IRON TOXICITY

TO WETLAND PLANTS

by

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DEDICATION

To my parents and the late
Edith Alice Cook.

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SUMMARY

1. This study investigated the toxicity of ferrous iron to 39 wetland species using two-week screening experiments in solution culture. These were found to be more satisfactory than a similar ten-week experiment, or trials at germination.

2. Growth measurements (including shoot and root length and dry weight, leaf size, and numbers and health of leaves) were made. Various tolerance indices were derived and compared, and one based on relative growth rate was selected as being most appropriate.

3. The assessed tolerance of species corresponded well with field measurements of iron concentrations in sites where they occurred, suggesting that iron availability may be important in influencing their field distribution.

4. Tolerance was conferred by exclusion, which was linked to ochre formation on the roots. For four species analysed, an inverse relationship between tolerance and shoot iron concentration was found; there was no evidence that internal tolerance was important.

5. X-ray fluorescence revealed that tolerant species grown in solution culture produced ferric oxide or hydroxide on their roots, while roots of intolerant species were frequently covered with a ferric phosphate precipitate.

6. Monocotyledon species were mostly more tolerant than dicotyledon species, probably due to an inherently superior oxidative detoxification system.

7. Iron tolerance and relative growth rate were inversely related.

8. Iron had both direct and indirect toxic effects, and disruption in phosphorus translocation and metabolism was a particularly important indirect effect.

9. Iron tolerance of *Iris pseudacorus*, *Lysimachia vulgaris*, *Juncus subnodulosus* and *Epilobium hirsutum* was largely unaffected by nitrogen source.

10. High bicarbonate concentrations ameliorated the effects of iron toxicity on *Juncus subnodulosus* and *Epilobium hirsutum*. This was indirect via pH effects on iron solubility.

11. Calcium availability did not influence the iron tolerance of *Juncus subnodulosus* or *Epilobium hirsutum*.

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CHAPTER SIX - THE INORGANIC BASIS OF IRON TOXICITY

CHAPTER ONE

INTRODUCTION

1.1 METAL TOXICITY

Metal toxicity and tolerance have been reviewed at length, e.g. Foy, Chaney and White (1978); Woolhouse (1983); Baker (1987). However iron toxicity has received relatively little attention, primarily because iron is generally plant-available in only small quantities in many natural environments. Indeed, more effort has been directed into studies of iron deficiency (e.g. Chen and Barak 1982; Vose 1982).

Little is known of the physiological basis of iron toxicity, though it may involve damage to cell membranes or to enzyme systems. Root stunting may be caused by inhibition of either cell division or elongation or both, and iron may affect root cell packing (Tibbetts, 1988). Hendry and Brocklebank (1985) presented a scheme outlining a biochemical mechanism for iron-mediated flood tolerance and intolerance in plants. Talbot, Etherington and Bryant (1987) found that in two Salix species photosynthesis was more sensitive to iron then was respiration, indicating that effects of iron may be specific rather than a general metabolic disturbance. It is possible that iron could affect photosynthesis by disrupting chloroplast structure, as manganese has been shown to do (Nazrul-Islam, 1976).

1.2 METAL TOLERANCE

There are many theories of metal tolerance and, since tolerance is usually specific to a particular metal, it is thought that different mechanisms may be responsible for the tolerance of different metals. These may also vary from species to species. There are two major types of tolerance strategy, that of exclusion, whereby metal uptake and transport to the shoot is restricted, and that of accumulation whereby metals are accumulated in a detoxified form (Baker 1981, 1987). There is however still poor understanding of the mechanisms involved at the subcellular level.

Internal detoxification may result from cell wall binding, active pumping of ions into vacuoles, complex formation by organic acids (e.g. malate, oxalate, citrate) and possibly by specific low molecular weight metal-binding proteins (phytochelatins), similar to metallothioneins found in animals. Enzymatic adaptations and effects on membrane permeability may also be involved (Baker, 1987).

Exclusion mechanisms may involve metal immobilisation at the cell wall, exudation of chelates or organic acids from roots, or the maintenance of a pH barrier or oxidation-reduction barrier at the plasma membrane (Taylor 1987). It is widely considered that the toxicity of ferrous iron and other reduced ion species may be ameliorated by the maintenance of a gradient of oxidation-reduction potential in the rhizosphere by diffusion of oxygen from the roots (e.g. Nagai and Matono 1959; Armstrong 1964, 1967, 1972, 1978, 1982; Tanaka, Loe and Navasero 1966; Teal and Kanwisher 1966; Martin 1968; Howeler 1973; Sheikh 1973; Bacha and Hossner 1977; Green and Etherington 1977; Keeley 1979; Drew and Lynch 1980; Chen *et al.* 1980a, b; Mendelssohn and Postek 1982). Thus iron-tolerant plants are excluders. However, this theory of iron tolerance has been called into question (Jayawardena *et al.* 1977 cited by Ando *et al.* 1983; Smirnoff 1981; Mansfield 1990 (see Section 1.6)). Hodgson (1972) noted that the tolerance of ferrous iron was peculiar to marsh plants.

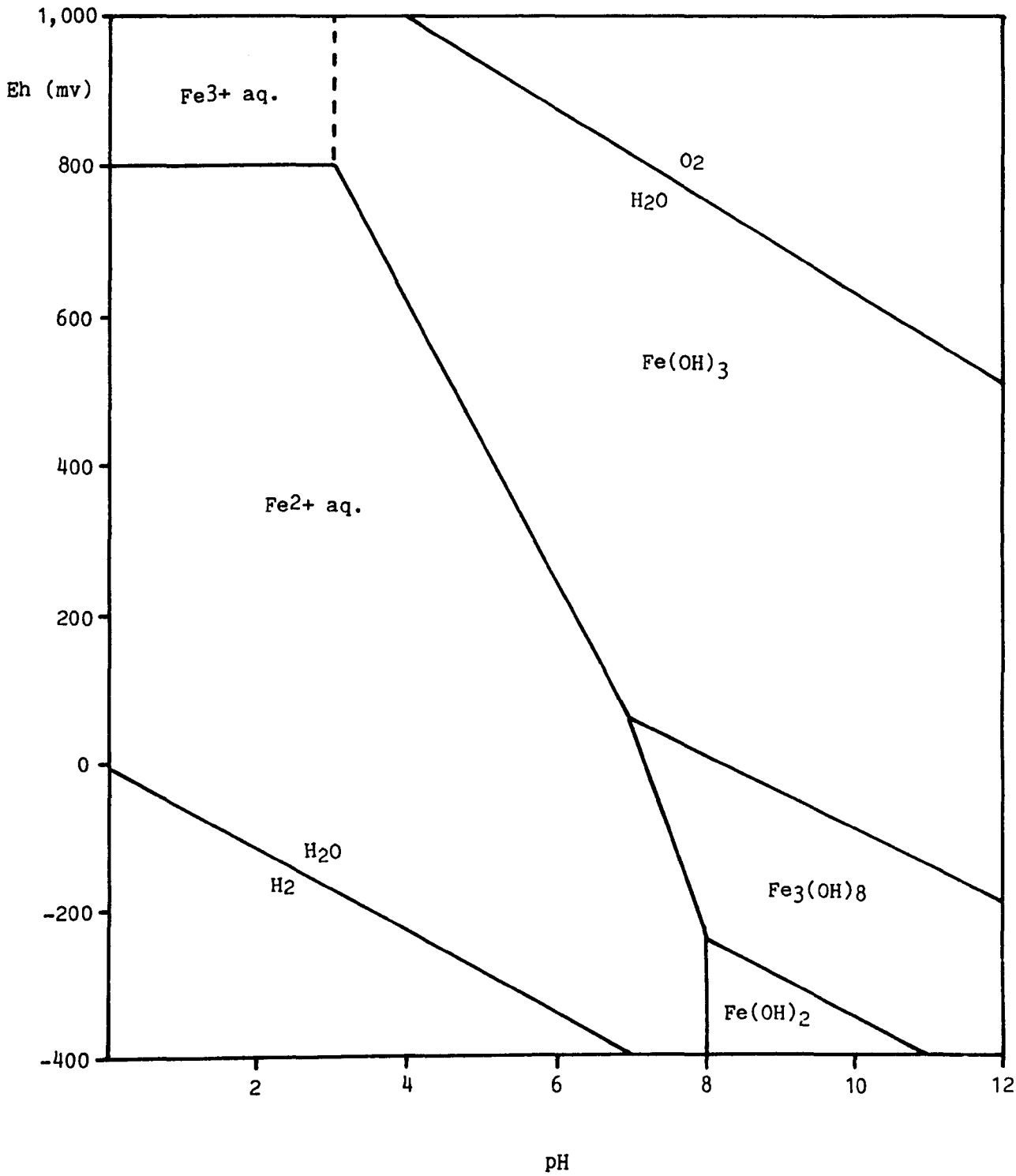
1.3 CONTROLS ON IRON AVAILABILITY IN THE ENVIRONMENT

Iron is an abundant element in most soils, but total iron does not relate reliably to solubility and plant availability (Hodgson 1972). In soils, a dominant oxidation-reduction couple is often Fe^{2+}-Fe(OH) (Armstrong 1982). The hydroxide is highly insoluble and the solubility of iron in such a system is governed by both pH and oxidation-reduction potential. Figure 1.1 shows the pH-Eh relationship for this redox couple (adapted from Armstrong 1982). There are two situations in which iron may become plant-available.

a. In very acid soils (pH 4 or less) (Olson 1947, Armstrong 1982) when iron is available in the ferric form.

b. Under reducing conditions (i.e. waterlogging) when iron is

Figure 1.1 pH-Eh Relationship for Fe^{2+}-Ferric Hydroxide at 25°C
(adapted from Armstrong 1982)



available in the ferrous form.

The study of ferrous iron toxicity under waterlogged conditions is the aim of the present work, though there is evidence that excess iron supplied either as Fe^{2+} or Fe^{3+} can be toxic (Skeen 1929, Olsen 1958, Jones and Etherington 1970, Brown and Jones 1977).

According to Gambrell and Patrick (1978) and Etherington (1983b) reduction of ferric iron is complete at an E_7 value of around 120 mV. This follows depletion of oxygen and sequential reduction of nitrate and manganic ions by soil microorganisms. If reduction is sufficient, sulphate and occasionally carbon dioxide may be reduced. Such changes are reviewed more fully by Ponnamperuma (1972), Gambrell and Patrick (1978), and Armstrong (1982).

Inorganic phosphorus may be released following the reduction of ferric iron compounds (Mortimer 1941; Patrick 1964; Patrick and Mahapatra 1968; Ponnamperuma 1972; Patrick and Khalid 1974; DeLaune, Reddy and Patrick 1981). This can occur if sufficient ferric phosphate is present in the soil (Marschner 1986) or if phosphate is adsorbed onto crystalline and particularly amorphous ferric solids (Patrick and Khalid 1974; Borggard 1983). Conversely, Waldren, Etherington and Davies (1987) suggest that phosphorus availability may be reduced by waterlogging since gel-like ferrous oxyhydroxides have a greater surface area than ferric oxyhydroxides for phosphorus adsorption.

Hutchinson (1957) reports that under reducing conditions ferrous iron may be soluble at pH values as high as 8. Thus it may be available at phytotoxic concentrations even in base-rich situations (Martin 1968; Wheeler, Al-Farraj and Cook 1985). Wheeler *et al.* (1985) suggest that Hutchinson's values may be conservative since more soluble iron solids ($Fe_3(OH)_8$, $FeCO_3$) may control Fe^{2+} equilibrium concentrations (Postma 1982; Schwab and Lindsay 1983)(see also Foy, Chaney and White 1978). Indeed supersaturation with siderite ($FeCO_3$) is possible in soil solution (Ponnamperuma 1972; Crowder and Macfie 1986).

Soluble organic-iron complexes which may be available to plants (Marschner and Barber 1975; Uren 1984) can also maintain iron in solution (Theis and Singer 1973) in organic substrata and may partly account for high dissolved-iron concentrations measured in dialysis cells (Wheeler and Giller 1984). In addition, some insoluble iron may be available to plant roots (Pozuelo and Grossenbacher 1965; Uren 1984) by contact reduction.

1.4 TOLERANCE TO WATERLOGGING

1.4.1 Theories of Waterlogging Tolerance

Explanations of waterlogging tolerance in plants have centred on 3 lines of research (Keeley 1979).

1. Metabolic Adaptations (Anaerobic Respiration)

One line of research holds that plants compensate for hypoxic conditions through metabolic changes in the root (i.e. anaerobic respiration). Within this field there has been controversy between two schools of thought, that of Crawford and co-workers (e.g. Crawford 1966, 1967, 1969, 1972, 1977; Crawford and McManmon 1968; McManmon and Crawford 1971; Garcia-Novo and Crawford 1973), and that of a number of other workers (e.g. Taylor 1942; John and Greenway 1976; Wignarajah and Greenway 1976; Avadhani *et al.* 1978; Smith and ap Rees 1979; Smith *et al.* 1984; ap Rees *et al.* 1987).

2. Internal Aeration

A second line of research maintains that waterlogging-tolerant plants are able to continue aerobic respiration in the roots by allowing atmospheric air to diffuse through the plant in a continuous system of aerenchyma (e.g. Conway 1937, 1940; Armstrong 1968, 1978).

3. Amelioration or Avoidance of Soil Toxins

A third line of study focuses on the toxicity of the reduced soil environment and argues that flood tolerance stems in part from the ability to avoid excessive accumulations of elements such as iron and manganese (e.g. Bartlett 1961; Martin 1968; Jones and Etherington 1970). The present study is aimed at this aspect of waterlogging tolerance.

It is likely that these three approaches to the study of waterlogging tolerance are linked, and indeed plants with a number of adaptations to

waterlogging tend to be the most successful in these situations (Keeley 1979; Hook and Scholtens 1978 ; Talbot, Etherington and Bryant 1987).

1.4.2 **Adaptations to Waterlogging**

1.4.2.1. Anaerobic Respiration

This may be a temporary waterlogging response (Keeley 1979; Drew and Lynch 1980), or it may occur continuously in parts of the root. Armstrong and Beckett (1987) have demonstrated by a diffusion-based mathematical model that anoxia in both stele and endodermis is possible and that this could serve to increase oxygen availability for rhizosphere oxidation.

1.4.2.2. Aerenchyma

Wetland species characteristically possess aerenchyma which is generally thought to aid the diffusion of oxygen from shoot to root, both for aerobic respiration requirements, and for amelioration of reduced soil toxins in the rhizosphere (e.g. Nagai and Matono 1959; Armstrong 1964, 1967, 1972, 1978, 1982; Tanaka, Loe and Navasero 1966; Martin 1968; Howeler 1973; Bacha and Hossner 1977; Green and Etherington 1977; Keeley 1979; Drew and Lynch 1980; Chen *et al*. 1980a, b; Mendelssohn and Postek 1982). Rhizosphere oxidation is thought to occur at the expense of root aeration (Armstrong and Beckett 1987), and it has been reported (Smirnoff 1981; Mendelssohn and McKee 1987) that oxygen transport is unable to maintain a fully aerobic metabolism in an anaerobic medium. In the root, diffusion occurs through intercellular spaces or aerenchyma in the cortex, though the gas space system is often distributed non-uniformly along the length of the root (Armstrong 1971, 1978). The mechanism of oxygen transport is undoubtedly gaseous diffusion rather than any sort of active transport (Evans and Ebert 1960; Barber *et al*. 1962; Greenwood 1967; Armstrong 1964, 1967, 1979; Teal and Kanwisher 1966; Luxmore *et al*. 1970), and mathematical models have been based on this (Armstrong 1978). Since aerenchyma formation both increases root porosity and reduces oxygen demand, it favours root extension in anoxic media (Armstrong 1979).

Table 1.1 Functions of Adventitious Roots Suggested in the Literature

Function	Reference
Absorbing organs	Jackson 1955
As a source of plant hormones (gibberellins) to the shoot	Gill 1975
To take over should the original root system become damaged	Gill 1975
Transfer of ethanol to environment	Keeley 1979
For mechanical support in unstable wet habitats	Etherington 1984
Oxygen transport (since they are aerenchymatous)	Hook and Scholtens 1978 Drew *et al.* 1979 Drew and Lynch 1980 Drew 1987
Since they grow near the surface, they are more likely to be within range of oxygen diffusion from the shoot, and less likely to be subject to reduced soil toxins than roots more deeply submerged	Armstrong 1968, 1982
They make a substantial contribution to water uptake, possibly reducing the rate at which dissolved toxins (Fe and Mn) arrive at the surface of deeper roots, and thus increasing the likelihood of their immobilisation.	Etherington 1984

1.4.2.3. Adventitious Roots

It has been noted that species or plants which produce adventitious roots tend to be better able to tolerate waterlogging than those which do not (e.g. Kramer 1951; Jackson 1955; Rowe and Beardsall 1973; Hook and Scholtens 1978; Keeley 1979; Drew and Lynch 1980; Etherington 1983a, 1984; Tang and Kozlowski 1984; Talbot, Etherington and Bryant 1987; Waldren, Davies and Etherington 1987a; Kransy et al. 1988). However their function (if they are not merely a symptom of waterlogging damage) seems in doubt. Etherington (1984) said they were effectively adaptive but did not know the mechanism by which they aided growth. Various suggested functions are listed in Table 1.1, and any signs that they might act as oxidative organs were noted in this study.

1.5 RHIZOSPHERE OXIDATION

1.5.1 Evidence

Deposits of a red-brown oxide/hydroxide (ochre or plaque) have been reported on or around the roots of a number of species grown under waterlogged conditions (Table 1.2). This provides evidence that rhizosphere oxidation is an exclusion mechanism for ferrous iron. Bartlett (1961) noted that though such precipitates occurred on roots of mesophytes, they were more common on hydrophytes.

1.5.2. Amelioration of Toxicity

It has been established that species vary in the rate at which oxygen diffuses from their roots and that this flux of oxygen into the rhizosphere is related to the intensity of anaerobiosis which a given plant can tolerate (Armstrong 1972). Differences in the capacity of wetland rice cultivars to release oxygen from the roots has been shown to correlate with resistance to iron toxicity diseases (Marschner 1986). The greater the flux of oxygen, the larger the oxygenated rhizosphere. This is important as many reduced soil toxins (e.g. Fe^{2+}, Mn^{2+}, S^{2-}) are only slowly oxidised and require a long residence time in oxidising conditions (Armstrong 1982). Armstrong (1972) showed that radial oxygen losses from

Table 1.2 Species with Ochreous Deposits reported on or around Roots

Species	Reference
Agrostis gigantea	McLaughlin et al 1985
Carex flacca	Heathcote, Davies and Etherington 1987
Carex rostrata	Macfie and Crowder 1987
Epilobium hirsutum	Etherington 1983b, 1984
Erica cinerea Erica tetralix	Jones and Etherington 1970
Juncus subnodulosus	Al-Farraj 1983
Oryza sativa (rice)	Ponnamperuma et al. 1955 Jeffery 1961 Howeler 1973 Green and Etherington 1977 Bacha and Hossner 1977 Chen, Dixon and Turner 1980a, b Boone, Bristow and VanLoon 1983
Phragmites communis	Taylor and Crowder 1983a Macfie and Crowder 1987 Mansfield 1990
Spartina alterniflora	Mendelssohn and Postek 1982
Typha latifolia	Taylor, Crowder and Rodden 1984 Crowder and Macfie 1986
Various	Armstrong and Boatman 1967

roots increased with root porosity up to high porosity levels.

1.5.3. Internal and External Ochre Precipitation

The size of the oxidised rhizosphere depends on the oxidising capacity of the plant and also on the oxidation-reduction potential of the substrate (Taylor, Crowder and Rodden 1984). Oxidation may be internal to the root as well as external (Armstrong 1967), and iron precipitates have been found inside the root (see also Botha et al. 1985). In Spartina alterniflora, Mendelssohn and Postek (1982) found the deposits restricted to the epidermal cell layer. Armstrong and Boatman (1967) noted that iron precipitates extended up to three cell layers into the outer cortex of Menyanthes trifoliata roots, while Green and Etherington (1977) found that deposits in rice roots extended into the cortex and along the diaphragms of the aerenchyma, but were not found in the endodermis or stele. They suggested that iron might travel along the middle lamellae following the transpiration stream pathway. It is thought likely that species with the poorest capacity for oxygenation may precipitate a greater proportion of the iron in, rather than on the roots, particularly in very reducing soils (Taylor, Crowder and Rodden 1984). There are some reports (e.g. Talbot, Etherington and Bryant 1987) that high iron concentrations in the root are associated with high phosphorus concentrations (see also Foy, Chaney and White 1978).

High concentrations of iron tend not to be translocated to the shoots and root iron concentrations are often an order of magnitude greater than in the shoot. High root/shoot ratios of iron have often been reported in plants which have been growing in waterlogged soil (e.g. Jones and Etherington 1970; Jones 1972a; Rozema and Blom 1977; Ernst 1978). Bartlett (1961) found that species which did not tolerate poor aeration (and were thus poor oxidisers) took up more iron into shoots than did the more efficient oxidisers (see also Davies and Singh 1983). Keeley (1979) reported that iron concentrations in the flooded roots of upland populations of Nyssa sylvatica were an order of magnitude greater than that found in the floodplain population, which was in turn an order of magnitude greater than that found in the swamp population.

1.5.4 Iron Toxicity and Nutrient Deficiency

Risk of iron toxicity may be increased by nutrient deficiency, since this may cause increased exudation of photosynthates from roots, increasing microbial activity and oxygen consumption, i.e. directly competing with its function to ameliorate the rhizosphere (Marschner 1986). Calcium- or nitrogen-deficient plants have a lower oxidising ability than do well-nourished plants (Ando et al. 1983), and the ability to exclude excess iron is reduced in plants that are deficient in calcium, magnesium, phosphorus, manganese and especially potassium (Foy, Chaney and White 1978). In rice and probably other wetland species, aerenchyma formation and stability (and hence tolerance to iron and manganese) depends on silica supply (Marschner 1986).

Howeler (1973) proposed that indirect iron toxicity in rice, due to deficiencies in phosphorus, potassium, calcium and magnesium, might be caused by the formation of an ochreous sheath which might diminish the root's capacity to absorb essential nutrients. There is a little evidence to support this (Otte et al. 1989; Mansfield 1990 cf Crowder et al. 1987). It has also been suggested that the ochreous barrier may have a beneficial effect in reducing uptake of harmful substances such as sulphide, copper and nickel (Armstrong and Boatman 1967; Taylor 1983; Taylor and Crowder 1983b), or may even have a role in micronutrient uptake (Otte et al. 1987, 1989; Conlin and Crowder 1989).

1.5.5. Root Porosity

From studies made on root porosity and the effect of waterlogging on porosity (Smirnoff 1981; Smirnoff and Crawford 1983; Justin and Armstrong 1987), it is apparent that species differ both in their inherent root porosity and in their ability to increase it on waterlogging. Generally wetland species have constitutively, or are able to develop, a higher root porosity on flooding than dryland species. Justin and Armstrong (1987) linked this to cortical cell packing configurations and ethene is thought to be the main growth regulator involved (e.g. Kawase 1978, 1979; Drew et al. 1979; Kawase and Whitmoyer 1980; Konings 1982). Environmental stimuli other than waterlogging are also able to increase root porosity (Smirnoff 1981; Smirnoff and Crawford 1983).

Justin and Armstrong (1987) found a general increase in root length

with increased root porosity, confirming findings of Yu, Stolzy and Letey (1969) and predictions of Armstrong's (1972) model. Since, in anaerobic media, gas transport from the shoot is essential to sustain root growth and root tip viability (Webb and Armstrong 1983), species with greater root porosity are able to root to a greater depth and thus exploit a larger soil volume than species with low root porosity.

1.5.6 **Oxidising Agent**

There is much evidence that atmospheric oxygen is the major oxidising influence forming rhizosphere sheaths, though it is possible that micro-organism-induced oxidation may occur (e.g. Hollis 1967; Pitts 1969; Pitts *et al*. 1972; Trolldenier 1988). Enzymatic oxidation has also been suggested. Yamada and Ota (1958) found that both extracts of rice roots and intact rice roots could oxidise ferrous iron in the presence of molecular oxygen. They attributed the activity to a peroxidase-like enzyme (see also Schreiner and Reed 1909). However Smirnoff (1981) was unable to demonstrate any link between iron tolerance and oxidase or catalase activities. Armstrong (1967) working with *Molinia caerulea* and *Menyanthes trifoliata* suggested enzymatic oxidation may be responsible for up to 90% of total oxidation, since radial oxygen loss only accounted for 10% of the activity measured. Smirnoff (1981) reviewed evidence that the oxidising power of roots could depend on metabolic activity and not simply oxygen diffusion from the roots, but Ando *et al*. (1983) showed that in rice, oxygen released from the roots was not biochemical in origin but was transported internally from the shoot.

It is thought that oxygen generated by photosynthesis may increase oxygen transport to the root since studies have shown that oxidation is greatest at high light intensities (Smirnoff 1981).

1.5.7 **Zonation of Oxidation**

Oxidising activity is reported to be highest around, or just behind the root tip, (which itself contains no air space), and to fall towards the base of the root (Armstrong 1971). This is because in subapical regions of the roots of many wetland species root wall permeability rapidly decreases, possibly to form a barrier to the influx of phytotoxic soil products (Yamasaki 1952), or more likely to maximise oxidising

activity around the root tip (Armstrong and Beckett 1987). There is no such tendency for reduced wall permeability in dryland species (Armstrong 1978). The zone of highest oxidising activity around the root tip often remains free from deposits while ochre tends to increase in intensity behind the tip, and then fall towards the base (Armstrong 1967). Fitter and Hay (1981) suggest that protection of the meristem ensures continued growth which could provide new sites for toxin chelation. More recent studies (Conlin and Crowder 1989; Laan *et al.* 1989) suggest that the pattern of oxidation along the length of a root differs in different wetland species.

Several diffusion-based mathematical and electrical models have been employed to help evaluate root aeration in saturated environments (e.g. Greenwood 1967; Luxmore *et al.* 1970; Armstrong 1972, 1979; Armstrong and Wright 1976; Armstrong and Beckett 1987). They have provided useful insights into the process of root aeration (e.g. extent of rhizosphere oxidation, zonation of oxidation, and effect of porosity and root length on radial oxygen loss), and have helped greatly in an assessment of its effectiveness.

1.5.8 **Models of Plaque Formation**

In plants grown in solution culture, ochre deposits (which only form if the iron source is ferrous iron) are usually amorphous and intimately associated with the root. However, in the field, ochre may be less evenly distributed on the root and have a more particulate nature (Taylor, Crowder and Rodden 1984 cf Mendelssohn and Postek 1982). Bacha and Hossner (1977) and Chen *et al.* (1980a) identified α-FeOOH (goethite) and γ-FeOOH (lepidocrocite) as the primary constituents of such plaques on the roots of *Oryza sativa*.

Based upon interpretation of scanning electron micrographs, Chen *et al.* (1980b) proposed two models of plaque formation. In one model, intact cell walls provide a template for the development of polyhedral iron oxide casts through the precipitation of FeOOH on both the internal and external surfaces of the cell wall. Subsequently the outer cell walls may decompose, permitting a junction to form between two adjacent casts. In the second model, the outer tangential cell walls decompose prior to the precipitation of FeOOH in open cell cavities. According to Marschner (1986) this process is enzymatic. Roots of *Typha latifolia* collected from

the field by Taylor et al. (1984) showed such cell cavities which were becoming infilled with FeOOH. Observations by these authors did not contradict the first model, but provided more support for the second model.

1.5.9 Seasonality and Site Dependence

Crowder and Macfie (1986) reported that deposition of ferric hydroxide plaque on the roots of three wetland species was seasonal (see also Crowder et al. 1987). On Typha latifolia, Carex rostrata and Phragmites australis roots, deposition increased rapidly in June and peaked in July-August which corresponded with peak above ground biomass production.

Ochre deposits were found on the roots of Typha latifolia at only some of the iron-rich wetlands studied, despite the fact that representatives from all the Typha populations were capable of producing ochre, when grown in the laboratory under anoxia, with a ferrous iron source (Macfie 1986). It was thus concluded that plaque formation was site-dependent but unrelated to oxidation-reduction potential.

Macfie and Crowder (1987) investigated a number of site factors known to affect iron solubility in wetlands and found that plaque formation correlated positively with extractable iron and pH (see also Tanaka and Navasero 1966e), and negatively with % organic matter and % inorganic carbonates in the soil. Soil texture had no effect. All the above correlations were significant but weak, though the strongest relationship was with % inorganic carbonates. In a stepwise multiple regression analysis these four factors accounted for 72% of the variance in plaque formation. More recent work (St-Cyr and Crowder 1988, 1989) has shown that the amount of plaque on P. australis roots was strongly correlated with the amount of iron-bound-to-carbonates fraction of the substrate. Additionally, greatest plaque accumulations were observed on roots which were bathed in flowing water.

1.6 ALTERNATIVE IRON TOLERANCE MECHANISMS

Smirnoff (1981) did not support the hypothesis that iron tolerance is a result of ferrous iron oxidation by oxygen diffusing from shoot to root. He noted that since the root tip was the primary site for toxic ferrous

iron action, tolerance mechanisms (physiological or metabolic adaptations) must take this into account. He could however find no evidence that malate or citrate accumulated in tolerant species to detoxify iron by chelation (see also Hodgson 1972; Keeley 1979; Etherington 1983b) although their ratio might be important (cf Hodgson 1972). Preliminary investigation into the possible removal of ferrous iron by root extracts gave inconclusive results. Some (but not all) of his results supported the hypothesis that there might be a link between iron tolerance and iron inefficiency (see also Hodgson 1972; Etherington and Thomas 1986).

Metabolic tolerance of iron at the cellular level may be an additional tolerance mechanism to exclusion by rhizosphere oxidation (Jones and Etherington 1970; Davies and Singh 1983; Talbot *et al.* 1987; Mansfield 1990), and the sensitivity of shoot tips of populations of *Carex flacca* to iron has been shown to be inversely related to their waterlogging tolerance (Etherington 1983b). Tolerance at the cellular level may be particularly important in situations where the exclusion mechanism is inadequate (e.g. if the external iron concentration is very high, if exposure is for a long period or if the shoot is transiently flooded). However, no mechanism for such tolerance has yet been identified, though Bienfait (1989) suggests that the possible role of ferritin warrants investigation. It is known that susceptibility to manganese tolerance is associated with tolerance of shoot tissues to high manganese concentrations rather than to its exclusion (Marschner 1986). Momon *et al.* (1980) found that manganese deposition took place in the extra-cellular compartment of the cell walls in the epidermis, mesophyll and bundle sheath in one manganese accumulating species.

There is limited evidence that reduction of the transpiration rate may slow the movement of reduced toxins to the root surface in the transpiration stream and permit more effective oxidative detoxification of iron (Jones 1971a; Armstrong 1982; Etherington 1983b). In bog species, plant characteristics which reduce transpiration rate (xeromorphic adaptations) can be attributed to nutrient deficiency (Eber 1982).

It seems likely that the species most tolerant to iron may possess more than one detoxification mechanism.

Table 1.3 Studies involving Toxicity or Tolerance of Iron on Native British Species

Reference	Species studied
Martin 1968	Brachypodium sylvaticum Carex sylvatica Circaea lutetiana Deschampsia caespitosa Endymion non-scriptus Geum urbanum Mercurialis perennis Primula elatior Primula vulgaris
Jones 1971a, b Jones and Etherington 1970	Erica cinerea Erica tetralix
Jones 1972a Jones and Etherington 1971	Agrostis stolonifera Carex flacca Carex nigra Festuca rubra
Hodgson 1972	Agrostis tenuis Briza media Carex flacca Deschampsia flexuosa Epilobium hirsutum Galium palustre Lathyrus montanus Leontodon hispidus Luzula multiflora Nardus stricta Poa trivialis Rumex acetosa Rumex hydrolapathum Scabiosa columbaria
Sanderson and Armstrong 1980a	Picea sitchensis Pinus contorta
Smirnoff 1981	Chamaenerion angustifolium Deschampsia caespitosa Eriophorum angustifolium Eriophorum vaginatum Glyceria maxima Myosotis scorpioides Nardus stricta Ranunculus flammula Schoenus nigricans Senecio aquaticus Senecio jacobea Zerna ramosa
Al-Farraj 1983 Wheeler, Al-Farraj and Cook 1985	Epilobium hirsutum Juncus subnodulosus
Davies and Singh 1983	Festuca rubra
Etherington and Thomas 1986	Dactylis glomerata
Waldren, Davies and Etherington 1987a	Geum rivale Geum urbanum
Talbot, Etherington and Bryant 1987 Talbot and Etherington 1987	Salix caprea Salix cinerea
Mansfield 1990	Eriophorum angustifolium Phragmites australis

1.7 PREVIOUS STUDIES ON IRON TOXICITY AND TOLERANCE

Iron toxicity has been reported in rice by many workers (e.g. Ponnamperuma, Bradfield and Peech 1955; Tanaka, Loe and Navasero 1966; Foy et al. 1978), often on acid substrata (e.g. Tanaka and Navasero 1966c). Studies of waterlogged soil however, usually involve potential toxicity from manganese and sulphide as well as iron. Solution culture work enables ferrous iron toxicity to be studied alone; however such studies have been rare.

Work on native species has considered both intraspecific tolerance (e.g. Davies and Singh 1983; Etherington and Thomas 1986), and interspecific tolerance (Table 1.3). Most of the latter studies have compared pairs of closely related or morphologically similar species (e.g. Jones 1971a, b; Jones and Etherington 1970; Sanderson and Armstrong 1980a; Talbot and Etherington 1987; Talbot, Etherington and Bryant 1987; Waldren, Davies and Etherington 1987a) or unrelated species (e.g. Al-Farraj 1983; Wheeler, Al-Farraj and Cook 1985; Mansfield 1990). Few studies have been made on a wide range of species (e.g. Martin (1968) - dryland (woodland) species; Hodgson (1972) - mainly dryland calcicoles, calcifuges and a few marsh species; Jones (1972a) - a small number of dune and dune slack species). The only study that compared several wetland species (Smirnoff 1981), failed to link iron tolerance with a number of proposed tolerance mechanisms including rhizosphere oxidation. Existing studies involving iron toxicity and/or tolerance in explaining the distribution of native species are summarised in Table 1.3.

1.8 OBJECTIVES AND OUTLINE OF THE PRESENT STUDY

The objectives of the present study were to investigate the toxicity of ferrous iron to a range of wetland species, to examine the mechanism(s) involved in iron tolerance, and to see to what extent variation in iron tolerance may explain the field distribution of the species.

Literature on iron and other metal tolerance is reviewed. The toxicity of ferrous iron to 39 wetland species was assessed and a number of ways of measuring and expressing iron tolerance were explored (Chapters 2, 3, 4 and 5). Iron tolerance was related to the nature of precipitates which formed on the root, to relative growth rate and to changes in

shoot/root ratio (Chapter 4). The direct or indirect characteristics of toxicity were investigated, and the relative importance of exclusion and internal detoxification was examined for selected species (Chapter 6). The field distribution of the 39 screened species was related to their tolerance to iron (Chapter 7); and possible interactions between iron and calcium, iron and bicarbonate, and iron and nitrogen source were also studied (Chapter 8).

The findings of these investigations are discussed fully in each chapter, and are summarised in the General Discussion (Chapter 9).

CHAPTER TWO

EXPERIMENTAL APPROACHES

2.1 PROBLEMS OF ASSESSING IRON TOLERANCE

2.1.1 Introduction

Any study aimed at assessing the response of a plant to a toxin must attempt to satisfy two conditions. First a known and constant amount of the toxin should be supplied, and secondly possible influence of other variables should be kept to a minimum.

2.1.2 Chemical Instability

There are problems trying to satisfy these conditions for the assessment of iron toxicity. Iron may oxidise and become less available (e.g. Green and Etherington 1977), and co-precipitation and/or adsorption of phosphates is likely to occur (Kuraev 1966, Hodgson 1972), making iron and particularly phosphorus potentially less available, especially if an inorganic iron source such as ferrous sulphate is used. Hodgson (1972), however, reported little oxidation of ferrous to ferric iron when titrated with potassium permanganate at pH 5.0. Kuraev (1966) states that most of the iron in his solutions was the reduced form even after aeration. In this study, it was thought that phosphorus precipitation from solution might be a potentially greater problem than iron precipitation. Another variable which cannot easily be kept constant is pH; it tends to fall with time as phosphorus precipitates and is replaced by sulphate (Hodgson 1972).

2.1.3 Choice of Iron Source

The use of FeNaEDTA as an iron source has none of these problems associated with it. There are however mixed reports in the literature as to how plants respond to chelated iron sources (e.g. Tiffin and Brown 1959; Hill-Cottingham and Lloyd-Jones 1961; Wallace and Hale 1961; Chaney

et al. 1972). Romheld and Marschner (1981) state that plants are able to take iron up both as an intact iron chelate molecule and as ionic iron after chelate splitting. The extent of chelate splitting in the roots, and thus the subsequent iron uptake, depend on the particular plant species (Brown et al. 1961; Marschner et al. 1978); dicotyledons with a higher root cation exchange capacity split chelates at a faster rate than do monocotyledons (Romheld and Marschner 1981). Chelate stability (Brown et al. 1961), chelate concentration (Beckett and Anderson 1973), the pH value of the nutrient medium (Romheld and Marschner 1981), and the plant's iron status (Hill-Cottingham and Lloyd-Jones 1965; Beckett and Anderson 1973) also affect a plant's response to chelated iron sources. Concentrations of chelated iron compounds or of chelating agents alone above 10 mg l^{-1} are reported to be distinctly toxic and may interfere with specific requirements for other micronutrients (Hewitt 1966). Wallace et al. (1957) refer to competition within the leaves between chelates and enzymes for micronutrients. EDTA is therefore likely to be of greater use in deficiency studies than in toxicity studies.

In a preliminary trial on Epilobium hirsutum, FeNaEDTA and $FeSO_4$ were used as iron sources in full strength Rorison solution (Hewitt 1966, Table 30c). Increasing concentrations of iron from both sources produced identical responses, although the supplied concentration of FeNaEDTA required to produce a given response was greater than that of $FeSO_4$. This is the reverse of the findings of Jones and Etherington (1970) working on cut shoots of Erica cinerea and Erica tetralix.

In view of the above evidence and the fact that sulphate is found naturally in wetlands, sometimes in high concentrations (Etherington 1983b) without adversely affecting plants, iron was supplied as ferrous sulphate. This had one further advantage of being comparable with the work of Kuraev (1966), Martin (1968), Hodgson (1972), Jones and Etherington (1970), Jones (1971a) and Smirnoff (1981).

2.1.4 **Phosphorus Deficiency**

To help minimise iron precipitation, low-phosphorus nutrient solutions can be used, though this might conceivably cause phosphorus deficiency. Kuraev (1966) working on oats and spring wheat investigated the amount of growth reduction due to direct iron toxicity and that caused by phosphorus deficiency. He worked at 6-8 mg P l^{-1} with small seedlings and

changed the solution every two days and, since the response was immediate, he concluded the effect was one of toxicity rather than of a deficiency. In a further experiment, he adjusted the amount of phosphorus supplied so that at equilibrium all treatments had the same phosphorus concentration in solution (approx. 4 mg l^{-1}), while the iron concentration was varied. He found the greatest yield reduction to occur between the two lowest iron treatments which contained practically the same concentration of phosphorus in solution. Results of a third experiment showed that the toxic effect on plants of iron in water culture was not eliminated by supplementary phosphorus nutrition of plants via the leaves. In a fourth experiment involving a split root technique where phosphate and other nutrients were fed to part of the roots and ferrous sulphate to the rest, root growth in both parts was severely reduced at high iron concentration and the decrease in yield with increasing iron concentrations was almost equal to that in preceding experiments. He concluded that iron toxicity was the cause of these symptoms and that phosphorus deficiency was not a problem. By a series of experiments, Al-Farraj (1983) arrived at the same conclusion (cf Chiu 1966). Etherington and Thomas (1986) grew tillers of Dactylis glomerata for 2 months in nutrient solution with additions of ferrous sulphate and manganese sulphate but lacking phosphorus, without the appearance of deficiency symptoms. They assumed that pretreatment in full nutrient solution would provide substantial reserves (see also McCain and Davies 1983). Indeed, there is often a tendency for experimenters to supply phosphorus concentrations which are several orders of magnitude greater than is typically found in soil solutions (Marschner 1986).

2.2 PRELIMINARY TRIALS TO DETERMINE SUITABLE EXPERIMENTAL CONDITIONS

2.2.1 Range of Iron Concentration and pH of Solution

Using Epilobium hirsutum as the test plant, iron was supplied as ferrous sulphate in 10% Rorison solution at 5, 10, 50, 100 and 250 mg Fe l^{-1}. Solutions were changed every two days and were adjusted to either pH 4.5 or pH 5.5.

Severity of symptoms increased with increasing iron concentration and, although the results were not quantified, there was no apparent difference between the pH treatments (see also Al-Farraj 1983). Table 2.1

shows the plant response after eight days (at either pH).

Table 2.1 Visual Response of Epilobium hirsutum seedlings grown for 8 days at different iron concentrations ($FeSO_4$, in 10% Rorison solution) at pH 4.5 and 5.5

	Iron concentration (mg l^{-1}) added as Ferrous Sulphate				
	5	10	50	100	200
Shoots	Healthy	Less healthy (smaller and darker green)	Many wilted, yellow and bruised.	Wilted or shrivelled. Some leaf reddening Some black necrotic patches.	Dead
Roots	White, long and prolific Adventitious roots with side roots	Old roots black. Adventitious roots white, shorter and less prolific.	Extensive blackening. No adventitious roots	Black and flaccid. No adventitious roots.	Black and flaccid. No adventitious roots

2.2.2. To Assess the Effect of Deoxygenating the Nutrient Solutions

Iron was supplied to Epilobium hirsutum as ferrous sulphate in 10% Rorison solution at 3.8, 50 and 100 mg Fe l^{-1} at pH 5.5 either deoxygenated ($-O_2$) with nitrogen, or not deoxygenated ($+O_2$), and at pH 4.5 not deoxygenated ($+O_2$). pH was monitored periodically throughout the experiment and the solutions were changed after two days. After four days the plants were harvested, rinsed in distilled water, dried (three days at 50°C) and weighed. Subsamples of each solution were centrifuged at 4,250 rpm for 5 minutes at 20°C and analysed for iron (Pye-Unicam SP190 atomic absorption spectrophotometer), and soluble reactive phosphorus (SRP). This was estimated by a molybdenum blue method (Stainton, Capel and Armstrong 1977), using an SP8-100 UV/VIS spectrophotometer at 710 nm. Results are shown in Table 2.2 a, b, c, and were analysed by one-way analysis of variance. Duncan's New Multiple Range test was used to distinguish significantly different mean values ($p < 0.05$).

Table 2.2 Yield of Epilobium hirsutum, and content of Soluble Iron and Soluble Reactive Phosphorus in Oxygenated and Deoxygenated Solutions
(mean of 5 replicates ± 1 SE)

Treatment (mg Fe l^{-1})	pH 5.5 ($-O_2$)	pH 5.5 ($+O_2$)	pH 4.5 ($+O_2$)
	a. Yield(mg)		
3.8	10.40 ± 0.45	12.80 ± 0.35	12.88 ± 0.10
50	7.90 ± 0.28	9.98 ± 0.18	9.74 ± 0.35
100	7.94 ± 0.23	8.84 ± 0.23	9.18 ± 0.31
	b. Iron in solution at end of experiment (mg l^{-1})		
3.8	2.02 ± 0.64	0.92 ± 0.05	1.71 ± 0.04
50	45.75 ± 1.97	45.38 ± 0.37	48.04 ± 0.52
100	98.00 ± 0.67	95.65 ± 0.59	96.51 ± 1.47
	c. SRP at end of experiment (mg l^{-1}) (Added concn. = 3.1 mg P l^{-1})		
3.8	1.91 ± 0.01	1.46 ± 0.03	1.79 ± 0.01
50	0.72 ± 0.01	0.31 ± 0.00	0.49 ± 0.00
100	0.40 ± 0.02	0.02 ± 0.00	0.28 ± 0.00

It was concluded that at all iron concentrations, plants grown in deoxygenated solution had a significantly lower yield than those grown in oxygenated solution, despite there being no more iron in solution and significantly more phosphorus. There was no significant difference in yield between the two oxygenated treatments (pH 4.5 and pH 5.5) indicating that this pH change had no obvious effect. (In fact pH values tended to fall with time to around 4.2 whatever the starting pH, though the deoxygenated solutions tended to have a lower pH than their oxygenated counterparts.)

2.2.3 Choice of Solutions for Assessment of Iron Tolerance

In view of the above pilot experiments it was decided to use 10% Rorison solution as a basal culture solution (Table 2.3), adjusted to pH 5.5 with 1M NaOH or 0.5M H_2SO_4. This pH was chosen to be in the middle of the range at which species to be screened naturally grow, while still maintaining iron solubility.

Table 2.3 Concentration of Elements Supplied in 10% Rorison Nutrient Solution

Element	Concentration ($mg\ l^{-1}$)
Calcium	8.0
Nitrogen	5.6
Magnesium	2.4
Potassium	7.8
Phosphorus	3.1
Manganese	0.05
Boron	0.05
Molybdenum	0.01
Zinc	0.01
Copper	0.01
Iron	Various (3.8 - 100)

10% Rorison solution was deemed adequate in terms of phosphorus supply so long as solutions were changed frequently. Small seedlings were grown in full strength Rorison solution for a minimum of two weeks prior to the experiment to ensure adequate starting phosphorus supply. Solution

culture was used rather than sand culture so that the solutions could be changed frequently and root growth could be observed, and solutions were neither deoxygenated nor aerated.

Iron was supplied as ferrous sulphate, and concentrations used were 3.8 mg Fe l^{-1} (control, - the iron concentration in 100% Rorison solution which is supposedly optimal for plant growth), 10, 25, 50, 75, and 100 mg l^{-1}. Selected aspects of solution chemistry are reported in Section 2.5.

2.3 METHODS OF ASSESSING IRON TOLERANCE

The tolerance of a species to iron (or to any metal or toxin) can be assessed in a variety of ways (see also Baker and Walker 1989a), though ultimately it is the ability to establish, survive and reproduce in an iron-rich environment which is important. Methods of tolerance assessment include:-

a. Yield response, or other growth measurement such as leaf number, shoot height etc. after a fixed time period (e.g. Kuraev 1966, Jones and Etherington 1971).

b. Plant mortality with time.

c. Root elongation over time (e.g. Wilkins 1957, 1978; Hodgson 1972; Smirnoff 1981; Wong and Bradshaw 1982; Al-Farraj 1983; Waldren, Davies and Etherington 1987b).

d. Germination (e.g. Wong and Bradshaw 1982), or seedling survival (Walley *et al*. 1971; Karataglis 1980).

It was decided not to use the most accepted method of metal tolerance measurement, that of root elongation, despite the fact that one of the first toxic effects of high concentrations of most metals is to stop root development (Marschner 1986). Al-Farraj (1983) had demonstrated that *Juncus subnodulosus* showed reduced root elongation yet survived in high iron concentrations while *Epilobium hirsutum*, the more sensitive species, had shown greater root elongation but died in the same iron concentrations. He concluded that root elongation is not always a reliable toler-

ance indicator for iron. Hodgson (1972) found Rumex hydrolapathum to be a quite tolerant species to iron when using root elongation as the indicator, but in the present study it was found to be one of the most sensitive species.

Use of a number of variables was investigated for their suitability in assessing iron tolerance over a range of iron concentrations for a fixed time period. Two weeks was found to be sufficient to show up differences in response between species, and thus a number of species could be screened in a relatively short time.

Two alternative approaches, that of germination, and that of plant mortality with time were tested on a selection of species and the results were compared with those from the Standard Screening Experiment (Chapters 3, 4 and 5).

2.4 METHODS

2.4.1 Choice of Species

A total of 39 species were screened for iron tolerance, 23 dicotyledons and 16 monocotyledons including wetland and non-wetland seed sources of Molinia caerulea (see Table 2.4). All plants were raised from seed, and these particular species were selected because they germinated relatively easily. Attempts were made to screen a number of other species (Table 2.5), but they could not be germinated in sufficient quantity. Nomenclature follows Clapham, Tutin and Warburg (1981) throughout.

2.4.2 Germination of Seeds

Seed was sown on 15 cm Whatman no. 1 filter paper in petri dishes, watered with distilled water and germinated in a growth room (Temp. 20-30°C, 16 h day). Seeds of certain species would not germinate fresh or after storage (5°C, dry, in the dark); these were cold pretreated (3°C, wet) for various lengths of time, depending on the species (see Appendix I).

Table 2.4 Species Screened for Iron Tolerance
(Monocotyledons are indicated by bold type)

Agrostis stolonifera	**Juncus subnodulosus**
Briza media	Lotus uliginosus
Caltha palustris	Lychnis flos-cuculi
Carex appropinquata	Lysimachia vulgaris
Carex diandra	Lythrum salicaria
Carex echinata	**Molinia caerulea (dryland source)**
Carex lepidocarpa	**Molinia caerulea (wetland source)**
Carex pulicaris	Parnassia palustris
Epilobium hirsutum	Pedicularis palustris
Epilobium palustre	**Phalaris arundinacea**
Eriophorum latifolium	Potentilla palustris
Eupatorium cannabinum	Primula farinosa
Filipendula ulmaria	Ranunculus flammula
Galium aparine	Rumex acetosa
Galium palustre	Rumex hydrolapathum
Holcus lanatus	Scrophularia auriculata
Iris pseudacorus	Thalictrum flavum
Juncus articulatus	Trifolium pratense
Juncus effusus	Valeriana dioica
Juncus inflexus	Valeriana officinalis

Table 2.5 Species which did not Germinate in Sufficient Quantity to be Screened for Iron Tolerance
(Monocotyledons are indicated by bold type)

Carex acutiformis	Pinguicula vulgaris
Carex dioica	Saxifraga aizoides
Cladium mariscus	**Tofieldia pusilla**
Eleocharis quinqueflora	**Typha angustifolia**
Hypericum tetrapterum	**Typha latifolia**
Linum catharticum	Viola palustris
Menyanthes trifoliata	

2.4.3 Growth of Seedlings Prior to Screening

Seedlings were grown sufficiently to be handled easily, and transferred into clear perspex sandwich boxes (27.5 x 15.5 x 9 cm) containing a 2.5 cm layer of alkathene beads, kept moist with 100% Rorison solution. The lid was initially kept closed and then gradually opened to accustom the plants to lower humidity. Some of the slower growing species (e.g. Juncus subnodulosus) were grown on 10% Rorison solution, (in some cases on sand initially - Appendix I), before being given 100% Rorison solution for a minimum of two weeks.

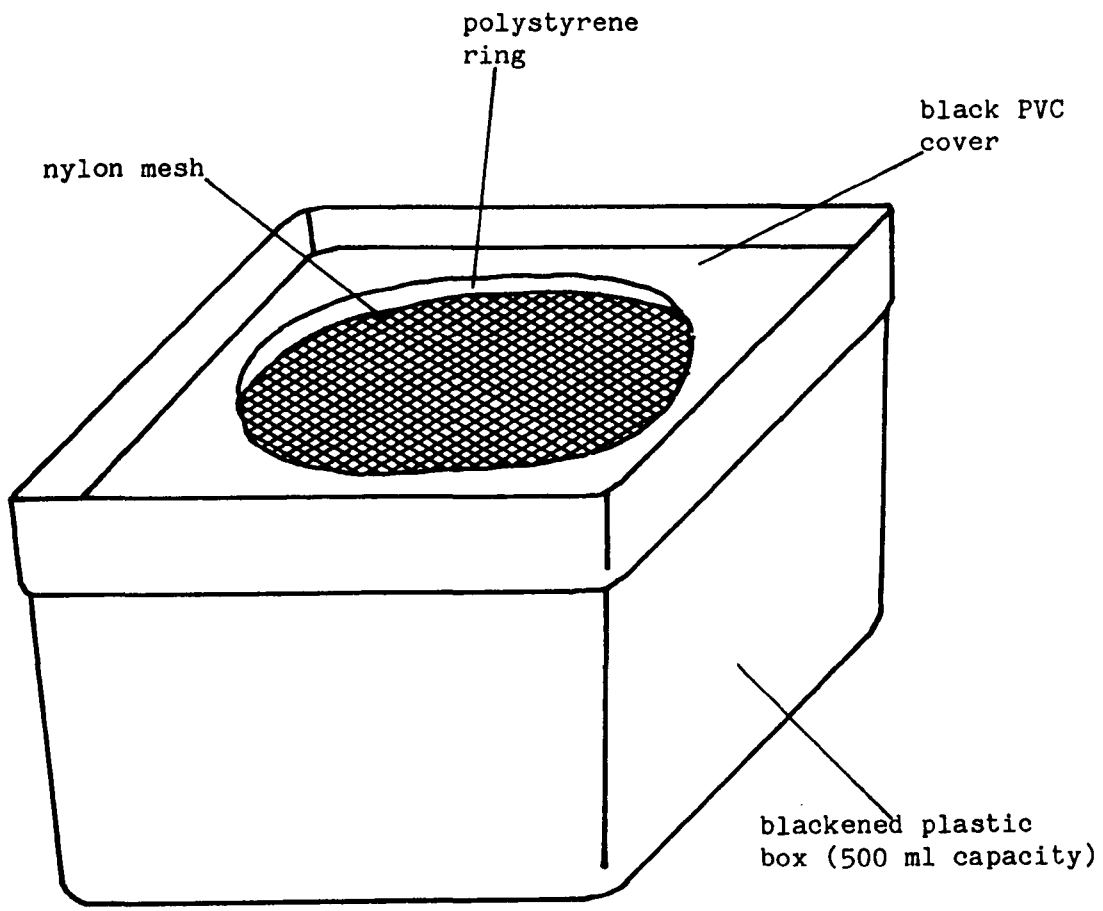
2.4.4 Standard Screening Method

Seedlings greater than 18 days of age (see Appendix I) were used. Size of plants was more important than age. The roots of the seedlings were threaded into floating rafts made from nylon mesh sandwiched between two polystyrene rings. Alkathene beads were placed on top of the mesh, around the plants. This partly supported the shoots so that they did not trail in the solution, eliminated light from the roots and helped prevent algal growth. A black PVC ring was placed over the float to stop light penetrating down the sides of the pot. The float was placed on the solution in a square plastic tub (capacity 500 ml) which had been painted black to stop light penetration (Figure 2.1). The plants were put in 10% Rorison solution with no extra iron for the first day to allow them to become established before being given iron additions. The solutions used were as described in Section 2.2.3.

The experimental design was 6 treatments x 5 replicates x 2 species. Between 3 and 5 plants (depending on size and number available) were used per replicate pot, and one replicate set was harvested at the start of screening, to give information on initial shoot and root weights. The growing period was 2 weeks, and the solutions were changed every 2-3 days to reduce potential phosphorus depletion, maintain iron concentrations and ameliorate effects of any spontaneous acidification of the solutions.

The pots were randomised at each solution change. Day length was 16 hours and temperature ranged from about 20°C at night up to 28-30°C in the day. Illumination was supplied by a mixture of white and warm white tubes (25-30 W m^{-2}, 380-750 nm) (Figure 2.2). The spectral composition contained no far-red light and less green light than would occur naturally,

Figure 2.1 Pot used in the Standard Screening Experiment



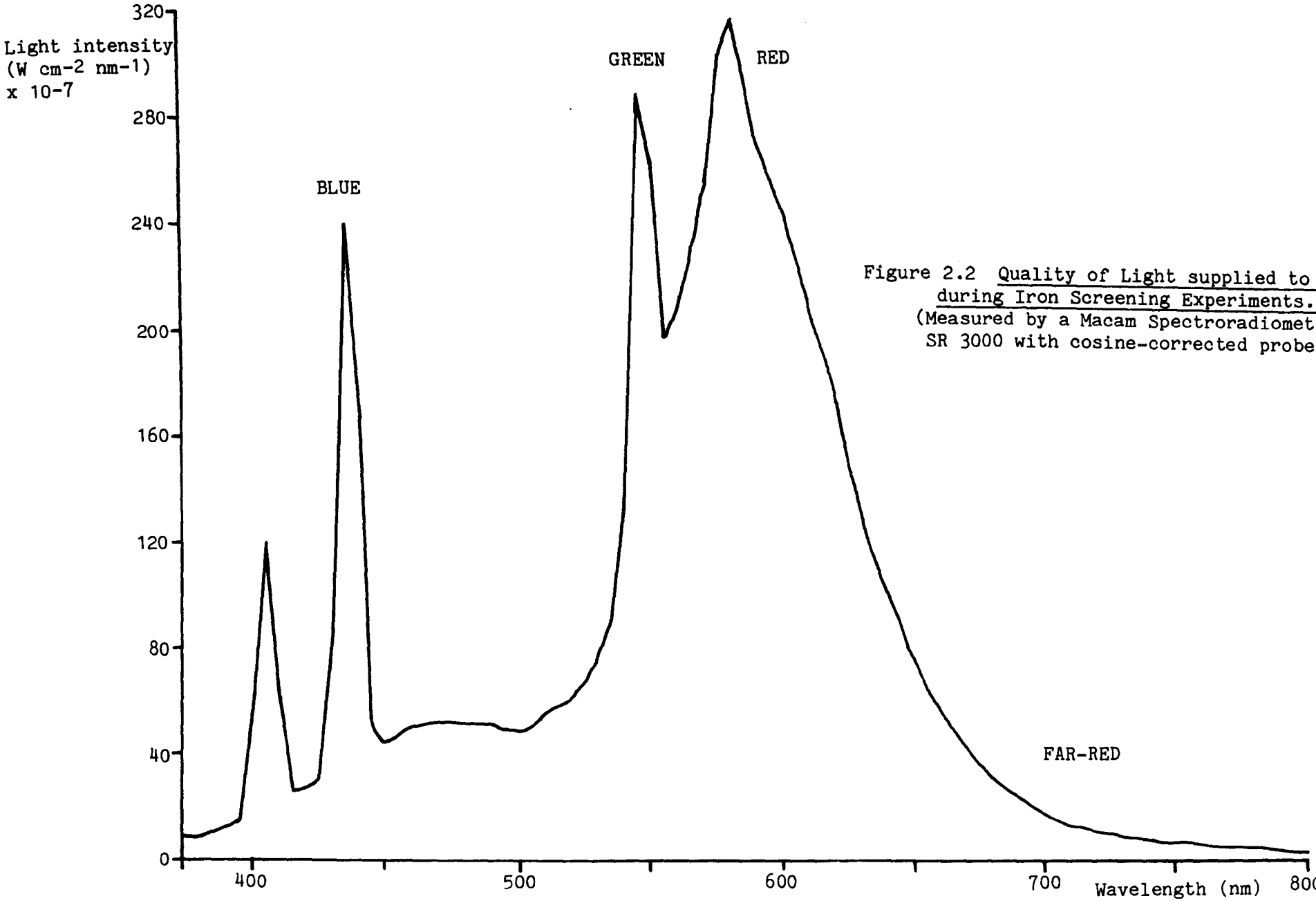


Figure 2.2 Quality of Light supplied to Plants during Iron Screening Experiments.
(Measured by a Macam Spectroradiometer SR 3000 with cosine-corrected probe)

Figure 2.2

so neither light quality nor temperature matched normal environmental conditions very closely.

2.4.5 Observations and Measurements on Seedlings

Visual observations of root and shoot condition were made approximately every two days, and after two weeks the following measurements were made (where relevant) on each plant: shoot length, root length, number of tillers (monocotyledons only), numbers of live, sick and dead leaves, presence/absence of adventitious roots, and some measure of leaf size (width or length).

The roots and shoots were harvested separately (bulk harvest for a pot), washed in distilled water and dried (3 days at 50°C). Shoot and root dry weight were used to calculate shoot/root ratio and relative growth rate (RGR) on the dry weight difference from starting material.

2.5 MEASUREMENT OF SOLUTION CHEMISTRY

A standard screening experiment was set up (using Epilobium hirsutum as the test plant), and over a four day period, (i.e. longer than the maximum time between solution changes) selected aspects of the solution chemistry were monitored.

Each morning (8 a.m.) oxidation-reduction potential and pH (initially 5.5) were measured in situ. A 15-20 ml subsample was taken, centrifuged at 4,250 rpm for 5 minutes at 20°C, and iron and soluble reactive phosphorus concentration were measured in the supernatant (as in Section 2.2.2). Additional pH and oxidation-reduction potential measurements were made after the first 12 hours.

Figure 2.3 shows how iron concentrations in solution remained remarkably steady over the four day period, particularly over the first two days after which the solutions were usually changed. A significant rise in soluble iron concentration was observed at the higher iron concentrations between the third and fourth day (i.e. after the maximum time between solution changes).

Soluble reactive phosphorus concentrations fell fairly rapidly, especially over the first 24 hours (Figure 2.4). At 100 mg Fe l^{-1}, phosphorus in solution fell from 0.4 mg P l^{-1} initially to 0.03 mg l^{-1}

Figure 2.3 Concentration of Iron in solution over Time.
(10% Rorison solution (pH 5.5) with iron additions.)
Solutions were centrifuged at 4,250 rpm for 5 minutes prior to analysis. Means of 5 replicates ± 1 SE.

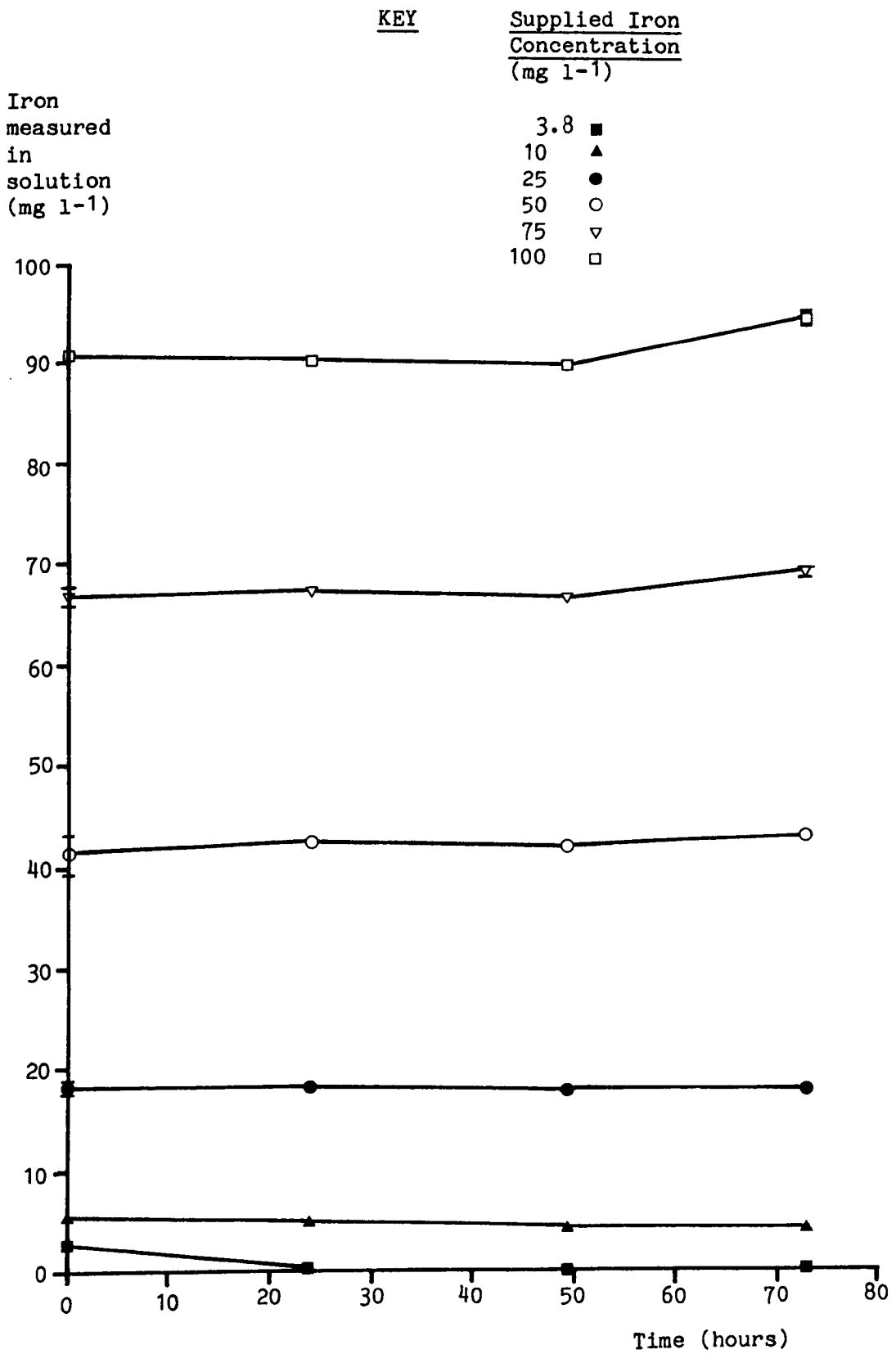


Figure 2.4 Changes in Soluble Reactive Phosphorus Concentration over Time (initially 3.1 mg P l^{-1}), in 10% Rorison solution at pH 5.5, with Iron additions.
Solutions were centrifuged at 4,250 rpm for 5 minutes prior to analysis.
(Means of 5 replicates ± 1 SE.)

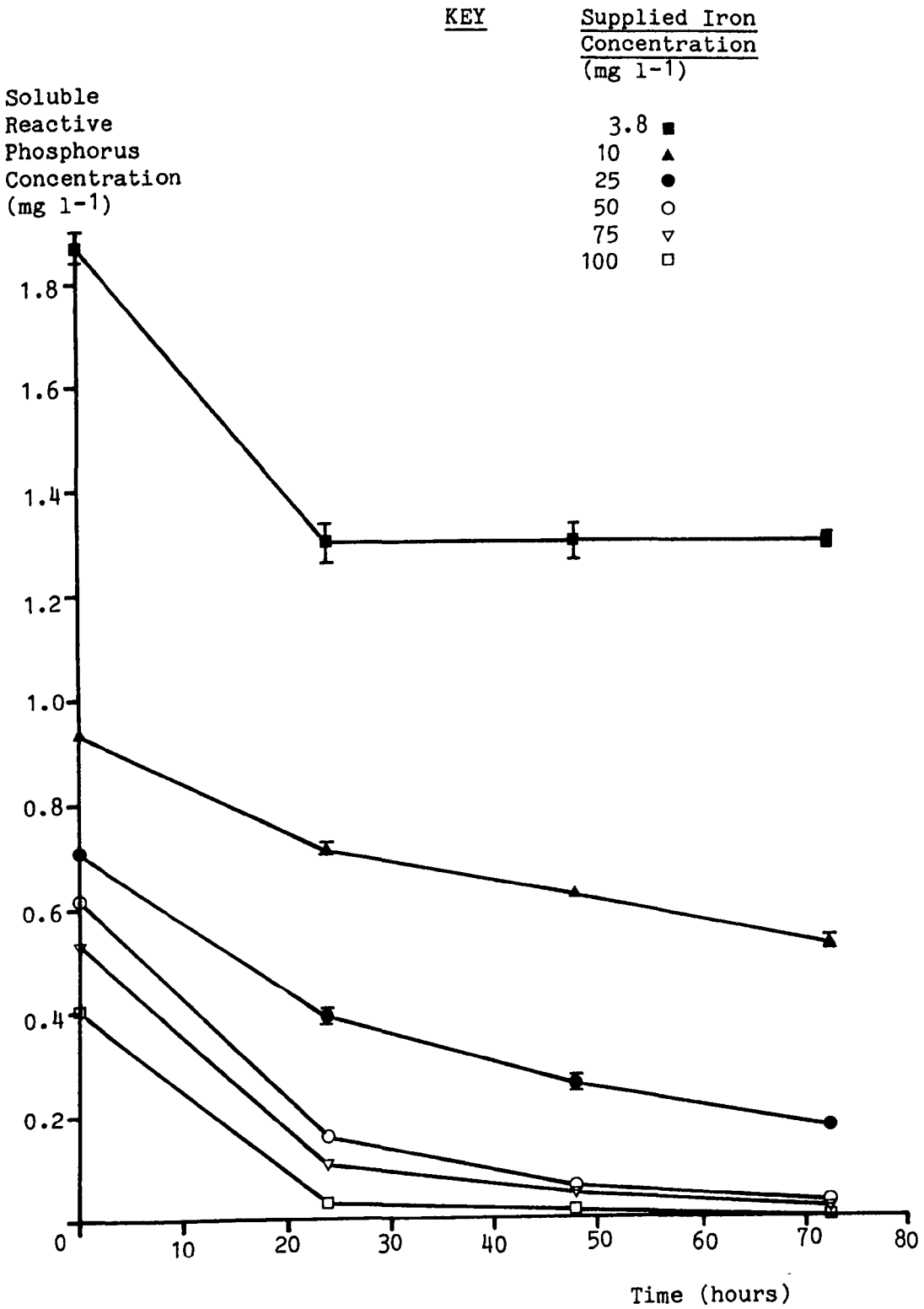
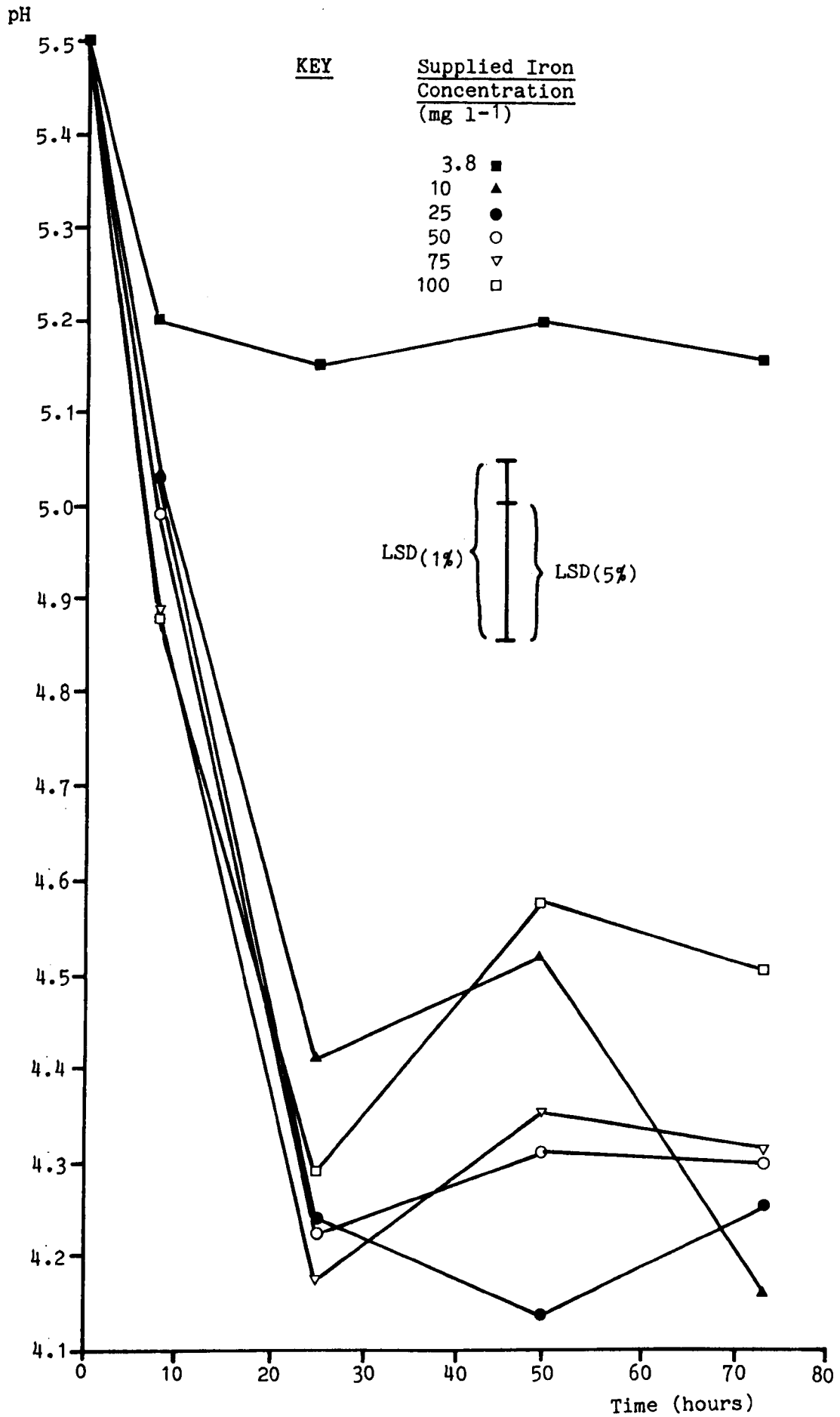


Figure 2.5 pH Changes with Time (initially 5.5) of 10% Rorison solution with Iron additions. (Mean of 5 replicates ± 1 SE)



after one day, and to 0.01 mg l^{-1} on the next two days. Large plants of Epilobium hirsutum are found growing in sites with as little as 0.02 mg P l^{-1} in solution (Al-Farraj 1983) so it was judged that phosphorus would not be limiting particularly as small seedlings were being used and the solutions were changed frequently.

Figure 2.5 shows that the pH in the control solution fell to around 5.2 within the first 12 hours and then remained constant. In all other treatments, pH decreased over the first 24 hours to 4.2-4.5 where it remained. pH values of this magnitude are thought not to be damaging to plants in themselves provided nutrient supply is adequate (Somers and Shive 1942; Olsen 1958; Hackett 1965; Al-Farraj 1983).

In all treatments the oxidation-reduction potential was roughly 285± 25 mV for the whole experiment. This is much higher than was found in a later experiment containing no plants in the pots.

CHAPTER THREE

RESPONSE OF A RANGE OF WETLAND SPECIES TO HIGH CONCENTRATIONS OF IRON

(Results of Standard Screening Experiment)

3.1 VISUAL EFFECTS OF HIGH IRON CONCENTRATIONS

3.1.1 Introduction

One of the problems of assessing iron toxicity to plants is the range of visual symptoms that have variously been regarded as indicating toxicity.

Much of the literature on iron toxicity and tolerance relates to work on crop species, especially rice, a crop of great economic importance (Ponnamperuma, Bradfield and Peech 1955; Tanaka and Navasero 1966c; Tanaka, Loe and Navasero 1966; Howeler 1973; Tadano 1975; Green and Etherington 1977; Ottow, Benckiser, Watanabe and Santiago 1983). However, a number of workers have researched into the effect of iron on native species (Table 1.3), though toxicity symptoms are not always mentioned (e.g. Jones 1971a; Jones 1972a).

Foy, Chaney and White (1978) state that iron toxicity symptoms may be expressed differently depending on the species and variety. Rice cultivars are known to vary in their tolerance to iron, and at different ages. Tadano (1975) reports that the rice plant is more susceptible to iron toxicity at early and late growth stages, while Tanaka, Loe and Navasero (1966) state that in solution culture, symptoms develop more quickly in the ripening phase than in the reproductive phase, and least of all in the vegetative phase. Foy, Chaney and White (1978) report that young plants are more susceptible than older ones.

Much of the literature is confused (Woolhouse 1983) and soil conditions which give rise to ferrous iron toxicity may also give rise to toxic concentrations of manganese or aluminium (e.g. Jones and Etherington 1970; Jones 1971b; Jones 1972a; Brady 1974). However, similar symptoms may be produced in the same plant in solution culture containing high concentrations of only one of these metals. Jones and Etherington (1970) observed different symptoms in Erica cinerea (i.e. different colours in

dying cut shoots) depending on whether the iron source was ferric KEDTA, ferrous sulphate or ferric citrate, though they report that ferrous and ferric iron sources produced a similar response. There may also be complications of symptoms induced by flooding per se and interactions leading to deficiencies of other elements. (e.g. phosphorus or manganese).

Physiological disease of rice under waterlogging conditions, variously known in different parts of the world as 'yellowing', 'oranging', 'red disease', 'bronzing', 'browning disease', 'red wilting' or 'suffocating' has been attributed to iron toxicity (Ponnamperuma, Bradfield and Peech 1955). Howeler (1973) differentiates between bronzing caused by direct iron toxicity, and oranging caused by deficiencies in potassium, phosphorus, calcium and magnesium (indirect toxicity), and states that a mixture of symptoms of the two disorders may be found together. He noted that larger plants were more affected by indirect toxicity owing to a greater nutrient demand. Ottow et al. (1983) support the view that iron toxicity is the result of a multiple nutritional soil stress involving an insufficient supply of potassium, phosphorus, calcium, magnesium and zinc. However, Wheeler, Al-Farraj and Cook (1985) present evidence that symptoms produced in Epilobium hirsutum are due to direct iron toxicity. The basis of iron toxicity is explored in Chapter 6.

There are some reports of antagonism in uptake of iron and manganese. Thus in certain species, e.g. soya beans and Dactylis glomerata, iron toxicity symptoms may be confused with manganese deficiency symptoms (Somers and Shive 1942; Etherington and Thomas 1986), though Tanaka and Navasero (1966d) were able to differentiate between the two responses in rice (see also Vlamis and Williams 1964).

Iron toxicity is difficult to identify by plant symptoms alone (Tadano 1975), possibly because there are so many potential interactions involved. Symptoms from plants grown in solution culture experiments may, however, be easier to interpret than those from plants grown in soil. Fitter and Hay (1981) state that toxicity symptoms may be part of a plant's resistance mechanism, e.g. necrotic patches on leaves are accumulations of iron in bronzing of rice, (Tanaka et al. 1966; Tanaka and Yoshida 1970).

3.1.2 **Visual Effects on Plants Screened**

The visual effects associated with high iron concentrations on the shoot, root, and growth responses of each of the species tested in this study are summarised in Table 3.1. Each species may show a variety of symptoms, some unique to that particular species. However, there are a number of effects which were common to many of the species. These include growth retardation, reduction in leaf size, deepening of green leaf colour (particularly in the youngest leaves), reddening or purpling of stems and older leaves, wilting of shoots, yellowing or dieback of oldest leaves especially from the tips or margins, brown or black speckles or larger necrotic patches on leaves, blackening of leaf tips and stem bases, stiffening of stems, root stunting (particularly of adventitious roots), lack of root branching, root flaccidity, root blackening (particularly of the apices), and formation of precipitates on roots.

In some species the visual effects may be symptoms of direct iron toxicity (or induced deficiencies of other elements owing to a disruption in the elements's metabolism within the plant). In other species, the observed response may be part of the plant's resistance mechanism. Figure 3.1a is an example of the response of a species sensitive to iron, while Figure 3.1b shows the typical response of a more tolerant species. Figure 3.2 shows 'top-bending', which was only observed in the two Galium species screened, and Figure 3.3 demonstrates clearly that root stunting may be very severe even at low iron concentrations.

3.1.3 **Comparison of Observed Visual Effects of High Iron Concentrations with Previously Reported Iron Toxicity Symptoms**

3.1.3.1 Growth Response

In all species growth was reduced at least to some extent by high iron concentrations. Carex echinata showed the least growth reduction of all the species tested, and few other symptoms. Conversely, in the most sensitive species, a reduction in shoot or leaf size relative to the control was apparent after only one or two days, and this was accompanied or closely followed by a number of other symptoms. This agrees with the findings of Wheeler, Al-Farraj and Cook (1985) who reported that after 8 days in solution culture reduced growth (reduction in shoot dry weight and

Table 3.1 Visual Effects of High Concentrations of Iron on Seedlings of a Range of Wetland Species
(Early symptoms are presented in bold type)

Species and Growth Reduction	Shoot Response	Root Response
Agrostis stolonifera Noticeable	**Wilting.** Leaves smaller and narrower. Dieback of leaves from tip.	**Covered in a yellow precipitate.** Flaccidity at high iron concentrations. Reduced vigour.
Briza media Marked	**Wilting.** Leaves less bright green, smaller and narrower. Reddening of some leaves from bases, and some stems. Dieback of older leaves from tips i.e. twisting and shrivelling. Stems felt stiff.	**Covered in a yellow-grey precipitate.** Flaccidity. Stunting. Darkened apices. Adventitious root formation reduced at higher iron concentrations.
Caltha palustris Marked	**Shrivelling of some leaves from edge,** and of cotyledons (which were tinged copper-blue coloured). Stunting. Smaller leaves. Stems shortened and dark purple-brown, (veins of some leaves were the same colour). Leaf yellowing. Some leaves had a dark patch in the centre, radiating outwards.	**Pale ochreous at low iron concentrations.** Stunting. Lack of root hairs. Progressive stunting of adventitious roots. Flaccidity and browning, especially at higher iron concentrations.
Carex appropinquata Marked	**Stunting.** Narrowing of leaves and more inrolling. Slight wilting. Less bright green. Older leaves blackened particularly at bases, with brown or black specks along the length. Stems felt stiff.	**Yellowing of roots,** (became brown later). Stunting. Reduction in growth and production of adventitious roots with increasing iron supply. Distortion of adventitious roots. Little root branching.
Carex diandra Marked	**Black or brown specks or blotches on older leaves, particularly near tips.** Less bright green. Slight wilting at high iron concentrations. Shrivelling or browning of older leaves from tip.	**Covered in a pale yellow or ochreous precipitate.** Flaccidity. Loss of vigour. Stunting, particularly of adventitious roots.

Table 3.1 continued

Carex echinata Slight	Eventual loss of vigour at highest iron concentration. Blackened leaf tips and bases. Black or brown blotches along length of leaf.	**Coated in ochreous precipitate.** Slight stunting.
Carex lepidocarpa Marked	Slight stunting and leaf narrowing. Yellowing and dieback of older leaves, or from leaf tips. Stems felt stiff.	**Yellowing with dark apices.** Flaccidity. Stunting, particularly of adventitious roots. Distortion of adventitious roots at high iron concentrations. A little ochreous precipitate at base of some roots.
Carex pulicaris Noticeable	**Blackening of leaf tips and bases.** Black blotches along leaf. Shoots felt stiff.	**Coated with a yellow precipitate** which later became grey-green. Flaccidity. No stunting. A little ochreous precipitate formed near the seed.
Epilobium hirsutum Severe	**Shoot smaller and darker green.** Wilting. Leaf shrivelling (starting from the oldest). Stem reddening. A few deaths at 50-100 mg Fe l^{-1}.	**Root flaccidity and blackening, (particularly of tips).** Adventitious roots failed to grow. A little yellow/grey precipitate on the roots.
Epilobium palustre Severe	**Smaller and darker green.** Metallic or brown patches on some leaves. Pale areas on leaves too. Reddening of stems and undersides of leaves. Young leaves very dark green. Older ones yellow with red veins. Oldest leaves wilted, or shrivelling from edges. Reduced side shoot production. Stem blackening at higher iron supply. Stems felt stiff and hard. Flower buds were forming at lowest iron concentrations only.	**Flaccidity and greying of roots with black tips.** Later became black all over at high iron concentrations. Yellow/grey precipitate. Stunting. Adventitious roots failed to grow. Many root primordia at base of stem.

Eriophorum latifolium Noticeable	**Browning or blackening of leaf tips with dieback.** Blackening of leaf bases. Brown or black specks along some leaves.	**Covered in a pale ochreous precipitate** (deepened with time). Darkened apices of a few adventitious roots. Little root stunting.
Eupatorium cannabinum Severe	**Brown spots on undersides of leaves.** Shoot rapidly outgrown by control plants. Top leaves dark green. Lower leaves yellowing, some with a pinkish tinge. Wilting. Stems brown and stiff. Hypocotyl hard and black.	**Grey/green colouration with black apices.** Flaccidity. Stunting, particularly of side-roots, producing peg-like roots. No precipitates.
Filipendula ulmaria Severe	**Leaf shrivelling from margins.** Wilting at high iron supply. Leaves smaller with pink undersides and sometimes red veins. Stems red. Death at 50-100 mg Fe l^{-1}.	**Blackening of apices.** Flaccidity and blackening of older roots. Stunting, particularly of adventitious roots. Reduced production of adventitious roots at high iron concentrations. Covered in a yellow/grey precipitate, especially early on.
Galium aparine Severe	**Lower leaf whorls yellowed.** Leaves then turned brown and died, proceeding up the stem. A typical response was for the stem to bend and almost break below the 2nd whorl, and to go black at this point (Fig. 3.2). This occurred more commonly at lower iron supply. Brown blotches or reddening on top whorl of some plants. A few deaths at 10-100 mg Fe l^{-1}.	**Flaccidity.** Browning. Stunting Stunting most marked between 3.8 and 10 mg Fe l^{-1}. Became fragile
Galium palustre Marked	**'Top-bending' as for G. aparine.** Some 'twisting' of main and side shoots. Top leaves dull green. Reddening of lower leaves and stem. Black veins on a few plants. Shrivelling of bottom leaf whorls. Stems stiff and brittle.	**Covered in a yellow precipitate.** Stunting of main and side roots. Flaccidity. Browning of some roots.

Table 3.1 continued

Holcus lanatus Marked-Severe	**Wilting, especially of older leaves.** Leaves shorter and narrower. Less bright green at high iron supply. Dieback from tips of older leaves. 'Crinkling' of some leaves.	**Covered in a pale ochreous precipitate which became yellow.** Flaccidity. Stunting of main, adventitious and side roots. Blackening of apices.
Iris pseudacorus Noticeable	Eventually leaves of plants at high iron concentrations were narrower and shorter than control leaves. A little browning at tips of older leaves. Shrivelling of extreme tips.	**Ochreous precipitate, became very intense.** Root stunting, especially at higher concentrations. Distortion of adventitious roots, i.e. bent, with curled tips or zig-zag ends. Very fragile.
Juncus articulatus Noticeable	**Blackening and then shrivelling of some leaf tips.** Less bright green at higher iron concentrations. Yellowing and shrivelling of older leaves.	**Creamy-coloured precipitate, became pale ochreous.** Flaccidity. Dark apices. Stunting, particularly of adventitious roots.
Juncus effusus Noticeable	**Small black specks on leaf bases.** Eventually leaves at higher iron concentrations were narrower and less glossy than those of control plants.	**Pale ochreous precipitate became more intense with time** Slight stunting, and reduction in root hair cover.
Juncus inflexus Marked	**Blackening and dieback of leaf tips.** Leaf 'crinkling' in some cases. Black blotches formed on older leaves starting near tips. Older leaves shrivelled and died.	**Flaccid and coated with a creamy-yellow precipitate.** Became pale ochreous in time. Stunting, with dark apices. Roots had a 'stringy' appearance.
Juncus subnodulosus Marked-Severe	**Shrivelling of leaves, especially older ones, from tips.** Leaves less bright green at high concentrations. Older leaves yellowed from tips before dieing. A few deaths, especially at 100mg Fe l^{-1}.	**Yellow precipitate on roots.** Darkened apices and stunting, particularly of adventitious roots. Became flaccid with time.

Lotus uliginosus Marked-Severe	**Leaves duller and darker green.** Shoots reduced in size with fewer leaves. Yellowing and shrivelling of lower leaves; some became brittle and fell off. Stems more brittle with increasing iron supply. Stems pinkish or brown. A few plants had red veins. Brown spots on some leaves, especially lower ones. Eventual loss of turgor A few deaths at 50-100 mg Fe l^{-1}.	**Flaccidity. Roots coated in a heavy yellow precipitate which became less intense with time.** Stunting. Reduced number and length of adventitious roots.
Lychnis flos-cuculi Severe	**Immediate wilting and stunting.** Reduced leaf size. 'Metallic' patches or brown blotches on some leaves. Older leaves became yellow and shrivelled. Some had black or dark brown veins. Several deaths at 25-100 mg Fe l^{-1}, particularly at the highest concentration.	**Flaccid and coated with a pale yellow precipitate.** Stunting. Loss of vigour. Reduced branching.
Lysimachia vulgaris Marked	**Shorter and less bright green.** Reduced leaf size. Young leaves were dark green tinged with red/brown. Older leaves became yellow and wilted, some developed red veins. Petioles of lower leaves became brittle, and leaves fell off. Hypocotyl became black, hard and brittle.	**Ochreous precipitate on roots, became intense with time.** Stunting of main and side roots gave root system a 'bristley' appearance. Became brittle/fragile. Not all plants had ochreous roots.
Lythrum salicaria Marked	**Leaf ends went brown.** A little wilting. Young leaves became bronzed and failed to expand fully. Lower leaves developed brown patches black veins or red margins. Lowest leaves were yellow or shiny. Stems reddened, and some became blackened towards the base.	**Root blackening, particularly of apices.** Flaccidity developed with time. Stunting, loss of vigour and reduced branching.

Table 3.1 continued

Molinia caerulea (wetland and dryland) Noticeable	**Blackening of leaf tips.** Leaves shorter and less bright green. Yellowing or browning of some older leaves. Stems felt very stiff.	**Covered in a pale ochreous precipitate which intensified with time.** Stunting. Distortion (crinkling) of some roots. Reduced branching.
Parnassia palustris Noticeable	**Leaf yellowing, with browning from tips which spread around margins.** Dark speckles appeared on leaf lamina. Blackening of some stems. Wilting and shrivelling of some leaves and stems.	**Roots developed a variety of colours, pale yellow or ochreous, or grey with black apices.** Eventually became flaccid at higher iron concentrations.
Pedicularis palustris Noticeable	Eventually cotyledons shrivelled and developed 'metallic' patches. A few leaves also shrivelled at high iron concentrations. A few deaths at 75-100 mg Fe l^{-1}.	**Pale ochreous colour - deepened with time, and then went deep brown.** Apices then became grey/black. Eventually became flaccid or fragile and brittle.
Phalaris arundinacea Marked	**Leaves became pale and blotchy, and shrivelled from tip while still green, (especially the young leaves).** Wilting at high iron concentrations Leaves were smaller and narrower than in the control. Shrivelling and death of older leaves.	**Bright orange/ ochreous precipitate.** Slight flaccidity. Stunting and loss of vigour, particularly at high iron concentrations Stunting of adventitious roots.
Potentilla palustris Marked	**Oldest leaves became brown/dark around margins, and shrivelled.** Younger leaves were small and dark green with short red stems, and sometimes red veins and leaf undersides. Some leaves failed to uncurl properly. Wilting at high iron concentrations.	**Darkening of apices. Flaccidity.** Roots became grey/black with a little pale yellow precipitate on them. Stunting, and lack of branching.

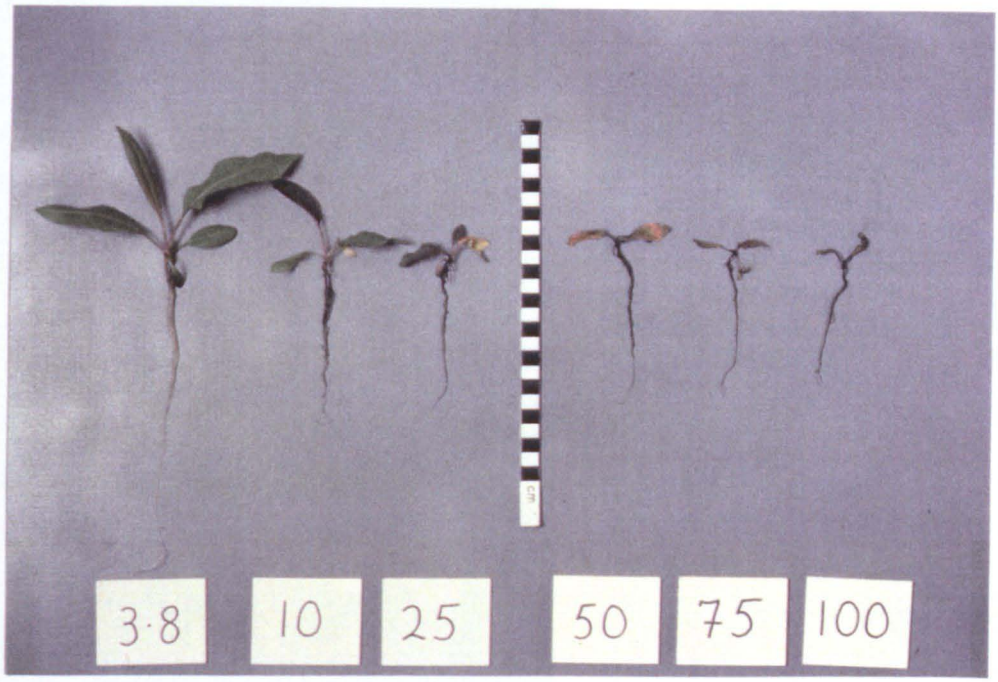
Table 3.1 continued

Primula farinosa Severe	**Wilting and yellowing of older leaves, while veins remained green.** Young leaves small and very dark green. Leaf shrivelling from tip.	**Pale brown and flaccid.** A yellow precipitate formed later and then diminished. Stunting of main, side and adventitious roots, i.e. lack of branching. Side roots developed dark tips
Ranunculus flammula Marked	**'Metallic' patches or veins appeared on leaves.** Leaves smaller and darker green. Some stem and leaf reddening. Yellowing and shrivelling of some leaves. Wilting at high iron concentrations. Some stems felt stiff.	**A pale ochreous precipitate formed on roots.** This became restricted to larger roots with a pale yellow precipitate covering the more fibrous roots. Stunting and lack of branching. Stunting of adventitious roots.
Rumex acetosa Severe	**Leaf shrivelling from tips.** Leaves smaller and wilted, especially at high iron supply. Leaves became red, yellow and wilted, or brown and shrivelled at high iron supply; few were green. Many deaths in 50-100 mg Fe l^{-1}.	**Immediate and severe flaccidity, and greying with blackening of apices.** At high iron concentrations, all roots became black with time. Roots coated in a yellow precipitate. Stunting and loss of vigour. Adventitious roots failed to form at high iron supply.
Rumex hydrolapathum Very Severe	**Noticeably reduced size** Wilting and shrivelling, particularly of older leaves. Some went black Reddening of stems and leaf margins. Some leaves were dull green with red blotches, others were yellow. Stems felt stiff. Many deaths at 50-100 mg Fe l^{-1}.	**Immediate flaccidity.** Roots became black or grey with darkened apices. Covered in a very heavy pale yellow precipitate. Stunting and reduced vigour. No Adventitious roots developed at higher iron concentrations.
Scrophularia auriculata Severe	**Immediate wilting, especially of older leaves.** Young leaves very dark green. Older leaves were yellow, sometimes with blackened veins and petiole. Some leaf shrivelling. Stems felt stiff.	**Immediate flaccidity and apical blackening.** Roots became grey, but were coated in a heavy yellow precipitate. Stunting and lack of branching. Adventitious root failed to grow at high iron concentrations.

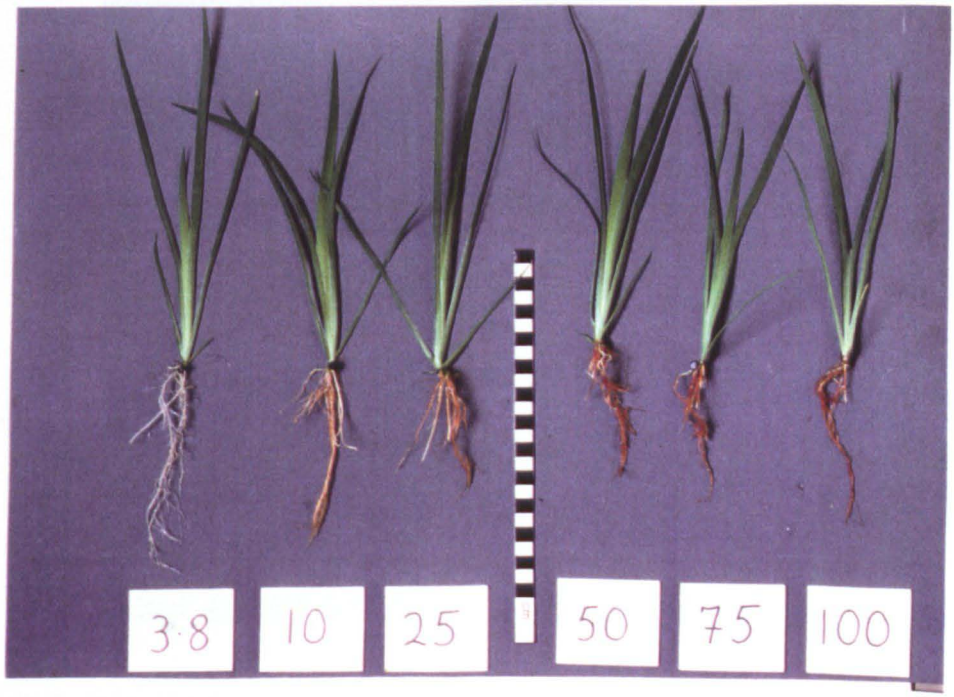
Table 3.1 continued

Thalictrum flavum Severe	**Darkening of veins.** Stunting, with darker green young leaves and yellowing or reddening of older leaves. Wilting. Shrivelling of older leaves from edges. Purpling of veins and leaf margins. Stems brown/purple and very stiff. A few deaths at 75 and 100 mg Fe l^{-1}.	**Darkening of apices.** Covered with a yellow precipitate which became ochreous and then went a more dull brown. Roots became flaccid or fragile with time. Stunting of main and adventitious roots. Branching reduced.
Trifolium pratense Severe	**Wilting.** Smaller and darker green with red stems. Brown or black specks on leaves starting from edges (Fig. 3.). Older leaves yellowed from edge. Shrivelling of stems and older leaves. A few deaths at 25-100 mg Fe l^{-1}.	**Flaccidity. Roots coloured pale pinky-brown.** Stunting and reduced branching. Few plants had nodules in control or at high iron supply. Nodules were most abundant in the 10 mg Fe l^{-1} treatment. No precipitates.
Valeriana dioica Marked	**Small brown/black blotches on some leaves.** Older leaves went yellow. Blackening of petioles of youngest leaves which spread onto leaf itself. Wilting (and reduced growth at high iron supply). Stems very stiff.	**Pale ochreous precipitate formed at high iron supply.** Apices soon went grey and roots became flaccid. At high iron concentrations whole root went grey, while an ochreous precipitate developed at lower iron concentrations. Slight stunting. Reduced branching. No adventitious roots at high iron supply.
Valeriana officinalis Severe	**Small brown/black or metallic patches on leaf.** Younger leaves smaller and darker green Older leaves became yellow-green and wilted slightly. Stems red-brown.	**Pale ochreous precipitate.** Flaccidity at high iron concentrations. Precipitate faded with time and roots became brown. Stunting.

Figure 3.1 Typical Responses of Plants to High Iron Concentrations (14 days in 10% Rorison solution (pH 5.5) with various iron additions (mg l^{-1}))



a. Rumex hydrolapathum, a species sensitive to iron shows reduced shoot and root growth, root flaccidity and blackening, and shoot colouration, flaccidity and shrivelling



b. Iris pseudacorus, a species tolerant of high iron concentrations shows little growth reduction and increasing ochreous root deposits with increasing iron supply

Figure 3.2
'Top-bending' response, and leaf-shrivelling from lower whorls upwards observed in Galium aparine (pictured) and Galium palustre, supplied with high iron concentrations.

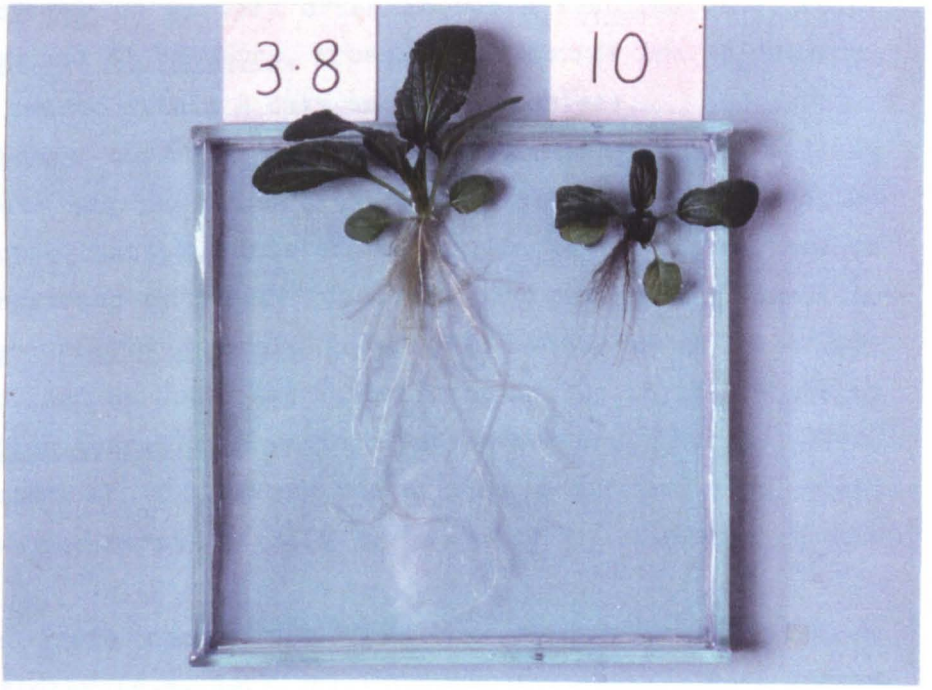


Figure 3.3 Low iron concentrations (10 mg l^{-1}) can have a marked effect on roots of particularly iron-sensitive species (Scrophularia auriculata after 14 days in 10% Rorison solution at pH 5.5 with 3.8 (control) or 10 mg Fe l^{-1})

root length) was the only effect observed in Juncus subnodulosus, whereas in Epilobium hirsutum growth reduction was more obvious and accompanied by many other symptoms.

Ponnamperuma, Bradfield and Peech (1955), and Kuraev (1966) reported growth reduction as a sign of iron toxicity in rice, and oats and spring wheat respectively.

3.1.3.2 Shoot Response

3.1.3.2.1 General Observations

Kuraev (1966) noticed that when oats were grown at high iron concentrations the leaves became a darker green colour and the leaf blades were reduced in width. Both symptoms were observed in a considerable number of species in this study; most often it was the youngest leaves on the plant which had the dark green colouration, and in some cases they also became tinged with red or purple. Millikan (1949) had noted the formation of dark green leaves in flax grown at high iron concentrations; phosphorus deficiency intensified the green colour and excess phosphorus reduced it (Foy et al. 1978). In this study, the dark green colouration may be linked to a disrupted phosphorus metabolism but it is unlikely to be phosphorus deficiency per se as very often it was a fast response, (e.g. in Epilobium hirsutum and E. palustre, greening of younger leaves occurred within the first 48 hours; within 4 days in other species).

A typical response to high iron concentrations was leaf wilting (particularly of older leaves) which often became yellow, and shrivelled to brown or grey subsequently. This disruption in the water balance of the shoot has been reported by Kuraev (1966) in oats and spring wheat, by Martin (1968) in Mercurialis perennis growing in wetter areas in a Cambridgeshire woodland, and by Jones and Etherington (1970) in cut shoots of Erica cinerea and Erica tetralix grown in solution culture. Kuraev (1966) noted that the number of shrivelled leaves and sometimes even stems increased with iron concentration; this agrees with the findings of the present study.

Brown spots or large necrotic patches are frequently reported as symptoms of iron toxicity (e.g. in rice, Ponnamperuma et al. 1955; Tanaka and Navasero 1966d; Tadano 1975; and in E. hirsutum, Wheeler et al. 1985). These usually form on the older leaves first and then spread to the

younger ones; the older leaves then become desiccated and die. In the present study, spots or patches were found on the leaves of less than 50% of the species studied; they were sometimes black or brown in colour but often of 'metallic' appearance, and were not always confined to the older leaves. In many monocotyledons, the leaf tips became brown or black and shrivelled or died back, usually starting with the oldest leaves. This was one of the few visual effects of iron on Carex echinata. Reduced tillering, late heading, and production of sterile florets are reported as symptoms of iron toxicity in rice (Ponnamperuma et al. 1955; Ottow et al. 1983). As all the plants in this study were in the vegetative phase these latter observations could not be verified, though flower buds were beginning to form on Epilobium palustre plants grown in up to 10 mg Fe l^{-1} but not at higher iron concentrations.

Foy, Chaney and White (1978) report that tobacco leaves become brittle, tender and dark brown to purple with poor burning qualities and flavour when plants were subjected to excess iron. Talbot, Etherington and Bryant (1987) and Talbot and Etherington (1987) noted that the lower leaves of Salix caprea became blackened and/or brittle and some were shed. In the present study, stems of many of the species grown at high iron concentrations became noticeably stiff, and in many monocotyledons the leaf bases were blackened and hard.

3.1.3.2.2 Formation of a Crystalline Deposit on the Shoots

During the course of the experiment, crystals of a white crystalline deposit appeared on the stems or leaves of a number of species (Table 3.2), particularly in the higher iron treatments. The deposit was found up to half way up the plant, and was occasionally tinged with ochre.

Table 3.2 Species with a White Crystalline Deposit on Shoots of Seedlings grown in 10% Rorison Solution with Elevated Iron Concentration
(Monocotyledons are indicated by bold type)

Agrostis stolonifera
Carex appropinquata
Carex diandra
Carex echinata
Carex lepidocarpa
Carex pulicaris
Eriophorum latifolium
Iris pseudacorus
Juncus articulatus
Juncus effusus
Juncus subnodulosus
Molinia caerulea
Parnassia palustris
Rumex acetosa
Rumex hydrolapathum

Most of the species with the deposit on their leaves were monocotyledons. The 3 dicotyledons had only very small quantities on their shoots; monocotyledons had varying amounts with Juncus effusus having the most. It was not clear whether this deposit formed as a result of capillary movement up the stem or whether the plants were excreting it (in which case it might conceivably be an exclusion mechanism). To test this the bases of a number of Juncus subnodulosus plants were smeared with vaseline to allow crystallisation to occur above this level should the transport system be internal, and to prevent salt movement up the outside of the stem should it be external. No salt was formed above the vaseline ring, indicating that this process was not being actively performed by the plant, nor was it a mechanism of tolerance. Presumably the morphology of monocotyledon stems is particularly suitable for this process to occur.

A sample of the deposit was taken and analysed by X-ray diffraction. It was found to be composed mainly of $CaSO_4.2H_2O$ (gypsum) mixed with smaller amounts of $FeSO_4.nH_2O$ and $K_2MgSO_4.nH_2O$. All these elements are abundant in the culture solutions, and it is likely that the growth-room temperature of 20-30°C was encouraging crystallisation to occur on the stems or leaves of certain species, especially in the high iron solutions which were more concentrated.

3.1.3.3 Root Response

3.1.3.3.1 General Observations

Root stunting has been reported as a major symptom of iron toxicity. Length of individual roots is reduced, with poor development of the root system (Ponnamperuma et al. 1955; Ottow et al. 1983), owing to inhibition and death of lateral root primordia (Martin 1968). In addition, Kuraev (1966) reports reduced root hair cover in oats and spring wheat. Root stunting and lack of branching was observed in the majority of species, though the effects were less marked in the more tolerant species. Reduction of root hair cover was also apparent in many of the species tested, even in Juncus effusus which was generally less affected by high iron concentrations. Sanderson and Armstrong (1980a) reported that if the iron concentration was sufficiently low, no permanent damage was caused, but higher iron concentrations were lethal to root apices of Picea sitchensis (Sitka spruce) and Pinus contorta (Lodgepole pine).

Root blackening (Ottow et al. 1983; Wheeler et al. 1985) occurred over much of the root in the more sensitive species, but more often blackening or darkening was confined to the root apices as noted by Smirnoff (1981) and Talbot and Etherington (1987). Blackening often occurred in the first 24 hours. Root flaccidity, with or without blackening, was another common effect of excess iron which usually developed very quickly and had previously been noted by Martin (1968), and Wheeler, Al-Farraj and Cook (1985).

Martin (1968) found that death of the root system of species studied was the major symptom of iron toxicity, particularly in the more susceptible species such as Mercurialis perennis. Hodgson (1972) found root measurements more sensitive to iron toxicity than shoot measurements, length moreso than weight, which prompted the use of root length increment as his tolerance index. In many species in the present study, visual changes in or on the root system occurred before changes were apparent in the shoot system, or before growth was reduced (Table 3.1). The most sensitive species were exceptions to this rule and shoot symptoms such as wilting and growth reduction occurred as quickly as changes to the root.

3.1.3.3.2. Root Precipitates

In the species most sensitive to iron, root flaccidity and/or blackening soon developed, but in other species the roots quickly became coated in a precipitate, often within the first 24 hours. Two main types were noted:-

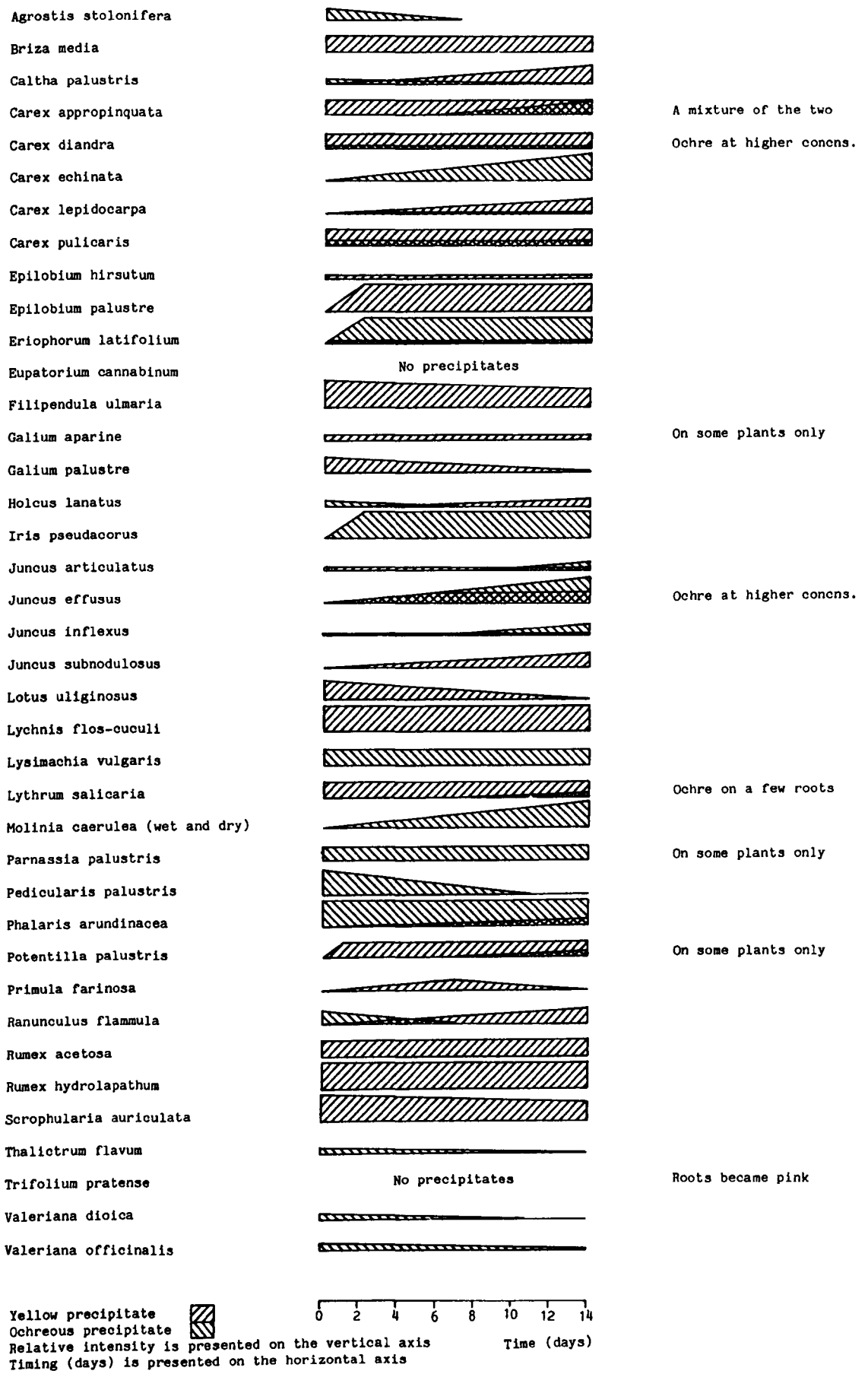
a. An ochreous precipitate,

b. A pale yellow, or sometimes yellow/grey precipitate.

The two precipitates were not mutually exclusive and sometimes one developed first and then the other, or sometimes one developed early in the experiment and then faded. The quantity of the two precipitates varied from species to species and with time (Figure 3.4). The ochreous precipitate was never found on blackened or flaccid roots, though darkened apices were sometimes associated with it, but the pale yellow precipitate often occurred on blackened roots in abundance.

The ochreous precipitate generally formed in definite zones on the root. Deepest ochre occurred at the base of the root with paler ochre towards the tip while the root apex itself remained white (Figure 3.5). However in some species, with time, the root tip also became ochreous.

Figure 3.4 Timing, Intensity and Nature of Root Precipitates on Seedlings Grown in 10% Rorison Solution (pH 5.5) with Iron additions



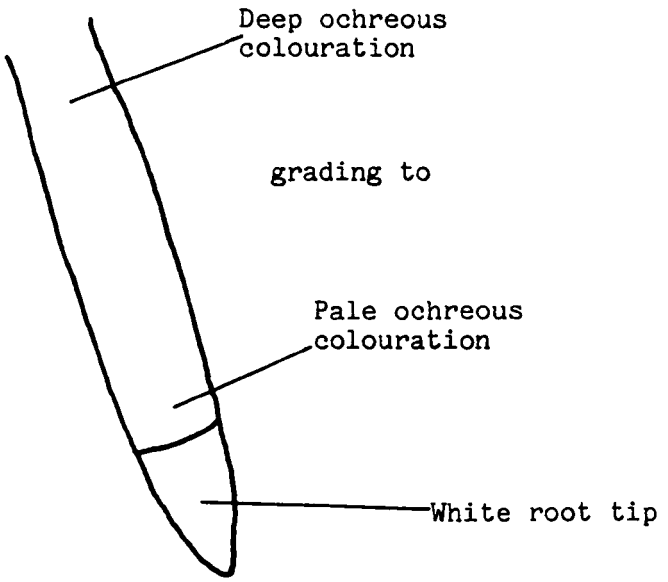


Figure 3.5 Typical Zonation of Ochreous Precipitate on a Root

White adventitious roots formed in some species; often these soon developed the typical zonation pattern of ochre precipitation. This pattern is similar to the findings of other workers, though Armstrong (1967) and Taylor, Crowder and Rodden (1984) noted that ochre intensity diminished towards the white tip zone and towards the base of the root. Diminution towards the base was only noted in Pedicularis palustris in the present study.

In some species (e.g. Pedicularis palustris and Thalictrum flavum) the zonation pattern was apparent early in the experiment and then the root tip became blackened. Subsequently the precipitate lost its ochreous colour and turned a darker brown. Browning or blackening then proceeded from the tip towards the base of the root, and roots finally became flaccid, or fragile and brittle.

The pale yellow or yellow/grey precipitate did not have any such zonation pattern but tended to cover the whole root, especially the more fibrous roots. In a few species, ochre was found on the larger roots (often adventitious) and the pale yellow precipitate on the more fibrous ones (e.g. Juncus articulatus, Juncus inflexus).

At harvest, roots were rinsed in distilled water before drying. Neither precipitate was removed much by rinsing, though the pale yellow one had a slightly greater tendency to dissolve. Levan and Riha (1986) noted black precipitates of mixed iron/manganese oxides on the flooded

roots of several conifer species. The black precipitate did not form any protective mechanism against reduced metals, and the ratio of iron to manganese in these precipitates was only 2:1 compared to 45:1 found in more ochreous coloured precipitates often associated with plant roots (Bacha and Hossner 1977; Mendelssohn and Postek 1982). In this study, root blackening in the species more sensitive to iron was not due to any obvious external precipitate. However, Tibbetts (1988) thought that similar blackening on roots of Epilobium hirsutum was a precipitate, since it was removable by 10% HCl. Further tests failed to confirm the precipitate as FeS or a tannic acid compound and it was suggested that it might be a polyphenolic iron compound, possibly an allomorph of ferric hydroxide which may have been excreted by stressed cells.

3.1.3.3.3 Investigation of Chemical Nature of Root Precipitates

3.1.3.3.3.1 Methods

A number of species were selected whose roots had either the ochreous precipitate or the yellow precipitate on them (Table 3.3). Preliminary tests had suggested that one might be a ferric oxide/hydroxide and the other ferric phosphate. The precipitates were analysed semi-quantitatively by X-ray fluorescence on a Camscan scanning electron microscope attached to a Link system computer. Segments of dried root were mounted on aluminium stubs and coated liberally with carbon to eliminate electrostatic 'glow'.

Table 3.3 Species used for Root Precipitate Analysis
(Monocotyledons are indicated by bold type)

Species	Precipitate
Juncus effusus	Ochreous
Iris pseudacorus	Ochreous
Holcus lanatus	Yellow
Juncus subnodulosus	Yellow
Rumex hydrolapathum	Yellow
Epilobium hirsutum	Yellow

3.1.3.3.3.2 Results

Figure 3.6 shows the composition of the yellow precipitate on roots of Rumex hydrolapathum. Comparable results were obtained from analysis of precipitates on roots of Epilobium hirsutum, Holcus lanatus and Juncus subnodulosus. All showed a large iron content and a slightly smaller phosphorus content. Conversely, for Juncus effusus (Figure 3.7) and Iris pseudacorus root precipitates, the phosphorus peak was virtually absent while a small potassium peak was present (possibly due to potassium leakage during drying). **N.B.** The y axes of the two graphs are not quantitatively comparable since background counts differ.

3.1.3.3.3.3 Discussion

The results of X-ray fluorescence support conclusions from preliminary tests that the ochreous precipitate was ferric oxide/hydroxide while the yellow one was ferric phosphate.

There is much evidence that plants are able to oxidise iron on the root surface in peat, water and agar (Jones and Etherington 1970; Howeler 1973; Green and Etherington 1977; Chinnery and Harding 1980; Taylor et al. 1984; Macfie and Crowder 1987). Electron probe analysis of deposits on rice roots yielded a very similar X-ray spectrum to Figure 3.7 (Green and Etherington 1977). However, little mention has been made of any other types of precipitate, though Jones (1968) (cited by Chinnery and Harding 1980) isolated a ferric phosphate precipitate from the roots of her plants. This precipitate may only form in solution culture and not under field conditions.

It is of interest to note that when grown in 10% Rorison solution with iron additions, Juncus subnodulosus precipitated ferric phosphate on its roots. However, when phosphorus was omitted from the culture solution, ochre formed instead. Clearly under suitable circumstances Juncus subnodulosus is capable of oxidising iron on the root, and indeed is frequently found in the field with ochreous sheaths around its root.

Figure 3.6 Composition of Yellow Precipitate found on roots of Rumex hydrolapathum after 14 days in 10% Rorison solution (pH 5.5) with Iron additions.
(Analysis was performed by X-ray fluorescence on a Camscan Scanning Electron Microscope attached to a Link system computer.)

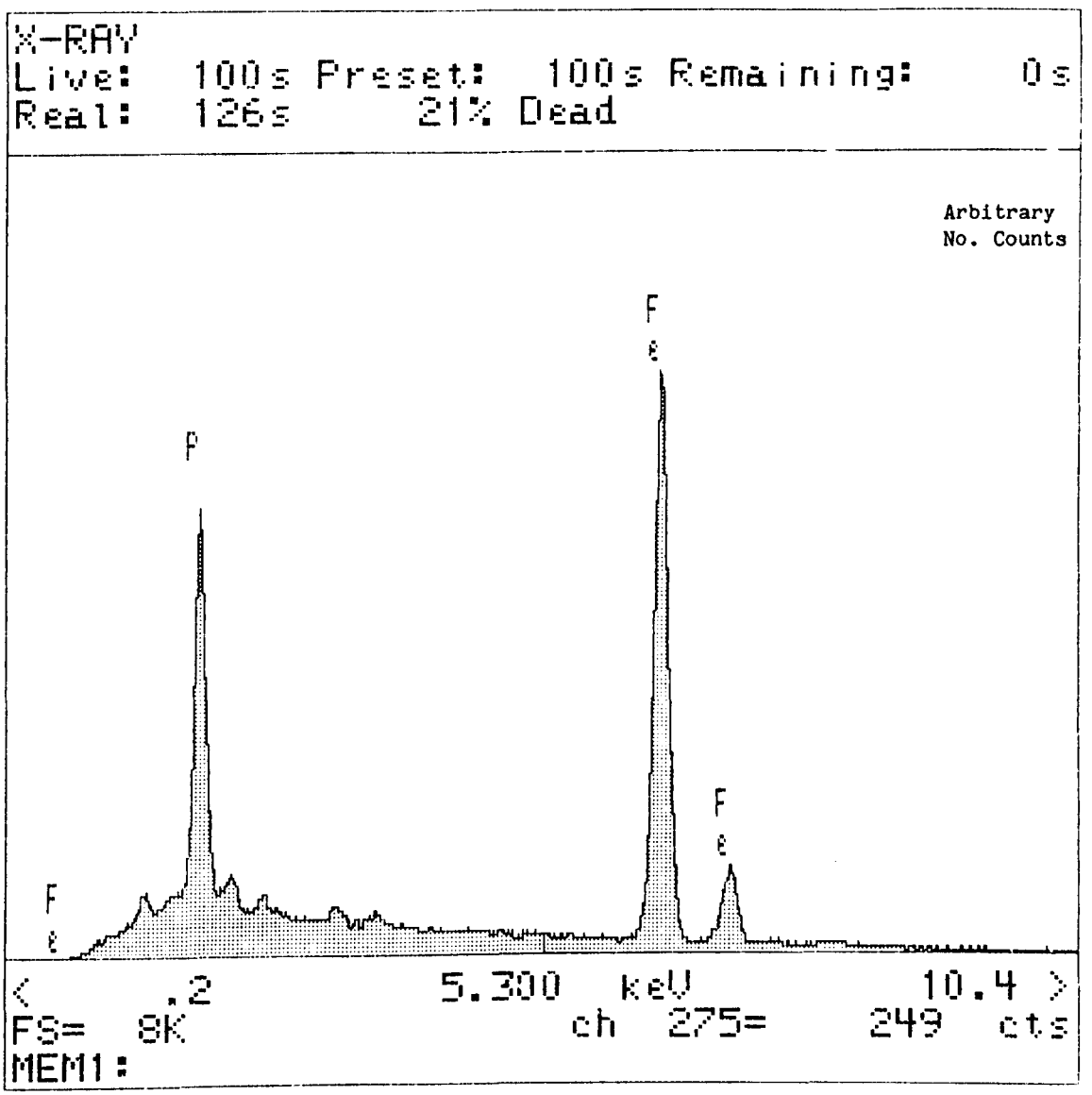
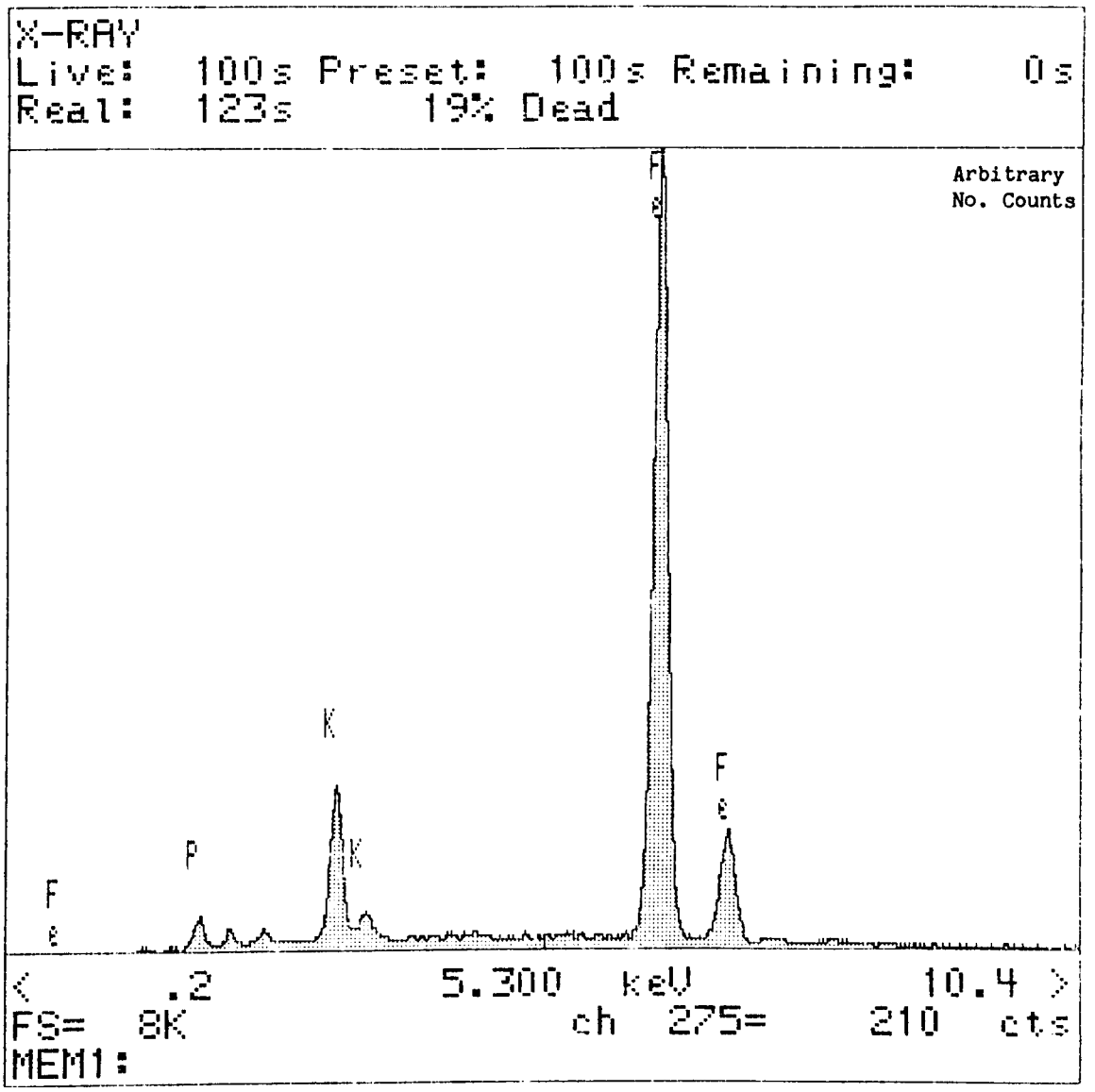


Figure 3.7 Composition of Ochreous Precipitate found on roots of Juncus effusus after 14 days in 10% Rorison solution (pH 5.5) with Iron additions.
(Analysis was performed by X-ray fluorescence on a Camscan Scanning Electron Microscope attached to a Link system computer.)



3.1.4 Comparison of Iron Toxicity Symptoms with those Reported for other Metals

3.1.4.1 Symptoms of other Metal Toxicities (Table 3.4)

Foy, Chaney and White (1978) report that metal toxicities in plants are often not clearly identifiable and may be the results of complex interactions of the major toxic ions in question with other essential or non-essential ions, and with other environmental factors. Symptoms may vary in different species owing to a diversity of biochemical pathways involved, and differential cultivar tolerances may involve differences in structure and function of the membranes.

There are two symptoms characteristic of a number of metal toxicities and these are stunting, which also occurs in iron toxicity, and chlorosis, which does not. These symptoms may be due to the specific toxicity of the metal to the plant, to antagonism with other nutrients in the crop, or to inhibition of root penetration into the soil.

For most metals, including iron, toxicity is first experienced in the root tips, so lateral root development is severely restricted limiting uptake of nutrients such as phosphorus, potassium and iron which are generally absorbed by diffusion rather than convection (Foy et al. 1978).

Wong and Bradshaw (1982), germinated and grew Lolium perenne seeds in solution culture containing a range of metals, and found that for all metals root growth (length) was affected more than shoot growth (length).

Thus, apart from the fact that stunting (especially of the roots) is common to many metal toxicities, symptoms of iron toxicity are unlikely to be confused with those of other metal toxicities (other than possibly manganese and aluminium) since chlorosis is not involved.

3.1.4.2 Manganese Toxicity Symptoms (Table 3.4)

Manganese toxicity symptoms are diverse among plant species (Foy et al. 1978; Woolhouse 1983) and include marginal or interveinal chlorosis and necrosis of leaves (Jones 1972b; Tanaka and Navasero 1966b), leaf puckering or marginal inrolling and necrotic spots on the leaves (Mulder and Gerretsen 1952; Wallace 1961; Tanaka and Navasero 1966b, c; Martin 1968 and Jones 1972b). Necrotic spots may also be a symptom of iron toxicity.

Table 3.4 Summary of Metal Toxicity and Phosphorus
N.B. Some or all of the symptoms may be present,

	Iron Toxicity	Aluminium Toxicity
SHOOTS	Stunting. Reduced leaf size. Wilting and desiccation (disrupted water balance) Reduced tillering. Late maturity and sterility of florets. Leaves darker green. Brown spots or necrotic patches on older leaves, spreading to younger leaves. Brittle leaves and stems. Dieback of leaf tips (monocotyledons).	Curling/rolling of young leaves, and collapse of growing point or petiole (Ca deficiency). Stunting. Small dark green leaves. Late maturity. Purpling of stems, leaves and leaf veins. Yellowing and death of leaf tips (P deficiency). Leaf spotting/necrotic streaks, initially on lower leaves.
ROOTS	Stunting. Reduced branching. Reduced root hair cover. Blackening of whole root or apices. Root flaccidity.	Characteristically brittle and distorted. Roots lack fine branching. Stubby - cell division in tip and elongation of main axis and laterals are inhibited. May become thickened and brown.
GROWTH	Growth usually affected before shoot symptoms become apparent.	Growth usually affected before shoots symptoms become apparent.
PART MOST AFFECTED	Root > Shoot	Root > Shoot

Deficiency Symptoms Collated from the Literature depending on the species and metal concentration.

Manganese Toxicity	Other Metal Toxicities	Phosphorus deficiency
Leaf spotting (brown/ black) and necrosis - may appear on stems in severe cases. Marginal or interveinal chlorosis and necrosis. Leaf puckering or marginal inrolling. Dieback of leaf tips (monocotyledons).	Stunting. Leaf chlorosis.	Reduced leaf size. Leaves darker green. Leaves may be tinted with red or purple. Lower leaves become yellow, and dry green. Lateral shoot production reduced. Flower initiation delayed, and fewer flowers produced. Purple or brown spots on leaves occasionally reported.
Stunting. Little branching of lateral roots. Short root hairs Roots turn brown in severe cases.	Stunting. Lateral root development is restricted.	Elongation and increased root hair formation (foraging).
Shoot symptoms usually appear at stress levels which produce little or no reduction in vegetative growth.	Concentration causing growth reduction depends on the metal.	Reduced
Shoot > Root (usually)	Root usually affected > shoot, depending on both the metal and the species.	Older leaves are affected more than younger ones. Shoot/ root dry ratio may fall as root growth rate increases.

In iron toxicity, growth is often obviously affected before other symptoms appear; the reverse is usually true of manganese toxicity (Foy et al. 1978). In manganese toxicity, plant tops are generally affected more severely than roots (e.g. Martin 1968), though in very severe cases the roots turn brown, usually after the tops have been badly injured (Foy et al. 1978). Indeed, genotypical differences in manganese tolerance are related to tolerance of the shoot rather than to differences in uptake or transport to the shoot (Marschner 1986).

Thus, both iron and manganese toxicity produce leaf spotting in a number of plants. However, yield is likely to be reduced before spotting becomes apparent in iron toxicity, whereas the reverse is normally true for manganese toxicity. Also, shoots are usually, though not exclusively, affected more than roots by excess manganese while high concentrations of iron tend to affect roots more than shoots (Table 3.4).

3.1.4.3 Aluminium Toxicity Symptoms (Table 3.4)

As for iron toxicity, aluminium toxicity affects roots first, or more severely than shoots (Clymo 1962; Tanaka and Navasero 1966a; Hodgson 1972; Wong and Bradshaw 1982; Scaife and Turner 1983; Woolhouse 1983; Marschner 1986). Yields can be greatly decreased before the production of clearly identifiable symptoms in the plant tops (Foy et al. 1978). Root systems become characteristically brittle (Foy et al. 1978) and may be distorted (Skeen 1929), whereas excess iron causes root flaccidity in susceptible species.

In some species, foliar symptoms may resemble those of phosphorus deficiency (overall stunting, small dark green leaves and late maturity, purpling of stems, leaves and leaf veins, and yellowing and death of leaf tips) (Foy et al. 1978; Scaife and Turner 1983). Such symptoms are frequently also associated with iron toxicity. In other species, curling or rolling of young leaves and collapse of the growing point or petioles may develop, possibly due to induced calcium deficiency or a calcium transport problem (Foy et al. 1978), though this is not symptomatic of iron toxicity. Leaf spotting and necrosis have also been reported in rice (Tanaka and Navasero 1966a).

3.1.4.4 Phosphorus Deficiency Symptoms (Table 3.4)

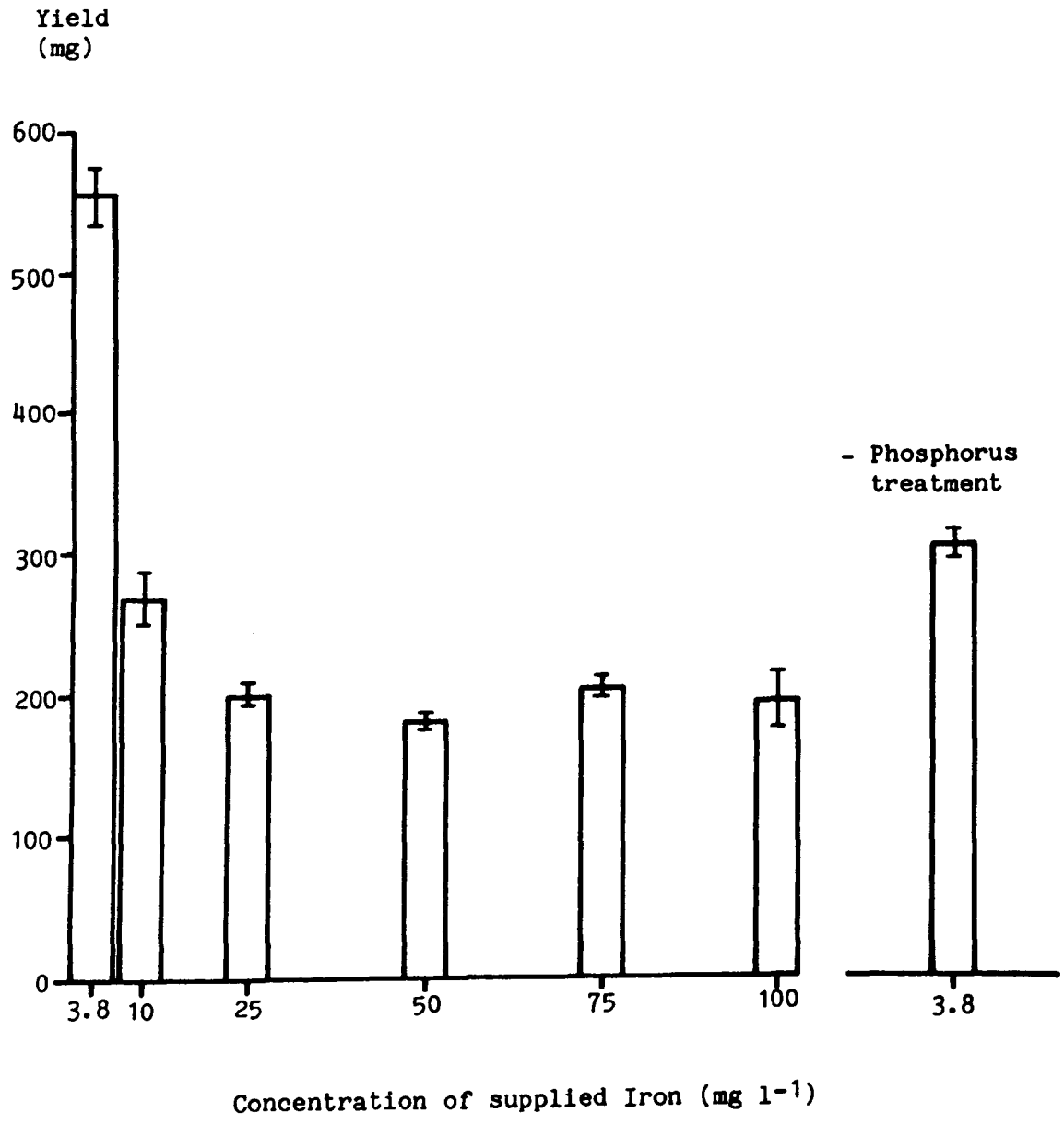
Visible symptoms of nutrient deficiency are usually much more specific than are those of nutrient toxicity unless the toxicity of one mineral nutrient induces a deficiency of another (Marschner 1986). In phosphorus-deficient plants growth is reduced (Wallace 1961; Scaife and Turner 1983; Marschner 1986) and growth habit may be thin and erect (Wallace 1961; Hewitt and Smith 1974). Leaves of such plants are usually smaller and darker or duller green than those of healthy plants (Wallace 1961; Bidwell 1974; Marschner 1986). This may be because cell and leaf expansion are more retarded than is chlorophyll formation and thus the chlorophyll content per unit leaf area is higher (Hecht-Bucholz 1967 cited by Marschner 1986), though photosynthetic efficiency per unit of chlorophyll is reduced (Tombesi et al. 1969). Leaves may also be characteristically tinted with purple or red (Wallace 1961; Armstrong and Boatman 1967; Bidwell 1974; Hewitt and Smith 1974; Scaife and Turner 1983), which may be due to enhanced anthocyanin formation (Marschner 1986).

Lower leaves may become yellow, drying to darker green when phosphorus is deficient (Bidwell 1974), and premature defoliation beginning at the lower leaves may occur (Wallace 1961). Older leaves are typically more affected than younger ones, since phosphorus is a mobile element within the plant (Bidwell 1974).

Production of lateral shoots is often reduced since lateral buds may die or remain dormant (Wallace 1961; Hewitt and Smith 1974), fewer flowers may be produced (Wallace 1961, Bould and Parfitt 1973), and flower initiation may be delayed (Rossiter 1978), since phosphorus affects the phytohormone balance (Marschner 1986).

Many of the above symptoms, particularly growth reduction, dark green colouration of young leaves, purpling or reddening of leaves and stems, yellowing and premature senescence of older leaves, and reduced side shoot production were found in the species screened for iron tolerance. Such symptoms are unlikely to be caused by lack of supplied phosphorus (Sections 2.1.4, 2.5), though a further test was carried out to investigate this (Section 3.1.5). Since similar symptoms are found for aluminium toxicity (Foy et al. 1978; Scaife and Turner 1983; Marschner 1986), it is possible that aluminium and iron many operate in a similar way.

Figure 3.8 Yield of Eupatorium cannabinum after 14 days in 10% Rorison solution (pH 5.5) with various Iron additions, or lacking Phosphorus.
(Mean of 5 replicate pots ± 1 SE)



3.1.5 Investigation of Phosphorus Deficiency

To investigate the possibility of phosphorus deficiency, an extra control was made during the routine screening of Eupatorium cannabinum. Control levels of iron were supplied (3.8 mg Fe l^{-1}), but no phosphorus, and the growth response is presented in Figure 3.8. The results were complicated by the fact that Eupatorium cannabinum is very sensitive to iron (even at 10 mg Fe l^{-1}) and that omission of phosphorus from the nutrient solution allowed more of the 3.8 mg Fe l^{-1} to be in solution than would be found in a normal control solution where co-precipitation of iron and phosphorus would occur.

Growth of phosphorus-free plants was intermediate to that of plants supplied with 3.8 and 10 mg Fe l^{-1} (Figure 3.8). Growth reduction could be explained in terms of direct iron toxicity, since P-free plants were larger than those grown at higher iron concentrations but which had a small supply of phosphorus in solution at equilibrium.

Within two days, small brown spots had developed on the undersides of the leaves in all treatments (except the control), including that lacking phosphorus. Yellowing and wilting did not develop to the same extent as in the higher iron treatments which contained phosphorus in solution. Nor were root stunting or tip blackening so marked. It is therefore likely that these may be symptoms of iron toxicity rather than of phosphorus deficiency.

3.2 MEASURED EFFECTS OF HIGH CONCENTRATIONS OF IRON ON A NUMBER OF GROWTH VARIABLES

3.2.1. Introduction

The response to iron of the following variables was studied for each species, where relevant:

Shoot Dry Weight (SWT)
Root Dry Weight (RWT)
Total Dry Weight (TOTWT)
Shoot Relative Growth Rate (SRGR)
Root Relative Growth Rate (RRGR)
Relative Growth Rate (RGR)
Shoot/Root Dry Weight Ratio (SR)
Shoot Length (SLNGTH)
Root Length (RLNGTH)
Leaf Size (LSIZE)
No. Tillers (monocotyledons only) (TILLS)
No. Side Shoots (SSHTS)
Total No. Leaves (TOTLF)
No. Healthy Leaves (HLVS)
No. Sick Leaves (SLVS)
No. Dead Leaves (DLVS)
Presence or Absence of Adventitious Roots (ADV)
(In some cases length or number were recorded)

Table 3.5 shows which variables were measured on which species, and any problems involved in the data analysis (e.g. low replication, missing treatments). Results were analysed in a number of ways in order to describe and compare the response of the various species.

3.2.2 Standardisation of Variables (%V)

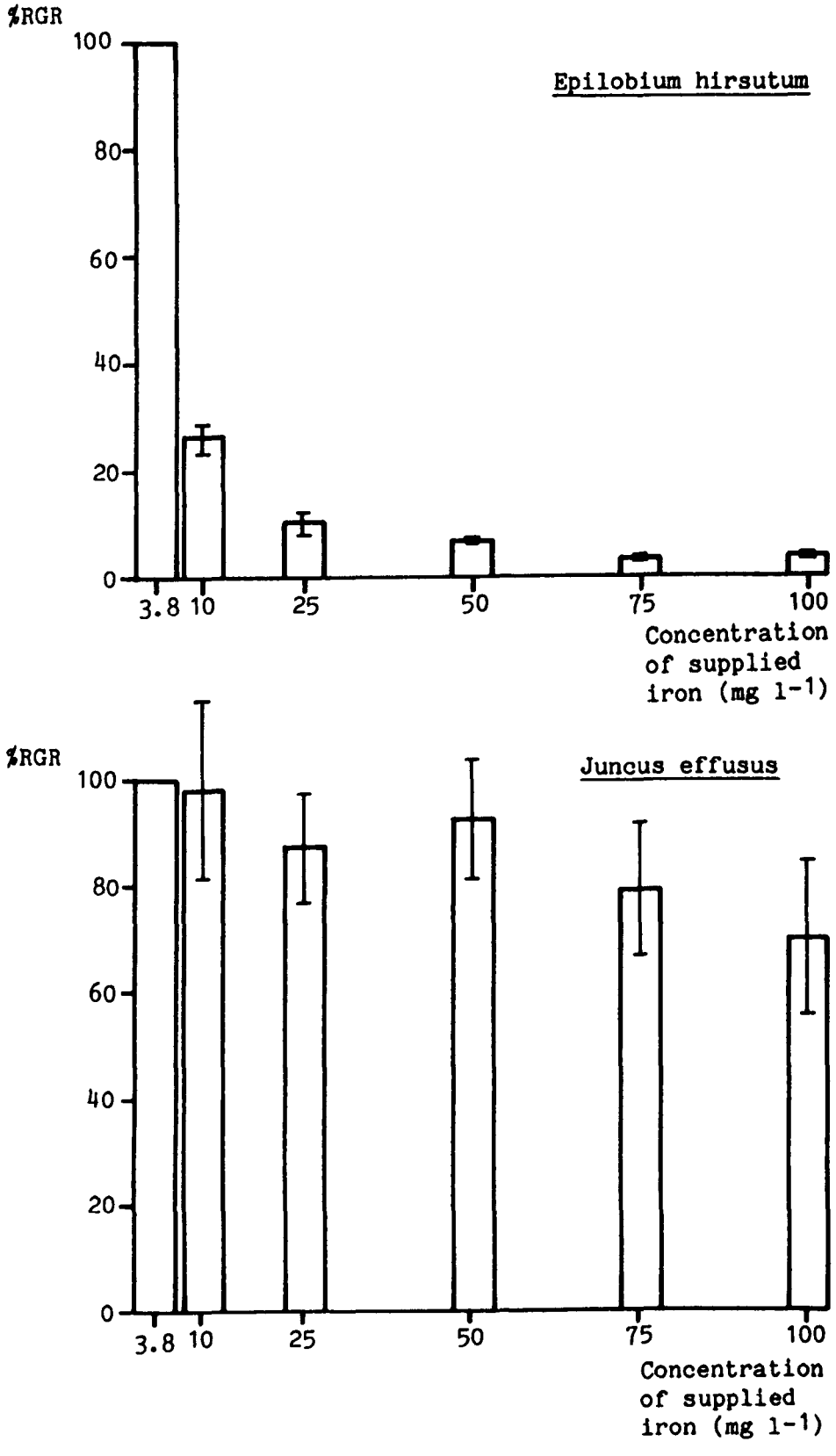
To compare the performance of the species, all data were standardised. For most variables, individual measurements were expressed as a percentage of the mean value of the variable under control conditions. However, for shoot/root ratio measurements, standardisation was made

Table 3.5 Summary Table of Variables Measured on each Species

Species	No. rep. pots	SWT/RWT	SLNGTH	RLNGTH	LSIZE	TILLS	TOTLF	LIVE	SICK	DEAD	ADV	SSHTS	Notes
Agrostis stolonifera	3	+	+	+	+	+	+						No 75 treatment
Briza media	4	+	+	+	+		+	+		+	+		
Caltha palustris	5	+	+	+			+	+		+	+		
Carex appropinquata	5	+	+	+		+	+				+		
Carex diandra	5	+	+	+		+	+	+	+	+	+		
Carex echinata	5	+	+	+		+	+						No 75 treatment
Carex lepidocarpa	5	+	+	+		+	+	+		+	+		
Carex pulicaris	5	+	+	+		+	+	+		+			No 10/75 treatments
Epilobium hirsutum	5	+	+	+	(+)		+	+	+	+	+		
Epilobium palustre	5	+	+	+			+	+	+	+	+		
Eriophorum latifolium	5	+	+	+		+	+				+		
Eupatorium cannabinum	5	+		+	+		+	+		+			
Filipendula ulmaria	5	+	+	+	+		+	+	+	+	+		
Galium aparine	5	+	+	+			+	+		+		+	
Galium palustre	5	+	+	+								+	
Holcus lanatus	5	+	+	+	+	+	+	+		+	+		
Iris pseudacorus	5	+	+	+	+		+			+	+		
Juncus articulatus	5	+	+	+		+	+	+		+			
Juncus effusus	5	+	+	+									
Juncus inflexus	5	+	+	+		+	+	+		+			
Juncus subnodulosus	5	+	+	+			+	+		+	+		
Lotus uliginosus	5	+	+	+			+	+		+			
Lychnis flos-cuculi	5	+	(+)	+			+	+	+	+			
Lysimachia vulgaris	5	+	+	+			+						
Lythrum salicaria	3	+	+		+							+	No 10/75 treatment
Molinia caerulea (dry)	5	+	+	+			+	+	+	+			
Molinia caerulea (wet)	5	+	+	+			+	+	+	+			
Parnassia palustris	5	+											
Pedicularis palustris	5	+		+			+	+	+	+			
Phalaris arundinaceae	5	+	+	+	+	+	+	+	+	+	+		
Potentilla palustris	2	+	+	+	+		+	+		+			2 replicates only
Primula farinosa	5	+		+									
Ranunculus flammula	5	+	+	+	+		+	+		+	+		
Rumex acetosa	5	+	(+)	+	(+)		+	+	+	+	+		
Rumex hydrolapathum	5	+	(+)	+	(+)		+	+	+	+	+		
Scrophularia auriculata	5	+	+	+	+		+	+	+	+	+		
Thalictrum flavum	5	+	+	+			+	+	+	+	+		
Trifolium pratense	5	+	+	+			+	+		+			
Valeriana dioica	3	+	+	+	+		+	+	+	+	+		3 replicates only
Valeriana officinalis	2	+	+	+	+		+	+	+	+			2 replicates only
No. species		40	36	38	16	11	35	29	15	30	19	3	

+ = Variable measured on that species
(+) = shrivelled at high iron concentrations

Figure 3.9 Growth Response of 2 Species Extreme in their Sensitivity/Tolerance to Iron.
(Relative Growth Rate standardised against control Relative Growth Rate (%RGR), with supplied Iron Concentration.)
Mean of 5 replicates ± 1 SE



against the maximum mean value, which was not necessarily in the control treatment. The numbers of healthy, sick and dead leaves were standardised against mean total leaf number in the control. Thus proportions were represented in relation to total leaf number which usually decreased itself with increasing iron concentrations. Total leaf number was also standardised in the same way. For presence/absence of adventitious roots, standardisation was made against an optimum of all plants having adventitious roots. All methods of analysis were performed on standardised data.

3.2.3 **Methods of Data Analysis**

3.2.3.1 One-way Analysis of Variance

One-way analysis of variance was used to establish whether iron had a significant effect on each of the variables in turn, for each species. Logarithmic transformation of data was necessary to reduce heterogeneity of variance.

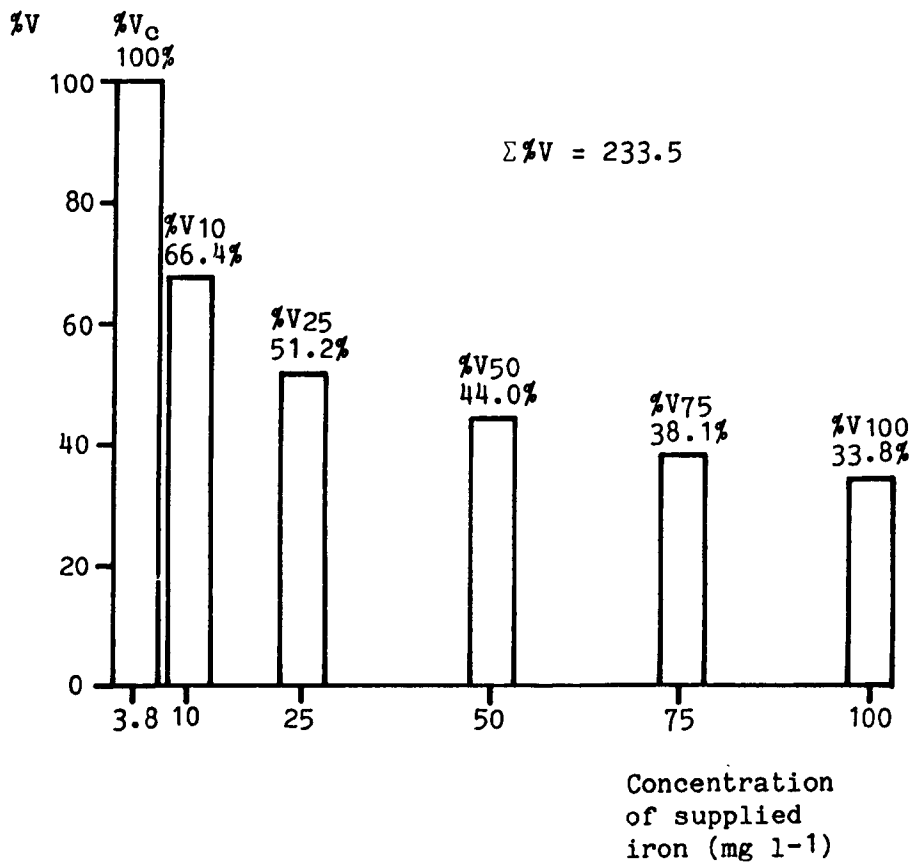
This method of analysis was not suitable for discontinuous data, and in these cases t-tests were performed on measurements from the control and the 100 mg Fe l^{-1} treatment.

3.2.3.2 Summation of Standardised Response of Each Variable (Σ%V)

Results were plotted graphically as histograms of mean value of standardised variable (%V) against added iron concentration. This made it possible to compare visually the response of the species. Figure 3.9 is an example of two such histograms demonstrating the extreme RGR response of two species.

For each variable considered, the effect or lack of effect of iron on each species was quantified by summing the standardised response over all the treatments (Σ%V)(see Figure 3.10). This summation excluded the 'control' value since it was always 100%. Each species thus had a specific score (Σ%V) representing its iron tolerance, and from these values league tables were drawn up of the species ranked in order of tolerance.

Figure 3.10 Calculation of the Iron Tolerance Index $\Sigma\%V$.
(Sum of standardised response of a variable)



$$\Sigma\%V = \%V_{10} + \%V_{25} + \%V_{50} + \%V_{75} + \%V_{100}$$

$$= 66.4 + 51.2 + 44.0 + 38.1 + 33.8 = 233.5$$

where $\%V_c$, $\%V_{10}$, $\%V_{25}$, $\%V_{50}$, $\%V_{75}$ and $\%V_{100}$ are the mean values of the variable measured in that iron concentration, standardised against the value measured under control conditions.

3.2.3.3 Multivariate Analysis

For each variable, comparison of the species' responses was made using multivariate ordination and classification techniques (Principal Components Analysis and Ward's method of hierarchical fusion (Ward 1963)). The species were treated as individual cases for classification and were clustered by 5 attributes corresponding to the standardised mean results (%V) from each of the 5 iron treatments. Thus, clustering based on standardised yield (TOTWT) data, for example, used mean values of %TOTWT at each iron concentration as attributes (excluding the control value which was 100%) (Table 3.6).

Table 3.6 Example of Input for Multivariate Classification (%TOTWT)
(Each entry is the value of %TOTWT for that species in a particular treatment)

Attribute no.	1	2	3	4	5
Iron concentration ($mg\ l^{-1}$)	10	25	50	75	100
Species					
Caltha palustris	80.7	65.5	51.9	53.4	43.1
Epilobium hirsutum	33.8	19.9	16.6	13.2	13.1
Juncus effusus	99.1	94.2	96.2	90.6	86.6

In view of the fact that standardised data were used, multivariate analysis was thought to be suitable for discrete data, e.g. leaf and tiller numbers. However, for leaf numbers or proportions 6 attributes were input, since the control value was not always 100% of the mean total no. leaves in the control, against which standardisation had been made. 6 attributes were also used for presence/absence of adventitious roots.

Since the attributes are sequential, it was possible to plot the cluster diagnostics from Ward's method, i.e. for each cluster the mean value of the standardised variable in question was plotted against iron concentration. This provided a visual representation of species similarity in response to iron concentration (Figure 3.11).

This approach is effectively an attempt to use response curve shape

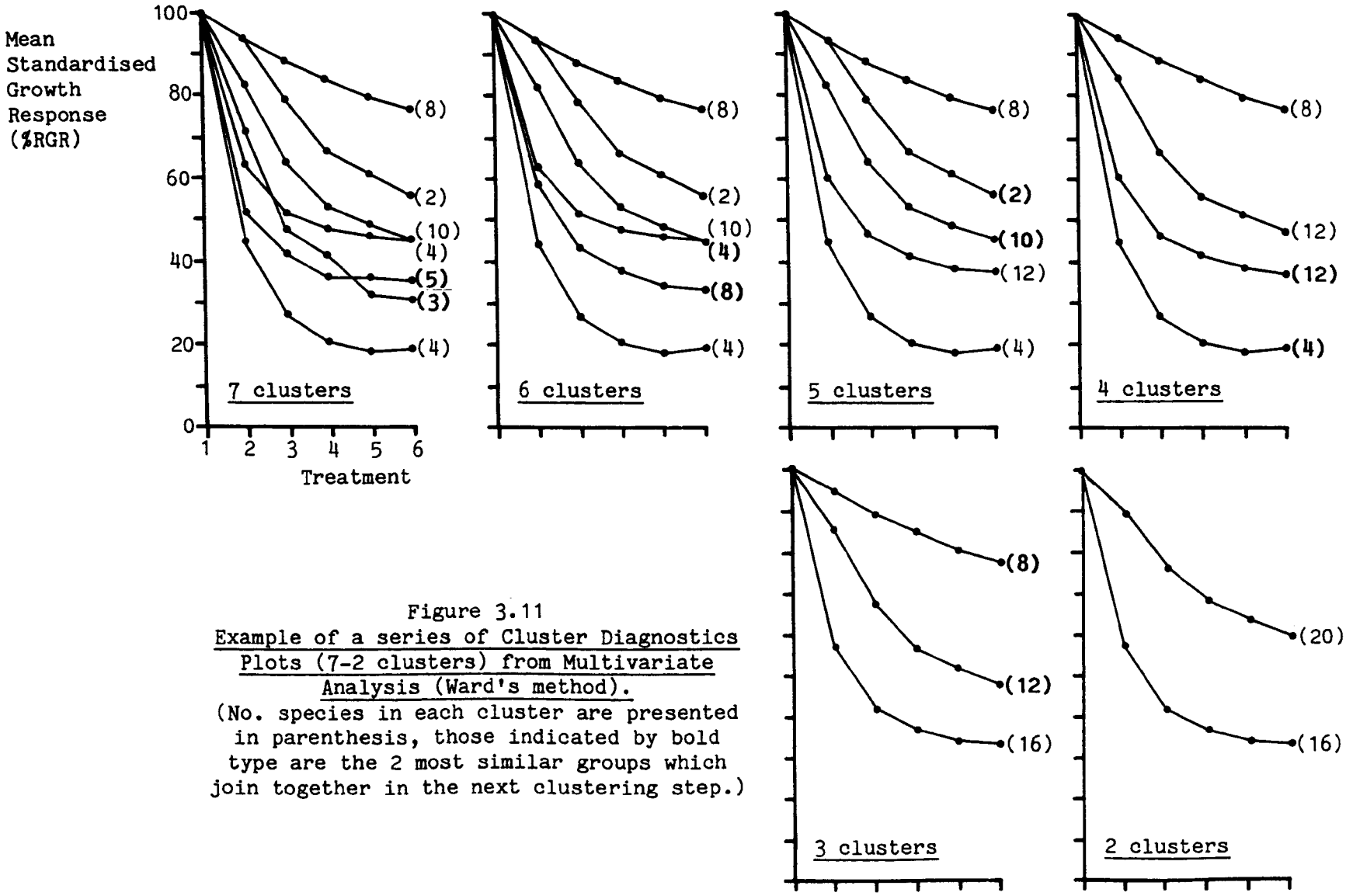


Figure 3.11
Example of a series of Cluster Diagnostics Plots (7-2 clusters) from Multivariate Analysis (Ward's method).
(No. species in each cluster are presented in parenthesis, those indicated by bold type are the 2 most similar groups which join together in the next clustering step.)

Figure 3.11

(from the histograms) rather than sum of standardised variable (Σ%V). It differs from the latter in that species are grouped together on similarity of response rather than ranked on an absolute value, and helps overcome the problem that any one value of Σ%V may result from a number of different curve shapes.

Ward's method grouped the 40 species downwards from 39 to 2 clusters. At each successive step two of the clusters fuse with a resulting change in curve shape. Figure 3.11 is an example of cluster diagnostic plots from 7 down to 2 clusters (in this case based on yield (TOTWT) data). When too many clusters are considered, individual 'odd' responses are no longer masked (see 7 cluster stage). For the majority of variables, greatest change in dissimilarity occurred at the 4 cluster stage, and so 4 clusters were considered in more detail. Cluster 1 refers to species showing greatest tolerance to iron, while clusters 2, 3, and 4 contain species of progressively greater sensitivity.

3.2.3.4 Regression of Species Response with Supplied Iron Concentration

For each standardised variable, linear regression was made of response of each species with supplied iron concentration. Logarithmic transformation of the data was necessary to linearise the response. Two derived parameters were noted, (see Figure 3.12), and species were ranked according to the values obtained:

a. The slope of the Regression line (Davies and Snaydon 1973)

The response of those species upon which iron has little effect would be expected to have a lower slope than that of species upon which iron has a greater effect.

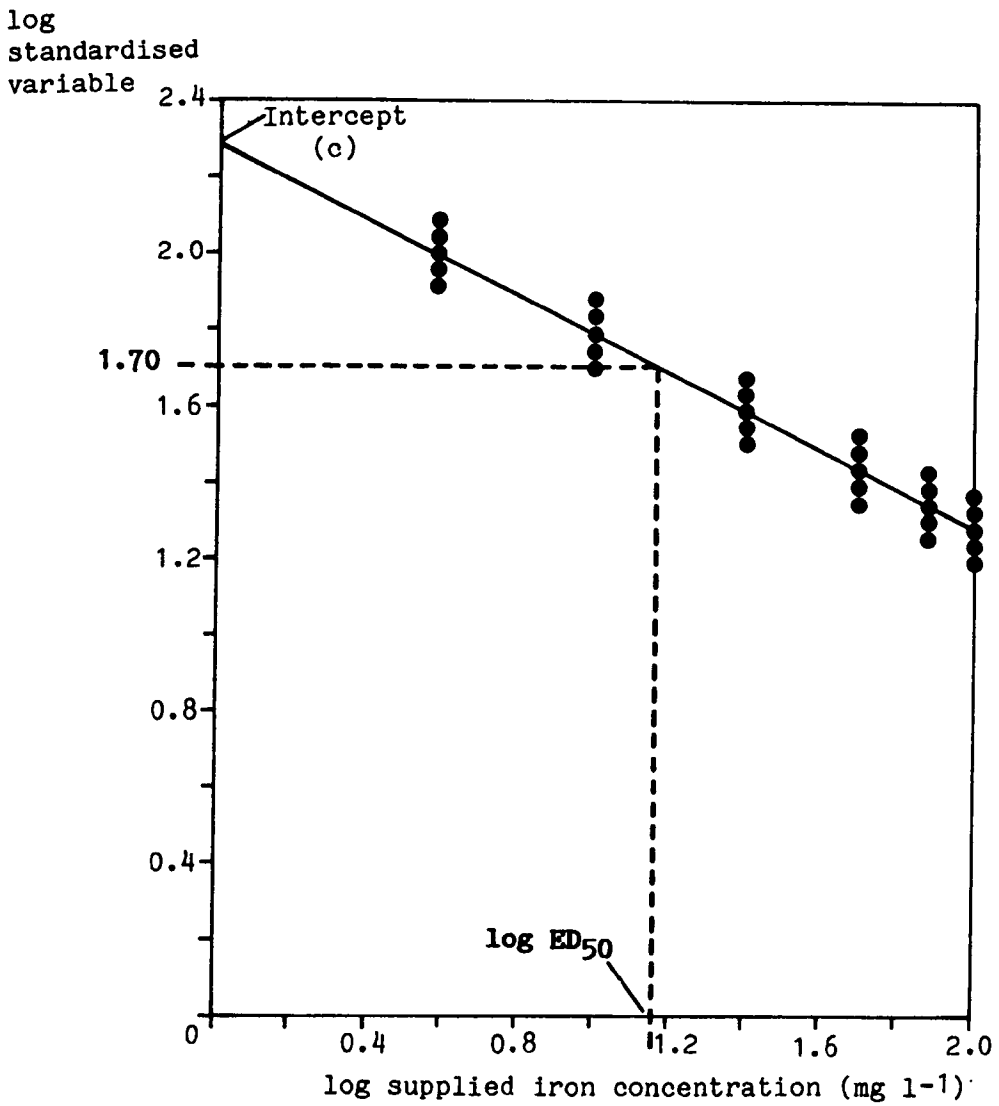
b. Calculated value of ED_{50} (Craig 1977)

i.e. The Effective Dose of iron which would cause 50% reduction in a variable when compared to control conditions.

A number of problems were encountered with these approaches (cf Davies and Snaydon 1973):

i. Since only 6 concentrations of iron had been supplied, there were only 6 points on the x axis (fewer in those species where treatments

Figure 3.12 Derivation of Slope and ED_{50} values from Regression of log standardised variable on log supplied Iron concentration



$$\text{Slope (m)} = \frac{\Delta \text{ log standardised variable}}{\Delta \text{ log supplied iron concn.}}$$

$$\text{log standardised variable} = m \text{ log supplied iron concn.} + c$$

therefore,

$$\text{log supplied iron concn.} = \frac{\text{log standardised variable} - c}{m}$$

The ED_{50} is the effective dose of iron which causes 50% reduction in the variable when compared to control conditions, (i.e. when log standardised variable = 1.70).

Therefore,

$$\log ED_{50} = \frac{1.70 - c}{m} \quad \text{and} \quad ED_{50} = \text{antilog} \left(\frac{1.70 - c}{m}\right)$$

were omitted). Thus, particularly for those species with low replication, there were few points with which to form the regression.

ii. A straight line response was assumed when in fact this was not always the case, particularly for the less tolerant species (even after logarithmic transformation of the data). This tended to be a greater problem for some variables than for others.

iii. In some cases, variance increased with increasing iron concentration.

iv. This method was not suitable for the analysis of discontinuous data.

3.2.4 **Results**

3.2.4.1 Introduction

In Tables 3.7 to 3.26, the effect of iron concentration on each measured variable is given for all species.

i. Species are ranked by decreasing value of $\Sigma\%V$.

ii. Results of multivariate analysis are presented; species are grouped into cluster 1, 2, 3, or 4. Those in cluster 1 are least affected by iron, while those in cluster 4 are most affected.

iii. Lack of significant effect on the response of any 1 species is noted.

iv. For those continuous variables upon which regression analyses were made, Spearman's rank correlation tests were performed to compare the species rankings based on $\Sigma\%V$, ED_{50} and regression line slope. Results of these comparisons are presented.

v. % agreement between the results of multivariate analysis and ranking based on $\Sigma\%V$, ED_{50} and slope, are given.

For those species with missing treatments (C. echinata, C. pulicaris, L. salicaria, and A. stolonifera), $\Sigma\%V$ could not be calculated, nor could multivariate analysis be performed; they are therefore not presented.

Table 3.7 Ranking and Ward's Method Clustering of Species based on Standardised Shoot Weight Data (%SWT)
(Species are presented in order of decreasing Σ%SWT i.e. decreasing tolerance)

	Σ%SWT	Cluster
+ **Juncus effusus**	**446.87**	1
Iris pseudacorus	**438.42**	1
+ Parnassia palustris	427.86	1
Molinia caerulea (dry)	**424.50**	1
Molinia caerulea (wet)	**409.79**	1
Eriophorum latifolium	**399.90**	1
Pedicularis palustris	382.28	1
Juncus articulatus	**372.52**	2
Carex lepidocarpa	**355.50**	2
Trifolium pratense	354.44	2
Briza media	**323.87**	2
Potentilla palustris	322.77	2
Juncus inflexus	**318.93**	2
Lysimachia vulgaris	314.03	2
Carex diandra	**310.41**	2
Primula farinosa	309.11	2
Valeriana dioica	308.42	2
Caltha palustris	308.27	2
Juncus subnodulosus	**298.47**	2
Phalaris arundinacea	**281.31**	2
Ranunculus flammula	277.05	3
Carex appropinquata	**271.95**	3
Galium palustre	266.67	3
Galium aparine	249.79	3
Filipendula ulmaria	244.75	3
Lotus uliginosus	233.50	3
Thalictrum flavum	224.74	3
Holcus lanatus	**222.86**	3
Eupatorium cannabinum	218.36	3
Epilobium palustre	216.72	3
Valeriana officinalis	209.43	3
Lychnis flos-cuculi	190.73	3
Scrophularia auriculata	158.66	4
Rumex acetosa	154.61	4
Rumex hydrolapathum	121.63	4
Epilobium hirsutum	94.19	4

Relationship between Σ%SWT Tolerance Ranking and other Assessments of Tolerance

Spearman's Rank Correlation of the 3 Ranking Methods (%SWT data)

(n=36)	ED_{50}	Slope
Σ%SWT	NS	*
ED_{50}	-	NS

% Agreement between Ward's Method Clustering on %SWT Data and the 3 Rankings

Ranking Method	Clustering
Σ%SWT	100%
ED_{50}	97%
Slope	86%

*** = $p < 0.001$
** = $p < 0.01$
* = $p < 0.05$
NS = no significant correlation

Monocotyledons are indicated by bold type
+ Iron concentration had no significant effect on shoot weight in this species.

3.2.4.2 Shoot Dry Weight Response (SWT)

Iron significantly reduced shoot dry weight in all but Parnassia palustris and Juncus effusus. Both these species appeared near the top of all 3 rankings as assessed by Σ%SWT, ED_{50} and slope, and in all 3 cases, shoot weights of Epilobium hirsutum, Rumex acetosa, Rumex hydrolapathum and Scrophularia auriculata were most affected (Table 3.7). There was a general tendency for shoots of dicotyledonous species to be affected more than those of monocotyledonous species.

Agreement between the results of multivariate analysis and species ranking based on Σ%SWT was exact, and agreement between clustering and ED_{50} ranking was also good. However, Spearman's rank correlation tests showed that there was significant correlation between the rankings based on Σ%SWT and slope only ($p < 0.05$).

Table 3.8 Ranking and Ward's Method Clustering of Species based on Standardised Root Weight Data (%RWT)
(Species are presented in order of decreasing Σ%RWT i.e. decreasing tolerance)

		Σ%RWT	Cluster
+	**Juncus effusus**	551.62	1
+	**Juncus articulatus**	496.40	1
+	**Eriophorum latifolium**	476.16	1
	Juncus inflexus	466.29	1
+	**Molinia caerulea (dry)**	463.09	1
+	**Molinia caerulea (wet)**	451.16	1
	Pedicularis palustris	449.16	1
	Carex lepidocarpa	438.66	2
	Iris pseudacorus	428.39	1
	Phalaris arundinacea	415.04	2
	Juncus subnodulosus	360.73	2
	Parnassia palustris	359.10	2
	Carex diandra	266.09	3
	Caltha palustris	256.74	3
	Holcus lanatus	256.74	3
	Ranunculus flammula	249.71	3
	Carex appropinquata	247.77	3
	Rumex hydrolapathum	238.22	3
	Valeriana officinalis	231.28	3
	Briza media	211.91	3
	Lychnis flos-cuculi	196.46	4
	Lotus uliginosus	193.16	3
	Galium aparine	180.60	4
	Primula farinosa	180.18	4
	Potentilla palustris	176.63	3
	Lysimachia vulgaris	170.15	3
	Trifolium pratense	165.94	4
	Thalictrum flavum	163.63	4
	Valeriana dioica	163.59	4
	Epilobium palustre	148.58	4
	Rumex acetosa	143.21	4
	Filipendula ulmaria	129.40	4
	Epilobium hirsutum	115.19	4
	Galium palustre	93.35	4
	Eupatorium cannabinum	83.60	4
	Scrophularia auriculata	54.57	4

Relationship between Σ%RWT Tolerance Ranking and other Assessments of Tolerance

Spearman's Rank Correlation of the 3 Ranking Methods (%RWT Data)

(n=36)	ED_{50}	Slope
Σ%RWT	***	*
ED_{50}	-	***

% Agreement between Ward's Method Clustering on %RWT Data and the 3 Rankings

Ranking Method	Clustering
Σ%RWT	86%
ED_{50}	89%
Slope	69%

*** = $p < 0.001$
** = $p < 0.01$
* = $p < 0.05$
NS = no significant correlation

Monocotyledons are indicated by bold type
+ Iron concentration had no significant effect on root weight in this species (also true for Carex echinata)

3.2.4.3 Root Dry Weight Response (RWT)

Iron significantly reduced root dry weight in the majority of species (Table 3.8). There was, however, no significant effect on Juncus effusus, Carex echinata, Juncus articulatus, Eriophorum latifolium, or Molinia caerulea (both wetland and non-wetland sources). These are all monocotyledons, and again it is apparent that monocotyledons were less affected than dicotyledons.

Agreement between all 3 methods of ranking was significant ($p < 0.05$), though agreement with results of multivariate analysis was not exact, presumably because different curve shapes can give the same value of $\Sigma\%V$. Ranking based on regression line slope correlated least well with all other methods of grouping/ranking of species. For this variable, linear regression with iron was particularly problematical since, despite logarithmic transformation of both axes, the response remained a curve for the majority of species. Roots of Juncus inflexus were significantly heavier in 10 mg Fe l^{-1} than in the control, suggesting that growth stimulation had occurred.

Table 3.9 Ranking and Ward's Method Clustering of Species based on Standardised Yield Data (%TOTWT)
(Species are presented in order of decreasing Σ%TOTWT i.e. decreasing tolerance)

		Σ%TOTWT	Cluster
+	**Juncus effusus**	**466.64**	**1**
	Iris pseudacorus	**436.53**	**1**
	Molinia caerulea (dry)	**431.51**	**1**
	Parnassia palustris	418.92	1
	Molinia caerulea (wet)	**417.30**	**1**
	Eriophorum latifolium	**410.30**	**1**
	Pedicularis palustris	407.30	1
	Juncus articulatus	**393.51**	**1**
	Carex lepidocarpa	**370.51**	**2**
	Juncus inflexus	**344.75**	**2**
	Juncus subnodulosus	**313.82**	**2**
	Carex diandra	**303.77**	**2**
	Briza media	**302.47**	**2**
	Trifolium pratense	301.20	2
	Phalaris arundinacea	**300.10**	**2**
	Caltha palustris	294.50	2
	Potentilla palustris	294.29	2
	Lysimachia vulgaris	291.26	2
	Valeriana dioica	278.17	2
	Primula farinosa	272.10	3
	Ranunculus flammula	271.05	2
	Carex appropinquata	**266.24**	**3**
	Galium palustre	244.22	3
	Galium aparine	238.09	3
	Holcus lanatus	**229.05**	**3**
	Lotus uliginosus	228.00	3
	Filipendula ulmaria	215.46	3
	Valeriana officinalis	214.48	3
	Thalictrum flavum	211.27	3
	Epilobium palustre	202.90	3
	Lychnis flos-cuculi	191.58	3
	Eupatorium cannabinum	190.57	3
	Rumex acetosa	152.56	4
	Rumex hydrolapathum	141.17	4
	Scrophularia auriculata	129.95	4
	Epilobium hirsutum	96.63	4

Relationship between Σ%TOTWT Tolerance Ranking and other Assessments of Tolerance

Spearman's Rank Correlation of the 3 Ranking Methods (%TOTWT data)

(n=36)	ED_{50}	Slope
Σ%TOTWT	**	NS
ED_{50}	-	*

% Agreement between Ward's Method Clustering on %TOTWT Data and the 3 Rankings

Ranking Method	Clustering
Σ%TOTWT	97%
ED_{50}	94%
Slope	81%

*** = $p < 0.001$
** = $p < 0.01$
* = $p < 0.05$
NS = no significant correlation

Monocotyledons are indicated by bold type
+ Iron concentration had no significant effect on yield in this species.

3.2.4.4 Total Dry Weight (Yield) Response (TOTWT)

Iron produced significant yield reduction in all species except Juncus effusus ($p < 0.05$). Indeed, this species was positioned at the top of all 3 rankings, i.e. those based on Σ%TOTWT, ED_{50} and slope, while Epilobium hirsutum, Rumex acetosa, Rumex hydrolapathum and Scrophularia auriculata were at or near the bottom of all 3 (Table 3.9). Again, a differential response between monocotyledons and dicotyledons was apparent.

There was significant correlation between these 3 methods of ranking except between Σ%TOTWT and slope. Agreement between clustering and ranking was also good, except when ranking was based on slope of regression line.

Table 3.10 Ranking and Ward's Method Clustering of Species based on Standardised Shoot Relative Growth Rate data (%SRGR)
(Species are presented in order of decreasing Σ%SRGR i.e. decreasing tolerance)

		Σ%SRGR	Cluster
	Iris pseudacorus	**405.81**	1
+	**Juncus effusus**	**389.01**	1
	Molinia caerulea (dry)	**372.46**	1
	Molinia caerulea (wet)	**355.37**	1
+	Parnassia palustris	350.77	1
	Juncus articulatus	**320.34**	1
	Eriophorum latifolium	**318.78**	1
	Pedicularis palustris	294.72	2
	Lysimachia vulgaris	282.97	2
	Carex lepidocarpa	**280.60**	2
	Potentilla palustris	278.51	2
	Briza media	**261.98**	2
	Juncus inflexus	**261.66**	2
	Ranunculus flammula	241.68	3
	Valeriana dioica	239.33	3
	Phalaris arundinacea	**234.67**	3
	Caltha palustris	233.74	3
	Carex diandra	**230.73**	3
	Trifolium pratense	223.63	3
	Galium palustre	215.65	3
	Juncus subnodulosus	**209.54**	3
	Primula farinosa	198.75	3
	Carex appropinquata	**193.78**	3
	Holcus lanatus	**193.45**	3
	Lotus uliginosus	177.87	3
	Epilobium palustre	166.01	4
	Eupatorium cannabinum	155.08	4
	Filipendula ulmaria	154.28	3
	Galium aparine	149.23	4
	Thalictrum flavum	146.19	4
	Valeriana officinalis	109.45	4
	Lychnis flos-cuculi	103.21	4
	Scrophularia auriculata	96.53	4
	Rumex acetosa	83.30	4
	Epilobium hirsutum	43.89	4
	Rumex hydrolapathum	35.30	4

Relationship between Σ%SRGR Tolerance Ranking and other Assessments of Tolerance

Spearman's Rank Correlation of the 3 Ranking Methods (%SRGR data)

(n=36)	ED_{50}	Slope
Σ%SRGR	***	NS
ED_{50}	-	NS

% Agreement between Ward's Method Clustering on %SRGR Data and the 3 Rankings

Ranking Method	Clustering
Σ%SRGR	97%
ED_{50}	97%
Slope	75%

*** = $p < 0.001$
** = $p < 0.01$
* = $p < 0.05$
NS = no significant correlation

Monocotyledons are indicated by bold type

+ Iron concentration had no significant effect on shoot relative growth rate in this species.

3.2.4.5 Shoot Relative Growth Rate Response (SRGR)

Iron had a significant effect on shoot relative growth rate response in all species except Juncus effusus and Parnassia palustris (Table 3.10). These two species were among the top five in all three methods of ranking along with Iris pseudacorus and Molinia caerulea (both wetland and non-wetland sources). Species with SRGR most affected by iron (by all three methods of assessment) were Epilobium hirsutum, Lychnis flos-cuculi, Rumex acetosa, Rumex hydrolapathum and Scrophularia auriculata. Again, dicotyledonous species were affected more than monocotyledons.

There was significant correlation between the Σ%SRGR and ED_{50} rankings only. Agreement between results of multivariate analysis and these two methods of ranking was good, though for slope ranking, agreement was poor, except that species least affected were in cluster 1 and those most affected were in cluster 4.

For Epilobium hirsutum and Rumex acetosa variance tended to increase with iron concentration, and for Rumex hydrolapathum regression could only be made up to 25 mg Fe l^{-1}, since above this concentration there had been no shoot growth. Shoots of this species were so badly affected by iron that final dry weight was less than mean initial shoot dry weight.

Table 3.11 Ranking and Ward's Method Clustering of Species based on Standardised Root Relative Growth Rate data (%RRGR)
(Species are presented in order of decreasing Σ%RRGR i.e.decreasing tolerance)

		Σ%RRGR	Cluster
+	**Juncus effusus**	**672.80**	**1**
+	**Juncus articulatus**	**494.84**	**1**
	Juncus inflexus	**456.94**	**1**
+	**Molinia caerulea (dry)**	**455.44**	**1**
+	**Eriophorum latifolium**	**453.54**	**1**
+	**Molinia caerulea (wet)**	**437.00**	**1**
	Carex lepidocarpa	**409.86**	**1**
	Pedicularis palustris	404.09	1
	Phalaris arundinacea	**399.39**	**1**
	Iris pseudacorus	**393.12**	**1**
	Holcus lanatus	**230.59**	**2**
	Juncus subnodulosus	**223.37**	**2**
	Carex diandra	**221.72**	**2**
	Caltha palustris	219.08	2
	Ranunculus flammula	217.44	2
	Parnassia palustris	208.20	2
	Rumex hydrolapathum	181.20	3
	Carex appropinquata	**169.43**	**2**
	Valeriana officinalis	152.27	2
	Lysimachia vulgaris	133.99	3
	Potentilla palustris	129.07	3
	Thalictrum flavum	116.31	3
	Briza media	**114.68**	**3**
	Lotus uliginosus	104.41	3
	Epilobium palustre	104.02	3
	Rumex acetosa	97.34	3
	Epilobium hirsutum	79.57	3
	Primula farinosa	78.84	3
	Trifolium pratense	74.53	3
	Galium palustre	41.95	4
	Valeriana dioica	32.63	4
	Scrophularia auriculata	24.64	4
	Eupatorium cannabinum	24.37	4
	Galium aparine	13.46	4
	Filipendula ulmaria	12.26	4
	Lychnis flos-cuculi	3.27	4

Relationship between Σ%SWT Tolerance Ranking and other Assessments of Tolerance

Spearman's Rank Correlation of the 3 Ranking Methods (%RRGR data)

(n=36)	ED_{50}	Slope
Σ%RRGR	***	**
ED_{50}	-	*

% Agreement between Ward's Method Clustering on %RRGR Data and the 3 Rankings

Ranking Method	Clustering
Σ%RRGR	97%
ED_{50}	97%
Slope	89%

*** = $p < 0.001$
** = $p < 0.01$
* = $p < 0.05$
NS = no significant correlation

Monocotyledons are indicated by bold type

\+ Iron concentration had no significant effect on root relative growth rate in this species (also true for Carex echinata).

3.2.4.6 Root Relative Growth Rate Response (RRGR)

Iron significantly affected root relative growth rate in all species except Juncus effusus, Carex echinata, Juncus articulatus, Eriophorum latifolium and both sources of Molinia caerulea, (i.e. as with root dry weight). The above species are high in all three types of ranking and again, the species least affected by iron tended to be monocotyledons whereas those most affected were dicotyledons (Table 3.11).

There was significant correlation ($p < 0.05$) between all three methods of ranking. There was also good agreement between clustering and ranking except where ranking was based on regression line slope.

Increasing variance with iron concentration occurred in almost 40% of the species considered, despite logarithmic transformation of both axes, and in a few species the response of %RRGR to iron remained a curve. Thus, use of regression line statistics to rank species may not be particularly meaningful.

Table 3.12 Mean Relative Growth Rate of Seedlings under Control Conditions
(Species are ranked by mean RGR)

Species	Relative Growth Rate (per day)
Holcus lanatus	**0.6715**
Epilobium hirsutum	0.5942
Scrophularia auriculata	0.4827
Ranunculus flammula	0.4699
Lysimachia vulgaris	0.4534
Epilobium palustre	0.4251
Rumex acetosa	0.3727
Phalaris arundinacea	**0.3679**
Galium palustre	0.3469
Eupatorium cannabinum	0.3455
Lotus uliginosus	0.3260
Rumex hydrolapathum	0.3155
Potentilla palustris	0.2920
Agrostis stolonifera	**0.2907**
Lythrum salicaria	0.2881
Thalictrum flavum	0.2852
Juncus inflexus	**0.2309**
Caltha palustris	0.2250
Lychnis flos-cuculi	0.2194
Valeriana officinalis	0.2150
Carex echinata	**0.2135**
Carex appropinquata	**0.2132**
Briza media	**0.2050**
Carex diandra	**0.2023**
Valeriana dioica	0.1949
Filipendula ulmaria	0.1935
Juncus articulatus	**0.1729**
Galium aparine	0.1583
Carex pulicaris	**0.1465**
Primula farinosa	0.1449
Carex lepidocarpa	**0.1403**
Iris pseudacorus	**0.1367**
Molinia caerulea (wet)	**0.1334**
Molinia caerulea (dry)	**0.1242**
Juncus subnodulosus	**0.1221**
Trifolium pratense	0.1070
Pedicularis palustris	0.0899
Eriophorum latifolium	**0.0863**
Parnassia palustris	0.0668
Juncus effusus	**0.0572**

Monocotyledons are indicated by bold type

3.2.4.7 Relative Growth Rate (RGR)

3.2.4.7.1 Relative Growth Rate under Control Conditions

The species tested are ranked by mean relative growth rate recorded under control conditions (Table 3.12). For all species except Carex echinata, maximum mean RGR occurred in the control (and even for Carex echinata the maximum value at 10 mg Fe l^{-1} was not significantly more than in the control).

Many of the dicotyledonous species tested tended to have high RGRs while many of the monocotyledonous species had low RGRs, (in fact 82% of monocotyledons tested had less than average RGR). This is reflected in their median, lower and upper quartile values (Table 3.13). However, since there were some very fast-growing monocotyledons (notably Holcus lanatus and Phalaris arundinacea) and also some very slow-growing dicotyledons (Parnassia palustris and Pedicularis palustris), the range of RGRs covered by monocotyledons and dicotyledons was not markedly different, nor did mean RGR values differ significantly.

Grime and Hunt (1975) measured the mean RGR of a large number of species from the Sheffield region over a period of five weeks from germination (and at a lower temperature than in the present study). Analysis of their data reveals no significant difference in the mean or median value of RGR between dicotyledons and monocotyledons, though few of their species were from wetlands. The fact that many of the monocotyledons in the present study had a lower RGR than many of the dicotyledons may reflect a bias in the choice of species.

Of the 40 species tested in this study, only 10 had had their RGR values measured by Grime and Hunt and, although values of RGR in the present work were greater than had been found in the earlier study, there was significant correlation ($r=0.83$, $p < 0.001$) between the values obtained.

Species which grow in non-wetland sites as well as wetland sites had RGR values from all parts of the range, and the wetland and non-wetland sources of Molinia had very similar RGR values despite the fact that the non-wetland plants were much larger and had come from much larger seed.

The relationship between species RGR and fertility of sites at which each species occurred was investigated. Fertility data (assessed phytometrically using Epilobium hirsutum as test plant) was used, from a survey

Table 3.13 Mean, Median and Range of Relative Growth Rate values for Monocotyledons, Dicotyledons and all species (per day)
(Data refer to seedlings grown under control conditions in 10% Rorison solution)

	Monocotyledons	Dicotyledons	All species
Minimum	0.06	0.07	0.06
Maximum	0.67	0.59	0.67
Mean	0.21±0.03	0.29±0.03	0.25±0.14
Median	0.17	0.29	0.21
Lower Quartile	0.13	0.19	0.14
Upper Quartile	0.22	0.37	0.34

Table 3.14 Ranking and Ward's Method Clustering of Species based on Standardised Relative Growth Rate Data (%RGR)
(Species are presented in order of decreasing Σ%RGR i.e. decreasing tolerance)

	Σ%RGR	Cluster
+ **Juncus effusus**	**426.33**	1
Iris pseudacorus	**402.91**	1
Molinia caerulea (dry)	**392.75**	1
Molinia caerulea (wet)	**373.68**	1
Juncus articulatus	**349.13**	1
Eriophorum latifolium	**337.21**	1
Pedicularis palustris	333.34	1
Parnassia palustris	331.34	1
Carex lepidocarpa	**304.28**	2
Juncus inflexus	**296.98**	2
Lysimachia vulgaris	258.51	2
Phalaris arundinacea	**258.19**	2
Potentilla palustris	247.29	2
Ranunculus flammula	235.95	2
Briza media	**233.17**	2
Carex diandra	**229.79**	2
Caltha palustris	229.26	2
Juncus subnodulosus	**212.21**	2
Holcus lanatus	**200.01**	2
Valeriana dioica	196.93	2
Galium palustre	191.35	3
Carex appropinquata	**188.26**	3
Lotus uliginosus	168.22	3
Trifolium pratense	168.10	3
Primula farinosa	159.31	3
Epilobium palustre	152.95	3
Thalictrum flavum	138.95	3
Galium aparine	127.65	3
Eupatorium cannabinum	126.88	3
Valeriana officinalis	119.08	3
Filipendula ulmaria	117.39	4
Lychnis flos-cuculi	91.78	4
Rumex acetosa	85.53	4
Scrophularia auriculata	75.15	4
Rumex hydrolapathum	60.32	4
Epilobium hirsutum	47.98	4

Relationship between Σ%RGR Tolerance Ranking and other Assessments of Tolerance

Spearman's Rank Correlation of the 3 Ranking Methods (%RGR data)

(n=36)	ED_{50}	Slope
Σ%RGR	***	***
ED_{50}	-	**

% Agreement between Ward's Method Clustering on %RGR Data and the 3 Rankings

Ranking Method	Clustering
Σ%RGR	100%
ED_{50}	92%
Slope	81%

*** = $p < 0.001$
** = $p < 0.01$
* = $p < 0.05$
NS = no significant correlation

Monocotyledons are indicated by bold type

+ Iron concentrations had no significant effect on relative growth rate in this species.

of 331 rich-fen sites in England, Scotland and Wales (Wheeler and Shaw 1987). There was a significant correlation ($r = 0.36$, $p < 0.05$) between RGR under control conditions and site fertility, i.e. those species with high RGR values tended to grow on sites with high fertility.

3.2.4.7.2 Relative Growth Rate Response to Iron

Iron significantly reduced the RGR of all species except *Juncus effusus* ($p < 0.05$) which leads the ranking based on Σ%RGR, and is only exceeded by *Carex echinata* on the regression rankings. In all cases, *Epilobium hirsutum*, *Rumex acetosa*, *Rumex hydrolapathum*, *Scrophularia auriculata* and *Lychnis flos-cuculi* were among the species with RGRs most affected by iron (Table 3.14). There was again a strong tendency for dicotyledons to be affected more markedly than monocotyledons.

Rank correlation between the three types of ranking method was highly significant ($p < 0.01$). However, for *Rumex acetosa*, variance increased with iron concentration and, for *Rumex hydrolapathum*, RGR was actually negative at 50-100 mg Fe l^{-1}, (i.e. plants were so badly affected by iron that final yield was less than mean initial yield).

Agreement between results of multivariate analysis and Σ%RGR ranking was exact. There was also good agreement between clustering, and ranking based on ED_{50}, though for ranking based on slope, agreement was less good.

3.2.4.8 Shoot/Root Dry Weight Ratio (SR)

3.2.4.8.1 Shoot/Root Ratio under Control Conditions

Table 3.15 shows the species ranked in order of decreasing shoot/root dry weight ratio measured under control conditions. There is wide variation between species, and those with both the highest and the lowest values of shoot/root ratio are dicotyledons. In high iron concentrations the maximum value of SR attained was 25.4 (*Galium palustre*). Note that the two sources of *Molinia caerulea* had very similar values of SR under control conditions.

Table 3.15 Mean Values of Shoot/Root Dry Weight Ratio of Seedlings grown under control Conditions (10% Rorison solution)
(Species are presented in order of decreasing shoot/root ratio)

Species	Shoot/Root Ratio
Epilobium hirsutum	7.68
Parnassia palustris	7.25
Galium palustre	6.69
Lotus uliginosus	6.49
Eriophorum latifolium	**6.37**
Phalaris arundinacea	**6.21**
Lychnis flos-cuculi	5.74
Carex diandra	**5.68**
Agrostis stolonifera	**5.66**
Lysimachia vulgaris	5.36
Rumex hydrolapathum	5.08
Galium aparine]	4.90
Juncus articulatus]	**4.90**
Juncus inflexus	**4.74**
Carex pulicaris	**4.65**
Molinia caerulea (wet)	**4.64**
Rumex acetosa	4.61
Carex lepidocarpa	**4.58**
Molinia caerulea (dry)	**4.57**
Holcus lanatus	**4.48**
Iris pseudacorus	**4.35**
Juncus effusus	**4.34**
Briza media	**4.29**
Potentilla palustris	4.14
Epilobium palustre	3.94
Eupatorium cannabinum	3.87
Valeriana dioica	3.80
Ranunculus flammula	3.62
Thalictrum flavum	3.54
Valeriana officinalis]	3.44
Lythrum salicaria]	3.44
Carex appropinquata	**3.28**
Juncus subnodulosus	**3.06**
Carex echinata	**3.02**
Filipendula ulmaria	2.95
Caltha palustris	2.75
Scrophularia auriculata	2.64
Trifolium pratense	2.55
Primula farinosa	2.49
Pedicularis palustris	1.69

Monocotyledons are indicated by bold type

Table 3.16 Clustering of Species According to Seedling Shoot/Root Dry Weight Ratio Response to Increasing Iron Supply

Species	Response
+ **Carex appropinquata** **Carex diandra** Epilobium hirsutum **Eriophorum latifolium** + **Iris pseudacorus** **Juncus effusus** **Juncus subnodulosus** Lychnis flos-cuculi **Molinia caerulea (wet and dry)** Pedicularis palustris Valeriana officinalis	Cluster 1 Shoot/root ratio remained constant or decreased, i.e. shoots were affected more than roots
Carex lepidocarpa **Holcus lanatus** **Juncus articulatus** **Juncus inflexus** Parnassia palustris **Phalaris arundinacea** Rumex acetosa Rumex hydrolapathum	Cluster 2 Large reduction in shoot/root ratio between 3.8 and 10 mg Fe l^{-1}, (i.e. shoots were affected more than roots). Thereafter, the ratio remained constant or rose slightly (i.e. roots were equally affected).
Caltha palustris Filipendula ulmaria Galium aparine Primula farinosa Valeriana dioica	Cluster 3 Shoot/root ratio rose with increasing iron concentration (i.e. roots were more affected than shoots).
Briza media Epilobium palustre Eupatorium cannabinum Galium palustre Lotus uliginosus Lysimachia vulgaris Potentilla palustris Ranunculus flammula Scrophularia auriculata Thalictrum flavum Trifolium pratense	Cluster 4 Shoot/root ratio rose with increasing iron concentration. The rise was greater for species in this group than for those in cluster 3, (i.e. roots were affected to an even greater degree than shoots).

Monocotyledons are indicated by bold type

+ Iron concentration had no significant effect on shoot/root dry weight ratio in this species.

3.2.4.8.2 Shoot/Root Ratio Response to Iron (SR)

One-way analysis of variance revealed a significant effect of iron on the shoot/root ratio of all species except Carex appropinquata and Iris pseudacorus. Table 3.16 shows the response patterns and grouping of the species produced by multivariate analysis, when four clusters were considered. Cluster diagnostics for the four clusters are presented in Figure 3.13.

For most of the dicotyledons tested, shoot/root ratio increased with increasing iron concentration, though the increase was less marked in some species (cluster 3) than in others (cluster 4). For the monocotyledonous species, shoot/root ratio generally decreased with increasing iron concentration.

In view of the non-linearity of the response to iron, regression was not employed as a method of data analysis in this instance; nor would calculation of $\Sigma\%V$ be meaningful.

Figure 3.13 Cluster Diagnostics (Ward's Analysis) of Mean Shoot/Root Dry Weight Ratio (standardised against maximum mean shoot/root ratio) with Treatment (4 cluster stage)

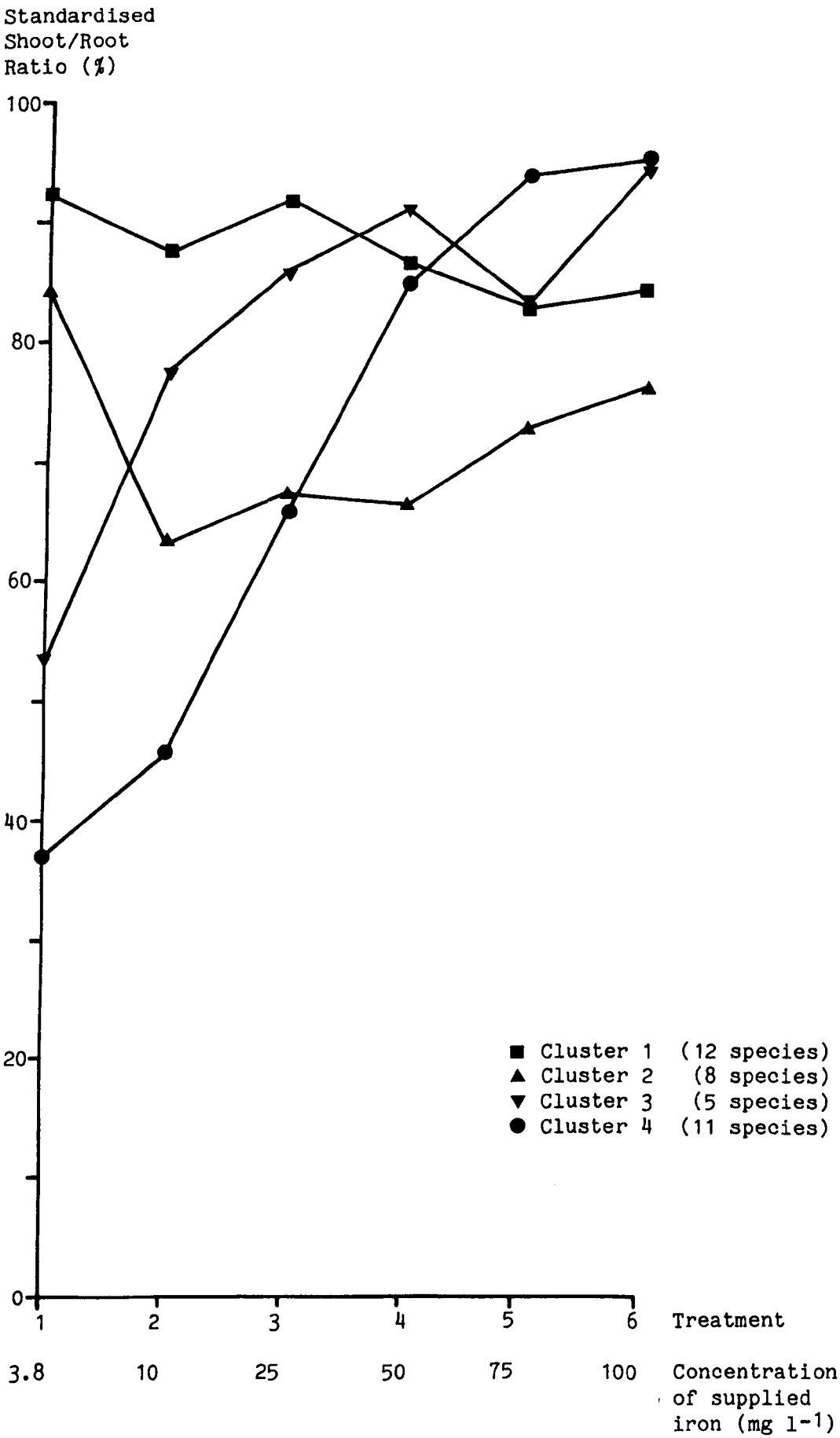


Table 3.17 Ranking and Ward's Method Clustering of Species based on Standardised Shoot Length Data (%SLNGTH)
(Species are presented in order of decreasing Σ%SLNGTH i.e. decreasing tolerance)

		Σ%SLNGTH	Cluster
+	**Eriophorum latifolium**	494.97	1
	Molinia caerulea (wet)	452.14	1
+	**Juncus effusus**	449.28	1
	Iris pseudacorus	449.06	1
+	Trifolium pratense	447.90	1
	Molinia caerulea (dry)	435.32	1
	Carex lepidocarpa	418.64	1
	Phalaris arundinacea	386.04	2
	Juncus articulatus	385.19	2
	Valeriana officinalis	378.74	2
	Thalictrum flavum	373.19	2
	Juncus inflexus	369.23	2
	Carex appropinquata	365.89	2
	Carex diandra	363.93	2
	Potentilla palustris	363.32	2
	Briza media	355.63	2
	Lotus uliginosus	353.61	2
	Galium palustre	348.38	2
	Holcus lanatus	329.86	3
	Lysimachia vulgaris	327.29	3
	Juncus subnodulosus	308.19	3
	Valeriana dioica	307.01	4
	Caltha palustris	300.00	3
	Ranunculus flammula	299.86	3
	Galium aparine	277.91	4
	Scrophularia auriculata	264.63	4
	Filipendula ulmaria	257.84	4
	Epilobium palustre	237.81	4
	Epilobium hirsutum	194.94	4
	Lychnis flos-cuculi	]Omitted because shoots	
	Rumex acetosa	]were shrivelled at high	
	Rumex hydrolapathum	]iron concentrations	

Relationship between Σ%SLNGTH Tolerance Ranking and other Assessments of Tolerance

Spearman's Rank Correlation of the 3 Ranking Methods (%SLNGTH data)

(n=29)	ED_{50}	Slope
Σ%SLNGTH	**	NS
ED_{50}	-	NS

% Agreement between Ward's Method Clustering on %SLNGTH Data and the 3 Rankings

Ranking Method	Clustering
Σ%SLNGTH	97%
ED_{50}	93%
Slope	83%

*** = $p < 0.001$
** = $p < 0.01$
* = $p < 0.05$
NS = no significant correlation

Monocotyledons are indicated by bold type

\+ Iron concentration had no significant effect on shoot length in this species. (also true for Carex pulicaris).

3.2.4.9 Shoot Length Response (SLNGTH)

High iron concentration significantly reduced the shoot length of all measured species, except Eriophorum latifolium, Carex pulicaris, Juncus effusus and Trifolium pratense (Table 3.17). These species were also ranked highly by Σ%SLNGTH, ED_{50} and slope. The species whose shoot lengths were most affected by iron (as shown by ranking based on Σ%SLNGTH and ED_{50}) are Epilobium hirsutum, Epilobium palustre, Filipendula ulmaria and Scrophularia auriculata. Although shoot length of Trifolium pratense was little affected, there was a general tendency for dicotyledonous species to be affected more than monocotyledons, and this is borne out by a further group of species, Lychnis flos-cuculi, Rumex acetosa and Rumex hydrolapathum. The shoots of these became so shrivelled in high iron concentrations that measurement was not possible. For these species, regression could only be made over the lower iron concentrations and, for Rumex acetosa, variance tended to increase with iron concentration. Also for some of the most sensitive species, the curvilinear response was not straightened by logarithmic transformation.

Correlation between ranking by Σ%SLNGTH and ED_{50} was significant ($p < 0.01$), and in both cases agreement with results of multivariate analysis was good. However, agreement between clustering and ranking based on slope was poor, and there was no correlation with either of the other two rankings.

Table 3.18 Ranking and Ward's Method Clustering of Species based on Standardised Root Length Data (%RLNGTH)
(Species are presented in order of decreasing Σ%RLNGTH i.e. decreasing tolerance)

		Σ%RLNGTH	Cluster
+	**Eriophorum latifolium**	**453.73**	1
+	**Carex appropinquata**	**444.72**	1
+	**Juncus effusus**	**434.00**	1
	Carex diandra	**420.45**	1
	Juncus subnodulosus	**416.71**	1
+	Valeriana officinalis	410.50	1
	Lychnis flos-cuculi	402.27	1
+	Galium aparine	398.29	1
+	Valeriana dioica	374.19	2
	Iris pseudacorus	**369.32**	2
	Phalaris arundinacea	**358.28**	2
	Primula farinosa	354.21	2
	Carex lepidocarpa	**346.67**	2
	Molinia caerulea (wet)	**345.45**	2
	Briza media	**340.67**	2
	Filipendula ulmaria	331.60	2
	Molinia caerulea (dry)	**330.89**	2
	Lotus uliginosus	328.99	2
	Juncus inflexus	**324.37**	2
	Thalictrum flavum	303.71	3
	Juncus articulatus	**302.98**	3
	Trifolium pratense	301.32	3
	Lysimachia vulgaris	286.53	3
	Eupatorium cannabinum	267.08	3
	Holcus lanatus	**265.49**	3
	Potentilla palustris	253.09	3
	Pedicularis palustris	252.86	4
	Ranunculus flammula	242.95	4
	Rumex hydrolapathum	238.15	4
	Epilobium hirsutum	235.22	4
	Epilobium palustre	226.71	4
	Caltha palustris	223.63	4
	Rumex acetosa	219.07	4
	Scrophularia auriculata	216.15	4
	Galium palustre	201.60	4

Relationship between Σ%RLNGTH Tolerance Ranking and other Assessments of Tolerance

Spearman's Rank Correlation of the 3 Ranking Methods (%RLNGTH data)

(n=35)	ED_{50}	Slope
Σ%RLNGTH	*	NS
ED_{50}	-	NS

% Agreement between Ward's Method Clustering on %RLNGTH Data and the 3 Rankings

Ranking Method	Clustering
Σ%RLNGTH	100%
ED_{50}	91%
Slope	63%

*** = $p < 0.001$
** = $p < 0.01$
* = $p < 0.05$
NS = no significant correlation

Monocotyledons are indicated by bold type

\+ Iron concentration had no significant effect on root length in this species. (also true for Carex pulicaris).

3.2.4.10 Root Length Response (RLNGTH)

High iron concentrations significantly reduced the root length of all species except Carex pulicaris, Eriophorum latifolium, Carex appropinquata, Juncus effusus, Galium aparine, Valeriana officinalis and Valeriana dioica (Table 3.18). For the last two species, lack of significance may partly reflect reduced replication.

Generally, root length of monocotyledons was affected less than that of dicotyledons. It is surprising to find little effect on root length of V. officinalis, L. flos-cuculi and G. aparine, as other criteria showed these species to be iron-sensitive. Juncus subnodulosus too was usually one of the more sensitive monocotyledonous species.

Correlation between the Σ%RLNGTH and ED_{50} rankings was significant, and for Σ%RLNGTH there was exact agreement with results of multivariate analysis. For ED_{50} ranking, agreement with clustering was good, but for ranking based on regression line slope there was very poor agreement.

Table 3.19 Ranking and Ward's Method Clustering of Species based on Standardised Leaf Size Data (%LSIZE)
(Species are presented in decreasing order of Σ%LSIZE i.e.decreasing tolerance)

	Σ%LSIZE	Cluster
Iris pseudacorus	**411.56**	1
Phalaris arundinacea	**319.40**	2
Briza media	**292.92**	2
Holcus lanatus	**254.76**	3
Potentilla palustris	229.02	3
Valeriana dioica	221.49	3
Ranunculus flammula	215.10	3
Eupatorium cannabinum	200.94	4
Scrophularia auriculata	148.49	4
Filipendula ulmaria	146.24	4
Valeriana officinalis	129.99	4
Epilobium hirsutum	]Omitted because of	
Rumex acetosa	]shrivelled leaves	
Rumex hydrolapathum	]at high iron]concentrations.	

Relationship between Σ%LSIZE Tolerance Ranking and other Assessments of Tolerance

Spearman's Rank Correlation of the 3 Ranking Methods (%LSIZE data)

(n=11)	ED_{50}	Slope
Σ%LSIZE	***	*
ED_{50}	-	NS

% Agreement between Ward's Method Clustering on %LSIZE Data and the 3 Rankings

Ranking Method	Clustering
Σ%LSIZE	100%
ED_{50}	100%
Slope	64%

*** = $p < 0.001$
** = $p < 0.01$
* = $p < 0.05$
NS = no significant correlation

Monocotyledons are indicated by bold type
Iron concentration significantly affected leaf size in all the above species.

3.2.4.11 Leaf Size Response (LSIZE)

One-way analysis of variance revealed significant reduction in leaf size with increasing iron concentration ($p < 0.05$) on all 14 species tested (Table 3.19). Above 25 mg Fe l^{-1}, leaves of Rumex acetosa and Rumex hydrolapathum were shrivelled making measurement impossible. The same was true for Epilobium hirsutum above 50 mg Fe l^{-1}. Thus, Σ%LSIZE could not be calculated for these species, nor could regressions include data for the higher iron concentrations.

In all three rankings, Iris pseudacorus was the least affected species, while Filipendula ulmaria, Eupatorium cannabinum, Scrophularia auriculata, and Valeriana officinalis were the most affected (at least in the Σ%LSIZE and ED_{50} rankings). There was exact agreement between these two rankings and clustering, but agreement was poor with slope ranking. Correlation was significant between the rankings, except between ED_{50} and slope.

Although a relatively small number of species had leaf size measurements recorded, there is evidence that leaf size reduction was greater in the dicotyledons than in the monocotyledons.

Table 3.20 Ranking and Ward's Method Clustering of Species based on Standardised Number of Tillers (%TILLS)
(Species are presented in order of decreasing Σ%TILLS, i.e. decreasing tolerance)

	Σ%TILLS	Cluster
Carex lepidocarpa	519.45	1
Juncus articulatus	431.43	2
Eriophorum latifolium	380.39	2
Carex appropinquata	373.59	2
Carex diandra	353.73	2
Juncus inflexus	314.89	3
Phalaris arundinacea	305.88	3
Holcus lanatus	221.67	4

All species are monocotyledonous and so are presented in bold type

In all the above species, there was a significant effect on tiller number with increasing iron supply. For the majority, increasing iron concentration significantly reduced tiller number, however, in Carex lepidocarpa tiller number increased significantly with iron supply.

There was 100% agreement between Ward's Method Clustering on %TILLS data and Σ%TILLS Tolerance Ranking.

3.2.4.12 Tiller Number Response (TILLS)

This variable could only be measured on the monocotyledons and was only recorded on 8 species upon which iron appeared to be having an effect. It was not measured on species such as Iris pseudacorus where there was no apparent effect.

t-tests revealed a significant reduction ($p < 0.05$) in tiller number in 7 of the species measured, and in Carex lepidocarpa there was a tendency for tiller number to increase with increasing iron concentration. For this reason it appears at the top of the Σ%TILLS ranking and is grouped into cluster 1 alone (Table 3.20).

There was exact agreement between Σ%TILLS ranking and results of multivariate analysis, and Holcus lanatus was the species upon which iron had greatest effect in terms of tiller number reduction.

Table 3.21 Ranking and Ward's Method Clustering of Species based on Percentage of Plants having Adventitious Roots (%ADV)
(Species are presented in order of decreasing Σ%ADV i.e. decreasing tolerance)

		Σ%ADV	Cluster	Iron Concentration which Arrested Adventitious Root Production (mg l^{-1})
+	**Iris pseudacorus**	**586.67**	1	
+	**Phalaris arundinacea**	**585.00**	1	
+	Caltha palustris	569.26	1	
+	**Juncus subnodulosus**	**584.00**	1	
	Holcus lanatus	**584.00**	1	
	Carex lepidocarpa	**552.00**	1	
+	**Eriophorum latifolium**	**465.00**	1	
	Carex appropinquata	**445.00**	2	
	Ranunculus flammula	416.00	2	100
	Filipendula ulmaria	320.00	3	75
	Rumex hydrolapathum	265.00	3	75
	Carex diandra	**260.00**	3	**100**
	Epilobium palustre	255.00	3	50
	Thalictrum flavum	200.00	3	75
	Rumex acetosa	186.67	4	50
	Briza media	**181.25**	4	**50**
	Valeriana dioica	175.00	4	50
	Epilobium hirsutum	168.00	4	50
	Scrophularia auriculata	140.00	4	25

Monocotyledons are indicated by bold type

+ Iron concentration had no significant effect on the percentage of plants which had adventitious roots in this species.

There was 100% agreement between Ward's Method Clustering on %ADV data and Σ%ADV Tolerance Ranking.

3.2.4.13 Response of Adventitious Roots to Iron (ADV)

Species are ranked in order of increasing effect of iron on adventitious root production (Table 3.21). Only those species are included where counts were made of presence/absence of adventitious roots on each plant. Other species produced adventitious roots but these were not counted. It can be seen that production of adventitious roots in monocotyledonous species tends to be less affected than in dicotyledonous species. Results of multivariate analysis agree exactly with ranking based on Σ%ADV.

t-tests revealed no significant treatment effect on the percentage of plants having adventitious roots for Iris pseudacorus, Phalaris arundinacea, Caltha palustris, Juncus subnodulosus and Eriophorum latifolium. In all other species tested there was a significant reduction, and in some cases adventitious root production ceased altogether. The concentration of iron which stopped adventitious root production is also presented in Table 3.21. Those species for which the threshold iron concentration for adventitious root production was reached tended to be in clusters 3 and 4.

For a few species, number of adventitious roots per plant and/or length of longest adventitious root was also measured. In all cases there was a significant reduction ($p < 0.05$) in length of longest adventitious root with increasing iron concentration, though the number of adventitious roots per plant was not always significantly affected (Table 3.22).

3.2.4.14 Side Shoot Production Response (SSHTS)

In all three species investigated, Galium aparine, Galium palustre and Lythrum salicaria, side shoot production, as assessed by mean number of shoots per plant, fell significantly with increasing iron concentration. In some plants at the higher iron concentrations, no side shoots were produced at all.

Table 3.22 Summary of Effect of Increasing Iron Concentrations on Number and Length of Adventitious Roots of Seedlings grown in 10% Rorison solution

Species	Effect on no. adventitious roots per plant	Effect on length of longest adventitious root
Iris pseudacorus	NS	sig. reduced
Phalaris arundinacea	-	sig. reduced
Juncus subnodulosus	-	sig. reduced
Eriophorum latifolium	NS	sig. reduced
Carex appropinquata	sig. reduced	sig. reduced
Ranunculus flammula	-	sig. reduced
Epilobium palustre	-	sig. reduced
Briza media	-	sig. reduced

Monocotyledons are indicated by bold type
- measurement not made
NS - no significant effect

Table 3.23 Ranking and Ward's Method Clustering of Species based on Standardised Total Number of Leaves (%TOTLF)
(Species are presented in order of decreasing Σ%TOTLF i.e. decreasing tolerance)

	Σ%TOTLF	Cluster
Molinia caerulea (wet)	**487.29**	1
Molinia caerulea (dry)	**481.58**	1
+ **Juncus articulatus**	**477.05**	1
Iris pseudacorus	**469.91**	1
Pedicularis palustris	458.54	1
Valeriana dioica	428.70	2
Lysimachia vulgaris	422.88	2
Juncus subnodulosus	**419.21**	2
Trifolium pratense	416.42	2
Caltha palustris	416.00	2
Juncus inflexus	**413.72**	2
Lychnis flos-cuculi	411.30	2
Carex lepidocarpa	**410.39**	2
Briza media	**402.23**	2
Valeriana officinalis	400.03	2
Rumex hydrolapathum	399.07	2
Eriophorum latifolium	**396.96**	2
Scrophularia auriculata	394.05	2
Ranunculus flammula	384.44	2
Epilobium palustre	383.37	2
Eupatorium cannabinum	379.43	2
Thalictrum flavum	343.00	3
Carex appropinquata	**337.07**	3
Filipendula ulmaria	332.79	3
Phalaris arundinacea	**331.39**	3
Potentilla palustris	330.37	3
Carex diandra	**329.63**	3
Galium aparine	319.88	3
Rumex acetosa	319.30	3
Epilobium hirsutum	309.09	3
Holcus lanatus	**294.87**	3
Lotus uliginosus	225.90	4

Monocotyledons are indicated by bold type

+ Iron concentration had no significant effect on total number of leaves in this species.

There was 100% Agreement between Ward's Method Clustering on %TOTLF data and Σ%TOTLF Tolerance Ranking.

3.2.4.15 Response of Total Number of Leaves (TOTLF)

A significant reduction in total number of leaves was found for all species, except Juncus articulatus, with increasing iron concentration. There was a smaller differential in response between monocotyledons and dicotyledons than for most other variables measured. Results of multivariate analysis and ranking based on Σ%TOTLF agreed exactly (Table 3.23).

Table 3.24 Ranking and Ward's Method Clustering of Species based on Proportion of Healthy Leaves (%HLVS) i.e. standardised against Mean Total Number of Leaves in Control
(Species are presented in order of decreasing Σ%HLVS i.e. decreasing tolerance)

	Σ%HLVS	Cluster
Molinia caerulea (dry)	**569.30**	1
Molinia caerulea (wet)	**544.92**	1
Pedicularis palustris	513.41	1
Carex lepidocarpa	**483.16**	1
Juncus articulatus	**479.23**	1
Caltha palustris	443.00	2
Valeriana dioica	440.74	2
Briza media	**433.01**	2
Ranunculus flammula	418.33	2
Juncus inflexus	**408.57**	2
Eupatorium cannabinum	379.43	2
Holcus lanatus	**369.74**	2
Potentilla palustris	365.81	2
Juncus subnodulosus	**348.31**	2
Lotus uliginosus	293.29	3
Valeriana officinalis	292.33	3
Filipendula ulmaria	286.89	3
Epilobium palustre	282.28	3
Scrophularia auriculata	277.98	3
Trifolium pratense	274.63	3
Phalaris arundinacea	**260.46**	3
Thalictrum flavum	257.00	3
Carex diandra	**250.74**	3
Epilobium hirsutum	236.36	3
Rumex hydrolapathum	183.18	4
Galium aparine	180.43	4
Lychnis flos-cuculi	136.82	4
Rumex acetosa	127.27	4

Monocotyledons are indicated by bold type

Iron concentration significantly affected the number of healthy leaves in all the above species.

There was 100% agreement between Ward's Method Clustering on %HLVS data and Σ%HLVS tolerance ranking.

3.2.4.16 Response of Number of Healthy Leaves (HLVS)

For all species tested there was a significant reduction in the number of healthy leaves with increasing iron concentration (Table 3.24). There was still a tendency for dicotyledons to be affected more than monocotyledons, though the differential in response was smaller than for many other variables. Rumex acetosa plants had no healthy leaves at 50 mg Fe l^{-1} and above; the same was true for Lychnis flos-cuculi at 75 mg Fe l^{-1}. There was perfect agreement between results of multivariate analysis and ranking based on Σ%HLVS.

Table 3.25 Ranking and Ward's Method Clustering of Species based on Proportion of Sick Leaves (%SLVS) i.e. standardised against Mean Total Number of Leaves in Control

(Species are presented in order of **increasing** Σ%SLVS i.e. decreasing tolerance)

	Σ%SLVS	Cluster
Molinia caerulea (dry)	**12.28**	**1**
+ Pedicularis palustris	18.29	1
Valeriana dioica	33.33	1
Molinia caerulea (wet)	**42.37**	**1**
Rumex hydrolapathum	42.99	1
Filipendula ulmaria	45.08	1
Thalictrum flavum	74.00	1
Valeriana officinalis	84.62	2
Epilobium hirsutum	92.56	2
Rumex acetosa	104.54	4
Lychnis flos-cuculi	114.23	4
Phalaris arundinacea	**121.51**	**3**
Epilobium palustre	124.40	3
Scrophularia auriculata	130.36	3
Carex diandra	**130.37**	**3**

Monocotyledons are indicated by bold type

\+ Iron concentration had no significant effect on the number of sick leaves in this species.

There was 87% agreement between Ward's Method Clustering on %SLVS data and Σ%SLVS Tolerance Ranking; clusters 3 and 4 were reversed.

3.2.4.17 Response of Number of Sick Leaves (SLVS)

Species are ordered in Table 3.25 by increasing value of Σ%SLVS, i.e. those species least affected by iron are at the top. Although only 15 species were measured, there was no great difference in response between monocotyledons and dicotyledons; monocotyledons were among the most and the least affected. Results of multivariate analysis did not correlate with ranking based on Σ%SLVS; the most sensitive species in cluster 4 (Table 3.25 and Figure 3.14) have lower values of Σ%SLVS than the less sensitive species in cluster 3. This is because the production of sick leaves is rarely a linear response with increasing iron concentration (see cluster diagnostics - Figure 3.14), and so Σ%SLVS is not a good measure of a plant's response. The number of sick leaves tends to increase to a maximum and then fall again as the leaves become dead at higher iron concentrations. The more sensitive the species, the lower the iron concentration at which the maximum occurs (see Cluster 4, Figure 3.14). In the least sensitive species (Cluster 1, Figure 3.14) ill health of leaves does increase linearly with increasing iron concentrations as the peak in number of sick leaves would theoretically occur above 100 mg Fe l^{-1}.

Thus in this instance, where the response is not a simple curve, multivariate analysis is a better tool for understanding species behaviour than is Σ%SLVS.

Figure 3.14 Cluster Diagnostics (Ward's Analysis) of Mean Number of Sick Leaves (standardised against mean total no. leaves in the control) with Treatment (4 cluster stage)

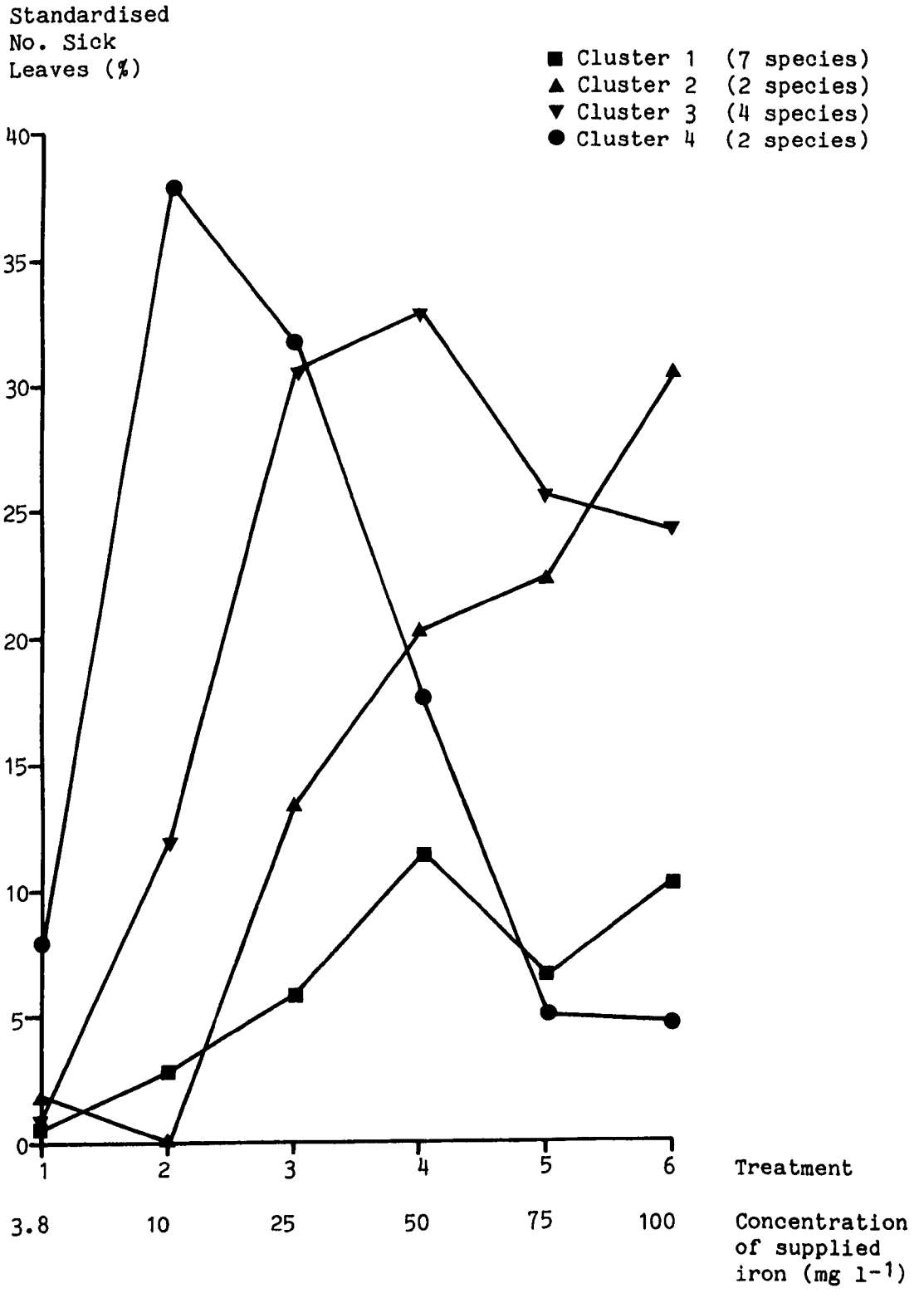


Table 3.26 Ranking and Ward's Method Clustering of Species based on Proportion of Dead Leaves (%DLVS) i.e. standardised against Mean Total Number of Leaves in Control

(Species are presented in order of **increasing** Σ%DLVS i.e. decreasing tolerance)

		Σ%DLVS	Cluster
+	**Iris pseudacorus**	0	1
+	**Molinia caerulea (dry)**	0	1
+	**Molinia caerulea (wet)**	0	1
	Holcus lanatus	**25.13**	2
X	Pedicularis palustris	26.83	2
	Carex lepidocarpa	**27.24**	2
X	Lotus uliginosus	30.87	2
	Carex diandra	**48.52**	2
	Phalaris arundinacea	**49.42**	2
	Valeriana dioica	54.63	2
	Potentilla palustris	64.56	2
X	Ranunculus flammula	66.11	2
	Briza media	**69.24**	2
	Caltha palustris	73.00	3
X	Epilobium palustre	76.69	3
X	Epilobium hirsutum	80.17	3
	Scrophularia auriculata	85.71	3
	Juncus articulatus	**97.81**	3
	Eupatorium cannabinum	100.00	3
X	Filipendula ulmaria	100.82	3
X	**Juncus inflexus**	**105.14**	3
X	Thalictrum flavum	112.00	3
	Valeriana officinalis	123.09	3
X	**Juncus subnodulosus**	**170.90**	4
X	Rumex acetosa	187.49	4
X	Galium aparine	239.44	4
X	Trifolium pratense	241.79	4
X	Lychnis flos-cuculi	260.25	4
X	Rumex hydrolapathum	272.90	4

Monocotyledons are indicated by bold type

\+ Iron concentration had no significant effect on the number of dead leaves in this species.

X Whole plants of this species died during the 2-week experiment.

There was 100% agreement between Ward's Method Clustering on %DLVS data and Σ%DLVS Tolerance Ranking.

3.2.4.18 Response of Number of Dead Leaves (DLVS)

Species are ranked in Table 3.26 by increasing value of Σ%DLVS. Iron concentration had no significant effect on the number of dead leaves in Iris pseudacorus, or Molinia caerulea (both sources). In all other species the number of dead leaves increased significantly with iron concentration. Leaves of the monocotyledons tended to be less affected than those of the dicotyledons, though as for other leaf health measurements, the differential between monocotyledons and dicotyledons was smaller than for most other variables. In some cases, whole plants died (marked X in the table); these were largely dicotyledonous species though some plants of Juncus subnodulosus and Juncus inflexus (the two most sensitive monocotyledons in the ranking) also died. In fact, 52% of the dicotyledons tested suffered whole plant deaths, as compared to only 12.5% of the monocotyledons tested. Agreement between results of multivariate analysis and ranking based on Σ%DLVS was exact.

3.2.5 Summary of Plant Responses to Iron

Sections 3.2.4.2 to 3.2.4.18 show that different parts of different species respond differently to increasing concentrations of iron. Thus, the relative performance or tolerance of species can depend upon the variable measured, and to some extent on the method of data analysis.

It is however, very noticeable (from Tables 3.7 to 3.26) that the dicotyledonous species tested tended to be affected more severely by iron than were the monocotyledonous species. Monocotyledons are very rarely found among those species most affected by iron (i.e. in Cluster 4). Where numbers and health of leaves were considered, the differential in response between monocotyledons and dicotyledons was less marked than for the majority of variables investigated.

It is obvious that some variables show similar responses to iron while others respond very differently. Comparison of the response of variables is made in the next chapter, in particular to derive an index of tolerance to iron.

CHAPTER FOUR

TOLERANCE TO IRON

4.1 SELECTION OF AN INDEX OF TOLERANCE TO IRON

4.1.1 Comparison of Response of the Different Variables to Iron and Selection of a Suitable Tolerance Indicator

4.1.1.1 Introduction

Since different parts of different species responded differently to iron, it was necessary to determine how closely the response of different variables agreed, to establish which variable or variables might produce effective indices of tolerance. The following are desirable features of an efficient tolerance index.

1. The variable should be measurable on all species. Thus, for example, tiller number would be of no value since it can only be measured on monocotyledons.

2. The response of the variable should correspond closely with the response of other variables and thus reflect the response of the whole plant rather than just a part.

3. The variable should provide a wide range of tolerance indices across the species, to facilitate discrimination.

4.1.1.2 Spearman's Rank Correlation of Response of Variables

4.1.1.2.1 Methods

Within each of the four methods of data analysis (i.e. tolerance ranking based on ED_{50}, slope of regression line, or $\Sigma\%V$, and multivariate analysis), Spearman's rank correlations were made between the species

rankings or groupings based on the different variables measured, to determine closeness of agreement.

4.1.1.2.2. Correlation of ED_{50} Tolerance Rankings

The method of species tolerance ranking based on ED_{50} can only be considered for continuous variables. These variables were measured on the majority of species.

Table 4.1 shows that rankings based on root weight (RWT) and relative growth rate (RGR) agree significantly with rankings based on the response of the greatest number of other variables. Ranking by root length response (RLNGTH) only agrees significantly with ranking based on one other variable and so does not reflect the response of a species as a whole. Ranking by leaf size response only correlates significantly with that based on three other variables, but this may partly be because it was only measured for a few species.

4.1.1.2.3 Correlation of Slope Tolerance Rankings

This method of species tolerance ranking was again only suitable for continuous variables. Species ranking by root relative growth rate (RRGR) in particular (and also by root weight (RWT)) agrees most closely with ranking based on the greatest number of other variables. However RLNGTH ranking agreed least well with other variables (as for ED_{50} ranking). Comparison of Table 4.2 with Table 4.1 shows that the number of significant correlations between species ranking based on different variables is generally lower with slope rankings than with ED_{50} rankings. This is probably because ED_{50} utilizes the y axis intercept as well as the slope and so is a more precise measure of species response.

4.1.1.2.4 Correlation of Σ%V Tolerance Rankings

With Σ%V used as a tolerance index, there were few significant correlations between rankings based on number and health of leaves (TOTLF, HLVS, SLVS, DLVS), leaf size (LSIZE), tiller number (TILLS), presence or absence of adventitious roots (ADV) and those based on other variables (Table 4.3). However, measurements of these variables had only been made on a few species and so their suitability as tolerance indicators was not

Table 4.1 Spearman's Correlation of Species Iron Tolerance Rankings Based on ED_{50} Projected from Regression Lines of Log. Variable on Log. Iron Concentration
(n = 40 unless stated)

	SWT	RWT	TOTWT	SRGR	RRGR	RGR	SLNGTH	RLNGTH	LSIZE	No.Significant Correlations
SWT		.3321 *	NS	.4220 **	NS	.3962 **	NS	NS	NS	3 (38%)
RWT			.3285 *	.4985 ***	.4467 **	.2983 *	.5586 ***	NS	.4676 *	7 (88%)
TOTWT				NS	.4692 ***	.3081 *	.3781 *	.4010 **	NS	5 (63%)
SRGR					NS	.3826 **	.5578 ***	NS	NS	4 (50%)
RRGR						.4527 **	.4906 ***	NS	NS	4 (50%)
RGR							.3748 *	NS	.8088 ***	7 (88%)
SLNGTH (n=36)								NS	.5893 **	6 (75%)
RLNGTH (n=38)									NS	1 (13%)
LSIZE (n=16)										3 (38%)

See Section 3.2.1 for key to variables.

$p < 0.001$	***
$p < 0.01$	**
$p < 0.05$	*
NS	No significant correlation

Table 4.2 Spearman's Correlations of Species Iron Tolerance Rankings Based on Slope of Regression Lines of Log. Variable on Log. Iron concentration
(n = 40 unless stated)

	SWT	RWT	TOTWT	SRGR	RRGR	RGR	SLNGTH	RLNGTH	LSIZE	No. Significant Correlations
SWT		NS	NS	NS	.3407 *	NS	.3012 *	NS	NS	2 (25%)
RWT			.3694 **	.3932 **	.3099 *	.4482 **	.5236 ***	NS	NS	5 (63%)
TOTWT				NS	NS	NS	NS	NS	NS	1 (13%)
SRGR					.3522 *	NS	NS	.3822 **	NS	3 (38%)
RRGR						.2985 *	.4628 **	NS	.4941 *	6 (75%)
RGR							.6525 ***	NS	NS	3 (38%)
SLNGTH (n=36)								NS	NS	4 (50%)
RLNGTH (n=38)									NS	1 (13%)
LSIZE (n=16)										1 (13%)

See Section 3.2.1 for key to variables.

$p < 0.001$	***
$p < 0.01$	**
$p < 0.05$	*
NS	No significant correlation.

Table 4.3 Spearman's Correlation of Species Iron Tolerance Ranking Based on $\Sigma\%V$ for Different Variables
(n = 36 unless stated)

	SWT	RWT	TOTWT	SRGR	RRGR	RGR	SLNGTH	RLNGTH	LSIZE	ADV	TILLS	TOTLF	HLVS	SLVS	DLVS	No. Significant Correlations	
SWT		.6118 ***	.5012 ***	NS	.3614 *	.2983 *	.3232 *	NS	NS	NS	NS	.4626 **	.3207 *	NS	NS	7	(50%)
RWT			.5763 ***	NS	.6430 ***	.3717 *	.4645 **	.3964 **	.6091 *	.4000 *	NS	.3350 *	NS	NS	.3842 *	10	(71%)
TOTWT				NS	.4329 **	.3802 *	.5897 ***	.3532 *	.5364 *	.4361 *	NS	.3618 *	NS	NS	NS	9	(64%)
SRGR					.4371 **	.4849 ***	.5897 ***	.3353 *	NS	NS	-.9048 ***	NS	NS	NS	NS	5	(36%)
RRGR						.4615 **	.6310 ***	.3644 *	NS	NS	NS	NS	.3859 *	NS	.4448 **	9	(64%)
RGR							.4833 **	.5468 ***	.5636 *	NS	NS	NS	NS	NS	NS	8	(57%)
SLNGTH (n=29)								.3310 *	NS	.5397 **	-.7619 ***	NS	NS	NS	NS	9	(64%)
RLNGTH (n=35)									NS	.4030 *	NS	NS	.4116 *	NS	NS	8	(57%)
LSIZE (N=11)										NS	NS	.6000 *	NS	NS	.6636 *	5	(36%)
ADV (n=20)											NS	NS	NS	NS	.4159 *	5	(36%)
TILLS (n=8)												NS	NS	NS	NS	2	(14%)
TOTLF (n=32)													NS	NS	NS	4	(29%)
HLVS (n=28)														NS	.3180 *	4	(29%)
SLVS (n=15)															NS	0	(0%)
DLVS (n=29)																5	(36%)

See Section 3.2.1 for key to variables.

$p < 0.001$	***
$p < 0.01$	**
$p < 0.5$	*
NS	No significant correlation.

fully tested.

Variables showing significant correlation with the greatest number of other variables were essentially those which had been measured on the greatest number of species, i.e. TOTWT, RGR, SLNGTH, RLNGTH, RRGR and in particular RWT.

4.1.1.2.5 Correlation of Species Clustering from Ward's Method of Hierarchical Fusion (Multivariate Analysis)

For each variable measured, multivariate analysis was used to group species into four clusters (1-4). The clustering changed for different variables (Table 4.4). Spearman's rank correlation test was used to compare clustering of the species across pairs of variables in turn. Results are presented in Table 4.5.

Since clustering involves grouping species with a similar response to iron rather than ranking them, there tended to be a greater number of significant correlations between the variables than for Σ%V rankings. The only exception to this was root length (RLNGTH), the response of which correlated with a number of other variables when species were ranked by Σ%V but which showed poor agreement with other variables when clustering was considered. Root length response to iron tended to be different from the response of other variables; for example root length of Galium aparine, Lychnis flos-cuculi and Valeriana officinalis was little affected by iron whereas other parts were sensitive. Conversely, root length of Pedicularis palustris, and to a lesser extent Molinia caerulea (both sources), was affected by iron while other parts were not.

When species are ranked, the omission of a species does not affect the tolerance ranking of other species, but when clustered by multivariate analysis, the omission of one or more species may alter the clusters to which other species belong. Thus for example, clustering by tiller number (TILLS) (which could only be measured on monocotyledons) led to a markedly different clustering from when other variables were used (Table 4.4).

Particular care should therefore be taken when comparing results of multivariate analysis between the different variables (Table 4.4). Even so, some variables with missing records (e.g. leaf size, or presence or absence of adventitious roots) gave similar clustering to variables for which more complete data were available.

Table 4.5 shows that the variables whose species clustering agreed

Table 4.4 Clustering of Species Based on Different Variables
(Results of Multivariate Analysis)

Species	SWT	RWT	TOTWT	RGR	SRGR	RRGR	SLNGTH	RLNGTH	LSIZE	ADV	TILLS	SR	TOTLF	LIVE	SICK	DEAD
	Cluster															
Briza media	2	3	2	2	2	3	2	2	2	4		4	2	2		2
Caltha palustris	2	3	2	2	3	2	3	4		1		3	2	2		3
Carex appropinquata	3	3	3	3	3	2	2	1		2	2	1	3			
Carex diandra	2	3	2	2	3	2	2	1		3	2	1	3	3	3	2
Carex lepidocarpa	2	2	2	2	2	1	1	2		1	1	2	2	1		2
Epilobium hirsutum	4	4	4	4	4	3	4	4		4		1	3	3	2	3
Epilobium palustre	3	4	3	3	4	3	4	4		3		4	2	3	3	3
Eriophorum latifolium	1	1	1	1	1	1	1	1		1	2	1	2			
Eupatorium cannabinum	3	4	3	3	4	4		3	4			4	2	2		3
Filipendula ulmaria	3	4	3	4	3	4	4	2	4	3		3	3	3	1	3
Galium aparine	3	4	3	3	4	4	4	1				3	3	4		4
Galium palustre	3	4	3	3	3	4	2	4				4				
Holcus lanatus	3	3	3	2	3	2	3	3	3	1	4	2	3	2		2
Iris pseudacorus	1	1	1	1	1	1	1	2	1	1		1	1			1
Juncus articulatus	2	1	1	1	1	1	2	3			2	2	1	1		3
Juncus effusus	1	1	1	1	1	1	1	1				1				
Juncus inflexus	2	1	2	2	2	1	2	2			3	2	2	2		3
Juncus subnodulosus	2	2	2	2	3	2	3	1		1		1	2	2		4
Lotus uliginosus	3	3	3	3	3	3	2	2				4	4	3		2
Lychnis flos-cuculi	3	4	3	4	4	4		1				1	2	4	4	4
Lysimachia vulgaris	2	3	2	2	2	3	3	3				4	2			
Molinia caerulea (dry)	1	1	1	1	1	1	1	2				1	1	1	1	1
Molinia caerulea (wet)	1	1	1	1	1	1	1	2				1	1	1	1	1
Parnassia palustris	1	2	1	1	1	2						2				
Pedicularis palustris	1	1	1	1	2	1		4				1	1	1	1	2
Phalaris arundinaceae	2	2	2	2	3	1	2	2	2	1	3	2	3	3	3	2
Potentilla palustris	2	3	2	2	2	3	2	3	3			4	3	2		2
Primula farinosa	2	4	3	3	3	3		2				3				
Ranunculus flammula	3	3	2	2	3	2	3	4	3	2		4	2	2		2
Rumex acetosa	4	4	4	4	4	3		4		4		2	3	4	4	4
Rumex hydrolapathum	4	3	4	4	4	3		4		3		2	2	4	1	4
Scrophularia auriculata	4	4	4	4	4	4	4	4	4	4		4	2	3	3	3
Thalictrum flavum	3	3	3	3	4	3	2	3		3		4	3	3	1	3
Trifolium pratense	2	4	2	3	3	3	1	3		3		4	2	3		4
Valeriana dioica	2	4	2	2	3	4	4	2	3	4		3	2	2	1	2
Valeriana officinalis	3	3	3	3	4	2	2	1	4			1	2	3	2	3

See Section 3.2.1 for key to variables.

Cluster 1 denotes greatest tolerance of iron; cluster 4 denotes greatest sensitivity

Species for which treatments were omitted are not included.

Table 4.5 Spearman's Correlation of Results of Multivariate Analysis from Different Variables
(n = 36 unless stated)

	SWT	RWT	TOTWT	SRGR	RRGR	RGR	SLNGTH	RLNGTH	LSIZE	ADV	TILLS	SR	TOTLF	HLVS	SLVS	DLVS	No.Significant Correlations	
SWT		.7227 ***	.9442 ***	.8740 ***	.6625 ***	.8938 ***	.6889 ***	.3703 *	.8383 ***	.5418 **	NS	.3499 *	.5602 ***	.7336 ***	NS	.5918 ***	13	(87%)
RWT			.7849 ***	.7801 ***	.9133 ***	.8367 ***	.6944 ***	NS	.7766 **	.8012 ***	NS	.5222 ***	.4746 **	.6759 ***	.4644 *	.5436 ***	13	(87%)
TOTWT				.8921 ***	.7137 ***	.9474 ***	.6541 ***	.2855 *	.8691 ***	.5604 **	NS	.3214 *	.6182 ***	.7876 ***	NS	.5994 ***	13	(87%)
SRGR					.6844 ***	.8797 ***	.6850 ***	NS	.8224 ***	.5562 **	NS	NS	.4838 **	.8167 ***	.5018 *	.6845 ***	12	(80%)
RRGR						.7707 ***	.6749 ***	NS	.6576 *	.8576 ***	NS	.6008 ***	.4176 **	.6199 ***	NS	.5119 **	12	(80%)
RGR							.5788 ***	NS	.9170 ***	.6492 ***	NS	.3373 *	.5528 ***	.8561 ***	NS	.7051 ***	12	(80%)
SLNGTH (n=29)								.3586 *	.6633 *	.4875 *	.7742 *	.3605 *	.3539 *	.4404 *	NS	.4738 **	14	(93%)
RLNGTH (n=35)									NS	NS	NS	.5011 ***	NS	NS	NS	NS	4	(27%)
LSIZE (n=11)										NS	NS	NS	NS	NS	NS	.9378 ***	8	(53%)
ADV (n=20)											NS	.3904 *	NS	.4414 *	NS	NS	9	(60%)
TILLS (n=8)												NS	NS	NS	NS	NS	1	(7%)
SR (n=36)													NS	NS	NS	NS	8	(53%)
TOTLF (n=32)														.5976 ***	NS	NS	8	(53%)
HLVS (n=28)															.6064 **	.6404 ***	11	(73%)
SLVS (n=15)																NS	3	(20%)
DLVS (n=29)																	9	(60%)

See Section 3.2.1 for key to variables.
SR = trend in shoot/root dry weight ratio

$p < 0.001$	***
$p < 0.01$	**
$p < 0.05$	*
NS	No significant Correlation.

Figure 4.1 Range of the value of the Iron Tolerance Index (Σ%V) for examined species, based on different Variables measured on those species.
(□ mean, ○ median, | upper and lower quartile)

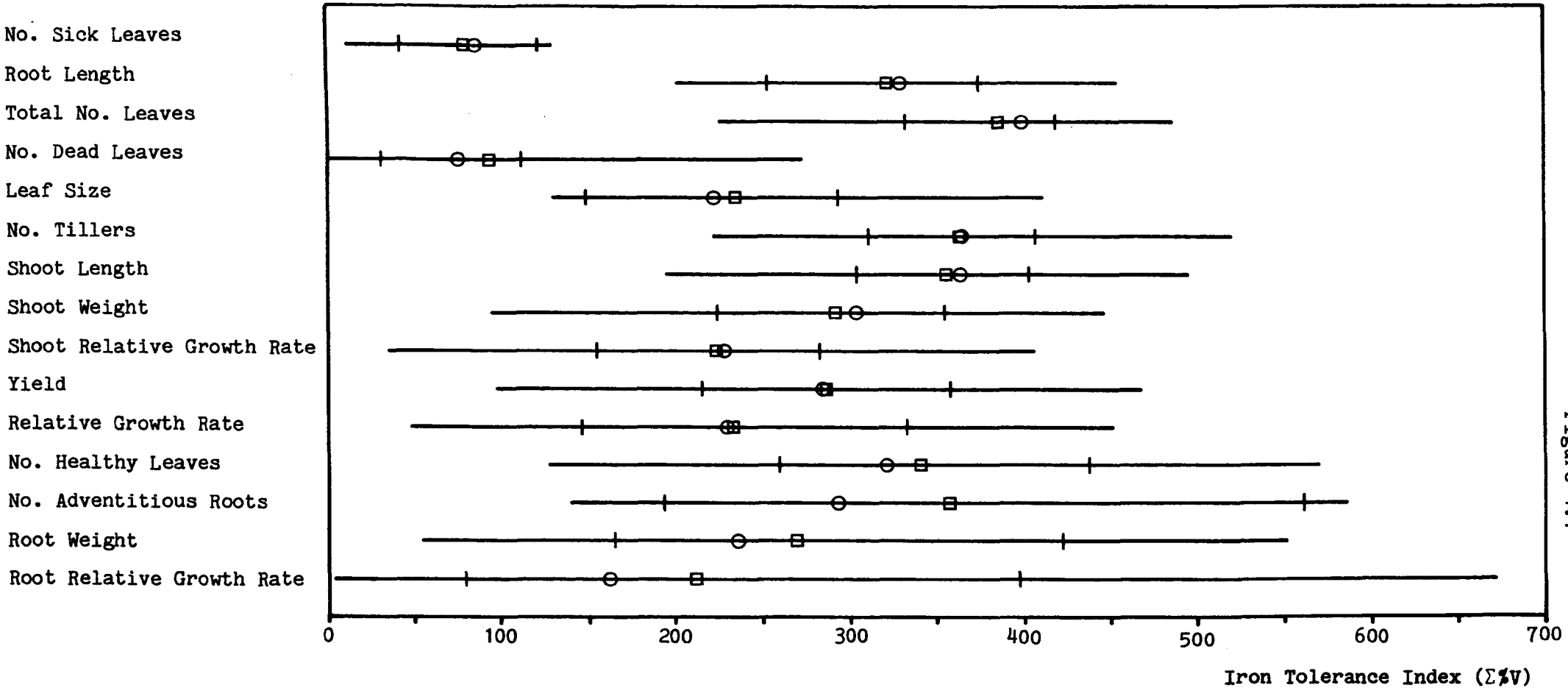


Figure 4.1

significantly with clustering based on the greatest number of other variables were shoot weight (SWT), root weight (RWT), yield (TOTWT), relative growth rate (RGR), shoot and root relative growth rates (SRGR, RRGR) and in particular shoot length (SLNGTH).

4.1.1.3 Range of the Value of the Tolerance Index Σ%V

Figure 4.1 shows the range of the value of Σ%V obtained for each variable from the species for which the variable was measured (see also Tables 3.7 - 3.26). The variables are ranked in order of increasing range of Σ%V.

The most sensitive indicators of iron toxicity are those variables which are most affected by iron and so give low values of Σ%V in at least some species. RGR, SRGR, RRGR and RWT all fall into this category. Number of sick leaves and number of dead leaves are different in that a low value of Σ%V indicates tolerance rather than sensitivity.

The range of a tolerance index is likely to be of greater importance than its relative sensitivity since a variable with a wide range of indices is likely to discriminate well between species. Conversely, a variable from which the range of tolerance indices is small is obviously affected by iron to a similar extent in all species and is unlikely to discriminate between species sufficiently to be a good tolerance indicator. Thus variables at the top of Figure 4.1 (e.g. no. sick leaves, root length) are likely to be poorer indices of tolerance than those at the bottom (e.g. root relative growth rate, root dry weight).

However, a contributory factor to the range of RRGR and RWT indices is that some of the tolerant species' roots had precipitated ochre on them which unavoidably increased their measured weight (or growth rate). Moreover, the very large range of these variables is primarily due to the contribution of one species (*Juncus effusus*) in which heavy ochre deposits on all except control roots caused the dry weight of the treatment roots to be always (non-significantly) greater than the dry weight of the control roots. Thus, values of Σ%RWT or Σ%RRGR which should be maximal at 500 with all treatments equal to the control, are as high as 552 and 673 (respectively) in this one case. Crowder and Macfie (1986) cite McLaughlin *et al.* (1985) as noting that up to 8% of the total root dry weight (and 98% of root iron) was due to the ochre precipitate on the roots of *Agrostis gigantea*.

Table 4.6 Summary of Suitability of Variables as Indices of Iron Tolerance

	No. spp. Measured	Method of Analysis: ED_{50}	Slope	Clustering	Σ%V	Range of Σ%V	Problems
Variable							
SWT	40	38%	25%	87%	50%	353	
RWT	40	**88%**	63%	87%	**71%**	497	Precipitate
TOTWT	40	63%	13%	87%	64%	370	
SRGR	40	50%	38%	80%	36%	371	
RRGR	40	50%	**75%**	80%	64%	**670**	Precipitate
RGR	40	**88%**	38%	80%	57%	378	
SLNGTH	33	75%	50%	**93%**	64%	300	Shoot shrivelling at high iron concentrations in some species.
RLNGTH	38	13%	13%	27%	57%	252	
TOTLF	35	Discontinuous Data		53%	29%	261	
HLVS	29			73%	29%	442	
DLVS	30			60%	36%	273	

See Section 3.2.1 for key to variables; (those which were only measured on a few species are not considered).

Percentages refer to the % of other variables with which response correlated significantly (see Tables 4.1, 4.2, 4.3 and 4.5).
Values **in bold type** indicate the variable(s) most suitable as a tolerance indicator for that particular method of analysis, i.e. highest correlation with other variables, or greatest range of indices.
Values underlined indicate the variable having least correlation with other variables, or with the smallest range of indices (i.e. the variables least suitable as a tolerance indicator (see Section 4.1.1.1))

Thus, for iron tolerance assessments, these two variables, RWT and RRGR are unsatisfactory as the ochre deposits cannot be readily removed. Alternative variables which give a wide range of tolerance index are presence/absence of adventitious roots, and number of healthy leaves. However, the large range of these indices may partly be an artefact of the method of data analysis since, for these two variables, standardisation of data was not made against the control (Section 3.2.2) and so the response of all 6 treatments (including the control) were summed, giving a theoretical maximum of 600. RGR, TOTWT, SRGR and SWT also all give a wide range of tolerance indices, and in these the weight of precipitated ochre on the roots is either inapplicable or masked, to some extent.

4.1.1.4 Discussion of Choice of Variable as an Index of Tolerance

As Sections 4.1.1.2.2 to 4.1.1.3 show, the different methods of data analysis result in different indications as to which variable might be a good index of tolerance to iron. This is summarised in Table 4.6. Those variables which were only measured on a few species (leaf size, tiller number, presence/absence of adventitious roots, and number of sick leaves) are not considered. One reason these were not measured on all species was that on casual observation iron had no obvious effect.

Table 4.6 shows that, except for the problem of the weight of ochre on the roots, RWT and RRGR might be good indices of tolerance since their response correlates with that of a number of other variables and they both have a wide range of values of $\Sigma\%V$. Leaf number and health variables (discontinuous variables) tended to correlate poorly with the response of other variables, particularly when species were ranked on $\Sigma\%V$. Root length (RLNGTH) is shown to be a poor index of tolerance since it generally correlates poorly with other variables and has a relatively low range of values of $\Sigma\%V$. This backs up evidence presented by Al-Farraj (1983) that root length increment was not a reliable index of iron tolerance. Shoot length (SLNGTH) is also unsuitable since at high iron concentrations shoot shrivelling occurred in the most sensitive species, making measurement impossible.

It is apparent from trends in shoot/root ratio response (Table 3.16) that shoot and root dry weights of any one species respond differently to iron. Since a good index of tolerance should reflect the response of the whole plant rather than just a part, any variable which considers shoot or

root alone is not ideal, unless perhaps to compare the response of genotypes of one species.

The only two variables to integrate the response of the whole plant are TOTWT and RGR and both variables were measured on all species in this study. Tolerance assessments based on these variables generally agreed well with assessments based on many other variables, and both covered a reasonably large range of Σ%V tolerance indices. The weight component of precipitated ochre is masked to some extent though not eliminated in these measurements. Since shoot/root ratios were never lower than 1.4 and usually much higher, it has been estimated that given an error in root weight measurement of 8% due to ochre (McLaughlin et al. 1985), the maximum adjustment over the weight of the whole plant would be 3.3% and would normally be much less. Thus the effect of ochre deposition on these methods of tolerance assessment is minimal.

Of the two variables, RGR was chosen as the better index of tolerance since it took into consideration the initial weight of each species, enabling a direct comparison to be made between them.

4.1.2 **Selection of Method of Tolerance Assessment**

4.1.2.1 Introduction

Tables 3.7 to 3.26 show that the apparent relative tolerance of species to iron may depend to some extent on the method of tolerance assessment (i.e. multivariate clustering, or tolerance ranking based on Σ%V, ED_{50} or slope of regression of variable on iron concentration). For all variables, there was significant correlation between the results of some methods of tolerance assessment but rarely between all methods. Selection of the most suitable method of tolerance assessment is thus discussed below.

4.1.2.2 Tolerance Ranking based on Slope of Regression of Variable on Iron Concentration

For almost all variables considered, the use of regression line slope to rank the species agreed least well with results from the other three methods of data analysis (Tables 3.7 - 3.19). This may be partly because even after logarithmic transformation a linear response could not always

be achieved, particularly for some species more than others. This was also a greater problem for some variables (e.g. SWT, TOTWT, RLNGTH and in particular RWT and LSIZE) than for others (e.g. RGR).

When comparison was made between the tolerance rankings of species based on different variables, but using slope as the ranking criterion (Table 4.2), there were fewer significant correlations between variables than for any other method of data analysis. This would imply that regression line slope is not very suitable as an indicator of tolerance in this study.

4.1.2.3 Tolerance Ranking based on ED_{50} projected from Regression of Variable on Iron Concentration

For the majority of variables considered, tolerance ranking based on ED_{50} agreed significantly with that based on $\Sigma\%V$ despite the fact that in some cases the regressions from which ED_{50} was calculated were not linear. There was also usually close, though rarely exact, agreement with results of multivariate analysis (Tables 3.7 - 3.19). The superiority of ED_{50} ranking over slope ranking must be due to the fact that ED_{50} takes into account the intercept on the y axis as well as the slope of the regression.

Problems of increased variance at higher iron concentrations, plus the fact that discontinuous data could not be analysed by this method, limited the use of regression line statistics to assess tolerance. However, ranking of species by ED_{50} value could be used in cases where treatments had been omitted.

4.1.2.4 Tolerance Ranking based on $\Sigma\%V$

For the majority of variables considered, tolerance ranking based on $\Sigma\%V$ agreed significantly with that based on ED_{50}. There was also very close agreement with the results of multivariate analysis, though discrepancies probably arise because different curve 'shapes' can give the same value of $\Sigma\%V$. This is particularly true in the middle orders of tolerance, whereas for the very tolerant or very intolerant species, less variation of curve 'shape' is possible.

An important advantage of using $\Sigma\%V$ over ED_{50} as a tolerance index is that it is simple to calculate and involves little manipulation of the raw data other than standardisation. It can also be used for discontinuous

data. However, Σ%V cannot be calculated in cases where treatments are omitted, without extrapolation of the data.

4.1.2.5 Tolerance as Assessed by Multivariate Analysis

The two methods of data analysis, multivariate analysis and Σ%V may be useful in conjunction with each other. The latter quantifies the effect iron has on each species allowing them to be ranked in tolerance sequence while the former graphically describes the response of a group of species showing similar behaviour.

For variables which do not show a linear response to iron concentration e.g. SR and SLVS, multivariate analysis is invaluable in assessing how the species are responding.

4.1.3 Summary of Choice of an Index of Iron Tolerance

The index Σ%V was adopted as the main method of ranking tolerance. Multivariate analysis was used in conjunction with the ranking method, to help visualise the species response to iron, and to group species which had a similar response.

These methods of data analysis were performed on relative growth rate (RGR) data as, for a number of reasons outlined earlier, this variable seemed the most suitable indicator of tolerance to iron.

4.2 IRON TOLERANCE OF WETLAND PLANTS

4.2.1 Histograms of Standardised RGR Response (%RGR)

Histograms of mean value of standardised relative growth rate response (%RGR) against supplied iron concentration are presented in Figure 4.2 for each species.

4.2.2 Omission of Treatments

For those species which were not grown in all treatments, it was not possible to calculate Σ%RGR or to enter the species into multivariate analysis. However, in order to quantify the response of these four species, values of %RGR for the omitted treatments were extrapolated by eye from curves drawn along the histograms, and used for the two methods of data analysis (Table 4.7).

Table 4.7 Missing Values of %RGR Extrapolated from Histograms of %RGR on supplied Iron Concentration
(for those Species not grown in all 6 Treatments)

Treatment (mg Fe l^{-1})	3.8	10	25	50	75	100	Σ%RGR Tolerance Index
Species							
Agrostis stolonifera	100	87.29	77.66	70.45	**59**	49.48	343.88
Carex echinata	100	101.42	100.00	99.05	**85**	71.56	457.03
Carex pulicaris	100	**92**	76.71	78.08	**67**	63.7	377.49
Lythrum salicaria	100	**83**	57.99	45.49	**39**	36.11	261.59

Measured values of mean %RGR
Extrapolated values of %RGR

The extrapolated values for the 75 mg Fe l^{-1} treatment are likely to be the more accurate since at this concentration the slope of the response curve is shallow. This is also true for Carex pulicaris in the 10 mg Fe l^{-1} treatment. The value least likely to be accurate is that of Lythrum salicaria at 10 mg Fe l^{-1}, since extrapolation was made on the steepest

Figure 4.2 Histograms of Mean value (± 1 SE) of Relative Growth Rate standardised against Mean control value (y), against Supplied Iron Concentration (x). (Species are presented in order of decreasing tolerance to iron (Σ%RGR), and monocotyledons are indicated by bold type)

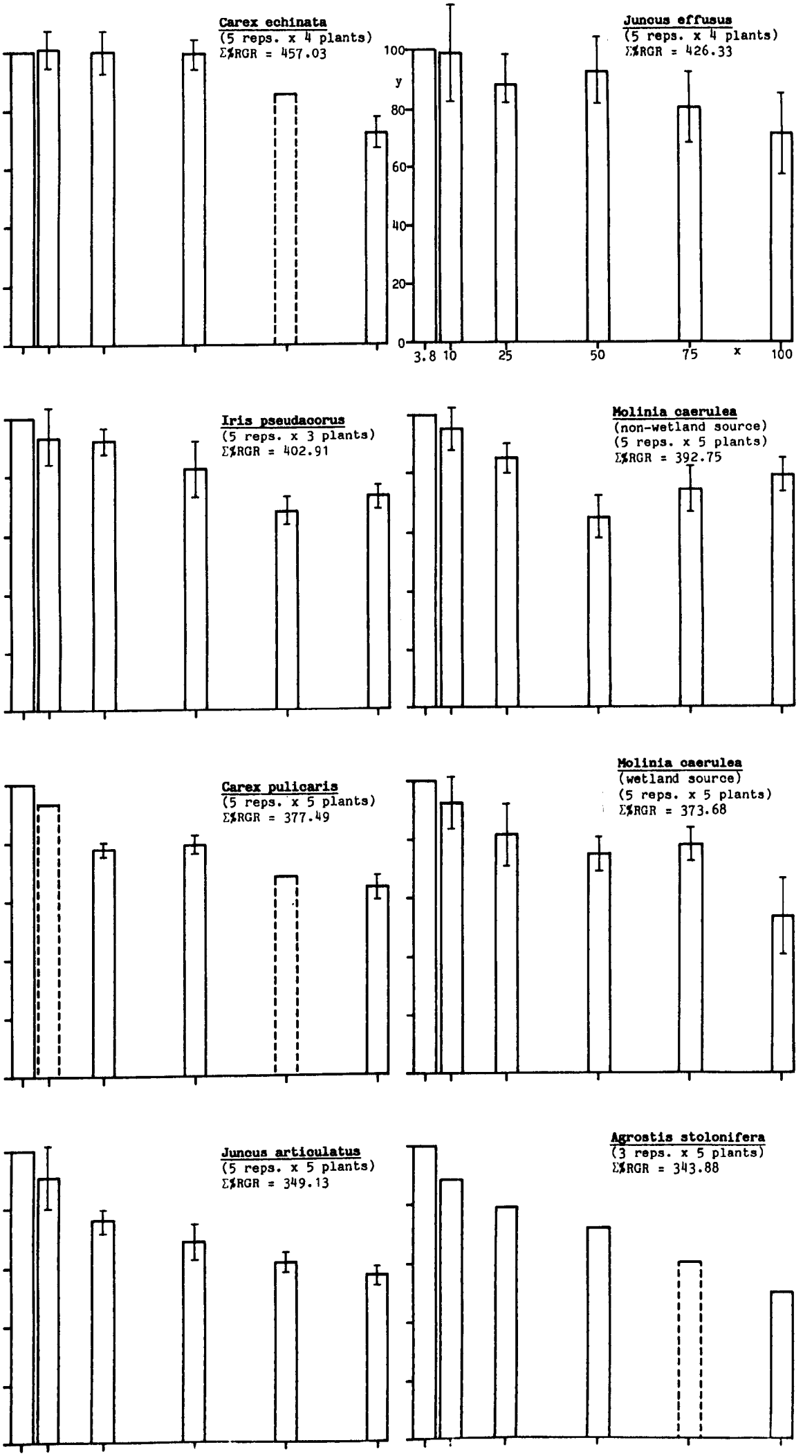


Figure 4.2 continued

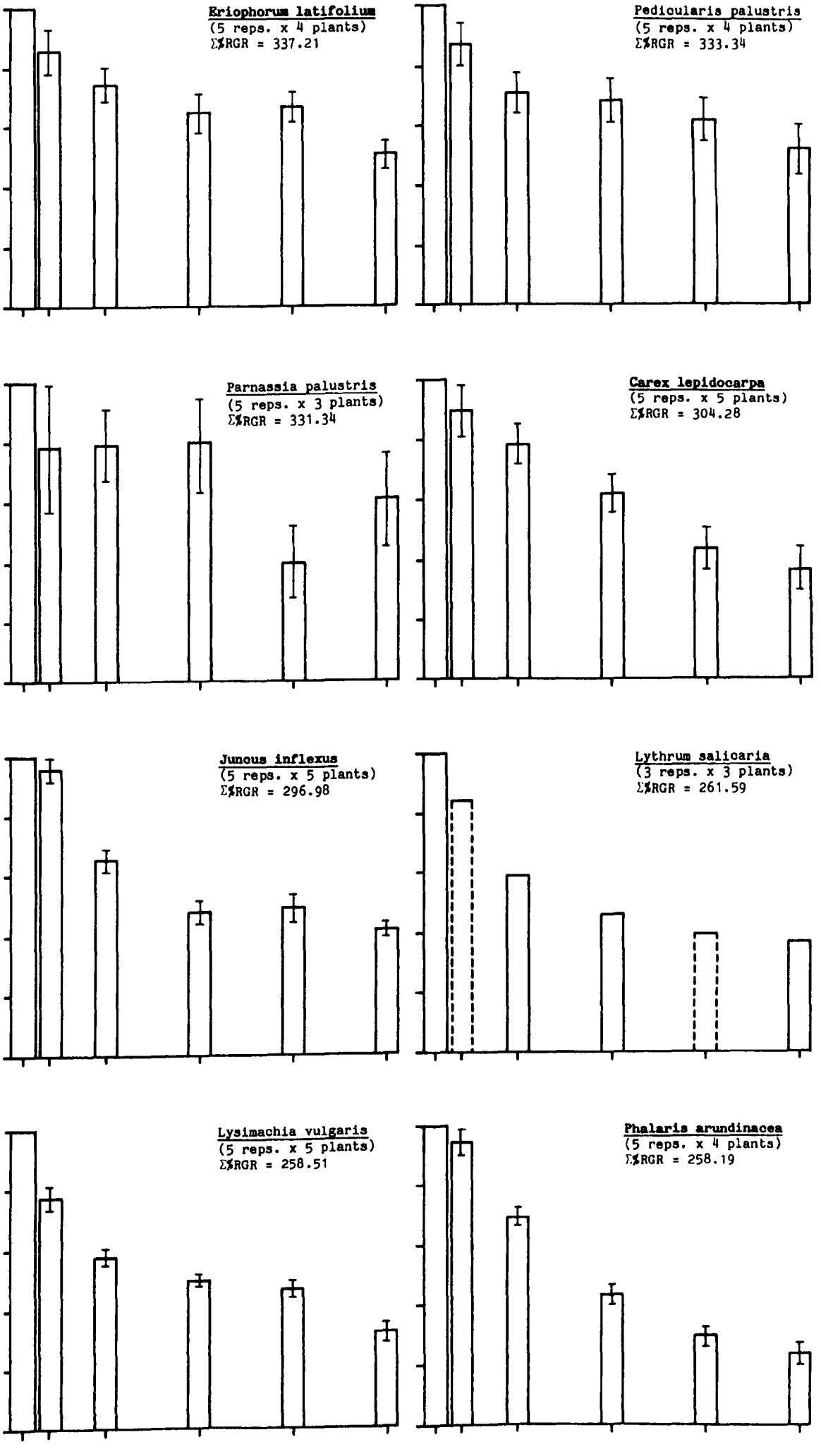
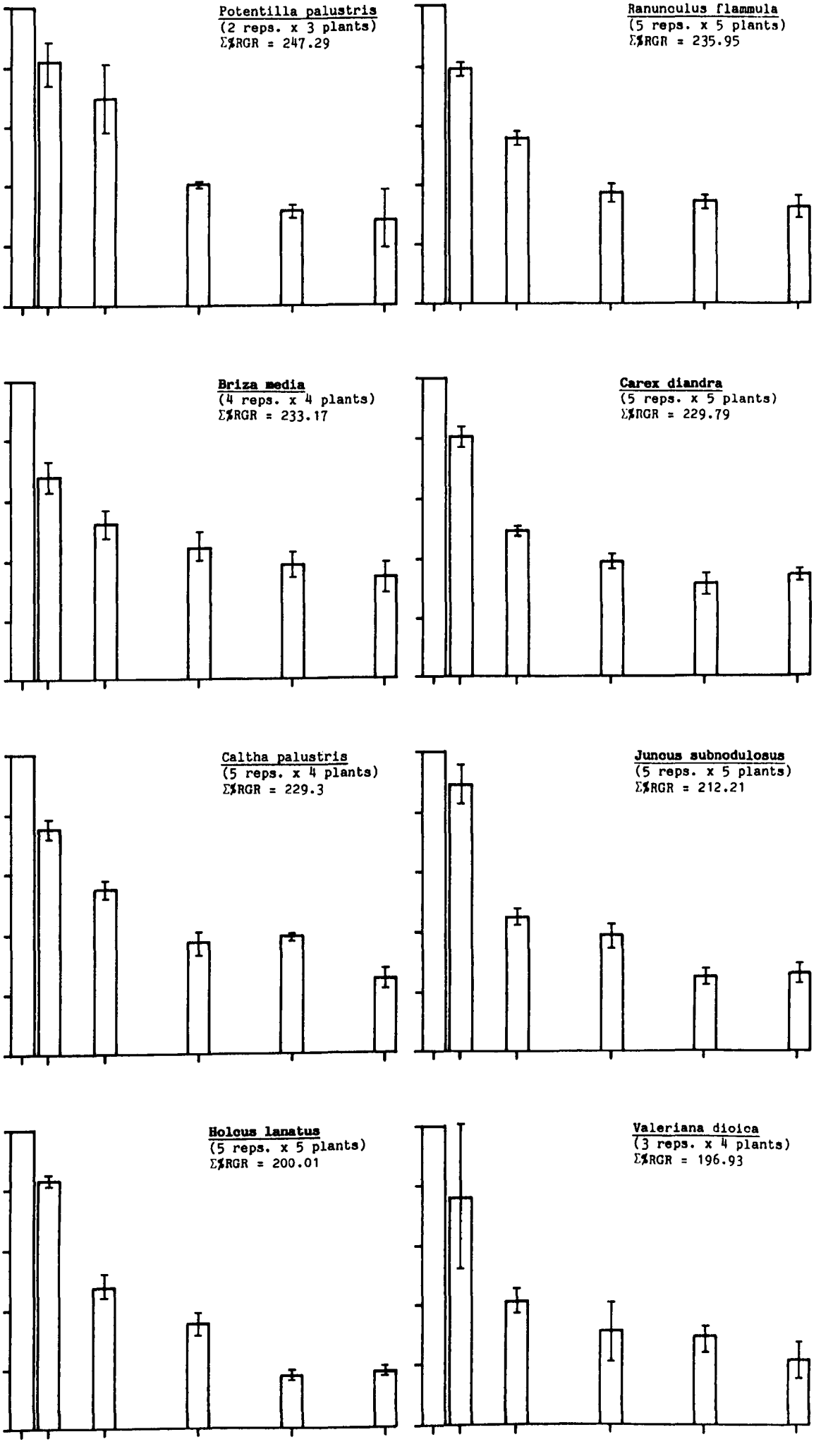
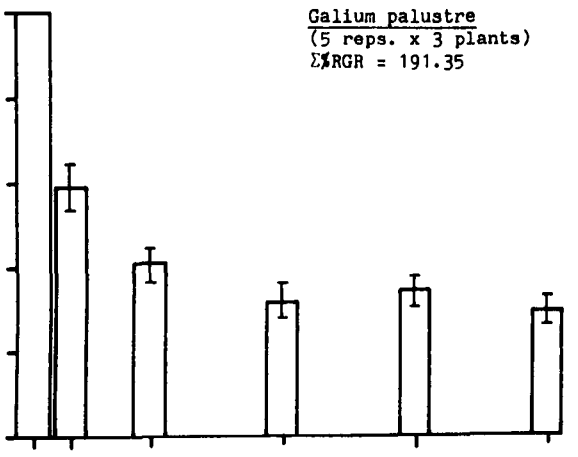
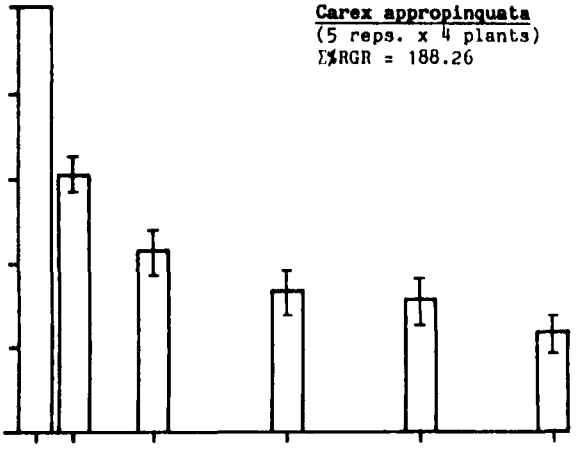
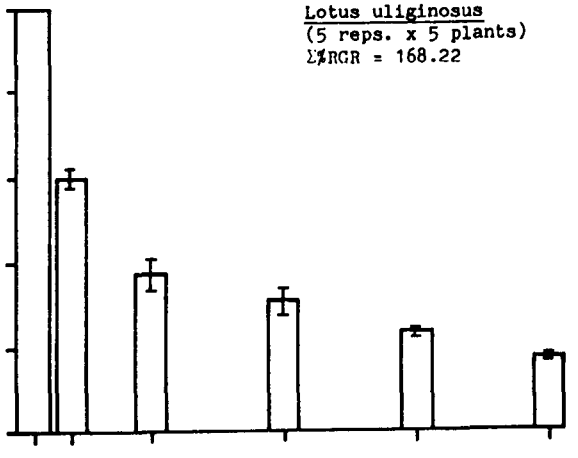


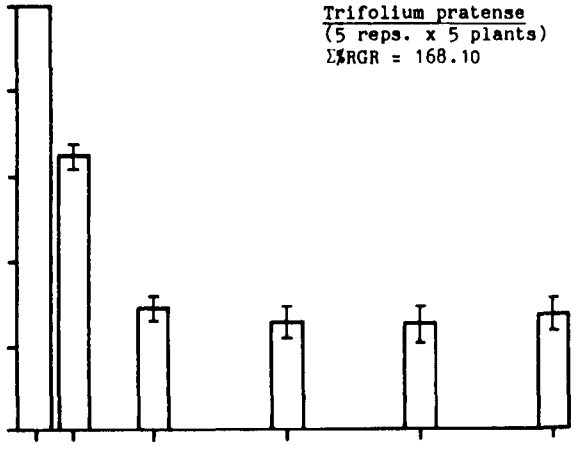
Figure 4.2 continued

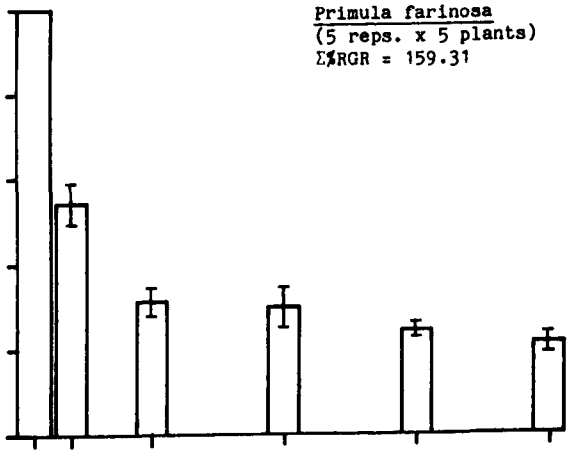


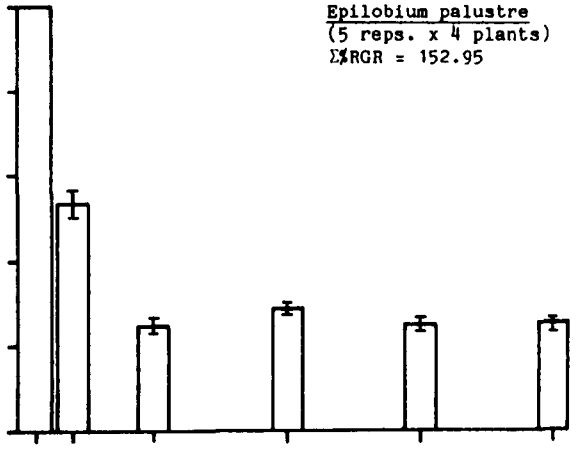

Galium palustre
(5 reps. x 3 plants)
ΣRGR = 191.35

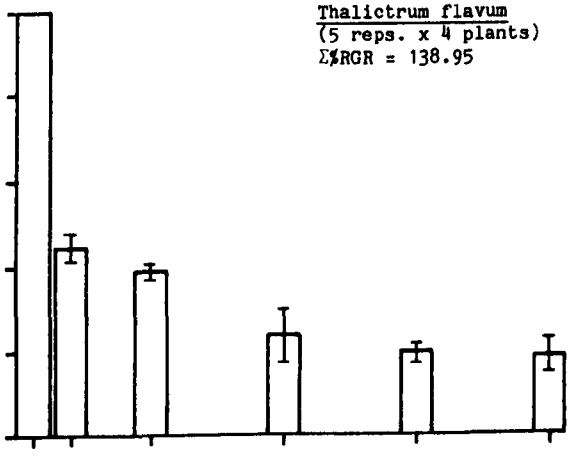

Carex appropinquata
(5 reps. x 4 plants)
ΣRGR = 188.26


Lotus uliginosus
(5 reps. x 5 plants)
ΣRGR = 168.22


Trifolium pratense
(5 reps. x 5 plants)
ΣRGR = 168.10


Primula farinosa
(5 reps. x 5 plants)
ΣRGR = 159.31


Epilobium palustre
(5 reps. x 4 plants)
ΣRGR = 152.95


Thalictrum flavum
(5 reps. x 4 plants)
ΣRGR = 138.95

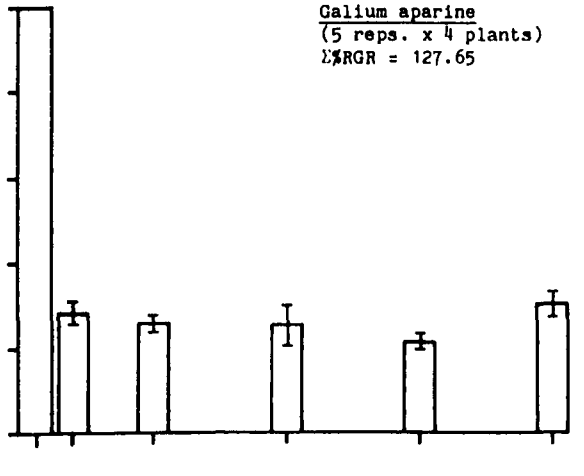
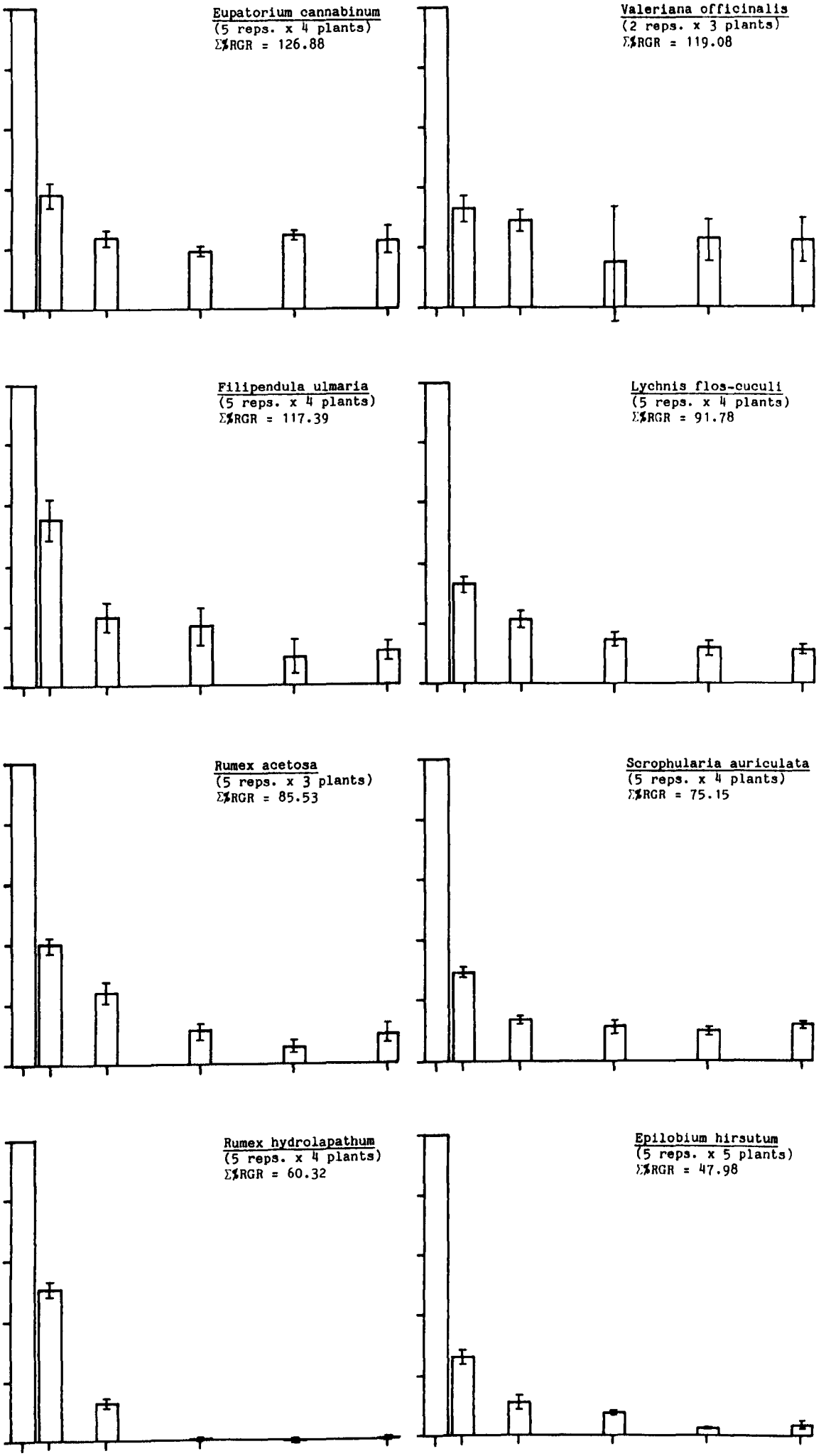

Galium aparine
(5 reps. x 4 plants)
ΣRGR = 127.65

Figure 4.2 continued



part of the response curve, of indeterminate shape at this point.

Inputting the above extrapolated data into multivariate analysis did not affect the grouping of the other species. When ranking species on Σ%RGR, these four species occurred very close to where they had been when ranked on ED_{50}. Since there had been close correlation ($p < 0.001$) between these two methods of ranking when the other 36 species were considered, it is likely that the above extrapolated data were acceptable estimates. However, it should be borne in mind that extrapolated data were used when considering the results from these four species.

4.2.3 Tolerance "League Tables"

Figures 4.3a, b and c show the species ranked by Σ%RGR in decreasing tolerance to iron. In Figure 4.3a, the tolerance index was calculated for the whole range over which the plants were tested, i.e. up to 100 mg Fe l^{-1} (Σ%RGR100). Figures 4.3b and c are the result of summing the standardised response of the species to 50 and 25 mg Fe l^{-1} only (Σ%RGR50 and Σ%RGR25 respectively). It is thought that the ceiling values of 50 mg Fe l^{-1} and, in particular 25 mg Fe l^{-1} might relate more closely to iron concentrations normally found in solution in a base-rich fen, though much higher concentrations have been reported for acid sites (Section 7.1.3).

4.2.4 General Trends in the "League Tables" (Figures 4.3a, b and c)

All three "league tables" showed that most monocotyledons were more tolerant of iron than were most dicotyledons, though there was some overlap in the ranges (Table 4.8). In all three cases, the mean tolerance index for dicotyledons as a whole was significantly less than for monocotyledons as a whole ($p < 0.001$). This trend had already been apparent when considering the response of the various parts of the plants (Sections 3.2.4.2 to 3.2.4.18).

The lower the maximum iron concentration considered, the more obvious was the monocotyledon/dicotyledon split i.e. there was less overlap in the ranges, and greater significant difference between mean values of Σ%RGR. This is because monocotyledons generally suffer very little growth reduction (relative to the control) at lower iron concentrations, while dicotyledons showed more, and in many cases most, of their growth reduction over this range.

Figure 4.3a Iron Tolerance "League Table"
Species are ranked by $\Sigma\%RGR_{100}$ in order of decreasing tolerance to iron.
(Monocotyledons are indicated by bold type and shading)

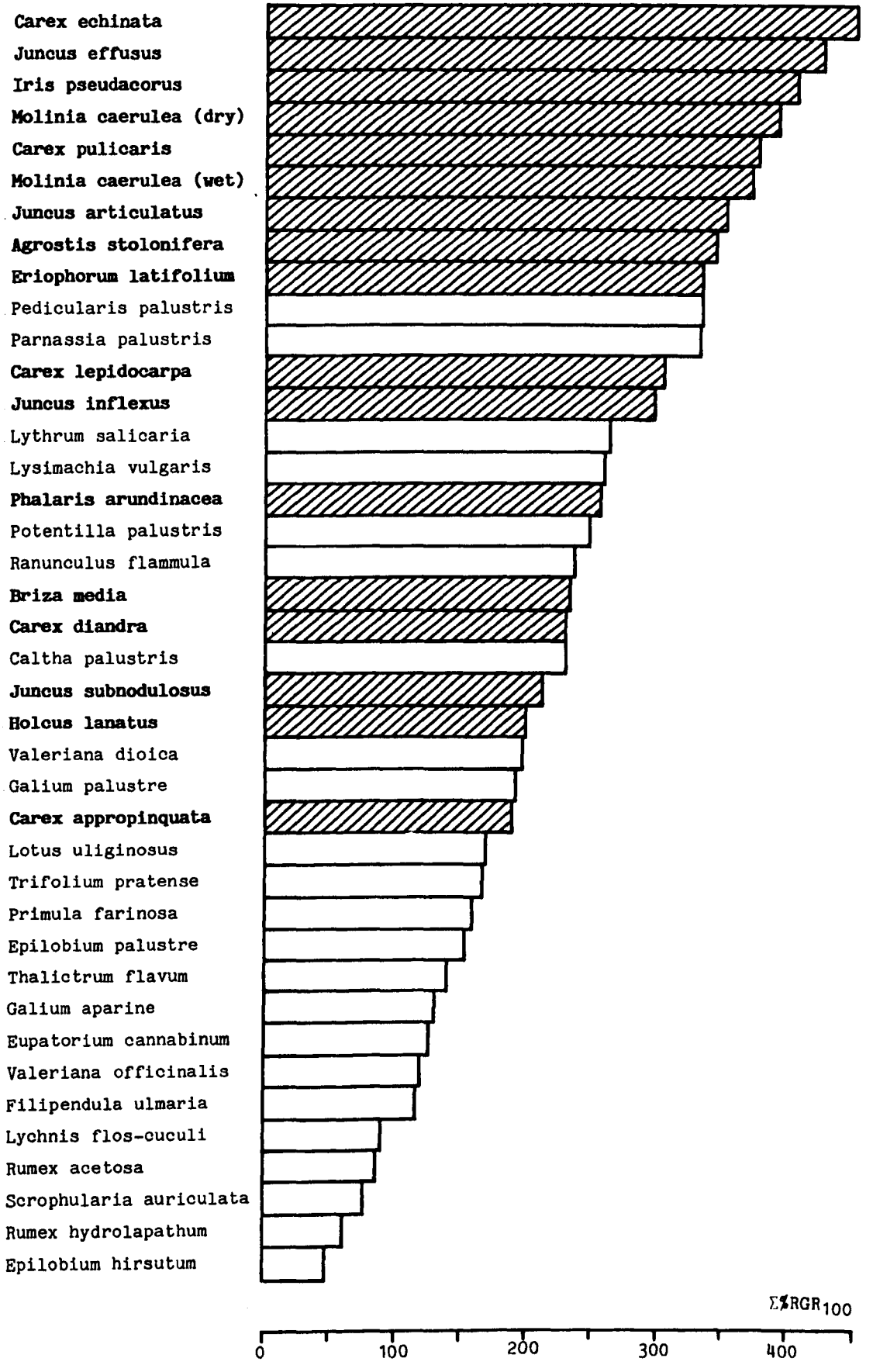


Figure 4.3b Iron Tolerance "League Table"
Species are ranked by $\Sigma\%RGR_{50}$ in order of decreasing tolerance to iron.
(Monocotyledons are indicated by bold type and shading)

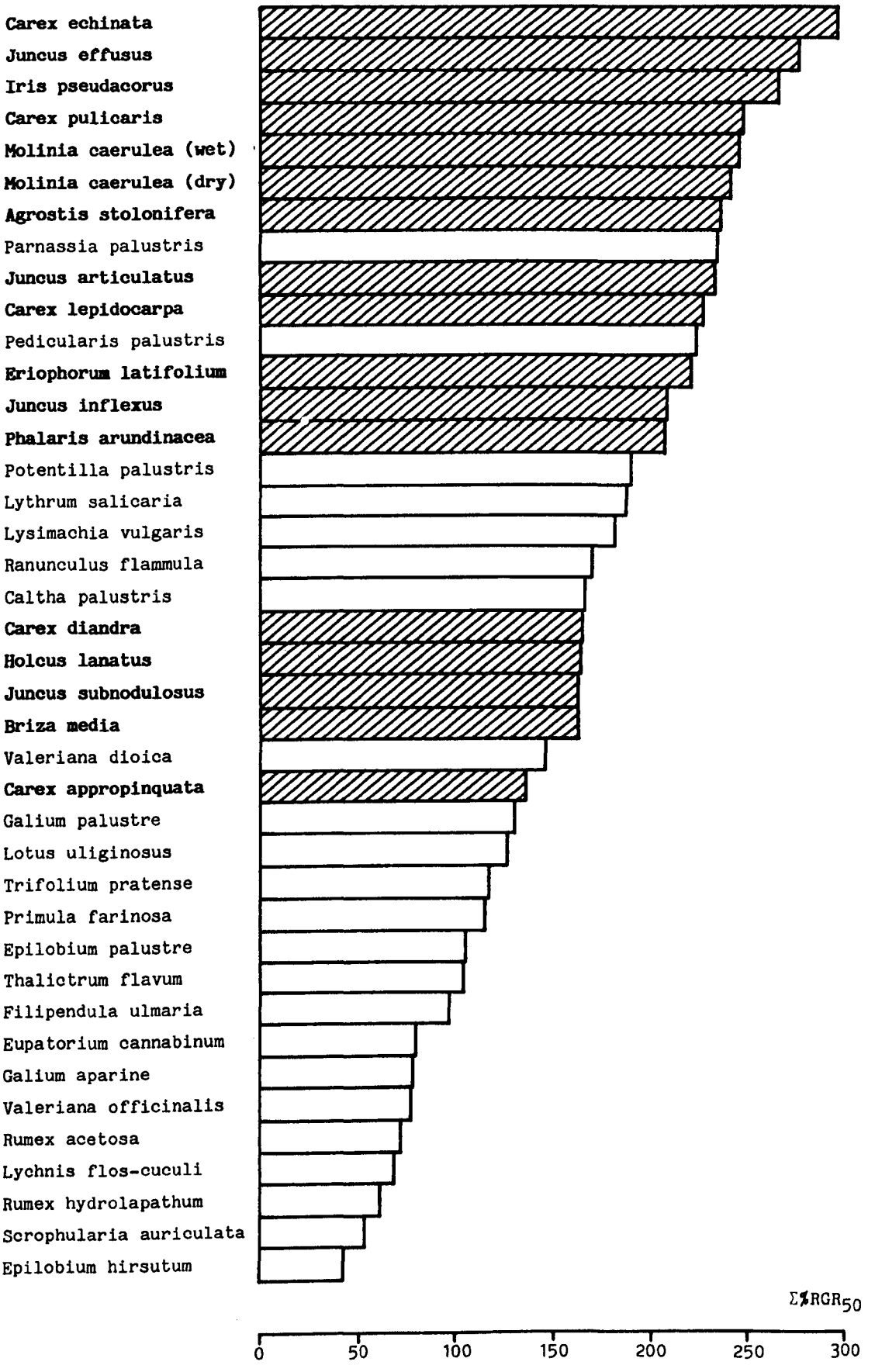
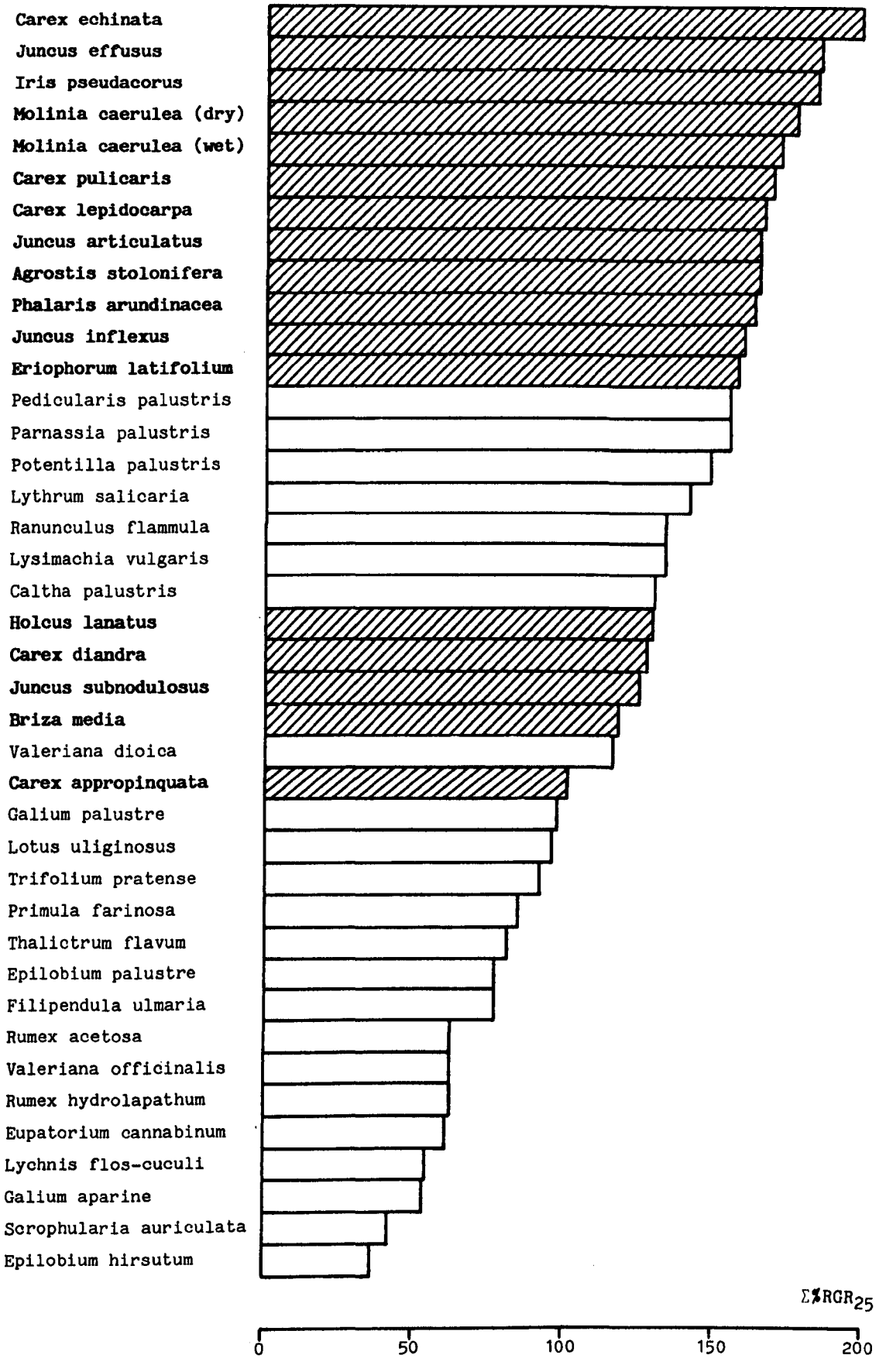


Figure 4.3c Iron Tolerance "League Table"
Species are ranked by $\Sigma\%RGR_{25}$ in order of decreasing tolerance to iron.
(Monocotyledons are indicated by bold type and shading)



The precise sequence of the species differed slightly between the tables though there was significant correlation ($p < 0.005$) between all three tolerance rankings. Hereafter, Σ%RGR refers to the Σ%RGR100 index which was adopted as the main method of tolerance ranking, since it considered a plant's response over the full range of iron concentrations used.

Table 4.8 Minimum, Maximum and Mean Values of the 3 RGR Iron Tolerance Indices (Σ%RGR100, Σ%RGR50 and Σ%RGR25) for Monocotyledons and Dicotyledons

Tolerance Index	Monocots/ Dicots	Minimum Σ%RGR	Maximum Σ%RGR	Mean Σ%RGR	Standard Error of Mean
Σ%RGR100	MONOCOTS	188.26	452.00	316.34	±20.32
	DICOTS	47.98	333.34	170.67	±16.84
Σ%RGR50	MONOCOTS	135.20	300.47	217.16	±11.23
	DICOTS	42.60	232.83	122.50	±11.46
Σ%RGR25	MONOCOTS	102.34	201.42	156.76	± 6.58
	DICOTS	36.03	155.56	93.32	± 7.92

4.2.5 Relationship Between Iron Tolerance (Σ%RGR) and Relative Growth Rate (RGR)

Some of the more tolerant species (e.g. Parnassia palustris, Pedicularis palustris, Juncus effusus, Eriophorum latifolium, Molinia caerulea, and Iris pseudacorus) also had low relative growth rates under control conditions. Similarly, some of the least tolerant species (Epilobium hirsutum, Scrophularia auriculata, Epilobium palustre, Rumex acetosa) had high relative growth rates (Table 3.12). Holcus lanatus had the highest relative growth rate of all species and was among the least tolerant of the monocotyledons. The relationship between tolerance and relative growth rate was examined using the Spearman's rank correlation test. There was a significant negative correlation ($p < 0.05$) between tolerance

ranking based on both Σ%RGR100 and Σ%RGR50, and ranking based on relative growth rate in the control. The relationship was non-significant with tolerance based on Σ%RGR25.

For each "league table" the relationship was further investigated for monocotyledons only, for dicotyledons only, and for both together. The tolerance indices Σ%RGR100, Σ%RGR50, and Σ%RGR25 were regressed on RGR (Table 4.9).

For each of the three tolerance indices there was no significant relationship between tolerance and RGR when monocotyledons and dicotyledons were considered separately. However, for all species together, (in all 3 cases) there was a significant negative relationship ($p < 0.01$), though less than 25% of the variation in tolerance could be explained by RGR. The strength of the relationship was Σ%RGR100 > Σ%RGR50 > Σ%RGR25, ($r = 0.50$, 0.45, and 0.42 respectively).

Table 4.9 Regression Equations Relating Tolerance Indices (y) to Relative Growth Rate (x)

Tolerance Index	Group	Regression Equation	Significance	% fit ($100r^2$)
	ALL SPECIES	y=327.9-376.1x	p < 0.01	24.51
Σ%RGR100	MONOCOTS	y=374.9-283.6x	NS	23.10
	DICOTS	y=236.8-229.4x	NS	15.24
	ALL SPECIES	y=218.2-219.0x	p < 0.01	20.50
Σ%RGR50	MONOCOTS	y=242.6-123.9x	NS	14.65
	DICOTS	y=164.9-147.0x	NS	13.48
	ALL SPECIES	y=154.0-132.9x	p < 0.01	17.19
Σ%RGR25	MONOCOTS	y=168.7-58.8x	NS	9.60
	DICOTS	y=119.6-90.7x	NS	10.78

4.2.6 **Multivariate Analysis**

4.2.6.1 Principal Components Analysis (PCA) based on %RGR Data

Figure 4.4 is a 3-dimensional plot of PCA axes 1, 2 and 3 based on standardised RGR data (%RGR). Four distinct clusters can be seen and are presented as four different colours.

91% of the variance in the data set was accounted for by axis 1; axes 2 and 3 accounted for only 5.9 and 1.4% respectively. Axis one species scores correlated strongly with their tolerance indices (Σ%RGR) ($r=0.9999$ at $p < 0.001$) suggesting that axis 1 may be an axis of iron tolerance. There was no significant relationship between tolerance and axis 2 or 3.

4.2.6.2. Ward's Method of Hierarchic Fusion

The dendrogram obtained from Ward's method of hierarchic fusion on standardised RGR data (%RGR) is presented in Figure 4.5. Inflection in a plot of number of clusters against dissimilarity coefficient occurred at the four cluster stage suggesting this is an appropriate termination of fusion.

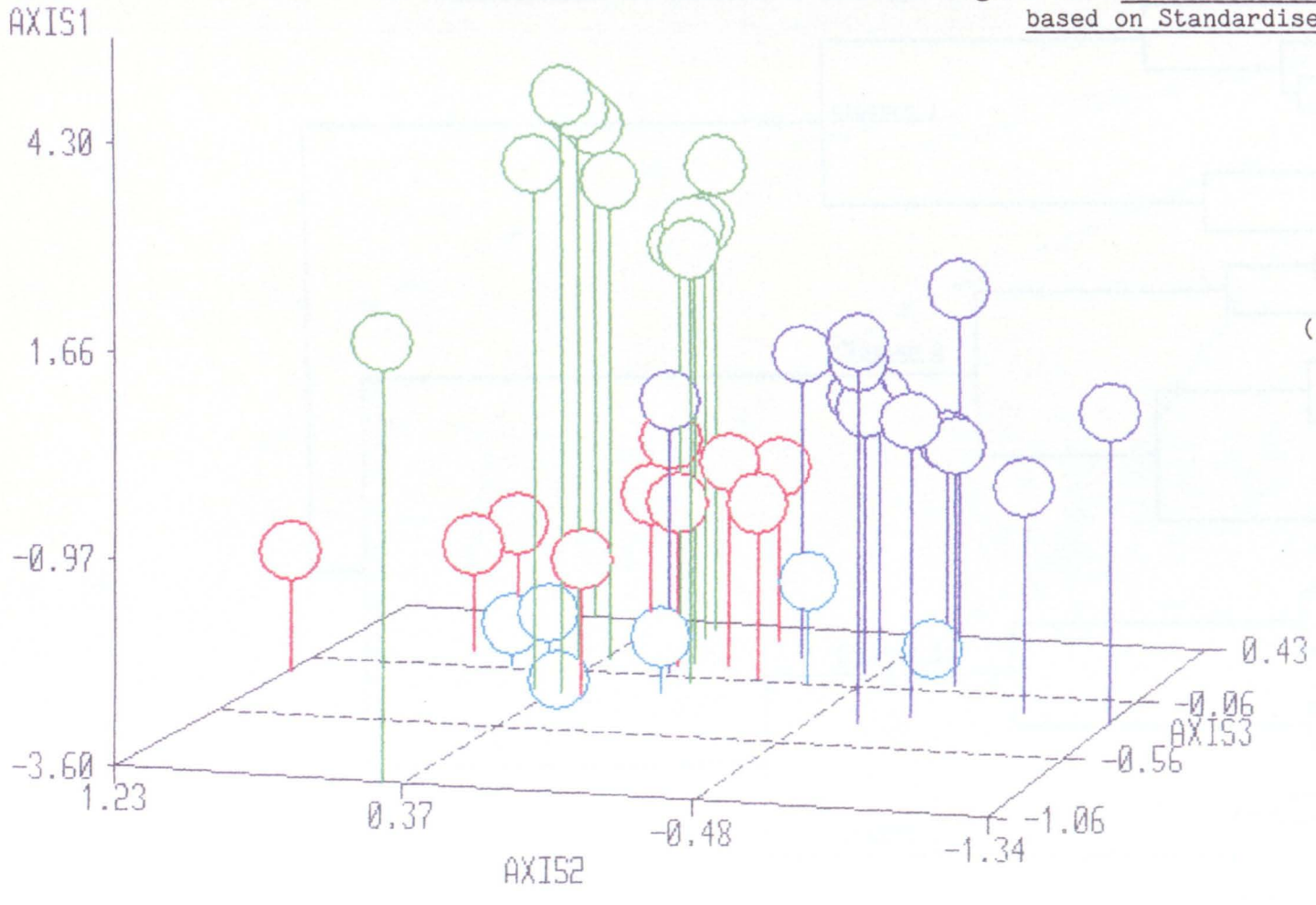
Figure 4.6 shows the mean response of standardised RGR to iron for each cluster. The 11 species showing least growth reduction with increasing iron concentration (Cluster 1) are the most tolerant of iron, while species in clusters 2 and 3 are progressively less tolerant, and the 6 species in cluster 4 are the most sensitive. In this cluster mean RGR of species is reduced by an average of 60% between the control and 10 mg Fe l^{-1} treatments. There is greatest differential in response between the four groups of species at these low iron concentrations.

This clustering of species agreed exactly with Σ%RGR tolerance ranking and, if the dendrogram was manipulated to obtain as close a ranking as possible to that of Σ%RGR, a Spearman's rank correlation coefficient of 0.81 ($p < 0.001$) was obtained. The species in each cluster are presented in Σ%RGR tolerance order in Table 4.10.

4.2.7 **Tolerance in Relation to Ecological Factors**

Table 4.10 shows the 40 species ranked in decreasing order of tolerance (Σ%RGR) and grouped into the 4 clusters obtained by multivariate

Figure 4.4 Plot of Principal Components Analysis Axes 1, 2, and 3 based on Standardised Relative Growth Rate Data (%RGR).



Key to Clusters
(in order of decreasing iron tolerance)

Green	Cluster 1
Blue	Cluster 2
Red	Cluster 3
Cyan	Cluster 4

Figure 4.4

Figure 4.5 Dendrogram of Ward's Method of Hierarchic Fusion on Standardised Relative Growth Rate Data (%RGR)
(Monocotyledons are indicated by bold type)

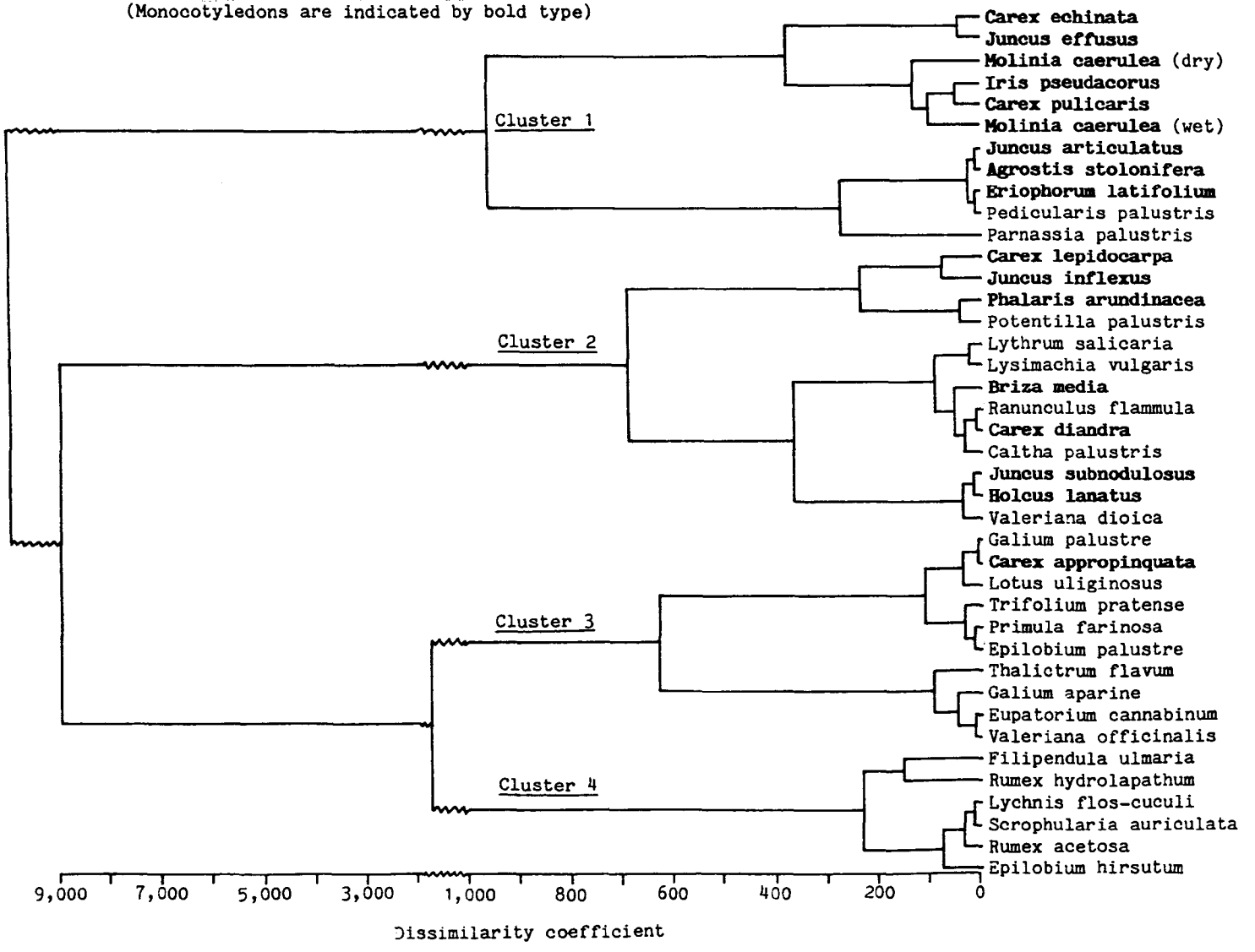


Figure 4.5

Figure 4.6 Cluster Diagnostics (Ward's Analysis) of Mean Relative Growth Rate (standardised against control mean RGR) with Treatment (4 cluster stage)

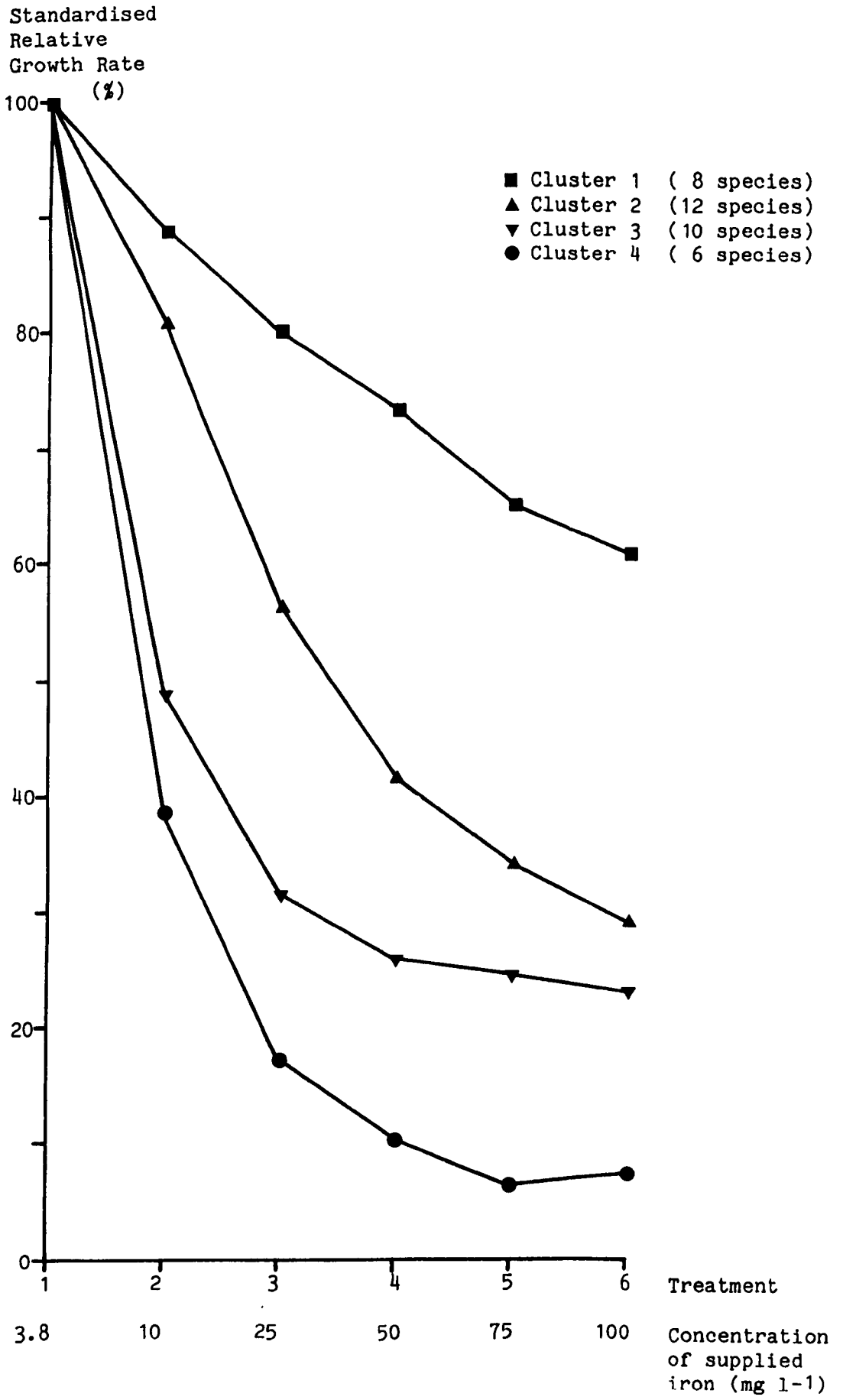


Table 4.10 Clustering of Species based on Standardised Relative Growth Rate Data (%RGR) (i.e. Results of Multivariate Classification)

(Species are presented in Σ%RGR tolerance order, and monocotyledons are indicated by bold type)

Carex echinata	Cluster 1
Juncus effusus	
Iris pseudacorus	'VERY TOLERANT'
Molinia caerulea (dry)	
Carex pulicaris	Mean value of Σ%RGR
Molinia caerulea (wet)	= 374.56 ± 12.20
Juncus articulatus	
Agrostis stolonifera	
Eriophorum latifolium	
Pedicularis palustris	
Parnassia palustris	
Carex lepidocarpa	Cluster 2
Juncus inflexus	
Lythrum salicaria	'SEMI-TOLERANT'
Lysimachia vulgaris	
Phalaris arundinacea	Mean value of Σ%RGR
Potentilla palustris	= 243.40 ± 9.10
Ranunculus flammula	
Briza media	
Carex diandra	
Caltha palustris	
Juncus subnodulosus	
Holcus lanatus	
Valeriana dioica	
Galium palustre	Cluster 3
Carex appropinquata	
Lotus uliginosus	'MODERATELY SENSITIVE'
Trifolium pratense	
Primula farinosa	Mean value of Σ%RGR
Epilobium palustre	= 154.08 ± 8.08
Thalictrum flavum	
Galium aparine	
Eupatorium cannabinum	
Valeriana officinalis	
Filipendula ulmaria	Cluster 4
Lychnis flos-cuculi	
Rumex acetosa	'VERY SENSITIVE'
Scrophularia auriculata	
Rumex hydrolapathum	Mean value of Σ%RGR
Epilobium hirsutum	= 79.69 ± 10.00

classification techniques.

Species in cluster 1 (the most tolerant of iron) are almost exclusively monocotyledons with the exception of Pedicularis palustris and Parnassia palustris which are both very slow-growing dicotyledons. Cluster 2 contains both monocotyledonous and dicotyledonous species. The less tolerant species in cluster 3 are mainly dicotyledons, the only exception being Carex appropinquata. Cluster 4, the species most sensitive to iron, are exclusively dicotyledons.

Species which are found in dry situations as well as wetland ones are found in all 4 clusters, e.g. Molinia caerulea, Agrostis stolonifera, Briza media, Holcus lanatus, Trifolium pratense, Galium aparine, Valeriana officinalis, Rumex acetosa, (and Carex pulicaris and Parnassia palustris (Clapham 1969)). The wetland and dryland sources of Molinia caerulea had a very similar tolerance to iron and are found in the same cluster.

The relationship between iron tolerance and fertility of sites at which each species occurred was investigated using the same data as in Section 3.2.4.7.1. There was significant negative correlation ($r = -0.42$, $p < 0.01$) between iron tolerance and site fertility, i.e. those species which normally occur in fertile sites are less tolerant of iron than species normally occurring in nutrient-poor sites. Similarly the RGR (Fe = 3.8 mg l^{-1}) of the species measured in this study correlated significantly ($r = 0.36$, $p < 0.05$) with mean site fertility, i.e. those species with the fastest relative growth rates generally grow on the more fertile sites.

4.2.8 **Tolerance in Relation to Response of Shoot/Root Ratio to Iron**

Table 4.11 shows the species presented in order of tolerance to iron. The numbers refer to clusters 1-4 corresponding to the 4 types of response of shoot/root ratio (SR) revealed by multivariate classification (Section 3.2.4.8.2).

In those species having type 1 response, SR ratio remained mostly constant over the range of iron concentrations. Generally, species with this response are very tolerant of iron (mostly monocotyledons) with neither shoot or root appreciably affected, or very sensitive, i.e. both shoot and root are severely affected (e.g. Epilobium hirsutum, Lychnis flos-cuculi, and Valeriana officinalis).

Species with type 2 response showed a marked reduction in SR at low

Table 4.11 Trends in Shoot/Root Dry Weight Ratio with Iron Tolerance (i.e. Results of Multivariate Classification)
(Species are presented in Σ%RGR Tolerance order, and Monocotyledons are indicated by bold type)

	Response Type
Juncus effusus	1
Iris pseudacorus	1
Molinia caerulea (dry)	1
Molinia caerulea (wet)	1
Juncus articulatus	2
Eriophorum latifolium	1
Pedicularis palustris	1
Parnassia palustris	2
Carex lepidocarpa	2
Juncus inflexus	2
Lysimachia vulgaris	4
Phalaris arundinacea	2
Potentilla palustris	4
Ranunculus flammula	4
Briza media	4
Carex diandra	1
Caltha palustris	3
Juncus subnodulosus	1
Holcus lanatus	2
Valeriana dioica	3
Galium palustre	4
Carex appropinquata	1
Lotus uliginosus	4
Trifolium pratense	4
Primula farinosa	3
Epilobium palustre	4
Thalictrum flavum	4
Galium aparine	3
Eupatorium cannabinum	4
Valeriana officinalis	1
Filipendula ulmaria	3
Lychnis flos-cuculi	1
Rumex acetosa	2
Scrophularia auriculata	4
Rumex hydrolapathum	2
Epilobium hirsutum	1

In species with type 1 response, shoot/root ratio (SR) remained constant or decreased slightly with increasing iron concentration. In those with type 2 response, SR fell markedly between the control and 10 mg Fe l^{-1} treatments, and then remained constant with increasing iron supply. In species with types 3 and 4 response, SR increased with increasing iron concentration; the increase was greater in species with type 4 response (see Table 3.16).

iron concentrations (i.e. shoots were most affected); thereafter the ratio remained constant or rose slightly (i.e. roots were then equally affected in the higher iron concentrations). These were also largely monocotyledons but Rumex acetosa and Rumex hydrolapathum, again very sensitive species, responded in this way.

Apart from Briza media, all species with types 3 and 4 response were dicotyledons (Section 3.2.4.8.2). In these species roots were affected by iron more severely than shoots (i.e. SR increased with increasing iron concentration), though in those with type 4 response the effect was more marked.

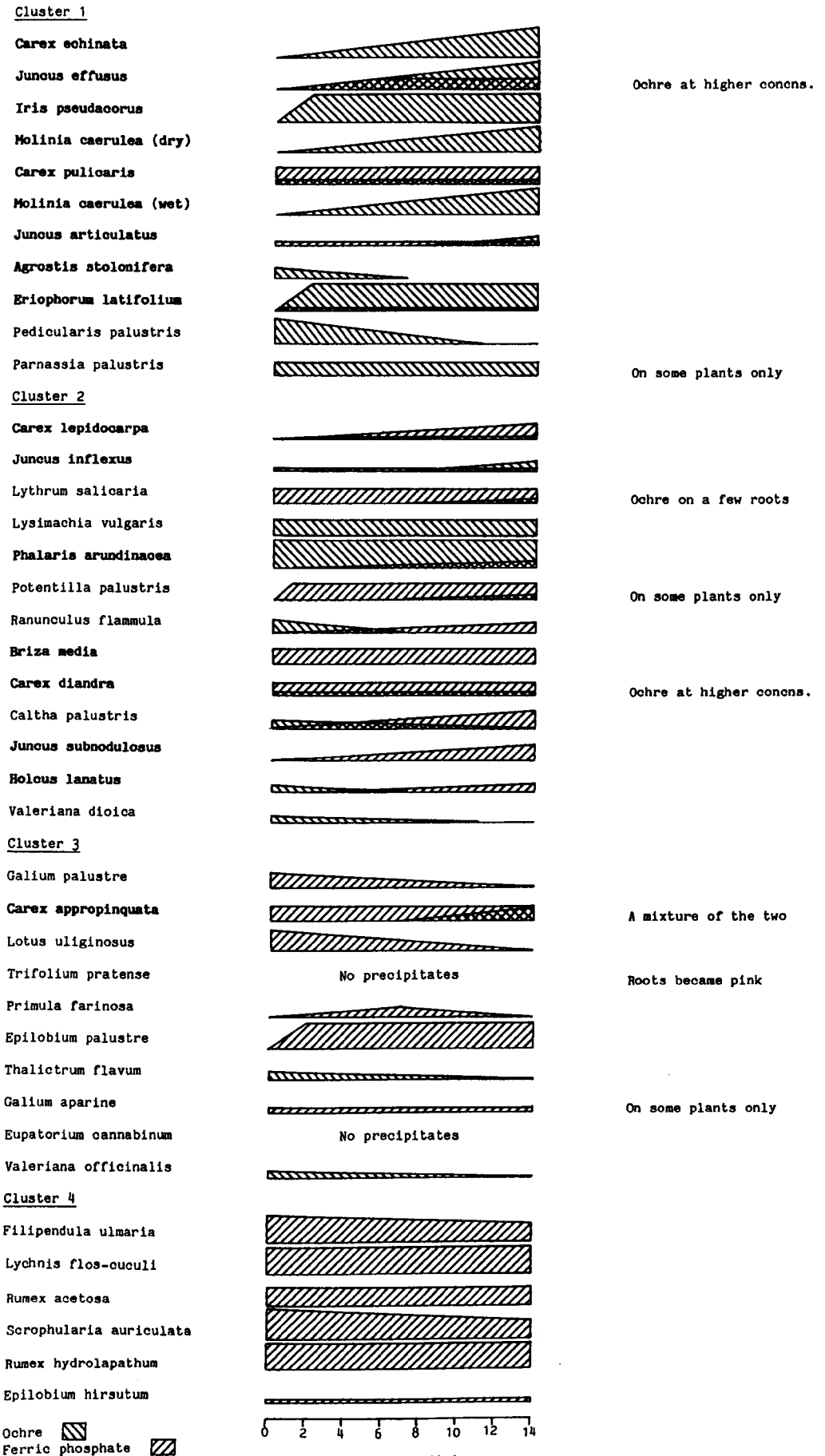
To summarise, in most dicotyledonous species, roots were affected by iron more severely than were shoots. Those dicotyledons which did not respond in this way were either very tolerant of iron (e.g. Pedicularis palustris and Parnassia palustris, type 1 and 2 response respectively), or very sensitive to iron, (e.g. Rumex acetosa and Rumex hydrolapathum (type 2 response), Epilobium hirsutum, Valeriana officinalis and Lychnis flos-cuculi (type 1 response).

When clustering based on iron tolerance (Σ%RGR) was compared with clustering based on SR trends, correlation was significant ($r_s = 0.34$, $p < 0.05$). Thus, in the species more sensitive to iron, roots are generally affected more than shoots, whereas shoots tend to be affected slightly more than roots in species more tolerant of iron. However, at the tolerant end of the ranking, SR may only appear to stay constant or fall slightly since many of these species produce heavy deposits of ochre on their roots. This would increase the apparent weight of the root (possibly even masking a fall in actual root weight) and thus make it appear that shoots are affected more than roots (see Section 4.1.1.3).

4.2.9 **Tolerance in Relation to Size and Age of Plants**

It has been previously reported that younger seedlings of a given species may be more sensitive to toxic metals than older ones (Hodgson 1972; Tadano 1975; Foy Chaney and White 1978; Wheeler, Al-Farraj and Cook 1985). As seedlings of the different species used in this study were of different age or size, this could potentially affect their measured tolerance. However, no significant correlation was found between iron tolerance and seedling age or size (initial dry weight).

Figure 4.7 Nature of Root Precipitates in Relation to Iron Tolerance
(Species are presented in ΣRGR tolerance order and monocotyledons are indicated by bold type)



4.2.10 Iron Tolerance in Relation to Nature of Root Precipitates

Figure 4.7 shows the relationship between iron tolerance of a species (Σ%RGR) and the precipitates which were found on their roots. There was a strong tendency for the most tolerant species (cluster 1) to have ochre (i.e. ferric oxide/hydroxide) on their roots, frequently at high intensity. Occasionally a little of the pale yellow precipitate (i.e. ferric phosphate) was mixed in with it (e.g. Eriophorum latifolium). Species in cluster 2 also tend to produce ochre but less intensely and ferric phosphate frequently occurs on roots of species in this cluster.

In cluster 3, many species had ferric phosphate on their roots and ochre was rare, while the most sensitive species (cluster 4) all had ferric phosphate on their roots, usually in large quantities, without any sign of ochre.

Thus the precipitate found on the roots of plants given high concentrations of ferrous iron in solution culture correlates strongly with their relative tolerance to iron. Species which produced ochre on their roots tended to be the most tolerant.

4.2.11 Discussion

4.2.11.1 Comparison with Results of Previous Studies

As there have been only few previous studies on iron toxicity to wetland plants, few comparisons can be made. However, results from this study agree with those of Al-Farraj (1983) and Wheeler, Al-Farraj and Cook (1985) that Juncus subnodulosus is more tolerant of iron than is Epilobium hirsutum, though Juncus subnodulosus was by no means among the most tolerant species.

The marsh species Hodgson (1972) screened for iron tolerance were among the most sensitive in the present study (except Agrostis stolonifera). It is difficult to say whether there is agreement between results in view of this. Hodgson consistently found Rumex acetosa and Rumex hydrolapathum among the more tolerant species whereas in the present study they were found to be very sensitive. A possible reason for this discrepancy is discussed later (Section 5.3.5). Use of different criteria of tolerance may also be important.

Only one species was common to both Smirnoff's (1981) study and the

present investigation. There is agreement that Ranunculus flammula is among the middle orders of tolerance.

4.2.11.2 Association between Iron Tolerance in Wetland Plants and the ability to Oxidise Iron on the Roots

Results of this study on wetland plants confirm the previous findings of many workers that iron tolerance is associated with the ability to oxidise iron on the roots. This is further supported by comparing tolerance with root porosity values presented by Justin and Armstrong (1987). Of the 91 species they studied, 13 were also tested here for iron tolerance. For these species, there was a significant correlation ($r = 0.53$, $p < 0.05$) between tolerance (Σ%RGR) and root porosity under both drained and flooded conditions. This agrees with findings of Martin (1968) that tolerance to iron and ability to inhabit wet soils are linked with the extent of inter-cellular spaces. However, not all workers have observed a link between iron tolerance and root oxidising power. Ando et al. (1983) report that Jayawardena et al. (1977) could find no correlation between root oxidising power (α-naphthylamine oxidation) and resistance to iron toxicity in rice. Smirnoff (1981) also found no correlation between root porosity and iron tolerance of a range of wetland species, though he did find a significant relationship between porosity and the ability to exclude iron.

4.2.11.3 Relative Tolerance of Monocotyledons and Dicotyledons to Iron

The fact that most monocotyledons were more tolerant of iron than were most dicotyledons was apparent for all variables used to assess response of the species, and broadly agrees with the results of Smirnoff (1981). Since tolerance is associated with the ability to oxidise iron on the roots, monocotyledons may be better oxidisers than dicotyledons. In this study, 88% of monocotyledons produced some signs of ochre on the roots compared with 43% of dicotyledons.

Much of the early work on oxidising ability was presented as species lists in order of oxidising power (e.g. Doi 1952a; Fukui (1953) cited by Armstrong 1978; Bartlett 1961). In these three studies, monocotyledons were usually among the strongest oxidisers and were never among the poorest oxidisers. Martin (1968) showed that the oxidising ability of the

Table 4.12a Differences in Root Porosity between Monocotyledons and Dicotyledons under Drained and Flooded conditions
(t-tests on data from Justin and Armstrong 1987)

	Dryland	Intermediate	Wetland	All Types
Drained	NS	NS	***	***
Flooded	NS	NS	***	***

*** = $p < 0.001$
NS = No significant difference in root porosity of monocotyledons and dicotyledons

Table 4.12b Effect of Flooding on Root Porosity of Monocotyledon and Dicotyledon Species
(t-tests on data from Justin and Armstrong 1987)

	Dryland	Intermediate	Wetland	All Types
Monocotyledons	NS	NS	NS	NS
Dicotyledons	NS	NS	**	***

*** = $p < 0.001$
** = $p < 0.01$
NS = No significant difference in root porosity under drained and flooded conditions

monocotyledon Deschampsia cespitosa was much greater than that of the dicotyledon Mercurialis perennis and it was able to tolerate iron more effectively. This was linked with the extent of their intercellular spaces. Smirnoff (1981) noted that monocotyledons generally developed more air space in their roots than did dicotyledons. Olsen (1958) found that the monocotyledons Deschampsia flexuosa and Secale cereale (rye) were less susceptible to ferric iron toxicity than were the dicotyledons Cannabis sativa (hemp) and Sinapis alba (mustard).

Re-analysis of data presented by Justin and Armstrong (1987) on the root porosity of a wide range of dryland, wetland and intermediate species reveals that under drained conditions, wetland monocotyledons and monocotyledons as a whole inherently have a more porous root system than do wetland dicotyledons and dicotyledons in general, respectively ($p < 0.001$). However, among dryland and intermediate species (sensu Justin and Armstrong 1987), monocotyledons and dicotyledons did not differ in root porosity (partly owing to a high degree of variability among monocotyledon species) (Table 4.12a).

Flooding tended to increase root porosity in all types of plant, though the effect was not significant in any group of monocotyledon or in monocotyledons as a whole. Dicotyledons had a greater tendency for increased root porosity after flooding, but the effect was only significant in wetland dicotyledons ($p < 0.01$) and dicotyledons as a whole ($p < 0.001$) (Table 4.12b). Although dicotyledons were more able to increase root porosity upon flooding, monocotyledons as a whole still had greater root porosity than dicotyledons under such conditions ($p < 0.001$). The difference between flooded monocotyledons and dicotyledons was also highly significant for wetland species ($p < 0.001$) but non-significant for dryland and intermediate groups (Table 4.12a).

Closer inspection of Justin and Armstrong's (1987) species classification based on cortical and aerenchyma types of flooded roots, revealed that many of the monocotyledons in the study, and in particular the wetland ones, had mixed cortical cell packing, i.e. hexagonal non-radial packing in the outer cortex, with cubic and radial packing of cells in the inner cortex. The aerenchyma in these species had either been formed by lysigeny (i.e. "Graminean") as in all Juncus species, or they had been formed tangentially (i.e. "Cyperacean") as in all Carex and Eriophorum species studied (see also Smirnoff and Crawford 1983). It must be remembered however, that in many of these species, the aerenchyma were consti-

tutive and were not induced or increased significantly by flooding.

Conversely, in the dicotyledons, aerenchyma formation was increased by flooding. This is probably because most dicotyledons in their study had cubic cortical cell packing, while in the wetland and intermediate species it was cubic and radial, a feature which tends to predispose a root to aerenchyma formation (Justin and Armstrong 1987). Various types of aerenchyma formed, but in some dicotyledons, root porosity could also be increased by suppression of secondary growth or by development of a highly porous phelloderm.

Thus the capacity of wetland monocotyledons for oxidative precipitation of iron on their roots appears to be linked with the aerenchymatous nature of their roots. Genetic factors seem to be more important here than environmental factors (see also Smirnoff 1981). Conversely, airspace formation in wetland dicotyledon roots has been shown to be under environmental control in those species pre-disposed to their formation. Porosity is rarely as high as that found inherently in wetland monocotyledon roots which may explain why dicotyledons are less effective at oxidation. It is possible that in mixed culture, monocotyledons may aid the growth of other species by rhizosphere oxidation. Talbot, Etherington and Bryant (1987) cite Schat (1984) as noting that several dune-slack species grew better in a waterlogged sward of Juncus maritimus than in pure culture. He attributed this to the root porosity of J. maritimus and oxygen leakage to the soil (see also Doi, 1952b).

Additionally, many wetland monocotyledons have a subapical secondarily-thickened exodermis, and it has been shown (Armstrong and Beckett 1987) that this modification can help conserve oxygen supplies for apical consumption and oxidative detoxification at the tip (see Section 1.5.7). Endodermal lignification has also been noted in many graminean species, in particular the Juncaceae, and Armstrong and Beckett (1987) have shown that this may serve as another method of conserving oxygen supplies by promoting stelar anoxia (see Sections 1.4.2.1 and 1.4.2.2). Neither modification has been noted in dicotyledonous species.

It seems therefore that monocotyledons are much better adapted for rhizosphere oxidation than are dicotyledons, and this explains why they are more tolerant of iron and why they typically dominate many wetland ecosystems. Etherington (1983b) and Crawford (1978) also note this fact and state that absence of stem tissue between roots and leaves, or a hollow stem may also ease the oxygen transport problem. They both point

out that monocotyledons produce adventitious roots freely (see also Waldren, Davies and Etherington 1987a), and these may perhaps also have a role in iron tolerance.

4.2.11.4 Function of Adventitious Roots

In this study, monocotyledons were no more prone to adventitious root formation than were dicotyledons (87% of dicotyledons and 88% of monocotyledons produced them).

One physiological function that has been suggested for adventitious roots (see Section 1.4.2.3) is that of oxidation. Indeed in 11 of the species studied, adventitious roots became ochreous, exhibiting the typical zonation pattern (Sections 1.5.7 and 3.1.3.3.2). However, in all but one of these species (Lythrum salicaria) the original root system already had this ability. Thus there was no evidence that adventitious roots were any better at oxidation than was the existing root system (cf Laan et al. 1989). In species lacking ochre, if the original root system suffered, any adventitious roots which formed tended to become stunted at the same concentration, again indicating that adventitious roots were no better adapted to a high iron environment than was the original root system. It is likely, therefore, that their role may be to take over from the normal functions of the original root system if it has been damaged by excessively high iron concentrations (see Gill 1975). However, as they also suffer damage at the same concentration it is questionable how long they can survive. Thus, very sensitive species which had their roots damaged at very low iron concentrations, failed to produce adventitious roots of any consequence (except under control conditions) and may have become unhealthy at high iron concentrations because no part of the root system was operating effectively. It is possible that, in the field, adventitious roots may function in the better oxidised surface areas so avoiding the highest concentrations of reduced iron in deeper layers (Armstrong 1968, 1982). Solution culture work would not model such a situation very closely.

Circumstantial evidence from this study therefore suggests that the main role of adventitious roots under high iron conditions may be to maintain a root system functioning as normally as possible for as long as possible (see also Drew 1987).

4.2.11.5 Relationship to Smirnoff's Study (1981)

As already mentioned (Section 4.2.11.2), Smirnoff (1981) did not support the view that radial oxygen loss is important in a species' tolerance to iron, since he found no correlation between root porosity and iron tolerance. He did however find a significant relationship between porosity and the ability to exclude iron. This discrepancy may arise through his method of assessing iron tolerance. Damage to intact roots on plants grown for 4 days in 4 mM deoxygenated $Ca(NO_3)_2$ with $FeSO_4$ additions was estimated in 3 ways:-

1. The occurrence of blackened root tips,

2. A qualitative assessment of root growth inhibition,

3. Loss of the ability of excised root tips to reduce triphenyl tetrazolium chloride (TTC).

These all gave similar results, and Smirnoff noted that the primary effect of iron toxicity was on the root tips; shoot toxicity symptoms rarely appeared within the 4 days. A similar tolerance ranking could be obtained when using excised roots, in this case measuring :-

1. Potassium leakage from excised root tips,

2. Elongation of excised root tips,

3. Viability of root tips (reduction of TTC as above).

He thus concluded that shoots were not necessary to maintain differential tolerance and that oxygen diffusion from shoots to roots was not important. However, it is quite possible that assessment of damage to roots alone is not a good index of tolerance. Al-Farraj (1983) found root elongation tests to be unreliable when assessing iron tolerance of *Epilobium hirsutum* and *Juncus subnodulosus*, two species of very dissimilar growth habit, and in the present study (Sections 4.1.1.2 - 4.1.1.4) it was shown that root length corresponded least well with the response of other variables measured. Further, although root tip blackening did tend to

occur in the more sensitive species, it did not always occur (e.g. Lychnis flos-cuculi, Valeriana officinalis, Galium aparine) and was sometimes observed on roots of the more tolerant species (e.g. Eriophorum latifolium, Carex pulicaris). It is clear that TTC reduction may well be linked to root tip blackening but not necessarily to iron tolerance.

By measuring root damage after 4 days, Smirnoff failed to take into account the fact that some species may have the ability to respond to root damage (e.g. by production of adventitious roots). Four days would have been barely sufficient time for new roots to develop and this may be critical in assessing a plant's long-term tolerance. However, it should be remembered that in the present study, in solution culture, no evidence was found to suggest that adventitious roots were in any way better adapted to high iron conditions than was the original root system. It was only in the very sensitive species, whose original root system was badly damaged by even low iron concentrations (i.e. flaccid but not necessarily with black tips), that adventitious roots failed to develop above control concentrations, and thus were of no help in increasing tolerance by replacing the original root system.

It was previously suggested (Section 4.1.1.4) that it is important to consider the response of the whole plant rather than parts, particularly when comparing a range of different types of species such as monocotyledons and dicotyledons. It is thus possible that Smirnoff was measuring root damage rather than iron tolerance and that this may explain his lack of correlation of tolerance with a number of possible mechanisms of tolerance including root porosity. Unfortunately, so few of Smirnoff's species were also used in the present study that reassessment of his data collected on these various alternative tolerance mechanisms was not possible.

4.2.11.6 Relationship Between Iron Tolerance and Relative Growth Rate

Significant negative correlation ($p < 0.01$) was found between iron tolerance ($\Sigma\%RGR$) and the relative growth rate of species grown under control conditions. This is contrary to the findings of Hodgson (1972) who noted that species capable of a high RGR appeared to depend on high external iron concentrations to attain maximum growth. Such iron-inefficient plants also tended to be tolerant of iron.

As monocotyledons were more tolerant of iron than were dicotyledons

it might be suspected that monocotyledons have a lower RGR than dicotyledons. Although mean RGR of monocotyledons and dicotyledons in this study did not differ significantly, and data from Grime and Hunt (1975) showed that there was no inherent tendency for monocotyledons to have lower RGRs than dicotyledons, 82% of the monocotyledons tested had less than average RGR (see Section 3.2.4.7). Thus, unless wetland monocotyledons have a lower RGR than dryland monocotyledons, there may have been a bias in selection of species for this study, which could be remedied by screening more wetland monocotyledons with high RGR for iron tolerance e.g. Typha latifolia and Phragmites communis. If these species were found to be iron-tolerant, it could be due to differences in oxidation capacity between monocotyledons and dicotyledons, and if they were less tolerant, the relationship with RGR would be strengthened. Phragmites is known to grow in iron-rich situations and produce ochreous roots (Mansfield 1990) and Typha produces ochreous roots (Taylor et al. 1984).

A possible source of error involving RGR may be the length of the experiment. In those species with low RGR, a greater margin of error was introduced in calculating growth reduction as compared with the faster growing species since (by definition) the control plants of these species grew very little during the 2 week period. Since tolerant species tended to have low RGRs (and were also often monocotyledons) this error might cause them to appear more tolerant than they actually were. Additionally it is possible that some of the tolerant species may have become less tolerant had the experiment lasted longer. These points could be tested by growing a selection of species for a longer period. However, links between metal tolerance and RGR have already been reported elsewhere (e.g. Ernst 1976; Wilson 1988). Indeed dwarfness in mine populations has been noted by many authors (see Cox and Hutchinson 1981). Baker (1987) cites Ernst (1976) as suggesting that the slower growth rates and lower biomass production of many tolerant plants by comparison with their non-tolerant counterparts is a corollary of the energy expenditure for operation of the mechanisms of tolerance involved.

4.2.11.7 Indirect Iron Toxicity

Indirect iron toxicity (Howeler 1973, Ottow et al. 1983), in the form of iron-induced P-immobilisation at some stage in the uptake or translocation of phosphorus, may be one mechanism by which relative growth rate

and iron tolerance are linked. It is likely that species with a high RGR may also have a high phosphorus requirement (Clarkson 1967; Rorison 1968) and, although Al-Farraj (1983) and Wheeler, Al-Farraj and Cook (1985) were able to show that direct iron toxicity was the main reason for poor performance of Epilobium hirsutum under high iron conditions, there was some evidence to suggest that indirect toxicity might also be involved.

To some extent iron toxicity and phosphorus deficiency are inextricably interlinked, but in this study phosphorus was not considered to be in limited supply (Sections 2.1.4 and 2.5). Symptoms observed in some species might suggest phosphorus deficiency (e.g. darkening of leaf colour, possibly with leaf purpling), and this may well be due to a failure in phosphorus uptake or translocation linked with high iron concentrations. It has already been noted that many species intolerant of iron have ferric phosphate precipitated on their roots, at least in solution culture (Section 4.2.10). Additionally high concentrations of aluminium are known to disrupt phosphorus metabolism in a number of species (e.g. Rorison 1965; Bennet et al. 1986; de Miranda and Rowell 1989). It is possible that iron might act in a similar manner (e.g. Hodgson 1972). Tissue analysis (Chapter 6) may clarify these points.

Iron-induced phosphorus deficiency may serve to make species with a high RGR and high nutrient requirement appear intolerant of iron. Thus although Phalaris arundinacea has a very strong capacity for oxidising iron on the roots, it appears lower down the tolerance ranking than might be expected on this basis. Poor shoot growth, and leaf drying while still green are possibly signs that phosphorus was not reaching the shoots. Similarly, Epilobium hirsutum, which has no obvious exclusion mechanism for iron and therefore suffers from direct iron toxicity, may also suffer indirectly from iron-induced phosphorus deficiency, the sum of which make it the least tolerant species tested. Use of a split root method might be one way to distinguish between direct and indirect iron toxicity. However, Kuraev (1966), working with two cereal crop species (of high nutrient requirement) showed that growth reduction was due to direct iron toxicity rather than to phosphorus deficiency (see Section 2.1.4). Conversely, Hodgson (1972) suggested that iron tolerance might be associated with the ability to maintain a normal phosphorus metabolism (thereby implying that sensitivity might be due to an iron-induced upset of phosphorus metabolism). Such points could be clarified by radiotracer studies.

4.2.11.8 Effect of Shoot/Root Ratio (SR) Changes brought about by Iron

The more tolerant species, particularly monocotyledons, showed little change in SR with increased iron concentrations. The possible influence of root ochre on this has already been noted (Section 4.2.8). In some of the very sensitive dicotyledons, SR was also little affected by iron since the shoot and the root were both affected severely. However, in the majority of dicotyledons, root growth was restricted more severely by iron than was shoot growth and it has already been noted that any factor which reduces the root surface area reduces phosphorus uptake (Marschner 1986). Since it is possible that a reduced root weight would result in a reduced root surface area, a major effect might be a reduction in phosphorus uptake. This would reduce yield and hence iron tolerance index, and is a further possible indirect toxicity mechanism which would compound the existing division between the iron tolerance of monocotyledons and dicotyledons, particularly in this study where dicotyledons tended to have a higher RGR (and hence higher nutrient requirement) than monocotyledons.

4.2.11.9 Summary

It seems that monocotyledons are inherently more suited to an iron-rich wetland environment, and indeed to the wetland environment in general than are dicotyledons; hence their dominance (see also Crawford 1978 and Etherington 1983b). They have a number of adaptations which serve to increase their success in rhizosphere oxidation and which may be linked to their evolutionary history. Crawford (1978) reports that monocotyledons are thought to have evolved from an amphibious or aquatic pre-monocotyledon dicotyledon group. Monocotyledon adaptations include an inherently higher root porosity than dicotyledons and exodermal or endodermal lignification, both of which act to conserve oxygen supplies for rhizosphere oxidation around the root tip. Dicotyledons solely have the ability to increase root porosity to some extent upon flooding. Flooding tolerance is also thought to be associated with adventitious root production. There was however no evidence to suggest that monocotyledons were any more able to produce adventitious roots than were dicotyledons. In experiments in solution culture, adventitious roots did not appear to have any role in iron tolerance over and above the normal functions of the root system of the species in question, which may or may not include rhizosphere oxi-

dation. Nor did they seem to be any better adapted to a high iron environment than was the original root system. It was suggested that their function might be to operate in the more oxidised surface layers of the soil and so avoid the highest concentrations of reduced toxins in deeper layers (Armstrong 1968, 1982). Solution culture would not model this situation adequately.

Species with a low RGR may also be (or appear to be) tolerant of iron. Presumably a low growth rate is linked with a low nutrient requirement which would firstly keep iron uptake low, and secondly minimise any effects of indirect iron toxicity such as an upset in the metabolism or uptake of phosphorus or other essential nutrient. Alternatively the low growth rate could be the result of energy expenditure needed for operation of tolerance mechanisms (Ernst 1976). The fact that roots of dicotyledons are usually affected by iron more than are shoots may reduce phosphorus uptake and further accentuate the indirect effects of iron toxicity, causing a greater split between monocotyledon and dicotyledon tolerance to iron. Tissue analysis might clarify the extent to which iron toxicity is direct or indirect.

There is undoubtedly a real difference between monocotyledon and dicotyledon iron tolerance and also a probable RGR effect, possibly linked to indirect toxicity. However, the effect of bias in the selection of species tested, plus the short duration of the experiment, plus the weight of ochre on the roots (marginally increasing apparent tolerance) could all act together to make the monocotyledon/dicotyledon split even more apparent in this study.

Further evidence that monocotyledons are generally more suited to the wetland habitat than are dicotyledons is presented by Braendle and Crawford (1987). Detached rhizomes of many wetland monocotyledons were able to tolerate longer periods of anoxia than were those of many dicotyledonous species, which are usually found nearer the soil/water interface. There appeared to be an ecological relationship between carbohydrate conservation and the degree of anoxia encountered in the preferred habitats of the species tested. Rhizomes were shown to have a greater range of tolerance to anoxia than had roots.

CHAPTER FIVE

ALTERNATIVE METHODS OF ASSESSING IRON TOLERANCE

5.1 INTRODUCTION

Two other approaches to the assessment of iron tolerance were tested on selected species; that of plant mortality with time, and that of germination (Wong and Bradshaw 1982). The response of the species used was compared with that found in the Standard Screening Method.

5.2 PLANT MORTALITY WITH TIME

5.2.1 Introduction

For some species, in the Standard Screening Experiment, deaths occurred (at least in the higher iron concentrations) during the two-week screening period (see section 3.2.4.18). More of the dicotyledons tested (52%) had deaths occurring at at least one iron concentration as compared to only 12.5% of the monocotyledons, namely Juncus subnodulosus and Juncus inflexus. This conforms with other evidence that monocotyledons are more tolerant of iron than are dicotyledons. It was thus thought that timing of death might be an additional way of assessing a species tolerance to iron, since ultimately it is the ability to reproduce successfully which is the most important criterion for true iron tolerance.

5.2.2 Methods

A selection of species (Rumex hydrolapathum, Epilobium hirsutum, Epilobium palustre, Eriophorum latifolium, Lysimachia vulgaris, Juncus subnodulosus, Agrostis stolonifera, Carex appropinquata and Eupatorium cannabinum) were grown at high iron concentration, and the number of days until death occurred was noted.

Seedlings were germinated in distilled water, grown in 100% Rorison

Table 5.1 Number of Days until Death of Each Plant of Each Species supplied with 100 mg Fe l^{-1} in 10% Rorison Solution
(Species are ranked in tolerance order, according to mean no. of days until death)
Monocotyledons are indicated by bold type

Species	Days until Death of Each Plant					Mean No. Days±1SE
	First	Second	Third	Fourth	Fifth	
Carex appropinquata	NO DEATHS					> 70
Agrostis stolonifera	NO DEATHS (but less healthy than C. appropinquata)					> 70
Juncus subnodulosus	22	42	42	65	70	48.2±8.7
Lysimachia vulgaris	36	42	46	49	56	45.8±3.4
Epilobium hirsutum	11	31	36	56	63	39.4±9.3
Eupatorium cannabinum	19	26	33	35	47	32.0±4.7
Eriophorum latifolium	14	14	22	25	63	27.6±9.1
Epilobium palustre	11	11	14	14	14	12.8±0.7
Rumex hydrolapathum	7	7	7	7	16	8.8±1.8

solution, and then suspended in floats in 500 ml pots (Section 2.4) with one plant per pot (n = 5). The experimental design was 2 treatments x 5 replicates x 9 species x 1 plant, and control and treatment plants were paired.

Iron was supplied as ferrous sulphate in 10% Rorison solution at pH 5.5, at 3.8 (control) and 100 mg Fe l^{-1}. This concentration was chosen to produce a rapid death in some species, and possible eventual death in others. Solutions were changed three times per week and the pots were randomised each time. Observations were made regularly, and when a 'high-iron' plant had apparently died it was harvested with its corresponding control, dried (3 days at 50°C) and weighed. The experiment was run for 10 weeks.

5.2.3 Results

Table 5.1 shows the number of days it took for each plant of each species to die. The species are ranked in tolerance order according to the mean number of days until death. However, there was great variation in the response of individual plants of any one species.

5.2.4 Discussion of Results

The exact point of death of each plant was not always clear. Some species, notably Epilobium hirsutum and Juncus subnodulosus looked unhealthy early in the experiment but a few plants survived in a poor state of health and without much growth, until almost the end of the experiment.

The trend for monocotyledons to be more tolerant of iron than dicotyledons which was shown by the Standard Screening Experiment (Sections 3.2.4, 3.2.5, 4.2.4) was broadly upheld by this experiment in that Epilobium palustre and Rumex hydrolapathum died early in the experiment, while Carex appropinquata, Agrostis stolonifera and some Juncus subnodulosus plants survived the full 70 days, albeit in a poor state of health.

There was no significant correlation between the tolerance rankings obtained from this experiment (based both on the mean number of days for death to occur, and on the number of days until the first death), and that obtained from the Standard Screening Experiment (Σ%RGR). Carex appropinquata was much more tolerant than had been found by the Standard Screening Method, although treatment plants weighed less than 5% of the control

plants at the end of 10 weeks.

Juncus subnodulosus and Epilobium hirsutum also seemed more tolerant than might have been expected since both species, more especially E. hirsutum, suffered some deaths within the 14 days of the Standard Screening Experiment. The large variation between individual plants, and problems associated with deciding the point of death, had given these species a higher position in the tolerance ranking than might have been expected. The reason for Eriophorum latifolium appearing so intolerant of iron in this experiment is not clear, but again there was considerable variation between plants.

The large variation in number of days to death observed in many species shows that within each species there may be a high degree of variability with respect to iron tolerance, and that five replicate plants were insufficient upon which to make an assessment. In the Standard Screening Experiment, each of the five replicate pots had contained 3-5 plants which were harvested as a pot replicate thus reducing the effects of variability.

5.2.5 **Bacterial Contamination**

An additional problem arose mid-way through this experiment when the iron-oxidising bacterium (Thiobacillus ferrooxidans) was found in the high iron solutions. The bacteria multiplied very rapidly and colonised the roots as well as the sides of the pots, making it difficult to tell which plants naturally produced ochre on the roots (though the bacterial ochre was generally darker and more intense than that normally found on roots). Since the seeds were not sterilised before germination it is possible that all the ochre observed in the Standard Screening Experiment was of bacterial origin. However, in this experiment, when the bacteria were present in any of the solutions, a film of 'oil' could be seen on the surface of the solutions. This was never observed in any of the Standard Screening Experiments suggesting that ochre production was not normally bacterial. Armstrong and Boatman (1967) reported a similar oily scum of bacterial origin on the water surface overlying a large accumulation of iron hydroxide and ferrous sulphide in the field. In this experiment, when the bacteria were present in large numbers they also had the effect of reducing the solution pH to as low as 2.8 in some cases, so that it was not clear whether effects on the plants had been caused directly or

indirectly by the bacteria or by the iron itself. However, as mentioned above, some species (Agrostis stolonifera, Carex appropinquata and two of the Juncus subnodulosus plants) survived both effects until the end of the experiment.

Dr. M. Wainwright, Department of Microbiology, confirmed the identity of the infection and suggested (pers. comm.) the use of sodium lauryl sulphate (Na dodecyl sulphate), a detergent, to control the bacteria (Dugan and Apel 1983). It was supplied to both control and treatment plants in three successive solution changes at 10 mg l^{-1}, 20 mg l^{-1} and then 10 mg l^{-1} again, and then discontinued. However, within a week the bacteria had returned, and so the treatment was restarted at 10 mg l^{-1} and continued for the remaining two weeks of the experiment. The detergent was thought to have no harmful effect on plants, and although it did not rid the solutions of the bacteria totally, it was able to keep the infection to a very low level, so that the pH remained above 4.0 for the rest of the experiment.

5.2.6 Suitability of the Use of Plant Mortality with Time for Iron Tolerance Assessment

This long-term experimental approach showed no advantages over the Standard Screening Method, and there were a number of disadvantages. Only a few species could be screened per unit time and space and, to reduce the variability of the results, much more replication would be required which was not practicable in view of these constraints. In shorter experiments, more species can be tested per unit time and space enabling greater replication of each to be carried out. There is also less risk of bacterial contamination, and less time and effort has been invested if something should go wrong. In view of the problems encountered, this method of iron tolerance assessment was not attempted again.

5.2.7 Tolerance in Relation to Relative Growth Rate

In the Standard Screening Experiment, significant negative correlation was found between iron tolerance ($\Sigma\%RGR$) and relative growth rate. It was suggested that the fact that the experimental period was only two weeks might have biased the tolerance assessment (Section 4.2.11.6). The measurements made might not represent the full expression of iron toxic-

ity, and species with a low RGR might show signs of iron intolerance, given a longer growth period.

There was no significant correlation between the tolerance ranking from the present experiment and the RGR ranking obtained from the Standard Screening Experiment. This could indicate that the two week experimental period was insufficient to distinguish between tolerant and intolerant species, though evidence already exists of a link between metal tolerance and RGR (Ernst 1976; Wilson 1988). However, in view of the variability, lack of replication and other problems associated with the present experiment, this conclusion should be treated with caution.

5.3 SCREENING OF SPECIES BY GERMINATION RESPONSE

5.3.1 Introduction

An alternative method of screening the species' sensitivity to iron examined the effect of iron concentration upon germination, using a method similar to Wong and Bradshaw (1982). It was thought that this might represent the field circumstance where plants may germinate in a high iron environment prior to growing in it.

5.3.2 Methods

A few seeds (10-25) of each of 29 species (see Table 5.3) were placed on 9 cm Whatman No. 1 filter paper in each of six small plastic petri dishes (and pretreated by refrigeration if necessary - Appendix I). 5 ml nutrient solution (10% Rorison solution at pH 5.5, containing 3.8, 10, 25, 50, 75, or 100 mg Fe l^{-1} as $FeSO_4$) was added to each petri dish and changed every 2-3 days. 10 ml was used for the much larger-seeded Iris pseudacorus. A control (3.8 mg Fe l^{-1}) was also made at pH 4.2 (the minimum pH normally reached by the 100 mg Fe l^{-1} solution), to see if this had any adverse effect. Complete nutrient solution was used rather than calcium nitrate which Wong and Bradshaw (1982) had used, to keep nutrient supply similar across all types of screening method.

The experiment was of three weeks duration, and general observations on the 29 species and counts of the number of seeds which had germinated, were made daily. Assessment was made of the condition of the seedlings,

whether they had ochre on their roots and, after 3 weeks, the length of the longest shoot and root per dish was recorded.

For each species, the shoot and root length data were standardised according to the maximum length attained (which was not necessarily in the control). From these standardised data, graphs were drawn of standardised shoot (%S) or root (%R) length, with supplied iron concentration. Tolerance indices were also produced for each species by summing the standardised shoot or root data at all iron concentrations (except that producing 100% growth) (see Section 3.2.3.2). Three indices were calculated:

i. based on standardised shoot length (STOL) i.e. Σ%S,

ii. based on standardised root length (RTOL) i.e. Σ%R,

iii. based on the sum of the two (TTOL) i.e. Σ%S + Σ%R.

"League tables" were drawn up from these data showing the relative tolerance of the species as assessed by the three methods.

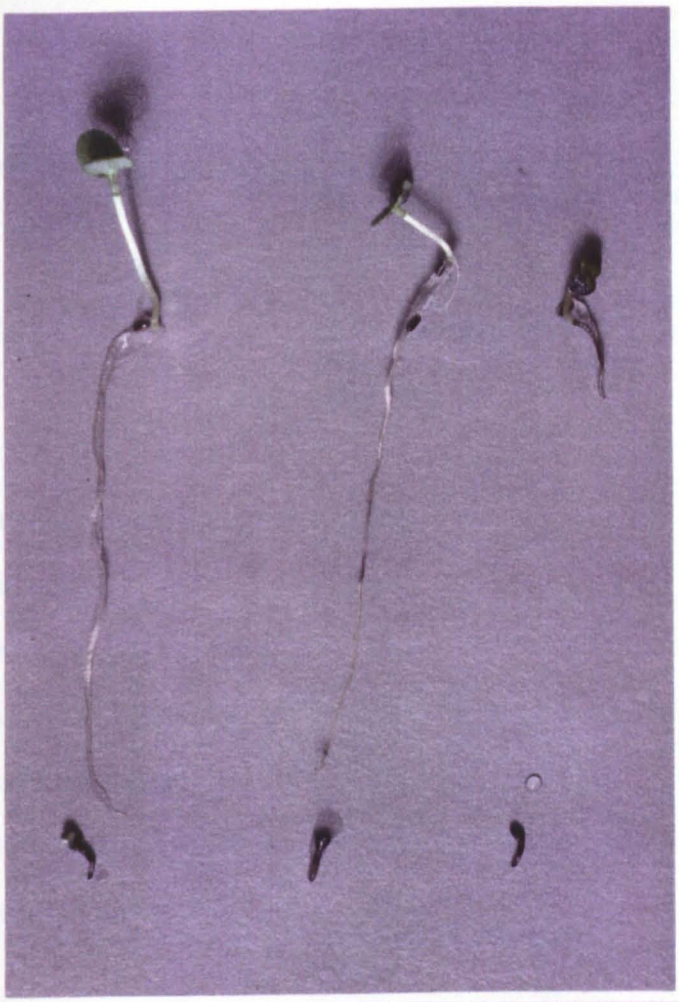
5.3.3 **Results**

5.3.3.1 Germination

For the majority of species, germination itself, i.e. shoot or root emergence, was little affected by iron concentration both in timing, and in terms of percentage germination. Most plants produced a very small root, though in some species this showed no further elongation (see Figure 5.1a). Therefore, germination itself cannot be used as an iron tolerance indicator (as Wong and Bradshaw 1982 had found when working on Lolium perenne). They did find however, that for each metal there was a concentration above which germination did not occur; this was as high as 250 mg Fe l^{-1} for Lolium perenne. A threshold was encountered for a very few species in this study. The iron concentration above which germination did not occur at all, and the iron concentration which caused a reduction in % germination as compared to the control are presented in Table 5.2 for the few species whose germination was affected. For the vast majority of species the iron concentrations which would cause reduced % germination and prevent germination were above 100 mg l^{-1}.

Table 5.3 shows that there was considerable variation in % germination across the treatments since there were only 10-20 seeds per treatment, which were not replicated. Generally, the control at pH 4.2 showed no reduction in germination as compared with the control at pH 5.5,

Figure 5.1 Condition of Seeds after 3 weeks Germination and Growth in 10% Rorison solution (pH 5.5) with a range of Iron additions. Left to right, 3.8, 10 and 25 mg Fe l^{-1} (top row), and 50, 75 and 100 mg Fe l^{-1} (bottom row)



a. Epilobium hirsutum, a dicotyledon sensitive to to iron. Note reduced growth, and shoot and root blackening at the higher iron concentrations.

b. Molinia caerulea, a monocotyledon tolerant of iron. Note the ochreous roots at the higher iron concentration, and the healthy shoot in all treatments.

Table 5.2 Iron Concentrations at which Seed Germination was Reduced, or Prevented Altogether (mg l^{-1})

Species	Iron Concentration which Caused Reduced Germination (mg l^{-1})	Iron Concentration which Prevented Germination (mg l^{-1})
Saxifraga aizoides	25	50
Briza media	25	50
Potentilla palustris	25+	50
Pinguicula vulgaris	50	100
Primula farinosa	25	>100
Juncus subnodulosus	75	>100
Epilobium palustre	75 (slight reduction only)	>100
21 remaining species	>100	>100
Lolium perenne (Wong and Bradshaw 1982)	62.5	250

Table 5.3 Percentage Germination of each Species at each Iron Concentration (where available)
(N.B. Values are from one replicate only)

Species	No Seeds	Supplied Iron Concentration (mg l^{-1})						
		3.8 pH 4.2/5.5		10	25	50	75	100
Agrostis stolonifera	20	-	44	52	28	52	48	28
Briza media	10	50	20	20	10	0	0	0
Carex echinata	25	100	92	100	96	84	88	80
Epilobium hirsutum	25	-	100	100	80	96	88	84
Epilobium palustre	25	-	100	88	92	88	72	76
Eriophorum latifolium	25	40	36	20	32	20	20	24
Eupatorium cannabinum	25	48	60	64	92	68	72	76
Filipendula ulmaria	many	high	high	high	high	high	high	high
Galium aparine	20	40	60	45	65	40	35	45
Holcus lanatus	15	87	73	80	93	67	100	100
Iris pseudacorus	10	50	90	70	90	100	80	80
Juncus effusus	many	high	high	high	high	high	high	high
Juncus subnodulosus	many	high	high	high	high	high	low	low
Lotus uliginosus	20	45	25	35	50	35	55	25
Lychnis flos-cuculi	25	92	96	100	100	100	100	100
Lysimachia vulgaris	25	-	100	92	72	88	100	92
Lythrum salicaria	20	-	35	50	35	35	40	55
Molinia caerulea	10	-	50	70	80	70	80	60
Pedicularis palustris	20	-	45	60	65	45	45	80
Pinguicula vulgaris	many	high	high	high	high	low	v.low	0
Potentilla palustris	10	50	30	50	30	0	0	0
Primula farinosa	many	high	high	high	low	0	v.low	v.low
Rumex acetosa	25	-	88	92	92	100	100	100
Rumex hydrolapathum	25	-	16	44	44	28	32	28
Saxifraga aizoides	25	-	48	32	8	0	0	0
Scrophularia auriculata	many	high	high	high	high	high	high	high
Scrophularia nodosa	many	high	high	high	high	high	high	high
Trifolium pratense	many	high	high	high	high	high	high	high
Valeriana dioica	20	60	55	75	50	45	50	60

- = test not done

Where seeds were very small (or not cleaned) an unknown (but approximately constant) number of seeds were used (i.e. many).

pH of solutions (10% Rorison solution) was 5.5 unless stated.

Table 5.4 Maximum Iron Concentration at which Species were Visually "Healthy" in the Germination Test

Monocotyledons	Max. Iron Concentration (mg l^{-1})	Dicotyledons
O Carex echinata	100	
O Molinia caerulea		
O Eriophorum latifolium	50	
O Iris pseudacorus		
Holcus lanatus	25	**Filipendula ulmaria**
O Juncus effusus		**Potentilla palustris**
		Rumex hydrolapathum
Agrostis stolonifera	10	Galium aparine
Briza media		Lotus uliginosus
		O Lysimachia vulgaris
		Lythrum salicaria
		Pinguicula vulgaris
		Trifolium pratense
		O Valeriana dioica
O Juncus subnodulosus	3.8	**Epilobium hirsutum**
		Epilobium palustre
		Eupatorium cannabinum
		Lychnis flos-cuculi
		Pedicularis palustris
		Primula farinosa
		Rumex acetosa
		Saxifraga aizoides
		Scrophularia auriculata
		Scrophularia nodosa

O denotes ochre was present on the roots (intense if in bold type).

suggesting that it was the high iron concentration rather than the low pH associated with it which had caused reduced germination in the few affected species.

5.3.3.2 Condition of Seedlings

Condition of seedlings after germination, (and growth if any) was very much affected by increasing iron concentration. This agrees with the findings of Walley *et al.* (1971) that % survival is a more useful measure of performance than is germination. There was a tendency for monocotyledons to survive at higher iron concentrations than did dicotyledons. Seedlings of some monocotyledonous species were visually healthy up to 100 mg Fe l^{-1} whereas dicotyledons were only visually healthy up to a maximum of 25 mg Fe l^{-1} (Table 5.4; see also Figures 5.1a, b).

Iron might have been concentrating in the filter paper when it was removed and drained at each solution change, and this might give an impression of lower tolerance than actually existed, though the effect would be likely to be the same for all species and should not affect their relative tolerance.

In the low-pH control treatment, seedlings were visually as healthy as in the control at pH 5.5 suggesting that it was the iron rather than the low pH associated with it which was detrimental to some species. With such small seedlings, nutrient deficiencies were unlikely and reduced size of shoot and root could be ascribed to iron toxicity.

5.3.3.3 Ochre Production

Some species that survived at the higher iron concentrations produced ochre on their roots (Table 5.4) (e.g. *Juncus effusus*, *Carex echinata*, *Molinia caerulea* (Figure 5.1b), *Eriophorum latifolium*,). This often developed soon after germination.

In monocotyledons the shoot tended to emerge before the root, whereas in dicotyledons the root tended to appear first. The significance of this to iron-toxicity is not clear, but the early development of a shoot may conceivably help prevent iron damage by promoting radial oxygen loss from the root.

Bacterial ochre production could not be ruled out as the seeds had not been sterilised, though there was no sign of any oily film on the

Table 5.5 League Tables of Iron Tolerance based on Shoot and Root Measurements made in the Germination Experiment

Ranking Based on Standardised Shoot Length Data (STOL)		Ranking Based on Standardised Root Length Data (RTOL)		Ranking based on Sum of STOL and RTOL (TTOL)	
STOL Index		RTOL Index		TTOL Index	
Carex echinata	**454**	**Molinia caerulea**	**284**	**Carex echinata**	**655**
Molinia caerulea	**339**	**Iris pseudacorus**	**230**	**Molinia caerulea**	**623**
Rumex hydrolapathum	329	Trifolium pratense	219	Trifolium pratense	494
Holcus lanatus	**322**	**Carex echinata**	**201**	Galium aparine	493
Galium aparine	310	Filipendula ulmaria	189	**Iris pseudacorus**	**487**
Filipendula ulmaria	287	Galium aparine	183	Filipendula ulmaria	476
Trifolium pratense	275	Lotus uliginosus	160	**Holcus lanatus**	**469**
Iris pseudacorus	**257**	**Agrostis stolonifera**	**159**	Rumex hydrolapathum	461
Rumex acetosa	250	**Holcus lanatus**	**147**	**Eriophorum latifolium**	**378**
Eriophorum latifolium	**242**	**Eriophorum latifolium**	**136**	**Agrostis stolonifera**	**356**
Valeriana dioica	227	Rumex hydrolapathum	132	Rumex acetosa	336
Pedicularis palustris	208	Lysimachia vulgaris	130	Lotus uliginosus	324
Juncus effusus	**200**	Potentilla palustris	122	Valeriana dioica	304
Agrostis stolonifera	**197**	Lythrum salicaria	113	**Juncus effusus**	**285**
Lythrum salicaria	166	Eupatorium cannabinum	93	Lythrum salicaria	279
Lotus uliginosus	164	Lychnis flos-cuculi	92	Lysimachia vulgaris	277
Epilobium hirsutum	156	Rumex acetosa	86	Potentilla palustris	256
Pinguicula vulgaris	151	**Juncus effusus**	**85**	Pedicularis palustris	241
Juncus subnodulosus	**150**	Valeriana dioica	77	**Juncus subnodulosus**	**224**
Lysimachia vulgaris	147	**Juncus subnodulosus**	**74**	Epilobium hirsutum	220
Epilobium palustre	146	Epilobium hirsutum	64	Pinguicula vulgaris	211
Potentilla palustris	134	Epilobium palustre	61	Epilobium palustre	207
Scrophularia nodosa	120	Pinguicula vulgaris	60	Eupatorium cannabinum	182
Briza media	**111**	Scrophularia auriculata	52	**Briza media**	**162**
Eupatorium cannabinum	89	**Briza media**	**51**	Lychnis flos-cuculi	156
Scrophularia auriculata	79	Pedicularis palustris	33	Scrophularia nodosa	153
Lychnis flos-cuculi	64	Scrophularia nodosa	33	Scrophularia auriculata	131
Primula farinosa	50	Primula farinosa	31	Primula farinosa	81
Saxifraga aizoides	0	Saxifraga aizoides	15	Saxifraga aizoides	15

Monocotyledons are indicated by bold type

surface of the solution, which had been observed in the long-term experiment when contaminated by iron-oxidising bacteria (Section 5.2.5).

5.3.3.4 Standardised Shoot Length and Root Length Data

Figure 5.2 shows the standardised response of shoots and roots for each species. Some of the between-treatment variation may be because only the maximum shoot and root length were measured per treatment and there were no replicate treatments. Nevertheless, trends in species response can be seen; the species at the top of Figure 5.2 have greater iron tolerance than do those at the bottom. (i.e. they suffer smaller reductions in shoot and root growth than do the less tolerant species). However, one unusually large seedling could result in under-estimation of tolerance owing to lack of replication.

5.3.3.5 "League Tables" of Tolerance Indices

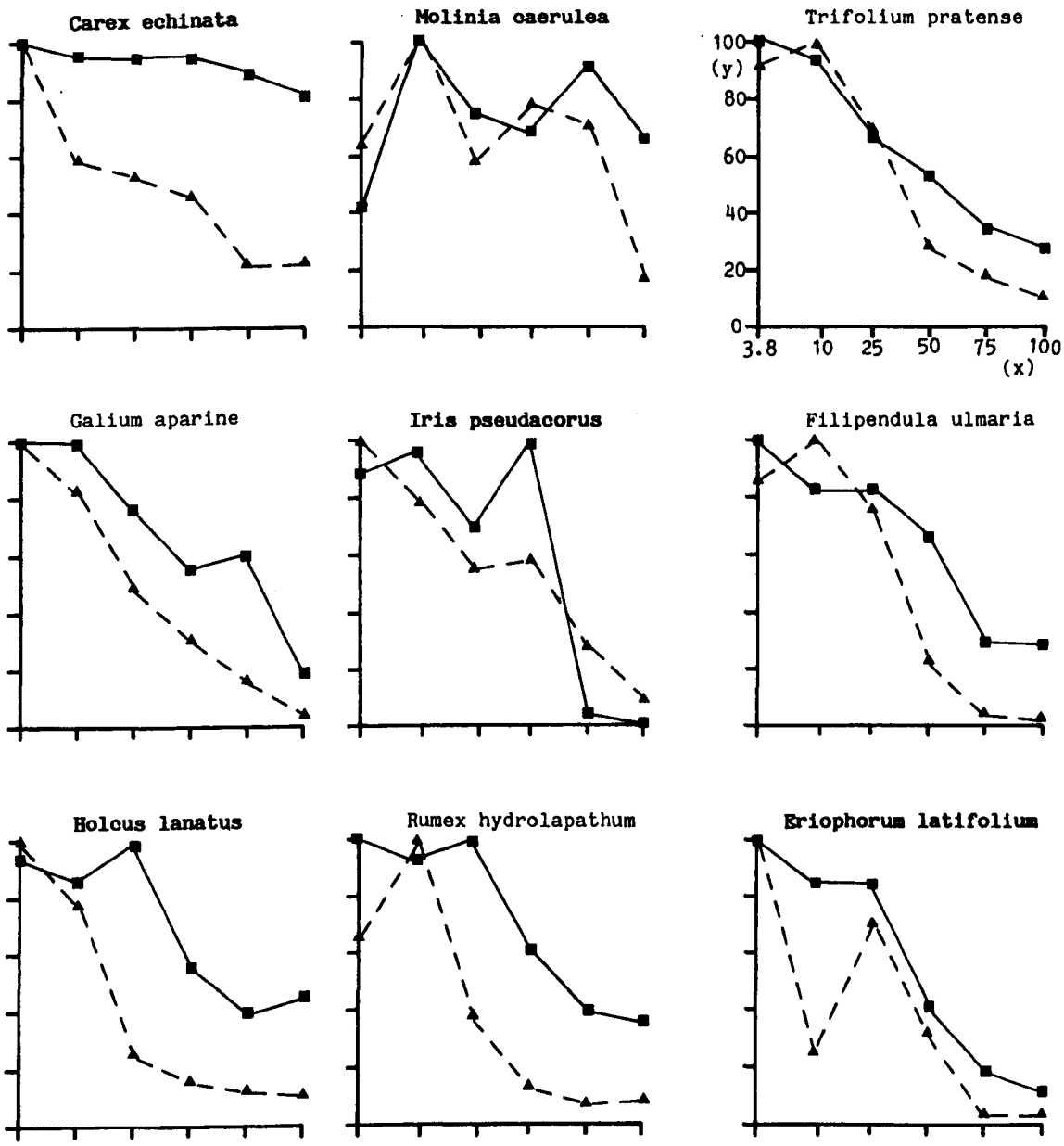
Table 5.5 shows the relative tolerance of the species tested. They are ranked on the 3 tolerance indices produced (i.e. STOL, RTOL, and TTOL; see Section 5.3.2). It is clear that for most species, root growth was affected more by iron than was shoot growth over the whole range, since RTOL is usually less than STOL. Wong and Bradshaw (1982) also reported this for *Lolium perenne* tested over a range of different metals. They found iron to be less toxic to this species than other metals, which may be expected since they were working at pH 7 where little iron would be in solution.

However, in some very sensitive species, e.g. *Eupatorium cannabinum*, *Lychnis flos-cuculi*, and *Saxifraga aizoides*, shoots failed to emerge at high iron concentrations, and only a very small root showed that germination had occurred at all.

5.3.4 **Relationship between Seed Size and Response of Seedlings to Iron Concentration at Germination**

Although observation of the condition of the seedlings suggested that some monocotyledons could tolerate higher iron concentrations than could dicotyledons, this relationship was not apparent in the "league tables" (Table 5.5). In fact, there was no significant correlation between these

Figure 5.2 Graphs of Standardised Maximum Shoot Length (—■—) and Standardised Maximum Root Length (– ▲ –) (y), from Seeds Germinated at Different Supplied Iron Concentrations (x) (Measurements were taken after 3 weeks growth, and standardisation was made against maximum length recorded. Species are presented in order of decreasing tolerance to iron as assessed by shoot + root growth (TTOL - see Section 5.3.2).) Monocotyledons are indicated by bold type N.B. The x axis is not to scale.



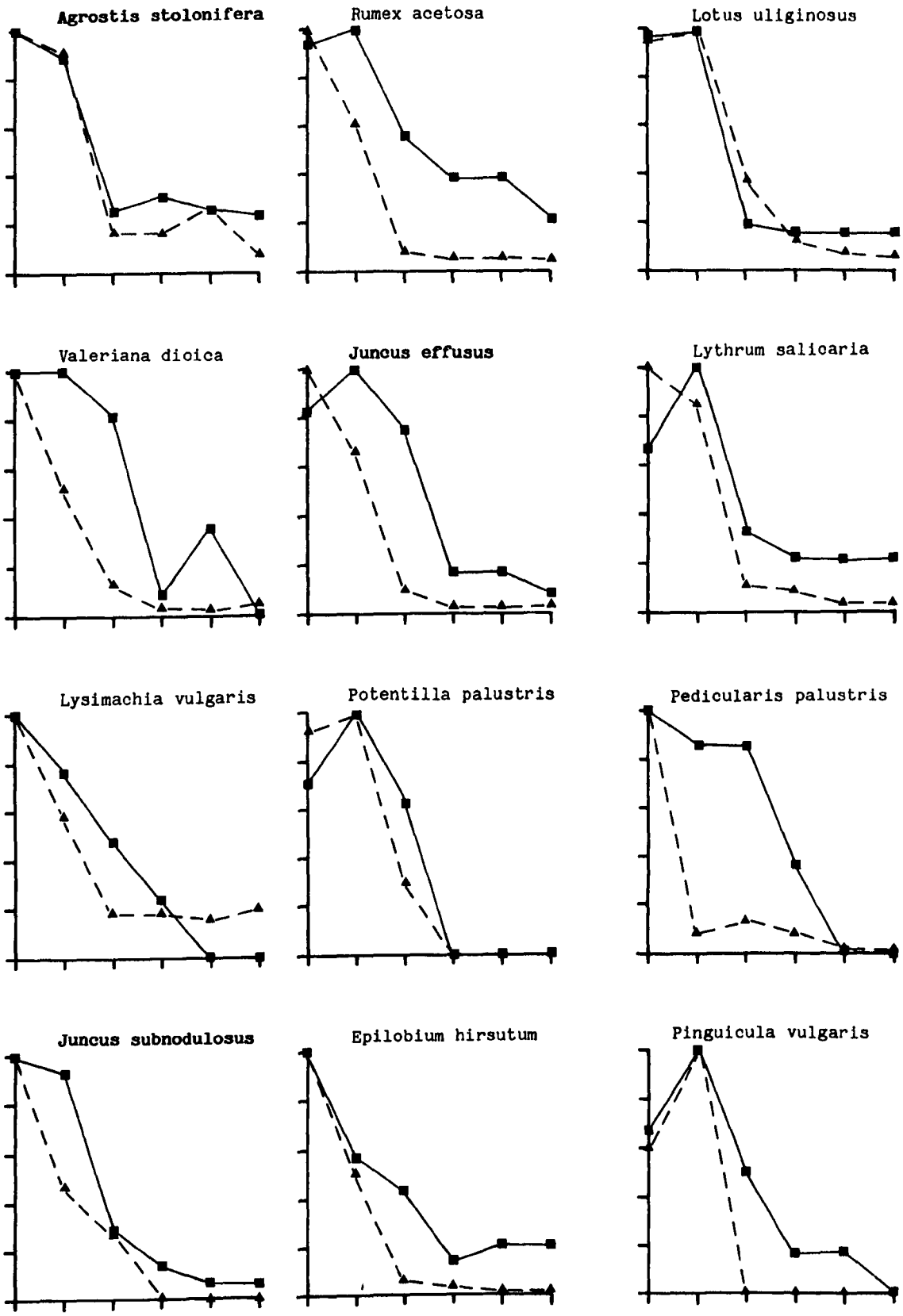


Figure 5.2 continued

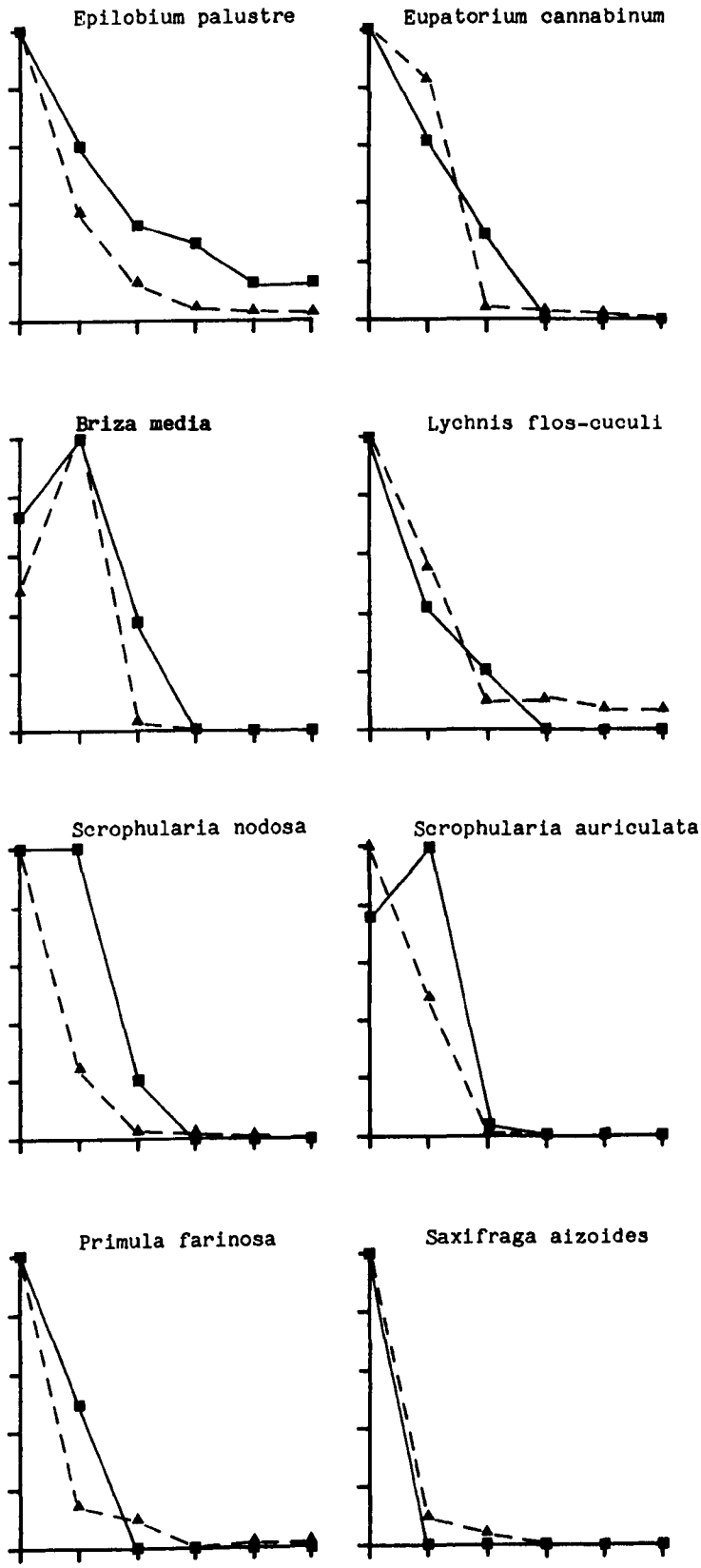


Figure 5.2 continued

tolerance rankings and that of Σ%RGR (Table 3.14), though there was significant correlation (r_s = 0.36, $p < 0.05$) between STOL and RTOL ranking only. Lack of correlation with the Σ%RGR tolerance ranking, which had been obtained with older plants, might indicate that iron tolerance varies with age. The tolerance of Pedicularis palustris was much lower than when tested by the Standard Screening Method, because a less tolerant seed source was used for this experiment. Plants from this source had died within 4 days in a pilot screening experiment, whereas plants from the more tolerant source remained healthy after the full 14 days.

It was noted that some species (Holcus lanatus, Filipendula ulmaria, Galium aparine and Rumex hydrolapathum) seemed to tolerate higher concentrations of iron in the present experiment than might have been predicted from the Standard Screening Experiment. These species tended to have fairly large seeds. Conversely, where a species performed less well than expected it generally had a small seed (e.g. Agrostis stolonifera, Juncus effusus, and Juncus subnodulosus). Regressions were made between tolerance indices (Table 5.5) and log. mean seed weight data. These data (Table 5.6) were obtained from the Unit of Comparative Plant Ecology, Sheffield University (where available), and were not weights of the seed actually used in this test. For all three relationships, there was significant correlation between iron tolerance and log. seed weight ($p < 0.01$ for STOL and TTOL, $p < 0.05$ for RTOL). However, less than a third of the variation in tolerance could be accounted for by log. seed weight. The closest correlation was with total tolerance (TTOL) i.e. sum of shoot and root tolerance.

These results suggest that there is a tendency for species with larger seeds to produce relatively longer roots and shoots at high iron concentrations than do plants with smaller seeds. They thus appear more tolerant. A large seed might be able to support a seedling for longer than a smaller one, making the seedling less reliant on the growth medium.

5.3.5 Suitability of use of Response at Germination for Iron Tolerance Assessment

Although germination response is a quick and simple method of assessing iron tolerance, the major drawback is that seed size is obviously very important, and what is measured may not be a true reflection of actual tolerance of the species. Thus, one reason why Hodgson (1972)

Table 5.6 Mean Seed Weight for Each Species examined for Iron Tolerance

Species	Seed Weight (mg)
Iris pseudacorus	21.274
Galium aparine	7.246
Rumex hydrolapathum	2.61
Pedicularis palustris	1.74
Trifolium pratense	1.351
Eriophorum latifolium	1.024
Filipendula ulmaria	1.04
Valeriana dioica	1.009
Carex echinata	0.832
Rumex acetosa	0.737
Molinia caerulea	0.527
Lotus uliginosus	0.400
Potentilla palustris	0.378
Holcus lanatus	0.361
Eupatorium cannabinum	0.271
Lychnis flos-cuculi	0.213
Lysimachia vulgaris	0.114
Scrophularia auriculata	0.071
Epilobium hirsutum	0.052
Epilobium palustre	0.043
Agrostis stolonifera	0.022
Juncus subnodulosus	0.017
Juncus effusus	0.015

N.B. These are not weights of the seed actually used for the germination test, data were obtained from Unit of Comparative Plant Ecology, Sheffield University.

found 7 day old seedlings of Rumex hydrolapathum to be moderately tolerant of high iron concentrations (by root elongation) may have been that they were still attached to the seed. However for Rumex acetosella he reported increasing iron tolerance with increasing age of seedlings from 2-6 days, when they might be expected to become progressively less dependent on the seeds and hence less iron-tolerant.

Germination itself was shown to be of no use as an indicator of tolerance, since iron concentration did not usually affect germination. However, casual observation of the condition of seedlings, though hard to quantify, did tend to uphold the monocotyledon/dicotyledon split found in the Standard Screening Experiment. This was not apparent in the STOL, RTOL and TTOL league tables based on maximum length of shoot and root.

5.4 CONCLUSION

It is clear that the Standard Screening Method of assessing iron tolerance has fewer problems associated with it than do the alternative methods tested.

The use of a long-term experimental approach recording plant mortality with time means that only a few species can be screened per unit time and space, since more replication is required to reduce the variance of the mean than was used in this study. Some variability was due to the fact that it was often difficult to assess the exact point at which a plant had died. The longer the duration of an experiment the greater is the chance of a problem occurring such as contamination by iron oxidising bacteria. The Standard Screening Method could be completed in two weeks thus reducing this risk.

Despite being a quick and simple method of assessing iron tolerance, the germination method also had its drawbacks. Germination itself was little affected by iron concentration, and the shoot and root growth response of the individual species was greatly influenced by seed size. Those species with larger seeds appeared more tolerant of iron than did those species with smaller seeds. Thus, measurements made during this type of test, e.g. shoot and root length, are unlikely to reflect the true iron tolerance of the species.

CHAPTER SIX

THE INORGANIC BASIS OF IRON TOXICITY

6.1 INTRODUCTION

The iron (and other ion) content of a selection of species of differing tolerance to iron, which had been supplied with a range of iron concentrations, was investigated. It was thought this might help establish whether toxicity was direct or indirect (e.g. Howeler 1973; Tadano 1975; Ottow et al. 1983), and whether tolerance was purely due to iron exclusion or also to an internal detoxification mechanism (e.g. Jones and Etherington 1970; Davies and Singh 1983; Talbot et al. 1987; Mansfield 1990).

Four species were selected, two monocotyledons and two dicotyledons; one of each was relatively tolerant of iron and the other was more sensitive (Table 6.1). However, since monocotyledons in this study had been found to be generally more tolerant than dicotyledons, Juncus effusus was considerably more tolerant, and Rumex hydrolapathum considerably more sensitive, than the other two species.

Table 6.1 Species Analysed for Elemental Composition
(Monocotyledons are indicated by bold type)

Species	Σ%RGR Tolerance Index	Type of Root Precipitate	Age at Start of Expt.(Days)
Juncus effusus	426	Ochre	57
Lysimachia vulgaris	258	Ochre	35
Holcus lanatus	200	Yellow	28-32
Rumex hydrolapathum	60	Yellow	26

Figure 6.1 Experimental Set-up for growing Plants supplied with Different Iron Concentrations, for Chemical Analysis

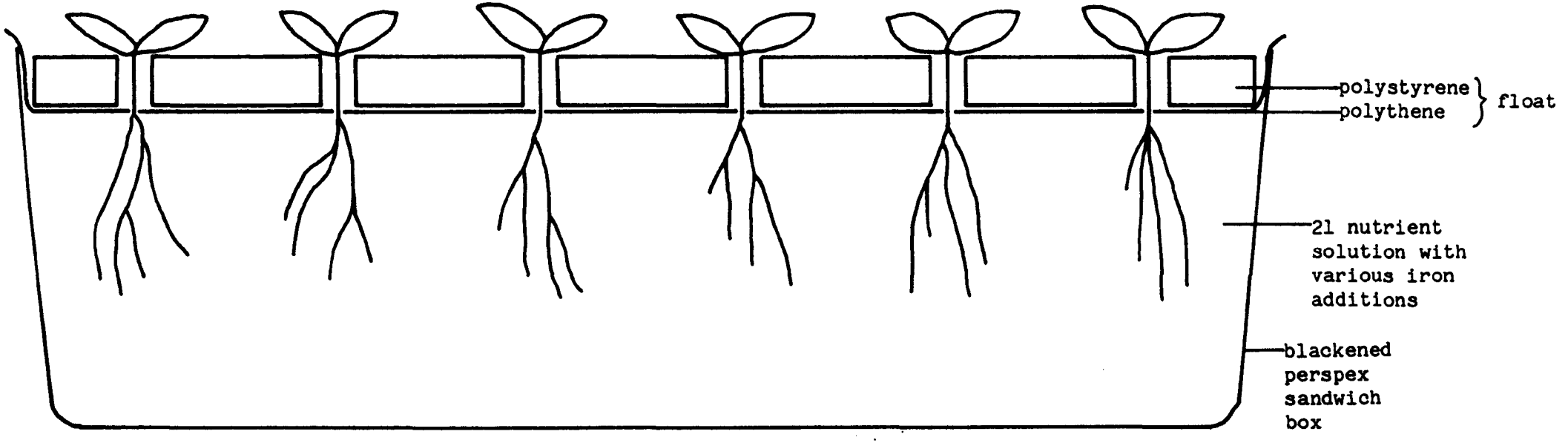


Figure 6.1

6.2 METHODS

Seedlings were germinated and raised as in Section 2.4. The age of seedlings used depended on the species (Table 6.1), though this had previously been shown to be unrelated to tolerance (Section 4.2.9).

The plant roots were threaded into holes in floating rafts made from polystyrene with a sheet of black plastic attached beneath (Figure 6.1). The polystyrene held the plants upright, while the polythene kept the roots in darkness, minimising algal growth in the nutrient solution and on the float, and prevented solution concentration in the polystyrene. The float was placed on the solution in a rectangular (27.5 x 15.5 x 9 cm) perspex sandwich box (capacity 2 l) which had been painted black to stop light penetration. Iron was supplied at 3.8 (control), 10, 25, and 100 mg l^{-1} as ferrous sulphate in 10% Rorison solution at pH 5.5.

The growing period was one week only. This allowed sufficient time for iron uptake without totally killing the most sensitive species, Rumex hydrolapathum. Solutions were changed twice during the week, and boxes were randomised at each solution change. Environmental conditions were as in Section 2.4.4. The experimental design was 4 treatments x 10 replicates (2 per box) x 4 species (1 at a time) x 20 plants.

At harvest, fresh weight measurements of the shoots were made immediately. Roots and shoots were washed separately in distilled water twice, and dried at 50°C for 3 days prior to weighing, digestion, and subsequent analysis. Plant material was digested in a mixture of H_2SO_4 and H_2O_2 (Allen et al. 1974), by heating slowly to 360°C, and maintaining temperature for 100 minutes. The digest was filtered and made up to 50 ml.

Calcium, magnesium, iron, manganese and zinc were analysed on a Pye Unicam SP190 atomic absorption spectrophotometer. Phosphorus was estimated by a modified molybdenum blue method (Stainton et al. 1977), specifically adapted for acid digests. (1 ml of digest was taken and made up to 40 ml, the pH was adjusted with 2,4-dinitrophenol, 5M NaOH and 2M HCl). Absorbance was measured at 710 nm on an SP8-100 UV/VIS spectrophotometer. Total nitrogen was determined on a Buchi Kjeldahl instrument, while sodium and potassium were analysed on a Corning 410 flame photometer.

Shoots and roots of the ten individual replicates were analysed separately except where root material was insufficient. In this case, root replicates were bulked into five replicates (or only two in the Rumex hydrolapathum treatments). One-way analysis of variance, and Duncan's

Table 6.2 Primary Location within the Plant of each of the Elements Analysed

Shoot	Root
Nitrogen (in both monocotyledons)	Nitrogen (in both dicotyledons)
Potassium	Phosphorus
Calcium	Iron
Magnesium (except *L.vulgaris*)	Manganese (generally)
	Sodium (generally, except the intolerant species at high iron concentrations)
	Zinc (generally)

N.B. Root analyses included any deposit (e.g. ochre, ferric phosphate) which was not removed by rinsing in distilled water.

range test were performed on the raw data. Logarithmic transformation did not help reduce heterogeneity of variance.

6.3 RESULTS

6.3.1 Shoot and Root Yields

6.3.1.1 Yield (Figure 6.2)

One-way analysis of variance revealed a significant reduction in yield with increasing iron concentration in all but Juncus effusus. Figure 6.2 confirms that Juncus effusus is the most tolerant and Rumex hydrolapathum the most sensitive to iron of the 4 species, (even though the experimental period was only one week).

6.3.1.2 Shoot/Root Ratio (Figure 6.3)

Earlier findings that the shoot/root ratio of dicotyledons increases with increasing external iron concentration while that of monocotyledons tends to fall were confirmed (Figure 6.3). One-way analysis of variance showed there was a significant effect in each case.

6.3.1.3 Shoot Fresh/Dry Weight (Figure 6.4)

One-way analysis of variance revealed a significant effect of external iron concentration on shoot fresh/dry weight ratio in all 4 species. There was greatest effect in Rumex hydrolapathum, the species most sensitive to iron, and least effect in the most tolerant species Juncus effusus (Figure 6.4).

6.3.2 Distribution of Elements within the Plant

Chemical analysis of shoot and root tissues revealed a differential shoot:root distribution of elements. Table 6.2 lists the primary location of each of the elements analysed, based on the shoot/root ratios of the elements.

Figure 6.2 Mean Yield (standardised against maximum mean yield) of Plants supplied with Different Iron Concentrations (± 1 SE)

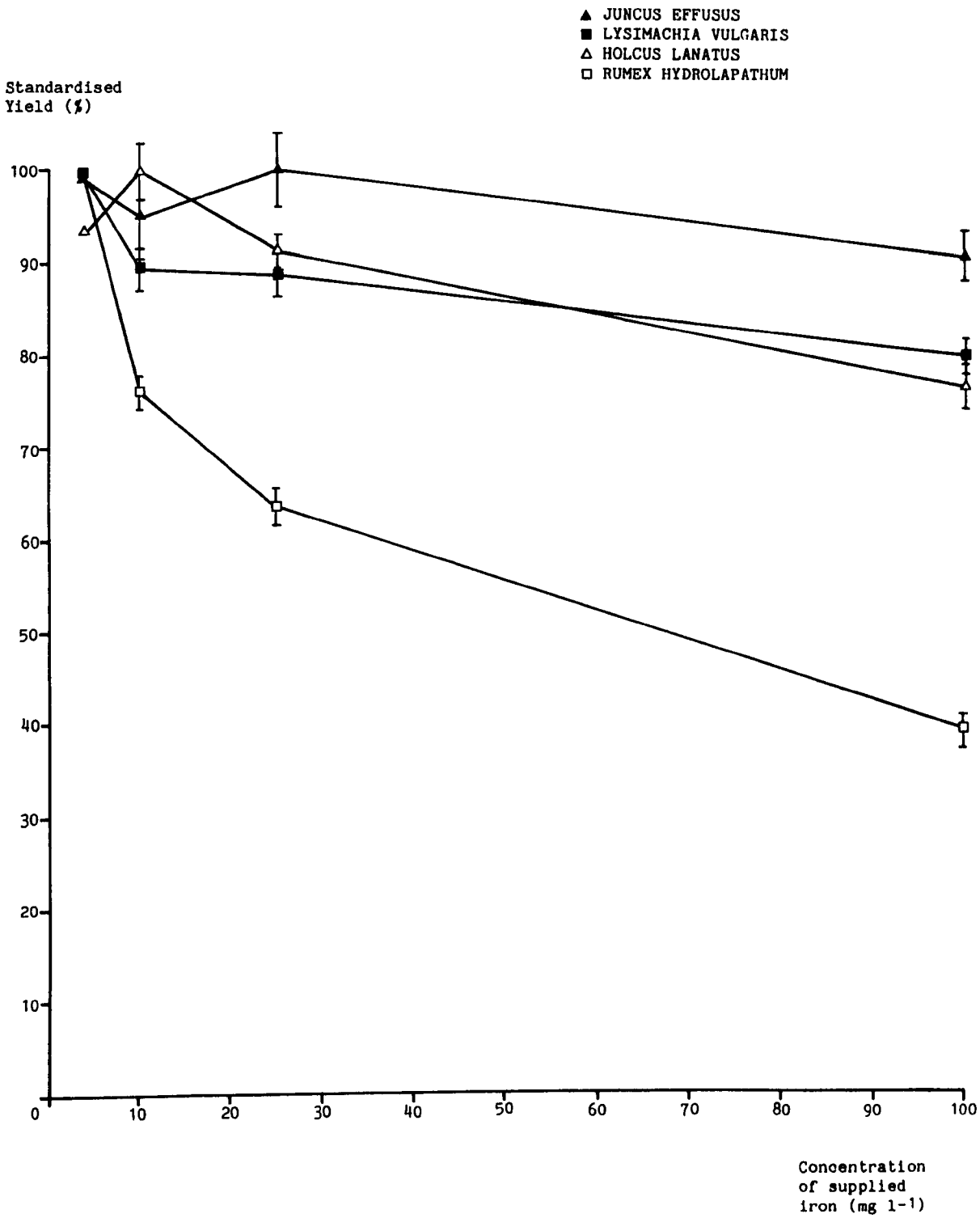


Figure 6.3 Mean Shoot/Root Dry Weight Ratio of Plants supplied with Different Iron Concentrations (± 1 SE)

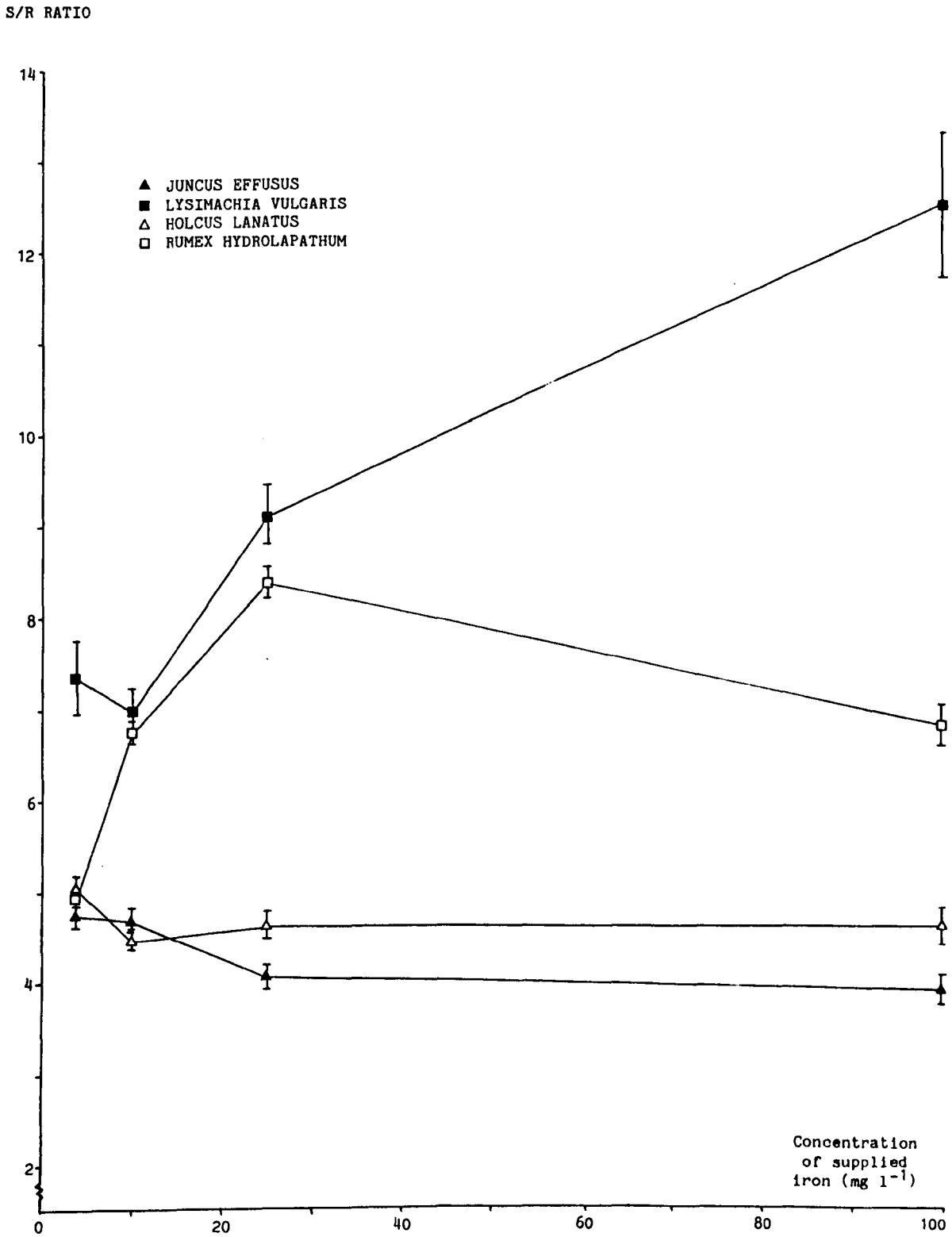


Figure 6.4 Mean Fresh/Dry Shoot Weight Ratio (standardised against control mean fresh/dry shoot weight ratio) of Plants supplied with Different Iron Concentrations (± 1 SE)

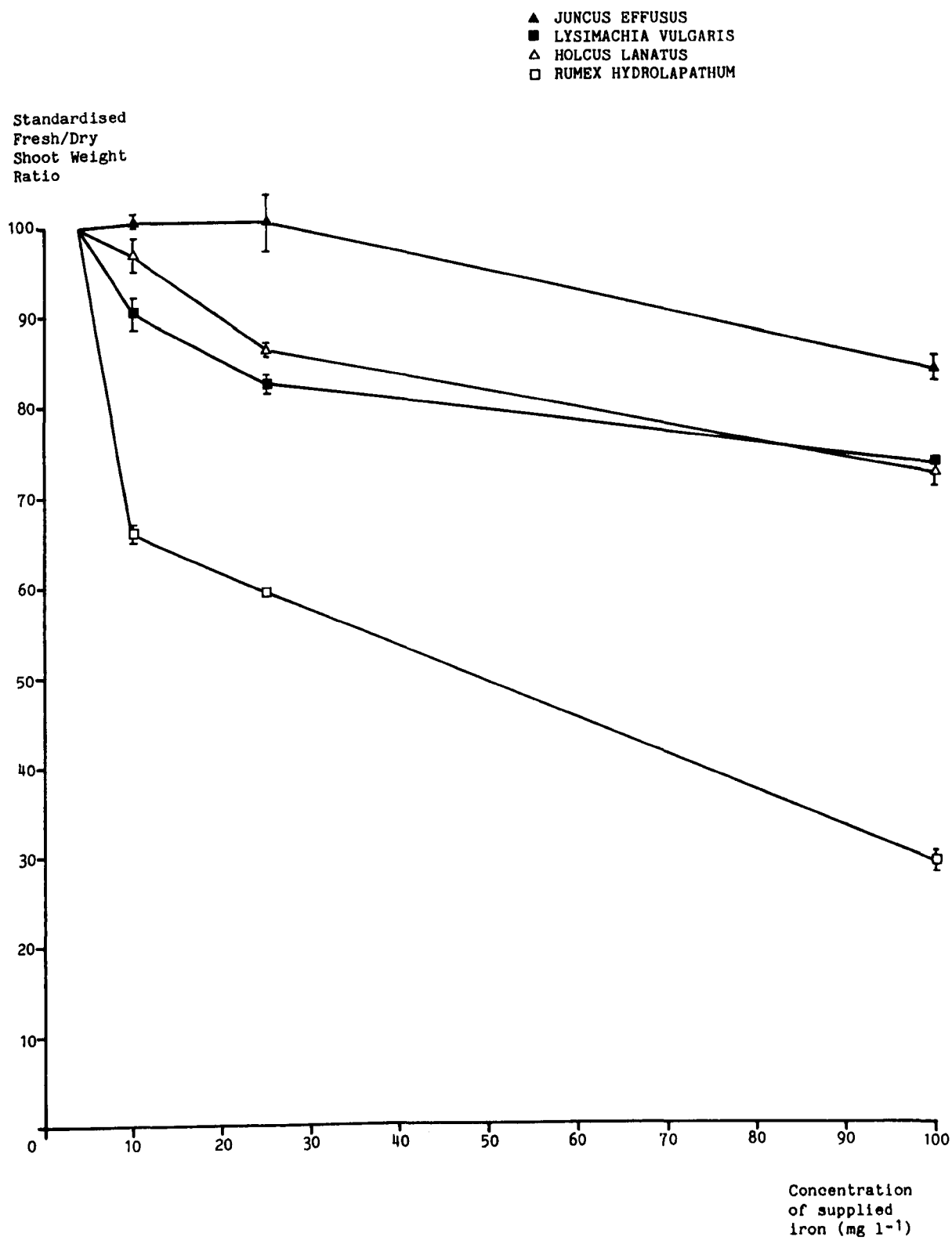


Figure 6.5 Mean Iron Concentration (mg g^{-1} dry wt) in Shoot and Root of 4 Species with varying Iron Supply (± 1 SE)

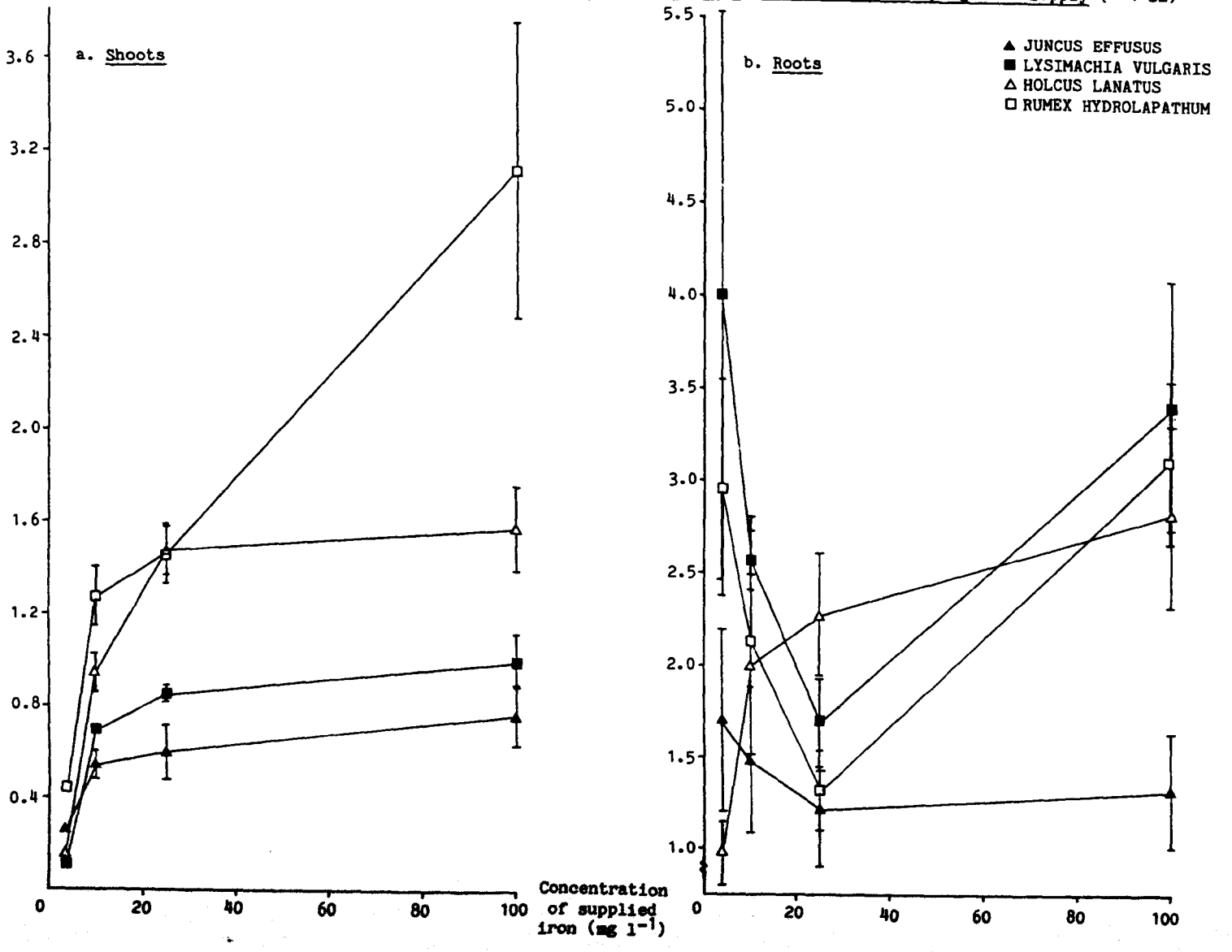


Figure 6.5

6.3.3 **Trends in Elemental Concentrations with Increasing External Iron Concentration** (Figures 6.5 to 6.13)

6.3.3.1 Iron (Figure 6.5)

6.3.3.1.1 Shoot Iron

In all four species there was a significant increase in mean shoot iron concentration with increasing external iron concentration. Figure 6.5a shows that the greatest increase occurred in *Rumex hydrolapathum*, followed by *Holcus lanatus*, *Lysimachia vulgaris* and *Juncus effusus*.

6.3.3.1.2 Root Iron

Figure 6.5b suggests that in *Rumex hydrolapathum*, *Lysimachia vulgaris* and *Juncus effusus* there is a tendency for mean root iron concentration to be reduced up to an external iron concentration of 25 mg l^{-1} and then to rise at 100 mg l^{-1}. However, one-way analysis of variance revealed the effect was not significant in any of these species. *Holcus lanatus* showed a completely different response; with increasing external iron concentration, mean root iron concentration also increased. This effect was significant.

Figure 6.6 Mean Nitrogen Concentration (mg g^{-1} dry wt) in Shoot and Root of 4 Species with varying Iron Supply (± 1 SE)

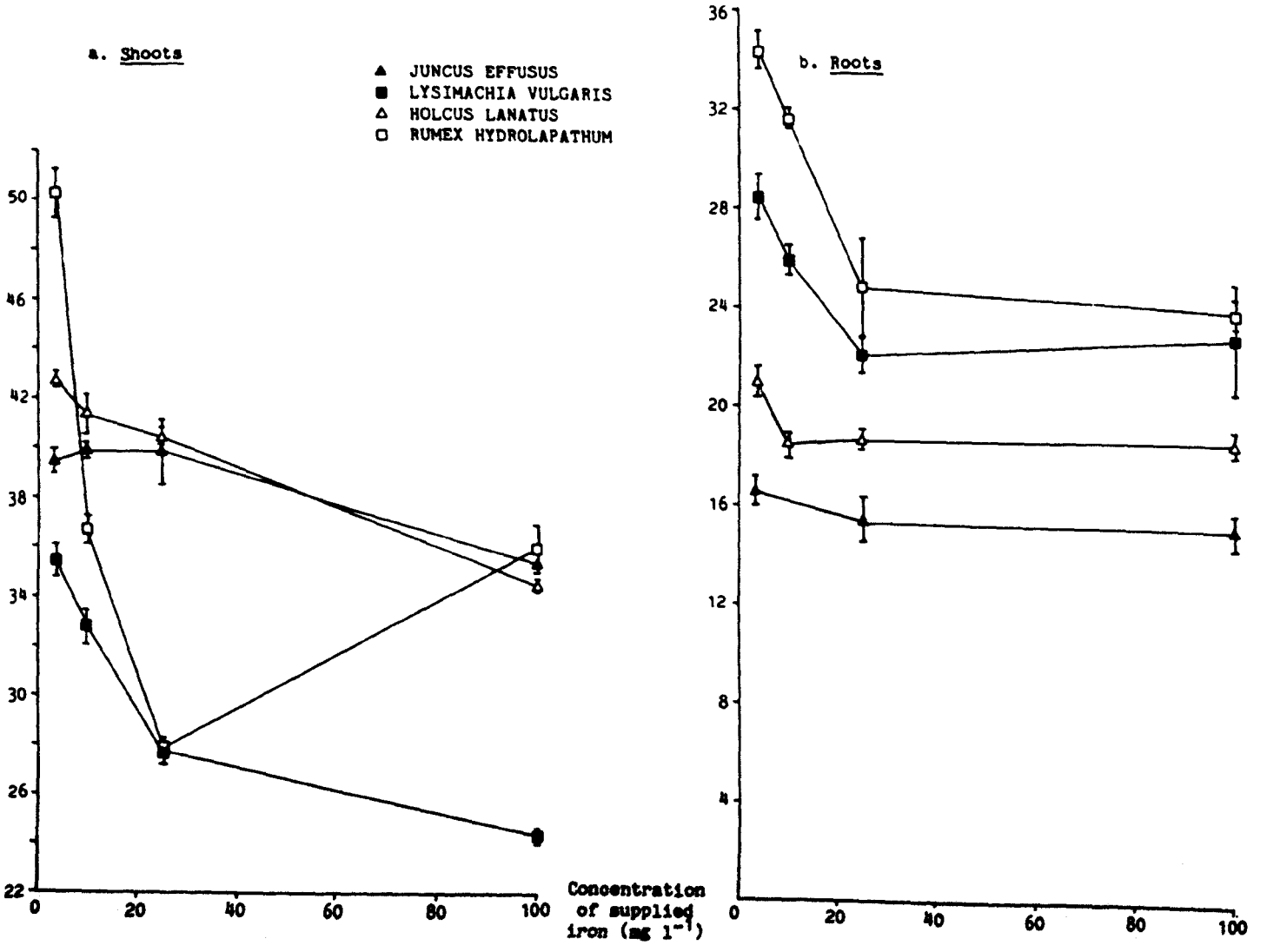


Figure 6.6

6.3.3.2 Nitrogen (Figure 6.6)

6.3.3.2.1 Shoot Nitrogen

In all four species there was a significant reduction in mean shoot nitrogen concentration with increasing external iron concentration. This effect was particularly marked in Rumex hydrolapathum, and to a lesser extent in Lysimachia vulgaris. In these two dicotyledons, greatest reduction occurred between the control and 25 mg Fe l^{-1} treatments, whereas in the monocotyledons there was a more gradual reduction over the range of treatments. Figure 6.6a shows that between 25 mg Fe l^{-1} and 100 mg Fe l^{-1} there was a marked increase in mean shoot nitrogen concentration in Rumex hydrolapathum.

6.3.3.2.2 Root Nitrogen

In all four species there was a significant reduction in mean root nitrogen concentration with increasing iron in solution. Greatest reduction occurred at the lower iron concentrations, and the effect was more marked in the dicotyledons than in the monocotyledons (Figure 6.6b).

Figure 6.7
Mean Phosphorus Concentration (mg g^{-1} dry wt) in Shoot and Root of 4 Species with varying Iron supply.
(± 1 SE)

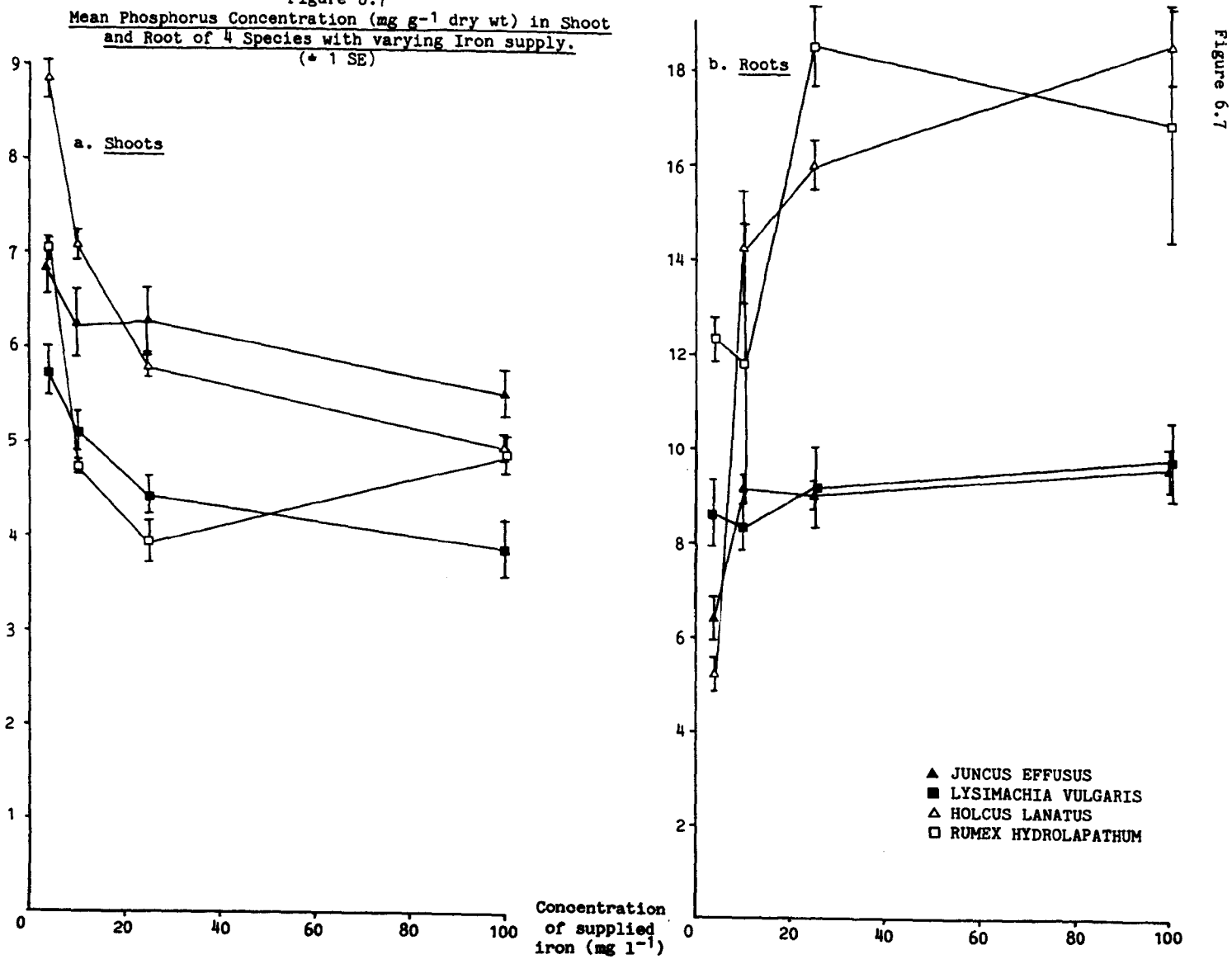


Figure 6.7

6.3.3.3 Phosphorus (Figure 6.7)

6.3.3.3.1 Shoot Phosphorus

In all four species there was a significant reduction in mean shoot phosphorus concentration with increasing external iron concentration. There was greatest reduction in Holcus lanatus and Rumex hydrolapathum. In the latter species, mean shoot phosphorus concentration rose again between the 25 and 100 mg Fe l^{-1} treatments, whilst in the other three species it continued to fall (Figure 6.7a).

6.3.3.3.2 Root Phosphorus

In all but Lysimachia vulgaris there was a significant increase in mean root phosphorus concentration with increasing external iron concentration. This was particularly marked in Holcus lanatus and Rumex hydrolapathum. Mean root phosphorus concentration of Lysimachia vulgaris was not significantly affected (Figure 6.7b).

Figure 6.8 Mean Potassium Concentration (mg g^{-1} dry wt) in Shoot and Root of 4 Species with varying Iron Supply (± 1 SE)

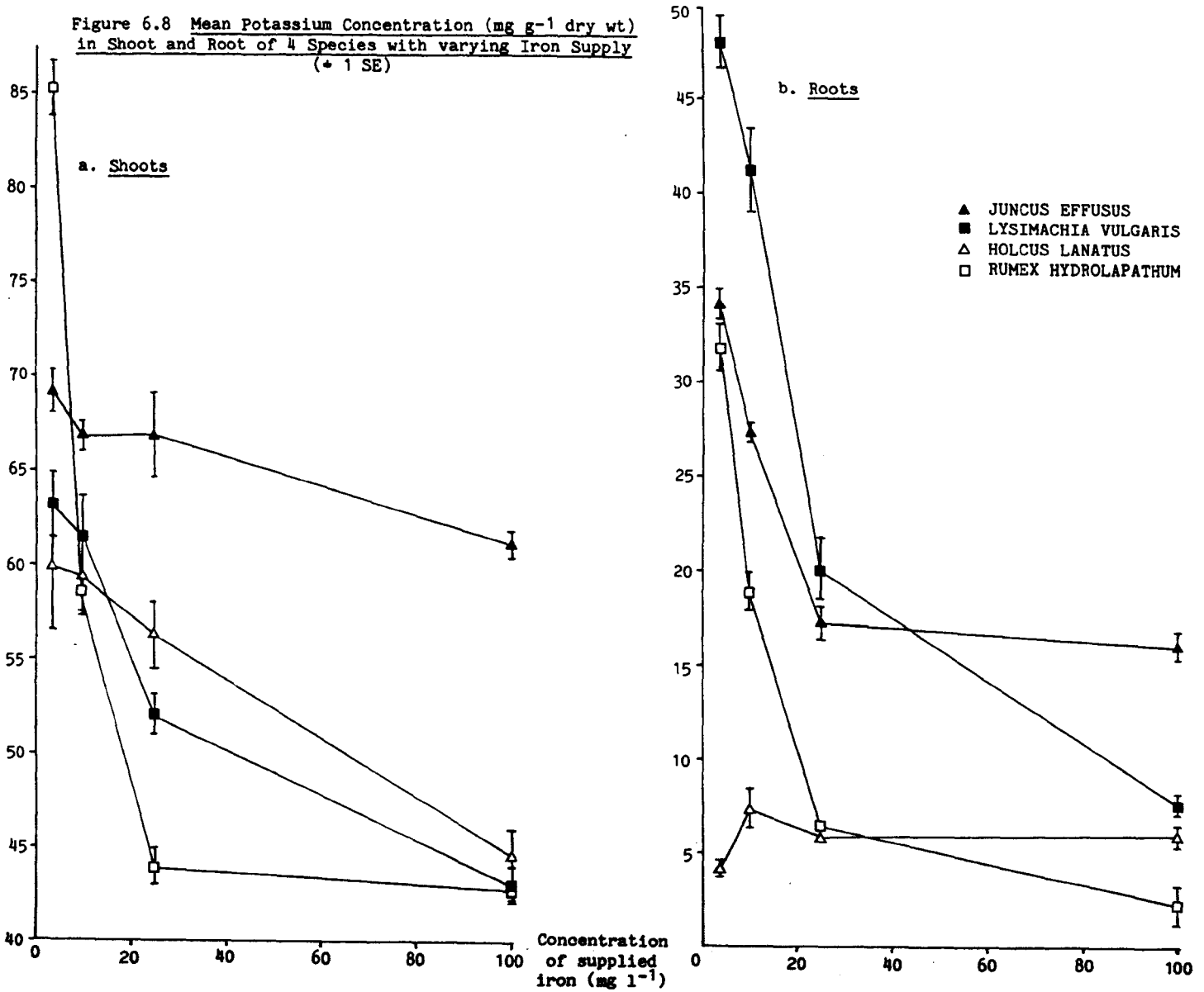


Figure 6.8

6.3.3.4 Potassium (Figure 6.8)

6.3.3.4.1 Shoot Potassium

In all four species there was a significant reduction in mean shoot potassium concentration with increasing external iron concentration. The effect was particularly marked in Rumex hydrolapathum shoots, which showed a dramatic fall in potassium concentration between the control and 25 mg Fe l^{-1} treatments, but no further significant reduction at 100 mg Fe l^{-1} (Figure 6.8a). There was least effect on Juncus effusus.

6.3.3.4.2 Root Potassium

In all species except Holcus lanatus there was a significant reduction in mean root potassium concentration with increasing external iron supply. The effect was especially marked between the control and 25 mg Fe l^{-1} treatments (Figure 6.8b).

Figure 6.9 Mean Calcium Concentration (mg g^{-1} dry wt) in Shoot and Root of 4 Species with varying Iron Supply (± 1 SE)

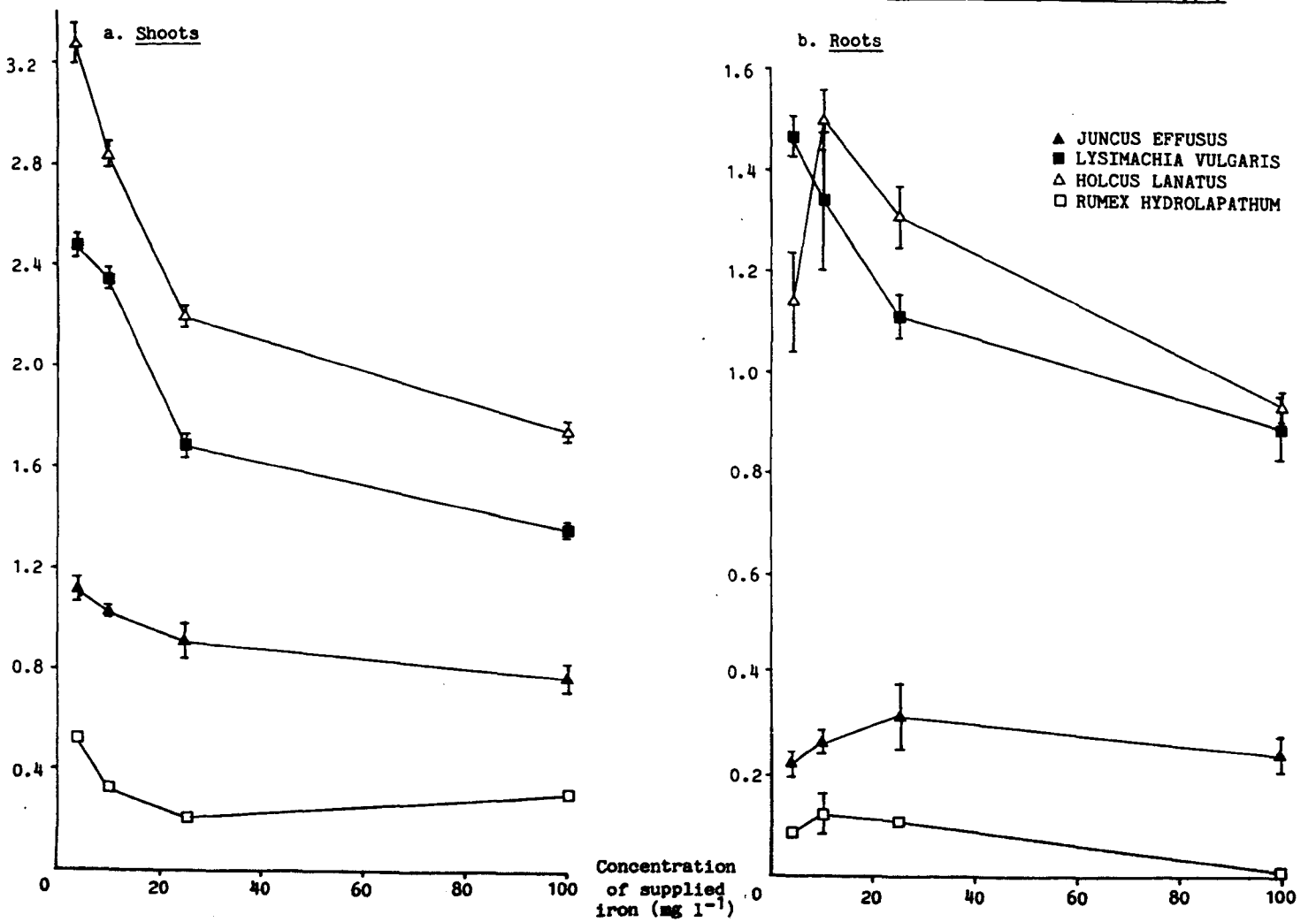


Figure 6.9

6.3.3.5 Calcium (Figure 6.9)

6.3.3.5.1 Shoot Calcium

In all four species, mean shoot calcium concentration fell significantly with increasing external iron concentration. The effect was most marked at the lower iron concentrations. In Rumex hydrolapathum at 100 mg Fe l^{-1} there was a significant increase in shoot calcium concentration over that found at 25 mg Fe l^{-1}, whereas in all other species, calcium concentration continued to fall significantly (Figure 6.9a)

6.3.3.5.2 Root Calcium

External iron concentration had no significant effect on the mean calcium concentration in roots of Juncus effusus. In all other species root calcium concentration fell significantly with increasing iron supply, sometimes after an initial increase (Figure 6.9b).

Figure 6.10 Mean Magnesium Concentration (mg g^{-1} dry wt) in Shoot and Root of 4 Species with varying Iron Supply (± 1 SE)

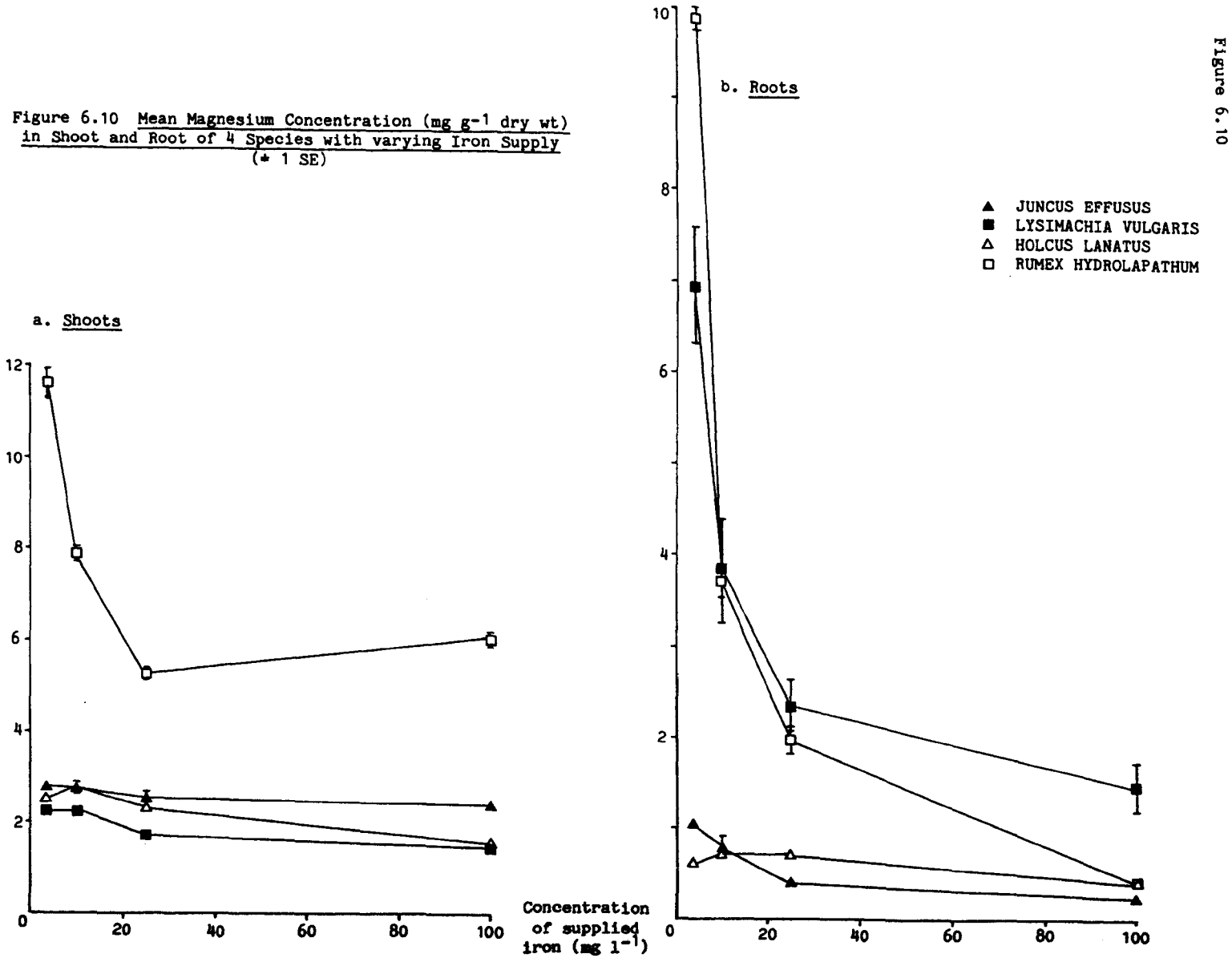


Figure 6.10

6.3.3.6 Magnesium (Figure 6.10)

6.3.3.6.1 Shoot Magnesium

In all species except Juncus effusus there was a significant reduction in mean shoot magnesium concentration with increasing external iron supply. The effect was particularly dramatic in Rumex hydrolapathum between the control and 25 mg Fe l^{-1} treatments, and was followed by a significant increase in magnesium concentration at 100 mg Fe l^{-1} (Figure 6.10a).

6.3.3.6.2 Root Magnesium

In the two dicotyledonous species, mean root magnesium concentration fell severely with increasing external iron concentration, particularly between the control and 25 mg Fe l^{-1} treatments (Figure 6.10b). However, in the monocotyledons, the reduction was much smaller and more gradual (but still significant).

Figure 6.11 Mean Manganese Concentration (μg g-1 dry wt) in Shoot and Root of 4 Species with varying Iron Supply (± 1 SE)

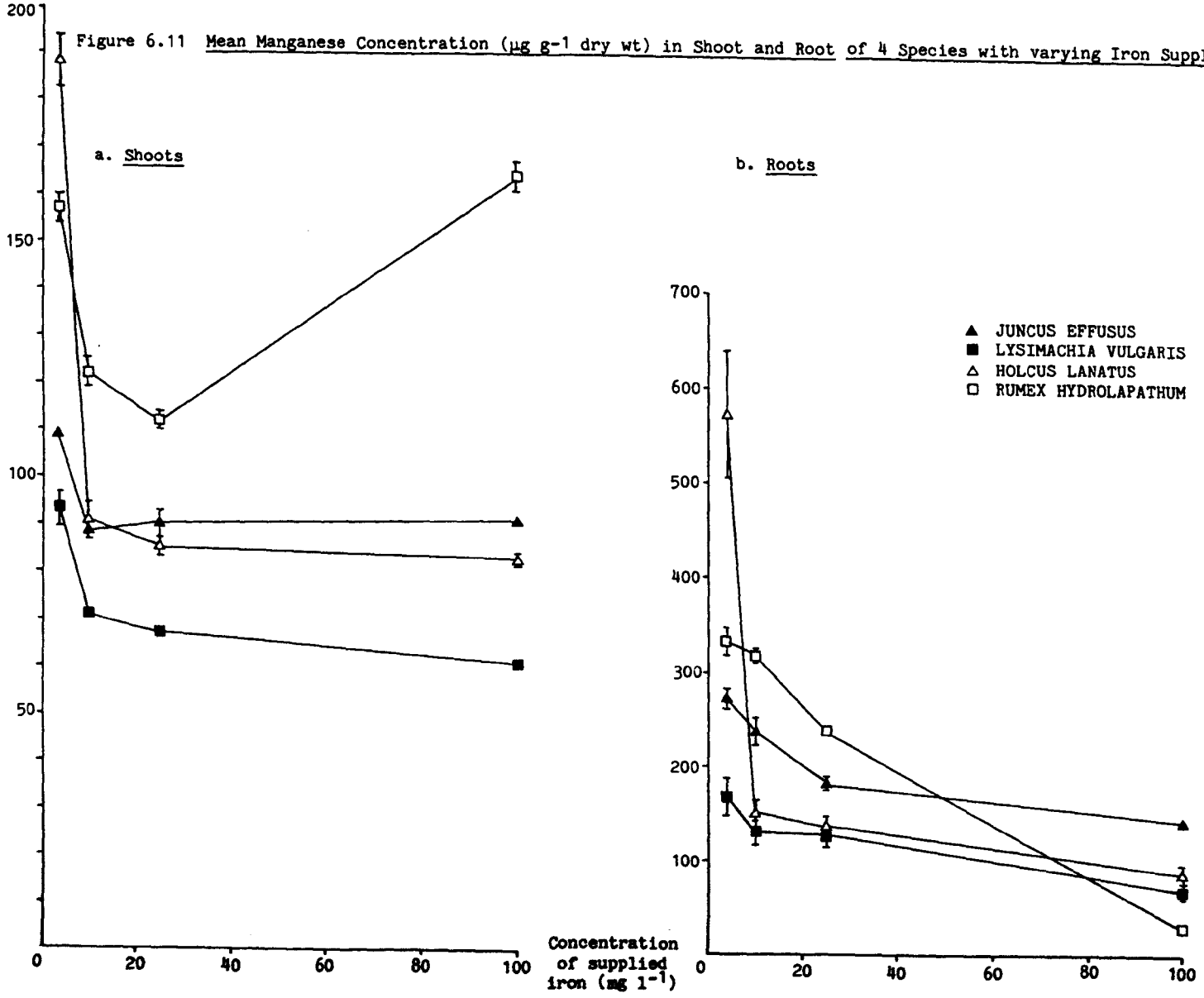


Figure 6.11

6.3.3.7 Manganese (Figure 6.11)

6.3.3.7.1 Shoot Manganese

In all four species, mean shoot manganese concentration fell significantly with increasing external iron concentration; greatest change occurred between the control and 10 mg Fe l^{-1} treatments. In the monocotyledons, there was no further significant decrease in manganese concentration with increasing external iron supply. However, in Lysimachia vulgaris mean manganese concentration continued to fall, and in Rumex hydrolapathum it fell to the 25 mg l^{-1} treatment, and then rose markedly in the 100 mg l^{-1} treatment (Figure 6.11a).

6.3.3.7.2 Root Manganese

Mean root manganese concentration also fell significantly with increasing external iron concentration, in all four species. There was a particularly large reduction in Holcus lanatus roots between the control and 10 mg Fe l^{-1} treatments (Figure 6.11b).

Figure 6.12 Mean Zinc Concentration (μg g^{-1} dry wt) in Shoot and Root of 4 Species with varying Iron Supply (± 1 SE)

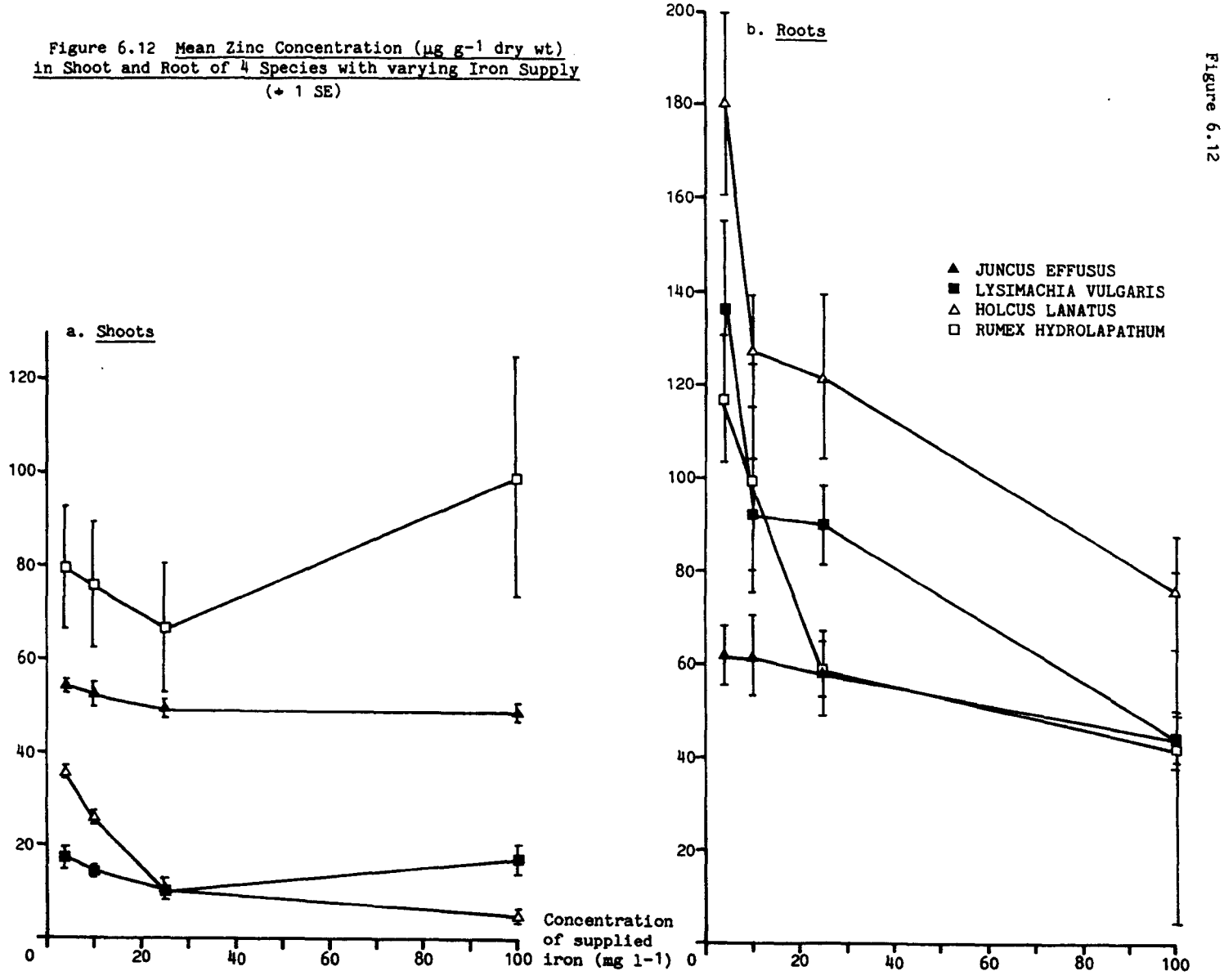


Figure 6.12

6.3.3.8 Zinc (Figure 6.12)

6.3.3.8.1 Shoot Zinc

Mean shoot zinc concentration decreased significantly with increasing external iron concentration, in Holcus lanatus. In the other species it tended to decrease, particularly between the control and 25 mg Fe l^{-1} treatments. In the dicotyledon shoots, however, zinc concentration tended to increase again above 25 mg Fe l^{-1} (Figure 6.12a). Lack of significance in Rumex hydrolapathum may be due to unusually high within-treatment variability.

6.3.3.8.2 Root Zinc

In all four species, there tended to be a reduction in mean root zinc concentration with increasing external iron supply, though this effect was only significant in Holcus lanatus and Lysimachia vulgaris. Lack of significance in Rumex hydrolapathum may be due to lack of replication (n=2 at 10, 25, and 100 mg Fe l^{-1}). Roots of Juncus effusus were least affected (Figure 6.12b).

Figure 6.13 Mean Sodium Concentration (μg g-1 dry wt) in Shoot and Root of 4 Species with varying Iron Supply (± 1 SE)

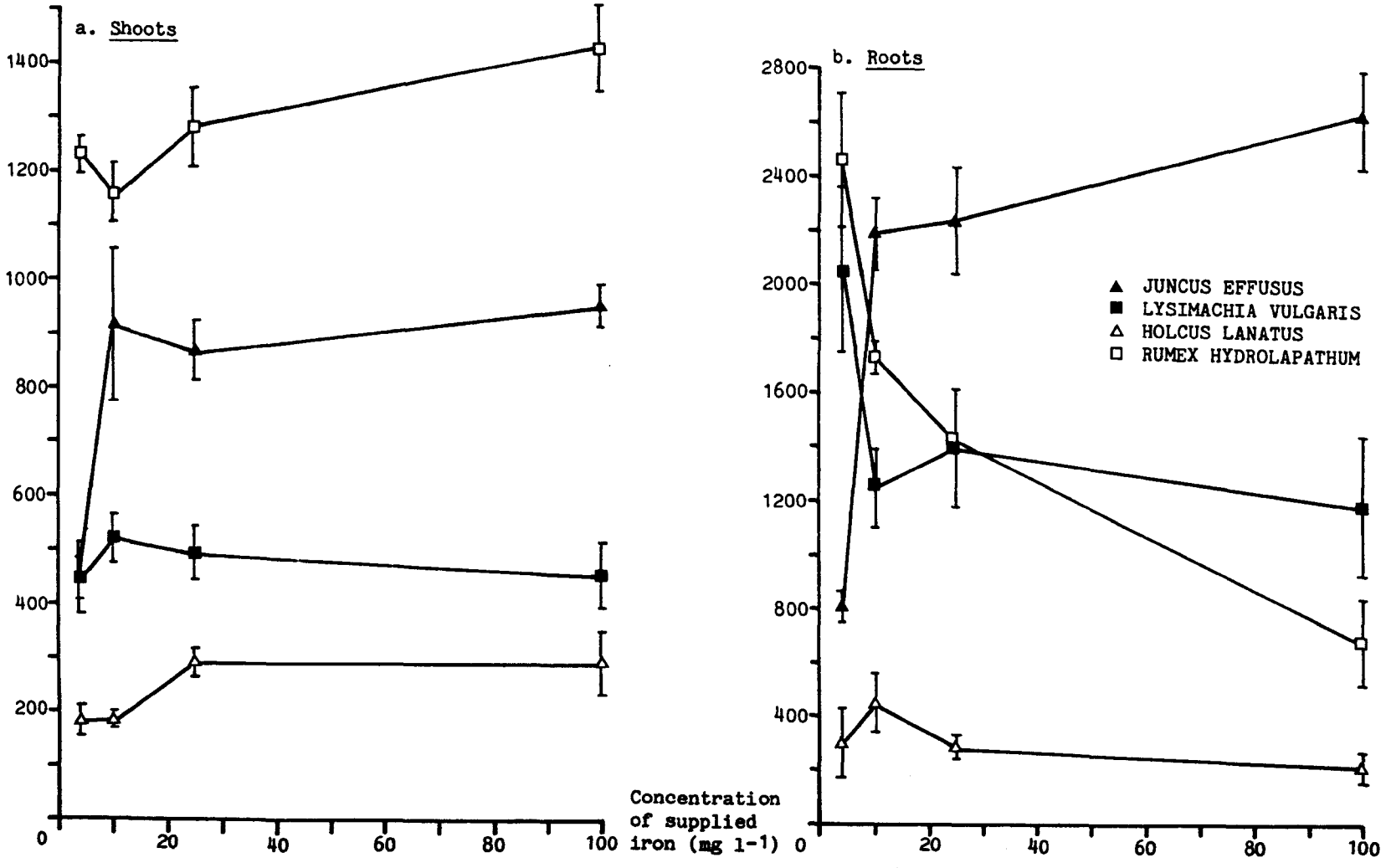


Figure 6.13

6.3.3.9 Sodium (Figure 6.13)

6.3.3.9.1 Shoot Sodium

Mean shoot sodium concentration increased significantly with external iron supply in all species except Lysimachia vulgaris. The effect was most marked in Juncus effusus (Figure 6.13a).

6.3.3.9.2 Root Sodium

In the two dicotyledonous species, mean root sodium concentration tended to fall with increasing external iron concentration, though the effect was only significant in Rumex hydrolapathum. Conversely, root sodium concentration increased significantly in Juncus effusus, particularly between the control and 10 mg Fe l^{-1} treatments (Figure 6.13b), while there was no significant effect on Holcus lanatus roots.

6.4 DISCUSSION

6.4.1 Comparison with Response in Standard Screening Experiment

Findings of this experiment agree broadly with the Σ%RGR tolerance ranking of the species (Tables 3.14 and 4.10), despite the fact that this study lasted only one week, tolerance was based on yield rather than RGR, and two of the higher iron treatments had been omitted. Rumex hydrolapathum yield was very much reduced by increasing iron supply, Juncus effusus yield was least severely affected, and Lysimachia vulgaris and Holcus lanatus were affected to an intermediate degree.

Trends in shoot/root ratios confirm earlier findings (Sections 3.2.4.8.2 and 4.2.8) that the ratio tends to fall in monocotyledons with increasing iron concentrations, while in dicotyledons it rises, since dicotyledonous roots tend to be more affected by iron than do monocotyledonous roots. Only at the very high iron concentration (100 mg Fe l^{-1}) did shoot/root ratio of Rumex hydrolapathum, the most sensitive species, fall since the shoot was also very adversely affected.

6.4.2 Direct Iron Toxicity

In all four species, mean shoot iron concentration increased significantly with increasing external iron supply (Figure 6.5a). This agrees with the findings of a number of other workers using solution culture techniques (Table 6.3a), and with results of some waterlogging studies (Table 6.3b), and demonstrates that root oxidation cannot fully exclude iron (Talbot and Etherington 1987; Talbot et al. 1987). It should be noted that iron concentrations supplied in the present study are within the range used in other studies, except that of Somers and Shive (1942) (Table 6.3a).

Many of the workers in Tables 6.3a, b have also reported increasing root iron concentrations with increasing iron supply. This may occur particularly under low nutrient levels (Smirnoff 1981) and increases are generally greater than in shoots. However, in the present study, Holcus lanatus was the only species to demonstrate such an increase, and indeed was the only species to have root iron concentrations significantly affected by external supply. In roots of the other three species there was a tendency for iron concentrations to fall between the control and 25

Table 6.3 Evidence from the Literature of an Increase in Tissue Iron Concentration with External Supply

Reference	Species	Tissue	Range of Supplied Fe Concentrations (mg l^{-1})
a. Solution Culture Studies			
Somers and Shive (1942)	Soybean	Leaf Root	.005 - 3
Tanaka Loe and Navasero (1966)	Rice	Shoot Root	2 - 600
Tanaka and Navasero (1966d)	Rice	Shoot Root	2 - 150
Jones and Etherington (1970)	Erica tetralix Erica cinerea (cut shoots)	Leaf Stem	1 - 100
Foy et al. (1978)	Any plant supplied with iron for a long period	Shoot	
Chinnery and Harding (1980)	Juncus effusus	Shoot Root	0.5 - 128
Al-Farraj (1983); Wheeler, Al-Farraj and Cook (1985)	Juncus subnodulosus Epilobium hirsutum	Shoot Root	0 - 200
Etherington and Thomas (1986)	Dactylis glomerata	Leaf	0.5 - 50
Talbot and Etherington (1987)	Salix caprea Salix cinerea	Leaf	0 - 320
b. Waterlogging Studies (involving increased iron supply upon flooding)			
Bartlett (1961)	Various grasses and legumes	Shoot	
Jones and Etherington (1970) Jones (1971a, b)	Erica tetralix Erica cinerea	Leaf Root	
Jones (1972a)	Agrostis stolonifera Festuca rubra Carex flacca Carex nigra	Shoot Root (live and dead)	
Nazrul-Islam (1976)	Rumex acetosa Eriophorum angustifolium Phalaris arundinacea Epilobium hirsutum Deschampsia flexuosa	Shoot Root Shoot Root Shoot Root Shoot Root	

Table 6.3 continued

Ladiges and Kelso (1977)	Eucalyptus ovalis	Leaf	
	Eucalyptus viminalis	Leaf	
Rozema and Blom (1977)	Agrostis stolonifera	Shoot	Root
	Juncus gerardii	Shoot	Root
Ernst (1978)	Holcus lanatus	Shoot	Root
	Juncus articulatus	Shoot	Root
	Stellaria media		Root
Keeley (1979)	Nyssa sylvatica		Root
Smirnoff (1981)	Deschampsia cespitosa	Shoot	Root
	Glyceria maxima	Shoot	Root
Davies and Singh (1983)	Agrostis stolonifera	Shoot	
	Festuca rubra	Shoot	
Wheeler, Al-Farraj and Cook (1985)	Epilobium hirsutum (from field)	Shoot	
Etherington and Thomas (1986)	Dactylis glomerata	Leaf	
Talbot et al. (1987)	Salix caprea	Leaf	Root
Talbot and Etherington (1987)	Salix cinerea	Leaf	Root
Waldren, Etherington and Davies (1987)	Geum urbanum	Leaf	Root
	Geum rivale	Leaf	Root
Heathcote et al. (1987)	Carex flacca		Root

mg Fe l^{-1} treatment and thereafter to rise (Figure 6.5b). This may suggest some kind of exclusion mechanism which breaks down above 25 mg Fe l^{-1}, more particularly in the dicotyledons. However, since root deposits (i.e. ochre and ferric phosphate) were included in the analyses, these results are not as might be expected, because intensity of precipitates increased with iron supply.

Etherington and Davies (1978) note that iron analyses of plant material are very often highly variable and attribute this to the loss of iron in volatile form (probably as pentacarbonyl which boils at 103°C). They suggest a low-temperature digestion method as the solution. In the present study, plant material was taken to 360°C, and shoot analyses show meaningful trends without particularly high replicate variability. Root analyses, on the other hand, are more variable, though this may be partly due to lower replication than for shoot material. Nicholas, Lloyd-Jones and Fisher (1957) mentioned that erratic iron results may be due to the adsorption of iron on silica, and to the formation of soluble iron complexes, but went on to demonstrate complete iron recovery over a wide silica content.

Smirnoff (1981) found a biphasic uptake pattern for iron, from a hypoxic culture solution, in roots and shoots of a number of species (see also Zhiznevskaya 1972, Thenabadu 1974). He noted that up to 1 or 2 mM (i.e. 56-112 mg Fe l^{-1}) iron supply, root iron concentration increased with supply. Thereafter saturation was indicated. This may hold true for Holcus lanatus only, in the present work, though at 100 mg Fe l^{-1} iron supply, the saturation phase had not been reached. On the other hand, Smirnoff (1981) found a greater increase in shoot iron concentration above 56 mg Fe l^{-1} (1.0 mM) than below it. Figure 6.5a shows the reverse to be true for the four species under study, i.e. the greatest increase in shoot iron concentration generally occurred at the lower end of the range of iron supply. This is compatible with an effective exclusion mechanism rather than with the breakdown of an exclusion mechanism which root analyses might imply.

In the four species studied, an inverse relationship was found between iron concentration in the shoot (and increase in shoot iron concentration) and iron tolerance, i.e. those species with greater tolerance to iron translocated less to the shoot (Figure 6.5a). Other authors have also reported that increasing sensitivity to iron is related to increased internal iron concentration, though not necessarily in the shoot (Table 6.4). Many of these studies however are circumstantial, relating

Table 6.4 Evidence from the Literature that Differential Sensitivity to Iron is Associated with Differential Iron Uptake into the Tissues

Reference	Species in order of decreasing tolerance to iron	Tissue	Notes
Olsen (1958) (solution culture)	Deschampsia flexuosa Rye Hemp	Leaf	Hemp was thought to have the greatest iron absorption rate, and D. flexuosa the lowest.
Jones and Etherington (1970) (waterlogging)	Erica tetralix Erica cinerea	Leaf Root	Iron sensitivity was related to a greater increase in leaf iron concentration rather than to the absolute concentration.
Jones (1972a) (waterlogging)	Carex nigra Carex flacca Agrostis stolonifera Festuca rubra	Shoot Root	Grasses suffered both a higher shoot iron concentration and a greater increase in iron concentration than did carices.
Foy et al. (1978) (solution culture)	Soybean vars. Corn vars.	Shoot "Tissues"	Iron toxicity is generally associated with increased iron uptake and translocation by plant tops.
Keeley (1979) (waterlogging)	Nyssa sylvatica (swamp, floodplain and upland population)	Root	Plants from the upland population (i.e. least tolerant of waterlogging) took up more iron into the roots than did the floodplain population; this in turn took up more iron than did the swamp population.
Smirnoff (1981) (solution culture)	Ranunculus flammula Senecio aquaticus	Shoot	Unhealthy replicates had much higher shoot iron concentrations than did healthy replicates.
Davies and Singh (1983) (waterlogging)	Saltmarsh and inland populations of Agrostis stolonifera and Festuca rubra	Shoot	
Al-Farraj (1983) Wheeler, Al-Farraj and Cook (1985) (solution culture)	Juncus subnodulosus Epilobium hirsutum	Shoot Root	
Etherington and Thomas (1986) (waterlogging and solution culture)	Wetland clone and dryland clone of Festuca rubra	Shoot	
Talbot et al. (1987) (waterlogging)	Salix cinerea Salix caprea	Leaf	Higher leaf iron concentration plus a greater increase in leaf iron concentration were associated with waterlogging sensitivity in S. caprea.

Table 6.5 References which Report that Root Iron Concentrations are Greater than Shoot Iron Concentrations

Reference	Species
Somers and Shive (1942)	Soybean
Tanaka and Navasero (1966a, d)	Rice
Jones and Etherington (1970) Jones (1971a, b)	Erica tetralix Erica cinerea
Jones (1972a, 1975)	Agrostis stolonifera Carex flacca Carex nigra Festuca rubra
Nazrul-Islam (1976)	Deschampsia flexuosa Epilobium hirsutum Eriophorum angustifolium Phalaris arundinacea Rumex acetosa
Rozema and Blom (1977)	Agrostis stolonifera Juncus gerardii
Ernst (1978)	Holcus lanatus Juncus articulatus Stellaria media
Smirnoff (1981)	Chamaenerion angustifolium Deschampsia caespitosa Eriophorum angustifolium Eriophorum vaginatum Glyceria maxima Myosotis scorpioides Nardus stricta Oryza sativa Ranunculus flammula Schoenus nigricans Senecio aquaticus Senecio jacobea Zerna ramosa
Al-Farraj (1983)	Epilobium hirsutum Juncus subnodulosus
Etherington (1984)	Chamaenerion angustifolium Epilobium hirsutum
Heathcote et al. (1987)	Carex flacca
Talbot et al. (1987) Talbot and Etherington (1987)	Salix caprea Salix cinerea
Waldren, Etherington and Davies (1987)	Geum rivale Geum urbanum

increasing tissue iron concentrations to waterlogging sensitivity.

In all four species, root iron concentrations were always considerably higher than shoot iron concentrations, as previously noted by many workers (Table 6.5). Jones (1972a, 1975) also noted that dead leaves of four monocotyledonous species contained more iron than live ones, while Jones and Etherington (1970) found stems contained higher iron concentrations than leaves from cut shoots of two Erica species. Tanaka, Loe and Navasero (1966) report that for rice, absorbed iron is translocated to the growing leaves when it is in short supply, while at higher concentrations it is deposited at the root surface. If the amount reaching the roots exceeds their depositing capacity, iron enters the culm and is deposited there (particularly at the nodes). Further increases in concentration lead to translocation of iron to the old leaves more readily than to the young ones (see also Mansfield 1990). Thus iron concentrations were highest in roots > culm > old leaves > young leaves.

If the iron content of the leaves exceeds a critical level, iron is deposited and forms the typical brown spots. This critical level is thought to be about 300 ppm, i.e. 0.3 mg g^{-1} dry weight for rice leaves (Tanaka, Loe and Navasero 1966; Ottow et al. 1983; Benckiser et al. 1984) though it may vary according to leaf age and variety, and presumably may differ between species. Ottow et al. (1983) found that all iron-toxic rice leaves had high iron concentrations, while a number of authors have reported that high shoot iron concentrations do not necessarily produce iron toxicity symptoms (e.g. Tanaka et al. 1966; Howeler 1973; Benckiser et al. 1984). Howeler (1973) differentiated between bronzing (direct iron toxicity) which occurred above a threshold iron concentration of 0.3-0.5 mg g^{-1} in leaves, and oranging (indirect iron toxicity) which could occur with leaf iron concentrations as low as 0.1 mg g^{-1}. It is thus doubtful whether upper critical level analysis (Beckett and Davis 1977) would be applicable to iron toxicity studies, since they found critical leaf concentrations of heavy metals (i.e. those causing 10% yield reduction) were independent of the growing conditions of barley plants.

Critical concentrations in iron supply are equally difficult to pinpoint (Foy et al. 1978) since they may vary depending on the method of solution culture (Tanaka, Loe and Navasero 1966; see also Shaw 1984), and with the age of the plant. Values of 10 mg Fe l^{-1} (Tanaka et al. 1966) and 25 mg Fe l^{-1} (Waldren, Etherington and Davies 1987) have been reported, which are in keeping with concentrations supplied in this study.

However, Tadano (1975) found no relationship between iron concentration in the growth medium and the condition of the rice plant, and suggested that susceptibility to iron toxicity must be influenced by the physiological status of the plant.

Internal iron tolerance has been suggested as an additional mechanism to its exclusion, i.e. tolerant species or clones may tolerate higher internal iron concentrations than more sensitive species or clones. This is true for manganese tolerance where manganese oxalate complexes are found in tolerant species, and the genotype of the scion is most important in grafting experiments (Marschner 1986). A little evidence for internal iron tolerance has been presented by Jones and Etherington (1970), Davies and Singh (1983), Talbot *et al.* (1987), and Mansfield (1990). Foy *et al.* (1978) note that plants with high calcium and SiO_2 concentrations can tolerate higher internal levels of iron, aluminium and manganese, possibly by preventing localisation into toxic spots. Silica may act primarily as a detoxifier, and is non-essential for sugar-cane in the absence of toxic factors.

In the present study, all species suffered increased shoot iron concentrations indicating that exclusion was not total. For each species, the critical iron concentration in solution relating to a 10% reduction in yield was extrapolated from Figure 6.2. This value was then used to find the shoot concentration which occurred at that particular level of iron supply (from Figure 6.5a). Table 6.6 shows that in three of the four species, a 10% yield reduction was associated with a critical shoot tissue iron concentration of 0.7-0.8 mg g^{-1} dry weight. The slightly higher concentration (1.5 mg g^{-1}) in *Holcus lanatus* may be due to the anomalous yield response demonstrated by this species in this experiment (Figure 6.2 cf Figure 4.2).

These results imply that the tolerance of *J. effusus* and *L. vulgaris* to iron is due solely to their excluding ability. There is no evidence to suggest that they can tolerate higher tissue iron concentrations than the more sensitive *R. hydrolapathum*. This is contrary to what is reported for manganese toxicity where each species has a critical tissue manganese concentration above which toxicity symptoms occur (Oulette and Dessureaux 1958; Foy *et al.* 1978; McGrath and Rorison 1982).

Table 6.6 Shoot Iron Concentrations Associated with a 10% Yield Reduction
(Monocotyledons are indicated by bold type)

Species	Iron supply (mg Fe l^{-1}) causing a 10% Yield Reduction (from Fig. 6.2)	Shoot Iron Concentration (mg Fe g^{-1}) associated with 10% Yield Reduction (from Fig. 6.5a)
Juncus effusus	100	0.76
Lysimachia vulgaris	9.7	0.70
Holcus lanatus	30.5	1.48
Rumex hydrolapathum	6.4	0.82

6.4.3 Indirect Iron Toxicity

6.4.3.1 Introduction

Concomitant with increased iron transport to the shoots was found a reduction in the fresh/dry shoot weight ratio, i.e. a disruption in the water balance of the shoot (see 6.4.3.2), and a reduction in shoot concentrations, and general disturbance in root concentrations of many other essential ions.

In Rumex hydrolapathum shoots there was a tendency for concentrations of many elements to fall between the control and 25 mg Fe l^{-1} treatments. However, in the very high iron treatment, concentrations frequently increased again. This increase was significant for nitrogen, phosphorus, calcium, magnesium and manganese. Presumably the plant was so badly affected by the iron that it was unable to control elemental uptake. A similar effect had been observed for calcium, magnesium, potassium and phosphorus concentrations in shoots of Epilobium hirsutum (another very iron-sensitive species) at 200 mg Fe l^{-1} (Al-Farraj 1983; Wheeler, Al-Farraj and Cook 1985).

6.4.3.2 Water Balance of the Shoot

The water balance of the shoot (fresh/dry weight ratio) was disrupted in all four species with increasing external iron supply. Other workers

(e.g. Olsen 1958; Martin 1968; Jones and Etherington 1970; Jones 1971a, b; Talbot *et al.* 1987; Talbot and Etherington 1987) have reported leaf wilting or desiccation in plants suffering from iron toxicity. Wilting is usually an early symptom, and loss of turgor must inhibit assimilation and cell expansion (Bannister 1964; Talbot *et al.* 1987).

Biddappa *et al.* (1980) noted that high iron concentrations reduced the amount of water absorbed by a number of rice varieties. This is thought to be a result of death of the root system (Martin 1968) rather than impairment of the ability to take up water (Bannister 1964). However, Marschner (1986) states that wilting is caused by a decrease in the water permeability of roots, which may result from phosphorus deficiency. Alternatively, reduced potassium uptake may be responsible for loss of turgor since potassium salts are important in maintaining the osmotic potential of cells.

Greatest sensitivity to iron was associated with the greatest disturbance in shoot water relations (as well as highest shoot iron concentrations), while tolerance was associated with the least disturbance (and lowest shoot iron concentrations). This agrees with the findings of Talbot *et al.* (1987) working on two *Salix* species. They found that the iron-sensitive *Salix caprea* took up more iron into the shoot, suffered reduced tissue water content and had a generally reduced photosynthetic capacity. *Salix cinerea* was less affected by much higher external (and possibly internal) iron concentrations. It is not clear whether reduction in photosynthetic capacity is directly due to excess iron in the tissues (possibly causing reduced magnesium and chlorophyll content), or indirectly due to reduced water potential. Iron toxicity, however, specifically reduces photosynthesis rather than causing general metabolic disturbance, and photosynthesis is known to be more sensitive to reduced leaf water potential than is respiration (Talbot and Etherington 1987).

6.4.3.3 Nitrogen

In all four species there was a significant reduction in both shoot and root nitrogen concentration with increasing external iron supply (Figures 6.6a, b,). The dicotyledons were affected most severely (*Rumex hydrolapathum* in particular) and the monocotyledons much less markedly (especially *Juncus effusus*). In all species, shoot concentrations were affected more than root concentrations indicating that translocation was

affected more than nitrogen uptake.

In all species, except Juncus effusus, there was significant ($p < 0.05$) negative correlation between shoot iron concentration and both shoot and root nitrogen concentrations. Disruption in nitrogen metabolism is unlikely to be particularly important in indirect iron toxicity since tolerant and intolerant species were affected in a similar way (e.g. Juncus effusus and Holcus lanatus). However, yellowing of older leaves (a symptom observed in many species in the Standard Screening Experiment) could be indicative of nitrogen deficiency, since it is readily translocated to younger leaves.

Scaife and Turner (1983) report that the critical leaf concentration for nitrogen deficiency in vegetables is 35 mg g^{-1} (3.5%); 25 mg g^{-1} is considered critical in rice leaves at the tillering stage (Ottow et al. 1983; Benckiser et al. 1984). However, Marschner (1986) suggests that an average concentration of 1.5% (15 mg g^{-1}) in plant shoot dry matter is adequate for growth. Presumably the critical value is largely species-dependent, and in any case total nitrogen content does not reflect the form of nitrogen in the plant. In Lysimachia vulgaris and particularly Rumex hydrolapathum, the fall in shoot nitrogen content was large and would be likely to upset the nitrogen metabolism of the plant even if it was not strictly deficient (minimum 24.4 mg l^{-1} in Lysimachia vulgaris).

There is little mention in the literature of the involvement of nitrogen in iron toxicity studies, even in those papers suggesting toxicity is the result of induced deficiencies of other ions (e.g. Howeler 1973). However, Biddappa et al. (1980) noted that under high iron conditions in solution culture, nitrogen uptake was reduced by about 15-25% in a number of rice varieties as compared with control plants. Of the seven elements investigated, nitrogen uptake was affected least severely by high concentrations of iron (see also Tanaka, Loe and Navasero 1966). In waterlogging studies, Nazrul-Islam (1976) found reduced shoot and root nitrogen concentrations associated with elevated shoot iron concentrations in Phalaris arundinacea and Rumex acetosa. Eber (1982) noted a reduction in nitrogen content of plant tissues which he linked to a higher degree of xeromorphism. On the other hand, Al-Farraj (1983) found that shoot iron and nitrogen were positively correlated in Juncus subnodulosus.

Ottow et al. (1983) noted iron toxicity symptoms in rice leaves having a range of nitrogen concentrations (even well above the deficiency threshold). Tadano (1975) stated that plants deficient in nitrogen were

more able to exclude iron than were those deficient in calcium, magnesium, phosphorus, manganese or particularly potassium. In fact, nitrogen-deficient plants translocated less iron to the shoots than did well-nourished plants. Results of work by Benckiser et al. (1984) suggest that potassium, calcium and magnesium fertilisation are more effective in counteracting iron toxicity in rice than are nitrogen or phosphorus fertilisation. Plants given a mixed fertiliser showed no signs of iron toxicity despite having higher leaf iron concentrations than those suffering from toxicity. This would suggest some kind of internal tolerance mechanism in well-nourished plants. In such plants however, only potassium and nitrogen levels in the leaves were also elevated. On the other hand, Howeler (1973) noted that fertilisation with high rates of nitrogen, phosphorus and potassium resulted in large rice plants which had more symptoms of indirect iron toxicity (oranging) than had small nutrient-deficient plants.

Thus, general evidence from the literature plus the present results suggest that the plant's nitrogen content is less important in iron toxicity than is the content of other nutrients.

6.4.3.4 Phosphorus

Figure 6.7 shows that the greatest reduction in shoot phosphorus concentration with increasing external iron supply occurred in Rumex hydrolapathum and Holcus lanatus, the species most sensitive to iron. These species also had the greatest increase in root phosphorus concentration (Figure 6.7b). Conversely Juncus effusus and Lysimachia vulgaris, the more iron-tolerant species, had a smaller reduction in shoot phosphorus concentration (non-significant for Juncus effusus), which was associated with a smaller increase in root phosphorus concentration (significant in all species). Results of similar work by Al-Farraj (1983) (see also Wheeler, Al-Farraj and Cook 1985) on Epilobium hirsutum and Juncus subnodulosus were inconclusive, though Tanaka, Loe and Navasero (1966) found significant reduction in phosphorus in rice with increasing iron supply.

Despite falling markedly with increasing iron supply, mean shoot phosphorus concentrations remained above 3.5 mg g^{-1} (0.35%) (at least after one week's growth), reported by Scaife and Turner (1983) as critical for phosphorus deficiency in vegetable leaves. Indeed in the two mono-

cotyledons, shoot phosphorus concentrations were always above that considered optimal for growth (Marschner 1986). However, either a comparison of shoot and leaf phosphorus concentrations is not valid (particularly after only one week's growth) or much of the phosphorus in the shoot is present in an unusable form, since Rumex hydrolapathum shoots showed visual symptoms suggestive of phosphorus deficiencies (i.e. retarded growth, dull green shoot colouration and reddening of leaves) despite having above-threshold phosphorus concentrations. Similar symptoms had been noted in many of the iron-sensitive species screened in the Standard Screening Experiment (Table 3.1). These observations highlight the fact that gross tissue analyses are of limited use, and more precise information on the location and form of an element within a tissue is required.

Howeler (1973) reported that oranging disease in rice was not due to direct iron toxicity, but to deficiencies particularly of magnesium and phosphorus. It could be ameliorated by foliar application of phosphorus. Ottow et al. (1983) report that iron-toxic soils are generally deficient in available phosphorus and even suggest that a low phosphorus supply is an essential pre-requisite for iron toxicity. Phosphorus-deficient rice plants are less able to exclude iron than are normal plants (Tadano 1975; Ottow et al. 1983). However, Benckiser et al. (1984) found that the addition of a mixed fertiliser to rice reduced iron toxicity effects. Since the phosphorus content of the plants had not increased much, amelioration of toxicity was ascribed to other nutrients rather than to an improved phosphorus supply.

Biddappa et al. (1980) showed that high concentrations of iron reduced phosphorus uptake in rice, and waterlogging of Festuca rubra plants resulted in reduced shoot phosphorus concentrations (Jones 1975; Davies and Singh 1983). Flooding plants of Nyssa sylvatica caused root phosphorus concentrations to increase (Keeley 1979). Findings of the present experiment suggest that toxic concentrations of iron may act by disrupting the translocation of phosphorus from root to shoot (see also Somers and Shive 1942; Ota and Yamada 1960). Similar effects have been found in aluminium toxicity studies (Foy et al. 1978). The fact that iron and phosphorus tend to co-precipitate is well-known, and such precipitation may occur on the surface and/or inside the root, and possibly within the stem and leaves. Thenabadu (1974) reports that phosphorus can inactivate iron in the plant, and it is known to disrupt the normal translocation of iron by citrate in the xylem (Tiffin 1970).

It should be noted that since root analyses in this study included any external root precipitates not removed by distilled water rinsing, it is not possible to distinguish between phosphorus precipitation inside and on the surface of the roots. The great increase in root phosphorus concentrations in Holcus lanatus and Rumex hydrolapathum may be largely due to the creamy-yellow ferric phosphate which formed on the roots of these and other iron-intolerant species in solution culture. However, iron and phosphorus analyses of Holcus lanatus roots correlate significantly ($r = 0.61$, $p < 0.01$), while those of Rumex hydrolapathum do not.

Jones (1975), and Waldren, Etherington and Davies (1987) thought that intolerance of waterlogging in Festuca rubra and Geum urbanum respectively may be due to phosphorus immobilisation in the roots, (though the reverse may be true in woody species (Talbot et al. 1987, Good and Patrick 1987)). Jones (1975) noted that in F. rubra, root iron concentrations and shoot phosphorus concentrations were significantly negatively correlated. The same was true for Geum urbanum (Waldren, Etherington and Davies 1987), and is also the case for Holcus lanatus. Waldren et al. (1987) thus suggested precipitation with phosphorus in the root might be an iron immobilisation mechanism employed by G. urbanum, but that G. rivale must have a different mechanism which did not result in shoot phosphorus deficiency.

The present findings suggest that iron-tolerant species are able to oxidise iron on their roots. This limits iron uptake to the shoot while maintaining a normal or near-normal phosphorus metabolism. Conversely, intolerant species cannot oxidise iron externally (or at least did not under present experimental conditions), and are unable to exclude it so effectively from the shoot. Iron and phosphorus co-precipitate on the root surface (and probably also inside the root) disrupting phosphorus translocation to the shoot. This agrees with the findings of Hodgson (1972) who stated that iron tolerance was associated with the ability to maintain a normal phosphorus metabolism.

6.4.3.5 Potassium

There is evidence that high concentrations of iron act by inducing deficiencies of potassium and other major nutrients in rice. The most severe symptoms of iron toxicity have been observed in plants with low or deficient leaf potassium concentrations (Tanaka, Loe and Navasero 1966; Howeler 1973; Ottow et al. 1983). Similarly, necrosis only occurred in

Epilobium hirsutum leaves if they had low potassium concentrations as well as high iron concentrations (Nazrul-Islam 1976). In this study, shoot potassium concentration was significantly reduced in all four species, with increasing external iron supply (Figure 6.8a). Rumex hydrolapathum, the species most sensitive to iron was particularly affected, while the most tolerant species (Juncus effusus) was affected least severely. Conversely, under iron deficiency, Olsen (1958) noted elevated leaf potassium concentrations.

Biddappa et al. (1980) found that in a number of rice varieties, potassium uptake was greatly reduced by a high iron supply. Potassium uptake was also reduced in this study, since root potassium concentrations fell even more than did shoot potassium concentrations, apart from in Holcus lanatus which had unaffected root potassium concentrations (Figure 6.8b). The two dicotyledonous species suffered most severe reduction in root potassium concentrations. This may be because iron tends to cause greater stunting of dicotyledonous roots than of monocotyledonous roots, and any factor reducing root surface area will decrease potassium uptake (Marschner 1986). Howeler (1973) suggests that the capacity of rice roots to absorb enough nutrients may be reduced by the coating of iron oxide on them; however, this would only apply to Juncus effusus and Lysimachia vulgaris in the present study.

In the two dicotyledonous species, shoot iron concentrations correlated inversely ($p < 0.05$) with both shoot and root potassium concentrations. In Holcus lanatus, there was negative correlation with shoot potassium concentration only, and in Juncus effusus with root potassium concentration only. Reduction in both shoot and root potassium concentrations with increasing iron supply and leaf iron content had also been noted in the iron-sensitive Epilobium hirsutum (but not in Juncus subnodulosus) by Al-Farraj (1983).

Potassium was supplied at 7.8 mg l^{-1} (and at 78 mg l^{-1} during the pretreatment period), and, although shoot concentrations had fallen considerably after one week's growth at high iron supply, they were still well above the critical level for deficiency (20 mg g^{-1}) reported for vegetable leaves (Scaife and Turner 1983).

Potassium salts make a major contribution to the osmotic potential of cells and tissues of glycophytic species (i.e. non-halophytes) (Marschner 1986). It is therefore possible that the disturbance in water balance observed, particularly in the more iron-sensitive species, was due to

reduced potassium uptake. Loss of turgor and wilting are typical symptoms of potassium deficiency (Marschner 1986).

As well as high iron concentrations inducing potassium deficiency, there is evidence from rice that plants insufficiently supplied with potassium are more prone to iron toxicity (Foy et al. 1978), and have higher shoot iron concentrations as a result of both increased uptake (Howeler 1973; Benckiser et al. 1984) and translocation (Tadano 1975). This may be because deficiency of potassium (or phosphorus or calcium) increases metabolic leakage from roots, which in turn increases microbial activity and oxygen consumption in the rhizosphere (Ottow et al. 1983; Marschner 1986). Indeed, fertilisation of rice plants with potassium and other major nutrients improved the iron exclusion mechanism (Benckiser et al. 1984; cf Howeler 1973). Higher internal iron concentrations could also be accommodated without adverse effect, suggesting some kind of internal tolerance mechanism in well-nourished plants. This may involve potassium (possibly through improved shoot turgor), since leaves from well nourished rice plants had increased potassium and nitrogen levels, and remained visually healthy despite having iron concentrations above the normal toxicity threshold.

6.4.3.6 Calcium

With increasing external iron supply, a significant reduction was found in calcium concentration in both shoots and roots of all four species (except roots of Juncus effusus, the species most tolerant to iron) (Figures 6.9a, b). This trend had also been observed in Epilobium hirsutum but not Juncus subnodulosus by Al-Farraj (1983), though at the highest iron concentration (200 mg Fe l^{-1}) an increase in shoot calcium concentration was noted. This was also observed in Rumex hydrolapathum and is probably because these two species are very sensitive to iron and their ion uptake regulation was damaged.

In waterlogging studies, shoot calcium concentrations fell with increasing iron concentrations in Rumex acetosa, Eriophorum angustifolium and Phalaris arundinacea (Nazrul-Islam 1976); root calcium concentrations fell in flooded plants of Nyssa sylvatica as root iron concentrations rose (Keeley 1979). Conversely, increased leaf calcium concentrations have been noted in iron deficient plants (Olsen 1958).

At 10 mg Fe l^{-1}, Holcus lanatus roots had a significantly higher

calcium content than in the control, suggesting stimulated uptake. A similar response was observed at low iron concentrations in Rumex hydrolapathum and Juncus effusus roots, but it was not statistically significant.

In all four species, shoot iron and shoot calcium concentrations were negatively correlated, while in the two dicotyledons, root calcium concentrations correlated inversely with shoot iron concentrations. In Holcus lanatus there was also significant negative correlation between root iron and shoot calcium concentrations.

It is likely that calcium uptake may be reduced as a result of competition with iron for binding sites (see also Tanaka and Navasero 1966a; Zhiznevskaya 1972). However, in all four species, shoot calcium concentrations were affected more than root concentrations, implying that translocation was affected more than uptake. Organic acids may be important for the translocation of calcium as well as of heavy metals (Marschner 1986), and again competitive interaction is suggested.

According to Scaife and Turner (1983), whole leaf analyses for calcium are not particularly meaningful, and critical deficiency concentrations are very variable in vegetable crop species. Ottow et al. (1983) report that 2.0 mg g^{-1} is the deficiency threshold for rice leaves, and noted that iron toxic leaves sometimes had calcium deficiency as well as high iron and manganese concentrations. Clearly, shoots of Juncus effusus and, in particular, Rumex hydrolapathum had shoot calcium concentrations well below this, even under control conditions (Figure 6.9a), though strictly speaking whole shoot and leaf analyses cannot be compared. These two species are extreme in their tolerance to iron, had lowest shoot and root calcium concentrations, and suffered less disruption in calcium concentration than the two species intermediate in iron tolerance. This suggests that neither absolute calcium concentration nor degree of lowering of calcium concentration are particularly important features in iron tolerance. However, Howeler (1973) found that induced deficiencies of calcium were important in indirect iron toxicity to rice. Oranging symptoms could not be related to leaf iron content, but the more severely affected plants were deficient in calcium, though deficiencies in phosphorus and magnesium were more important. Calcium deficiency is a common feature of both manganese and aluminium toxicity in some species (Marschner 1986). Waldren, Etherington and Davies (1987) and Talbot et al. (1987) reported no interpretable changes in leaf calcium concentrations in either

Geum or Salix species upon flooding, despite considerable increases in leaf iron concentrations. They noted however, that leaf calcium concentrations differed markedly between species, as was the case in this study.

Iron toxic soils are generally low in exchangeable calcium (Ottow et al. 1983; Benckiser et al. 1984) and, according to Ottow et al., an insufficient calcium supply is one of the essential prerequisites inducing iron toxicity in rice. Plants deficient in calcium are less able to exclude iron than are normal plants (Tadano 1975; Ottow et al. 1983; Benckiser et al. 1984). This may be a result of increased root membrane permeability which enhances metabolic leakage. This can increase microbial activity in the rhizosphere, increasing oxygen consumption and reductive dissolution of the protective Fe_2O_3 coatings on roots, resulting in excessive iron uptake by mass flow (Ottow et al. 1983; Benckiser et al. 1984). Similarly, results of fertilisation studies by Benckiser et al. (1984) suggest that sufficient calcium supply is important to protect against iron toxicity, and Marschner (1986) states it is very effective in detoxifying high concentrations of some elements in plants.

6.4.3.7 Magnesium

In all four species, there was reduction in both shoot and root magnesium concentration with increasing external iron supply (Figures 6.10a, b). This was significant in all cases except shoots of Juncus effusus. In a similar study, Al-Farraj (1983) found an inverse trend in iron and magnesium concentrations in shoots of Epilobium hirsutum. Shoot concentrations were particularly affected in Rumex hydrolapathum (the most sensitive species to iron), as were root concentrations in both the dicotyledons, possibly because iron affects dicotyledonous root growth more severely than monocotyledonous root growth.

Shoot iron concentration was inversely correlated ($p < 0.05$) with both shoot magnesium concentration and root magnesium concentration in the two dicotyledons. In Holcus lanatus plants this was true for shoot magnesium concentrations only, and in Juncus effusus plants for root magnesium only. Juncus effusus root iron and magnesium concentrations were positively correlated (a trend which Juncus subnodulosus roots had also tended to show) (Al-Farraj 1983).

In waterlogging studies, Nazrul-Islam (1976) found reduced shoot magnesium concentrations as shoot iron content rose in Phalaris arundin-

acea and Rumex acetosa, and in similar studies, Al-Farraj (1983) noted reduced magnesium concentrations in roots of Epilobium hirsutum and Juncus subnodulosus as root iron concentrations rose. Conversely, under iron deficiency, Olsen (1958) noted increased shoot magnesium concentrations.

In all species (except Holcus lanatus) root magnesium concentrations were more severely affected than shoot magnesium concentrations suggesting that magnesium uptake is affected more than translocation. Biddappa et al. (1980) noted that magnesium uptake was very severely reduced under high iron conditions in a number of rice varieties. Marschner (1986) states that the rate of Mg^{2+} uptake can be strongly depressed by a number of other cations (e.g. Mn^{2+}) and thus magnesium deficiency induced by competing cations is a fairly widespread phenomenon. Howeler (1973) reported that magnesium and phosphorus were the two most important iron-induced deficiencies causing oranging in rice and that severity was inversely related to phosphorus, potassium, calcium, and magnesium concentration in the leaf.

The average magnesium concentration in the shoot sufficient for adequate growth is 2.0 mg g^{-1}, though 1.5 mg g^{-1} may suffice in some cereal shoots, and 5.0 mg g^{-1} may be optimum in vegetative parts (Marschner 1986). Scaife and Turner (1983) report that 2.0 mg g^{-1} is the deficiency threshold in leaves of vegetable crops, while in rice leaves this may be as low as 1.0 mg g^{-1} (Ottow et al. 1983). Clearly, magnesium concentrations in Rumex hydrolapathum shoots did not approach such low levels despite a very marked reduction, while concentrations in shoots of the other three species were much lower, even under control conditions. Chlorosis of fully-expanded leaves is the most obvious symptom of magnesium deficiency (Marschner 1986), although this was not apparent in any of the 39 species screened in the Standard Screening Experiment, even after two weeks' growth.

Talbot et al. (1987) observed a significant reduction in leaf magnesium concentration and photosynthetic capacity accompanying a marked increase in leaf iron concentration in the waterlogging-sensitive Salix caprea. Conversely, in the waterlogging tolerant species Salix cinerea, magnesium concentration and photosynthesis were largely unaffected, and increase in leaf iron concentration was less drastic. Talbot and Etherington (1987) report that in both species, photosynthesis was more sensitive to high iron concentrations than was respiration, indicating a specific effect rather than a general metabolic disturbance. The involvement

of a lowered tissue water content in reduced photosynthetic capacity is not clear and warrants further study.

Typical iron toxic soils have low phosphorus, potassium, calcium, and magnesium supply (Benckiser et al. 1984). Insufficient magnesium supply is one of the essential prerequisites inducing iron toxicity, though iron toxic rice leaves were not always deficient in this element (Ottow et al. 1983). Plants deficient in magnesium, calcium, phosphorus, manganese and especially potassium were less able to exclude iron than were normal rice plants, and had higher translocation rates of iron to the shoot (Tadano 1975). Work by Benckiser et al. (1984) suggests that sufficient magnesium supply is important in counteracting iron toxicity in rice, both by improving its iron exclusion capacity and by increasing internal tolerance since, contrary to Tadano (1975), these authors report that iron translocation is improved in well-nourished plants.

6.4.3.8 Manganese

Both shoot and root manganese concentrations fell significantly in all four species, with increasing external iron concentrations (Figures 6.11a, b). This agrees with the findings of Al-Farraj (1983) for Juncus subnodulosus (but not for Epilobium hirsutum). In all four species there was significant negative correlation ($p < 0.05$) between shoot iron concentration and both shoot and root manganese concentrations (except in Rumex hydrolapathum shoots). In Holcus lanatus there was also negative correlation between shoot and root manganese concentrations and root iron concentration, whereas in the dicotyledonous species, root iron concentration correlated positively with shoot manganese concentration only.

This agrees with findings of solution culture work by Somers and Shive (1942), Tanaka and Navasero (1966d), Biddappa et al. (1980) and Chinnery and Harding (1980) that iron and manganese uptake are inversely related, and suggests that one way iron may act is by reducing manganese uptake and possibly causing deficiency (see also Tanaka, Loe and Navasero 1966). However, the minimum manganese concentration measured in shoot tissues after one week's growth in high iron concentrations was well above the levels reported as deficient in leaf tissues (20 $\mu g\ g^{-1}$, Scaife and Turner 1983, Marschner 1986) and indeed well above optimum levels (50 $\mu g\ g^{-1}$, Marschner 1986). A longer growth period might have caused a continued reduction in shoot manganese concentrations.

In addition to competing for absorption sites, iron and manganese also compete at the cellular level (Zhiznevskaya 1972; Marschner 1986), and are thought to be interrelated in metabolic function (Somers and Shive 1942). On the other hand, manganese translocation, which occurs as the free Mn^{2+} ion (Marschner 1986), may be promoted by high iron concentrations (Tanaka and Navasero 1966d). Indeed in the present study, uptake was affected more than translocation, since root manganese concentrations fell considerably more than did shoot concentrations, particularly in the two iron-sensitive species.

In some waterlogging studies (e.g. Jones and Etherington 1970; Howeler 1973; Al-Farraj 1983), tissue manganese concentrations also fell with increasing tissue iron concentrations (as was the case in solution culture). Tanaka and Navasero (1966d) noted that environments which cause iron toxicity may also cause manganese deficiency, but thought it unlikely that the plant's mechanisms involved were interrelated. However, in many waterlogging studies (e.g. Jones 1972a; Ottow et al. 1983; Davies and Singh 1983; Heathcote et al. 1987; Waldren, Etherington and Davies 1987), internal iron and manganese concentrations both increase so that toxic concentrations of both are potentially possible. In such situations, antagonism between the two is not obvious. It is likely that findings will vary depending on the amount of manganese in the substrate (see also Talbot et al. 1987). In most solution culture work with varying iron supply, manganese supply is constant and low.

Manganese is known to ameliorate iron toxicity to some extent (e.g. Tanaka and Navasero 1966d; Etherington and Thomas 1986; cf Foy et al. 1978), and manganese-deficient plants are more susceptible to iron toxicity since they are less able to exclude iron, and may translocate more iron to the shoot (Tadano 1975 cf Tanaka and Navasero 1966d). Somers and Shive (1942) found that for soya bean, absolute concentrations of iron or manganese supplied was immaterial, it was the ratio of the two elements which was important for optimum health. They noted that iron toxicity and manganese deficiency symptoms (which they were unable to distinguish, but which were distinguishable in rice (Tanaka and Navasero 1966d)) appeared whenever the external iron/manganese ratio exceeded 2. Conversely, manganese toxicity and iron deficiency occurred below this value. However, Tanaka and Navasero (1966d) and Chinnery and Harding (1980) have queried the importance of a ratio of 2 in rice and Juncus effusus respectively. In the present study, manganese was supplied at one rate only (0.05 mg l^{-1});

thus plants were subject to an external iron/manganese ratio of between 76 and 2,000. Since no species tested showed any toxicity or deficiency symptoms in control solutions (Fe/Mn = 76) there was no evidence to support the theory that a ratio of 2 is optimal. Somers and Shive (1942) were, however, working at iron concentrations below control values in the present study.

A sap (i.e. soluble) Fe/Mn ratio of 1.5-2.5 has also been reported as critical (Somers and Shive 1942). In the present study, total shoot ratios ranged from about 1 to 3 in the control, and at high iron concentrations those species more severely affected had higher total Fe/Mn ratios than did the less affected species. Reduced performance could be explained by the increase in shoot iron concentration alone, though in the longer term induced manganese deficiency may become more important (see also Etherington and Thomas 1986).

6.4.3.9 Zinc

Ottow et al. (1983) reported that leaves of rice plants suffering from iron toxicity were often deficient in zinc (as well and potassium and phosphorus) in addition to having high iron concentrations. In the present study, mean shoot zinc concentrations were reduced in all four species, especially between the 3.8 and 25 mg Fe l^{-1} treatments. This reduction was only significant in Holcus lanatus.

In all four species, root zinc concentrations fell more markedly than did shoot zinc concentrations (though root changes were only significant in Holcus lanatus and Lysimachia vulgaris) (Figures 6.12a, b). This suggests that zinc uptake may be affected more than translocation by high concentrations of iron, and it is likely that the two elements compete for uptake sites, since hydrated Zn^{2+} and Fe^{2+} ions have similar ionic radii (Marschner 1986).

High concentrations of iron may partly act by inducing zinc deficiency, which is reported to occur below 20 μg Zn g^{-1} leaf tissue (Scaife and Turner 1983; Marschner 1986). If this threshold applies to all species, Holcus lanatus shoots became deficient as iron supply increased which could help explain its iron sensitivity. However, Lysimachia vulgaris was more tolerant of iron despite shoots having very low zinc concentrations (below deficiency threshold) even in control plants. On the other hand, Rumex hydrolapathum (also very sensitive to iron) had

highest shoot zinc concentrations of all species, well above optimum levels. It is possible that high concentrations of iron may act by reducing zinc concentrations in the more sensitive species and that the degree of reduction may be of greater importance than absolute zinc concentrations.

6.4.3.10 Sodium

With increasing external iron supply, sodium concentration increased significantly in the shoots of all species except Lysimachia vulgaris (Figure 6.13a). This was the only element analysed to actually increase in concentration with iron supply (apart from iron itself), and greatest increase occurred in shoots of Juncus effusus, the species most tolerant of iron. Results of similar analyses by Al-Farraj (1983) on Epilobium hirsutum and Juncus subnodulosus did not reveal any such increase in shoot sodium concentrations, and Biddappa et al. (1980) report that at high iron concentrations sodium uptake was reduced in rice.

In the dicotyledonous roots, sodium concentrations fell with increasing iron supply, though this effect was only significant in Rumex hydrolapathum. Conversely, in Juncus effusus there was a marked increase in sodium uptake (Figure 6.13b).

Iron tolerance in Juncus effusus and Lysimachia vulgaris is apparently afforded by iron oxidation in the rhizosphere, resulting in low uptake of iron to the shoot. Juncus effusus is considerably more tolerant of iron than is Lysimachia vulgaris and this is likely to be because monocotyledons are more efficient at oxidation than are dicotyledons. However, it is possible that increased sodium uptake to the shoot may help ameliorate some of the indirect effects of iron toxicity, further increasing the tolerance of Juncus effusus over Lysimachia vulgaris. Iron sensitivity is associated with increased shoot iron concentrations, reduced shoot potassium levels, and a disruption in the water balance of the shoot. Since the sodium content of both shoots and roots of Juncus effusus increased with iron supply it is probably a natrophile. In such plants the sodium ion (Na^+) can substitute for the potassium ion (K^+) in many of its functions including enzyme activation and osmoregulation (Marschner 1986). It is possible that a near-normal shoot water balance can be maintained in this species under conditions of high iron concentration, due to a high shoot sodium concentration.

Table 6.7 Variance in Shoot Dry Weight (y) Accounted for by Shoot Iron Concentration (x)
(Monocotyledons are indicated by bold type)

Species in Tolerance Order	Regression Equation	r	Variance Accounted for by Shoot Iron Concentration	Significance
Juncus effusus	y = -47.08x + 434.88	-0.32	10%	$p < 0.05$
Lysimachia vulgaris	y = -50.05x + 464.22	-0.47	22%	$p < 0.01$
Holcus lanatus	y = -31.61x + 347.77	-0.51	26%	$p < 0.001$
Rumex hydrolapathum	y = -25.20x + 208.65	-0.68	46%	$p < 0.001$

Table 6.8 Variance in Shoot Water Content (y) Accounted for by Shoot Iron Concentration (x)
(Monocotyledons are indicated by bold type)

Species in Tolerance Order	Regression Equation	r	Variance Accounted for by Shoot Iron Concentration	Significance
Juncus effusus	y = -0.436x + 8.22	-0.19	3.4%	NS
Lysimachia vulgaris	y = -2.67x + 12.82	-0.76	57%	$p < 0.001$
Holcus lanatus	y = -1.22x + 10.51	-0.66	43%	$p < 0.001$
Rumex hydrolapathum	y = -2.09x + 14.06	-0.68	46%	$p < 0.001$

6.4.4 Quantitative Assessment of Direct and Indirect Toxicity

6.4.4.1 Introduction

An attempt was made to quantify the relationship between shoot chemical variables and the observed shoot growth reduction under high iron conditions, using regression methods. Similarly the chemical variables were related to the observed disruption of the shoot water balance.

6.4.4.2 Results and Discussion

Single regression of shoot dry weight (y) on shoot iron concentration (x) showed that with increasing sensitivity of the species to iron, both the strength (r and r^2) and the significance of the relationship increased (Table 6.7). This suggests that direct iron toxicity may be responsible for part of the reduction in shoot dry weight, and that the more sensitive species may be affected to a greater extent by direct iron toxicity. The amount of variance accounted for ranged from only 10% in Juncus effusus to 46% in Rumex hydrolapathum, perhaps because indirect iron toxicity (i.e. iron-induced deficiencies of other elements) may also be important. However, results of multivariate regression analysis did not help explain the indirect toxicity, since no consistent trends were found, i.e. in the different species different combinations of elements could most closely account for the reduction in shoot yield.

For all species (except Juncus effusus) variance in shoot water content accounted for by shoot iron concentration was greater than (or as great as) the variance in shoot dry weight which could be accounted for by shoot iron concentration (max. = 57%) (Table 6.8). In all but Juncus effusus the relationship was highly significant. This suggests that shoot iron concentration may directly affect the water balance of the shoot. Another possibility is that high iron supply causes root stunting reducing the surface area for both water and nutrient uptake, thus disrupting the plant's metabolism generally, since the concentration of other elements, namely potassium and nitrogen, gave closer correlation with shoot water content.

6.5 SUMMARY

Iron toxicity in plants is a complex phenomenon, though at least part of the toxic effect of high concentrations of iron is thought to be direct. Iron can affect root growth markedly, and may be damaging if the oxidative exclusion mechanism is not sufficiently effective to keep its uptake to the shoot below a threshold value (reported as 0.3 mg g^{-1} in the literature for rice, but found to be 0.7-0.8 mg g^{-1} in three of the four species under test in the present experimental conditions). High concentrations of iron in the tissues could directly disrupt many cell processes and, although all four species suffered some increase in shoot iron concentration, those species least tolerant of iron tended to translocate greater amounts into the shoot. No evidence of internal tolerance mechanisms was found.

Indirect iron toxicity may also be important, since high concentrations of iron may reduce the uptake of a number of essential elements (e.g. potassium, magnesium, manganese, calcium, zinc and to a lesser extent nitrogen), and may induce their deficiency. This may be partly a result of the effect of iron on root growth, and partly due to competition or more specific inhibition of uptake mechanisms. One important indirect effect of iron is to upset the phosphorus metabolism in intolerant species by making phosphorus unavailable in the plant, particularly by preventing translocation to the shoot. More direct effects on phosphorus metabolism are also possible. Disruption in the water balance of shoots, more especially in the iron-sensitive species, may be directly due to high iron concentrations therein, but is more likely to be a result of damage to and reduced growth of the root system. It may be linked to reduced potassium uptake, and natrophilic species, which tend to accumulate sodium and are able to substitute it for potassium, may avoid the disruption in water balance to some extent. Loss of shoot water potential (as well as reduced magnesium concentration in the leaves) is known to reduce photosynthetic capacity.

Iron toxicity can be aggravated by a low nutrient supply and ameliorated by fertilisation. Well-nourished plants may not display iron toxicity symptoms despite having high concentrations of iron in the shoot (Benckiser *et al.* 1984).

Deficiency of any one or a combination of the essential elements (either iron-induced, or due to low supply) can severely upset the plant's

metabolism in a variety of ways, including reducing the effectiveness of the oxidative detoxification mechanism. This may result in greater quantities of iron reaching the shoot. It is possible that deficiencies of different elements may occur in different species, or under different circumstances. Deficiency of any particular element is hard to define due to differing species requirements and variation in values reported in the literature. Strictly, shoot and leaf analyses should not be compared, and gross tissue analyses fail to demonstrate the exact location and form of an element within the tissues and cells.

It should be remembered that in this study, however, high concentrations of iron were maintained by keeping the pH of the nutrient solution low. Plants may respond differently in the field, since iron may be maintained in solution by low oxidation-reduction potentials (often at a high pH).

CHAPTER SEVEN

IRON IN THE WETLAND ENVIRONMENT

7.1 INTRODUCTION

7.1.1 General

The great variety of methods employed to analyse the iron content of peats and soils makes comparison of values quoted within the literature difficult. Total, extractable, active and soluble iron are frequently estimated, though results often do not correlate. The 'active iron' ratio (McKeague and Day 1966, Davies and Singh 1983) indicates past flooding history, while extractable and soluble iron most closely represent plant-available iron. These may vary seasonally with flooding (e.g. Jones 1972a, 1973; Nazrul-Islam 1976; Sanderson and Armstrong 1980b), though reduction of iron occurs only gradually on submergence (Jeffery 1961), while oxidation occurs much more rapidly on drying of soil (Ignatieff 1941).

Some workers (e.g. Proctor 1974) consider that measurement of the chemistry of the substratum may be more valuable than that of fen water, in terms of assessing vegetation-nutrient relations. However, greater chemical variation is often found among replicate peat samples than among replicate water samples (Wheeler 1983), and in some of the drier sites, water samples may be difficult to obtain.

The aim of this chapter was to test the relationship between iron tolerance as assessed experimentally, and species distribution in the field, using such field data as are available.

7.1.2 Extractable Iron

A variety of extractants may be used (e.g. Andersson 1975; Olson and Ellis 1982), and generally the amount of iron extracted is greater the lower the pH of the extractant (Ponnamperuma 1972; Andersson 1975). Some extractants specifically remove reduced iron only (e.g. $AlCl_3$, Ignatieff 1941; air-free ammonium acetate, Jones 1973, Davies and Singh 1983) while

others remove all soluble iron, exchangeable ferrous iron and part of the exchangeable ferric iron (e.g. ammonium acetate, Sanderson and Armstrong 1980b).

Some workers have extracted air- or even oven-dried samples (e.g. Olson 1947; McVean and Ratcliffe 1962; Jones 1971b), while others have used fresh peat or soil (e.g. Jones and Etherington 1970; Jones 1973; Sanderson and Armstrong 1980b; Giller 1982; Wheeler 1983; Al-Farraj 1983; Wheeler and Shaw 1987), though iron release differs between fresh and dry soils (Howeler 1973; Waldren, Davies and Etherington 1987a). Extractable iron concentrations increase with the relative wetness of a soil (Jones 1972a), and with the duration of flooding (Heathcote *et al.* 1987; Talbot *et al.* 1987), since submergence causes iron reduction. Indeed, alternate flooding and drying (see also Nhung and Ponnamperuma 1966) and mid-season drainage have been used effectively in keeping soluble iron concentrations low in paddy fields (Howeler 1973). Experimental flooding of a soil may not yield the same results as flooding in the field (Jones 1972a, 1973).

A further difficulty in the comparison of extractable chemical data arises since results may be expressed on a dry weight basis (e.g. Jones and Etherington 1970; Jones 1971b; Sanderson and Armstrong 1980b; Waldren, Davies and Etherington 1987a; Talbot *et al.* 1987; Heathcote *et al.* 1987), or on a volume basis (e.g. Giller 1982; Wheeler 1983; Al-Farraj 1983; Wheeler, Al-Farraj and Cook 1985; Giller and Wheeler 1986; Wheeler and Shaw 1987). Within each study, apart from the last, only a few samples were compared, involving a small selection of species.

7.1.3 Soluble Iron

Water-soluble iron may make comparison within the literature easier, though there are still differences in approach (e.g. acidification of samples, or filtration through millipore filters to remove colloidal iron), and many estimates may include colloidal iron. Concentrations rarely exceed 20 mg Fe l^{-1} in neutral and calcareous soils (Ponnamperuma 1972; see also Al-Farraj 1983; Wheeler and Giller 1984; Wheeler, Al-Farraj and Cook 1985), while values of 3,000 to > 5,000 mg l^{-1} can be obtained if pH and oxidation-reduction potential are sufficiently low (Gotoh and Patrick 1974), and if the dominant solid phase is $Fe_3(OH)_8$ (Nhung and Ponnamperuma 1966; Foy *et al.* 1978).

Soluble iron, a widely estimated index of iron availability, is

presumed to give the best comparison with iron concentrations supplied in solution culture. However, the complexity of natural soil solutions bears little relation to artificial nutrient solutions in which iron uptake is normally studied (Fitter and Hay 1981), and water-soluble iron may only be measurable in soils with pH < 5.5 (Olson 1947). Iron tends to precipitate from solution in culture work making the actual amount of iron supplied difficult to predict. There is also evidence from both iron and other metal toxicity studies that different solution culture methods can yield different results (e.g. Tanaka, Loe and Navasero 1966; Shaw 1984).

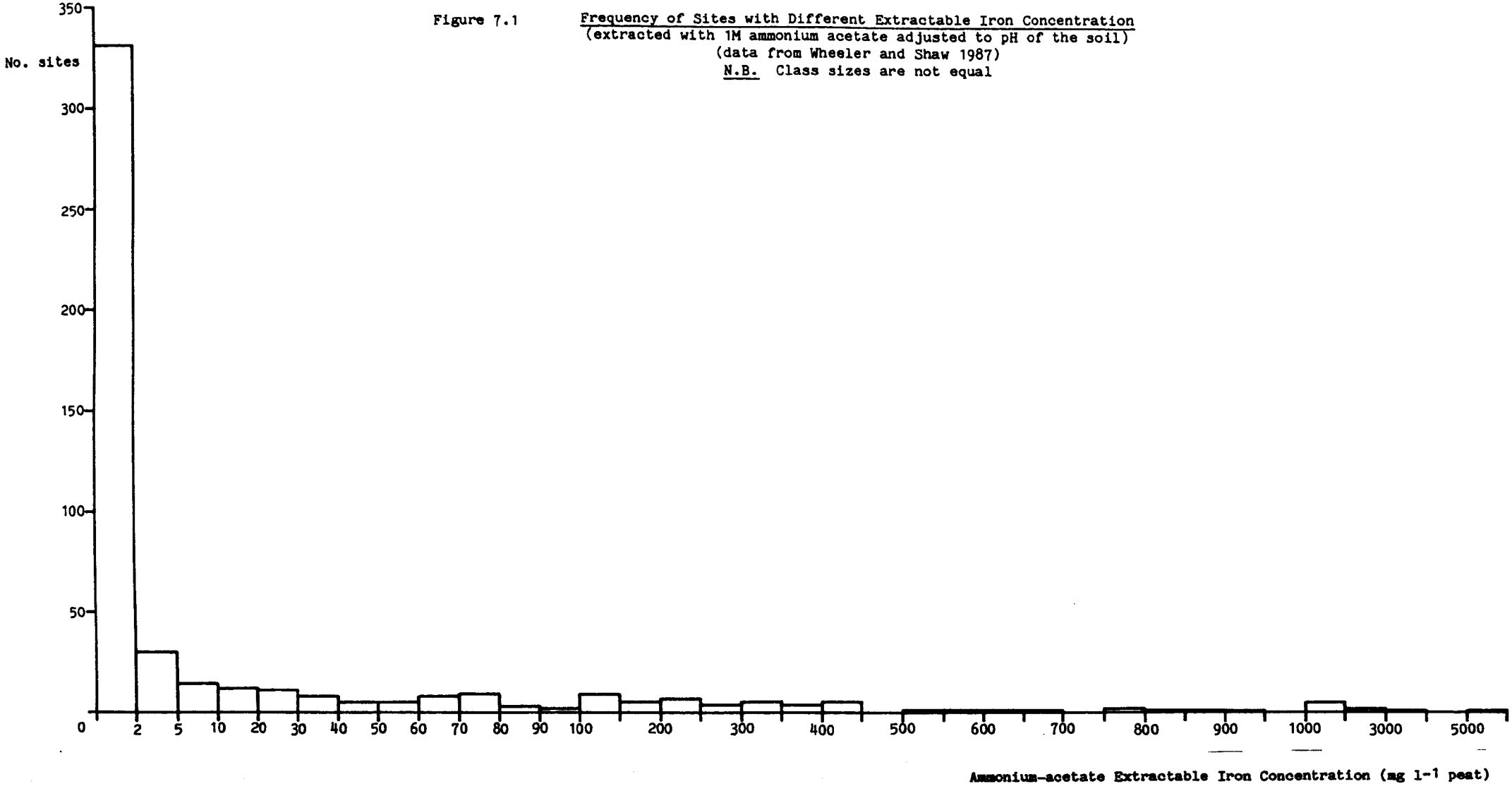
7.2 METHODS

As part of a survey of fens in England and Wales (Wheeler and Shaw 1987), 495 sites were visited in summer. Species composition was recorded at each, and a number of field measurements made, including oxidation-reduction potential and depth of the water table.

The sites were revisited in autumn, and peat samples were collected for chemical analysis, and for estimation of fertility using a phytometric method (Al-Farraj, Giller and Wheeler 1984). After measuring the pH of the fresh peat in a 1:1 slurry, soluble plus exchangeable iron was extracted by shaking 38 ml fresh peat for 1 hour with 200 ml 1M ammonium acetate adjusted to the pH of the sample (see Gotoh and Patrick 1974; Andersson 1975). Iron was determined by atomic absorption spectrophotometry; potassium, calcium, magnesium, manganese and sodium were estimated on the same extracts. Nitrogen and phosphorus were analysed in extracts of 2M KCl and 0.5M $NaHCO_3$ (pH 8.5) respectively. In each case, five replicate extracts were made and results were expressed on a volume basis (i.e. mg l^{-1} fresh peat). This made comparisons possible with the results of Giller (1982), Al-Farraj (1983), Wheeler (1983), and Wheeler, Al-Farraj and Cook (1985), who in addition used the same methodology.

The iron tolerance of a species (Σ%RGR) was compared with the amounts of extractable iron and other elements measured in the sites in which the species grew. It should be noted, however, that this survey was not undertaken with iron tolerance in mind, and does not necessarily represent the typical range of iron conditions. Indeed, highest exchangeable iron concentrations are frequently reported to occur in summer (e.g. Jones 1973; Nazrul-Islam 1976) when microbial activity is at its peak.

Figure 7.1 Frequency of Sites with Different Extractable Iron Concentration
(extracted with 1M ammonium acetate adjusted to pH of the soil)
(data from Wheeler and Shaw 1987)
N.B. Class sizes are not equal



7.3 RESULTS AND DISCUSSION

7.3.1 Extractable Iron

7.3.1.1 Extractable Iron Distribution among Sites

Mean ammonium acetate-extractable (i.e. soluble plus exchangeable) iron contents of the 495 sites are presented in Figure 7.1. The majority of sites (67%) had low iron concentrations (< 2 mg l^{-1}), a substantial number contained up to several hundred mg Fe l^{-1}, while only a few sites had very high extractable iron concentrations (max. 5,272 mg l^{-1} peat). Owing to the different methods of estimating iron availability, and in particular extractable iron content, the different ways of expressing the results, plus the fact that no other large-scale peat analyses had been published, it was difficult to compare the iron content of sites in the survey of Wheeler and Shaw (1987) with others in the literature. This highlights both the need for a standardised procedure, and the need to state precisely the techniques used.

7.3.1.2 Species Distribution in Sites with Different Extractable Iron Status

Table 7.1 shows the occurrence of each screened species in sites with different extractable iron contents (> 2 mg l^{-1} peat). Species are presented in rank order of iron tolerance (Σ%RGR), and the maximum extractable iron concentration at which each species occurred is also noted. The more iron-tolerant species tend to occur more frequently in sites with > 2 mg Fe l^{-1} than do sensitive species, particularly in those sites with the highest extractable iron contents. Conversely, the very sensitive species are largely confined to sites with less than 50 mg l^{-1} peat extractable iron. In fact, significant correlation was found between a species' iron tolerance index and the maximum ($r = 0.30$, $p < 0.05$) and mean ($r = 0.46$, $p < 0.01$) iron concentration at which the species was found. Likewise, regression of iron tolerance against mean extractable iron concentration was significant ($p < 0.05$), though regression of iron tolerance index on maximum iron concentration was not. This was partly because certain tolerant species were only found at low iron concentrations (e.g. *Iris pseudacorus* and *Parnassia palustris*), possibly because sites with high

Table 7.1 Number of Occurrences of each Screened Species in Sites Classified by Extractable Iron Concentration (> 2 mg Fe l^{-1} fresh peat)
(Raw Data from Wheeler and Shaw 1987)
(Species are presented in order of tolerance to iron, based on Σ%RGR tolerance index)

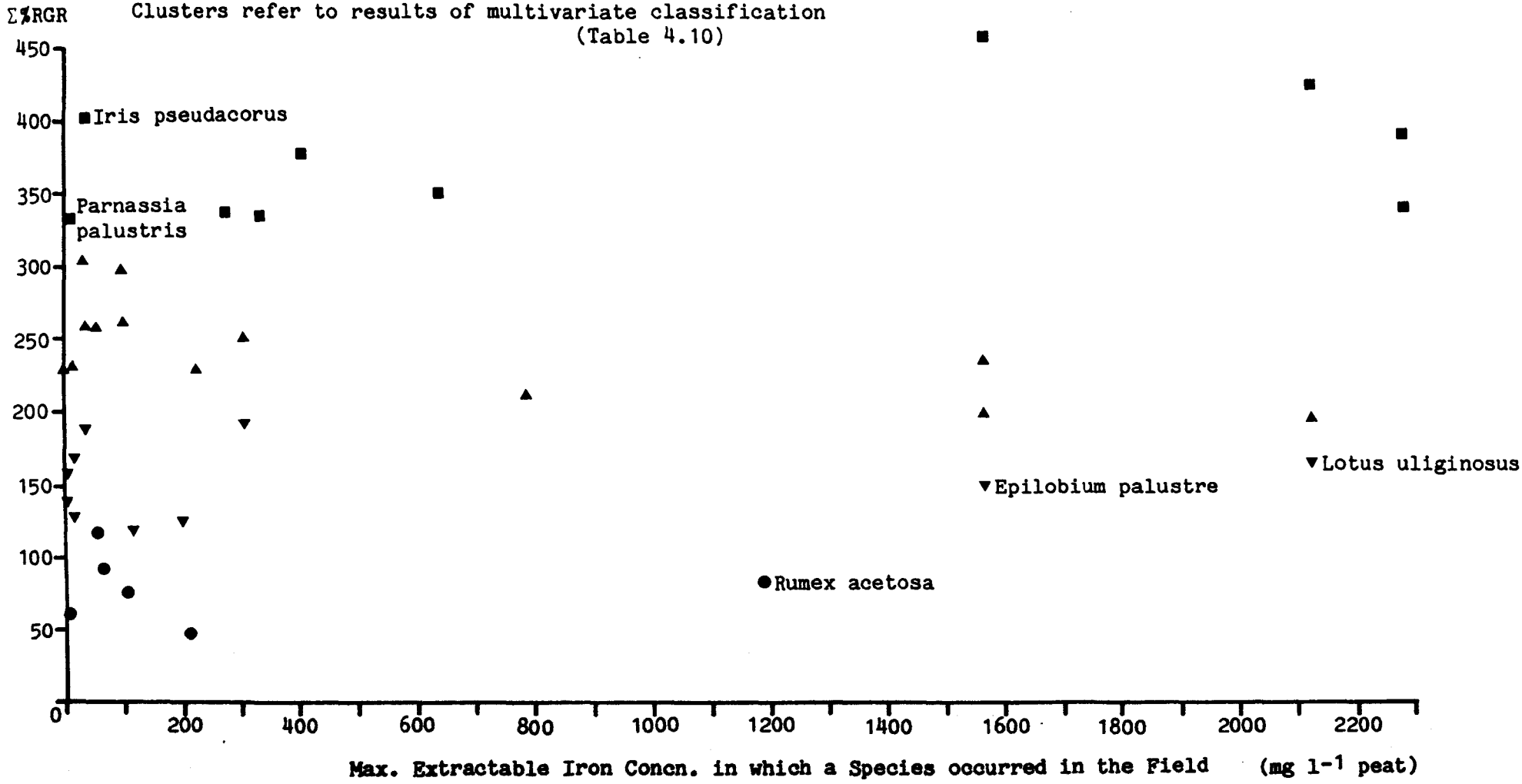
	Extractable Iron Concentration (mg l^{-1} fresh peat)				
Species	2-5	5-50	50-500	>500	Maximum
Carex echinata	1	10	12	6	1569.9
Juncus effusus	5	8	14	4	2124.8
Iris pseudacorus	2	1	0	0	36.2
Molinia caerulea	12	35	48	13	2278.4
Carex pulicaris	1	4	1	0	411.9
Juncus articulatus	2	4	2	1	639.2
Agrostis stolonifera	11	15	19	5	2278.4
Eriophorum latifolium	0	0	1	0	283.1
Pedicularis palustris	5	5	6	0	337.7
Parnassia palustris	0	4	0	0	12.3
Carex lepidocarpa	3	3	0	0	29.1
Juncus inflexus	1	1	1	0	98.7
Lythrum salicaria	5	0	1	0	103.4
Lysimachia vulgaris	2	2	0	0	36.2
Phalaris arundinacea	1	0	1	0	55.0
Potentilla palustris	7	14	6	0	305.5
Ranunculus flammula	4	7	12	1	1569.9
Briza media	1	3	0	0	17.7
Carex diandra	1	0	0	0	2.1
Caltha palustris	3	4	1	0	225.8
Juncus subnodulosus	9	4	3	1	787.2
Holcus lanatus	7	10	5	2	1569.9
Valeriana dioica	4	2	2	1	2124.8
Galium palustre	9	14	8	0	305.5
Carex appropinquata	0	2	0	0	34.8
Lotus uliginosus	5	7	6	2	2124.8
Trifolium pratense	0	1	0	0	17.7
Primula farinosa	0	1	0	0	7.1
Epilobium palustre	5	12	10	2	1569.9
Thalictrum flavum	1	0	0	0	4.1
Galium aparine	1	1	0	0	15.8
Eupatorium cannabinum	3	0	2	0	201.7
Valeriana officinalis	4	5	1	0	103.4
Filipendula ulmaria	8	4	1	0	54.2
Lychnis flos-cuculi	4	6	1	0	65.2
Rumex acetosa	1	2	0	1	1186.8
Scrophularia auriculata	1	1	1	0	103.4
Rumex hydrolapathum	0	1	0	0	6.9
Epilobium hirsutum	2	0	2	0	209.8

KEY

Cluster 1 ■
Cluster 2 ▲
Cluster 3 ▼
Cluster 4 ●

Figure 7.2 Plot of Iron Tolerance Index (Σ%RGR) against Maximum ammonium acetate-extractable Iron Concentration in which a Species occurred in the Field. (data from Wheeler and Shaw 1987)

Clusters refer to results of multivariate classification (Table 4.10)



iron concentrations are relatively rare. Lack of significance also occurred because some iron-sensitive species were found in sites with high extractable iron content (e.g. Lotus uliginosus, Rumex acetosa, Epilobium palustre) (Figure 7.2). The reason for intolerant species occurring in sites with a high extractable iron concentration was investigated further.

7.3.2 Soluble Iron

Samples with more than an arbitrarily chosen minimum concentration of extractable iron (2 mg l^{-1} peat) and which contained at least one of the 39 species screened for iron tolerance were selected (i.e. 126 sites, with a maximum of 2,278 mg Fe l^{-1} peat). These were then categorised on the iron-sensitivity of species growing in them (Table 7.2). Iron sensitivity was based on the four clusters obtained by multivariate classification techniques (Figure 4.10, see also Table 7.1). Species in cluster 1 are most tolerant and those in cluster 4 are most sensitive.

Table 7.2 Classification of Sites based on Iron-sensitivity of Species they Support

	Site Category			
Species Composition	A	B	C	D
Cluster 1	X	X	X	X
Cluster 2		X	X	X
Cluster 3			X	X
Cluster 4				X

Clusters 1-4 relate to clusters produced by multivariate analysis (Ward's Method) of standardised RGR data (Table 4.10 and Table 7.1). To belong to a given category (A-D), sites had to contain at least one species from a particular cluster, but none of the more sensitive species, (i.e. none from a cluster of higher number).

The drawback of this method of classifying sites was that the iron-sensitivity of species other than the 39 tested was not known and could have potentially affected the site classification, i.e. the occurrence of species more sensitive than those screened would take a site into a lower category (A to D) in Table 7.2.

Table 7.3 Sites Supporting Iron-Sensitive Species under Conditions Likely to Provide High Soluble Iron Concentrations

Site	Extractable Iron Concn. (mg l^{-1} peat)	Oxidation Reduction Potential (E_7)	pH	Iron-sensitive species present: Domin Value												
				Galium palustre	Carex appropinquata	Lotus uliginosus	Trifolium pratense	Primula farinosa	Epilobium palustre	Thalictrum flavum	Galium aparine	Eupatorium cannabinum	Valeriana officinalis	Filipendula ulmaria	Lychnis flos-cuculi	Rumex acetosa	Scrophularia auriculata	Rumex hydrolapathum	Epilobium hirsutum
Gormire 2	84.60	-105	4.2						1										
Gormire 1	140.54	-61	5.6	3					3										
Statts Bridge 3	171.16	+35	5.4			1													
Statts Bridge 4	203.88	-186	4.4	1					2										
Stoney Cross	288.24	-195	5.0			3													
Creason Wood	342.94	+23	4.4			2													
Fox Tor Mires	1186.8	-34	4.5													5			
East Ruston Common	2124.8	+116	2.2			1													

Oxidation-reduction potential and pH are strong factors influencing the solubility and hence plant availability of iron (e.g. Martin 1968; Gotoh and Patrick 1974; DeLaune, Reddy and Patrick 1981; Armstrong 1982), and may be more important than total iron content (Ponnamperuma 1972). The distribution between the water-soluble and exchangeable fractions is also highly pH-dependent (Gotoh and Patrick 1974). Figure 7.3 summarises the occurrence of species in relation to these three variables. It shows that sites containing the strongly iron-sensitive species (Group D) or moderately sensitive species (Group C) tend to have a combination of the following:-

a) low extractable iron content,

b) high pH,

c) high oxidation-reduction potential,

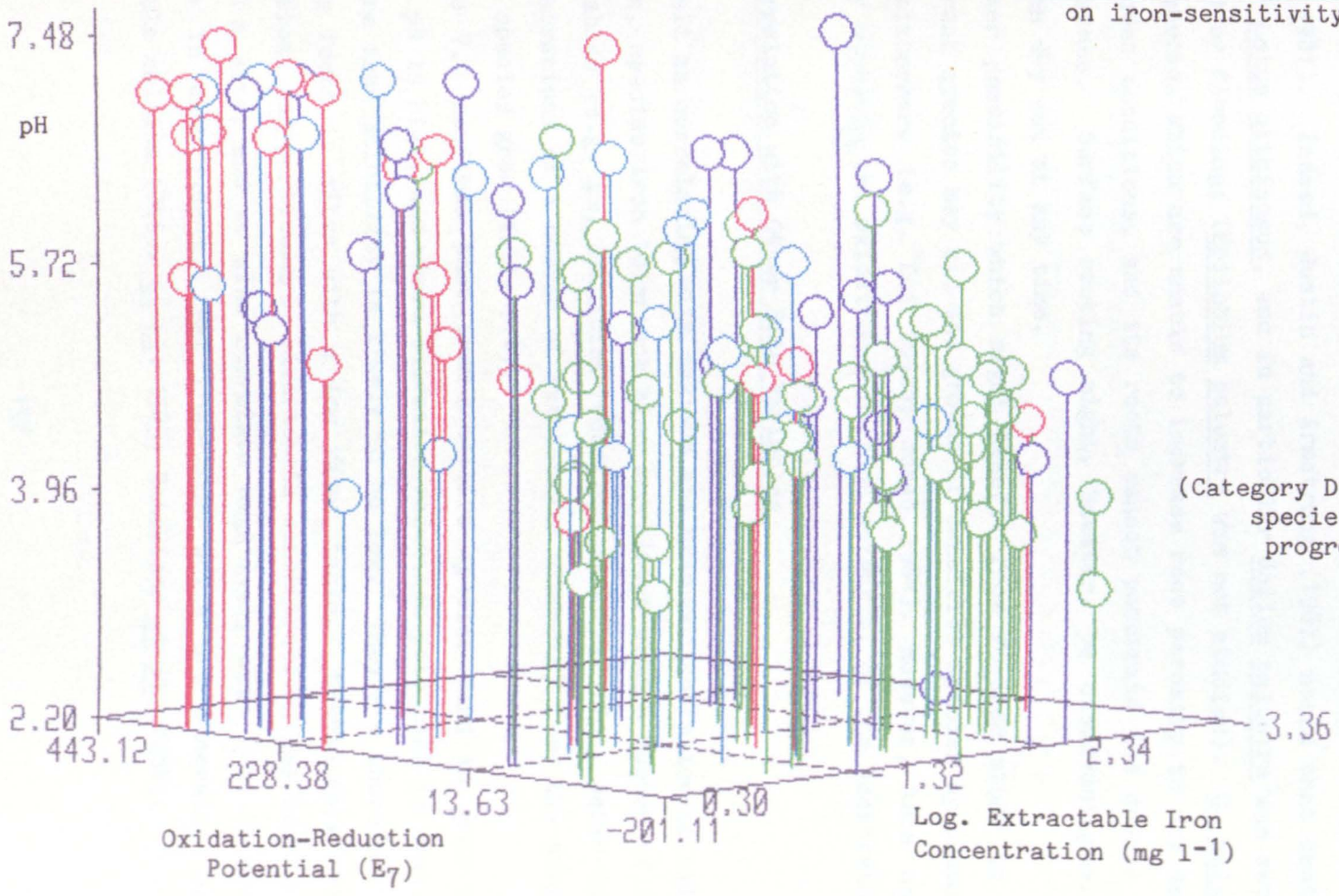
and hence low iron solubility. In contrast, those sites with only the more tolerant species, (Groups A and B) may have a lower pH and/or a low oxidation-reduction potential as well as a high extractable iron concentration, and so are more likely to have a higher content of soluble iron. Thus soluble iron (e.g. in dialysis tubing, Wheeler and Giller 1984) might have been a better measure of iron availability, and might have been more directly comparable with the solution culture results, though samples from some of the drier sites may have been hard to obtain.

The occurrence of iron-intolerant species in sites of high extractable iron content but with high oxidation-reduction potential and pH may mean that ammonium-acetate extractable iron can be a poor measure of iron availability.

In a very few sites (see Table 7.3 and Figure 7.3), iron-sensitive species were found under conditions where the extractable iron concentration with the measured combination of pH and oxidation-reduction potential might produce a relatively high soluble iron concentration. In three of the sites however, the only moderately sensitive species was represented by a domin value of one, and this could have been due to the presence of only one unhealthy plant.

It is of interest to note that of the 16 possible sensitive and moderately sensitive species, three in particular (i.e. Epilobium palustre, Galium palustre, and Lotus uliginosus) occurred at several of the sites, while many were not found at all. This could indicate an error in the tolerance measurement of these species, e.g. in the comparison of solution culture results with field observations, though Al-Farraj (1983)

Figure 7.3 Ammonium acetate-extractable Iron concentration, Oxidation-reduction potential and pH of Sites supporting Species screened for iron tolerance. (Sites are colour-coded on iron-sensitivity of the species they support.)



KEY

Colour	Site Category
Green	A
Blue	B
Cyan	C
Red	D

(Category D sites support the most iron-sensitive species, categories C to A only support progressively more tolerant species.)

Figure 7.3

had also noted E. palustre growing in iron-rich sites despite the fact he had found it to be iron-sensitive. Alternatively it may suggest the evolution of tolerant populations under high iron conditions, as has been demonstrated in other metal toxicity studies (e.g. Antonovics, Bradshaw and Turner, 1971). Differential tolerance of Galium palustre, Lotus uliginosus, and Epilobium palustre populations may warrant further study, although no difference was found in the tolerance of the two Molinia populations studied in this thesis.

An alternative and more likely explanation is that these species are able to inhabit iron-rich sites by confining their roots to the less-reducing surface layers (e.g. Martin 1968; Nazrul-Islam 1976; Justin and Armstrong 1987). Indeed, Justin and Armstrong (1987) noted that depth of rooting of Lotus uliginosus, and in particular Galium palustre was severely reduced by flooding; (Epilobium palustre was not studied). G. palustre is among species which are unable to increase root porosity to any degree under flooded conditions, and its roots cannot penetrate the deeper more reducing zones. Surface rooting might, however, be disadvantageous in sites which dry out at any time.

Another possibility which might benefit from further study is that iron-tolerant species may aid the growth of sensitive species by oxidation of the rhizosphere (e.g. Doi 1952b; Schat 1984). However, this should operate by increasing the oxidation-reduction potential of the substrate.

7.3.3 Correlation with Other Site Variables

As well as correlating with maximum and mean concentration of extractable iron, species iron tolerance also correlated with a number of other site variables (e.g. pH, oxidation-reduction potential (E_7), water table, and concentrations of a number of extractable elements) from the sites in which the species grew (Table 7.4).

Table 7.4 confirms that iron-sensitive species tend to grow where substrate pH is high, and where oxidation-reduction potential is high, and hence where iron solubility is likely to be low. They are therefore more frequently found in sites with a low water table. Extractable calcium concentration, and particularly bicarbonate concentration, may help govern the pH of a site, and so also correlate negatively with iron tolerance. This ties in with reports that iron-toxic soils are generally low in exchangeable calcium (Ottow et al. 1983; Benckiser et al. 1984).

Table 7.4 Correlation of Site Variables with Species Iron Tolerance Index

Variable	Correlation Coefficient (r)	Significance
a. Positive Correlation		
Mean extractable nitrogen concentration	+ .4050	$p < 0.01$
Mean extractable manganese concentration	+ .2935	$p < 0.05$
Mean water table	+ .2837	$p < 0.05$
b. Negative Correlation		
Mean oxidation-reduction potential (E_7)	- .5183	$p < 0.001$
Mean extractable phosphorus concentration	- .4649	$p < 0.001$
Mean bicarbonate concentration	- .4120	$p < 0.01$
Mean substrate pH	- .3715	$p < 0.01$
Mean extractable calcium concentration	- .3277	$p < 0.05$
c. No Significant Correlation		
Mean extractable magnesium concentration		
Mean extractable potassium concentration		
Mean extractable sodium concentration		

There are a number of reports that organic matter content is another important factor influencing iron availability (e.g. Robinson 1930; Ignatieff 1941; Mandal 1962; Kuraev 1966; Jones 1973; Nazrul-Islam 1976; Olson and Ellis 1982; Benckiser et al. 1984). This is due both to the fact that microorganisms in the organic matter rapidly consume available oxygen and cause iron reduction (Mandal 1962), and also to the large surface area for ferrous iron absorption (Ignatieff 1941). Indeed, minimum organic matter addition is advocated to avoid iron toxicity in rice (Ponnamperuma, Bradfield and Peech 1955). Unfortunately, organic matter content of the sites was not investigated and so the link with iron tolerance could not be tested.

High concentrations of extractable manganese tend to be found in sites supporting iron tolerant species, probably because manganese availability is controlled in a similar way to iron availability.

The occurrence of iron tolerant species in sites with higher extractable nitrogen content may not be genuine, since Wheeler and Shaw (1987) have shown that extractable nitrogen is not a good measure of plant availability. The involvement of nitrogen in ameliorating iron toxicity is thought to be unimportant, at least in rice (Section 6.4.3.3).

The lack of correlation between mean extractable potassium and magnesium concentrations and iron tolerance is interesting, since there is much evidence that plants insufficiently supplied with potassium are prone to iron toxicity (e.g. Tanaka, Loe and Navasero 1966; Howeler 1973; Nazrul-Islam 1976; Foy et al. 1978; Ottow et al. 1983; Benckiser et al. 1984) (see Section 6.4.3.5). Similarly, a low magnesium supply tends to induce iron toxicity in rice (Ottow et al. 1983; Benckiser et al. 1984) (Section 6.4.3.7). It would be of interest to study the effect of nutrient levels, particularly of potassium and possibly also of nitrogen, on iron tolerance of a range of native species.

7.3.4 Iron Tolerance and Site Fertility

Site fertility was assessed phytometrically (Al-Farraj, Giller and Wheeler 1984). However, for rich-fen sites Epilobium hirsutum was used as the phytometer, while for poor-fen sites Phalaris arundinacea was used. Percentage mortality for each species was also recorded. There was negative correlation between a species iron tolerance and the mean fertility of the sites in which it grew using either phytometer (Table 7.5).

Table 7.5 Correlation of Species Iron Tolerance Index with Mean Site Fertility in which Each Species Grew, and with Mean % Mortality of the 2 Phytometers.

Variable	Correlation Coefficient, r	Significance
Mean site fertility (*E. hirsutum*)	-0.46	$p < 0.01$
Mean site fertility (*P. arundinacea*)	-0.32	$p < 0.05$
Mean % Mortality (*E. hirsutum*)	+0.57	$p < 0.001$
Mean % Mortality (*P. arundinacea*)	-	NS

The fact that the less tolerant species tend to grow in the more fertile sites and in those sites with higher extractable phosphorus content (Table 7.4) is in accord with the fact that iron-sensitive species often have a high relative growth rate and also tend to be phosphorus-inefficient, suffering from external and internal phosphorus immobilisation by iron. Conversely, phosphorus uptake into iron-tolerant species in high-iron situations is much less affected, and they often have a low relative growth rate. This ties in with reports that iron toxicity symptoms in rice are more apparent in soils with low phosphorus supply (e.g. Ottow *et al.* 1983; Benckiser *et al.* 1984), though Wheeler and Shaw (1987) found that phosphorus values alone do not always give a reliable estimate of fertility. They found no negative correlation between extractable iron concentration and fertility (as assessed by *E. hirsutum* phytometry), and concluded that iron concentration may not have been a phytometric constraint of this species' response. However, the fact that iron-intolerant species tend not to grow in those sites which produced a high rate of mortality of *E. hirsutum* (which is itself sensitive to iron)(Table 7.5), suggests that this species may not be a suitable phytometer for sites rich in iron. The more iron-tolerant *Phalaris arundinacea* may be a better phytometer, since far fewer deaths occurred, and there was no correlation between percentage mortality and iron sensitivity of species growing in any particular site.

7.4 SUMMARY

Thus, iron availability has an important role in determining the distribution of wetland plant species. Indeed, Wheeler and Shaw (1987) showed that extractable iron concentration was the second most important factor after site fertility (or after extractable phosphorus concentration if only chemical factors are considered) in accounting for species richness in regression models in wetland sites.

However, soluble iron concentration may be a better measure of iron availability than was ammonium acetate-extractable iron concentration. Iron solubility is controlled by the oxidation-reduction potential and pH of a site, which is possibly more important than total iron content. These in turn may be influenced by the depth of the water table, organic matter content, and bicarbonate and extractable calcium concentrations at the site.

There is also evidence that site fertility and extractable phosphorus concentration may help control the distribution of iron-sensitive species.

CHAPTER EIGHT

INTERACTIONS OF IRON WITH OTHER IONS IN THE GROWTH MEDIUM

8.1 CALCIUM AND BICARBONATE

8.1.1 Introduction

Many high-pH fen soils (pH > 6.0) which contain large amounts of extractable iron also contain high concentrations of extractable calcium, or are irrigated by bicarbonate-rich water (Wheeler and Shaw 1987). Indeed, the bicarbonate ion often governs the pH of a site, and concentrations in the soil solution may exceed 600 mg l^{-1} (Marschner 1986). It is possible that both these ions may ameliorate iron toxicity to all or selected species, other than by pH effects.

There is some evidence that increasing calcium supplies can ameliorate iron toxicity (e.g. Skeen 1929; Olsen 1958), possibly by inhibiting iron uptake (Zhiznevskaya 1972). Iron-toxic soils frequently have low concentrations of exchangeable calcium (Ottow et al. 1983; Benckiser et al. 1984), and plants deficient in calcium are less able to exclude iron by rhizosphere oxidation (Ottow et al. 1983; Ando et al. 1983; Benckiser et al. 1984). Indeed, paddy soils are frequently limed to reduce the effects of iron toxicity (Tanaka and Navasero 1966c; Nhung and Ponnamperuma 1966; Tadano 1975). There is much evidence that manganese and aluminium toxicity may also be alleviated by high calcium concentrations (Skeen 1929; Robson and Loneragan 1970; Foy et al. 1978; Nazrul-Islam 1976, 1986; Alva et al. 1986; Marschner 1986; Kinraide and Parker 1987; Zhao et al. 1987), and Al^{3+} and Ca^{2+} have been shown to act antagonistically in altering root membrane permeability characteristics (Zhao et al. 1987). Although calcium amelioration of metal toxicity may be partly a pH effect, there is evidence that divalent ions are more effective than monovalent ones (Kinraide and Parker 1987).

At low iron supply, bicarbonate ions are known to antagonise the uptake, translocation and utilisation of iron in several plant species (e.g. Brown 1960; Woolhouse 1966; Venkatraju and Marschner 1981; Wallace and Abou-Zamzam 1984; Kolesch et al. 1984). Further, high bicarbonate

concentrations are thought to be a major factor contributing to the development of iron chlorosis (e.g. Boxma 1972; Venkatraju and Marschner 1981; Chen and Barak 1982; Coulombe et al. 1982, 1984; Marschner 1986), though there is controversy concerning the mechanisms involved (e.g. Brown 1960; Woolhouse 1966; Venkatraju and Marschner 1981; Marschner, Romheld and Kissel 1986).

Woolhouse (1966) presents evidence that at constant pH, bicarbonate affects the uptake and translocation of iron by four species of grass differentially. Similar differences have been shown in soybean cultivars (Fleming et al. 1984). Accordingly, it was decided to investigate whether bicarbonate might also have a differential effect on iron uptake under conditions of high iron supply, and so influence a species' iron tolerance, since tolerance seems to be a function of iron transport to the shoot. Two species were selected, Epilobium hirsutum, which is very sensitive to iron, and Juncus subnodulosus which is less sensitive (Section 4.2.3; Al-Farraj 1983; Wheeler, Al-Farraj and Cook 1985). The same two species were used to test for amelioration of iron toxicity by high calcium concentrations.

8.1.2 Pilot Experiment to Test the Effect of High Concentrations of Calcium and Bicarbonate separately on Iron Toxicity

8.1.2.1 Methods

A pilot experiment was set up to test the effect of high concentrations of calcium and bicarbonate separately on Juncus subnodulosus and Epilobium hirsutum growing in a nutrient solution of high iron concentration.

The experimental design was 3 treatments x 2 species x 3 replicates x 4 plants (Eh) or 5 plants (Js), and conditions were similar to that of the Standard Screening Method (Section 2.4.4). Solutions were placed in blackened plastic tubs (capacity 500 ml) with the plants floating in rafts on the solution. Solutions were modified 10% Rorison solution adjusted to pH 5.5 at each solution change with 0.5M H_2SO_4 and 1M NaOH. Iron was supplied as ferrous sulphate, and bicarbonate as sodium bicarbonate ($NaHCO_3$). In the high calcium treatment, calcium chloride ($CaCl_2.6H_2O$) was used in place of calcium nitrate ($Ca(NO_3)_2.4H_2O$), with sodium nitrate ($NaNO_3$) as the nitrogen source. Treatments are shown in Table 8.1, and

concentrations of calcium and bicarbonate were chosen to simulate concentrations measured in calcareous fens. Experimental duration was five weeks, with solution changes three times per week. The pH of the solutions was monitored over a 3-day period, and analyses of soluble iron were made on one occasion, after the plants had been growing in the solutions for three days. A small subsample was centrifuged at 5,000 rpm for 10 minutes prior to analysis by atomic absorption spectrophotometry. Observations were made on the condition of the plants at each solution change.

Table 8.1 Concentrations of Iron, Calcium and Bicarbonate (mg l-1) Supplied in Pilot Experiment Treatments

Treatment	Iron (as $FeSO_4$)	Calcium	Bicarbonate (as $NaHCO_3$)
High Iron	100	8 (as $Ca(NO_3)_2.4H_2O$)	]
High Calcium	100	100 (as $CaCl_2.6H_2O$)	]None added]
High Bicarbonate	100	8 (as $Ca(NO_3)_2.4H_2O$)	400

8.1.2.2 Results

8.1.2.2.1 pH

There was no significant difference between the pH changes in the high iron treatment and in the high calcium treatment. In both cases, the pH fell from 5.5 to around 4.2 in the first 24 hours. However, in the high bicarbonate treatment, the pH rose slightly initially and then fell gradually over the three days to about 4.9.

8.1.2.2.2 Soluble Iron

Concentrations of iron in solution after three days growth of plants are presented in Table 8.2. One-way analysis of variance showed that within each treatment there was no significant difference between the iron found in solution beneath each species; nor was there any significant difference between soluble iron concentrations in the high iron and high

calcium treatments. However, the high bicarbonate treatments contained significantly less soluble iron ($p < 0.05$) than did any other treatment.

Table 8.2 Iron Concentrations in Solution after 3 days Growth of Plants
(Means of 3 replicates unless stated, ± 1 SE)
(Originally supplied at 100 mg Fe l^{-1})

Treatment	Juncus subnodulosus	Epilobium hirsutum
High Iron	97.95 ± 2.05 (2 reps)	92.10 ± 6.45 (2 reps)
High Calcium	97.88 ± 0.18	94.05 ± 2.70
High Bicarbonate	14.37 ± 0.59	13.38 ± 0.41

8.1.2.2.3 General Condition of Plants (Table 8.3 and Figures 8.1a, b)

After five weeks, both species were unhealthy and had suffered a number of deaths in both the high iron and the high calcium treatments. However, in the high bicarbonate treatment there was a marked difference in response between the two species. Epilobium hirsutum plants were still mainly alive but looked unhealthy with red veins and more general leaf reddening; the oldest leaves were brown, papery and shrivelled. Roots were brittle, black and covered in an ochreous precipitate which was not easily removed by washing. In contrast, the Juncus subnodulosus plants were much more healthy and growing well, though the tips of all leaves, even the youngest, were blackened. Only the very oldest leaves had shrivelled and died. The roots were long, prolific and healthy and covered with the same ochreous precipitate as found on roots of Epilobium hirsutum, though adventitious roots were still white. The ochreous precipitate also covered the bottom of the pots of both species.

8.1.2.3 Discussion

8.1.2.3.1 Calcium Amelioration of Iron Toxicity

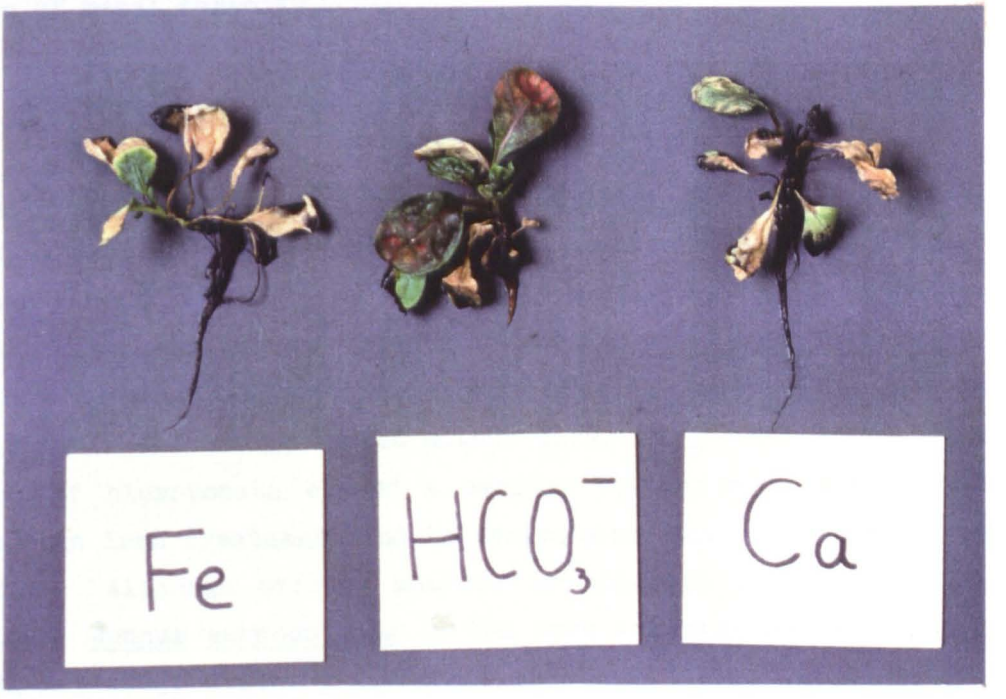
The addition of calcium at 100 mg l^{-1} to nutrient solution containing 100 mg Fe l^{-1} had no significant effect on the pH stability or the soluble iron concentration, when compared with a solution containing only 8 mg Ca

Table 8.3 Visual Observations of Seedlings after Five Weeks Growth in 10% Rorison Solution enriched with Iron

Treatment	Juncus subnodulosus	Epilobium hirsutum
High Iron	Unhealthy. Small and shrivelled. Roots short, pale and flaccid.	Unhealthy. Leaves shrivelled (particularly the older ones). Roots black and brittle.
High Calcium	Unhealthy, but marginally healthier than above. Roots pale and flaccid with a few short white adventitious roots.	As above, but roots slightly less brittle.
High Bicarbonate	Healthy, except for blackening of leaf tips and shrivelling of oldest leaves. Roots long, prolific, and covered in an ochreous precipitate. Some white adventitious roots.	Unhealthy. Leaf reddening (especially veins). Older leaves brown and shrivelled. Roots black, brittle and covered in an ochreous precipitate.

Figure 8.1 Condition of Juncus subnodulosus and Epilobium hirsutum seedlings after 5 weeks growth in 10% Rorison solution at pH 5.5 containing 100 mg iron l^{-1} (Fe) with either 400 mg bicarbonate l^{-1} (HCO_3^-) or 100 mg calcium l^{-1} (Ca).

a. Juncus subnodulosus



b. Epilobium hirsutum

l^{-1}. Furthermore, there was no amelioratory effect on the toxicity of ferrous iron to either Juncus subnodulosus or Epilobium hirsutum. This is contrary to the findings of Skeen (1929) and Olsen (1958). However, it is possible that the ratio of calcium to iron may be important. In a study on chlorosis, Lingle et al. (1963) noted that calcium, potassium and magnesium must be present at much greater molar concentrations than iron to depress iron uptake-transport to any degree. Similarly, a calcium : manganese molar ratio of between 2.2 and 1,100 was required for optimum growth of a number of species (Nazrul-Islam 1976). The optimum ratio was species-dependent and was higher for the more manganese-sensitive species.

In the present study, calcium was supplied at 2.5 mM (100 mg l^{-1}) while iron was supplied at 1.79 mM (100 mg l^{-1}) (Ca:Fe = 1.4). Thus, although the calcium levels were akin to those found in solution in the field (Al-Farraj 1983; Wheeler, Al-Farraj and Cook 1985) iron supply may have been excessive. Indeed, in a Buckinghamshire fen the maximum soluble iron concentration recorded was 10.9 mg Fe l^{-1} (0.●2 mM), while soluble calcium concentration was 165 mg Ca l^{-1} (4.12 mM) giving a molar ratio of approximately 20:1 (Ca:Fe).

It is therefore possible that failure to demonstrate an amelioration of iron toxicity to Epilobium hirsutum and Juncus subnodulosus by calcium could have been due to an imbalance in the Ca:Fe ratio, especially since neither species is particularly iron-tolerant. This may explain some of the controversy in the literature concerning the role of calcium in amelioration of metal toxicity.

8.1.2.3.2 Bicarbonate Amelioration of Iron Toxicity

Both Epilobium hirsutum and Juncus subnodulosus grew very poorly in a solution containing 100 mg Fe^{2+} l^{-1} and many plants of both species died. However, when 400 mg HCO_3^- l^{-1} was added to a similar solution there was a differential effect on the two species. Plants of Juncus subnodulosus were considerably more healthy than those of Epilobium hirsutum. This effect could have been brought about either directly or indirectly. First, the addition of bicarbonate caused a smaller reduction in the pH value than in the high iron treatment, and in consequence much iron precipitated from solution. Although neither species is tolerant of very high iron concentrations, Juncus subnodulosus is the more tolerant species (Section 4.2.3; Al-Farraj 1983; Wheeler, Al-Farraj and Cook 1985). It is possible

that the iron remaining in solution (approx. 14 mg l^{-1}) could be more detrimental to Epilobium hirsutum than to Juncus subnodulosus. Secondly, bicarbonate may act directly and differentially on the two species to reduce the rate of iron uptake and translocation into Juncus subnodulosus relative to Epilobium hirsutum (see Woolhouse 1966; Fleming et al. 1984). A further experiment was performed to examine whether the effect of bicarbonate on the iron tolerance of these two species was more than just an indirect effect on iron solubility mediated by pH.

8.2 INVESTIGATION OF THE EFFECT OF BICARBONATE ON IRON TOLERANCE OF JUNCUS SUBNODULOSUS AND EPILOBIUM HIRSUTUM

8.2.1 Methods

To investigate further the effect of the bicarbonate ion on the iron tolerance of Juncus subnodulosus and Epilobium hirsutum, plants were grown in a number of solutions containing iron and bicarbonate in varying amounts.

In the Standard Screening Experiment, greatest growth reduction in both species had occurred in solutions containing between 3.8 and 20 mg Fe l^{-1} (Figure 4.2). Over this range, the growth response approximated to linearity. Thus in this experiment, iron was supplied at a maximum concentration of 20 mg l^{-1} as ferrous sulphate (in 10% Rorison solution). A preliminary test had determined the range of concentrations of bicarbonate (as $NaHCO_3$) which would precipitate different amounts of the iron. The experimental design was 7 treatments x 10 replicates x 2 species x 4 plants. Floats and pots were used as in the pilot experiment (Section 8.1.2.1). There were two series of treatments (Table 8.4). In one, iron was supplied at 3.8, 7.5, 12.5 and 20 mg l^{-1}; in the other, iron was supplied at 20 mg l^{-1} with additions of bicarbonate at 20, 40, or 60 mg l^{-1} (so that the residual soluble iron concentrations in the two series approximately coincided).

Table 8.4 Iron and Bicarbonate Concentrations (mg l^{-1}) Supplied in the 7 Treatments in 10% Rorison Solution

Treatment	Iron (as $FeSO_4$)	Bicarbonate (as $NaHCO_3$)
1	3.8	-
2	7.5	-
3	12.5	-
4	20	-
5	20	20
6	20	40
7	20	60

In studies involving low iron concentrations, buffers are frequently used to keep the pH constant. However, in this work the high iron concentrations, frequent solution changes, and large number of replicates meant that this was impracticable as large quantities of buffer would be required. The effect of high buffer concentrations on Epilobium hirsutum or Juncus subnodulosus was also not known. The pH was originally adjusted to 5.5 with 1M NaOH and 0.5 M H_2SO_4, and at each solution change (3 times per week). The experiment was continued for three weeks with Epilobium hirsutum, and four weeks with Juncus subnodulosus. Environmental conditions were as for the Standard Screening Experiment (Section 2.4.4).

Additional replicates of the treatment pots were set up containing no plants, and selected chemical analyses were made of the solutions over a 4-day period (i.e. longer than the maximum time between solution changes). pH and oxidation-reduction potential were measured at 8 a.m. each morning, and each day a 30 ml subsample was taken, filtered (millipore 0.2 μm) and analysed for soluble iron concentration (atomic absorption spectrophotometry), soluble reactive phosphorus (Stainton, Capel and Armstrong 1977), and total alkalinity (Goltermann et al. 1978). Iron determinations were made from ten replicate pots in an attempt to reduce sample variance. Other analyses were made from five replicate pots only. For each variable, 'proportional means' were calculated, which represented the mean value over the whole experiment (see Tables 8.5, 8.6, 8.7).

Observations were made on the condition of the two species, and at

the end of the experiment, shoots and roots were harvested separately, washed in distilled water, dried (50°C for 3 days) and weighed. Correlation was made of yield with 'proportional mean' values of the solution chemistry.

8.2.2 **Results**

8.2.2.1 Solution Chemistry

8.2.2.1.1 Precipitates

Solutions lacking supplied bicarbonate (treatments 1-4) had a pale yellow precipitate (thought to be ferric phosphate) on the bottom of the pots. This increased in quantity with increasing added iron concentration, and had also been noted in the Standard Screening Experiment. In the solutions containing bicarbonate, a greater amount of a more ochreous precipitate was present, both on the bottom of the pots, and coating the roots of plants. In these treatments, an 'oily' film occupied the surface of the solution, similar to that which had been present when solutions had become contaminated with iron oxidising bacteria (Section 5.2.5). This had previously been thought to be of bacterial origin, but occurred more especially in the treatment with the highest bicarbonate addition (treatment 7).

8.2.2.1.2 pH (Table 8.5)

In most solutions, pH values fell over the 4-day period, except in the 20 mg Fe l^{-1} and 60 mg HCO_3^- l^{-1} treatment (7), where the pH value increased. The fall in pH tended to be greatest in solutions with the highest iron concentrations, except when bicarbonate had been added.

8.2.2.1.3 Soluble Iron (Table 8.6)

Addition of higher concentrations of bicarbonate caused more of the iron to precipitate from solution. In all treatments, soluble iron concentrations decreased during the first three days, but on the fourth day there was a significant rise again ($p < 0.05$) (except in solutions 1 and 7 which contained little soluble iron anyway).

Table 8.5 pH Changes with Time in 7 Nutrient Solutions Varying in Iron and Bicarbonate Concentration
(Means of 5 replicates ± 1 standard error)
(pH initially set to 5.5 ± 0.1 in bulked solution)

Treatment	Day 1	Day 2	Day 3	Day 4	Proportional Mean
1	5.5	4.7 ± 0.02	4.7 ± 0.02	4.61 ±0.02	5.1 ± 0.00
2	5.5	4.4 ± 0.03	4.3 ± 0.02	4.3 ± 0.02	4.8 ± 0.00
3	5.5	4.3 ± 0.03	4.2 ± 0.02	4.0 ± 0.02	4.8 ± 0.00
4	5.5	4.2 ± 0.02	4.1 ± 0.02	4.0 ± 0.02	4.7 ± 0.00
5	5.5	4.8 ± 0.04	4.5 ±0.02	4.4 ± 0.02	5.1 ± 0.00
6	5.5	5.2 ± 0.04	4.9 ± 0.04	4.7 ± 0.03	5.3 ± 0.00
7	5.5	5.8 ± 0.02	6.0 ± 0.08	6.3 ± 0.04	5.7 ± 0.00

Key to Treatments

	Iron (supplied concentrations, mg l^{-1})	Bicarbonate
1	3.8	-
2	7.5	-
3	12.5	-
4	20	-
5	20	20
6	20	40
7	20	60

Proportional Mean

This represents the 'mean' pH value over the whole experiment, taking into account the fact that twice a week solutions were changed on the second day, and once a week on the third day.

i.e. $\frac{3([1] + [2]) + [3]}{7 \text{ days}}$

[1] = mean value on 1st day
[2] = mean value on 2nd day
[3] = mean value on 3rd day

Table 8.6 Changes with Time in Soluble Iron Concentration (mg l^{-1}) in 7 Nutrient Solutions Varying in Iron and Bicarbonate Concentration
(Means of 10 replicates ± 1 standard error)
(Samples were filtered through a 0.2 μm millipore filter prior to analysis)

Treatment	Day 1	Day 2	Day 3	Day 4	Proportional Mean
1	1.9 ± 0.15	0.4 ± 0.04	0.3 ± 0.04	0.3 ± 0.02	1.0 ± 0.04
2	3.8 ± 0.02	2.9 ± 0.04	2.6 ± 0.04	3.2 ± 0.05	3.2 ± 0.03
3	7.6 ± 0.03	6.8 ± 0.05	6.2 ± 0.07	7.8 ± 0.11	7.1 ± 0.01
4	15.0 ± 0.02	13.9 ± 0.04	13.4 ± 0.06	16.7 ± 0.23	14.3 ± 0.01
5	9.9 ± 0.05	8.3 ± 0.06	7.8 ± 0.06	10.0 ± 0.08	8.9 ± 0.01
6	5.8 ± 0.03	3.4 ± 0.08	3.1 ± 0.08	3.6 ± 0.11	4.4 ± 0.02
7	2.9 ± 0.01	0.9 ± 0.04	0.2 ± 0.02	0.0 ± 0.01	1.7 ± 0.03

Key to Treatments

	Iron (supplied concentrations, mg l^{-1})	Bicarbonate
1	3.8	-
2	7.5	-
3	12.5	-
4	20	-
5	20	20
6	20	40
7	20	60

Proportional Mean

This represents the 'mean' iron concentration in solution over the whole experiment, taking into account the fact that twice a week solutions were changed on the second day, and once a week on the third day.

i.e. $\frac{3([1] + [2]) + [3]}{7 \text{ days}}$

[1] = mean concn. on 1st day
[2] = mean concn. on 2nd day
[3] = mean concn. on 3rd day

Table 8.7 Changes with Time in Soluble Reactive Phosphorus Concentration ($mg\ l^{-1}$) in 7 Nutrient Solutions Varying in Iron and Bicarbonate Concentration

(Means of 5 replicates ± 1 standard error)

(All solutions were filtered through a 0.2 μm millipore filter, and had 3.1 mg P l^{-1} added initially)

Treatment	Day 1	Day 2	Day 3	Day 4	Proportional Mean
1	2.4 ± 0.11	1.8 ±0.05	1.7 ± 0.01	1.7 ± 0.03	2.0 ± 0.01
2	1.6 ± 0.10	1.2 ± 0.01	1.1 ± 0.02	1.1 ± 0.00	1.4 ± 0.01
3	1.4 ± 0.05	1.0 ± 0.01	0.8 ± 0.01	0.7 ± 0.02	1.1 ± 0.00
4	1.2 ± 0.03	0.7 ± 0.03	0.5 ± 0.02	0.4 ± 0.02	0.9 ± 0.00
5	0.0 ± 0.01	nd	nd	nd	nd
6	nd	nd	nd	nd	nd
7	nd	nd	nd	0.1 ± 0.01	nd

nd = not detectable

Key to Treatments

	Iron (supplied concentrations, $mg\ l^{-1}$)	Bicarbonate
1	3.8	-
2	7.5	-
3	12.5	-
4	20	-
5	20	20
6	20	40
7	20	60

Proportional Mean

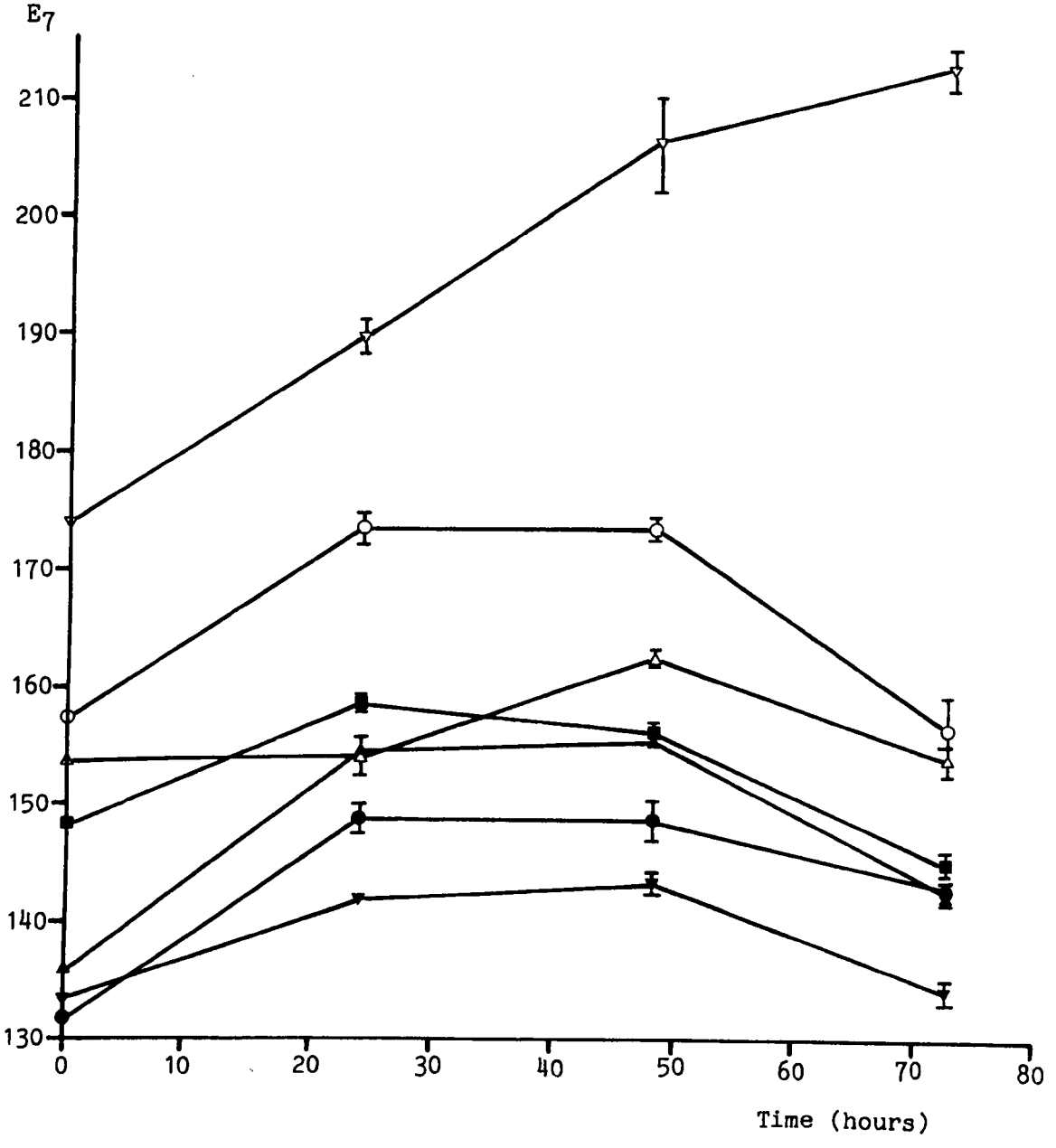
This represents the 'mean' soluble reactive phosphorus concentration over the whole experiment, taking into account the fact that twice a week solutions were changed on the second day, and once a week on the third day.

$$\text{i.e. } \frac{3([1] + [2]) + [3]}{7 \text{ days}}$$

[1] = mean concn. on 1st day
[2] = mean concn. on 2nd day
[3] = mean concn. on 3rd day

Figure 8.2 Changes in Oxidation-Reduction Potential (E_7) with Time of 7 solutions containing different amounts of Iron and Bicarbonate.
(Means of 5 replicates ± 1 SE)

Key to Treatments	Iron (mg l-1)	Bicarbonate (mg l-1)
▼ 1	3.8	-
● 2	7.5	-
▲ 3	12.5	-
■ 4	20	-
△ 5	20	20
○ 6	20	40
▽ 7	20	60



8.2.2.1.4 Soluble Reactive Phosphorus (Table 8.7)

With increasing concentrations of supplied iron, progressively less phosphorus remained in solution. In solutions containing bicarbonate, soluble reactive phosphorus was virtually undetectable.

8.2.2.1.5 Total Alkalinity

A maximum of less than 20% of the bicarbonate supplied was detected, presumably because the pH of the solutions had been adjusted to 5.5 initially. Traces of bicarbonate (dissolved from atmospheric carbon dioxide) were detectable in low-iron solutions which had not had bicarbonate added.

8.2.2.1.6 Oxidation-Reduction Potential (E_7) (Figure 8.2)

Those solutions containing bicarbonate had higher oxidation-reduction potentials than those without added bicarbonate. In treatment 7, containing 60 mg HCO_3^- l^{-1}, oxidation-reduction potential increased with time. In other treatments, the initial increase was followed by a decrease.

8.2.2.2 Visual Condition of Plants

8.2.2.2.1 Juncus subnodulosus

Precipitates (similar to those found on the bottom of the pot) were visible on Juncus subnodulosus roots within 24 hours. In treatments 1-4, the precipitate was creamy or pale yellow in colour, while in the bicarbonate treatments (5-7) it was initially golden, gradually becoming more ochreous. Shoots remained visually healthy for 8-10 days, after which time they were shorter in those treatments with more iron in solution. Leaf tip blackening became apparent at high soluble iron concentrations, and some of the older leaves began to shrivel in these treatments. Observations of shoot and root health at harvest are shown in Table 8.8.

Table 8.8 Visual Observations of Juncus subnodulosus at Harvest after Four Weeks Growth in Solutions Varying in Iron and Bicarbonate Concentration
(See Table 8.4 for key to treatments)

Treatment	Proportional Mean Iron Concentration in Solution (mg Fe l^{-1})	Shoots	Roots
1	1.0	Tall, bright green, healthy.	White or pale cream.
2	3.2	Healthy, but slightly shorter. A little tip dieback and some black leaf speckling was apparent.	Precipitate increased to pale yellow. Roots became more flaccid.
3	7.1	Much shorter. Tips of younger leaves were blackened and dieing back. Many of older leaves had shrivelled	
4	14.3	Short and less bright green. Older leaves were particularly shrivelled, while younger ones had much black speckling.	
5	8.9	Taller. Speckling and dieback of older leaves and tips of younger leaves.	Increasingly covered in a gelatinous ochreous precipitate. Roots became less flaccid.
6	4.4	Much taller and brighter green. Largely healthy with speckling on some leaves. Oldest leaves shrivelled.	
7	1.7	Tall, bright green, healthy. A little shrivelling of tips of older leaves, and very little speckling.	

Increasing Bicarbonate Concentration ↓

Table 8.9 Visual Observations of Epilobium hirsutum at Harvest after Three Weeks Growth in Solutions Varying in Iron and Bicarbonate Concentration
(See Table 8.4 for key to treatments)

Treatment	Proportional Mean Iron Concentration in Solution (mg Fe l^{-1})	Shoots	Roots
1	1.0	Large and bright green.	White, long and prolific (with black removable 'socks' on).
2	3.2	Smaller and darker green. Some stems were pink. Some brown patches on leaves.	Became shorter and more flaccid, with increased blackening ↓
3	7.1	Smaller. Youngest leaves very dark green. Oldest leaves yellowing and wilted. Red stems and veins in one or two plants.	
4	14.3	Very small and wilted. Oldest leaves were shrivelled.	
Increasing Bicarbonate Concentration ↓			
5	8.9	Larger. Youngest leaves very dark green. Oldest leaves yellowing and shrivelling. Red stems.	Blackening decreased. Roots became longer. All roots were covered in an ochreous precipitate, (intensity increased with increase in added bicarbonate concentration) ↓
6	4.4	Larger and more bright green, but most leaves had brown blotches on them. Reddening of stems and some veins.	
7	1.7	Larger and brighter green (though smaller than shoots in treatment 1). Some stems and veins were pink. Many leaves had brown blotches on them.	

8.2.2.2.2 Epilobium hirsutum

In each treatment, Epilobium hirsutum plants were less healthy than those of Juncus subnodulosus, confirming their relative sensitivity to iron.

After one day, root blackening was apparent in treatments 3, 4, and 5 (i.e. those containing most soluble iron). Otherwise the roots in treatments containing no bicarbonate were white, while those in treatments containing bicarbonate had a golden precipitate on them. By the second day, differences in shoot size were visible, decreasing from treatments 1 to 4 as soluble iron concentration increased, and increasing again to treatment 7 as soluble iron concentration decreased. At the highest iron concentration (treatment 4), shoots were already starting to wilt, and root blackening had now spread to treatments 7 and 2.

After three weeks, the plants in those solutions containing least soluble iron were considerably larger and more healthy than those subject to higher soluble iron concentrations (see Table 8.9).

8.2.2.3 Relationship between Yield and Measured Chemical Variables (Table 8.10)

8.2.2.3.1 Juncus subnodulosus

8.2.2.3.1.1 Soluble Iron

In each series of treatments, and in both series of treatments together, there was a significant negative correlation between yield and proportional mean soluble iron concentration ($p < 0.01$) (see Table 8.10). The plot of yield against proportional mean soluble iron concentration for the two series of treatments (with and without bicarbonate) followed virtually the same curve (Figure 8.3).

8.2.2.3.1.2 Soluble Reactive Phosphorus

There was no correlation between yield and proportional mean soluble reactive phosphorus concentration, except in the solutions containing no added bicarbonate. Where 60 mg HCO_3 l^{-1} was supplied, relatively high yields (up to 96% control yields) were found even when measured soluble

Table 8.10 Correlation of Yield with Measured Chemical Variables
(Proportional Means)

Variable	Correlation Coefficient for all Treatments
a. *Juncus subnodulosus*	
Soluble Iron	-.9834 ***
pH	NS
Soluble Reactive Phosphorus	NS
Total Alkalinity	NS
Oxidation-Reduction Potential	NS
b. *Epilobium hirsutum*	
Soluble Iron	-.7230 *
pH	NS
Soluble Reactive Phosphorus	NS
Total Alkalinity	NS
Oxidation-Reduction Potential	NS

$p < 0.05$ *
$p < 0.01$ **
$p < 0.001$ ***
NS Correlation was not significant

Figure 8.3 Yield of Juncus subnodulosus after 4 weeks growth in 7 nutrient solutions containing Iron with (- - -) or without (——) Bicarbonate additions (mean ± 1 SE) against Proportional Mean Iron Concentration. (mean ± 1 SE)
(For explanation of proportional means see Tables 8.5 - 8.7)

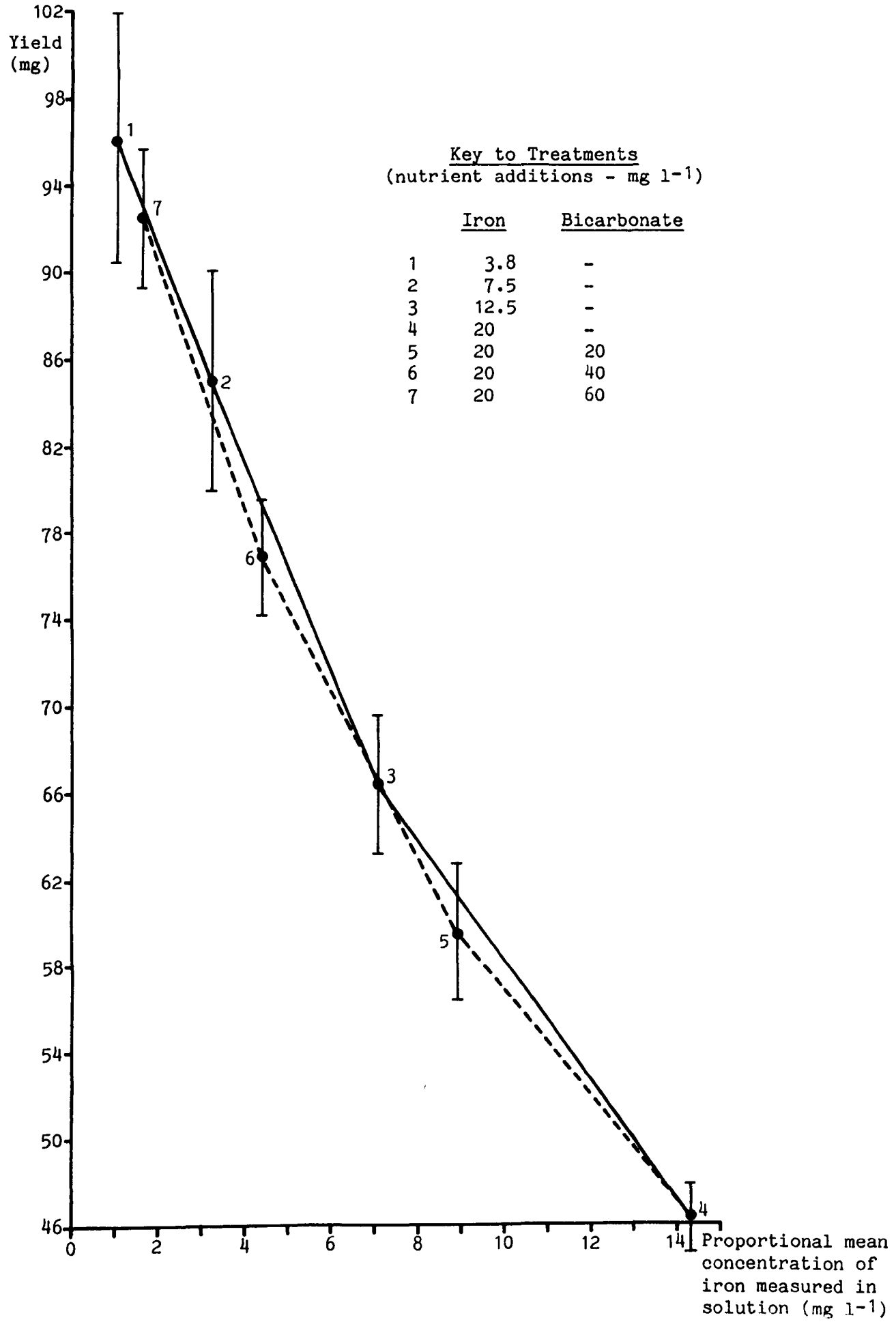
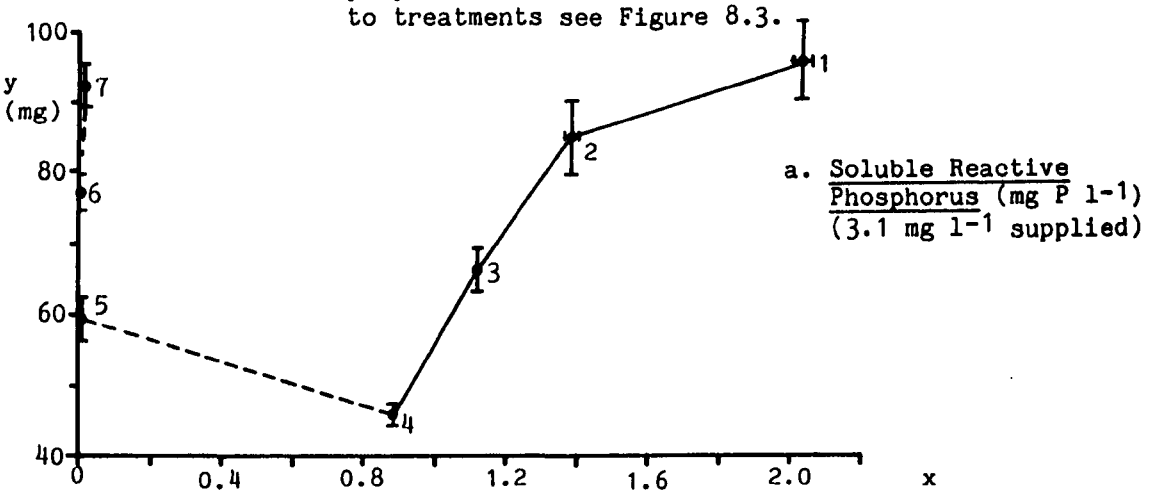
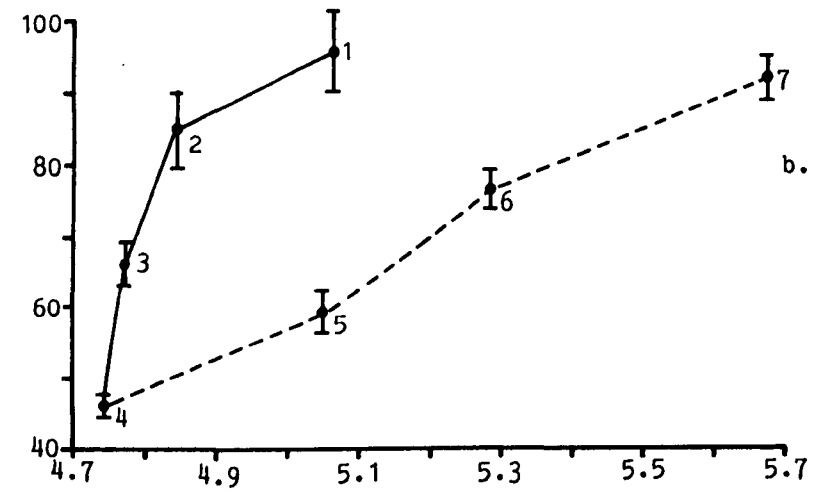


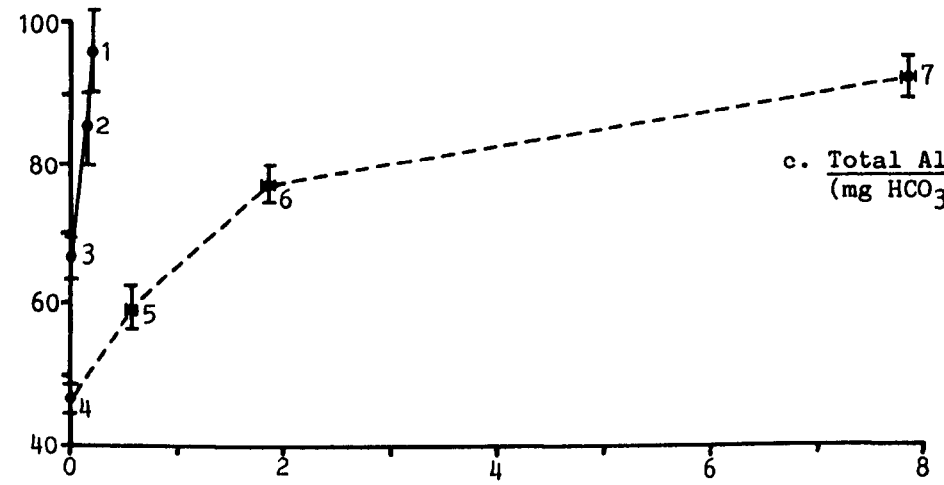
Figure 8.4 Yield of Juncus subnodulosus after 4 weeks growth in 7 nutrient solutions containing Iron with (---) or without (——) Bicarbonate additions (y)(mean ± 1 SE), against measured chemical variables (x)(proportional means ± 1 SE).
For explanation of proportional means see Tables 8.5 - 8.7; for key to treatments see Figure 8.3.



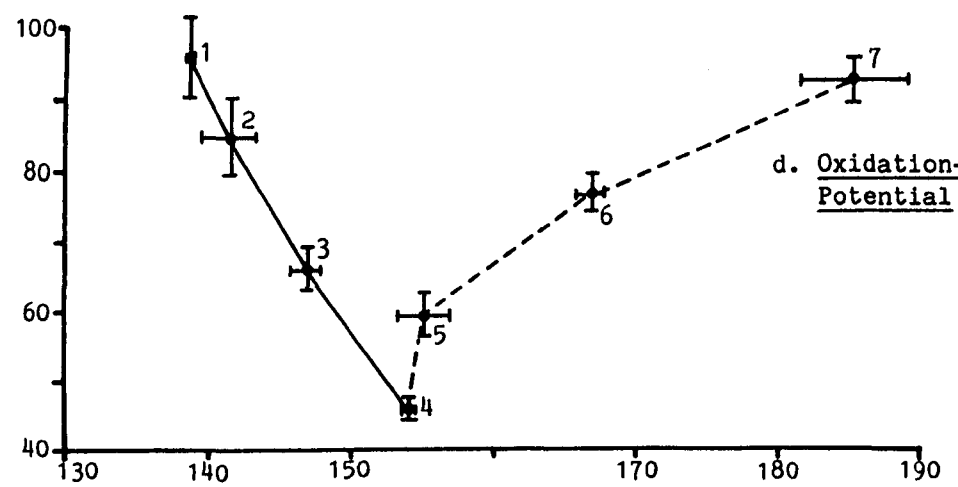
a. Soluble Reactive Phosphorus (mg P l^{-1}) (3.1 mg l^{-1} supplied)



b. pH (initially 5.5)



c. Total Alkalinity (mg HCO_3^- l^{-1})



d. Oxidation-Reduction Potential (E_7) (mV)

phosphorus concentration was negligible (Figure 8.4a).

8.2.2.3.1.3 pH

There was no significant correlation between yield and proportional mean pH (except in the series of treatments containing bicarbonate) (Figure 8.4b).

8.2.2.3.1.4 Total Alkalinity

For the treatments as a whole there was no correlation between yield and proportional mean total alkalinity (Table 8.10), and both maximum and minimum yields were found in treatments containing negligible bicarbonate (Figure 8.4c).

8.2.2.3.1.5 Oxidation-Reduction Potential

For treatments as a whole, there was no significant correlation between yield and proportional mean oxidation-reduction potential (Table 8.10). However, for the 'normal' series of treatments there was significant negative correlation ($p < 0.01$) while for the 'bicarbonate' series, correlation was positive ($p < 0.05$) (Figure 8.4d).

8.2.2.3.2 Epilobium hirsutum

8.2.2.3.2.1 Soluble Iron

For the 'bicarbonate' series of treatments and for the treatments as a whole, there was a significant negative correlation between yield and proportional mean soluble iron concentration ($p < 0.05$) (see Table 8.10). Figure 8.5 however, reveals that plots from the two series of treatments do not coincide quite as well as those for Juncus subnodulosus. Since Epilobium hirsutum is less tolerant of iron than is Juncus subnodulosus, its growth response plot with supplied iron is more curved (see Figure 4.2), with greatest growth reduction occurring at the lowest iron concentrations.

Figure 8.5 Yield of Epilobium hirsutum after 3 weeks growth in 7 nutrient solutions containing Iron with (- - -) or without (——) Bicarbonate additions (mean ± 1 SE) against Proportional Mean Iron Concentration. (mean ± 1 SE)
(For explanation of proportional means see Tables 8.5 - 8.7)

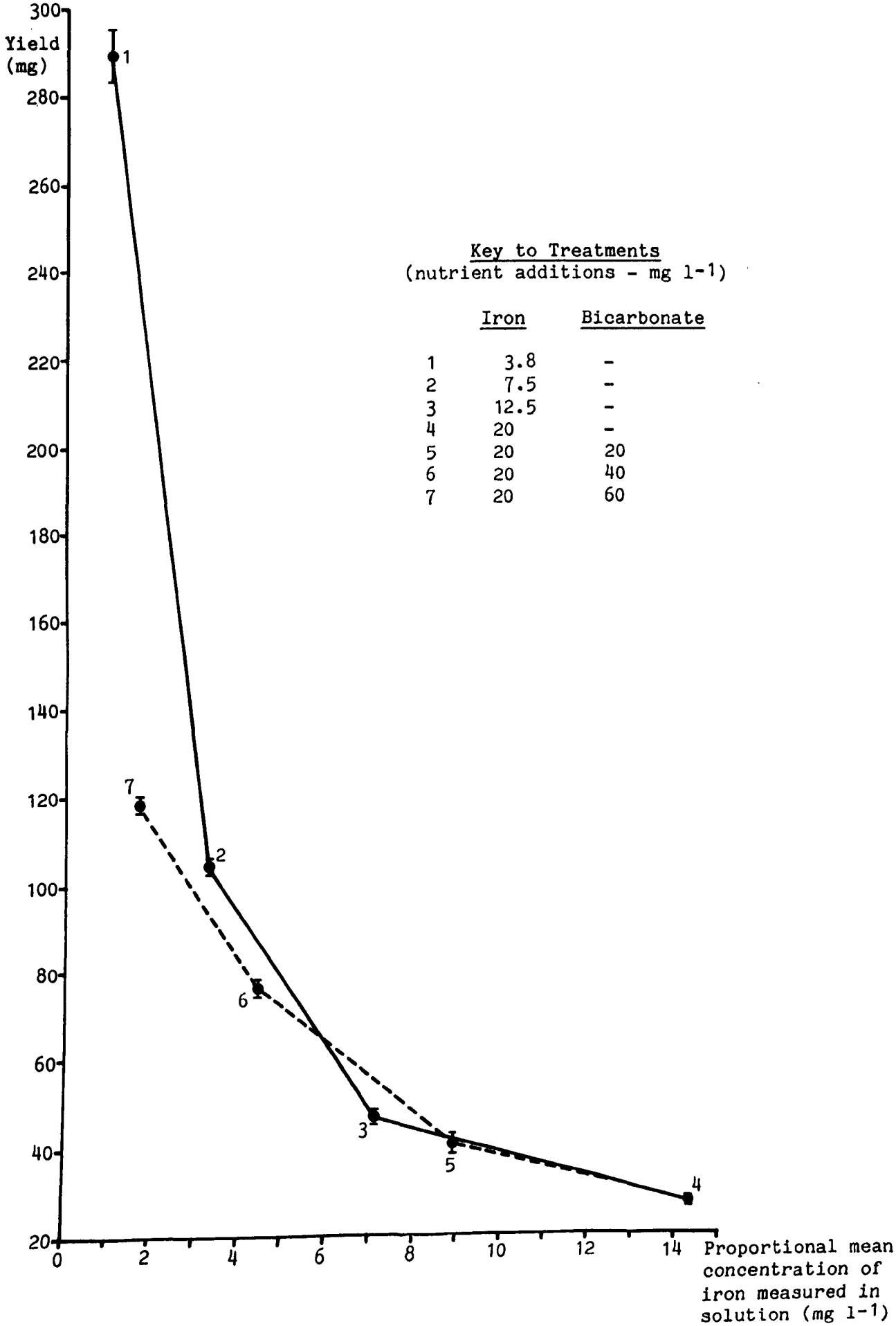
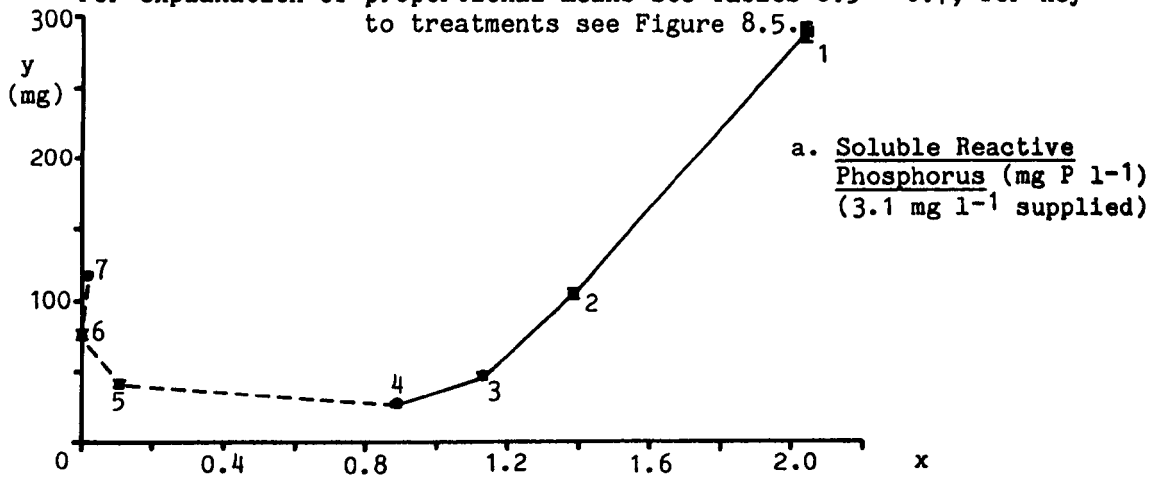
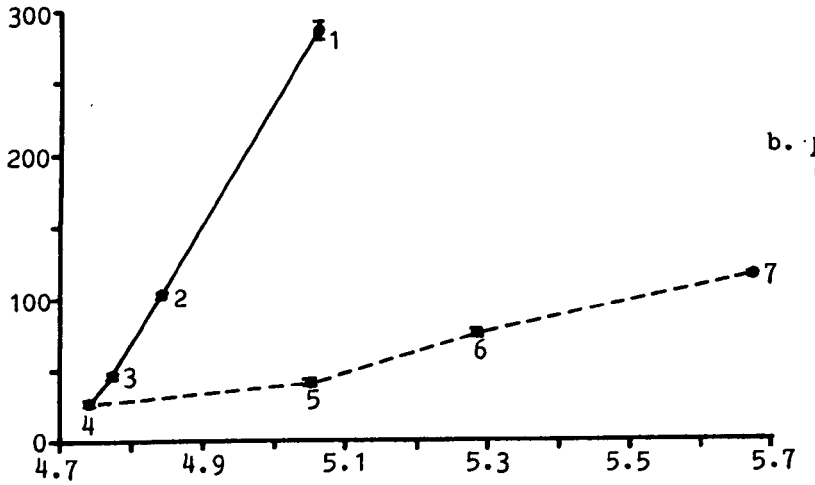


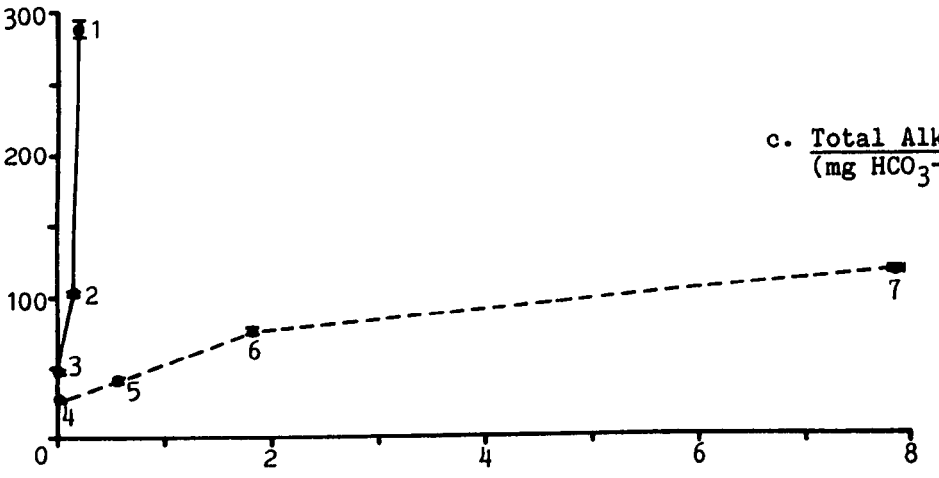
Figure 8.6 Yield of Epilobium hirsutum after 3 weeks growth in 7 nutrient solutions containing Iron with (- - -) or without (——) Bicarbonate additions (y)(mean ± 1 SE), against measured chemical variables (x)(proportional means ± 1 SE).
For explanation of proportional means see Tables 8.5 - 8.7; for key to treatments see Figure 8.5.



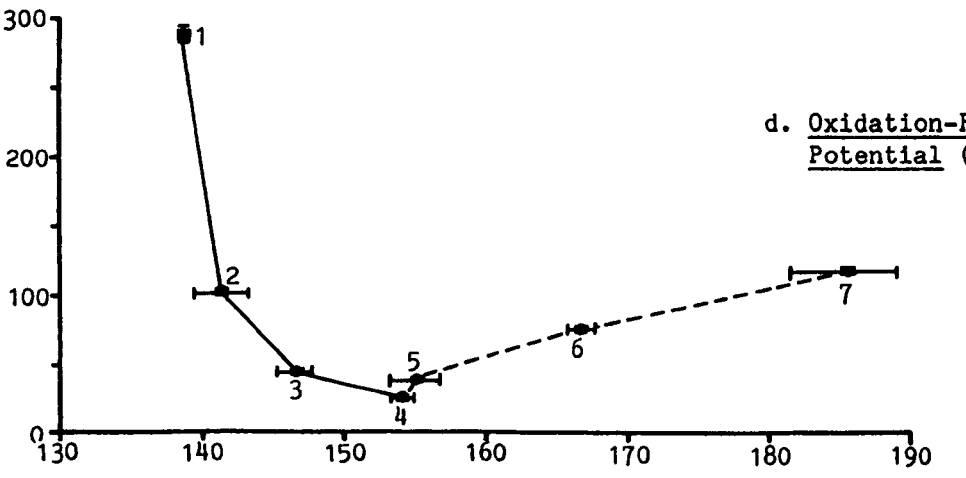
a. Soluble Reactive Phosphorus (mg P l^{-1}) (3.1 mg l^{-1} supplied)



b. pH (initially 5.5)



c. Total Alkalinity (mg HCO_3^- l^{-1})



d. Oxidation-Reduction Potential (E_7) (mV)

8.2.2.3.2.2 Soluble Reactive Phosphorus

There was no correlation between yield and proportional mean soluble reactive phosphorus (except in solutions containing no added bicarbonate). Yields up to only 40% of the control yield were obtained in solutions containing bicarbonate and thus negligible measured SRP (Figure 8.6a).

8.2.2.3.2.3 pH

In each series of treatments, yield correlated significantly with proportional mean pH, i.e. there was better growth at higher pH values. However, as for Juncus subnodulosus there was no significant correlation for the treatments as a whole (Table 8.10), since the two series had very different responses (Figure 8.6b).

8.2.2.3.2.4 Total Alkalinity

There was no correlation between yield and proportional mean total alkalinity except in the bicarbonate series of treatments and, as for Juncus subnodulosus, both maximum and minimum yields were found in solutions containing negligible bicarbonate (Figure 8.6c).

8.2.2.3.2.5 Oxidation-Reduction Potential

There was no correlation between yield and proportional mean oxidation-reduction potential except in the 'bicarbonate' series of treatments (Figure 8.6d).

8.2.3 Discussion

8.2.3.1 Correlation of Yield with Chemical Variables

In both species, yield was independent of pH, oxidation-reduction potential, soluble reactive phosphorus concentration and total alkalinity. There was however, close correlation with mean soluble iron concentration. 97% of the variation in Juncus subnodulosus yield could be accounted for by proportional mean soluble iron concentration, but for Epilobium hirsutum the value was only 52%.

8.2.3.2 Direct Effects of Bicarbonate

The idea behind this experiment was that if bicarbonate reduced iron uptake-translocation in either species, yields of plants grown in solutions containing bicarbonate should be significantly greater than yields of those grown in comparable concentrations of iron but without bicarbonate (since yield varies with iron tolerance which in turn varies with iron concentration in the shoot). Figures 8.3 and 8.5 show that this is not the case. There is thus no evidence to suggest that the bicarbonate ion has any direct amelioratory effect on the toxicity of iron supplied at high concentrations. Romheld et al. (1982), and Fleming et al. (1984) have shown that the bicarbonate ion may only affect iron uptake if the plants are iron-stressed, indicating that bicarbonate inhibits the plant's stress response rather than the normal uptake-translocation mechanisms.

A direct adverse effect of bicarbonate on Epilobium hirsutum may possibly be implicated since plants grew significantly less well in solutions containing the higher concentrations of bicarbonate (Figure 8.5). However, indirect effects are more likely to be causing the reduction in yield.

8.2.3.3 Indirect Effects of Bicarbonate

It seems from this study that bicarbonate ameliorates toxicity of high concentrations of iron by indirect means, i.e. by reduction of H^+ activity and precipitation of much supplied iron. This agrees with Thenabadu (1974) who found that in iron deficiency studies, iron inactivation by bicarbonate occurred external to the rice plant. The better health (and lower yield reduction) of Juncus subnodulosus compared with Epilobium hirsutum in any given treatment was due to its greater tolerance of iron.

Low yields of Epilobium hirsutum in treatment 7 may be both because of its sensitivity to iron, even at relatively low concentrations, and because of a further indirect effect of bicarbonate, namely reduced phosphorus solubility. Epilobium hirsutum has a higher RGR and hence a greater phosphorus requirement than does Juncus subnodulosus. Indeed, a test with Juncus subnodulosus in nutrient solution lacking phosphorus revealed little yield reduction as compared with control plants.

8.3 EFFECT OF NITROGEN SOURCE ON IRON TOLERANCE

8.3.1 Introduction

In waterlogged situations of low oxidation-reduction potential, plant-available nitrogen is likely to occur predominantly as the ammonium form. Mineralisation of organic matter produces ammonium which is not converted to nitrate since nitrification does not occur under oxygen-deficient conditions (DeLaune et al. 1981). Nitrate is not present appreciably in soils with an oxidation-reduction potential lower than 220 mV, since upon depletion of oxygen, nitrate is the first of a series of alternative electron acceptors used by soil micro-organisms for respiration (i.e. denitrification) (Gambrell and Patrick 1978; Etherington 1983b).

In the Standard Screening Experiment, nitrogen was supplied as nitrate. However, McGrath and Rorison (1982) and Rorison (1985) reported interactions between nitrogen source and manganese and aluminium tolerance respectively. It was therefore decided to test a small number of species, to see if their tolerance to supplied iron varied with nitrogen source.

8.3.2 Methods

Four species were selected for screening, two monocotyledons and two dicotyledons (Table 8.11). One of each had been found to be tolerant of iron and the other less tolerant, in the Standard Screening Experiment (using nitrate as nitrogen source).

Table 8.11 Species used for Assessment of the Effect of Nitrogen source on Iron Tolerance
(Monocotyledons are indicated by bold type)

Iron-Tolerant	Iron-Sensitive
Iris pseudacorus	**Juncus subnodulosus**
Lysimachia vulgaris	Epilobium hirsutum

The experimental design was 2 nitrogen sources x 3 iron concentrations x 4 species x 5 replicates x 4 plants (Eh & Js) or 3 plants (Lv and Ip). Boxes and floats were used as in Section 2.4.4, and the basal nutrient solution was 10% Rorison solution at pH 5.5. Since the majority of growth reduction tends to occur at the lower iron concentrations, more especially in the less tolerant species, iron concentrations chosen were 3.8 (control), 10 and 25 mg Fe l^{-1} supplied as ferrous sulphate. Nitrogen was supplied at 5.6 mg N l^{-1} as either calcium nitrate ($Ca(NO_3)_2.4H_2O$) (as in Rorison solution), or as ammonium sulphate ($(NH_4)_2SO_4$) with calcium as calcium chloride ($CaCl_2.6H_2O$). The experimental period was three weeks, and solutions were changed three times per week, with pH adjustment to 5.5 on each occasion. Environmental conditions were as for the Standard Screening Experiment (Section 2.4.4).

A repeat set of pots contained no plants, and selected aspects of solution chemistry were monitored over a four-day period (i.e. greater than the maximum time between solution changes). pH values and oxidation-reduction potential were measured in situ, and then a 10 ml subsample of solution was filtered (millipore 0.2 μm) and analysed for soluble iron (including particulate < 0.2 μm) (atomic absorption spectrophotometry) and soluble reactive phosphorus (UV-VIS spectrophotometry) by the molybdate blue method of Stainton, Capel and Armstrong (1977). For each variable, 'proportional means' were calculated which represented the mean value over the whole experiment (see Tables 8.12, 8.13, 8.14). In addition, the pH was recorded at the end of the experiment, after plants had been growing in the final change of solutions for four days.

At each solution change, observations were made of the condition of the plants. At the end of the experiment, shoots and roots were harvested separately, washed in distilled water, dried (3 days at 50°C) and weighed. Yield was related to chemical variables measured. Dry weight of a replicate set of each species was recorded at the beginning of the experiment, to calculate relative growth rate (RGR) and RGR tolerance indices.

8.3.3 **Results**

8.3.3.1 Solution Chemistry

8.3.3.1.1 Soluble Iron (Table 8.12)

t-tests (and analysis of variance) revealed that at any one time and at any one iron concentration, there was no significant difference in the iron measured in solution between the two nitrogen sources.

At all three iron concentrations, soluble iron concentration fell over the first two days, but at the two higher iron concentrations it subsequently increased. This increase, which was significant ($p < 0.05$) in the 25 mg Fe l^{-1} treatments, had been detected on all previous occasions when soluble iron had been measured (Sections 2.5, and 8.2.2.1.3).

8.3.3.1.2 Soluble Reactive Phosphorus (Table 8.13)

In all solutions, soluble reactive phosphorus concentration fell with time, particularly during the first 24 hours. Greatest reduction occurred in those solutions with the highest iron concentration. There was little difference in SRP concentration between nitrogen sources at any one iron concentration.

8.3.3.1.3 pH (Table 8.14)

In all iron concentrations and at each time, the pH of the ammonium-containing solution was significantly higher than that of the corresponding nitrate-containing solution, in the absence of plants. In all treatments, pH decreased with time, most particularly during the first 12 hours. The higher the supplied iron concentration, the greater the pH reduction (Table 8.14).

Solution pH was only measured once with plants present, i.e. at the end of the experiment, after plants had been in the final change of solutions for four days. For each species, nitrate-containing solutions had greater final pH values with than without plants, whereas ammonium-containing solutions had a lower final pH with plants present (Table 8.15). Greatest pH differential occurred in the lower iron concentrations, and larger plants produced greater pH changes in their solutions.

Table 8.12 Changes with Time, in Soluble Iron Concentration (mg l^{-1}) in Solutions Enriched With Different Amounts of Iron, with Nitrate or Ammonium as the Nitrogen Source
(Means of 5 replicates ± 1 standard error)
(Samples were filtered through a 0.2 μm millipore filter)

Treatment	Day 1	Day 2	Day 3	Day 4	Proportional Mean
NO_3/3.8Fe	2.0 ± 0.25	0.3 ± 0.01	0.2 ± 0.01	0.1 ± 0.01	1.0 ± 0.06
NO_3/10Fe	6.4 ± 0.09	4.5 ± 0.01	4.2 ± 0.03	4.2 ± 0.04	5.3 ± 0.01
NO_3/25Fe	20.2 ± 0.23	17.1 ± 0.12	16.8 ± 0.16	20.4 ± 0.19	18.6 ± 0.07
NH_4/3.8Fe	2.0 ± 0.18	0.3 ± 0.00	0.2 ± 0.01	0.2 ± 0.01	1.0 ± 0.03
NH_4/10Fe	6.4 ± 0.07	4.3 ± 0.09	4.0 ± 0.09	4.3 ± 0.09	5.1 ± 0.01
NH_4/25Fe	19.9 ± 0.05	17.6 ± 0.13	17.2 ± 0.16	20.1 ± 0.32	18.5 ± 0.02

Proportional Mean

This represents the 'mean' iron concentration in solution over the whole experiment, taking into account the fact that twice a week solutions were changed on the second day, and once a week on the third day.

$$\text{i.e. } \frac{3([1] + [2]) + [3]}{7 \text{ days}}$$

[1] = mean concn. on 1st day
[2] = mean concn. on 2nd day
[3] = mean concn. on 3rd day

Table 8.13 Changes with Time, in Soluble Reactive Phosphorus Concentration ($mg\ l^{-1}$) in Solutions Enriched With Different Amounts of Iron and Nitrate or Ammonium as the Nitrogen Source
(Means of 5 replicates ± 1 standard error)
(Solutions were filtered through a 0.2 μm millipore filter, and were all supplied with 3.1 mg P l^{-1} initially)

Treatment	Day 1	Day 2	Day 3	Day 4	Proportional Mean
NO_3/3.8Fe	2.6 ± 0.07	2.0 ± 0.05	2.0 ± 0.03	1.9 ± 0.02	2.2 ± 0.01
NO_3/10Fe	1.9 ± 0.06	1.3 ± 0.05	1.0 ± 0.02	0.8 ± 0.02	1.5 ± 0.01
NO_3/25Fe	1.5 ± 0.04	0.4 ± 0.01	0.2 ± 0.00	0.1 ± 0.01	0.8 ± 0.00
NH_4/3.8Fe	2.5 ± 0.13	1.7 ± 0.09	1.9 ± 0.04	1.9 ± 0.04	2.1 ± 0.02
NH_4/10Fe	1.7 ± 0.06	1.1 ± 0.07	0.9 ± 0.04	0.8 ± 0.02	1.3 ± 0.01
NH_4/25Fe	1.5 ± 0.01	0.4 ± 0.01	0.2 ± 0.01	0.2 ± 0.00	0.8 ± 0.00

Proportional Mean

This represents the 'mean' soluble reactive phosphorus concentration over the whole experiment, taking into account the fact that twice a week solutions were changed on the second day, and once a week on the third day.

$$\text{i.e. } \frac{3([1] + [2]) + [3]}{7 \text{ days}}$$

[1] = mean concn. on 1st day
[2] = mean concn. on 2nd day
[3] = mean concn. on 3rd day

Table 8.14 pH Changes with Time of Solutions Enriched With Different Amounts of Iron, with Nitrate or Ammonium as the Nitrogen Source
(Means of 5 replicates * 1 standard error)
(pH was initially adjusted to 5.5 * 0.1)

Treatment	Day 1	Day 2	Day 3	Day 4	Proportional Mean
NO_3/3.8Fe	5.5	5.0 ± 0.02	5.0 ± 0.02	4.9 ± 0.02	5.2 ± 0.00
NO_3/10Fe	5.5	4.5 ± 0.02	4.4 ± 0.01	4.3 ± 0.01	4.9 ± 0.00
NO_3/25Fe	5.5	4.3 ± 0.00	4.2 ± 0.00	4.1 ± 0.00	4.8 ± 0.00
NH_4/3.8Fe	5.5	5.1 ± 0.01	5.1 ± 0.01	5.0 ± 0.01	5.3 ± 0.00
NH_4/10Fe	5.5	4.5 ± 0.00	4.4 ± 0.01	4.3 ± 0.01	4.9 ± 0.00
NH_4/25Fe	5.5	4.3 ± 0.01	4.2 ± 0.01	4.1 ± 0.01	4.8 ± 0.00

Proportional Means

This represents the 'mean' pH value over the whole experiment, taking into account the fact that twice a week solutions were changed on the second day and once a week on the third day.

$$\text{i.e. } \frac{3([1] + [2]) + [3]}{7 \text{ days}}$$

[1] = mean value on 1st day
[2] = mean value on 2nd day
[3] = mean value on 3rd day

Table 8.15 Final pH of Solutions Containing Nitrate-N or Ammonium-N, enriched with Different Amounts of Iron, in the Presence or Absence of Plants
(Means of 5 replicates ± 1 standard error)
(pH was initially 5.5)

Iron Concn. ($mg\ l^{-1}$)	In Absence of Plants	+ Iris pseudacorus	+ Lysimachia vulgaris	+ Juncus subnodulosus	+ Epilobium hirsutum
a. Nitrate-N					
3.8	4.9 ± 0.02	5.8 ± 0.03	6.2 ± 0.09	5.5 ± 0.04	6.0 ±0.09
10	4.3 ± 0.01	5.0 ± 0.05	5.1 ± 0.08	4.5 ± 0.02	4.4 ±0.01
25	4.1 ± 0.00	4.2 ± 0.04	4.2 ± 0.01	4.3 ± 0.01	4.3 ±0.02
b. Ammonium-N					
3.8	5.0 ± 0.01	3.5 ± 0.06	3.5 ± 0.01	4.0 ± 0.04	4.1 ±0.04
10	4.3 ± 0.01	3.5 ± 0.02	3.5 ± 0.04	4.1 ± 0.04	4.2 ±0.07
25	4.1 ± 0.01	3.6 ± 0.02	4.0 ± 0.06	4.0 ± 0.02	4.0 ± 0.03

In either nitrogen source, there was a significant correlation between plant dry weight and both final pH and pH change ($p < 0.001$), when all species were considered together.

8.3.3.1.4 Oxidation-Reduction Potential (E_7)

In all solutions, oxidation-reduction potential ranged from 120 mV to 160 mV. There were no obvious trends either with nitrogen source or over time.

8.3.3.2 Visual Condition of Plants (Table 8.16)

8.3.3.2.1 Iris pseudacorus

The first visual effect of iron was ochre production on the roots of plants at the two higher iron concentrations. This occurred within the first 24-48 hours, in both nitrogen sources. Some leaf-tip dieback was apparent in nitrate-fed plants well before ammonium-fed plants.

After 3 weeks, slight reduction in shoot height with increasing iron concentration was noticeable in both nitrogen sources; plants grown in NO_3-N had a little leaf tip dieback whereas in NH_4-N fed plants this was negligible.

8.3.3.2.2 Lysimachia vulgaris

An ochreous root precipitate formed within the first 12-24 hours, more obviously on the roots grown in NO_3-N. Leaves of plants grown in NH_4-N were darker green than those grown in NO_3-N, and shoots and leaves in particular were much smaller in the NH_4-N control than in the NO_3-N control. NH_4-N control roots were also short (compared with those in the NO_3-N control), with stunted side roots and darkened apices. Visual observations at harvest are recorded in Table 8.16.

8.3.3.2.3 Juncus subnodulosus

The first visual effect of iron was the formation of a creamy precipitate (probably ferric phosphate) on roots of all except control plants. Slight blackening of leaf tips, and leaf tip dieback did not occur until

Table 8.16 Visual Observations at Harvest of the Performance of Species Grown in Solutions Enriched with Iron, with Different Nitrogen Sources

Iron Concentration (mg l^{-1})	*Iris pseudacorus*		*Lysimachia vulgaris*		*Juncus subnodulosus*		*Epilobium hirsutum*	
	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N
	SHOOTS							
3.8 (control)	Tall and healthy.	Tall and healthy.	Very large. Bright green. Healthy.	Large (but with smaller leaves than NO_3 control). Healthy. Dark green.	Tall and healthy. Bright green.	Tall and healthy. Dark green.	Very large. Healthy. Bright green. Stems green.	Much smaller than in NO_3 control. Darker green. Tougher and more hairy. Slight leaf reddening ± red veins. Red stems with many root primordia.
10	Slightly shorter. A little leaf tip dieback.	Slightly shorter. Healthy.	Smaller. Darker green. Healthy. 'Metallic' specks on 1 or 2 lower leaves.	Smaller. Dark green. Healthy.	Shorter. Less healthy. Black tips to some leaves. Shrivelling of tips of oldest leaves.		Very much smaller. Younger leaves dark green. Older leaves yellowing or reddening. Stems red, with many root primordia.	
25	Slightly shorter. Leaf blades less wide. Some leaf tip dieback.	Slightly shorter. Healthy.	Much smaller. Top leaves dark green. Lower leaves yellow and wilted. Stems stiff in NO_3-N.		Very short and unhealthy Yellowing of some leaves. Shrivelling of tips of oldest leaves.		Much smaller and unhealthy Wilted. Older leaves shrivelled, but no plant deaths. Stems red.	Smaller. Wilted Older leaves shrivelled. Stems red, with many root primordia.

Table 8.16 continued

	ROOTS							
3.8 (control)	Long, white, prolific.		Long and prolific. Covered in a pale creamy precipitate.	Covered in a creamy precipitate. Short with stunted side roots and dark apices.	Long and covered with a creamy precipitate. Adventitious roots long and white.	Long and white (i.e. no precipitate on root) White adventitious roots.	Long, white and prolific with adventitious roots. Some tips with black 'socks' on.	Long and blackened, but shorter and less prolific than in NO_3-N contro Side roots shor
10	Long. Ochreous with white tips.		Very short. Ochreous, with white tips.	Short. Pale ochreous with white tips.	Older roots flaccid, and covered in a dark cream precipitate. Adventitious roots shorter than in control concentrations and white.		Shorter. Grey or black.	Shorter. Black Black. Brittle.
25	Slightly shorter. Very ochreous with white tips.	Shorter. Very ochreous. with white tips.	Flaccid, and covered in pale ochre. The most flaccid roots had least ochre on them.	Short and flaccid. Ochreous with dark tips. The least ochreous roots were the most flaccid.	Thin and flaccid. Covered in a dark cream precipitate Adventitious roots short and brown if present.		Short. Thin. Black and brittle or flaccid.	Short, thin black and flaccid.
Shoot sizes (observed visually)	$NO_3$3.8 > $NO_3$10 > $NH_4$3.8 > $NH_4$10 > $NH_4$25 > $NO_3$25 (but size difference very small)		$NO_3$3.8 >> $NH_4$3.8 > $NH_4$10 > $NO_3$10 > NO_3 and $NH_4$25		$NH_4$3.8 > $NO_3$3.8 > NO_3 and $NH_4$10 > NO_3 and $NH_4$25		$NO_3$3.8 >> $NH_4$3.8 > NO_3 and $NH_4$10 > $NH_4$25 > NO_3 25	

several days later. Table 8.16 shows the visual observations recorded at harvest.

The most striking difference between plants from the two nitrogen sources was that shoots of plants grown in ammonium were darker green than those grown in nitrate. Other effects of iron on shoots were similar between the nitrogen sources.

Root precipitates were apparent on all except NH_4-N control plants; they were generally darker on roots of nitrate-grown plants than on the corresponding ammonium-fed plants.

8.3.3.2.4 Epilobium hirsutum

After only 24 hours at 25 mg Fe l^{-1}, shoots of plants in NO_3-N had wilted a little, and roots had become grey with blackened tips. However, after 48 hours, shoots of corresponding plants in NH_4-N had wilted more than the nitrate-fed plants. A shoot size reduction with increasing iron concentrations was soon apparent. Roots of plants in 10 and 25 mg Fe l^{-1} and NO_3-N became blackened earlier than their counterparts in NH_4-N. Visual observations recorded after 3 weeks are shown in Table 8.16. In both nitrogen sources, reduction in shoot size and root length with increasing iron concentration was apparent, though roots and shoots of plants in the NH_4-N control were considerably smaller than those in the NO_3-N control. Roots of nitrate-fed control plants were white, while those in the ammonium-fed controls were black. Shoots of plants grown in NH_4-N were darker green than those grown in NO_3-N.

8.3.3.3 Effect of Nitrogen Source on Yield Response

Yield of all four species fell with increasing iron supply, whether supplied with nitrate-N or ammonium-N (Figures 8.7a, b, c, d). Yield reduction was significantly greater in nitrate than in ammonium, except in Juncus subnodulosus.

Nitrogen source did not significantly affect yield of either monocotyledon at any one iron concentration, though there were some significant yield differences in the two dicotyledons. Control plants of both Lysimachia vulgaris and Epilobium hirsutum grew significantly less well ($p < 0.01$) in ammonium-N than in nitrate-N, though at 10 mg Fe l^{-1} the reverse was true for Epilobium hirsutum ($p < 0.05$).

Figure 8.7 Yield of Seedlings supplied with varying Iron Concentrations and Nitrate or Ammonium as the Nitrogen Source (Mean ± 1 SE)

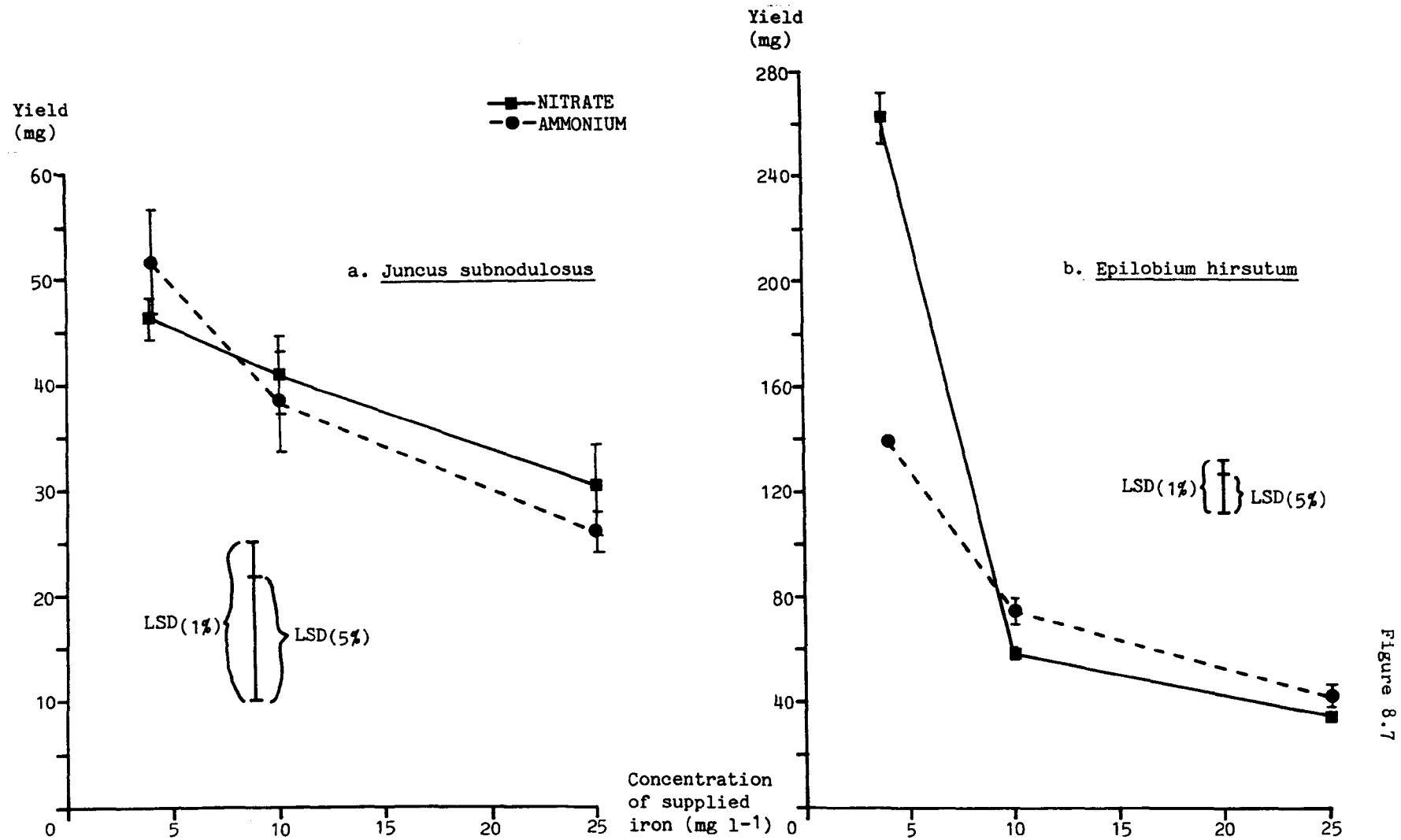


Figure 8.7

Figure 8.7 continued

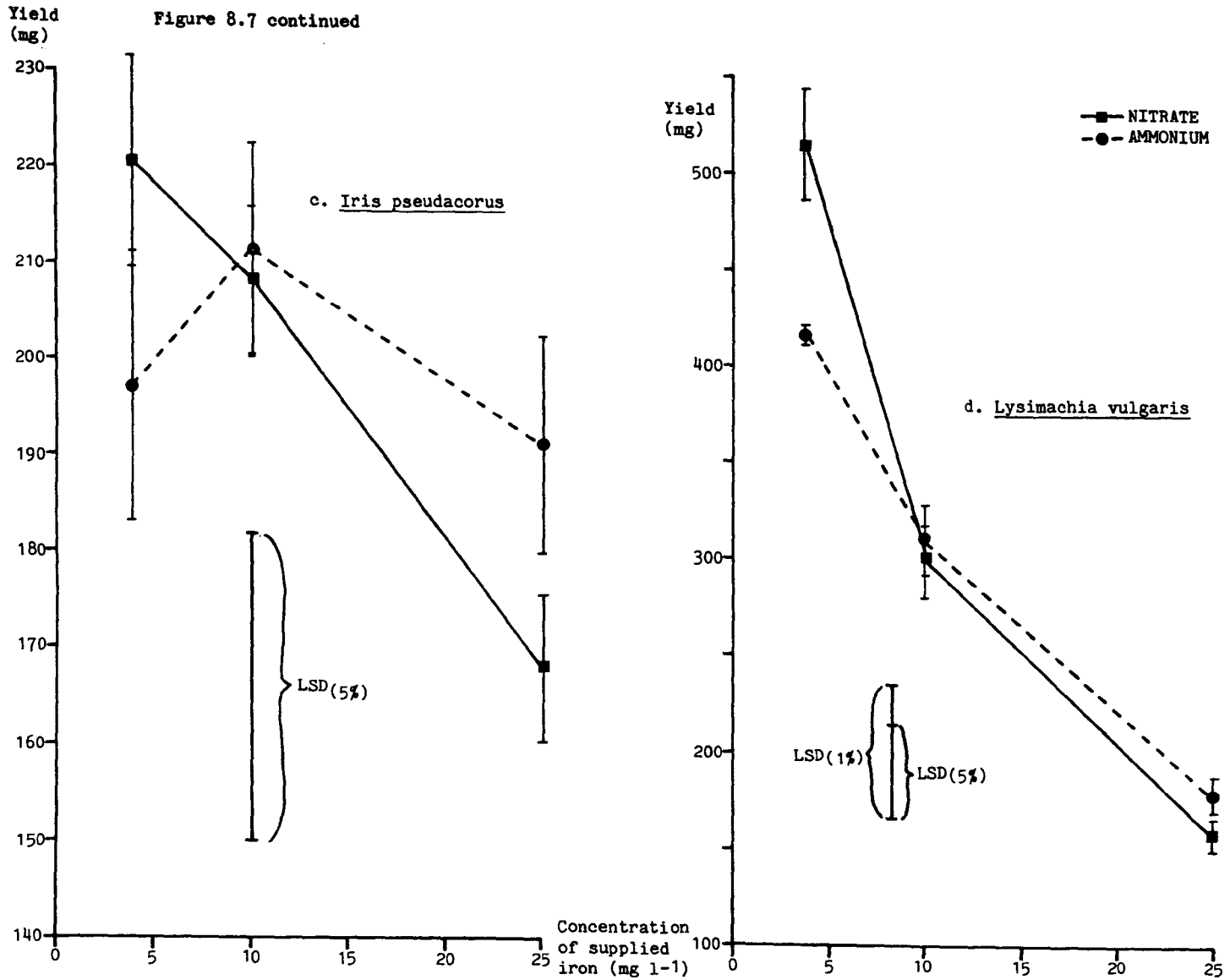


Figure 8.7 continued

Two-way analysis of variance (summarised in Appendix II), showed that yield of all 4 species was significantly affected by iron concentration, ($p < 0.05$ for the most tolerant I. pseudacorus, $p < 0.001$ for the other 3 species). However, the effect of nitrogen source was only significant on the yield of E. hirsutum ($p < 0.001$), and a significant interaction (N source x Fe concentration) was found in the two dicotyledons only.

8.3.3.4 Effect of Nitrogen Source on Absolute and Relative Tolerance to Iron

Σ%RGR25 iron tolerance indices were calculated in the same way as for the Standard Screening Experiment (Section 3.2.3.2). The NO_3-N treatments were in effect a repeat of this experiment, although the higher iron concentrations were omitted. For each nitrogen source, %RGR was calculated as a % of the control RGR in that particular source; thus %RGR for the control was always 100% and was therefore omitted from Σ%RGR25 calculations. Table 8.17 shows Σ%RGR25 for the species in each of the nitrogen sources, and in the original Standard Screening (SS) Experiment.

Although tolerance indices do not exactly coincide, there was a weakly significant correlation ($r = 0.91$; $p < 0.10$) between indices recorded in NO_3-N in the present experiment and those recorded in NO_3-N in the Standard Screening Experiment. Note, however, that only four species are being compared. Indices measured in NH_4-N did not correlate significantly with those from either of the NO_3-N tests, though species followed the same tolerance ranking as in the Standard Screening Experiment.

Table 8.17 Iron Tolerance Indices (Σ%RGR25) of the four species supplied with Nitrate or Ammonium Nitrogen
(Monocotyledons are indicated by bold type)

Species	NH_4-grown Plants	NO_3-grown Plants	SS Experiment (NO_3-grown)
I. pseudacorus	206	162	184
L. vulgaris	106	77	133
J. subnodulosus	94	132	125
E. hirsutum	67	24	36

8.3.4 Discussion

8.3.4.1 Effect of Nitrogen Source on Yield

Reduction in yield with increasing iron supply confirmed that in either nitrogen source iron was toxic to all four species to varying degrees. In the monocotyledons, nitrogen source had no effect on sensitivity, but at control iron concentrations the two dicotyledonous species grew significantly better in nitrate-N than in ammonium-N. This is comparable with some nitrogen effects on aluminium and manganese sensitivity (McGrath and Rorison 1982; Rorison 1985).

NH_4-fed control plants (of the dicotyledons in particular) had smaller shoots, leaves (darker green) and roots than nitrate-fed control plants, while their roots or root tips were blackened. Poor growth in NH_4-N has been linked to reduced potassium uptake (Gigon and Rorison 1972). However, at high iron concentrations, yields and visual observations did not differ significantly between the two nitrogen sources. Thus, since NH_4-fed control plants were themselves stunted, and this was the main reason for interaction between nitrogen source and iron, yield reduction brought about by high iron concentrations was less in NH_4-fed than in NO_3-fed plants (see also McGrath and Rorison 1982 (Mn); and Rorison 1985 (Al)).

Nitrate has been reported to increase metal uptake (Tanaka and Navasero 1966b; Jackson and Williams 1968; McGrath and Rorison 1982; Rorison 1985), while ammonium depresses it (McGrath and Rorison 1982). This may be because NH_4-N acts as an exchangeable cation (Robinson and Rorison 1983) and can compete with other cations for uptake (Marschner 1986). Thus, higher tissue metal concentrations, more severe metal toxicity symptoms, and an accompanying greater yield reduction are frequently found with NO_3-N than with NH_4-N (McGrath and Rorison 1982; Rorison 1985). Indeed, NH_4-N is reported to ameliorate manganese toxicity (Vlamis and Williams 1962; McGrath and Rorison 1982) and aluminium toxicity (Rorison 1975). Such interactions with iron were not apparent in the present study.

In all four species examined, the detrimental effect of high concentrations of iron was greater than that of NH_4-N. This contrasts with the findings of McGrath and Rorison (1982) for *Holcus lanatus* and *Bromus erectus*, and of Rorison (1985) for *B. erectus*, where the adverse effect of

NH_4-N largely masked any possible effect of manganese or aluminium respectively. Rorison (1985), on the other hand, found that aluminium significantly ameliorated the deleterious effect of NH_4-N on Holcus lanatus.

8.3.4.2 Effect of Nitrogen Source on Assessment of Iron Tolerance

The generally very close agreement between iron tolerance indices obtained from plants in the present experiment (NO_3-N) and in the Standard Screening Experiment helps confirm the reproducibility of the method. However, calculation of a Σ%RGR tolerance index depends upon the fact that control plants sustain 100% growth. Thus, reduced growth of the two dicotyledonous species in the NH_4-N control would influence their assessed iron tolerance, despite the fact that at high iron concentrations there was no absolute difference in a plant's performance.

Lack of correlation between iron tolerance indices obtained from ammonium-grown plants with those from nitrate-grown plants does not necessarily indicate that nitrogen source is important, for a number of reasons. The fact that only four species were being compared reduced the likelihood of significant correlation should one species respond anomalously, and in all assessments Iris pseudacorus was the most tolerant species and Epilobium hirsutum was clearly the most sensitive.

Thus effects of nitrogen source appear to be largely insignificant to iron toxicity. Nitrogen source was also reported to be unimportant for manganese toxicity in subterranean clover (Evans et al. 1987).

CHAPTER NINE

GENERAL DISCUSSION

9.1 Does Iron have a Direct Toxic Effect upon Plant Growth?

Iron toxicity is undoubtedly a complex phenomenon, and findings from this study suggest that its action on wetland plants may be both direct and indirect (see also Al-Farraj 1983, and Mansfield 1990). Particularly sensitive species suffer more severely from both the direct and the indirect effects, than more tolerant species.

(i) Evidence for Direct Iron Toxicity

The rate at which high concentrations of iron produced symptoms in many of the screened species (Sections 3.1.2 and 3.1.3) suggests that at least part of the toxic action of iron may be direct, as it may be supposed that deficiencies of other elements induced by high concentrations of iron (i.e. indirect toxicity) would take longer to produce symptoms.

In all the four species selected for chemical analysis, yield reduction was linked to elevated iron concentrations in the shoot (Section 6.3). Moreover, there was an inverse relationship between tolerance and both shoot iron concentration and increase in shoot iron concentration (Section 6.4.2) suggesting strongly that iron may be directly toxic. However, the mode of action of direct iron toxicity is unknown, though it almost certainly affects cell division or elongation in roots (as do many metal toxins), and may have a direct effect on the phosphorus metabolism in cells throughout the plant. Various other suggestions from the literature are reported in Section 1.1.

(ii) Evidence for Indirect Iron Toxicity

Reduced tissue concentrations of many elements (e.g. N, P, K, Ca, Mg, Mn, Zn) (Section 6.3.3), and the concomitant disruption in shoot water balance (Section 6.3.1.3), suggest that iron also acts indirectly to restrain plant growth. It may compete for uptake with these elements, or

inactivate specific uptake or transport processes, but it is also likely that water and nutrient uptake are more generally affected since iron causes root stunting and may affect membrane permeability.

Chemical analysis of roots and shoots of the four selected species was suggestive of a disruption in the plants' phosphorus metabolism (especially in the two intolerant species). Phosphorus was retained in or on the roots at high external iron supply. Iron and phosphorus have a great affinity, and may be precipitated in or on the roots. Indeed, the yellow precipitate found on the roots of the most iron-sensitive species (Section 4.2.10) was identified as ferric phosphate (Sections 3.1.3.3.2 and 3.1.3.3.3). The accompanying reduction in shoot phosphorus concentration implies that iron may interfere with phosphorus translocation, and, although shoot phosphorus concentrations were not considered to represent deficiency values, what appeared to be P-deficiency symptoms were visible in the shoot of many iron-sensitive species. It is likely that iron interferes both with phosphorus translocation in the xylem, and with phosphorus availability in the shoot (and perhaps root) tissues, possibly by co-precipitation. The two species that were more tolerant of iron showed less evidence for disruption in phosphorus metabolism.

Further work might help elucidate the exact physiological contribution of direct iron toxicity and indirect toxicity through phosphorus-immobilisation effects in the plant. However this may be immaterial ecologically, since high iron concentrations and low phosphorus supply often co-exist in the field. Wheeler and Shaw (1987) found a highly significant negative rank correlation (r_s = -0.30; $p < 0.001$) between extractable iron and phosphorus concentrations in both rich-fen and poor-fen sites.

9.2 Is the Toxic Action of Iron similar to that of Aluminium?

The toxic effects of iron are more similar to those of aluminium than of any other metal discussed in Section 3.1.4. In both cases, roots are affected before shoots, patterns of mineral uptake and accumulation are altered, and growth is usually reduced before shoot symptoms become apparent. In addition, phosphorus deficiency symptoms are a common feature of both iron and aluminium toxicity, due partly to the failure of phosphorus translocation to the shoot.

Since aluminium toxicity is a potential source of yield loss to crops on acid soils, research into its physiology is much more advanced than for iron toxicity (see Taylor 1988a). There is evidence that aluminium may also have both direct and indirect effects on plant growth, though it may act differently in different species. It is reported to inhibit cell division in root tips (Clarkson 1965), and to act on lipid and protein portions of cell membranes, affecting both permeability and ion transport systems located there (Jensen et al. 1989). The possible indirect effects of aluminium on phosphorus metabolism include reduced phosphorus uptake, owing to a small root surface area (Marschner 1986), and precipitation as $AlPO_4$ in the root (Foy et al. 1978). However, direct effects on phosphorus metabolism (e.g ATP hydrolysis, phosphate esterification, or the hydrolysis of phosphoric acid monoesters, possibly leading to a significant disruption of enzymatic processes in roots exposed to aluminium) may be more important (Rorison 1965; Taylor 1988a). Iron might perhaps act in a similar direct manner. Bennet et al. (1986) linked the accumulation of non-metabolised phosphorus in the roots (i.e. failure of its transport to shoots) to a decline in root respiration caused by aluminium. Such a mechanism could perhaps similarly explain why phosphorus accumulated in roots of Rumex hydrolapathum in iron-enriched culture while iron did not (Section 6.4.3.4).

Differential aluminium tolerance is less well understood, but it is thought to be a mixture of various exclusion and internal tolerance strategies. These are fully reviewed by Taylor (1988b).

9.3 Were the Methods used in this Study an Adequate Measurement of Iron Toxicity/Tolerance?

If a large number of species are to be screened for their response to any factor, it is desirable that the method used should be relatively rapid (see also Baker and Walker 1989a). However, it must also be accurate. Attempts to screen the species at germination (Section 5.3) revealed a) that germination per se was largely unaffected by iron concentration, and b) that assessed tolerance (based on standardised seedling size measurements) was largely dependent on seed size. Root elongation measurements are also reported to be unsuitable for iron (Al-Farraj, 1983).

Longer-term screening methods may give a more reliable assessment of

species' tolerance (since it is the ability of a species to grow, mature and produce viable offspring which is the ultimate test of tolerance (see also Baker and Walker 1989a)). However, attempts to measure plant mortality over time met with a number of additional difficulties (Section 5.2).

There is, of course, a further problem in actually assessing the "accuracy" of a given screening method. One approach is to relate screened tolerance to field distribution. This has several inherent difficulties (see Section 7.1), but it is notable that in this case, results from the Standard (2-week) Screening Method used (Section 2.4.4) tied in well with field observations (Section 7.3). The use of different culture solutions can yield different results (e.g. Tanaka, Loe and Navasero 1966; Shaw 1984), but since field distribution agreed closely with laboratory findings it is likely that 10% Rorison solution is an adequate substitute for conditions in the wetland environment. Frequent solution changes meant that the iron concentration was approximately constant (Section 2.5), and influence of other variables was kept to a minimum (Section 2.1.1).

Relative Growth Rate (the variable chosen as the indicator of tolerance) (Section 4.1.1) considered the response of the whole plant, allowed discrimination between the response of a range of different types of species, and made comparison between species possible (see Wilson 1988). The effect of ochre deposition on RGR measurement was deemed minimal (Section 4.1.1.4).

The development of a tolerance index (Σ%RGR), based on summation of the treatment RGRs over a range of iron concentrations, each standardised against the control RGR response, meant that species could be ranked by a unique index which was independent of the other species being assessed (Section 3.2.3.2; see also Section 4.1.2). The index was simple to calculate and involved little manipulation of the raw data, other than standardisation (Section 4.1.2.4). Multivariate Classification, (Section 3.2.3.3), which was used in conjunction with the tolerance index, gave a graphical representation of species behaviour, and allowed species to be grouped with others which had a similar response to iron.

9.4 Is Iron Tolerance Conferred by an Internal Mechanism, or by an Exclusion Mechanism?

(i) Evidence for Exclusion

In contrast to the findings of Jayawardena et al. (1977), Smirnoff (1981) and Mansfield (1990), this study suggests strongly that external detoxification of iron by rhizosphere oxidation is an important mechanism in the iron tolerance of wetland plants. Tolerance was closely linked with the production of ochre on the roots in solution culture (Sections 4.2.10 and 3.1.3.3.3). Tolerance also correlated significantly with root porosity measurements reported by Justin and Armstrong (1987) (Section 4.2.11.2). In addition, of the four species selected for tissue analysis, the two which produced ochre on their roots showed a smaller increase in shoot iron concentration than the two species which did not (Section 6.3.3). This indicated that oxidative detoxification acts by excluding iron from the shoot (cf St-Cyr and Crowder 1987; Mansfield 1990).

Although plaque formation is dependent on root porosity, it is also reported to be site-dependent (Macfie 1986). Macfie and Crowder (1987) and St-Cyr and Crowder (1988, 1989) have further elucidated reasons for site dependence (Section 1.5.9). In the present study, using solution culture, some species (e.g. Juncus subnodulosus; Lysimachia vulgaris) were found to produce ochre on their roots under some circumstances, and ferric phosphate in others. Thus more work needs to be done to establish the factors involved.

(ii) Evidence for an Internal Tolerance Mechanism (see Section 6.4.2)

The significant increase in shoot iron concentration shown by all four species analysed chemically, indicates that rhizosphere oxidation does not fully exclude iron (see also Talbot and Etherington 1987; Talbot et al. 1987), and might imply the presence of an internal tolerance mechanism. However, in three of these species, (Juncus effusus, Lysimachia vulgaris and Rumex hydrolapathum), a 10% yield reduction was brought about by a similar shoot iron concentration (0.7-0.8 mg g^{-1} dry weight). This suggests that the greater iron-tolerance of Juncus effusus and Lysimachia vulgaris over Rumex hydrolapathum is due solely to a more

effective exclusion mechanism rather than to internal tolerance, though gross tissue analyses do not locate the exact site of the iron. On the other hand, Jones and Etherington (1970), Davies and Singh (1983), Talbot et al. (1987) and Mansfield (1990) give evidence for internal tolerance mechanisms in a few species. The overall importance of such mechanisms in iron tolerance needs further investigation. Cell culture techniques might be useful to screen a large number of species for tolerance at the cellular level, rather than at the whole plant level (Taylor 1988a).

9.5 Does Iron Tolerance Confer Waterlogging Tolerance?

A significant correlation was found between the iron tolerance of a species and the mean depth of the water table at sites in which it grew (Section 7.3.3), i.e. the more tolerant species occurred on the wetter sites which were more reducing and would tend to have greater iron availability. This and other results from the present study support the idea that iron tolerance (i.e. exclusion) and waterlogging tolerance are linked (Section 1.4.1). In contrast, Laan et al. (1989) found that the most flood-tolerant of three Rumex species had highest shoot iron concentrations.

Possession of aerenchyma is a major adaptation to both waterlogging and to exclusion of reduced soil toxins (Section 1.4.2.2), although rhizosphere oxidation is thought to occur at the expense of root aeration (Armstrong and Beckett 1987 cf Mansfield 1990).

The development of adventitious roots is generally thought of as an adaptation to flooding, and aeration is one of their possible functions (Section 1.4.2.3). However, contrary to the findings of Laan et al. (1989), circumstantial evidence from this study suggested they were no more effective at rhizosphere oxidation than the existing root system; nor were they any better adapted to a high iron environment. It was therefore concluded that their role might be to operate in the more-aerated surface layers should the original root system become damaged or unable to function in the deeper, more reducing layers (Section 4.2.11.4).

To summarise, iron tolerance and waterlogging tolerance are linked, since they are both conferred by the possession of aerenchyma. The ability of roots to respire anaerobically is another adaptation to waterlogging which may help confer iron tolerance indirectly by conserving

oxygen supplies for rhizosphere oxidation. The presence of adventitious roots may also be beneficial in conferring iron tolerance, though, in this study at least, it seemed to be purely an adaptation to waterlogging. Internal iron tolerance mechanisms, on the other hand, would confer iron tolerance but not necessarily waterlogging tolerance.

9.6 Why are Slower-Growing Species More Tolerant of Iron than Faster-Growing Species?

Iron tolerance and relative growth rate are inversely linked (Section 4.2.5). This agrees with findings reported for other metal toxicities (e.g. Ernst 1976; Wilson 1988). Although it is possible that a low relative growth rate might of itself keep iron uptake low, Ernst (1976) suggested that reduced growth might be due to the energy expenditure required to operate a tolerance mechanism. This is unlikely since growth rates were measured under control conditions requiring no such mechanism. Also, Walker (1990) noted that cadmium-tolerant and non-tolerant genotypes of Holcus lanatus had the same relative growth rate. It is more likely that the reduced growth rate is a co-adaptation to other factors in an iron-rich environment (see also Baker and Walker 1989b).

A low relative growth rate gives plants the ability to grow under low nutrient supply (Clarkson 1967; Shipley and Keddy 1988 and references therein), although it may give competitive inferiority under more favourable conditions (Hickey and McNeilly 1975; Cox and Hutchinson 1981; Wilson 1988). In the present study there was a significant correlation between a species' relative growth rate and the fertility of the sites in which it grew (Section 3.2.4.7.1). Since iron can act as a sink for phosphorus, iron toxic environments may be infertile. Indeed, a negative rank correlation was found between extractable iron concentration and fertility of poor-fen sites ($r_s = -0.02$; $p < 0.01$) (Wheeler and Shaw unpublished data). Iron tolerance was also inversely correlated with site fertility, and with mean extractable phosphorus concentration (Section 7.3.4). This suggests that the slow-growing iron-tolerant species may be able to survive at lower phosphorus supply than the faster-growing iron-sensitive species (see Clarkson 1967; Rorison 1968). It has already been shown that one of the indirect ways in which iron acts is to disrupt the phosphorus metabolism within the plant (Section 9.1). Those species which are tolerant of

iron were less affected (Section 6.4.3.4), possibly due to greater phosphorus efficiency.

It is widely accepted that a slow growth rate is a strategy of stress-tolerant species (Grime 1974, 1977, 1979), but it appears that in this situation the stress in question may not be iron itself, but low external or internal nutrient availability. A slow growth rate alone would be unlikely to confer iron tolerance in the long term in the absence of an effective exclusion mechanism. Indeed, relative growth rate explained less than 25% of the variance in measured tolerance in the present study.

9.7 Why are Monocotyledons More Tolerant of Iron than are Dicotyledons?

It was evident from all the variables measured that iron tends to affect the growth of dicotyledons more severely than of monocotyledons (Section 3.2.4). It was also apparent that many monocotyledons are more effective at oxidative detoxification than are most dicotyledons; (88% of monocotyledons showed signs of ochre precipitation on their roots compared with 43% of dicotyledons) and this was found to be the major iron tolerance mechanism in wetland plants (Section 4.2.10). Monocotyledon shoots are frequently hollow, allowing ease of oxygen diffusion to the roots (Crawford 1978; Etherington 1983b). There is also evidence, from reanalysis of data presented by Justin and Armstrong (1987), that monocotyledons (and particularly wetland ones) have a more porous root system than do dicotyledons. Although dicotyledons (and particularly wetland ones) are more able to increase root porosity upon flooding than are monocotyledons, they do not attain the high values inherent in monocotyledonous roots. These differences are linked to cortical cell packing configurations (Section 4.2.11.3). Many wetland monocotyledons have additional adaptations (e.g. endodermal lignification, and a subapical secondarily-thickened exodermis) which help conserve oxygen supplies for apical consumption and oxidative detoxification at the root tip (Section 4.2.11.9). Such adaptations have not been found in dicotyledons.

The greater degree of iron tolerance in monocotyledons over dicotyledons may therefore be due to an inherently superior oxidative detoxification system which would also make monocotyledons more suited to the wetland environment in general. The above adaptations (and others dis-

cussed in Section 4.2.11.9) explain why monocotyledons typically dominate many wetland systems.

There are two further possible reasons why monocotyledons are more iron-tolerant than dicotyledons. Iron tended to affect roots of dicotyledons more severely than shoots, while there was less effect in monocotyledons (Section 4.2.8). This is likely to affect nutrient and water uptake and cause dicotyledons to suffer more severely from indirect iron toxicity. In addition, dicotyledons in this study tended to have faster growth rates than monocotyledons (Section 3.2.4.7.1) implying a higher nutrient requirement. This would also make indirect iron toxicity more likely in dicotyledons. However, it should be noted that this may well reflect a bias in the choice of species, since monocotyledons and dicotyledons in general do not inherently have different growth rates (Grime and Hunt 1975). Screening of more monocotyledons with high growth rates might clarify the relative importance of the monocotyledonous morphology and growth rate, if indeed they can be separated (see Section 4.2.5).

9.8 Do External Factors in the Environment Affect the Toxicity of Iron?

Iron solubility is essentially controlled by the pH and oxidation-reduction status of the substrate (Section 1.3). Therefore, any environmental factor which affects either of these two variables is likely to affect iron availability. The level of the water table, for example, helps control oxidation-reduction potential, whereas calcium and in particular bicarbonate concentrations may help control the pH of a site. Thus, presupposing that iron is primarily available in dissolved form (see Section 1.3), the availability of potentially toxic concentrations is governed by environmental factors as well as the iron concentration in the substrate.

There is considerable evidence to suggest that calcium is able to ameliorate many metal toxicity effects (Section 8.1.1). However, investigations on two species in this study did not support this contention for iron (Section 8.1.2) (see also Mansfield 1990).

There is also evidence in the literature that bicarbonate may differentially affect iron uptake (albeit at low concentrations) in different species (Section 8.1.1). This idea was extended to high iron concentrations. No evidence was found to suggest that bicarbonate differentially

affected iron uptake in Juncus subnodulosus and Epilobium hirsutum, indicating that at such iron concentrations, bicarbonate only affects iron uptake indirectly by pH effects (Sections 8.1 and 8.2).

Reports also suggest that metal toxicity in plants may vary according to the source of nitrogen (Section 8.3.1) and, although NH_4-N is more common in the wetland environment (Section 8.3.1), NO_3-N had been used in the screening experiments. The iron tolerance of four selected species was therefore compared in these two nitrogen sources, and was shown to be largely unaffected by it (Section 8.3.3.). The two dicotyledons were, however, more sensitive to NH_4-N per se than to NO_3-N, although the toxic effect of iron was far greater. If this sensitivity to ammonium can be extended to dicotyledons in general, it could be an additional way in which they are less suited to the wetland environment than are monocotyledons.

It is reported that plants well-supplied with nutrients such as phosphorus, potassium, calcium, magnesium and manganese are able to exclude iron more effectively from the tissues than those poorly supplied. Deficiency may increase metabolic leakage from the roots, which in turn increases microbial activity and oxygen consumption in the rhizosphere (Foy et al. 1978; Ottow et al. 1983; Benckiser et al. 1984; Marschner 1986; Mansfield 1990), (see also Sections 6.4.3.3 to 6.4.3.8). Silicon supply is also thought to be important for the formation and stability of aerenchyma (Marschner 1986).

9.9 Does the Nutritional Status of the Plant Affect Iron Tolerance?

External nutrient supply can affect iron availability and hence uptake, while a plant's nutritional status (a function of external supply) can affect the efficiency of the iron exclusion mechanism (Section 9.8). Additionally, high concentrations of supplied iron are known to cause a reduction in the concentration of other elements in plant tissues (Section 6.3.3). There is also evidence (e.g. Benckiser et al. 1984) that adequate nutrition of rice plants can prevent the development of symptoms of iron toxicity (despite the presence of high iron concentrations in the shoot). This may imply the existence of an internal detoxification system (which was not apparent in any of the four species analysed chemically in this study (Section 6.4.2)).

It would be interesting to assess the effects of a high iron supply with different concentrations of nitrogen, phosphorus and potassium (separately and in combination) on a number of native species. Use of a split-root technique would separate internal effects from those which might occur in the solution (such as precipitation and/or competition for uptake).

9.10 Summary

This study has shown that iron availability is important in controlling species distribution in wetlands and that it may act both directly and indirectly.

For a plant to survive and complete its life-cycle in an iron-rich wetland environment it needs at least two adaptations:-

(i) An iron-exclusion mechanism (sensu Baker 1981, 1987)
This is conferred by aerenchyma and other structural adaptations that increase the effectiveness of rhizosphere oxidation: these also adapt a plant to life in wetlands in general. Iron may be retained both on the root surface and inside the root, but translocation to the shoot is restricted. The importance of internal tolerance mechanisms for iron has not been fully investigated, but it may be important in some species.

(ii) The ability to grow where nutrient-availability (particularly phosphorus) is low
This may be conferred by a low relative growth rate, a phosphorus-efficient metabolism, or possibly by mycorrhizal associations, which are not commonly found in wetland species. Phosphorus efficiency, and a low relative growth rate might enable a plant to survive both low internal phosphorus availability (an indirect effect of iron toxicity) as well as a low external supply.

Wheeler and Shaw (1987) report that, after phosphorus, iron was the second most important measured chemical factor in accounting for species richness in wetlands.

BIBLIOGRAPHY

AL-FARRAJ, M.M. (1983) The Influence of Environmental Conditions on the Growth of Wetland Plants with Special Reference to Epilobium hirsutum L. and Epilobium palustre L. PhD Thesis, University of Sheffield.

AL-FARRAJ, M.M., GILLER, K.E. & WHEELER, B.D. (1984) Phytometric estimation of fertility of waterlogged rich-fen peats using Epilobium hirsutum L. Plant and Soil, **81**, 283-289.

ALLEN, S.E., GRIMSHAW, H.M., PARKINSON, J.A. & QUARMBY, C. (1974) Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, Oxford.

ALVA, A.K., EDWARDS, D.G., ASHER, C.J. & BLAMEY, F.P.C. (1986) Effects of phosphorus/aluminium molar ratio and calcium concentration on plant response to aluminium toxicity. Soil Science Society of America Journal, **50**, 133-137.

ANDERSSON, A. (1975) Relative efficiency of 9 different soil extractants. Swedish Journal of Agricultural Research, **5**, 125-135.

ANDO, T., YOSHIDA, S. & NISHIYAMA, I. (1983) Nature of oxidising power of rice roots. Plant and Soil, **72**, 57-71.

ANTONOVICS, J., BRADSHAW, A.D. & TURNER, R.G. (1971) Heavy metal tolerance in plants. Advances in Ecological Research, **7**, 1-85.

AP REES, T., JENKIN, L.E.T., SMITH, A.M. & WILSON, P.M. (1987) The metabolism of flood-tolerant plants. In: Plant Life in Aquatic and Amphibious Habitats. Special Publication No. 5 of the British Ecological Society. Blackwell Scientific Publications, London. 227-238.

ARMSTRONG, W. (1964) Oxygen diffusion from the roots of some British bog plants. Nature, **204**, 801-802.

ARMSTRONG, W. (1967) The oxidising activity of roots in waterlogged soils. Physiologia Plantarum, **20**, 920-926.

ARMSTRONG, W. (1968) Oxygen diffusion from the roots of woody species. Physiologia Plantarum, **21**, 539-543

ARMSTRONG, W. (1971) Radial oxygen losses from intact rice roots as affected by distance from the apex, respiration and waterlogging. Physiologia Plantarum, **25**, 192-197.

ARMSTRONG, W. (1972) A re-examination of the functional significance of aerenchyma. Physiologia Plantarum, **27**, 173-177.

ARMSTRONG, W. (1978) Root aeration in the wetland condition. In: Plant Life in Anaerobic Environments. (Ed. by Hook, D.D. & Crawford, R.M.M.) 269-297. Ann. Arbor.

ARMSTRONG, W. (1979) Aeration in higher plants. In: Advances in Botanical Research. (Ed. by Woolhouse, H.W.) 7, 225-332.

ARMSTRONG, W. (1982) Waterlogged soils. In: Environment and Plant Ecology. (Ed. by Etherington J.R.) John Wiley and Sons, Ltd. 290-327

ARMSTRONG, W. & BECKETT, P.M. (1987) Internal aeration and the development of stelar anoxia in submerged roots. A multishell mathematical model combining axial diffusion of oxygen in the cortex with radial losses to the stele, the wall layers and the rhizosphere. New Phytologist, 105, 221-245.

ARMSTRONG, W. & BOATMAN, D.J. (1967) Some field observations relating growth of bog plants to conditions of soil aeration. Journal of Ecology, 55, 101-110.

ARMSTRONG, W. & WRIGHT, E.J. (1976) An electrical analogue to simulate the oxygen relations of roots in anaerobic media. Physiologia Plantarum, 36, 383-387.

AVADHANI, P.N., GREENWAY, H., LEFROY, R., & PRIOR, L. (1978) Alcoholic fermentation and malate metabolism in rice germinating at low oxygen concentrations. Australian Journal of Plant Physiology, 5, 15-25.

BACHA, R.E. & HOSSNER, L.R. (1977) Characteristics of coatings formed on rice roots as affected by iron and manganese additions. Soil Science Society of America Journal, 41, 931-935.

BAKER, A.J.M. (1981) Accumulators and excluders - strategies in the response of plants to heavy metals. Journal of Plant Nutrition, 3, 643-654.

BAKER, A.J.M. (1987) Metal tolerance. New Phytologist, 106 (suppl.), 93-111.

BAKER, A.J.M. & WALKER, P.L. (1989a) Physiological responses of plants to heavy metals and the quantification of tolerance and toxicity. Chemical Speciation and Bioavailability, 1, 7-17.

BAKER, A.J.M. & WALKER, P.L. (1989b) Ecophysiology of metal uptake by tolerant plants. In: Heavy Metal Tolerance in Plants: Evolutionary Aspects. (Ed. by Shaw, A.J.) 155-177. CRC Press, Incorporated, Boca Raton, Florida.

BANNISTER, P. (1964) The water relations of certain heath plants with reference to their ecological amplitude. Journal of Ecology, 52, 499-509.

BARBER, D.A., EBERT, M. & EVANS, N.T.A. (1962) The movement of ^{15}O through barley and rice plants. Journal of Experimental Botany, 13, 397-403.

BARTLETT, R.J. (1961) Iron oxidation proximate to plant roots. Soil Science, 92, 372-379.

BECKETT, I.T. & ANDERSON, W.P. (1973) Ferric-EDTA absorption by maize roots. In: Ion Transport in Plants. (Ed. by Anderson, W.P.) Academic Press, London. 595-607.

BECKETT, P.H.T. & DAVIS, R.D. (1977) Upper critical levels of toxic elements in plants. New Phytologist, 79, 95-106.

BENCKISER, G., OTTOW, J.C.G., WATANABE, I. & SANTIAGO, S. (1984) The mechanism of excessive iron-uptake (iron toxicity) of wetland rice. Journal of Plant Nutrition, 7, 177-185.

BENNET, R.J., BREEN, C.M. & FEY, M.V. (1986) Aluminium toxicity and induced nutrient disorders involving the uptake and transport of P, K, Ca and Mg in Zea mays L. South African Journal of Plant and Soil, 3, 11-17.

BIDDAPPA, C.C., SAMANTARAY, R.N. & MOHAMTY, S.K. (1980) Influence of inorganic and organic toxicants on inhibition of nutrient uptake by rice. Oryza, 17, 58-62. (Central Rice Research Institute, Cuttack 753006.)

BIDWELL, R.G.S. (1974) Plant Physiology, 244-245. Macmillan.

BIENFAIT, H.F. (1989) Prevention of stress in iron metabolism of plants. Acta Botanica Neerlandica, 38, 105-129.

BOONE, C.M., BRISTOW, J.M. & VANLOON G.W. (1983) The relative efficiency of ionic iron (III) and iron (II) utilization by the rice plant. Journal of Plant Nutrition, 6, 201-218.

BORGGARD, O.K. (1983) The influence of iron oxides on phosphate adsorption by soil. Journal of Soil Science, 34, 333-341.

BOTHA, C.E.J., FURNESS, H.D. & MAGWA, M.L. (1985) Iron and manganese localisation in Cynodon dactylon and Carex acutiformis under waterlogged and well-drained conditions. Electron Microscopy Society of Southern Africa - Proceedings, 15, 75-76.

BOULD, C. & PARFITT, R.I. (1973) Leaf analysis as a guide to the nutrition of fruit crops. X. Magnesium and phosphorus sand culture experiments with apple. Journal of the Science of Food and Agriculture, **24**, 175-185.

BOXMA, R. (1972) Bicarbonate as the most important soil factor in lime induced chlorosis in Netherlands. Plant and Soil, **37**, 233-243.

BRADY, N.C. (1974) In: The Nature and Properties of Soils. 8th edn. Macmillan Publishing Co., Inc., New York; Collier Macmillan Publishers, London. 388-389.

BRAENDLE, R. & CRAWFORD, R.M.M. (1987) Rhizome anoxia tolerance and habitat specialization in wetland plants. In: Plant Life in Aquatic and Amphibious Habitats. (Ed. by R.M.M. Crawford) Special Publication No. 5 of the British Ecological Society. Blackwell Scientific Publications, London. 397-410.

BROWN, J.C. (1960) An evaluation of bicarbonate-induced iron chlorosis. Soil Science, **89**, 246-247.

BROWN, J.C. & JONES, W.E. (1977) Manganese and iron toxicities dependent on soybean variety. Communications in Soil Science and Plant Analysis, **8**, 1-15.

BROWN, J.C., TIFFIN, L.O. SPECHT, A.E. & RESNICKY, I.W. (1961) Stability and concentration of metal chelates; factors in iron chlorosis of plants. Agronomy Journal, **53**, 85-90.

CHANEY, R.L., BROWN, J.C. & TIFFIN, L.O. (1972) Obligatory reduction of ferric chelates in iron uptake by soybeans. Plant Physiology, **50**, 208-213.

CHEN, C.C., DIXON, J.B. & TURNER, F.T. (1980a) Iron coatings on rice roots: mineralogy and quantity influencing factors. Soil Science Society of America Journal, **44**, 635-639.

CHEN, C.C., DIXON, J.B. & TURNER, F.T. (1980b) Iron coatings on rice roots: morphology and models of development. Soil Science Society of America Journal, **44**, 1113-1119.

CHEN, Y. & BARAK, P. (1982) Iron nutrition of plants in calcareous soils. Advances in Agronomy, **35**, 217-240.

CHINNERY, L.E. & HARDING, C.P. (1980) The effect of ferrous iron on the uptake of manganese by Juncus effusus L. Annals of Botany, **46**, 409-412.

CHIU, T.F. (1966) Iron and manganese absorption by rice plants. Soils and Fertilizers in Taiwan, 1-6.

CLAPHAM, A.R. (1969) The Flora of Derbyshire. Derby Museum and Art Gallery.

CLAPHAM, A.R., TUTIN, T.G. & WARBURG, E.F. (1981) Excursion Flora of the British Isles. 3rd edn. Cambridge University Press, Cambridge.

CLARKSON, D.T. (1965) The effect of aluminium and some other trivalent metal cations on cell division in the root apices of Allium cepa. Annals of Botany, **29**, 309-315.

CLARKSON, D.T. (1967) Phosphorus supply and growth rate in species of Agrostis L. Journal of Ecology, **55**, 111-117.

CLYMO, R.S. (1962) An experimental approach to part of the calcicole problem. Journal of Ecology, **50**, 707-731.

CONLIN, T.S.S. & CROWDER, A.A. (1989) Location of radial oxygen loss and zones of potential iron uptake in a grass and two nongrass emergent species. Canadian Journal of Botany, **67**, 717-722.

CONWAY, V.M. (1937) Studies in the autecology of Cladium mariscus R. Br. Part III. The aeration of the subterranean parts of the plant. New Phytologist, **36**, 64-96.

CONWAY, V.M. (1940) Aeration and plant growth in wet soils. Botanical Review, **6**, 149-163.

COULOMBE, B.A., CHANEY, R.L. & WIEBOLD, W.J. (1982) Bicarbonate can induce iron chlorosis in 'Wayne' soybeans. Agronomy Abstracts, 204.

COULOMBE, B.A., CHANEY, R.L. & WIEBOLD, W.J. (1984) Bicarbonate directly induces iron chlorosis in susceptible soybean cultivars. Soil Science Society of America Journal, **48**, 1297-1301.

COX, R.M. & HUTCHINSON, T.C. (1981) Multiple and co-tolerance to metals in the grass Deschampsia caespitosa: adaptation, preadaptation and 'cost'. Journal of Plant Nutrition, **3**, 731-741.

CRAIG, G.C. (1977) A method of measuring heavy metal tolerance in grasses. Transactions of the Rhodesia Scientific Association, **58**, 9-16.

CRAWFORD, R.M.M. (1966) The control of anaerobic respiration as a determining factor in the distribution of the genus Senecio. Journal of Ecology, **54**, 403-413.

CRAWFORD, R.M.M. (1967) Alcohol dehydrogenase activity in relation to flooding tolerance in roots. Journal of Experimental Botany, **18**, 458-464.

CRAWFORD, R.M.M. (1969) The physiological basis of flooding tolerance. Bericht der Deutschen botanischen Gesellschaft, **82**, 111-114.

CRAWFORD, R.M.M. (1972) Some metabolic aspects of ecology. Transactions and Proceedings of the Botanical Society of Edinburgh, **41**, 309-322.

CRAWFORD, R.M.M. (1977) Tolerance of anoxia and ethanol metabolism in germinating seeds. New Phytologist, **79**, 511-517.

CRAWFORD, R.M.M. (1978) Biochemical and ecological similarities in marsh plants and diving animals. Naturwissenschaften, **65**, 194-201.

CRAWFORD, R.M.M. & McMANMON, M. (1968) Inductive responses of alcohol and malic dehydrogenases in relation to flooding tolerance in roots. Journal of Experimental Botany, **19**, 435-441.

CROWDER, A.A. & MACFIE, S.M. (1986) Seasonal deposition of ferric hydroxide plaque on roots of wetland plants. Canadian Journal of Botany, **64**, 2120-2124.

CROWDER, A.A., MACFIE, S.M., CONLIN, T., ST-CYR, L. & GREIPSSON, S. (1987) Iron hydroxide plaques on roots of wetland plants. In: Proceedings of the 6th International Conference on Heavy Metals in the Environment, New Orleans, (Ed. by Lindberg, S.E. & Hutchinson, T.C.), **1**, 404-406, CEP Consultants Ltd., Edinburgh.

DAVIES, M.S. & SINGH, A.K. (1983) Population differentiation in Festuca rubra L. and Agrostis stolonifera L. in response to soil waterlogging. New Phytologist, **94**, 573-583.

DAVIES, M.S. & SNAYDON, R.W. (1973) Physiological differences among populations of Anthoxanthum odoratum L. collected from the park grass experiment, Rothamstead. II. Response to Aluminium. Journal of Applied Ecology, **10**, 47-55.

DeLAUNE, R.D., REDDY, C.N. & PATRICK, W.H. jr. (1981) Effect of pH and redox potential on concentration of dissolved nutrients in an estuarine sediment. Journal of Environmental Quality, **10**, 276-279.

DOI, Y. (1952a) Studies on the oxidising power of roots of crop plants. I. The difference with species of crop plants and wild grasses. Proceedings of the Crop Science Society of Japan, **21**, 12-13.

DOI, Y. (1952b) Studies on the oxidising power of roots of crop plants. II. The interrelation between paddy rice and soy-bean. Proceedings of the Crop Science Society of Japan, **21**, 14-15.

DREW, M.C. (1987) Mechanisms of acclimation to flooding and oxygen shortage in non-wetland species. In: Plant Life in Aquatic and Amphibious Habitats. (Ed. by Crawford, R.M.M.) Special Publication No. 5 of the British Ecological Society. Blackwell Scientific Publications, London. 321-331.

DREW, M.C., JACKSON, M.B. & GIFFARD, S. (1979) Ethylene-promoted adventitious rooting and development of cortical air spaces (aerenchyma) in roots may be an adaptive response to flooding in Zea mays L. Planta, **147**, 83-88.

DREW, M.C. & LYNCH, J.M. (1980) Soil anaerobiosis, microorganisms, and root function. Annual Review of Phytopathology, **18**, 37-66.

DUGAN, P.R. & APEL, W.A. (1983) Bacteria and acidic drainage from coal refuse:- inhibition by sodium lauryl sulfate and sodium benzoate. Applied and Environmental Microbiology, **46**, 279-282.

EBER, W. (1982) The ecology of bogs and bog plants. Journal of Life Sciences, **3**, 243-254.

ERNST, W. (1976) Physiological and biochemical aspects of metal tolerance. In: Effects of Air Pollutants on Plants. (Ed. by Mansfield, T.A.). Cambridge University Press, Cambridge. 115-133.

ERNST, W.H.O. (1978) Chemical soil factors determining plant growth. In: Structure and Functioning of Plant Populations. (Ed. by Freyson, A.H.J. & Woldendorp, J.W.) North Holland Publishing Co., Amsterdam, Oxford, New York.

ETHERINGTON, J.R. (1983a) Control of germination and seedling morphology by ethene: differential responses, related to habitat of Epilobium hirsutum L. and Chamerion angustifolium (L.) J. Holub. Annals of Botany, **52**, 653-658.

ETHERINGTON, J.R. (1983b) Wetland Ecology. The Institute of Biology Studies in Biology No. 154. Edward Arnold.

ETHERINGTON, J.R. (1984) Comparative studies of plant growth and distribution in relation to waterlogging. X. Differential formation of adventitious roots and their experimental excision in Epilobium hirsutum and Chamerion angustifolium. Journal of Ecology, **72**, 389-404.

ETHERINGTON, J.R. & DAVIES, M.S. (1978) Analysis of iron in organic materials: an unrecognised problem of volatility. Plant and Soil, **50**, 497-502.

ETHERINGTON, J.R. & THOMAS, O.M. (1986) Response to waterlogging and differing sensitivity to divalent iron and manganese in clones of Dactylis glomerata L. derived from well-drained and poorly-drained soils. Annals of Botany, **58**, 109-119.

EVANS, J., SCOTT, B.J. & LILL, W.J. (1987) Manganese tolerance in subterranean clover (Trifolium subterraneum L.) genotypes grown with ammonium nitrate or symbiotic nitrogen. Plant and Soil, **97**, 207-215

EVANS, N.T.S. & EBERT, M. (1960) Radioactive oxygen in the study of gas transport down the root of Vicia faba. Journal of Experimental Botany, **11**, 246-257.

FITTER, A.H. & HAY, R.K.M. (1981) Ionic toxicity. In: Environmental Physiology of Plants. Academic Press. 200-231.

FLEMING, A.L., CHANEY, R.L. & COULOMBE, B.A. (1984) Bicarbonate inhibits iron-stress response and iron uptake-translocation of chlorosis-susceptible soybean cultivars. Journal of Plant Nutrition, **7**, 699-714.

FOY, C.D., CHANEY, R.L. & WHITE, M.C. (1978) The physiology of metal toxicity in plants. Annual Review of Plant Physiology, **29**, 511-566.

FUKUI, J. (1953) Studies on the adaptability of green manure and forage crops to paddy field conditions. Proceedings of the Crop Science Society of Japan., **22**, 110.

GAMBRELL, R.P. & PATRICK, W.H. jr. (1978) Chemical and microbiological properties of anaerobic soils and sediments. In: Plant Life in Anaerobic Environments. (Ed. by Hook D.D. & Crawford, R.M.M.) 375-423. Ann. Arbor.

GARCIA-NOVO, F. & CRAWFORD, R.M.M. (1973) Soil aeration, nitrate reduction and flooding tolerance in higher plants. New Phytologist, **72**, 1031-1039.

GIGON, A. & RORISON, I.H. (1972) The response of some ecologically distinct plant species to nitrate- and to ammonium-N. Journal of Ecology, **60**, 93-102.

GILL, C.J. (1975) The ecological significance of adventitious rooting as a response to flooding in woody species, with special reference to Alnus glutinosa (L.) Gaertn. Flora, **164**, 85-97.

GILLER, K.E. (1982) Aspects of the Plant Ecology of a Flood-Plain Mire in Broadland, Norfolk. PhD Thesis, University of Sheffield.

GILLER, K.E. & WHEELER, B.D. (1986) Peat and peat water chemistry of a flood-plain fen in Broadland, Norfolk, U.K. Freshwater Biology, **16**, 99-114.

GOLTERMANN, H.L., CLYMO, R.S. & OHNSTAD, M.A.M. (1978) Methods for Physical and Chemical Analysis of Fresh Waters. IBP Handbook No. 8. 2nd edn. Blackwell Scientific Publications, Oxford, London.

GOOD, B.J. & PATRICK, W.H. (1987) Gas composition and respiration of water oak (Quercus nigra L.) and green ash (Fraxinus pennsylvanica Marsh) roots after prolonged flooding. Plant and Soil, **97**, 419-427.

GOTOH, S. & PATRICK, W.H. jr. (1974) Transformation of iron in a waterlogged soil as influenced by redox potential and pH. Soil Science Society of America Proceedings, **38**, 66-71.

GREEN, M.S. & ETHERINGTON, J.R. (1977) Oxidation of ferrous iron by rice (Oryza sativa L.) roots: a mechanism for waterlogging tolerance? Journal of Experimental Botany, **28**, 678-689.

GREENWOOD, D.J. (1967) Studies on oxygen transport through mustard seedlings (Sinapsis alba L.). New Phytologist, **66**, 597-606.

GRIME, J.P. (1974) Vegetation classification by reference to strategies. Nature, **250**, 26-31

GRIME, J.P. (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. American Naturalist, **111**, 1169-1194.

GRIME, J.P. (1979) Plant Strategies and Vegetation Processes. Wiley, Chichester.

GRIME, J.P. & HUNT, R. (1975) Relative growth-rate: its range and adaptive significance in a local flora. Journal of Ecology, **63**, 393-422

HACKETT, C. (1965) Ecological aspects of nutrition of Deschampsia flexuosa (L.) Trin. II. The effect of Al, Ca, Fe, K, Mn, N, P and pH on growth of seedlings and established plants. Journal of Ecology, **53**, 299-314.

HEATHCOTE, C.A., DAVIES, M.S. & ETHERINGTON, J.R. (1987) Phenotypic flexibility of Carex flacca Schreb. Tolerance of soil flooding by populations from contrasting habitats. New Phytologist, **105**, 381-391

HECHT-BUCHOLZ, C. (1967) Uber die Dunkelfarbung des Blattgruns bei Phosphormangel. Zeitschrift fur Pflanzenernaehrung und Bodenkunde, **118**, 12-22.

HENDRY, G.A.F. & BROCKLEBANK, K.J. (1985) Iron-induced oxygen radical metabolism in waterlogged plants. New Phytologist, **101**, 199-206.

HEWITT, E.J. (1966) Sand and Water Culture Methods used in the Study of Plant Nutrition. Technical Communication No. 22 (revised 2nd edn.). Commonwealth Bureau of Horticulture and Plantation Crops, East Malling, Maidstone, Kent. Commonwealth Agric. Bureaux, Farnham Royal, Bucks., England.

HEWITT, E.J. & SMITH, T.A. (1974) Plant Mineral Nutrition. Unibooks, English Universities Press Ltd.

HICKEY, D.A. & McNEILLY, T. (1975) Competition between metal tolerant and normal plant populations: a field experiment on normal soil. Evolution, 29, 458-464.

HILL-COTTINGHAM, D.G. & LLOYD-JONES, C.P. (1961) Absorption and breakdown of FeEDTA by tomato plants. Nature, 189, 312.

HILL-COTTINGHAM, D.G. & LLOYD-JONES, C.P. (1965) The behaviour of iron chelating agents with plants. Journal of Experimental Botany, 16, 233-242.

HODGSON, J.G. (1972) A Comparative Study of Seedling Root Growth with respect to Aluminium and Iron supply. PhD Thesis, Sheffield University.

HOLLIS, J.P. (1967) Toxicant diseases of rice. Bulletin of the Louisiana Agricultural Experimental Station, 614.

HOOK, D.D. & SCHOLTENS, J.R. (1978) Adaptations and flood tolerance of tree species. In: Plant Life in Anaerobic Environments. (Ed. by Hook, D.D. & Crawford, R.M.M.) 299-331. Ann. Arbor.

HOWELER, R.H. (1973) Iron-induced oranging disease of rice in relation to physico-chemical changes in a flooded oxisol. Soil Science Society of America Proceedings, 37, 898-903.

HUTCHINSON, G.E. (1957) A Treatise on Limnology. Wiley, New York.

IGNATIEFF, V. (1941) Determination and behaviour of ferrous iron in soils. Soil Science, 51, 249-260.

JACKSON, W.A. & WILLIAMS, D.C. (1968) Nitrate-stimulated uptake and transport of strontium and other cations. Soil Science Society of America Proceedings, 32, 698-704.

JACKSON, W.T. (1955) The role of adventitious roots in recovery of shoots following flooding of the original root systems. American Journal of Botany, 42, 816-819.

JAYAWARDENA, S.D.G., WATANABE, T. & TANAKA, D. (1977) Relation between root oxidising power and resistance to iron toxicity in rice. The Report of the Society of Crop Science and Breeding in Kinki (Japan), No. 22, 38-47.

JEFFERY, J.W.O. (1961) Measuring the state of reduction of a waterlogged soil. Journal of Soil Science, 12, 317-325.

JENSEN, P., PETTERSSON, S., DRAKENBERG, T. & ASP, H. (1989) Aluminium effects on vacuolar phosphorus in roots of beech (Fagus sylvatica L.). Journal of Plant Physiology, 134, 37-42.

JOHN, C.D. & GREENWAY, H. (1976) Alcoholic fermentation and activity of some enzymes in rice roots under anaerobiosis. Australian Journal of Plant Physiology, 3, 325-336.

JONES, H.E. (1968) Some Chemical Effects of Waterlogging in Soils of low pH on the Growth of Erica cinerea and Erica tetralix. PhD Thesis, University of Wales, Cardiff.

JONES, H.E. (1971a) Comparative studies of plant growth and distribution in relation to waterlogging. II. Experimental study of relationship between transpiration and uptake of iron in Erica cinerea L. and Erica tetralix L. Journal of Ecology, 59, 167-178.

JONES, H.E. (1971b) Comparative studies of plant growth and distribution in relation to waterlogging. III. The response of Erica cinerea L. to waterlogging in peat soils of differing iron content. Journal of Ecology, 59, 583-591.

JONES, H.E. & ETHERINGTON, J.R. (1970) Comparative studies of plant growth and distribution in relation to waterlogging. I. The survival of Erica cinerea L. and Erica tetralix L. and its apparent relationship to iron and manganese uptake in waterlogged soil. Journal of Ecology, 58, 487-496.

JONES, R. (1972a) Comparative studies of plant growth and distribution in relation to waterlogging. V. The uptake of iron and manganese by dune and dune slack plants. Journal of Ecology, 60, 131-140.

JONES, R. (1972b) Comparative studies of plant growth and distribution in relation to waterlogging. VI. The effect of manganese on the growth of dune and dune slack plants. Journal of Ecology, 60, 141-145.

JONES, R. (1973) Comparative studies of plant growth and distribution in relation to waterlogging. VII. The influence of water table fluctuation on iron and manganese available in dune slack soils. Journal of Ecology, 61, 107-116.

JONES, R. (1975) Comparative studies of plant growth and distribution in relation to waterlogging. VIII. The uptake of phosphorus by dune and dune slack plants. Journal of Ecology, 63, 109-116.

JONES, R. & ETHERINGTON, J.R. (1971) Comparative studies of plant growth and distribution in relation to waterlogging. IV. The growth of dune and dune slack plants. Journal of Ecology, 59, 793-801.

JUSTIN, S.H.F.W. & ARMSTRONG, W. (1987) The anatomical characteristics of roots and plant response to soil flooding. New Phytologist, 106, 465-495.

KARATAGLIS, S.S. (1980) Selective adaptation to copper of populations of Agrostis tenuis and Festuca rubra (Poaceae). Plant Systematics and Evolution., 134, 215-228.

KAWASE, M. (1978) How plants adapt to waterlogging. Ohio Report, Ohio Agricultural Research and Development Center, 63, 14-15.

KAWASE, M. (1979) Role of cellulase in aerenchyma development in sunflower. American Journal of Botany, 66, 183-190.

KAWASE, M. & WHITMOYER, R.E. (1980) Aerenchyma development in waterlogged plants. American Journal of Botany, 67, 18-22.

KEELEY, J.E. (1979) Population differentiation along a flood frequency gradient: physiological adaptations to flooding in Nyssa sylvatica. Ecological Monographs, 49, 89-108.

KINRAIDE, T.B. & PARKER, D.R. (1987) Cation amelioration of aluminium toxicity in wheat. Plant Physiology, 83, 546-551.

KOLESCH, H., OKTAY, M. & HOFNER, W. (1984) Effect of iron chlorosis-inducing factors on the pH of the cytoplasm of sunflower. (Helianthus annuus). Plant and Soil, 82, 215-221.

KONINGS, H. (1982) Ethylene-promoted formation of aerenchyma in seedling roots of Zea mays L. under aerated and non-aerated conditions. Physiologia Plantarum, 54, 119-124.

KRAMER, P.J. (1951) Causes of injury to plants resulting from flooding of the soil. Plant Physiology, 26, 722-736.

KRANSY, M.E., ZASADA, J.C. & VOGT, K.A. (1988) Adventitious rooting of four Salicaceae species in response to a flooding event. Canadian Journal of Botany, 66, 2597-2598.

KURAEV, V.N. (1966) Effect of various concentrations of ferrous iron in solution culture on the growth and development of crop plants. Agrochmiya, 12, 110-117.

LAAN, P., SMOLDER, A., BLOM, C.W.P.M. & ARMSTRONG, W. (1989) The relative roles of internal aeration, radial oxygen losses, iron exclusion and nutrient balances in flooding-tolerance of Rumex species. Acta Botanica Neerlandica, 38, 131-145.

LADIGES, P.Y. & KELSO, A. (1977) The comparative effect of waterlogging on two populations of Eucalyptus viminalis Labill. and one population of E. ovata Labill. Australian Journal of Botany, 25, 159-169.

LEVAN, M.A. & RIHA, S.J. (1986) The precipitation of black oxide coatings on flooded conifer roots of low internal porosity. Plant and Soil, **95**, 33-42.

LINGLE, J.C., TIFFIN, L.O. & BROWN, J.C. (1963) Iron uptake-transport of soybeans as influenced by other cations. Plant Physiology, **38**, 71-76.

LUXMOORE, R.J., STOLZY, L.H. & LETEY, J. (1970) Oxygen diffusion in the soil-plant system. Agronomy Journal, **62**, 317-322.

MACFIE, S.M. (1986) Wetland Influence on Ferric Hydroxide Plaque Formation. MSc Thesis, Queen's University, Kingston, Ontario.

MACFIE, S.M. & CROWDER, A.A. (1987) Soil factors influencing ferric hydroxide plaque formation on roots of Typha latifolia. Plant and Soil, **102**, 177-184.

MANDAL, L.N. (1962) Levels of iron and manganese in soil solution and the growth of rice in waterlogged soils in relation to the oxygen status of the soil solution. Soil Science, **94**, 387-391.

MANSFIELD, S.M. (1990) Ecophysiological Effects of High Concentrations of Iron and other Heavy Metals on Eriophorum angustifolium Honch. and Phragmites australis (Cav.) Trin. ex Steudel. PhD Thesis, University of Sheffield.

MARSCHNER, H. (1986) Mineral Nutrition in Higher Plants. Academic Press, London.

MARSCHNER, H. & BARBER, D.A. (1975) Iron uptake by sunflower plants under sterile and non-sterile conditions. Plant and Soil, **43**, 515-518.

MARSCHNER, H., ROMHELD, V. & AZARABADI, S. (1978) Iron stress response of efficient and inefficient plant species. Proceedings of the 8th International Colloquium on Plant Analysis and Fertilizer Problems, Auckland, New Zealand. 319-327.

MARSCHNER, H., ROMHELD, V. & KISSEL, M. (1986) Different strategies in higher plants in mobilization and uptake of iron. Journal of Plant Nutrition, **9**, 695-713.

MARTIN, M.H. (1968) Conditions affecting the distribution of Mercurialis perennis in certain Cambridgeshire woodlands. Journal of Ecology, **56**, 777-793.

McCAIN, S. & DAVIES, M.S. (1983) Effects of pretreatment with phosphate in natural populations of Agrostis capillaris L. I Influences on the subsequent long term yield of, and accumulation of phosphorus in, plants grown from tillers. New Phytologist, **94**, 367-379.

McGRATH, S.P. & RORISON, I.H. (1982) The influence of nitrogen source on the tolerance of Holcus lanatus L. and Bromus erectus Huds. to manganese. New Phytologist, **91**, 443-452.

McKEAGUE, J.A. & DAY, J.H. (1966) Dithionite- and oxalate- extractable iron and aluminium as aids in differentiating various classes of soil. Canadian Journal of Soil Science, **46**, 13-22.

McLAUGHLIN, B.E., VANLOON, G.W. & CROWDER, A.A. (1985) Comparison of selected washing treatments on Agrostis gigantea samples from mine tailings near Copper Cliff, Ontario, before analysis for Cu, Ni, Fe and K content. Plant and Soil, **85**, 433-436.

McMANMON, M. & CRAWFORD, R.M.M. (1971) A metabolic theory of flooding tolerance: the significance of enzyme distribution and behaviour. New Phytologist, **70**, 299-306.

McVEAN, D.N. & RATCLIFFE, D.A. (1962) Plant Communities of the Scottish Highlands. H.M.S.O., London.

MENDELSSOHN, I.A. & McKEE, K.L. (1987) Root metabolic responses of Spartina alterniflora to hypoxia. In: Plant Life in Aquatic and Amphibious Habitats. (Ed. by Crawford, R.M.M.) Special Publication No. 5 of the British Ecological Society. Blackwell Scientific Publications, London. 239-253.

MENDELSSOHN, I.A. & POSTEK, M.T. (1982) Elemental analysis of deposits on the roots of Spartina alterniflora Loisel. American Journal of Botany, **69**, 904-912.

MILLIKAN, C.R. (1949) Effects on flax of a toxic concentration of Bo, Fe, Mo, Al, Cu, Zn, Mn, Co, or Ni in the nutrient solution. Proceedings of the Royal Society of Victoria, **61**, 25-42.

de MIRANDA, L.N. & ROWELL, D.L. (1989) Aluminium-phosphate interactions in wheat. New Phytologist, **113**, 7-12.

MOMON, A.R., CHINO, M., TAKEOKA, Y., HARA, K. & YATAZAWA, M. (1980) Distribution of manganese in leaf tissues of manganese accumulator Acanthopanax sciadophylloides as revealed by electronprobe X-ray microanalyser. Journal of Plant Nutrition, **2**, 457-476.

MORTIMER, M.H. (1941) The exchange of dissolved substances between mud and water in lakes. Journal of Ecology, **29**, 280-329.

MULDER, E.G. & GERRETSEN, F.C. (1952) Soil manganese in relation to plant growth. Advances in Agronomy, **4**, 221-277.

NAGAI, T. & MATONO, T. (1959) Cultivated rice varieties viewed from root characteristics. IV. Interrelationships amongst root activity,

ferrous iron coating formation and CEC. Proceedings of the Crop Science Society of Japan, 28, 208-210.

NAZRUL-ISLAM, A.K.M. (1976) Comparative Ecology of Plants in Relation to Soil Moisture and Mineral Nutrition. PhD Thesis, Dept. of Botany, University of Sheffield.

NAZRUL-ISLAM, A.K.M. (1986) Effects of interaction of calcium and manganese on the growth and nutrition of Epilobium hirsutum L. Soil Science and Plant Nutrition, 32, 161-168.

NHUNG, M-T.M. & PONNAMPERUMA, F.N. (1966) Effects of calcium carbonate, manganese dioxide, ferric hydroxide, and prolonged flooding on chemical and electrochemical changes and growth of rice in a flooded acid sulfate soil. Soil Science, 102, 29-41.

NICHOLAS, D.J.D., LLOYD-JONES, C.P. & FISHER, D.J. (1957) Some problems associated with determining iron in plants. Plant and Soil, 8, 367-377.

OLSEN, C. (1958) Iron uptake in different plant species as a function of the pH value of the nutrient solution. Physiologia Plantarum, 11, 889-905.

OLSON, R.V. (1947) Iron solubility in soils as affected by pH and free iron oxide content. Soil Science Society of America Proceedings, 12, 153-157.

OLSON, R.V. & ELLIS, R. jr. (1982) Iron. In: Methods of Soil Analysis. Part 2 - Chemical and Microbiological Properties. 301-312. Agronomy Series No. 9, Part 2, 2nd edn. (Ed. by Page, A.L., Miller, R.H. & Keeney, D.R.) American Society of Agronomy Inc. Soil Science Society of America Inc. Publisher Madison, Wisconsin, USA.

OTA, Y. & YAMADA, N. (1960) Physiological effect of iron on rice plants. I. Effect of iron in relation to soil aeration. Proceedings of the Crop Science Society of Japan, 28, 367-370.

OTTE, M.L., BUIJS, E.P., RIEMER, L. ROZEMA, J. & BROEKMAN, R.A. (1987) The iron-plaque on the roots of salt-marsh plants: a barrier to heavy metal uptake? In: Proceedings of the 6th International Conference on Heavy Metals in the Environment, New Orleans, 1, (Ed., by Lindberg, S.E. & Hutchinson, T.C.) CEP Consultants Ltd., Edinburgh.

OTTE, M.L., ROZEMA, J., KOSTER, L., HAARSMA, M.S. & BROEKMAN, R.A. (1989) Iron plaque on roots of Aster tripolium L.: interaction with zinc uptake. New Phytologist, 111, 309-317.

OTTOW, J.C.G., BENCKISER, G., WATANABE, I. & SANTIAGO, S. (1983) Multiple nutritional soil stress as the prerequisite for iron toxicity of wetland rice (Oryza sativa L.). Tropical Agriculture, Trinidad, **60**, 102-106.

OULLETTE, G.T. & DESSUREAUX, L. (1958) Chemical composition of alfalfa as related to tolerance of manganese and aluminium. Canadian Journal of Plant Science, **38**, 206-214.

PATRICK, W.H. jr. (1964) Extractable iron and phosphorus in a submerged soil at controlled redox potentials. 8th International Congress of Soil Science, Bucharest, Romania. 605-609.

PATRICK, W.H. jr. & KHALID, R.A. (1974) Phosphate release and sorption by soils and sediments: effect of aerobic and anaerobic conditions. Science, **186**, 53-55.

PATRICK, W.H. jr. & MAHAPATRA, I.C. (1968) Transformation and availability to rice of nitrogen and phosphorus in waterlogged soils. Advances in Agronomy, **20**, 323-359.

PITTS, G. (1969) Exploration in the Chemistry and Microbiology of Louisiana Rice Plant-Soil Relations. PhD Diss. Louisiana State University, Louisiana, USA.

PITTS, G., ALLAM, A.I. & HOLLIS, J.P. (1972) Beggiatoa: occurrence in the rice rhizosphere. Science, **178**, 990-992.

PONNAMPERUMA, F.N. (1972) The chemistry of submerged soils. Advances in Agronomy, **24**, 29-95.

PONNAMPERUMA, F.N., BRADFIELD, R. & PEECH, M. (1955) Physiological disease of rice attributable to iron toxicity. Nature, **175**, 265.

POSTMA, D. (1982) Pyrite and siderite formation in brackish and freshwater swamp sediments. American Journal of Science, **282**, 1151-1183.

POZUELO, M.R.E. & GROSSENBACHER, K.A. (1965) Uptake of insoluble iron by sterile and nonsterile alfalfa plants. Agrochimica, **9**, 361-367.

PROCTOR, M.C.F. (1974) The vegetation of the Malham Tarn fens. Field Studies, **4**, 1-38.

ROBINSON, D. & RORISON, I.H. (1983) Relationship between root morphology and nitrogen availability in a recent theoretical model describing nitrogen uptake from soil. Plant, Cell and Environment, **6**, 641-647.

ROBINSON, O.W. (1930) Some chemical phases of submerged soil conditions. Soil Science, **30**, 197-217.

ROBSON, A.D. & LONERAGAN, J.F. (1970) Sensitivity of annual Medicago species to manganese toxicity as affected by calcium and pH. Australian Journal of Agricultural Research, 21, 223-232.

ROMHELD, V. & MARSCHNER, H. (1981) Effect of Fe stress on utilization of Fe chelates by efficient and inefficient plant species. Journal of Plant Nutrition, 3, 551-560.

ROMHELD, V., MARSCHNER, H. & KRAMER, D. (1982) Responses to iron deficiency in roots of "Fe-efficient" plant species. Journal of Plant Nutrition, 5, 489-498.

RORISON, I.H. (1965) The effect of aluminium on the uptake and incorporation of phosphate by excised sainfoin roots. New Phytologist, 64, 23-27.

RORISON, I.H. (1968) The response to phosphorus of some ecologically distinct plant species. I. Growth rates and phosphorus absorption. New Phytologist, 67, 913-923.

RORISON, I.H. (1975) Nitrogen source and metal toxicity. Journal of the Science of Food and Agriculture, 26, 1426.

RORISON, I.H. (1985) Nitrogen source and the tolerance of Deschampsia flexuosa, Holcus lanatus and Bromus erectus to aluminium during seedling growth. Journal of Ecology, 73, 83-90.

ROSSITER, R.C. (1978) Phosphorus deficiency and flowering in subterranean clover (Trifolium subterraneum L.). Annals of Botany, 42, 325-329.

ROWE, W.H. & BEARDSALL, D.V. (1973) Waterlogging of fruit trees. Horticultural Abstracts, 443, 533-548.

ROZEMA, J. & BLOM, B. (1977) Effects of salinity and inundation on the growth of Agrostis stolonifera and Juncus gerardii. Journal of Ecology, 65, 213-222.

SANDERSON, P.L. & ARMSTRONG, W. (1980a) The responses of conifers to some of the adverse factors associated with waterlogged soils. New Phytologist, 85, 351-362.

SANDERSON, P.L. & ARMSTRONG, W. (1980b) Phytotoxins in periodically waterlogged forest soils. Journal of Soil Science, 31, 643-653.

SCAIFE, A. & TURNER, M. (1983) Diagnosis of Mineral Disorders in Plants. Vol. 2, Vegetables. London, H.M.S.O.

SCHAT, H. (1984) A comparative ecophysiological study on the effects of waterlogging and submergence on dune slack plants: growth, survival and mineral nutrition in sand culture experiments. Oecologia, 62, 279-286.

SCHREINER, O. & REED, H.S. (1909) Studies on the oxidising power of roots. Botanical Gazette, **47**, 355-388.

SCHWAB, A.P. & LINDSAY, W.L. (1983) Effect of redox on the solubility and availability of iron. Soil Science Society of America Journal, **47**, 201-205.

SHAW, S.C. (1984) Physiological Studies on Heavy Metal Tolerance in Plants colonizing Tideslow Rake, Derbyshire. PhD Thesis, Sheffield University.

SHEIKH, K.H. (1973) Effects of flooding and drainage and their alternation on the growth and uptake of nutrients by rice, (Oryza sativa L. indica var. IR-8). Journal of Experimental Botany, **24**, 64-75.

SHIPLEY, B & KEDDY, P.A. (1988) The relationship between relative growth rate and sensitivity to nutrient stress in twenty-eight species of emergent macrophytes. Journal of Ecology, **76**, 1101-1110.

SKEEN, J.R. (1929) The tolerance limit of seedlings for aluminium and iron and the antagonism of calcium. Soil Science, **27**, 69-80.

SMIRNOFF, N. (1981) Iron Tolerance and the Role of Aerenchyma in Wetland Plants. PhD Thesis, University of St. Andrews.

SMIRNOFF, N. & CRAWFORD, R.M.M. (1983) Variation in the structure and response to flooding of root aerenchyma in some wetland plants. Annals of Botany, **51**, 237-249.

SMITH, A.M. & AP REES, T. (1979) Pathways of carbohydrate fermentation in the roots of marsh plants. Planta, **146**, 327-334.

SMITH, A.M., KALSI, G. & WOOLHOUSE, H.W. (1984) Products of fermentation in the roots of alders (Alnus Mill.). Planta, **160**, 272-275.

SOMERS, I.I. & SHIVE, J.W. (1942) The iron-manganese relation in plant metabolism. Plant Physiology, **17**, 582-602.

STAINTON, M.P., CAPEL, M.J. & ARMSTRONG, F.A.J. (1977) The Chemical Analysis of Freshwater. Fisheries and Environment of Canada Miscellaneous Special Publication, No. 25, 2nd edn. Freshwater Institute, Winnipeg, Manitoba, Canada.

ST-CYR, L. & CROWDER, A.A. (1987) Relations between Fe, Mn, Cu, and Zn in root plaque and leaves of Phragmites australis. In: Proceeding of the 6th International Conference on Heavy Metals in the Environment, New Orleans., (Ed. by Lindberg, S.E. & Hutchinson, T.C.), **1**, 466-468. CEP Consultants, Edinburgh.

ST-CYR, L. & CROWDER, A.A. (1988) Iron oxide deposits on the roots of Phragmites australis related to the iron bound to carbonates in the soil. Journal of Plant Nutrition, **11**, 1253-1262.

ST-CYR, L. & CROWDER, A.A. (1989) Factors affecting iron plaque on the roots of Phragmites australis (Cav.) Trin. ex Steudel. Plant and Soil, **116**, 85-93.

TADANO, T. (1975) Devices of rice roots to tolerate high iron concentrations in growth media. Japan Agricultural Research Quarterly, **9**, 34-39.

TALBOT, R.J., ETHERINGTON, J.R. & BRYANT, J.A. (1987) Comparative studies of plant growth and distribution in relation to waterlogging. XII. Growth, photosynthetic capacity and metal ion uptake in Salix caprea and S. cinerea spp. oleifolia. New Phytologist, **105**, 563-574.

TALBOT, R.J. & ETHERINGTON, J.R. (1987) Comparative studies of plant growth and distribution in relation to waterlogging. XIII. The effect of Fe^{2+} on photosynthesis and respiration of Salix caprea and S. cinerea spp. oleifolia. New Phytologist, **105**, 575-583.

TANAKA, A., LOE, R. & NAVASERO, S.A. (1966) Some mechanisms involved in the development of iron toxicity symptoms in the rice plant. Soil Science and Plant Nutrition, **12**(4), 158(32)-(164(38).

TANAKA, A. & NAVASERO, S.A. (1966a) Aluminium toxicity of the rice plant under water culture conditions. Soil Science and Plant Nutrition, **12**(2), 55(9)-60(14).

TANAKA, A. & NAVASERO, S.A. (1966b) Manganese content of the rice plant under water culture conditions. Soil Science and Plant Nutrition, **12**(2), 67(21)-72(26).

TANAKA, A. & NAVASERO, S.A. (1966c) Growth of the rice plant on acid sulfate soils. Soil Science and Plant Nutrition, **12**(3), 107(23)-114(30).

TANAKA, A. & NAVASERO, S.A. (1966d) Interaction between iron and manganese in the rice plant. Soil Science and Plant Nutrition, **12**(5), 197(29)-201(33).

TANAKA, A. & NAVASERO, S.A (1966e) Chlorosis of the rice plant induced by high pH of culture solution. Soil Science and Plant Nutrition, **12**(6), 213(7)-219(13).

TANAKA, A. & YOSHIDA, S. (1970) Nutritional disorders of the rice plant in Asia. International Rice Research Institute Technical Bulletin, Makati, Rizal, **10**, 1-51.

TANG, Z.C. & KOZLOWSKI, T.T. (1984) Water relations, ethylene production and morphological adaptation of Fraxinus pennsylvanica seedlings to flooding. Plant and Soil, 77, 183-192.

TAYLOR, D.L. (1942) Influence of oxygen tension on respiration, fermentation and growth in wheat and rice, American Journal of Botany, 29, 721-738.

TAYLOR, G.J. (1983) Copper and Nickel Tolerance in Clones of Typha latifolia L. from Contaminated Wetlands near Sudbury, Ontario. PhD Thesis, Dept. of Biology, Queen's University, Kingston, Ontario.

TAYLOR G.J. (1987) Exclusion of metals from the symplasm: a possible mechanism of metal tolerance in higher plants. Journal of Plant Nutrition 10, 1213-1222.

TAYLOR, G.J. (1988a) The physiology of aluminium phytotoxicity. In: Metal Ions in Biological Systems - Volume 24 - Aluminium and its Role in Biology. (Ed. by Sigel, H. & A.) 123-163.

TAYLOR, G.J. (1988b) The physiology of aluminium tolerance. In: Metal Ions in Biological Systems - Volume 24 - Aluminium and its Role in Biology. (Ed. by Sigel, H. & A.) 165-198.

TAYLOR, G.J. & CROWDER, A.A. (1983a) Use of DCB technique for extraction of hydrous iron oxides from roots of wetland plants. American Journal of Botany, 70, 1254-1257.

TAYLOR, G.J. & CROWDER, A.A. (1983b) Uptake and accumulation of copper, nickel and iron by Typha latifolia L. grown in solution culture. Canadian Journal of Botany, 61, 1825-1830.

TAYLOR, G.J., CROWDER, A.A. & RODDEN, R. (1984) Formation and morphology of iron plaque on the roots of Typha latifolia L. grown in solution culture. American Journal of Botany, 71, 666-675.

TEAL, J.M. & KANWISHER, J.W. (1966) Gas transport in the marsh grass, Spartina alterniflora. Journal of Experimental Botany, 17, 355-361.

THEIS, T.L. & SINGER, P.C. (1973) The stabilization of ferrous iron by organic compounds in natural waters. In: Trace Metals and Metal Organic Interactions in Natural Waters. (Ed. by Singer P.C.) 301-320. Ann. Arbor.

THENABADU, M.W. (1974) Iron in the nutrition of the rice plant. Journal of the National Agricultural Society of Ceylon, 11/12, 87-102.

TIBBETTS, R.A. (1988) An Investigation to Determine the Effects of Different Ferrous Iron Concentrations on the Root Morphology of Species Tolerant and Intolerant of Waterlogging. Undergraduate Dissertation, Dept. of Plant Science, University College, Cardiff.

TIFFIN, L.O. (1970) Translocation of iron citrate and phosphorus in xylem exudate of soybean. Plant Physiology, **45**, 280-283.

TIFFIN, L.O. & BROWN, J.C. (1959) Absorption of iron from iron chelate by sunflower roots. Science, **130**, 274-275.

TOMBESI, L., CALE, M.T. & TIBORNE, B. (1969) Effects of nitrogen, phosphorus and potassium fertilizers on assimilation capacity of Beta vulgaris chloroplast. Plant and Soil, **31**, 65-76.

TROLLDENIER, G. (1988) Visualisation of oxidising power of rice roots and of possible participation of bacteria in iron deposition. Zeitschrift fur Pflanzenernaehrung und Bodenkunde, **151**, 117-121.

UREN, N.C. (1984) Forms, reactions and availability of iron in soils. Journal of Plant Nutrition, **7**, 165-176.

VENKATRAJU, K. & MARSCHNER, H. (1981) Inhibition of iron-stress reactions in sunflower by bicarbonate. Zeitschrift fur Pflanzenernaehrung und Bodenkunde, **144**, 339-355.

VLAMIS, J. & WILLIAMS, D.E. (1962) Ion competition in manganese uptake by barley plants. Plant Physiology, **37**, 650-655.

VLAMIS, J. & WILLIAMS, D.E. (1964) Iron and manganese relations in rice and barley. Plant and Soil, **20**, 221-231.

VOSE, P.B. (1982) Iron nutrition in plants: a world overview. Journal of Plant Nutrition, **5**, 233-249.

WALDREN, S., DAVIES, M.S. & ETHERINGTON, J.R. (1987a) Comparative studies of plant growth and distribution in relation to waterlogging. XI. Growth of Geum rivale L. and Geum urbanum L. and soil chemical condition in experimentally flooded soil. New Phytologist, **105**, 551-562.

WALDREN, S., DAVIES, M.S., & ETHERINGTON, J.R. (1987b) The effect of manganese on root extension of Geum rivale L., Geum urbanum L. and their hybrids. New Phytologist, **106**, 679-688.

WALDREN, S., ETHERINGTON, J.R. & DAVIES, M.S. (1987) Comparative studies of plant growth and distribution in relation to waterlogging. XIV. Iron, manganese, calcium and phosphorus concentrations in leaves and roots of Geum rivale L. and Geum urbanum L. grown in waterlogged soil. New Phytologist, **106**, 689-696.

WALKER, P.L. (1990) Genotypic and Phenotypic Aspects of Metal Tolerance in Holcus lanatus L. PhD Thesis, University of Sheffield.

WALLACE, A. & ABOU-ZAMZAM, A.M. (1984) Nitrogen and bicarbonate relationships with iron nutrition in plants. Journal of Plant Nutrition, 7, 587-594.

WALLACE, A. & HALE, V.Q. (1961) Effect of varying concentrations of iron and ethylenediaminedi(o-hydroxyphenylacetate) on concentrations of each in soybeans. Soil Science, 92, 404-407.

WALLACE, A., SHANNON, L.M., LUNT, O.R. & IMPEY, R.L. (1957) Some aspects of the use of metal chelates as micronutrient fertilizer sources. Soil Science, 84, 27-41.

WALLACE, T. (1961) The Diagnosis of Mineral Deficiencies in Plants by Visual Symptoms. A Colour Atlas and Guide. 3rd edn., H.M.S.O., London.

WALLEY, K.A., KHAN, M.S.I. & BRADSHAW, A.D. (1971) The potential for evolution of heavy metal tolerance in plants. I. Copper and zinc tolerance in Agrostis tenuis. Heredity, 32, 309-319.

WARD, J.H. (1963) Hierarchial grouping to optimise an objective function. Journal of the American Statistical Association, 58, 236-244.

WEBB, T. & ARMSTRONG, W. (1983) The effects of anoxia and carbohydrates on the growth and viability of rice, pea and pumpkin roots. Journal of Experimental Botany, 34, 579-603.

WHEELER, B.D. (1983) Vegetation, nutrients and agricultural land use in a North Buckinghamshire valley fen. Journal of Ecology, 71, 529-544.

WHEELER, B.D., AL-FARRAJ, M.M. & COOK, R.E.D. (1985) Iron toxicity to plants in base-rich wetlands: comparative effects on the distribution and growth of Epilobium hirsutum L. and Juncus subnodulosus Schrank. New Phytologist, 100, 653-669.

WHEELER, B.D. & GILLER, K.E. (1984) The use of dialysis cells for investigating pore water composition in wetland substrata, with particular reference to dissolved iron and sulphide. Communications in Soil Science and Plant Analysis, 15, 707-716.

WHEELER, B.D. & SHAW, S.C. (1987) Comparative survey of habitat conditions and management characteristics of herbaceous rich-fen vegetation types. Unpublished Report to the Nature Conservancy Council. Department of Botany, University of Sheffield, Sheffield.

WIGNARAJAH, K. & GREENWAY, H. (1976) Effects of anaerobiosis on activities of alcohol dehydrogenase and pyruvate decarboxylate in roots of Zea mays. New Phytologist, **77**, 575-584.

WILKINS, D.A. (1957) A technique for the measurement of lead tolerance in plants. Nature, **180**, 37-38.

WILKINS, D.A. (1978) The measurement of tolerance to edaphic factors by means of root growth. New Phytologist, **80**, 623-633

WILSON, J.B. (1988) The cost of heavy-metal tolerance: an example. Evolution, **42**, 408-403.

WONG, M.H. & BRADSHAW, A.D. (1982) A comparison of the toxicity of heavy metals, using root elongation of rye grass, Lolium perenne. New Phytologist, **91**, 255-261.

WOOLHOUSE, H.W. (1966) The effect of bicarbonate of the uptake of iron in four related grasses. New Phytologist, **65**, 372-375.

WOOLHOUSE, H.W. (1983) Toxicity and tolerance in the responses of plants to metals. In: Encyclopedia of Plant Physiology. New Series, Vol. 12c, Physiological Plant Ecology, III Chemical and Biological Environment, 242-300. (Ed. by Lange, O.L., Nobel, P.S., Osmond, C.B., and Zeigler, H.) Springer-Verlag, Berlin.

YAMADA, N. & OTA, Y. (1958) Study on the respiration of crop plants. 7. Enzymatic oxidation of ferrous iron by root of rice plant. Proceedings of the Crop Science Society of Japan, **26**, 205-210.

YAMASAKI, T. (1952) Studies on the 'excess moisture injury' of upland crops in overmoist soil from the viewpoint of soil chemistry and plant physiology. Bulletin of the National Institute of Agricultural Sciences, Japan, Series B, **1**, 1-92.

YU, P.T. STOLZY, L.H. & LETEY, J. (1969) Survival of plants under prolonged flooding. Agronomy Journal, **61**, 844-847.

ZHAO, X-J., SUCOFF, E. & STADELMANN, E.J. (1987) Al^{3+} and Ca^{2+} alteration of membrane permeability of Quercus rubra root cortex cells. Plant Physiology, **83**, 159-162.

ZHIZNEVSKAYA, G.Y. (1972) Iron in plant nutrition. Agrochimica, **17**, 46-68.

APPENDIX I

SEED PRE-TREATMENT AND GERMINATION OF SPECIES USED IN THE STANDARD IRON-SCREENING EXPERIMENT

N.B.

(i) As a normal procedure, seeds were germinated on damp filter paper and then transferred onto alkathene beads and Rorison solution (see Sections 2.4.2 and 2.4.3). Very small seedlings were first given 10% Rorison solution, or transferred to sand and 10% Rorison solution (see notes below). Seed was stored dry, in the dark at 5°C.

(ii) Chilling treatment involved storing seeds damp at 3°C in a refrigerator. The time quoted for chilling treatment may not be the minimum required to break dormancy, (indeed chilling was later found to be unnecessary in species marked *). Seed was usually left under refrigeration until required, unless germination during chilling necessitated their immediate use.

(iii) $ signifies that germination of seed was not simultaneous.

Species	Days chilled at 3°C	No. days until germination	Age at start of experiment (days)	Germination rate	Notes
Agrostis stolonifera	80*	3	45	50%	Some germinated while chilling.
Briza media	-	7	47	low	
Caltha palustris	-	10	38	high	
Carex appropinquata	>125*	6	70	90%$	Used sand and and 10% Rorison solution. 50 days chilling was inadequate.
Carex diandra	240	7	30	high	

Carex echinata	135*	3-4	18-19	100%	Some germinated while chilling.
Carex lepidocarpa	180	6	41	V.low	Some germinated while chilling. 50 days chilling was insufficient
Carex pulicaris	195	1	74	100%$	Some germinated while chilling.
Epilobium hirsutum	-	4	24	100%	
Epilobium palustre	-	4-5	32	100%	
Eriophorum latifolium	125	6	70	60%	% germination was lower for seeds chilled longer.
Eupatorium cannabinum	165	5	42	88%	40 days chilling only resulted in 40% germination.
Filipendula ulmaria	120	4	35	high	
Galium aparine	-	6	32	50%	
Galium palustre	210	4-7	35-38	$	
Holcus lanatus	-	4	23	high	
Iris pseudacorus	-	5-10	25-30	85%$	
Juncus articulatus	-	5	55	high	Supplied with 10% Rorison solution initially.

Juncus effusus	255*	5	103	high	Grown in sand and 10% Rorison soln. initially. Fresh germination rate was as high as that after 300 days chilling.
Juncus inflexus	-	7	50	high	Supplied with 10% Rorison solution initially.
Juncus subnodulosus	-	5	65	high	Grown in sand and 10% Rorison soln. initially. 250 days chilling did not reduce germination rate.
Lotus uliginosus	-	2	31	20%	
Lychnis flos-cuculi	-	5	51	high	Chilled seeds had a lower germination rate.
Lysimachia vulgaris	120	3	28	95%	Some germinated while chilling.
Lythrum salicaria	80*	3+	42	50%$	Husks caused mould problems.
Molinia caerulea	168	6	26	60%	Wetland and non-wetland sources had the same germination characteristics.

Parnassia palustris	-	7+	143	high$	Initially given 10% Rorison solution with sand, then beads, and finally full-strength Rorison solution.
Pedicularis palustris	75	3-7	20	60%	
Phalaris arundinacea	-	8+	29	82%$	Chilled seeds had lower germination
Potentilla palustris	169	various	52	30%$	
Primula farinosa	-	7	84	20%	
Ranunculus flammula	-	18+	36	$	Germination occurred over a very long time.
Rumex acetosa	-	5	28	high	
Rumex hydrolapathum	-	3-4	28	100%	
Scrophularia auriculata	-	5	42	26%	Supplied with 10% Rorison solution initially.
Thalictrum flavum	-	20-24	32	high$	Seed sown in the capsules.
Trifolium pratense	-	2	31		
Valeriana dioica	-	6	31-45	28%$	
Valeriana officinalis	-	5-9	50	60%	

APPENDIX II

Effect of Nitrogen Source and Iron Concentration on Yield of Four Wetland Species (see Section 8.3.3.3)
Two-way Analysis of Variance
(Monocotyledons are indicated by bold type)

Source of Variation	Df	Mean Square	F	p
Iris pseudacorus				
N source	1	6.5	.014	.906
Fe concn.	2	2,341	5.145	.017
N source x Fe concn.	2	1,096	2.408	.118
Residual	18	455		
Total	23	655		
Lysimachia vulgaris				
N source	1	3,667	2.65	.117
Fe concn.	2	222,543	160.78	.000
N source x Fe concn.	2	10,810	7.81	.002
Residual	24	1,384		
Total	29	17,365		
Juncus subnodulosus				
N source	1	1.1	.015	.902
Fe concn.	2	1,109	15.3	.000
N source x Fe concn.	2	65	.90	.420
Residual	24	72		
Total	29	141		
Epilobium hirsutum				
N source	1	8,148	64	.000
Fe concn.	2	74,509	591	.000
N source x Fe concn.	2	15,048	119	.000
Residual	24	126		
Total	29	6,562		