.4.4. Errors are one standard deviation from the mean and n = 3, unless otherwise stated.

6.3 Results

6.3.1 *Solution culture parameters*

Monitoring of solution cultures with ICP-AES and CIA (before and after plant growth) indicated that at no time were any nutrients in nutrient solutions limiting to plant growth.

6.3.2 *Modelling F species in solution*

In Experiment 6.1, addition of Al without F increased predicted Al_r activity to 37 μM before precipitation of $Al_2(SO_4)_3$ and $Al(OH)_3$ occurred at a total Al concentration of 185 μM. In Experiment 6.2, increasing concentrations of Al in nutrient solutions which contained 1684 or 3368 μM F led to a range of activities of AlF complexes (Figure 6.1, Table 6.1). The predicted activities of Al_r in this experiment were < 0.1 μM for all treatments containing < 1482 μM Al. For treatments containing 1684 μM F, the predicted activity of F^- decreased as the concentration of Al in these solutions increased (Table 6.1). Speciation of these solution with the computer modelling program MINTEQA2 gave slightly different activities of AlF for some treatments (Figure 6.1).

In Experiment 6.3, the molar ratio of Al:F was maintained constant (0.44), at the ratio which was found to be toxic in Experiment 6.2, and a series of concentrations added to solutions up to 1684 μM F. The predicted Al_r activity was 0.05 μM in the treatment containing the lowest concentration of Al (Table 6.1).

6.3.3 *Measurement of free fluoride concentrations in solution*

Fluoride ion concentrations in solution measured using the NCB correlated well with calculated values (Fig. 6.2a).

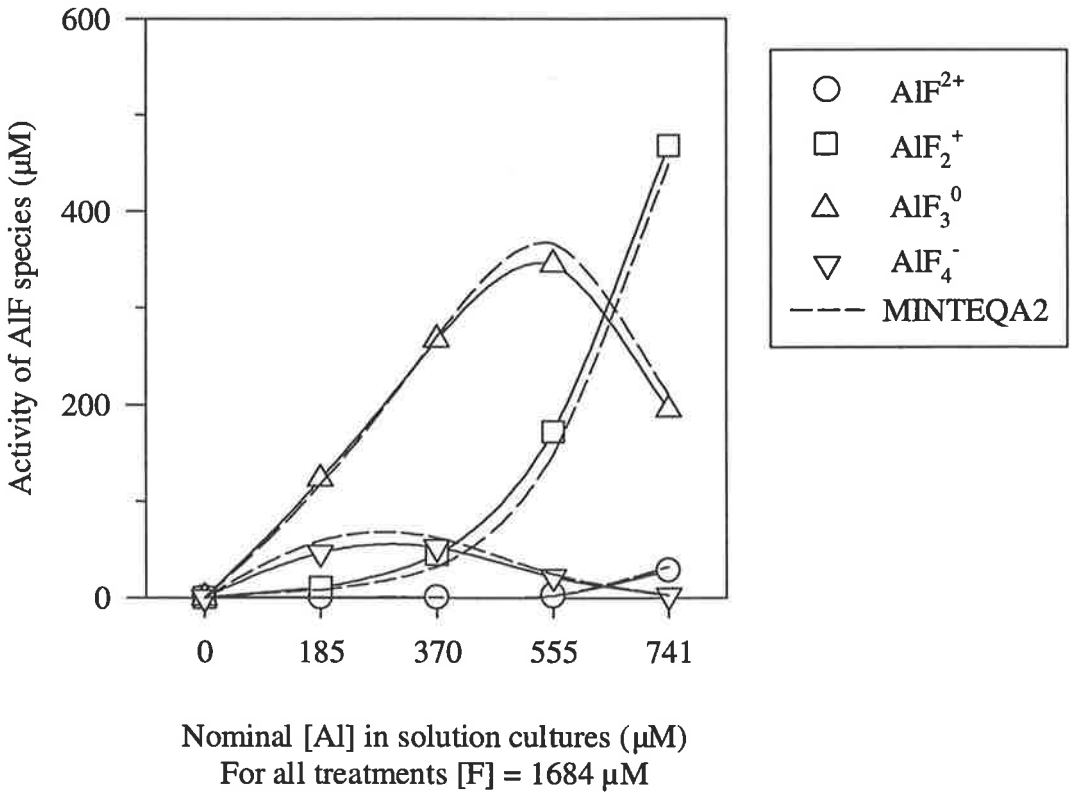


Figure 6.1 Changes in AlF speciation of solutions calculated with GEOCHEM-PC. Treatments are from Experiment 6.2, described in Table 6.1. Activity of $Al_r < 0.1$ μM for all treatments. Broken line represents values calculated with MINTEQA2.

6.3.4 *Measurement of reactive aluminium in solution*

Al_r measured with 8-hydroxyquinoline in solutions containing Al and F (Fig. 6.2b) was much greater than Al_r predicted by GEOCHEM-PC. Al_r measured with 8-hydroxyquinoline in solutions containing Al only (Fig. 6.2b) was also much greater than that predicted with GEOCHEM-PC (37 μM).

6.3.5 *Solution ageing effects*

For solutions containing no Al, ICP-AES analyses showed, that for all elements, equilibrium was reached within 0.5 hours (first sampling) of the solutions being prepared. However, for solutions containing 370 μM Al, P concentrations in solution significantly decreased over a 600 h period from addition of stock solutions (Figure 6.3).

6.3.6 *The effect of aluminium and aluminium-fluoride activity in solution on dry weights of plants*

Dry weights of oat shoots and roots were significantly ($p < 0.05$) decreased when predicted Al_r activities (in the absence of F) exceeded 37 and 23 μM respectively (Experiment 6.2, Figure 6.4). However, when both Al and F were present in solution, limitations to plant growth could not be explained solely by the predicted Al_r activities in solution (Figure 6.4). When the ratio of Al:F increased to 0.44, the presence of AlF complexes in solution (Experiment 6.2) led to significantly lower ($p < 0.01$) dry weights, despite predicted activities of Al_r being less than 0.1 μM, activities which were non-phytotoxic in the absence of F (Figure 6.4). Growth reductions in plants grown in solutions containing Al and F were accompanied by leaf tip chlorosis (yellowing) and necrosis (burning), general symptoms of F toxicity (Rauch, 1983). Mottled interveinal chlorosis was also

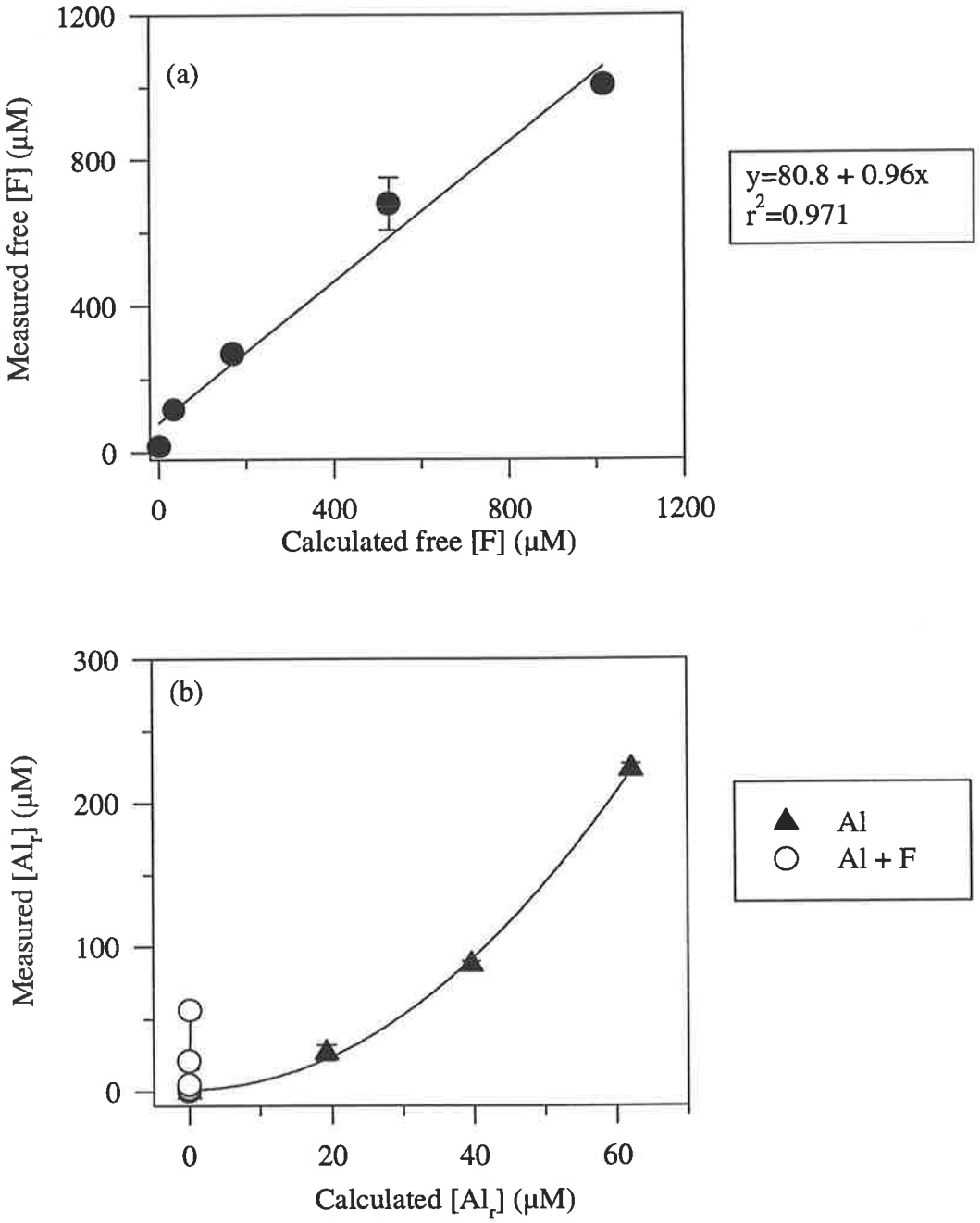


Figure 6.2 (a) Relationship between calculated (GEOCHEM-PC) F^- concentration in solutions containing Al with measured concentrations (see Section 6.2.5) (b) Comparison between measured (8-hydroxyquinoline) and calculated Al_r in solutions with and without F.
Error bars for measured concentrations represent one standard deviation from the mean and were generally less than the size of the symbol, n = 3.

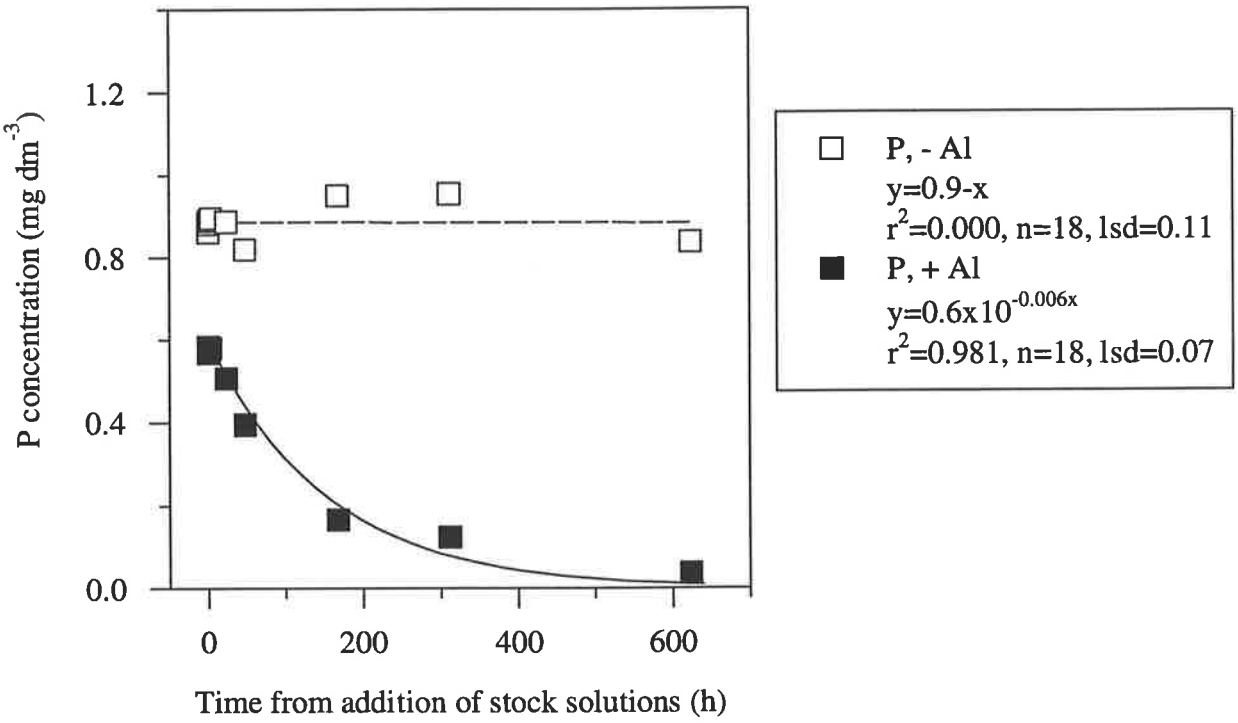


Figure 6.3 Effect of time on total P concentrations in solution with and without Al. Points are means, n = 2.

noted in oats grown as described in Experiment 6.2 (Plate 6.1). However, these symptoms were absent when either free F^- only, or Al_r only were present at activities which limited growth.

Dry weight decreases similar to those of oats were also obtained with tomatoes grown as described for Experiments 6.1 and 6.2. Dry weights of tomato shoots and roots were significantly ($p < 0.05$) decreased when predicted Al_r activities in the absence of F exceeded 12 μM (Experiment 6.2) (Figure 6.5). However, the poorer growth in solutions with Al and F was not accompanied by leaf tip necrosis.

Linear regression of plant dry weights from Experiment 6.2 with predicted Al_r and AlF species in solution showed plant dry weights to be negatively correlated with predicted activities of Al_r, AlF^{2+} and AlF_2^+ (Table 6.2). However, in the presence of F (Experiment 6.2, $Al_r < 0.1$), Al_r activities were at least 2 orders of magnitude lower than those found to be toxic in the absence of F (Experiment 6.1, Al_r = 12-37 μM).

Dry weights of tomato and oat shoots from plants grown in 741:3368 μM Al:F (Experiment 6.2) were not significantly different from controls (Figure 6.6). Activities of AlF_3^0 and AlF_4^- with this treatment were greater than all other treatments in Experiment 6.2.

Plate 6.1 Necrotic symptoms of oats grown in solution cultures containing 741:1684 µM Al:F.

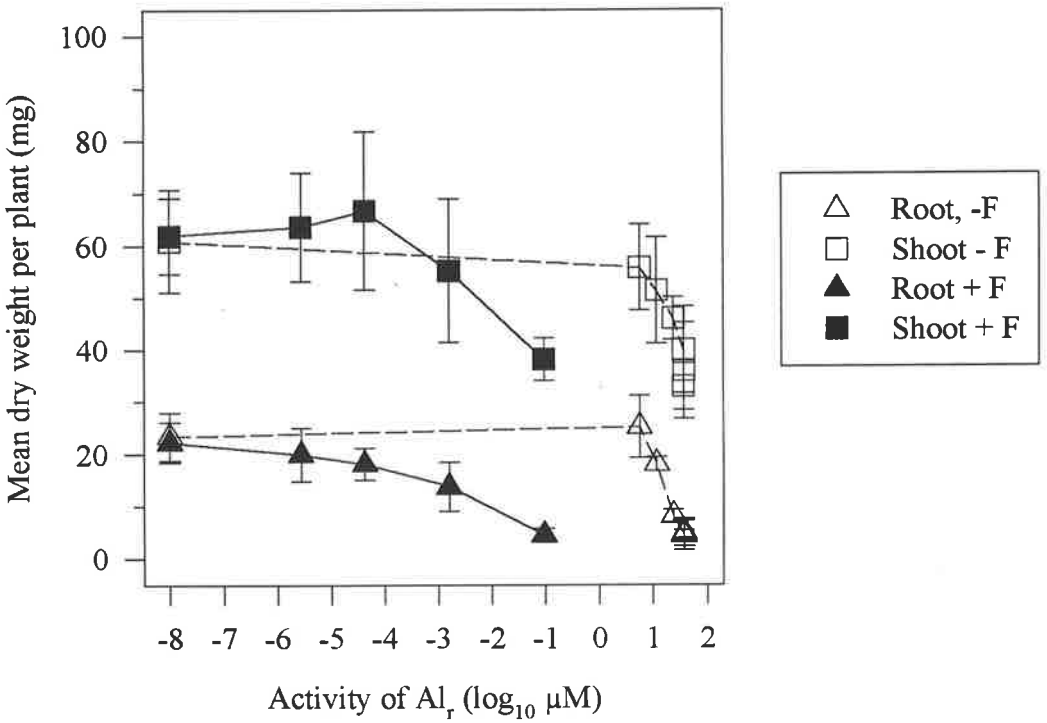


Figure 6.4 Effect of Al_r in solutions with and without F on dry weights of oats. Error bars represent one standard deviation from the mean, n = 3.

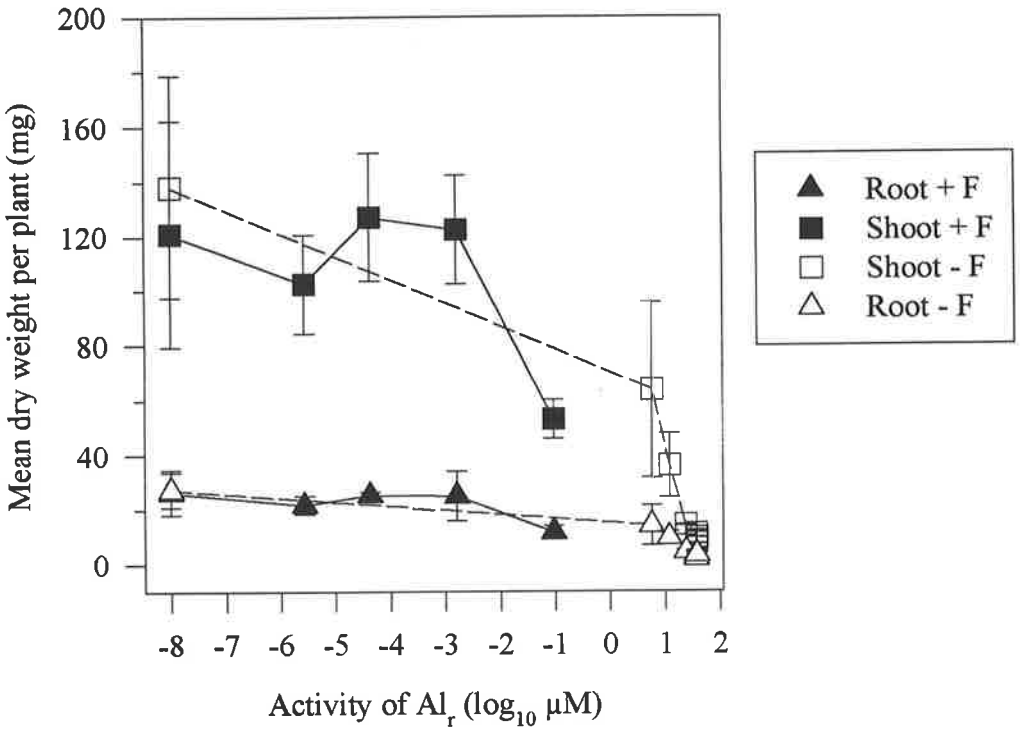


Figure 6.5 Effect of Al_r in solution cultures with and without F on dry weights of tomatoes. Error bars represent one standard deviation from the mean, n = 3.

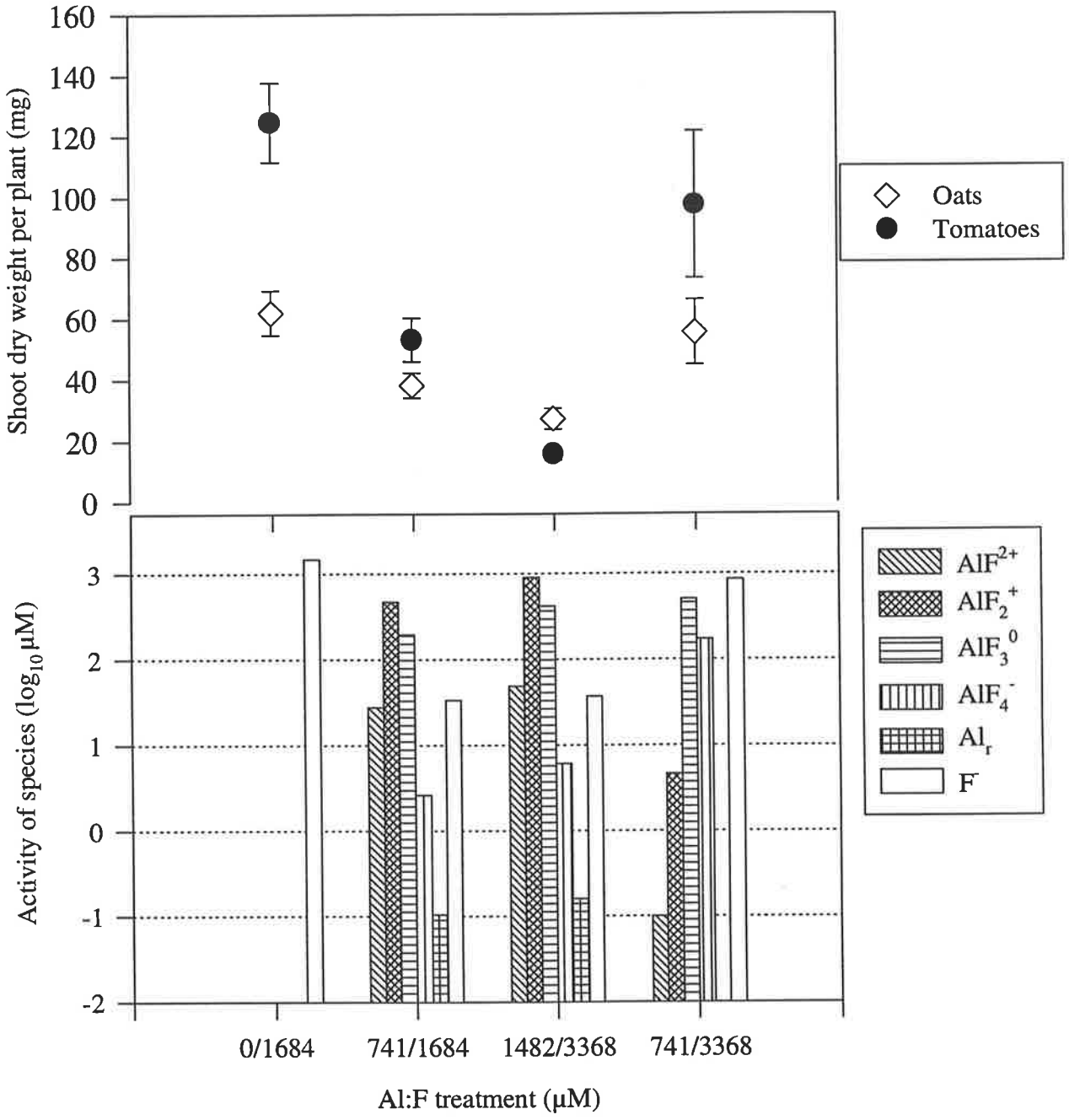


Figure 6.6 Effect of Al_r, F and AlF species in solution cultures on the dry weights of oat and tomato shoots (Experiment 6.2, Table 6.1). Error bars represent one standard deviation from the mean, n = 3.

Table 6.2 Regression coefficients (r^2) for Al and AlF species regressed with plant dry weights and concentrations of Al and F in shoots (Experiment 6.2, 1684 µM F).

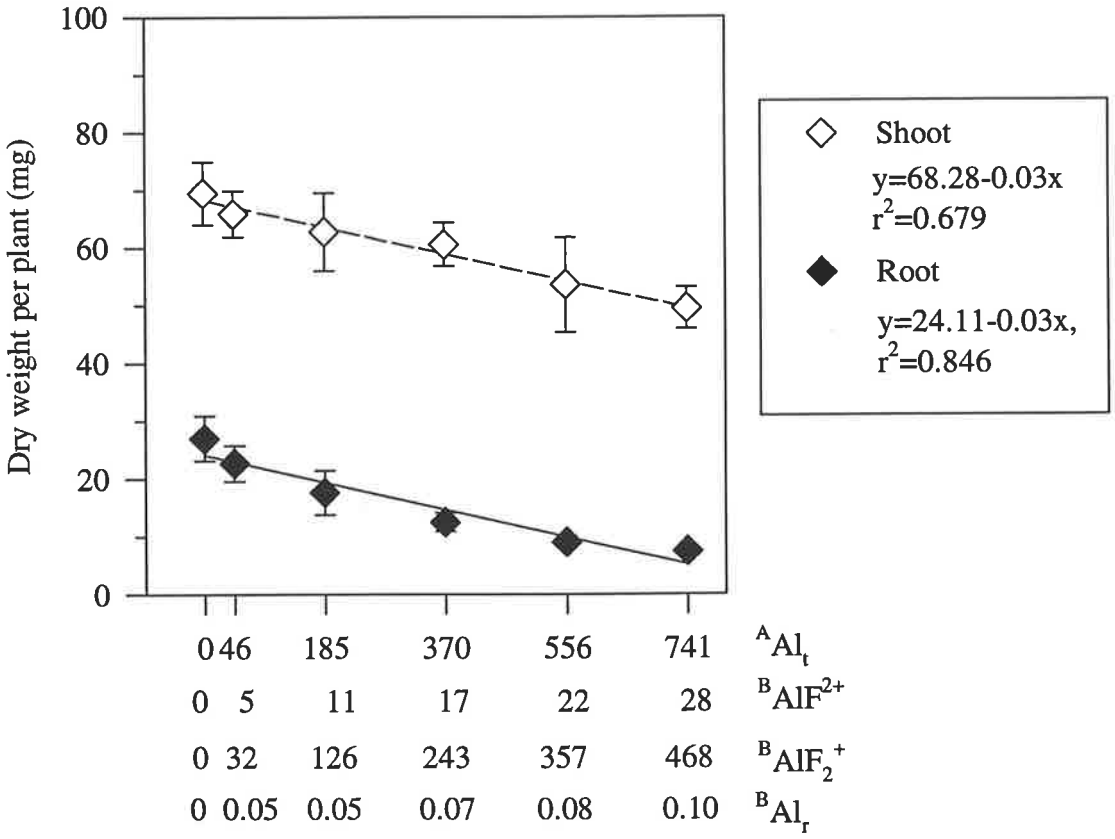
AlF Species	Linear correlation of AlF species in solution culture with plant dry weight.				Correlation of AlF species in solution culture with F and Al concentrations in shoots.[A]			
	Shoots		Roots		F		Al	
	Tomato	Oat	Tomato	Oat	Tomato	Oat	Tomato	Oat
Al_r	0.77[B]	0.65[B]	0.69[B]	0.62	0.91[B]	0.31	0.84[B]	0.62
AlF^{2+}	0.76[B]	0.67[B]	0.69[B]	0.70[B]	0.91[B]	0.64[B]	0.84[B]	0.91[B]
AlF_2^+	0.72[B]	0.68[B]	0.64[B]	0.67[B]	0.88[B]	0.77[B]	0.85[B]	0.93[B]
AlF_3^0	<0.1	<0.1	<0.1	0.05	<0.1	<0.1	0.18	0.26
AlF_4^-	0.39	0.35	0.34	0.23	<0.1	0.35	0.12	0.26

[A] Equation 6.1, see Figures 6.9 and 6.10
[B] significant $p < 0.01$

In Experiment 6.3, the ratio of Al:F was kept constant at the most phytotoxic ratio determined in Experiment 6.2 (0.44) and a series of low concentrations of Al and F employed. Growth of shoots and roots of oats were significantly limited ($p < 0.05$) even at lower Al_r activities, 0.083 and 0.053 µM respectively (Fig. 6.7). Shoot and root dry weights were significantly ($p < 0.05$) limited at AlF^{2+} and AlF_2^+ activities of 11 - 22 and 130 - 357 µM, respectively.

6.3.7 *Nutrient concentrations in plant shoots*

Nutrient concentrations in shoots were considered to be within the adequate ranges outlined by Reuter and Robinson (1986), except for P in oat shoots. Phosphorus concentrations in oat shoots were higher than the range considered adequate. However, as found in Experiments 5.1, 5.4 and 5.5 (Section 5.4.4), the range set by Reuter and Robinson (1986) (which was taken mainly from field or pot studies) as adequate, does not appear applicable to oat plants grown in solution culture (see Section 5.4.4).



[A] Total [Al] in solution culture. Al:F ratio = 0.44 (μM)
[B] Activity of species predicted with GEOCHEM-PC (μM)

Figure 6.7 Effect of positively charged AlF species on dry weights of oats. Al:F ratio constant at 0.44 (Experiment 6.3). Error bars represent one standard deviation from the mean, n = 3. Al_t = total Al added to solutions. Al_r = rhizotoxic Al (Al^{3+}, $AlOH^{2+}$ and $Al(OH)_2^+$)

6.3.8 *The effect of aluminium and aluminium-fluoride complexes in solution culture on fluoride and aluminium concentrations in plants.*

In the Al-only treatment, there was a significant relationship between the activity of Al_r in solution and the Al concentration in the shoots of oats and tomatoes ($r^2 > 0.88$). The highest two Al treatments were excluded from this regression as these concentrations led to significant precipitation of $Al_2(SO_4)_3$ and $Al(OH)_3$ (Figure 6.8).

To estimate Al and F taken up due to the presence of AlF complexes in solution (Experiment 6.2), the calculated contribution of free F^- and Al_r to Al and F concentrations in plants was first subtracted from the total Al and F concentrations in plants. Uptake of F and Al as the free ions were calculated from the regression lines of Experiments 5.1 (Figure 5.6) and 6.1 (Figure 6.8). Assuming no interaction between free F, Al_r and AlF occurred, the remaining concentrations of Al and F in the shoot would be from the presence of AlF complexes in solution. These values are referred to as corrected values. Aluminium and F concentrations in shoots of plants attributed to the presence of AlF complexes in solutions (1684 µM F treatment, Experiment 6.2), were regressed against the activities of the four major AlF complexes in solution (Table 6.2) with the Equation 6.1 where, a = the upper asymptote, b = a - y intercept and c = curvature of the line. The Al and F concentrations in both oat and tomato plants were found to correlate best with the activity of AlF^{2+} and AlF_2^+ in solution (Figure 6.9 and 6.10). Correlations with AlF_3^0 and AlF_4^- were not significant (Table 6.2).

$$y = a - (b \times 10^{(-cx)}) \qquad \text{(Equation 6.1)}$$

For solutions where AlF^{2+} and AlF_2^+ activities correlated best with F and Al concentrations in shoots, the molar ratio of F:Al concentrations in the shoots of oats and tomato plants taken up from AlF complexes in solution were compared by combining all data obtained from plants grown in solutions containing 1684 μM F (Experiment 6.2). Fluoride concentrations were linearly regressed against the Al concentrations (Fig. 6.11). For every mol of Al in the shoot tissue there was 1.54 mol of F.

Corrected concentrations of F and Al in tomato and oat shoots grown in solutions with the highest activities of AlF_3^0 (741:3368 μM Al:F, Experiment 6.2) showed significant decreases ($p < 0.05$) in Al concentrations, when compared with tomato and oat shoots grown in treatments with the highest activities of AlF_2^+ (Figure 6.12). The mean molar ratio of F:Al (calculated from corrected concentrations as described above) in oat and tomato shoots grown in solution with the highest activities of AlF_3^0 was 3.0 ± 0.2.

For plants grown in solutions with 741:1684 μM Al:F, assuming uptake of F by plants was due to the presence of AlF_2^+ (the dominant species in these solutions), $AlF_2{}^+$S-UCE of oats and tomatoes were 17 ± 0.4 and 40 ± 8 $dm^3\ kg^{-1}$, respectively. For plants grown in solutions with 741:3368 μM Al:F, assuming uptake of F by plants was due to the presence of AlF_3^0 (the dominant species in these solutions) AlF_3^0 S-UCE of oats and tomatoes were 18 ± 2 and 35 ± 2 $dm^3\ kg^{-1}$, respectively.

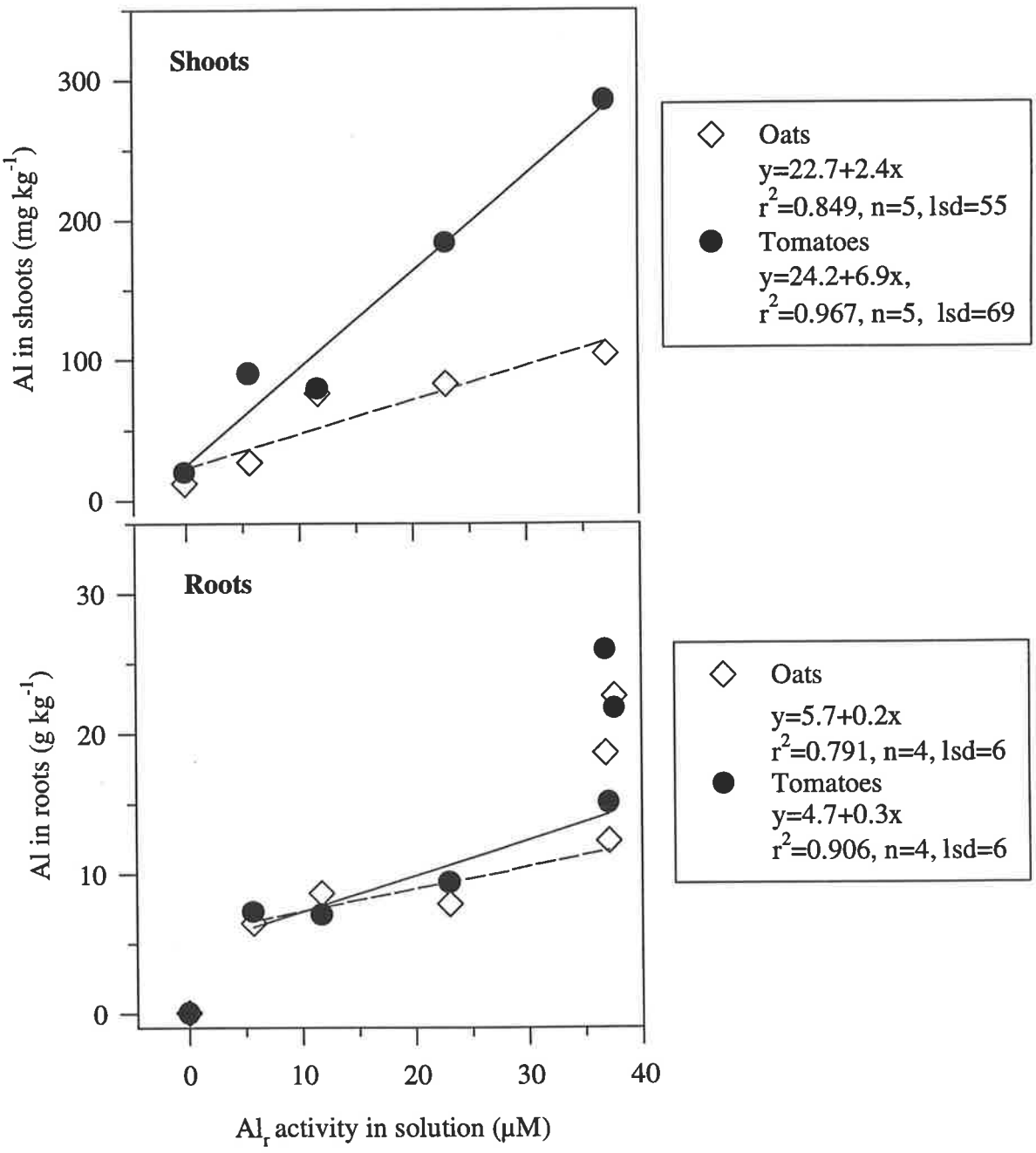


Figure 6.8 Effect of Al_r (rhizotoxic Al) on Al concentations in roots and shoots of oat and tomato plants. Points are means of bulked samples.

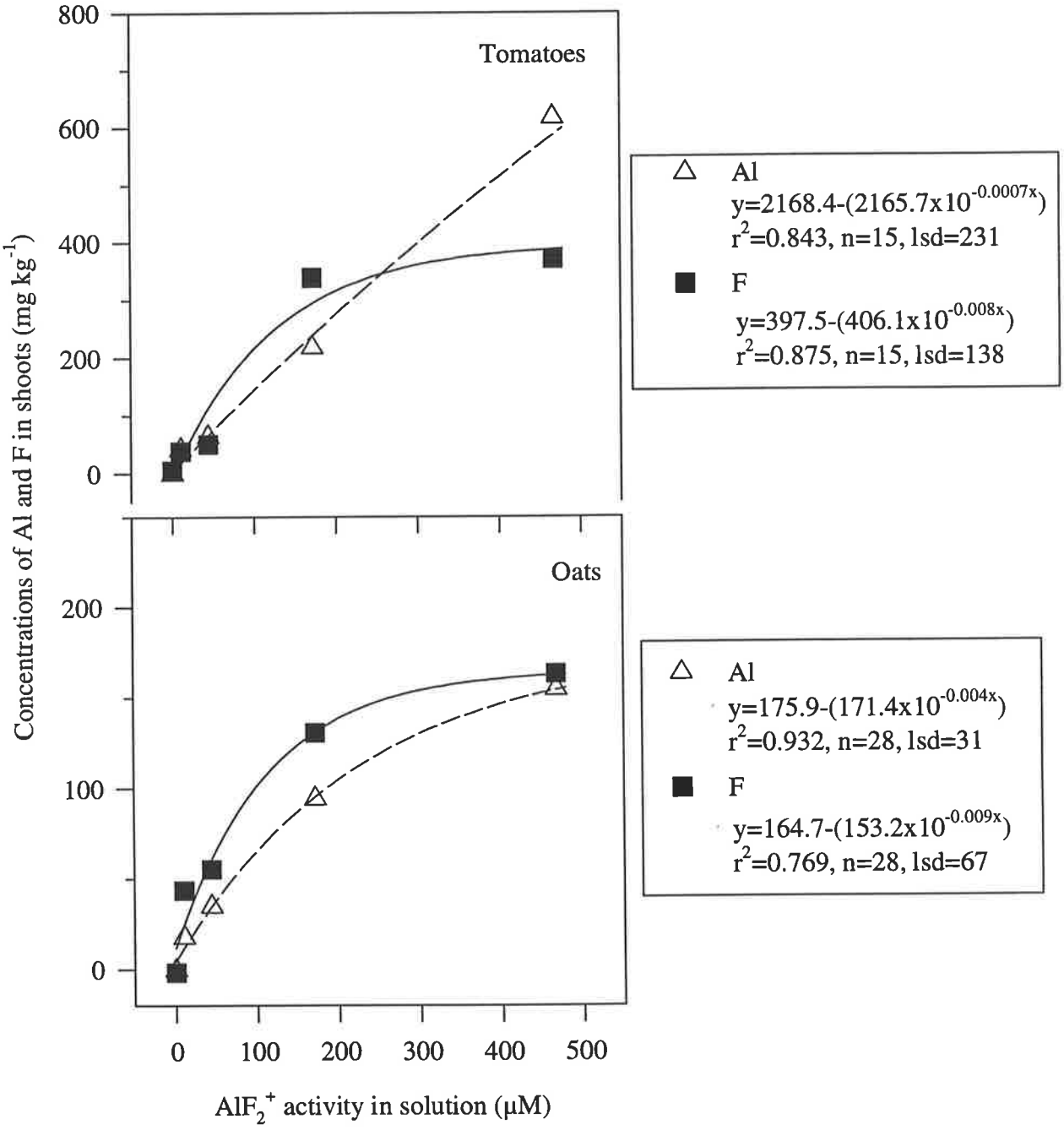


Figure 6.9 Relationship between AlF_2^+ activity in solution and Al and F concentrations in plant shoots. Plant concentrations of F and Al are corrected concentrations (*i.e.* exclusion of Al and F estimated to be taken up due to the free ion activity in solution). Points are means, n = 3 for tomatoes and n = 5-6 for oats.

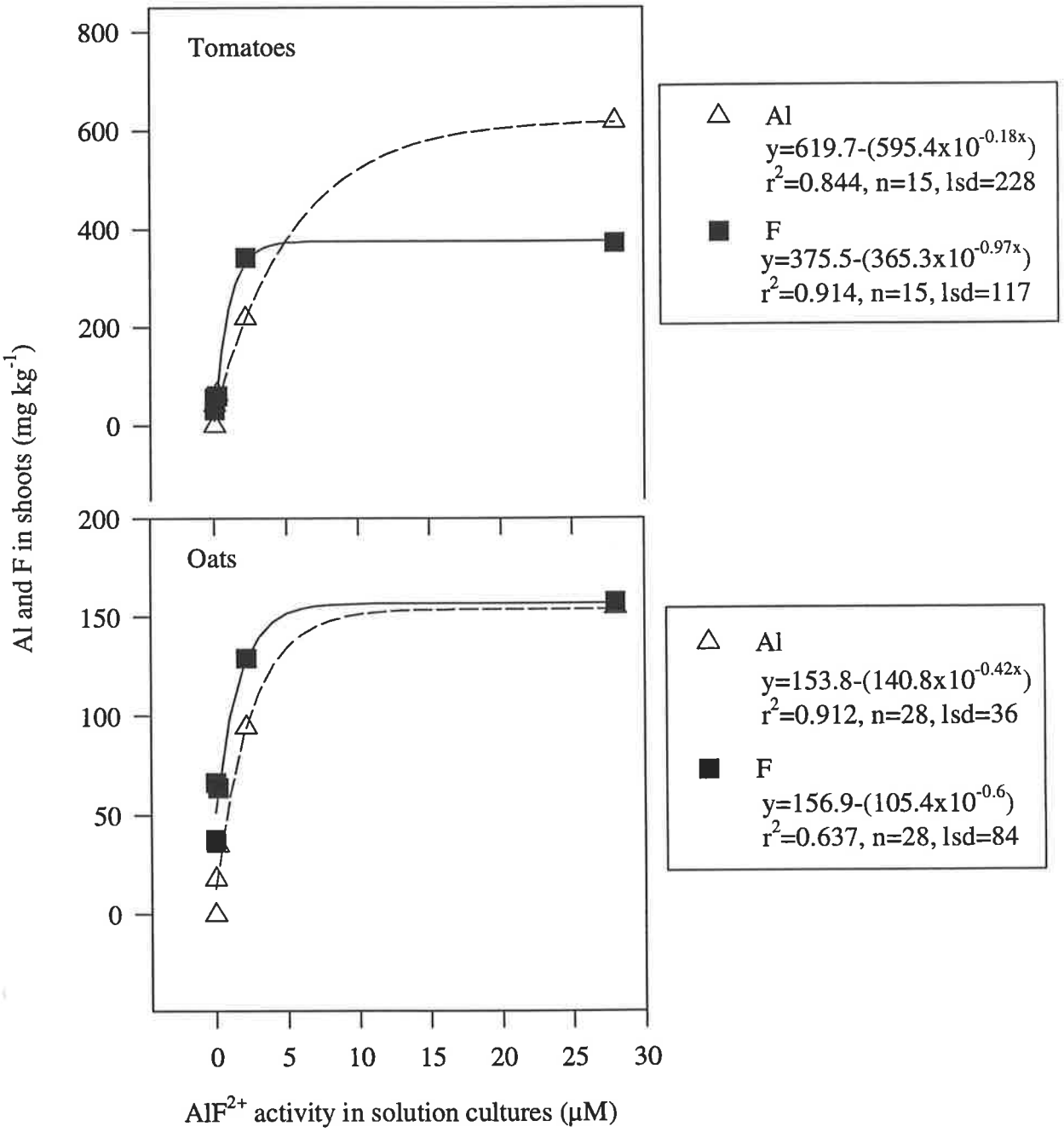


Figure 6.10 Relationship between AlF^{2+} activity in solution and Al and F concentrations in plant shoots. Plant concentrations of F and Al are corrected concentrations (*i.e.* exclusion of Al and F estimated to be taken up due to the free ion activity in solution). Points are means, n = 3 for tomatoes and 5-6 for oats .

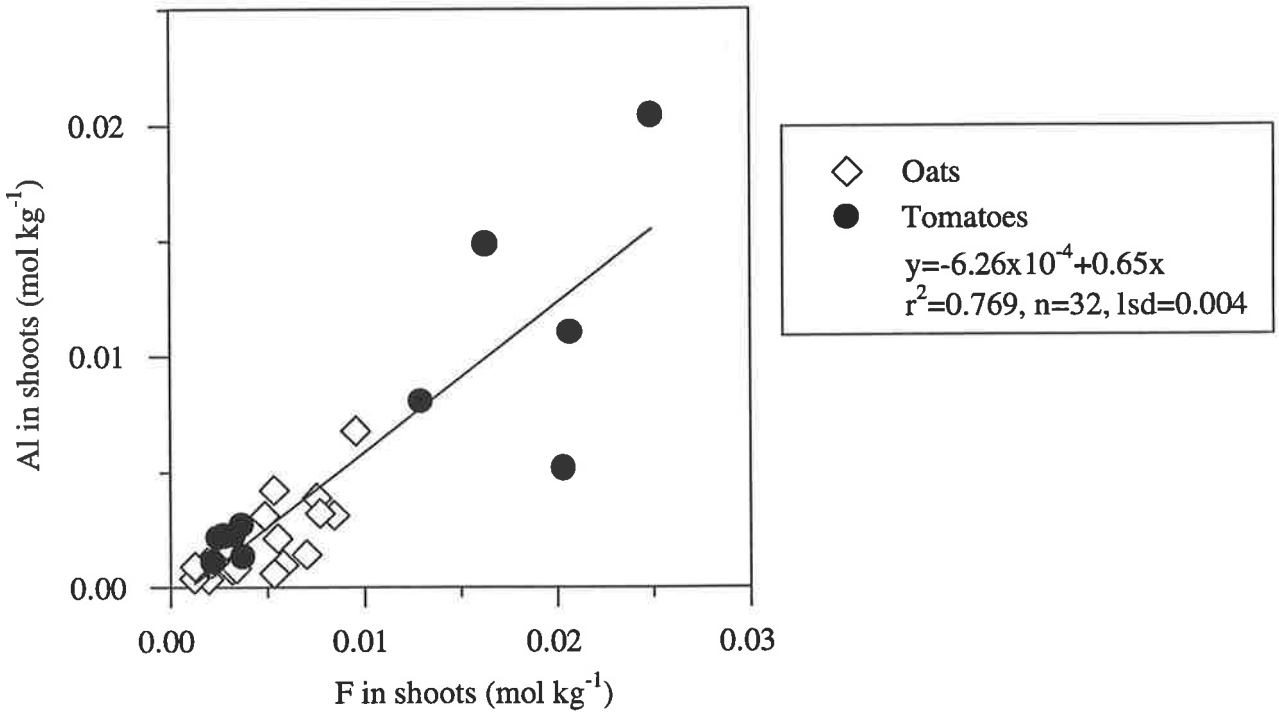


Figure 6.11 Relationship between F and Al concentrations in the shoots of oats and tomatoes grown in solutions containing 1684 μM F (Experiment 6.2).

Corrected Al and F concentrations in tomato and oat plants (Figure 6.13) differed from those in the shoots only (Figure 6.12). Corrected F concentrations (*i.e.* F taken up due to the activity of AlF in solution) in the plants grown in solutions from the treatments containing F only ranged from -48 to 890 mg F kg^{-1} (negative values are artifacts of the correction for Al^{3+} and F^- activity in solution as described above). However, if the F only treatment is excluded from the analysis of variance, concentrations of F in tomato plants grown in solutions containing 556:1684 and 741:3368 μM Al:F increased significantly from the plants grown in the solutions containing 185:1684 and 370:1684 Al:F. Fluoride concentrations in the tomato plants grown in the solutions of the 741:3368 μM Al:F treatment were significantly greater than F concentrations in plants from all other treatments. There was no significant difference between treatments of F concentrations of oat plants when plants from the F only treatment were excluded from the analysis of variance. Aluminium concentrations in both plants were similar to plant shoots, where plants grown in solutions from the 741:1684 μM Al:F treatment were significantly greater than Al concentrations in plants grown in all other treatments.

For plants grown in solutions with 741:1684 μM Al:F, assuming uptake of F by plants was due to the presence of AlF_2^+ (the dominant species in these solutions), AlF_2^+ P-UCE of oats and tomatoes were 16.1 ± 1.9 and 68.8 ± 10.9 $dm^3\ kg^{-1}$, respectively. For plants grown in solutions with 741:3368 μM Al:F, assuming uptake of F by plants was due to the presence of AlF_3^0 (the dominant species in these solutions) AlF_3^0 P-UCE of oats and tomatoes were 21.5 ± 6.9 and 33.3 ± 1.6 $dm^3\ kg^{-1}$, respectively.

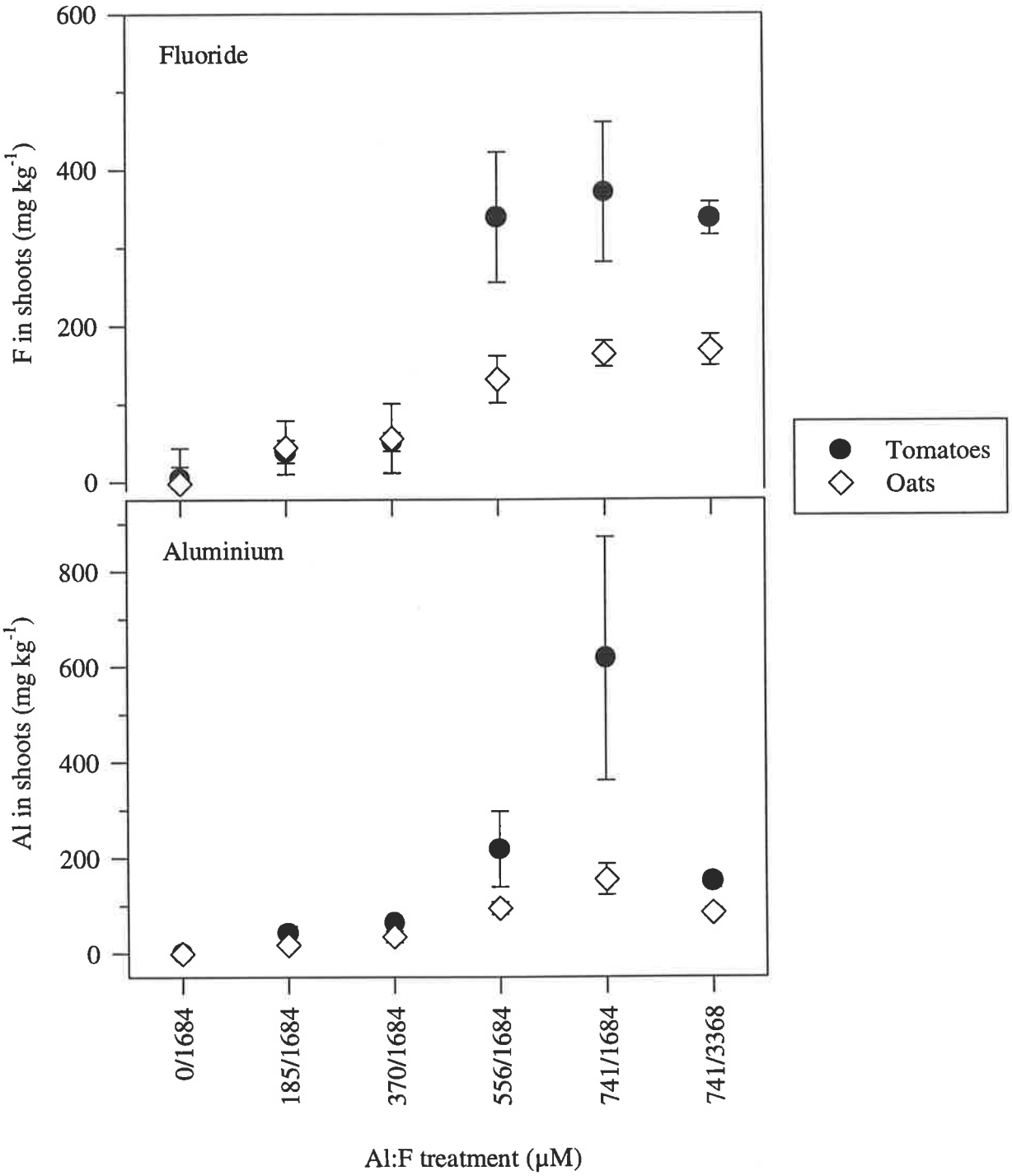


Figure 6.12 Differences in Al and F concentrations in the shoots of tomatoes and oats grown in solutions containing a variety of Al:F treatments. Plant concentrations of Al and F are corrected concentrations (*i.e.* exclusion of Al and F estimated to be taken up due to the free ion activity in solution). Error bars represent one standard deviation from the mean, n = 3 for tomatoes and n = 5-6 for oats.

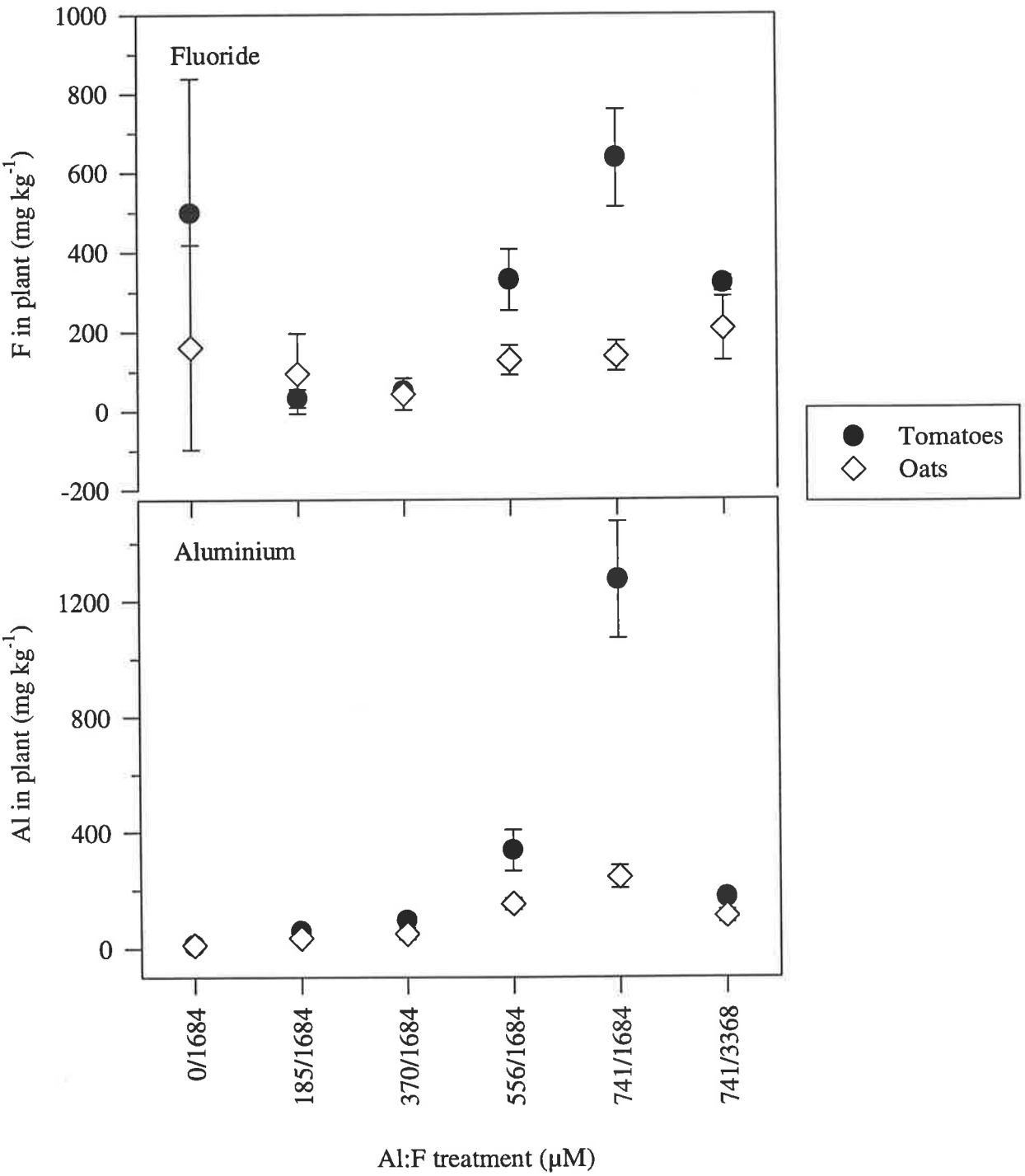


Figure 6.13 Differences in Al and F concentrations in tomato and oat plants grown in solutions containing a variety of Al:F treatments. Plant concentrations of Al and F are corrected concentrations (*i.e.* exclusion of Al and F estimated to be taken up due to the free ion activity in solution). Error bars represent one standard deviation from the mean, n = 3 for tomatoes and n = 5-6 for oats.

6.4 Discussion

6.4.1 *Ionic species of aluminium and fluoride in solution*

The agreement between measured and calculated free F concentrations in the solutions containing Al (Fig. 6.2a), support the calculations and stability constants used in GEOCHEM-PC for the speciation of F^- under these conditions. However, measurement of Al_r with the 8-hydroxyquinoline method of James *et al.* (1983), which has been found to be selective for Al_r in the presence of AlF (Alva *et al.*, 1989), did not agree well with predicted values for solutions containing Al, or Al and F. It is probable that the 8-hydroxyquinoline method allowed some decomplexation of AlF, $Al_2(SO_4)_3$ and $Al(OH)_3$ (Alva and Sumner, 1989; Noble *et al.*, 1988; Alva *et al.*, 1989). Other methods of quantifying cationic AlF species have given good agreement with computer speciation models (Willett, 1989), supporting the validity of computer modelling of chemical species under well defined conditions.

The small differences between activities of AlF species determined by GEOCHEM-PC and MINTEQA2 probably arise from differences in the thermodynamic constants (Table 3.3) used for the calculations, and because MINTEQA2 does not consider complexes of Al and phosphate (*e.g.* $AlHPO_4^+$) which are considered by GEOCHEM-PC.

6.4.2 *Solution ageing effects*

Decreases of P over time in solutions containing Al suggest that P undergoes a slow reaction with Al, forming a solid which is precipitated out of solution. The formation of this solid is not predicted by GEOCHEM-PC probably because, as a footnote in the thermodynamic database states, values for Al and PO_4^- complexes are estimates only and should not be considered reliable.

Decreases in available P in such solutions may affect plant dry weights and were reflected in lower P concentrations in plants grown in solutions containing Al but not F (*e.g.* oats grown in Al-only treatments with Al concentrations greater than 185 μM Al contained 0.04 ± 0.006 % P in their shoots). In solutions where Al and F were present, a significant proportion of the Al is complexed strongly with F and would not precipitate P. Phosphate concentrations in shoots of oats and tomatoes grown with AlF treatments suggested that these plants were not P deficient (*e.g.* concentrations of P in oat shoots ranged between 1.1 and 1.5 %, which were shown to be adequate in Table 5.2).

These data suggest that the growth decreases due to Al only in solution could be partly due to P deficiencies. Toxic responses by plants to Al are often expressed in symptoms similar to P deficiency (Foy 1974). Restriction of growth due to AlF treatments could not be due directly to P deficiencies (*i.e.* low P concentrations in plant shoots). However, the toxic response of plants to AlF could be due to increased Al concentrations in the shoots precipitating Al and P.

6.4.3 *The effect of aluminium fluoride complexes in solution on dry weights of plants*

The activities of Al_r in solutions containing F (1684 μM, Experiment 6.2) which were rhizotoxic were three orders of magnitude lower than those found to be rhizotoxic in solution cultures containing Al only (Experiment 6.1), indicating that Al_r was unlikely to have limited plant growth in these solutions. There were significant growth reductions for tomatoes and oats as the Al:F ratio approached 0.44 (Figure 6.4 and 6.5, 0.1 μM Al_r). These data suggest that Al species in the solution other than Al_r (*i.e.* AlF^{2+}, AlF_2^+, AlF_3^0 and AlF_4^-) limited the growth of tomato and oat plants.

The linear regressions of predicted Al, F and AlF complexes with plant dry weights were best correlated with Al_r, AlF^{2+} and AlF_2^+ (Table 6.2). The activities of both Al_r and F^- were below those determined to be phytotoxic (Experiments 5.1 and 6.1), suggesting that one or both of the positively charged AlF species are phytotoxic. Differences in visual symptoms of toxicity in oats produced by the different treatments, also lend support to the hypothesis that the toxicity produced by Al and F was not due to Al_r acting at the root surface, but could be due to: a) Al-induced F toxicity in the shoot, b) F-induced Al toxicity in the shoot, or c) toxicity of the AlF complexes within the root and shoot.

The visual symptoms observed in oat shoots (Experiment 6.2 and 6.3) were similar to those found by Woltz (1964a and b) in leaves of gladioli. Woltz (1964a) found that gladioli grown in soils of pH 4.3 - 6.1, which had been amended with superphosphate, developed symptoms of necrosis on inner areas of the leaves. With addition of superphosphate (which contains approximately 2-3 % F) to the low pH soils, AlF complexes would be present.

Nagata *et al.* (1993), using NMR spectrometry, found evidence that the AlF complex is taken up and transported in tea plants (know accumulators of F and Al) in this form until these reach the leaf where they are dissociated. Similar processes may affect all plants, and if so, it is unlikely that the leaf tip necrosis would be due the AlF complexes themselves. Aluminium tends to accumulate in the roots of plants and symptoms of Al toxicity are stubby roots with brown tips. Symptoms of Al toxicity in the shoot usually resemble those of P deficiency (abnormally dark green leaves; purpling of the stems and leaves), due to the precipitation of Al phosphates in the soil solution or within the root (Foy, 1974). Similar symptoms and mechanisms may also act in the shoot. The symptoms on the oats and tomatoes grown in Experiment 6.2 were not characteristic of P deficiency, suggesting that poor growth was not due to Al toxicity.

The toxicity shown by plants grown in the treatments with an Al:F ratio of 0.44 were alleviated by increasing the concentration of F which decreased the Al:F ratio to 0.22. In the 741:3368 μM Al:F treatment concentrations of AlF_3^0 and AlF_4^- were greater than all other treatments, discounting AlF_3^0 and AlF_4^- as the toxic species (Figure 6.6). These data support the hypothesis that one or both of the positively charged AlF species are phytotoxic.

Sikora *et al.* (1992) found that high amounts of F (170 mg F kg^{-1}) added to soils increased Al concentrations in soil water extracts to approximately 240 μM, a concentration which Sikora *et al.* (1992) suggested was probably responsible for decreasing the growth of maize by inducing Al toxicity. However the molar ratio of Al:F in these extracts, calculated from the data of Sikora *et al.* (1992), was 0.32. The data of Sikora *et al.* (1992) were analysed with GEOCHEM-PC. Due to the limited data presented by Sikora *et al.* (1992), ionic species were approximated using pH, and the concentrations of F and Al only. GEOCHEM-PC predicted Al_r activities to be less than 0.02 μM, also suggesting some complexes of AlF are phytotoxic. The activity of AlF_2^+ in these solutions was predicted to be approximately 70 μM, close to the critical activity for oats determined here (Figure 6.7). Sikora *et al.* (1992) studied *Zea mays.* Variation in species sensitivity of plants to F or AlF toxicity could explain the growth restrictions observed by Sikora *et al.* (1992) compared to oats and tomatoes studied in this thesis.

It is difficult to compare data from this thesis with other published data as the concentrations of Al and F have not be high enough in other studies to allow similar activities of AlF species to be present. Takmaz-Nisancioglu and Davison (1988) did not find any significant difference from controls in dry weights of beans (*Phaseolus vulgaris* L.) grown in solutions treated with 175 μM AlF_3^0. GEOCHEM-PC was used to calculate activities (μM) of ionic species in solution from the data of Takmaz-Nisancioglu and Davison (1988); $Al_r < 0.006$, $AlF^{2+} = 2$ $AlF_2^+ = 83$, AlF_3^0

= 87 and AlF_4 = 2. The 175 μM AlF_3^0 treatment tended to show lower dry weights than that of the control treatments, even though Al_r would be unlikely to limit growth. The activities of AlF_2^+ in AlF treatments of Takmaz-Nisancioglu and Davison (1988) were less than those which were found to restrict dry weights of oat shoots significantly in Experiment 6.3 (Figure 6.7).

GEOCHEM-PC was used to calculate the activities of AlF_2^+ or AlF^{2+} in solution from the data of Cameron *et al.* (1986) and MacLean *et al.* (1992) who used maximum concentrations of F ≤ 200 μM . Activities were less than 65 μM. The low Al and F concentrations, combined with the short growth periods in the experiments conducted by Cameron *et al.* (1986) and MacLean *et al.* (1992) would explain why toxic responses to AlF_2^+ or AlF^{2+} were not observed.

Changes in uptake and toxicity of both Al and F have also been found in mammals and bacteria (Kessabi *et al.*, 1986; Kraus and Forbes, 1992; Ahn and Jeffery, 1994).

It is difficult to identify the toxic species as all species in solution do not vary independently. The data in this thesis support the findings of others that AlF complexes are less toxic to plants than Al_r (Cameron *et al.*, 1986; Tanaka *et al.*, 1987; MacLean *et al.*, 1992). However, the data also show that some AlF species are potentially phytotoxic at concentrations which could exist in some polluted soils. Obviously, the complexity of the speciation of AlF is such that it is difficult to change one solution parameter without affecting others, and therefore difficult to identify one species of AlF which is taken up by the plant.

6.4.4 *The effect of aluminium and aluminium-fluoride complexes in solution culture on fluoride and aluminium concentrations in plants*

When plant roots were exposed to Al without F or F without Al in solution, the concentrations

of both elements in the shoots of tomato and oat were positively related to their activities in solution (Figures 5.6 and 6.8). However, when roots were exposed to a constant concentration of F (1684 μM) and an increasing concentration of Al (Experiment 6.2) where a range of AlF species were present (Fig 6.1), uptake and translocation of F to the shoots of tomatoes and oats was significantly increased as the Al:F ratio approached 0.44. This suggests that F uptake and translocation was influenced by the ionic species of AlF present in solution. Nagata *et al.* (1993) found that the concentration of F in tea leaves was greater when detached shoots were soaked in solutions where AlF^{2+} and AlF_2^+ were predominant when compared with solutions where AlF_3^0 was predominant. The data of Nagata *et al.* (1993) support the data presented in this thesis. However, the mechanisms involved in uptake AlF by the plant roots were by-passed in Nagata's work making direct comparisons difficult.

The AlF_2^+ ion is predicted to be the predominant species at an Al:F molar ratio of 0.44. Regressions of corrected Al and F concentrations in plants (attributed to uptake as AlF) against AlF species in solution correlated best with AlF^{2+} and AlF_2^+ activities (Table 6.2) (Experiment 6.2, 1684 μM F treatments). The molar ratio of Al:F concentrations in shoots of tomatoes and oats was 1.54 (Figure 6.11), suggesting that both AlF_2^+ and AlF^{2+} were taken up and translocated to the shoot. The curvilinear nature of these uptake relationships and the possibility of an upper asymptote, as described by Equation 6.1, suggests that uptake of these species is somehow regulated by the plant. Disruption of root function may slow or prevent uptake and translocation of AlF species.

Shoots of tomatoes and oats grown in the solutions with the highest activities of AlF_3^0 (500 μM) (741:3368 μM Al:F, Experiment 6.2) and low activities of AlF^{2+} and AlF_2^+ (< 50 μM), showed significant decreases ($p < 0.05$) in Al concentrations compared with plants grown with the

highest activities of AlF_2^+ in solution (741:1684 μM Al:F, Figure 6.12). These data indicate that AlF_3^0 and AlF_4^- complexes are not as readily taken up and translocated to the shoot as Al present in AlF^{2+} and AlF_2^+ complexes. There was no significant difference between F concentrations in tomato or oat shoots between these treatments (Figure 6.12). At such high activities of AlF_3^0 in solution, it could be postulated that F was taken up as this complex. The mean molar ratio of F:Al in the shoots of plants grown in these treatments was 3.0 ± 0.2, supporting this postulate. These data also suggest that the restricted growth and toxicity observed when the Al:F ratio is 0.44 may be due to translocation of AlF to the shoots where AlF dissociates and toxicity is due to the Al (*i.e.* AlF induced Al toxicity of the shoots). No P deficiency symptoms were observed in plants grown in solution containing Al:F ratios of 0.44, suggesting the toxicity is not due to Al precipitating P within the shoot.

Assuming either AlF_2^+ or AlF_3^0 is taken up as described in Section 6.3.8, S-UCE for oats and tomatoes averaged approximately 28 $dm^3\ kg^{-1}$, approximately two to three times greater than the S-UCE of F^- (Section 5.3.4) at F activities in solution greater than 1473 μM. This indicates that AlF complexes are more readily taken up and translocated to the shoot than F^-.

In general, concentrations of F and Al in tomato plants (roots + shoots) were similar to those in the shoots alone because the ratio of shoots:roots was high. However, F in oat plants did not follow this generalisation (Figures 6.12 and 6.13). There were no significant differences between F concentrations in whole plants. These difference could be due to either: a) differences in uptake by plant species, or b) incomplete removal of AlF species adsorbed to the roots by the washing procedure. Aluminium accumulates in plant roots, and oats have a more fibrous root system and a lower ratio of shoot:root dryweight than tomatoes. Therefore, the difference found with oats plants compared to oat shoots was probably due to incomplete removal of the AlF

complex absorbed to roots by this washing procedure.

Takmaz-Nisancioglu and Davison (1988) discussed the different processes involved in AlF uptake and suggested that the AlF complex may act as a carrier for F, overcoming repulsion of F^- by the negative charge in the Donnan free space (DFS) of the roots and increasing the concentration of F at sites where it may leak past the endodermis (Takmaz-Nisancioglu and Davison, 1988). AlF species with a positive charge would be preferentially attracted to the root surface and therefore be nearer to sites of uptake. The results presented here support this hypothesis in that positively charged AlF species seem to enhance F uptake and translocation to the shoots. Positively charged species can also suppress the negative charges of the DFS. The dominant role of divalent cations in this suppression has been demonstrated by Walker and Pitman (1976). Similarly, a complex with no charge (AlF_3^0) will not be repelled by the negative charge of the DFS, and could be lipophilic enabling it to diffuse freely across the membrane. However, the effects of ionic size and degree of hydration of these complexes on membrane transport are unknown (Hewitt and Smith, 1974).

The molar ratio of Al:F in the shoots of tomatoes and oats (1.54) is different from the ratio (0.42) found by Takmaz-Nisancioglu and Davison (1988). However, these authors studied beans (*Phaseolus vulgaris* L.) and treatments were imposed without nutrients which could cause changes to membrane permeability, making a direct comparison difficult (Hewitt and Smith, 1974).

The findings in this thesis are in contrast to those of MacLean *et al.* (1992) who suggested that AlF complexes are not readily translocated to the shoots. MacLean *et al.* (1992) found that when 200 μM F was present with and without 100 μM Al, more F was taken up by wheat (*Triticum*

aestivum) in the absence of Al (14.4 mg F kg^{-1}) compared to when Al was present (11 mg F kg^{-1}). However, MacLean *et al.* (1992) did not consider the possibility that HF may form in the F only solutions. With larger plants in the last two days of their experiments, the pH values of their solutions containing only F fluctuated from 4.2 - 3.9. Using the data of MacLean *et al.* (1992) GEOCHEM-PC was used to calculate the activity of ionic species in solution. At a pH of 3.9 and a F concentration of 200 µM, a significant proportion of F would be present as HF (20 µM). These concentrations of HF would be 30 times the activity in solutions containing Al and F. Gutknecht and Walter (1981) found the permeability coefficient of HF to be six orders of magnitude higher than that of the F^- ion. The presence of HF in the solution of McLean *et al.* (1992) would significantly increase the apparent uptake of the free F-ion. This could account for the small differences in F concentrations in plant material between F and AlF treatments found by these authors.

The results in this Chapter suggest both AlF^{2+} and AlF_2^+ are taken up and translocated to the shoots of the plant, and/or the positively charged or uncharged AlF species are not excluded from the negatively charged DFS, unlike the F^- ion. This would allow F as the AlF complex to approach closer to the sites of uptake. Extrusion of protons from the plasma membranes near the sites of uptake may change the ionic species of F and Al present, affecting the uptake of these ions (Takmaz-Nisancioglu and Davison, 1988). These results also suggest that AlF_3^0 may also be taken up by the plant at high F and Al concentrations in solution, when AlF_3^0 activities are high.

6.5 Conclusions

The direct measurement of Al_r in a multi-ligand system is complex, and there are limitations to the measurement of Al_r in such a system with the 8-hydroxyquinoline method when F is present. Plant growth experiments showed that Al_r is more toxic than Al complexed with F, confirming earlier studies. However, some complexes of AlF are toxic to tomatoes and oats. The toxic species could be either, or both, of the positively charged AlF complexes, AlF^{2+} and AlF_2^+. The results in the current study also suggest that complexation of F with Al to form AlF^{2+}, AlF_2^+ or AlF_3^0 can increase uptake and translocation of F and Al in plants. Increased uptake of F and toxic responses similar to that observed in solution culture would be expected in soil solutions which contained similar concentrations of F and Al, but further work is required to confirm this.

Chapter 7

7.0 Uptake of fluoride complexed with hydrogen by plants grown in solution culture

7.1 Introduction

The dissociation constant of hydrogen fluoride (HF), a weak acid, is 3.17 (Table 2.1). In very acid soils (pH 3 - 4) a large portion of the F in solution could therefore exist as the HF complex if the concentrations of Al in solution are low (see Section 2.7.1.5). There are limited data on uptake of HF through the plant root (Kronberger, 1988). The purpose of this chapter was to determine the phytoavailability and phytotoxicity of F in solution when it is exposed to the root as HF.

7.2 Materials and methods

7.2.1 *Solution culture parameters*

Solution culture parameters were as described in Section 3.2.2.

7.2.2 *Fluoride treatments added to solution*

Tomatoes and oats were grown in solution culture with a range of pH treatments (Table 7.1). Treatments with a solution pH which was a multiple of 0.5 were triplicated, all other treatments were not replicated. These treatments were not replicated due to limited growth cabinet space. To obtain sufficient tissue for analysis of plants grown at high HF activities (low pH), where plant dry weights were significantly lower than controls, extra replications of these treatments were undertaken.

7.2.3 *Modelling of the ionic species of fluoride in solution*

The activities of F and HF were modelled with GEOCHEM-PC as described in Section 3.4.

7.2.4 *Analysis of plant material*

Plant material was analysed as described in Section 4.2.1.5. Digests were analysed for F as described in Section 4.2.2.2, Method ME_2. Plant nutrients were analysed in these digests as described in Section 3.6.2. Where treatments greatly restricted growth, replicates were bulked to obtain sufficient sample for analysis.

7.2.5 *Statistical Analyses*

Data were fitted to either Equation 6.1 or an asymmetrical sigmoidal equation (Equations 7.1) and a lsd ($p < 0.05$) of the model determined. Variations between mean plant dry weights of triplicated experiments were determined using a one way analysis of variance and the Tukey's test was used to determine significant differences between treatment means. Errors are one standard deviation from the mean and $n = 3$, unless otherwise stated.

7.3 Results

7.3.1 *Modelling of HF species in solution cultures*

In solutions containing F, the HF activity increased from 0.2 µM (pH 7.0) to 376 µM (pH 3.5) as the pH neared the pKa of HF (3.17) (Table 2.1). There was a corresponding decrease in the F^- activity (Table 7.1). The ionic strength of solutions with F ranged from 4.28 - 4.65 mM. This range was similar to that in control solutions without F (Table 7.1)

Table 7.1 Treatments imposed in solution cultures on tomato and oat plants.

	Treatments											
NaF treatments												
pH	3.5	3.7	3.9	4.0	4.1	4.2	4.3	4.4	4.5	5.0	6.0	7.0
HF activity (µM)	376	255	169	137	111	88	71	57	46	15	2	0.2
F^- activity (µM)	1177	1269	1337	1363	1384	1402	1414	1426	1435	1461	1472	1473
Ionic strength (mM)	4.65	4.51	4.41	4.39	4.37	4.34	4.32	4.31	4.29	4.27	4.25	4.28
NaCl treatments (controls)												
pH (NaCl)	3.5			4.0					4.5	5.0	6.0	7.0
Ionic strength (mM)	4.74			4.53					4.40	4.39	4.39	4.42

7.3.2 *The effect of HF activity in solution on dry weights of plants*

Dry weights of tomato and oat shoots grown in control (NaCl) solutions at pH 3.5, were significantly ($p < 0.05$) less than plants grown at pH greater than 4.0 (Figure 7.1). At pH 3.5, oats were more tolerant than tomatoes of the acidic conditions. When F was present, dry weights of tomatoes and oats shoots were significantly reduced ($p < 0.05$) as pH of the solution culture decreased below 4.3 (HF activity = 71 µM) and 4.0 (HF activity = 137 µM), respectively (Figure 7.1). The dry weights of roots were affected in a similar manner (Figure 7.2). Severe leaf necrosis was observed only in plants grown in solutions with F at values which limited growth (Plate 7.1).

The relationship between plant dry weights and F activities in solution was best described by a sigmoidal model, as described in Section 5.3.4, with an extra parameter (e) which allowed the curve to be asymmetrical (Equation 7.1, where a = the first asymptote, b = the slope parameter, c = the value at the inflection point, d = the second asymptote and e = asymmetrical parameter).

$$y = \frac{(a-d)}{[1+(\frac{x}{c})^{b}]^{e}} + d \qquad \text{(Equation 7.1)}$$

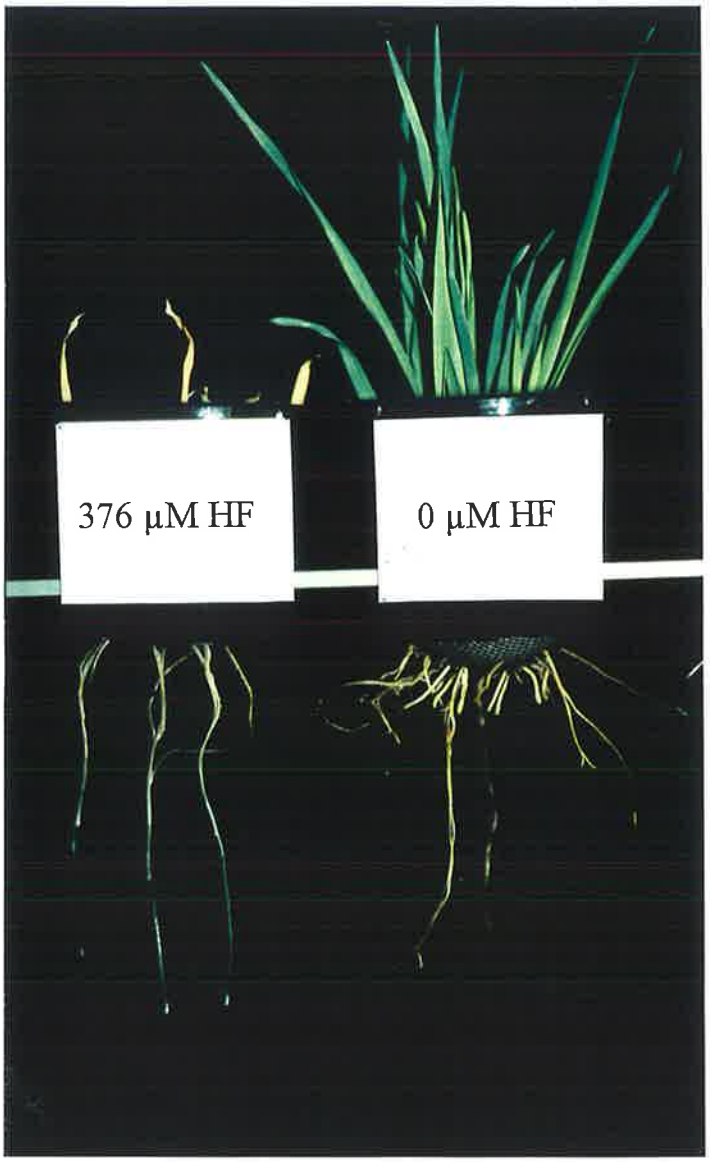


Plate 7.1 Necrotic symptoms of oats grown in solution cultures containing 376 (left) and 0 (right) µM HF, pH 3.5.

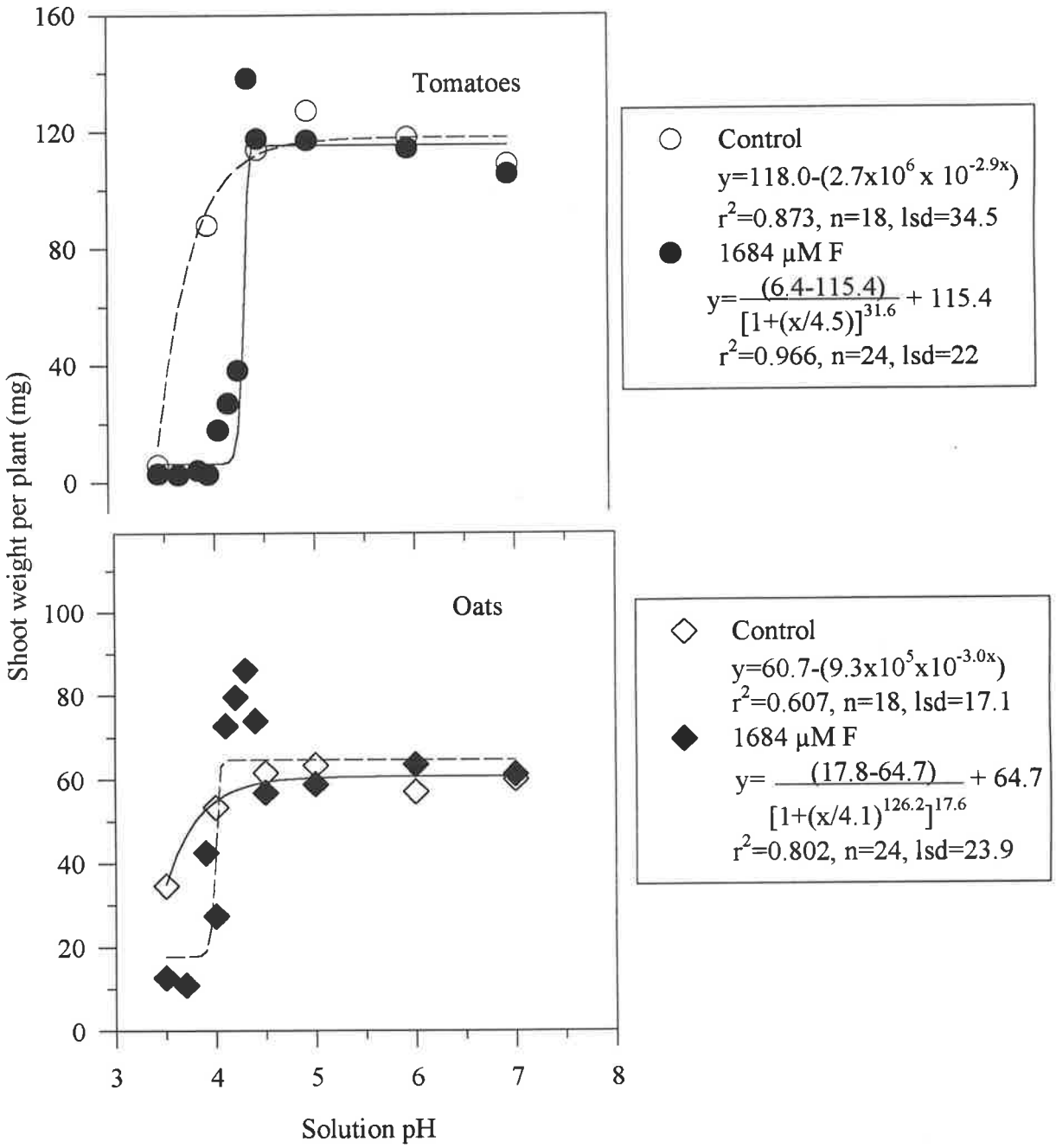


Figure 7.1 Effect of solution pH with and without F on shoot dry weights. Individual values except when pH is a multiple of 0.5, in which case they represent means, n = 3.

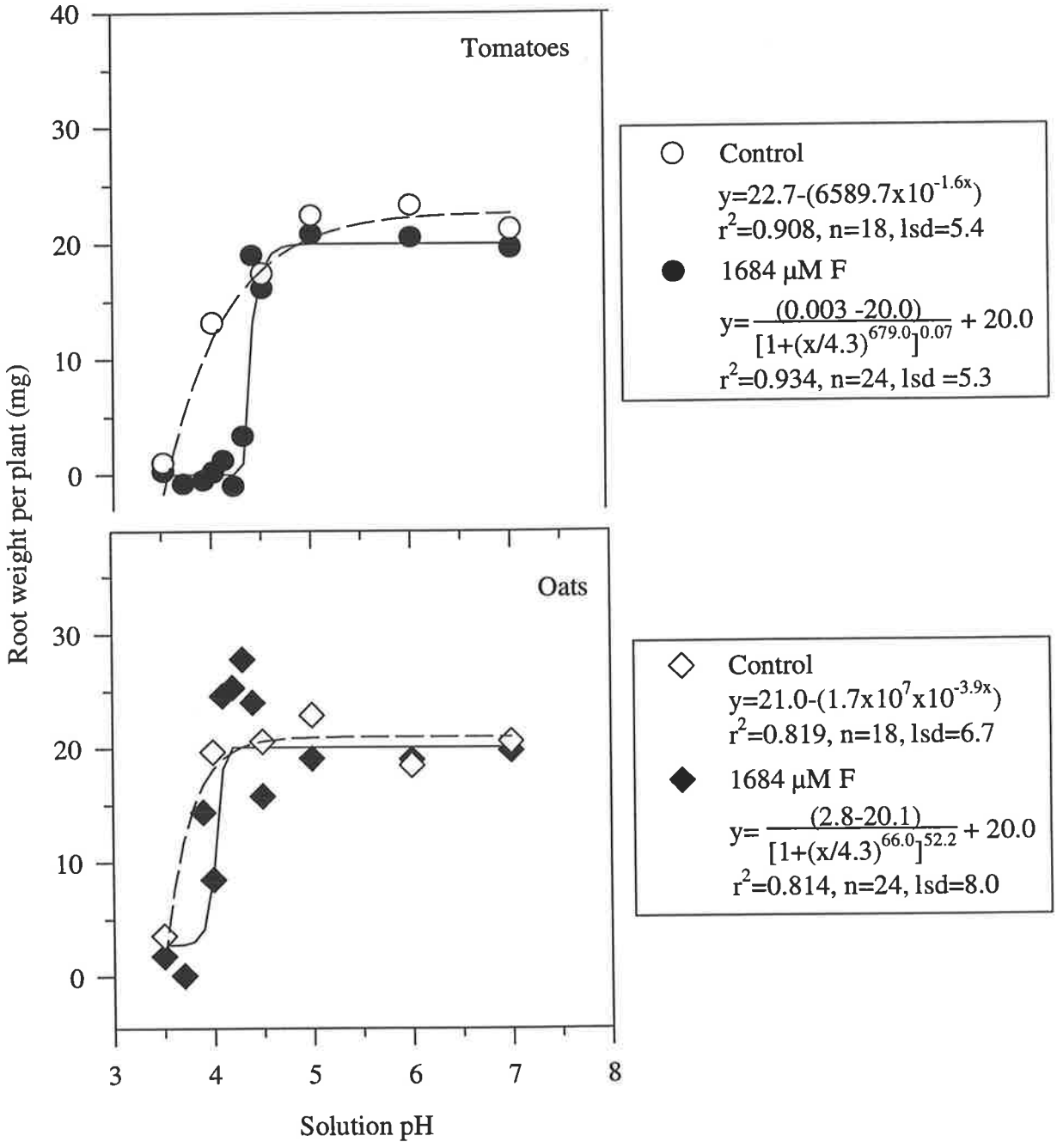


Figure 7.2 Effect of solution pH with and without F on root dry weights. Individual values except when pH is a multiple of 0.5, in which case they represent means, n = 3.

7.3.3 *The effect of HF activity in solution culture on fluoride concentrations in plants*

Fluoride concentrations in the shoots of oat and tomato plants increased significantly as HF activities in solutions exceeded 169 (solution pH = 3.9) and 111 μM (solution pH = 4.1), respectively. Maximum concentrations of F in oats and tomatoes shoots were 3860 and 7800 mg F kg^{-1}, respectively (Figure 7.3). Fluoride concentrations in plant materials were not corrected for the contribution of F taken up as the free ion (Section 6.3.8) because this represented an error of less than 2% at the highest HF activities in solution.

For all treatments, F concentrations in the shoots of oats and tomatoes increased to a point where plant dry weights were restricted. The F concentrations in tomato and oat shoots which corresponded with significant restriction in plant dry weights were 228 and 125 mg F kg^{-1}, respectively (calculated from Figure 7.3).

Concentrations of F in roots of tomatoes increased almost linearly with HF activities in solution, showing no signs of reaching a maximum uptake like that found in shoots (Figure 7.4). However, concentrations of F in roots of oats increased sharply at HF activities (73 μM) where root and shoot dry weights were restricted (Figure 7.4) and appeared to reach a maximum concentration of approximately 1100 mg F kg^{-1}. The ratio of F in roots:shoots of tomatoes was approximately 2:1. However, the ratio was approximately 3:1 in oats grown at higher activities of HF. S-UCEs for HF ranged between 500 - 2000 dm^3 kg^{-1} for shoots of plants grown in HF activities of 255 - 376 μM. For the highest two activities of HF, uptake coefficients for tomatoes and oats ranged between approximately 860 - 2000 and 500 - 800 dm^3 kg^{-1}, respectively. Differences between S-UCEs and P-UCEs were less than 30 dm^3 kg^{-1}.

7.4 Discussion

7.4.1 *Modelling of HF species in solution*

The thermodynamic stability constants for HF are well established (Table 3.3) and therefore it was considered unnecessary to check calculations with GEOCHEM-PC by F-ISE. Theoretically it is possible to check the speciation of HF in the solution using F-ISE with a range of non-complexing buffers (NCB) and determine HF by subtracting F in solution from total F added. However, to ensure accuracy of this measurement buffers would be required for each pH treatment with a range of standards for each.

7.4.2 *The effect of HF activity in solution on dry weights of plants*

At pH 3.5, the dry weights of both tomatoes and oats were restricted by H^+ activity alone (Figure 7.1). However, when F was also added to the nutrient solutions across the same pH range, dry weights were lower at pH values greater than 3.5. These data indicate that a) HF has a more deleterious affect on plant growth than H^+, and b) when compared with dry weights of plants exposed to F^- (Section 5.3.3) at the same activities of F^- and HF in solution, HF has a more deleterious affect on plant growth than F^-. The dry weights of oats and tomatoes shoots were significantly decreased at HF activities one order of magnitude less than F^- activities which decreased dry weights of tomato shoots. Oat shoots were not signifcantly decreased at the highest F^- activity in solution (5130 µM). This suggests that the uptake and mechanism of toxicity of HF is different to that of F^-. There are no data available from previous studies with which to compare these growth restrictions with HF activities in solution. The effects of HF and H^+ on plant dry weights (Figures 7.1 and 7.2) suggest that the toxic response of both plants to HF is different from H^+ (Plate 7.1). The asymmetrical sigmoidal curve, which described the growth restrictions of tomatoes and oats grown in solutions containing

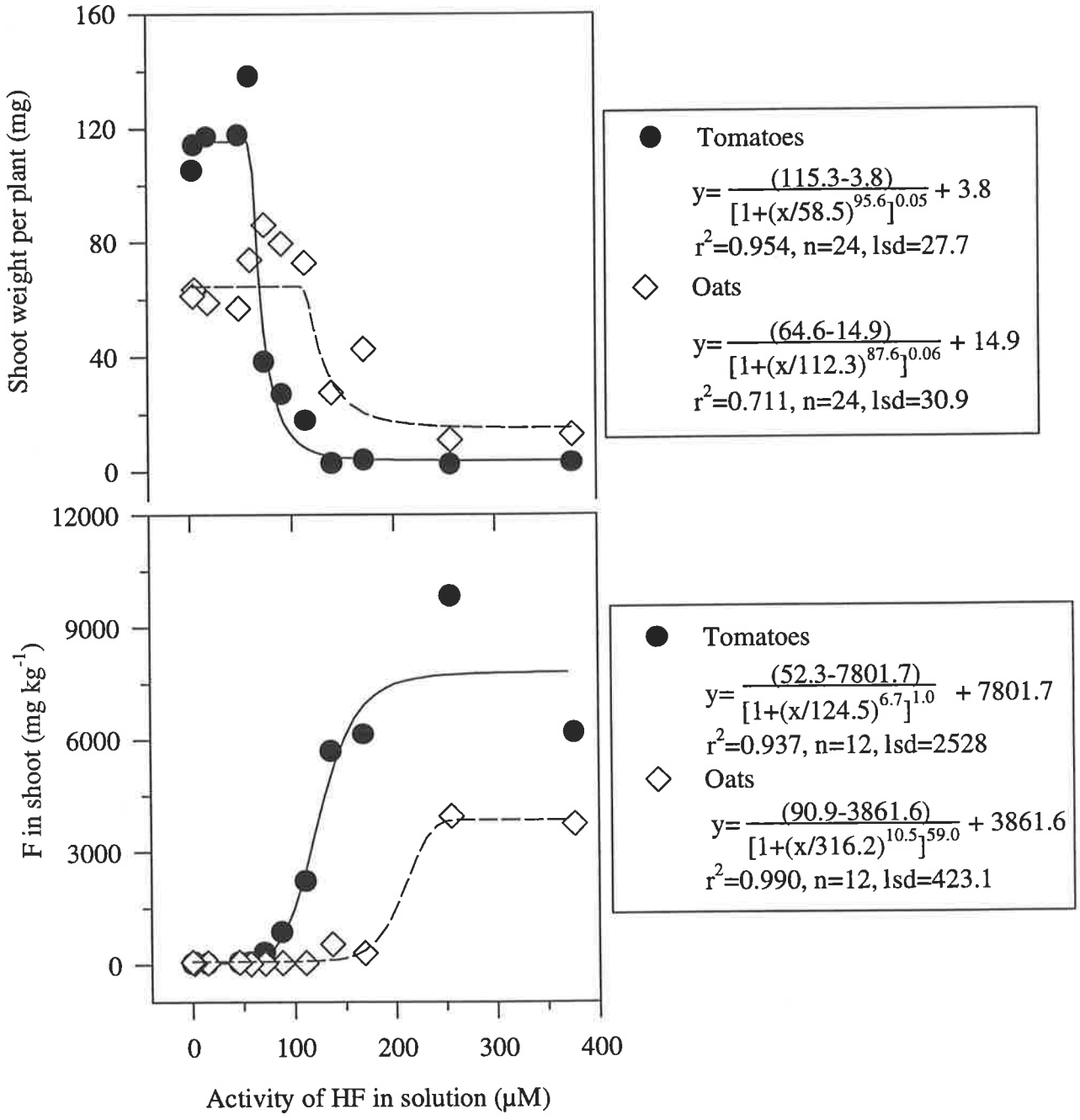


Figure 7.3 Effect of HF activity in solution on weights and F concentrations in shoots of oat and tomato plants. Points represent means, n = 3, or single values (see Section 7.2.2).

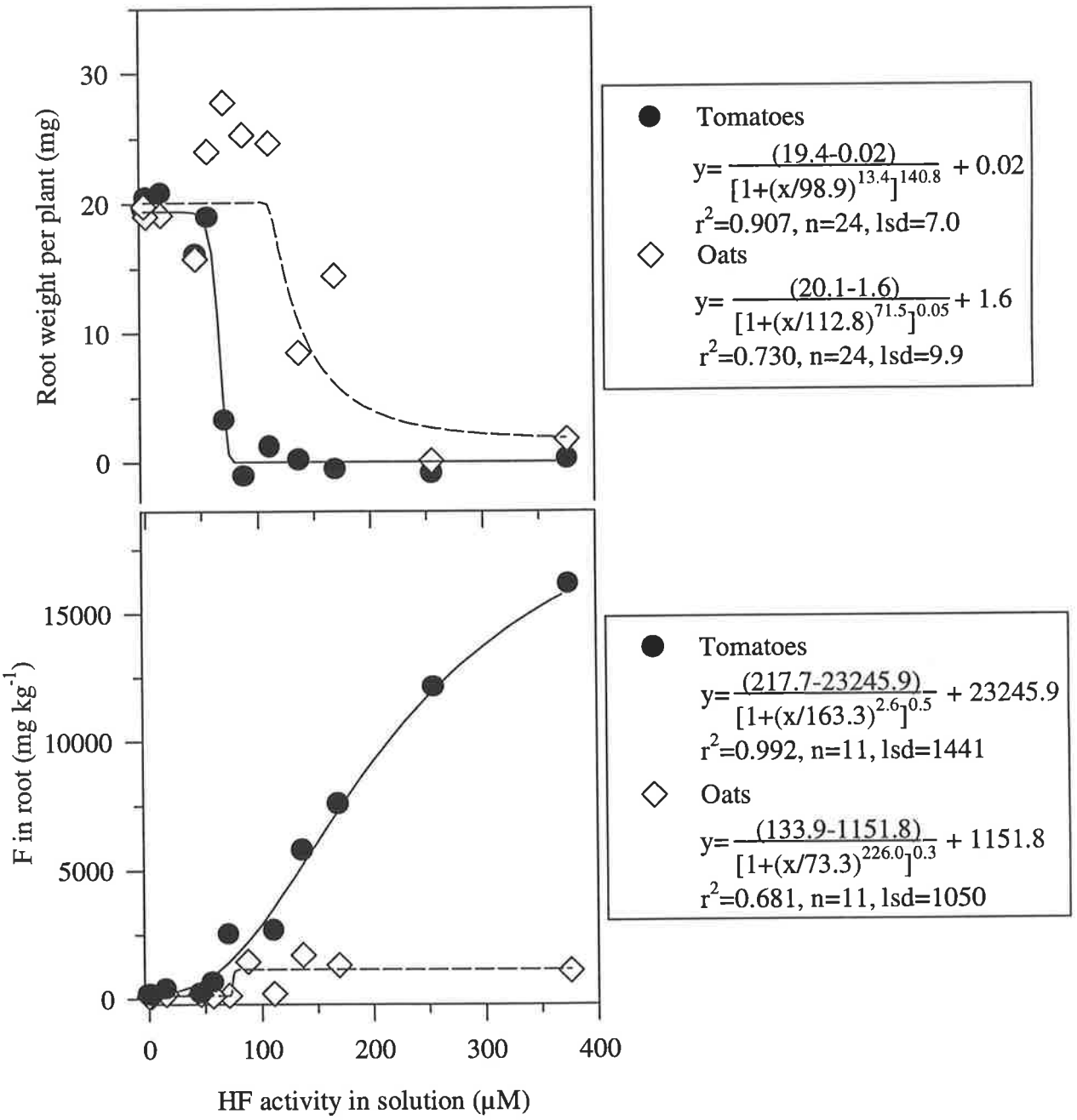


Figure 7.4 Effect of HF activity in solution on dry weights and F concentrations in roots of oat and tomato plants. Points represent means, n = 3, or single values (see Section 7.2.2).

HF, suggests that once a threshold of HF activity in solution is reached, toxicity to the plant is severe. However, the effects of H^+ on plant dry weights were best described by Equation 6.1, suggesting a more gradual increase in toxicity and the involvement a different mechanism.

7.4.3 *The effect of HF activity in solution on fluoride concentrations of plants*

Fluoride uptake by plants from solutions containing HF was much greater than from solutions containing F^- (Chapter 5). S-UCEs for F in tomatoes or oats attributed to F^- were approximately 2.3 $dm^3\ kg^{-1}$ (F activity ≤ 1476 µM, Section 5.3.4) and 16 - 49 $dm^3\ kg^{-1}$ for AlF species (AlF_2^+ or AlF_3 = 500 µM, Section 6.3.8). In comparison, the S-UCEs for HF are orders of magnitude higher (500 - 2000 $dm^3\ kg^{-1}$ for plants grown in HF activities of 255 - 376 µM). At similar ion activities, P-UCEs for F^-, AlF and HF were similar to S-UCEs (*i.e.* approximately 2 - 3, 16 - 69 and 500 - 2000 $dm^3\ kg^{-1}$ respectively). These data highlight that F is more readily taken up as HF compared with F^- and AlF species.

The increased F concentrations in the shoots of oat and tomatoes exposed to HF (Figure 7.3) could be explained by non-ionic diffusion of HF across the cell membrane. The permeability of HF has been studied thoroughly and found to be six orders of magnitude higher than for F^- (Kronberger, 1987).

Phytotoxic responses (decreases in plant dry weights) to HF were associated with concentrations of F in plants of approximately 100 to 200 mg F kg^{-1} (Figure 7.3, HF activity = 75 to 140 µM). For tomatoes, these concentrations are similar to those found in plants grown at much higher activities of F^- (1473 - 2412 µM) in solution (Figure 5.7) which caused growth limitations (72 - 400 mg F kg^{-1}, Section 5.3.3). However, when F was taken up as F^- by oats, F concentrations in shoots were as high as 1000 mg F kg^{-1} and no limitations to growth were observed.

The data from this chapter suggest that: a) tomatoes (F^- sensitive) have no mechanism for negating the toxic effects of F once taken up, and b) that the mechanism(s) by which oats (F^- tolerant) previously appeared to be able to cope with high internal F concentrations (Section 5.4.3), was not evident if the plant was exposed to F as HF. One explanation for the changes in F tolerance of oats could be that the mechanism by which oats negate the toxic effects of F becomes saturated and ineffective by the rapid influx of F as HF, the more permeable ionic species. The toxic action of F is thought to be the through the inactivation of metal ions (Ca, Mg, Mn and Zn) at their sites of physiological activity, once in the plant cell (Weinstein and Alscher-Herman, 1982; Suttie 1977).

Fluoride concentrations in plant roots associated with HF in solution suggested that oats, which have been found to be more tolerant to F^-, may also be able to restrict HF uptake at the root (Figure 7.4). However, this occurs to a much lesser degree than with F^- and AlF.

7.5 Conclusions

Fluoride, as the HF complex, is much more readily taken up by oats and tomatoes than F^- and AlF complexes. Oats are more resistant to uptake and toxicity of the HF complex than are tomatoes. However, HF is still toxic to both plant species at low activities (75 to 140 μM) relative to F^- and some AlF species.

Chapter 8

8.0 Uptake of fluoride complexed with boron (fluoroborate) by plants grown in solution culture

8.1 Introduction

Previous chapters have reported the effects of F as the free F ion or complexed with H or Al, on the uptake of F by tomatoes and oats. These species of F may form in soils at different soil pH. However, fluoroborate complexes are different in two ways from the F species previously studied: a) BF_4^- is considered to be relatively stable and capable of existing under a range of soil conditions, and b) BF_4^- will not form from F and B added separately to soils but must be added to the soil as fluoroborate salts (Section 2.6.1). There are limited data available on uptake of this ion through the plant root (Collet, 1969). The purpose of this chapter was to determine: a) the stability of BF_4^-, and b) the phytoavailability and phytotoxicity of F in solution when exposed to the root as BF_4^-.

8.2 Materials and methods

8.2.1 *Solution culture parameters*

Oats and tomatoes were grown in solutions as described in Section 3.2.2. Solution pH was 4.8 ± 0.2, low enough to avoid large fluctuations of pH, but high enough to avoid formation of HF.

8.2.2 *Fluoride treatments added to solutions*

Treatments and calculated activities of dominant F species in solution cultures are summarised in Table 8.1. All treatments were triplicated. At some of the higher activities of BF_4^- the plant dry weights were insufficient to allow analyses of individual samples, and replicates were bulked prior to analysis.

8.2.3 *Modelling of fluoroborate species in solutions*

Fluoroborate activities were calculated with MINTEQA2 version 3.11 (Allison *et al.*, 1991), with F and B concentrations entered as F^- and HBO_3^+. GEOCHEM-PC considers both B ($B(OH)_4^-$) and F (F^-) as a ligands and therefore cannot consider complexes of fluoroborate. While borate can exist in a number of forms in solution depending on pH, B will be referred to as $B(OH)_4^-$ for this thesis.

Table 8.1 Treatments imposed on tomato and oat plants grown in solution cultures.

Treatment salt and ion	Concentrations and ion activities in nutrient solutions (μM)					pH
$NaBF_4$		0	53	210	421	4.8 ± 0.2
BF_4^- activity	calculated[A]	0	6.3×10^{-8}	5.4×10^{-5}	1.7×10^{-3}	
	measured[B]	0	48	193	383	
H_3BO_3		0			421	4.8 ± 0.2

[A] Due to the slow dissolution of BF_4^-, correct activities in solution could not be calculated (MINTEQA2), only those at equilibrium (see Section 8.2.4).

[B] Values are an average for experiments, measured indirectly as described in the Section

8.2.4 *Measurement of free fluoride and fluoroborate*

Fluoroborate undergoes a slow hydrolysis as outlined in the following equations (Largent and Heyroth, 1949), complicating its measurement and the time taken to reach equilibrium in solution. Equations 8.2 - 8.4 are rapid in comparison to Equation 8.1 (Simons, 1954).

$$NaBF_4 + H_3O^+ \leftrightarrow 2H^+ + F^- + BF_3(OH)^- + Na^+ \quad \text{(Equation 8.1)}$$

$$BF_3(OH)^- + H_3O^+ \leftrightarrow 2H^+ + F^- + BF_2(OH)_2^- \quad \text{(Equation 8.2)}$$

$$BF_2(OH)_2^- + H_3O^+ \leftrightarrow 2H^+ + F^- + BF(OH)_3^- \quad \text{(Equation 8.3)}$$

$$BF(OH)_3^- + H_3O^+ \leftrightarrow H_2O + B(OH)_3 + H^+ + F^- \quad \text{(Equation 8.4)}$$

To determine the rate of decomposition in stage 1 (Equation 8.1) after addition of $NaBF_4$ to solutions, a 0.7 cm^{-3} aliquot was removed from each pot on days 5, 7, 9, 12 and prior to harvest (day 16). The F^- concentrations was determined immediately by Capillary Ion Analysis (CIA) (Section 3.3.1). By subtracting 0.25 × F^- (μM) measured from the total BF_4^- (μM) added, an indirect measure of BF_4^- concentration was obtained.

Because of the negative charge on the fluoroborate ion (BF_4^-), it is also possible to measure BF_4^- directly with CIA. However, this method was time consuming as standards needed to be made fresh daily because of the slow decomposition of BF_4^- (Wilde, 1973). To check the validity of the method for indirect measurement of BF_4^-, BF_4^- concentrations in all solutions were sampled on day 12 and 16 of Experiment 8.1 (tomatoes) and were determined directly (CIA) with fresh standards. Direct and indirect measures were then compared.

8.2.5 *Analysis of plant material*

Plant material was digested as described in Section 4.2.1.5. Digests were analysed for F as described in Section 4.2.2.2, Method ME_2. Plant nutrients were analysed in these digests as described in Section 3.6.2. Plants from each replicate were analysed separately, except where growth restrictions gave insufficient material for analysis. In these cases, samples from the same treatments were bulked prior to analysis.

8.2.6 *Statistical analyses*

Plant dry weights and elemental concentrations were analysed using a one way analysis of variance. Significant differences between treatment means were determined using Tukey's test. Plant dry weights and F concentrations were plotted against activity of BF_4^- in solution and data were fitted to a sigmoid function or regressed linearly where appropriate.

8.3 Results

8.3.1 *Modelling and measurement of ionic species of F and BF_4^-*

There was no difference between indirect and direct measurements of BF_4^- concentrations (Figure 8.1). When the BF_4^- concentrations were measured indirectly by CIA over the period that treatments were imposed (9 days) in this experiment, average concentrations of BF_4^- were much higher than those predicted at equilibrium (Table 8.1).

Measurement of BF_4^- and F^- concentrations over the duration of the experiments showed that BF_4^- was slowly decomposing to F^-. For all concentrations of BF_4^-, the time taken for complete hydrolysis of BF_4^- to $B(OH)_4^-$ and F^- would be approximately 72 days (Figure 8.2). The rate of this reaction does not vary with the concentration of the products. Therefore the reaction can be referred to as a zero order reaction with the rate law as defined in Equation 8.5 (Kneen *et al.*, 1972). The rate constant *k*, was 0.0136 ± 0.0004 for the conditions described here (Section 8.2.1). Changes in pH, ionic strength and temperatures would probably affect the decomposition of BF_4^-.

$$\frac{d[BF_4]}{dt} = k[BF_4] \qquad \text{(Equation 8.5)}$$

8.3.2 *The effect of fluoroborate activity in solution on plant dry weights*

Dry weights of tomato and oat shoots were significantly restricted ($p<0.05$) by BF_4^- activities in solution greater than 48 and 383 μM, respectively (Figure 8.3). Similar restrictions were found for root dry weights except significant reductions in dry weights of oat roots were found at

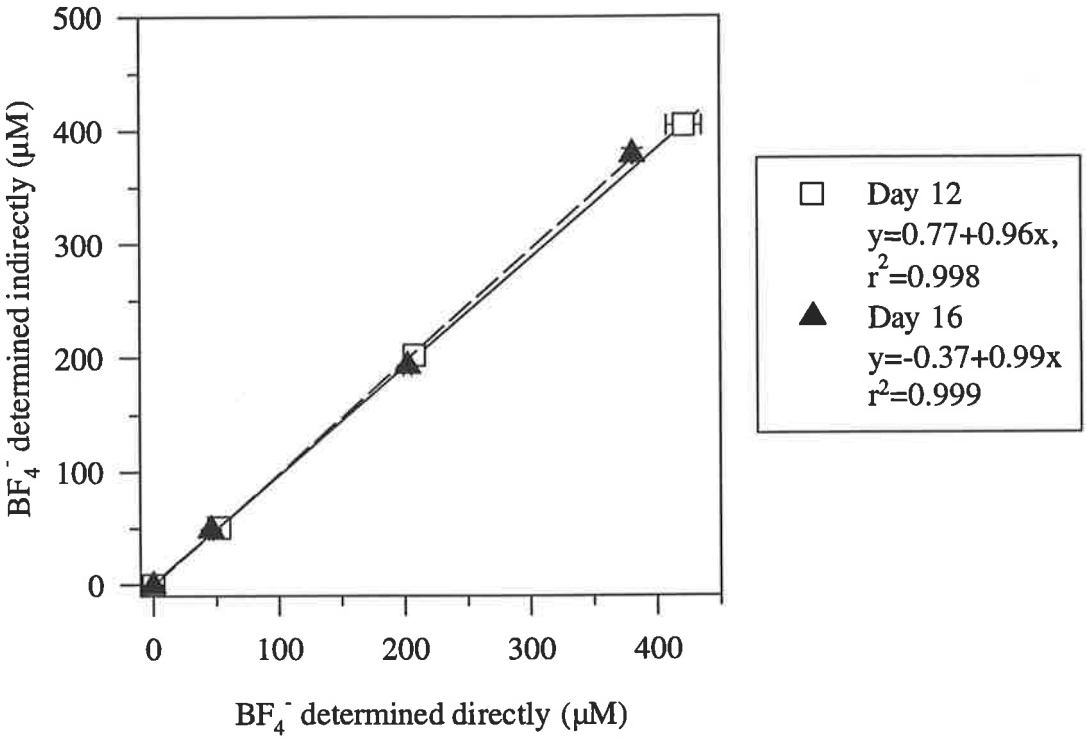


Figure 8.1 Comparison of indirect and direct measures of BF_4^- in solution on days 12 and 16. Error bars represent one standard deviation from the mean and were generally less than the size of the symbol, n = 3.

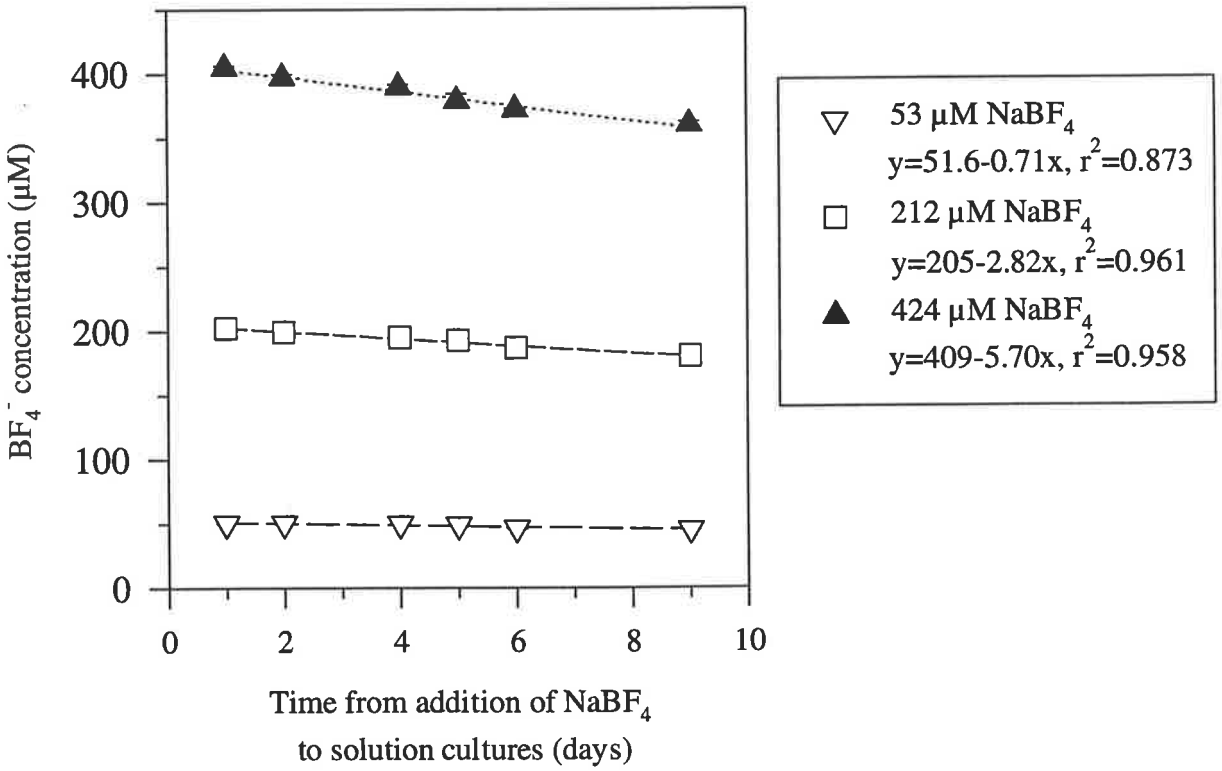


Figure 8.2 Decomposition of the BF_4^- ion in solutions (see Section 8.2.4) at room temperature (20°C). Error bars represent one standard deviation from the mean and are less than the size of the symbols, n = 3.

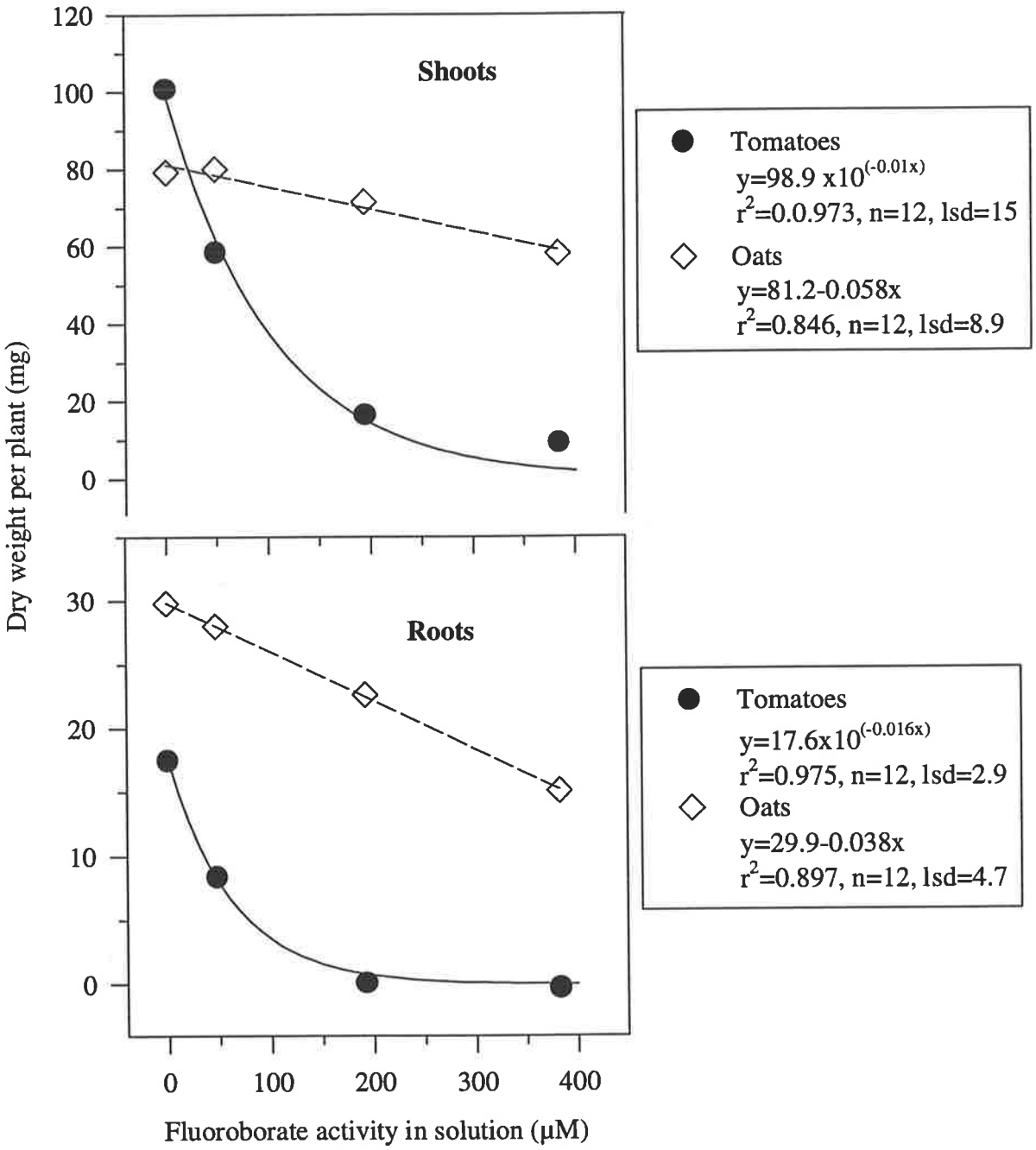


Figure 8.3 Effect of BF_4^- activity in solution on plant dry weights. Points represent means, n = 3.

approximately 200 μM BF_4^-. These activities were orders of magnitude lower than F^- activities which decreased tomato dry weights, or which had no effect on the dry weights of oats (Section 5.3.3). Concentrations of B added to solution cultures as H_3BO_4, equivalent to the highest concentration of B added as BF_4^-, had no effect on the dry weights of the shoots or roots of tomatoes or oats (Figure 8.4). Leaf tip necrosis of oats (Plate 8.1) was not as severe as in shoots of oats grown in solution containing phytotoxic activities of HF and AlF (Plates 6.1 and 7.1). Tomato shoots showed no visible necrosis of leaves.

8.3.3 *The effect of fluoroborate activity in solution on plant fluoride and boron concentration*

Fluoride concentrations in the roots of oats and tomatoes increased linearly with BF_4^- activity in solution (Figure 8.5). Fluoride concentrations in shoots of oats and tomatoes did not increase linearly (Figure 8.5). A second order polynomial curve was used to describe concentrations of F in shoots as BF_4^- activities increased. The concentration of F in the root, compared to the shoot, was approximately 1:2.

The mean F (BF_4^-) S-UCE for oats and tomatoes grown in all BF_4^- treatments were 4470 ± 1380 and 10878 ± 3410 $dm^3 kg^{-1}$ respectively (F in shoot/BF_4^- activity in solution), and mean F (BF_4^-) P-UCE were 3915 ± 1089 and 10141 ± 2832 $dm^3 kg^{-1}$, respectively. Boron concentrations in both plants were less than F, but root:shoot ratios for B concentrations in oat roots and shoots were similar to F root:shoot ratios (1:2) (Figure 8.6). The root:shoot ratio for B concentrations in tomatoes was much lower (approximately 1:6).

Plate 8.1 Necrotic symptoms of oats grown in solution cultures containing 383 μM BF_4^-.

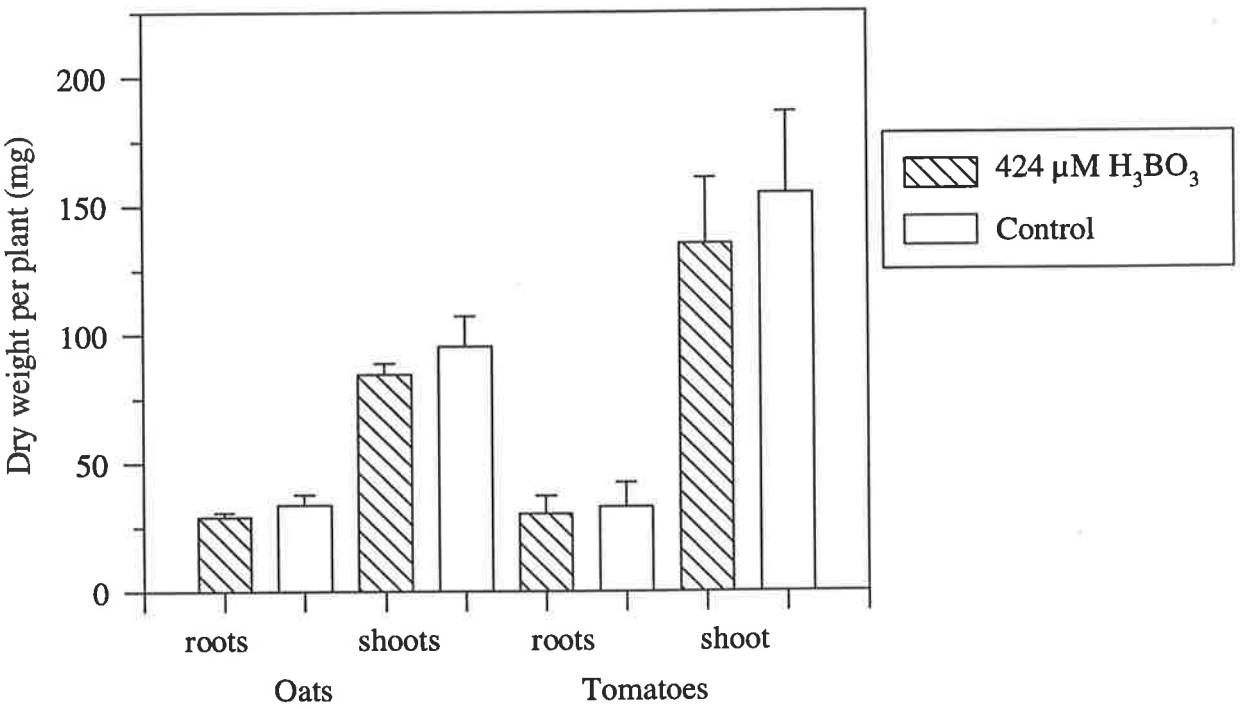


Figure 8.4 Effect of H_3BO_3 on plant dry weights. Error bars represent one standard deviation from the mean, n = 3.

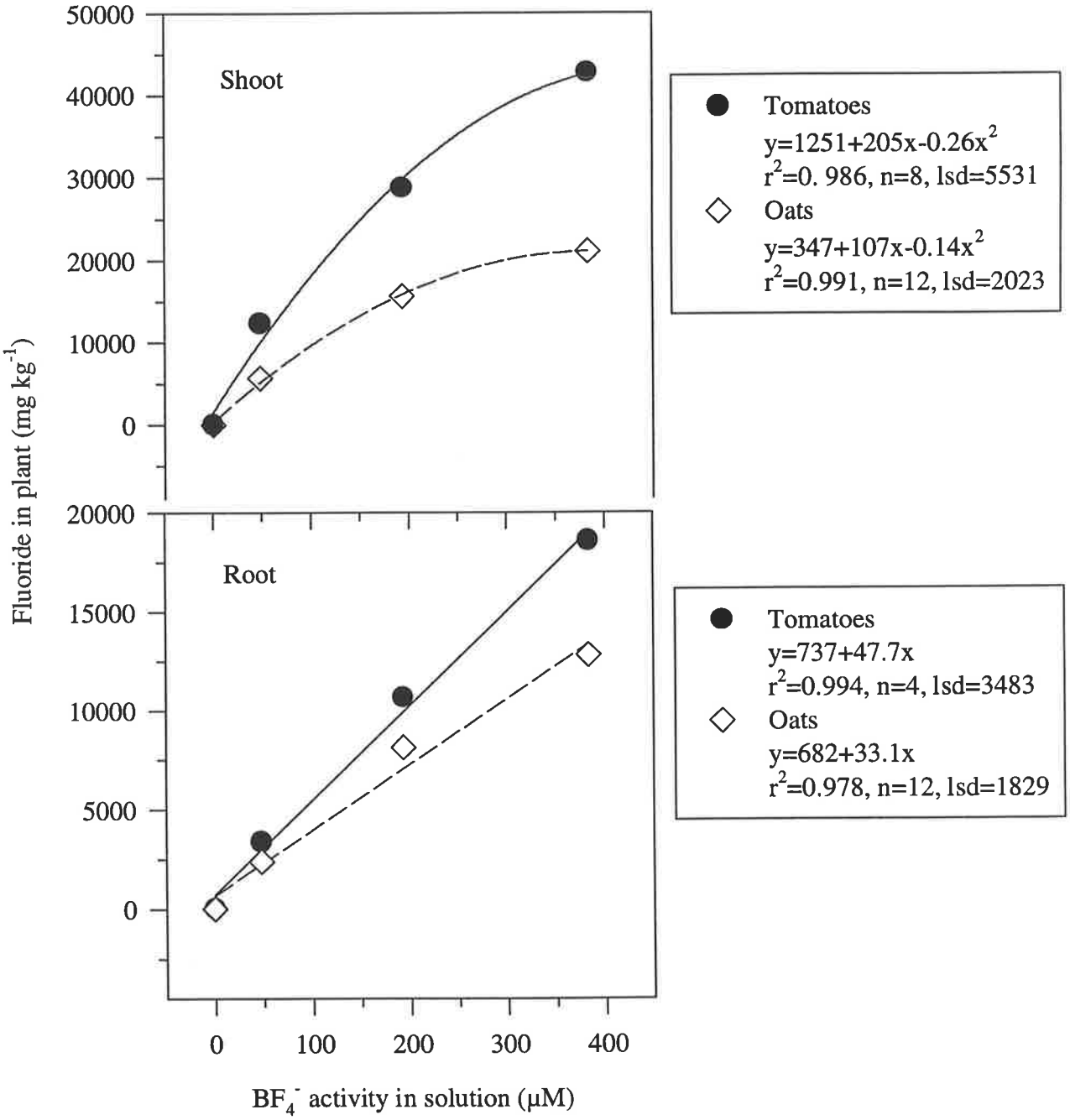


Figure 8.5 Effect of BF_4^- ion in solution on fluoride concentrations in plants. For oats, points represent means, n = 3. For tomatoes, symbols represent single values except for tomato shoots grown in solution containing less than 100 µM BF_4^-, where points represent means, n = 3.

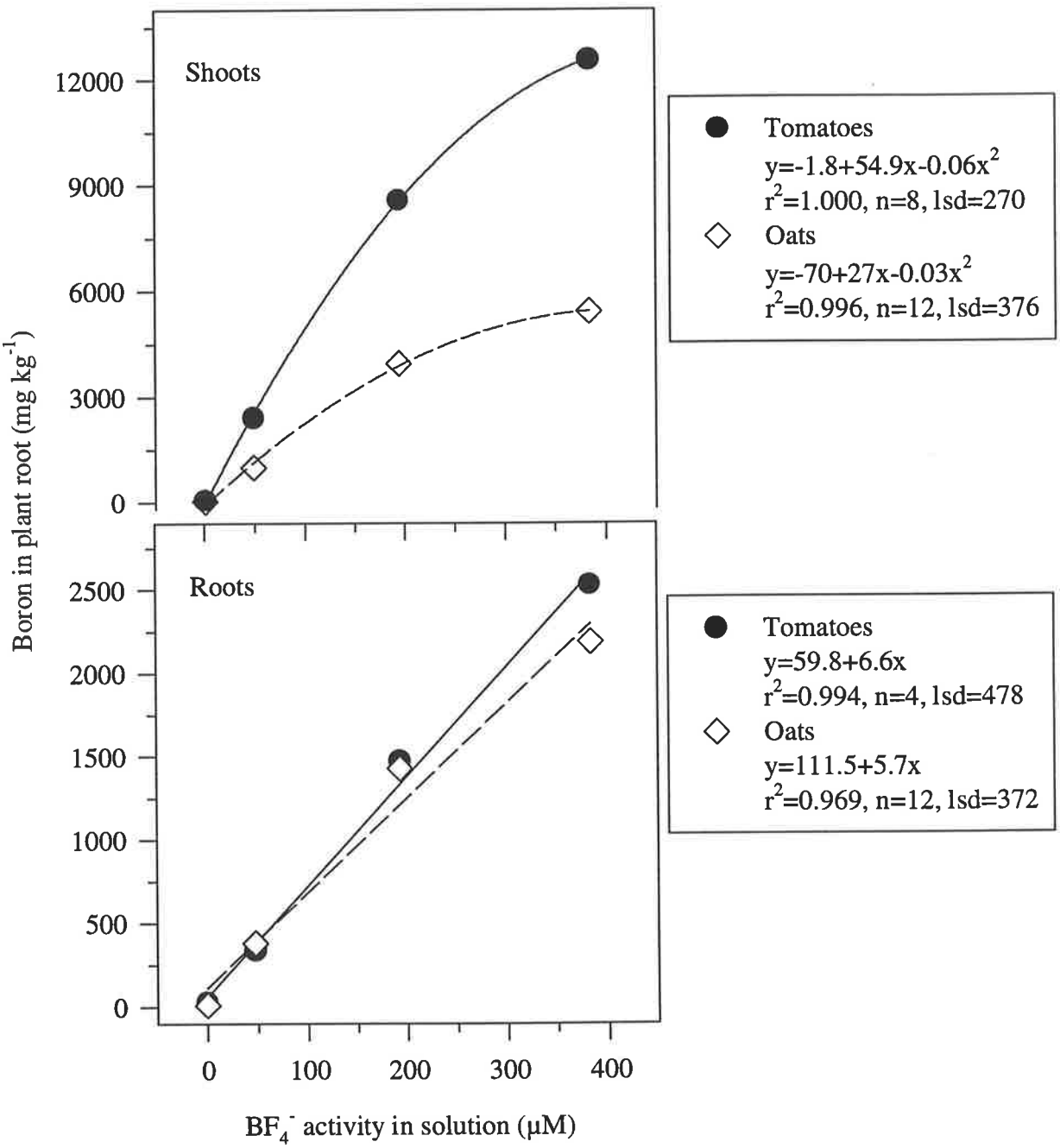


Figure 8.6 Effect of BF_4^- ion in solution on boron concentrations in plants. For oats, points represent means, n = 3. For tomatoes, symbols represent single values except for tomato shoots grown in solution containing less than 100 µM BF_4^-, where points represent means, n = 3.

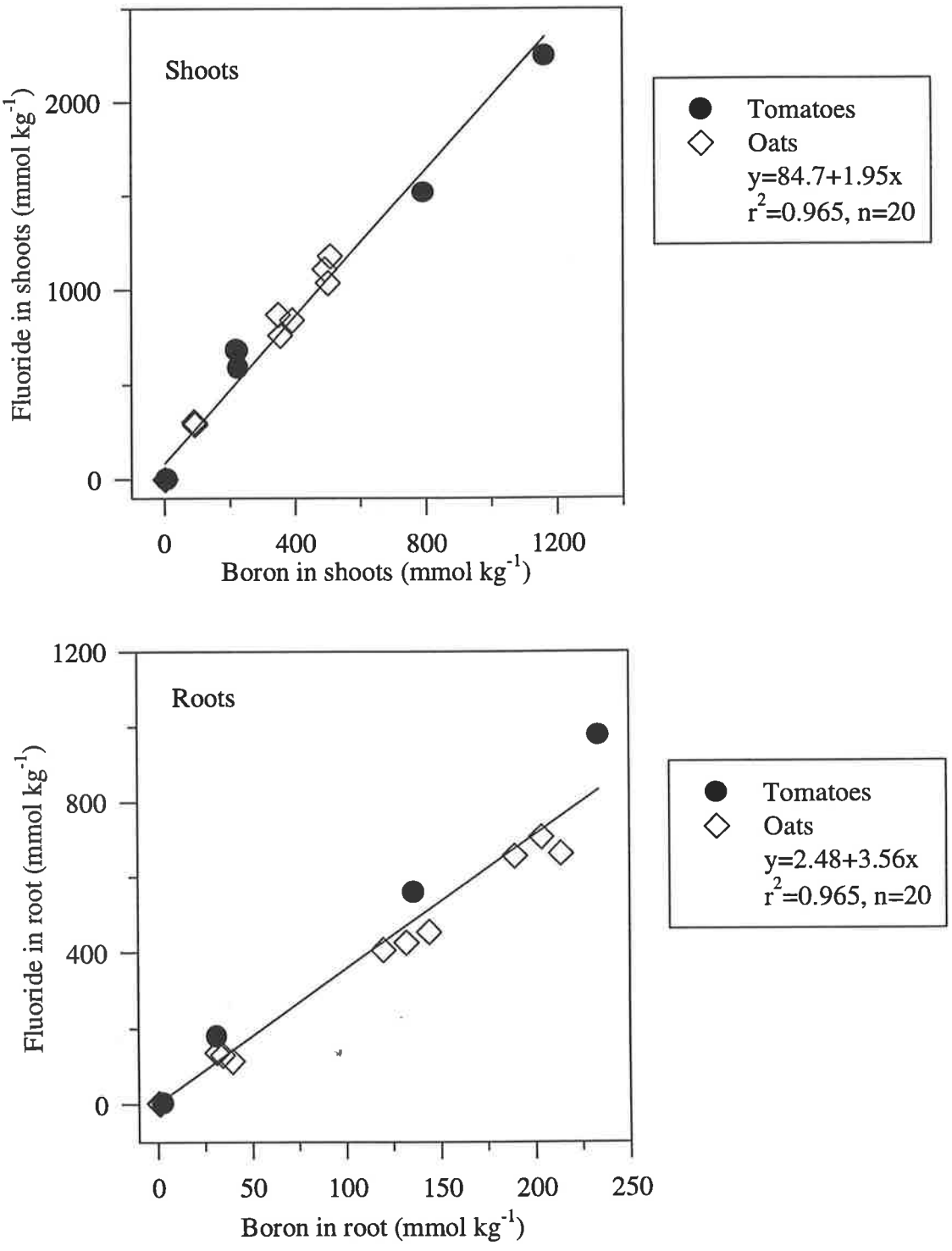


Figure 8.7 Ratio of boron:fluoride in roots and shoots of tomatoes and oats grown in solutions with BF_4^-. Points represent individual values.

The molar ratio of F:B in oat and tomato shoots was 1.95, and in roots 3.56 (Figure 8.7). No account was taken of F taken up from the presence of F^- or B from $B(OH)_4^-$ in solution as contribution of these ions to F and B in the plant would be less than 2.5% of total F and B concentrations in shoots.

Concentrations of B in shoots of plants grown in solutions containing 421 μM BF_4^- were approximately 13000 and 5000 mg B kg^{-1} for tomatoes and oats, respectively (Figure 8.6). These concentrations were much greater than the B concentrations of plants grown with equal concentrations of B present in solution as $B(OH)_4^-$ *viz* 348 and 164 mg B kg^{-1}, respectively (Figure 8.8).

When F concentrations exceeded approximately 12000 or 21000 mg F kg^{-1} in shoots of tomatoes and oats respectively (Figure 8.9), dry weights were adversely affected.

8.4 Discussion

8.4.1 *Modelling of fluoroborate species*

Modelling of chemical equilibria in pure or soil solutions predicts the final extent of the reaction but not the rate (Lumsdon and Evans, 1995). Because of the slow hydrolysis of BF_4^- outlined above (See Equations 8.1- 8.4), it was not possible to calculate the activities of BF_4^- to which the plant roots were exposed. Indirect measurements of BF_4^- indicated that under the experimental conditions, complete hydrolysis of BF_4^- (equilibrium) was reached in approximately 72 days (Figure 8.1). Wilde (1973) found it necessary to make BF_4^- standards fresh daily due to the slow hydrolysis of this complex, but the rate of hydrolysis was not stated.

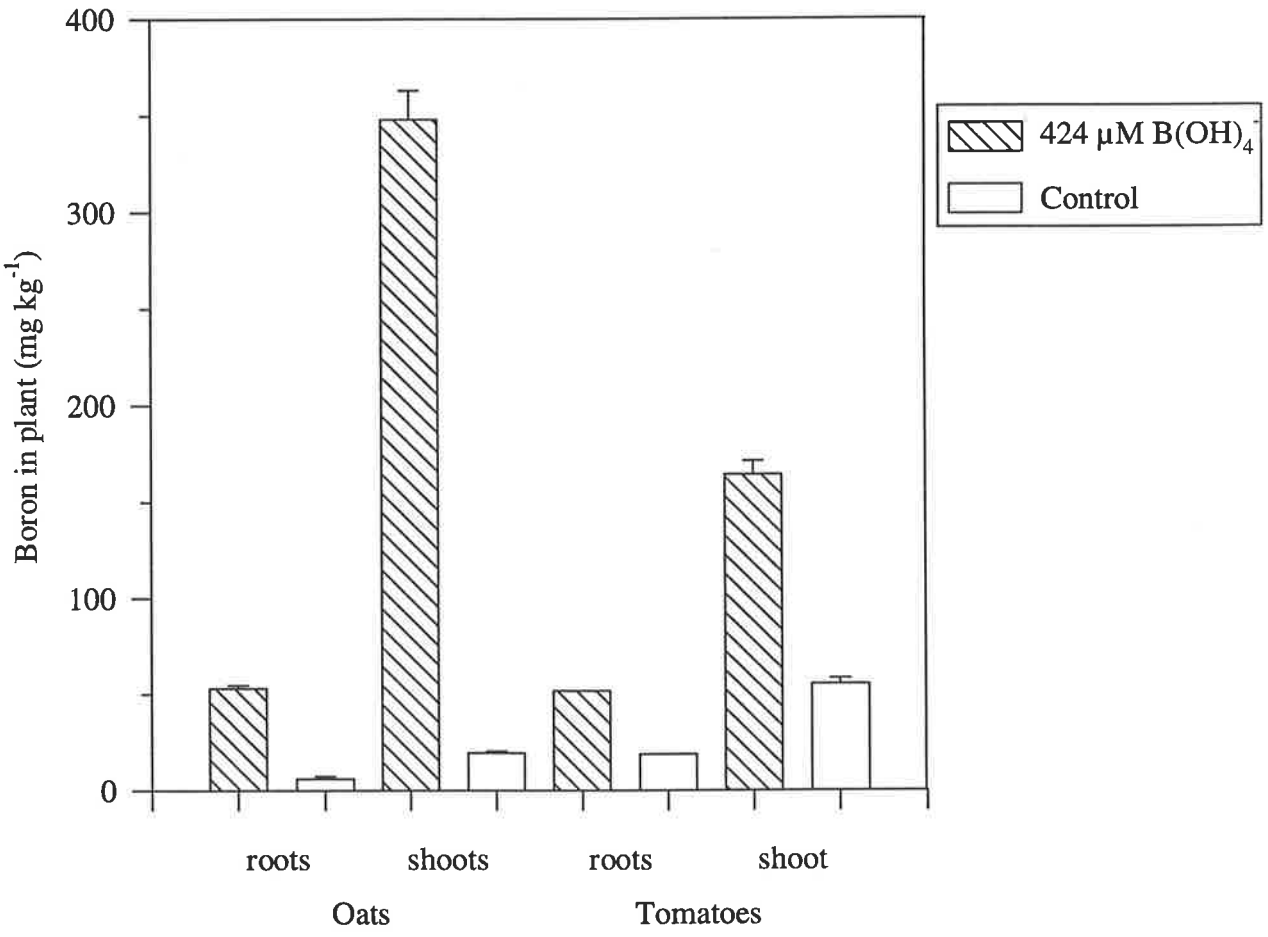


Figure 8.8 Boron concentrations in roots and shoots of tomatoes and oat grown in basal nutrient solution with $B(OH)_4^-$. Error bars represent one standard deviation from the mean, n = 3.

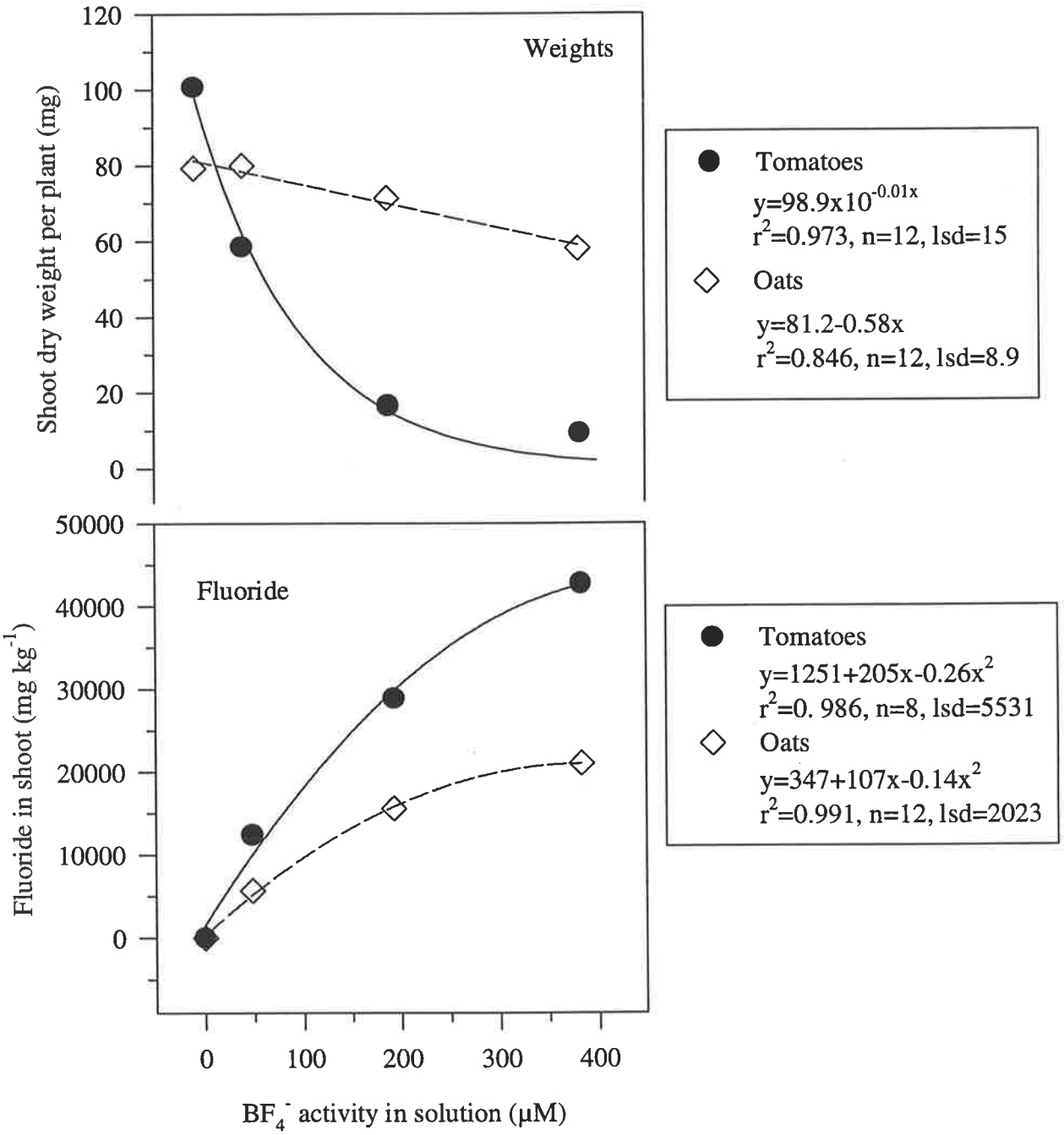


Figure 8.9 Effect of BF_4^- activity in solution on dry weights and fluoride concentrations in tomato and oat shoots. Points represent means, n = 3, except for tomato shoots (see Figure 8.5).

The direct and indirect method for measuring BF_4^- gave the same result. The 1:1 correlation between these methods shows that: a) reactions outlined in equations 2-4 are almost instantaneous and the presence of BF_x (where x = 1 to 3) should not contribute significantly to F uptake, and b) the less time consuming indirect method for quantifying BF_4^- can be used under these conditions.

8.4.2 *The effect of borate and fluoroborate activity in solution on plant dry weights*

Shoot dry weights were unaffected by B in solution present as $B(OH)_4^-$ in contrast to equivalent concentrations of B as BF_4^-. These data indicated that there was no impact of $B(OH)_4^-$ from dissociation of BF_4^- on plant growth.

Tomatoes were more sensitive than oats to the BF_4^- complex in solution. However, dry weights of oat and tomato shoots were significantly restricted at activities of BF_4^- approximately one to two orders of magnitude lower than F^- activities which restricted shoot weights (Figure 5.2), and at activities similar to HF activities which limited plant dry weights. These findings are contradictory to those of Collet (1969) who found that BF_4^- was less toxic than other salts of F (NaF and $(NH_4)_2SiF_6$) to apricot trees grown in solution culture.

There are two possible explanations for this disagreement between the data presented in this thesis and the work of Collet (1969). In the study of Collet (1969) plants were exposed to treatments for only 8 days, and there may not have been sufficient time for decomposition of the absorbed BF_4^- ion with in the plant to the physiologically active F^-. If stock solutions of KBF_4 were not prepared immediately before addition to the solution cultures, the BF_4^- would have undergone some hydrolyses and presented lower concentrations of this ion to the root than was calculated.

8.4.3 *The effect of the fluoroborate activity in solution on fluoride and boron concentrations in plants.*

The presence of the negatively charged BF_4^- ion in solution cultures caused large increases in B and F concentrations in tomato and oats (Figure 8.5 and 8.6) in comparison to $B(OH)_4^-$ controls (Figure 8.8) and similar activities of F^- (Figure 5.6). The ratio of F in roots:shoots (1:2) also suggests that there is very little restriction at the root to BF_4^- uptake. Fluoride UCEs were the highest (compated to F^-, AlF or HF) for oats and tomatoes grown in the solutions with the highest BF_4^- treatment; approximately 2890 and 5870 $dm^3 kg^{-1}$, respectively.

Uptake of F and B by tomato and oat plants was best described by a quadratic equation. However, if higher activities of BF_4^- were used over a longer period of time an upper asymptote would probably have been established. If so, Equation 6.1 (Section 6.3.8) may have been more appropriate for describing F and B uptake by both roots and shoots. At toxic activities of BF_4^-, uptake of F and B is probably governed by disruption of the function of the root slowing or preventing uptake and translocation of BF_4^-, similar to AlF (Section 6.4.4).

The electronegativity of the covalently bound BF_4^- complex would be expected to be much less than that of F^-. Changes in electroegativity would effect exclusion by the negative charge of the DFS, allowing BF_4^- closer to site of uptake. Less controlled studies than those in this thesis, applying fertilisers to soil containing BF_4^- (formed during the manufacturing process), suggest that BF_4^- is readily taken up by apricot trees and grape vines (Bovay, 1969), supporting the findings in this thesis. Studies on absorption from gastrointestinal tracts of rats have also shown BF_4^- is more readily absorbed than F^- and is physiologically inactive (Zipkin and Likins, 1957; Largent and Heyroth, 1949). It is not known if BF_4^- is physiologically inactive in plants.

If F is taken up and translocated as BF_4^- the concentrations of both these elements in the shoots should reflect this (*i.e.* molar F:B = 4:1). Collet (1969) calculated the F:B ratio, on a mg kg^{-1} basis to equal 4 and concluded incorrectly that F moves towards the leaf as BF_4^-. The molar ratio (F:B) in the plants of Collet (1969) was 2.4:1, similar to the data reported in this thesis (2.0:1).

These results show that, although the presence of BF_4^- in solution significantly increases uptake of F and B, these elements are not transported to the shoot as BF_4^- as originally suggested by Collet (1969). However, they may be held in the roots as BF_4^- (molar ratio of B:F = 3.56). There are dramatic changes in the chemical environment moving from solution culture, through the rhizosphere to the apoplast and symplast. It could be speculated that once BF_4^- has passed the barriers limiting uptake of F^-, changes in solution composition could increase the decomposition of BF_4^-, leading to a mixture of F^-, $B(OH)_4^-$ and BF_4^- being present. The transport of F in the xylem may be limited by precipitation with Ca in the root. There is some evidence which suggests that Ca increases the retention of F and lessens its rate of transport in the transpiration stream (reviewed by Weinstein and Alscher-Herman, 1982). However, research by Largent and Heyroth (1949) suggested that BF_4^- does not react with calcium. Therefore $B(OH)_4^-$ and BF_4^- would be transported to the shoots in the xylem and F^- would remain at the sites of uptake or at sites where decomposition of BF_4^- occurred. Theoretically, this could increase the F:B ratio in roots to greater than 4. However, this was not the case (molar F:B = 3.56), suggesting that uptake and transport may be more complex than this.

These data show that F concentrations in tomato and oat shoots exposed to BF_4^- in solution were greater than in plants exposed to similar activities of HF (Figures 8.5 and 7.3). This is unusual as BF_4^- is negatively charged and therefore would be unlikely to diffuse readily through membranes. There are three possible explanations for this contradiction to the hypothesis that

the negative charge of DFS would repel negative charged ions from sites of uptake: The negative charge of BF_4^- is less than that of F^- and is therefore less affected by the negative charge of DFS, the ionic radii of BF_4^- is similar to SO_4^{2-} and $H_2PO_4^-$ (Waddington, 1959) and BF_4^- could substitute for these ions, or during the hydrolysis of BF_4^- (Equations 8.1 - 8.4) HF is formed in micro-sites at the membrane due to proton flux and F is taken up as the membrane permeable HF complex (Kronberger, 1987).

However, high concentrations of B found in plant shoots grown in solutions containing BF_4^- (Figure 8.6) suggest that F is being taken up as a B-F complex and that F is not taken by diffusion of HF across the membrane, discounting formation of HF at micro-sites. There were no apparent differences between S and P concentrations in shoots of oat and tomatoes across the range of BF_4^- treatments, suggest that BF_4^- does not substitute for these ions.

The ratio of F concentations in roots:shoot for all other ionic species of F studied was greater than one, except for oats exposed to HF (Figures 7.3 and 7.4). However, for BF_4^- ratios of F concentrations in roots:shoot were 1:2, suggesting BF_4^- is readily translocated within the plant. Concentrations in the shoot were far in excess of the total amount which could have been translocated with the transpiration stream (under the assumptions in Section 5.3.8) suggesting that uptake of BF_4^- is an active process.

Concentrations of F in shoots of plants grown with treatments containing BF_4^- were orders of magnitude higher than those found in plants exposed to F^- and AlF (Figures 5.7 and 6.10) and approximately 5 times those found in plants exposed to solution containing HF, at same concentrations of F in solution. However, toxic responses to BF_4^- in solution occurred at similar activities to toxic responses to HF in solution. The data suggest that the BF_4^- ion is taken up rapidly, but dissociates slowly to form F^-, and hence more F is taken up before the physiological

activity of F^- is equal to that of the plants exposed to HF. Once inside the plant cell, the toxic action of F is thought to be the inactivation of metal ions (Ca, Mg, Mn and Zn) by F^- at their sites of physiological activity (Weinstein and Alscher-Herman, 1982)

8.5 Conclusions

Concentrations of BF_4^- in solution can be measured directly or indirectly using CIA. The indirect measure is simpler and is recommended for routine analysis of BF_4^- in samples of known initial concentrations of BF_4^-. Fluoroborate (BF_4^-) is actively taken up by oats and tomatoes and is phytotoxic to these plants. At equivalent activities in BF_4^- is taken up more than HF, AlF and F^- and it has a toxicity similar to that of HF, the most toxic of the other species.

Chapter 9

9.0 General discussion and conclusions

9.1 Introduction

The first part of this chapter discusses the general findings from solution culture studies, regarding ionic species of F in solution and differences in uptake of ionic species by plants. These findings were then combined with data from the literature on F solubility in soils. The aim was to use the data obtained in solution cultures (Chapters 5-8), with data already available in the literature, to identify soils which now, or in the foreseeable future, could have enough potentially phytoavailable F to produce plants with concentrations of F which are phytotoxic or zootoxic.

9.2 Discussion of solution culture experiments

Solution culture experiments (Chapters 5 - 8) have shown there is a variable relationship between ionic species of F (F^-, AlF, HF and BF_4^-) in solution and the amount of F taken up by oat and tomato plants. A summary of the results is presented in Table 9.1. These experiments have also shown differences in uptake and phytoavailabilty of F between tomatoes and oats, and extrapolation of the data to other plant species requires caution. There may be more distinct differences in plant species which are know accumulators (Section 2.7.3). Further work with such species is required to confirm this. However, the general trends found in the solution culture studies of this thesis should apply, to some degree, to all plant species.

Data from solution cultures showed that at low F^- activities, F^- was prevented from passing the root endodermis and F was not readily taken up by the plant. At high activities of F^-, uptake was increased (Table 9.1), suggesting some break down or saturation of the roots ability to take up F^-.

However, the shoot-uptake-coefficients (S-UCE) for F^- at low or high activities are less than all other ionic species of F investigated, confirming that F^- is the least readily taken up and translocated of the ionic species F^-, AlF, HF and BF_4^-.

Solution culture studies have also shown that F^- is the least toxic of the examined species, with significant decreases in dry weights of tomatoes recorded at activities in solution greater than 1472 μM F. No significant decreases in dry weights were recorded for oats at activities of 5130 μM F (Table 9.1).

decreases in plant dry weights at activities in solutions less than 500 μM, with the exception of AlF_3. Of the ionic species of F studied, HF and BF_4^- were found to be the most toxic. Tomatoes were more sensitive than oats for all ionic species in solution, except for AlF, where similar toxic responses were observed (Table 9.1) . In general, for all ionic species of F except for BF_4^-, when dry weights of F-treated plants were significantly lower than the controls F concentrations were between 72 - 400 mg F kg^{-1} on a dry weight basis. These values are similar to those reported in other studies (Figure 5.8, Section 2.9.1.2). However, they are orders of magnitude lower than concentrations at which BF_4^- significantly decreased dry weights, presumably because of the slow hydrolysis of BF_4 to the biologically active F^- once taken up (Section 8.4.2). Concentrations of F in the shoots of oats grown in solutions containing high activities of F^- were also significantly greater than the range quoted above (Table 9.1), suggesting that oats have some mechanism to inactivate F within the plant, preventing phytotoxicity. This would also explain oats greater tolerance of all ionic species of F studied when compared with tomatoes.

Table 9.1 Summary of findings form solution cultures: effects of ionic species of F in solution on uptake and toxicity of fluoride.

Ionic species or plant species	Activity of species in solution at or above which plant growth was restricted (μM)	F concentration in plant when plant growth was restricted (mg F kg^{-1})	Mean [A]S-UCE (dm^3 kg^{-1})	Ion activity for which the [A]S-UCE was calculated (μM)
F^-				
Tomatoes	> 1476	72 - 400	2.3	< 1476
			7.0	> 1476
Oats	> 5130[B]	[C]	2.3	< 1476
			12.4	> 1476
AlF_2^+				
Tomatoes	468	370	40	468
Oats	468	163	17	468
AlF_3				
Tomatoes	>502[B]	337	35	502
Oats	>502[B]	168	18	502
HF				
Tomatoes	111	228	1430	>169
Oats	169	125	650	>169
BF_4^-				
Tomatoes	48	12360	10878	>48
Oats	383	21081	4470	>48

[A] S-UCE - Uptake coefficient (mmol F kg^{-1}/mmol F dm^{-3}). Where F is present as a complex, the activity this complex is used in the calculation of the S-UCE.

[B] Highest treatment [C] Plant growth was not restricted in these solutions: maximum F concentration was 1010 mg kg^{-1}.

9.3 Identification of soils which may have the potential to increase F concentration of plants grown in them

To identify soils where concentrations of F in plants grown on these soils could increase to potentially phytotoxic or zootoxic levels, the data obtained in Chapters 5-7 were combined with the major soil parameter which affects the speciation of F in solution *viz.* pH. It was unnecessary to consider the presence of BF_4^-, because in soils the presence of this species of F is not dependent on the pH of the soil, but dependent on addition of the complex in boron-containing fertilisers. The BF_4^- complex is stable in solution for only approximately three months, and once hydrolysed will not re-form under normal conditions in soils.

A range of pH (3.0 - 9.0), F (0 - 3.3 mM) and Al concentrations (Al:F = 1:3) were modelled with GEOCHEM-PC using the composition of the nutrient solutions described in Chapter 3 with 712 μM Si added. Sikora (1992) found an Al:F ratio of 1:3 in soil solutions, and Si was used to determine if there were any interactions with Si and F under these conditions. Mean S-UCEs (mM F in plant shoot:mM ionic species of F in solution) for oats and tomatoes for HF, F^- and AlF from solution culture studies were combined with speciation of F in solution. Fluoride concentrations in plant shoots were calculated relative to the concentrations of F in solution.

GEOCHEM-PC predicted that significant concentrations of F^-, HF and AlF could exist in solution. No solid phases were considered. GEOCHEM-PC does not consider F complexed with Si or B. GEOCHEM-PC treats Si and B as ligands (SiO_4^{4-} and $B(OH)_4^-$). However, MINTEQA2 considered Si and B as the acids (H_4SiO_4 and H_3BO_3) and calculates complexation with F. Nethertheless, MINTEQA2 also predicted no significant concentrations of F complexed with Si or B in solution.

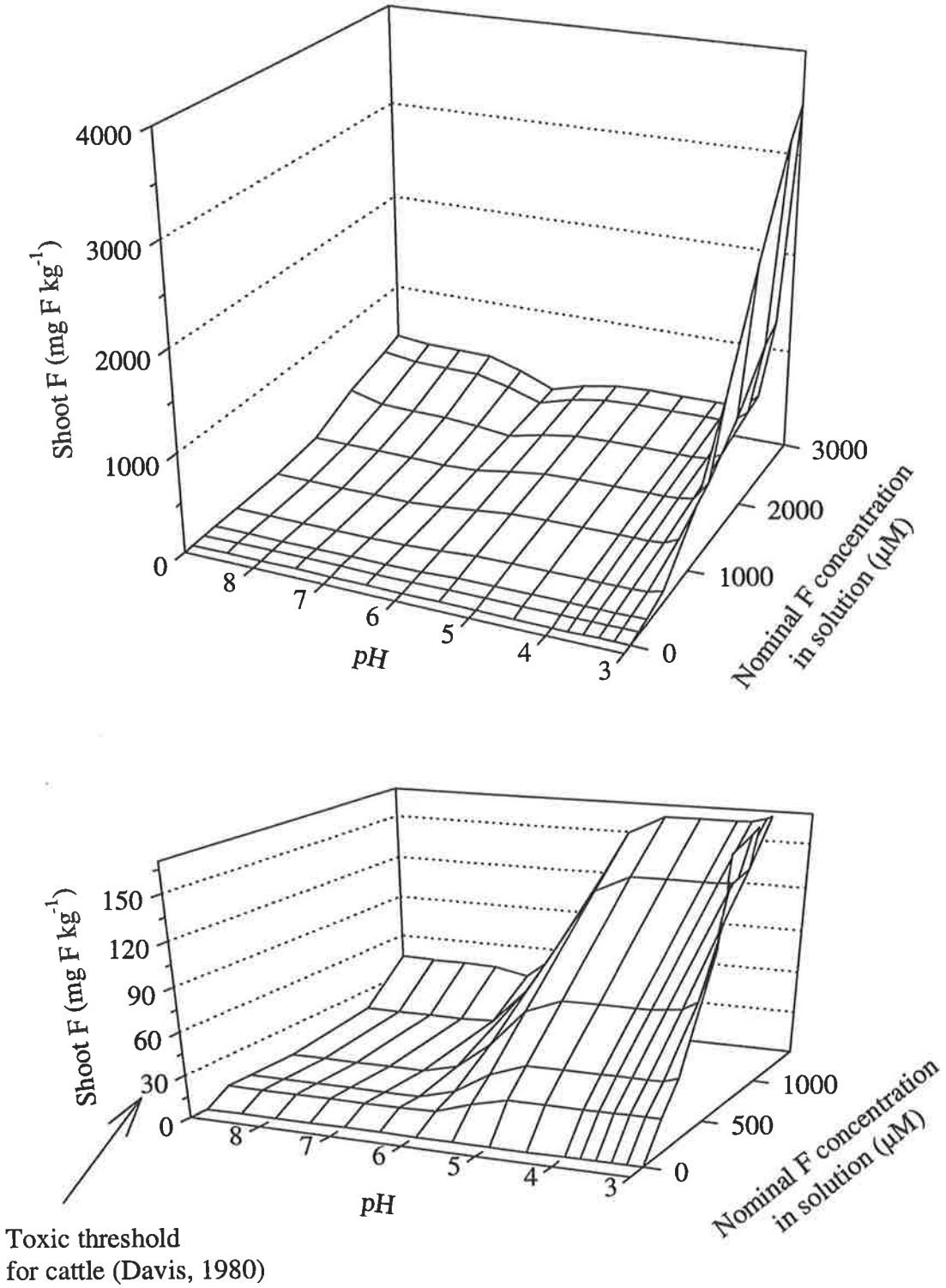


Figure 9.1 Calculated effects of pH and F concentrations in soil solution on F concentrations in plant shoots.

Uptake coefficients from solution cultures were combined with calculated activities of ionic species of F in solution to calculate mean F uptake from solution (Figure 9.1). In general, as the concentration of F in solution increased, calculated uptake of F by plants increased. However, as the ionic species of F in solution (which are pH-dependent) affect F uptake, the calcualted increases in F concentrations in plants were not linear across the pH range studied. At pH 3.6 - 6.0, F uptake is influenced by AlF. However, at lower pH (< 3.6) the higher activities of HF (which is the ionic species most readily taken up) in solution increased F uptake by plants. At high pH (7.0 - 9.0), F in solution is present as F^- which is readily taken up at high F activities in solution (> 1.5 mM), but not at low F activities.

There are several limitations to the general model presented in Figure 9.1. Uptake of F by plants is also affected by: variation between plant species (Section 2.9.1), composition of the soil solution (*i.e.* if there is low soluble Al, HF activities would be much greater), solution ionic strength (Section 5.4.3) and F solubility in soils (Section 2.6.4). Therefore each soil must be considered with these factors in mind. However, the model is proposed as a 'first approximation' method for identifying soils with the potential to contribute significant amounts of F to plant shoots through uptake of F through the roots. Once these soils have been identified, further tests on plants grown in these soils would be required to confirm if the soils do indeed produce plants with high concentration of F.

Fluoride is strongly adsorbed to soils at pH 6.0 ± 0.5 and solubility increases at pH < 5.5 and > 6.5 (Section 2.6.4.1). The species of F which are most readily taken up exist at the pH where F is most soluble. Increases in solubility of F in soil and the presences of ionic species of F which are more readily taken up are both factors which will increase the potential for uptake of F by plants. Fluoride concentrations in plants could exceed the toxic threshold for cattle (30 mg F kg^{-1}: Davis, 1980) or for *Gladiolus* spp. (Jacobson *et al.*, 1966) at total F concentrations in solution

above approximately 0.3 mM when pH is less than 5.5 and 1.5 mM when pH is greater than 7.5. These concentrations are typical of many heavily polluted soils (Wenzel and Blum, 1992).

Wenzel and Blum (1992) found concentrations of up to approximately 10 mmol F kg^{-1} of soil extracts of soils taken from nearby an aluminium smelter. There is limited data on soil solution F, and limited data relating soil solution F with F extracted from soils. However, from the data of Haidouti (1991) and Polomski *et al.*, (1982a) the ratios between F in soil solutions: soil extracts ranged between 1:10 to 1:20, therefore the concentration of F in the soil solution in the soils extracted by Wenzel and Blum (1992) would have been approximately 0.5 mM F. These concentrations could significantly affect plant growth by increasing plant uptake of F to phytotoxic levels (phytotoxic range = 20 - > 4000 mg F kg^{-1} in the plant, depending on the plant species) or increase concentations of F in plants to zootoxic levels (toxic threshold for cattle = 30 mg F kg^{-1}) (Section 2.9).

In soils where phosphatic fertilisers have been applied, concentrations of water soluble F are generally less than 0.250 mmol F kg^{-1} of soil (approximately 0.023 mM F in the soil solution (Haidouti, 1991)) and uptake of F by plants would have little effect on plant growth or grazing animals.

9.4 General conclusions

The data from this thesis have shown:

1. that there are limitations to sealed chamber acid digestion techniques for the dissolution of plants materials for the analyses of total F by F-ISE,
2. that the sealed chamber acid digestion technique could be used for routine, rapid analysis of biologically active F and multi-element analysis of plant materials,

3. that uptake of F through the plant roots and phytotoxicity of F is dependent on the ionic species of F in solution,
4. that the rate of F uptake at equivalent activities in solution is in the order: $BF_4^+ > HF > AlF^{2+} = AlF_2^+ > AlF_3 = AlF_4^- = F^-$, and
5. that phytotoxicity of F at equivalent activities in solution is in the order: $BF_4^+ = HF > AlF^{2+} = AlF_2^+ > AlF_3 = AlF_4^- = F^-$.

Fluoride uptake by plants is dependent on the ionic species of F in soil solution, which is essentially controlled by the pH of the soil. In the long term, continual high application of F to soils of pH < 5 or > 7.5 could significantly increase F concentrations of plants grown in these soils. With increasing amounts of F added to soils, uptake of F by plants may eventually reach levels which are toxic either to plants or to grazing animals.

9.5 Further studies

Further studies are required:

1. to confirm relationships between soil extactable and soil solution F and to develop a more appropriate method for the measurement of plant available F in soils,
2. to determine if uptake of F by plants is affected by F-organic or F-organo-mineral complexes,
3. to confirm the mechanisms responsible for uptake of F by plant roots,
4. to determine the ionic species of F which could exist in soil solution and confirm that similar toxicities to plants grown in solution cultures would be expressed by plants grown in particular soils, and
5. to verify the use of soil pH and F concentations in soil solution as a means of

identifying soils which may contain phytotoxic levels of F, or increase concentrations of F in plants to zootoxic levels.

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