Iron Toxicity to Fen Plant Species

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Summary

1. The toxicity of ferrous iron to seedlings of 44 fen plant species was examined using 2-week screening experiments in solution culture. These were found to be more practicable than alternative methods which were tested.

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 summation of standardized relative growth rates over a range of iron concentrations (\( \frac{\%RGR}{2} \)) was selected as being most appropriate. Standardization was achieved by expressing mean \( RGR \) for each treatment as a percentage of that in a low-iron control. An iron-tolerance species ranking was derived from this.

3. Ward’s classification (multivariate analysis) grouped the species into four main clusters on their standardized relative growth rate response. The very iron-tolerant species (cluster 1) were almost entirely monocotyledons; cluster 2 contained both monocotyledons and dicotyledons; all except one species in cluster 3 were dicotyledons; while the most sensitive species (cluster 4) were exclusively dicotyledons.

4. Evidence suggests that the greater iron tolerance of monocotyledonous species may be due to an inherently superior oxidative detoxification system reducing direct toxicity, and to a generally lower growth rate minimizing indirect toxicity (nutritional effects associated with high iron concentrations).

5. Analysis by Ward’s method of shoot:root dry weight ratio trends revealed that for most monocotyledons shoot:root ratio was little affected by iron concentration. Conversely, root growth of most dicotyledonous species was affected more severely than shoot growth by increased iron supply.

6. Assessed tolerance of species correlated positively with mean and maximum field measurements of iron concentrations in sites where they occurred.

7. Iron-sensitive species also tended to grow where substrate pH, extractable calcium concentration and titratable alkalinity were high, and where mean summer water table height, extractable manganese, aluminium and nitrogen concentrations were relatively low.

8. For those fen species tested, an inverse relationship was found between a species iron tolerance and the fertility and extractable phosphorus concentration of sites in which it grew.

9. Iron tolerance and relative growth rate (control treatment) were negatively correlated.

Key-words: fen plants, iron, iron tolerance, iron toxicity

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Introduction

There is evidence that high concentrations of reduced toxins such as iron may be responsible for the absence of dryland plant species from wetland environments (e.g. Bartlett 1961; Martin 1968; Jones & Etherington 1970; Jones 1971; Jones 1972a; Hodgson 1972; Waldren, Davies & Etherington 1987). There is also some evidence that high iron concentrations may influence the growth and distribution of various wetland plant taxa (e.g. Tanaka, Loe & Navasero 1966; Foy, Chaney & White 1978; Al-Farraj 1983; Wheeler, Al-Farraj & Cook 1985; Talbot & Etherington 1987; Talbot, Etherington & Bryant 1987).

In this study, the possibility of differential iron tolerance among wetland plants has been explored further by laboratory screening of the effects of high...
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Iron concentrations upon the growth of a range of species typical of fens. Their response was related to iron availability at sites where they occur.

Methods

PRETREATMENT OF SEEDLINGS

Forty-three British native plant species, all found in fens, were screened for iron tolerance. They included 23 dicotyledons and 20 monocotyledons (with wetland and non-wetland seed sources of Molinia caerulea). (Nomenclature follows Clapham, Tutin & Warburg (1981).) Rice (Oryza sativa IR 36) was also tested, for comparison.

Seed was sown on 15 cm Whatman no. 1 filter paper, watered with distilled water. Seeds of certain species would not germinate fresh or after storage (5°C, dry, in the dark); these were cold pretreated (3°C, wet) (Cook 1990; and unpublished data). Seedlings were grown sufficiently to be handled without damage. They were transferred into clear perspex sandwich boxes (27.5 x 15.5 x 9 cm), with a 2.5-cm layer of black alkathene beads kept moist with 100% Rorison solution, for a minimum of 2 weeks. The lid was initially kept closed and then gradually opened for acclimatization to lower humidity.

EXPERIMENTAL PROCEDURE (STANDARD SCREENING METHOD)

Seedlings more than 18 days old (Cook 1990; and unpublished data) were screened in blackened (500-ml) plastic tubs. Their roots were threaded into holes cut into polystyrene floats backed with perforated black plastic, and the floats were placed on the solution (polythene-side down). The plants were grown in 10% Rorison solution with no extra iron for one day to facilitate establishment, and then provided with 10% Rorison solution containing iron (as ferrous sulphate), at concentrations of 3.8 (control), 10, 25, 50, 75 and 100 mg Fe⁻¹ (at pH 5.5).

Between three and five plants (depending on size and number available) were used per replicate tub (n = 5); root and shoot dry weight of one additional replicate set were measured by harvesting at the start of screening. The growing period was 2 weeks, and solutions were changed three times per week. (This helped maintain phosphorus supply and iron concentrations, and compensated for other chemical changes (e.g. acidification) of the solutions.) The tubs were randomized at each solution change. Day length was 16 h and temperature ranged from about 20°C at night up to 28−30°C in the day.

Visual observations of root and shoot condition were made approximately every 2 days. After 14 days, measurements were made of shoot length, root length, number of tillers (monocotyledons only), numbers of live, moribund and dead leaves, presence of adventitious roots, and leaf size (width or length). Roots and shoots were harvested separately (bulk harvest per tub), washed and dried. Shoot and root dry weights were used to calculate shoot: root ratio and relative growth rate (rgr) for each replicate tub, and mean values for each treatment.

\[
gr (\text{per day}) = (\text{final dry weight} - \text{mean initial dry weight}) / (\text{mean initial dry weight} \times 14).
\]

Mean relative growth rate (RGR) = rgr/number of replicate tubs.

CHEMICAL COMPOSITION OF CULTURE SOLUTIONS

A standard screening experiment was set up (using Epilobium hirsutum as the test plant), to monitor selected aspects of the solution chemistry over a 4-day period (i.e. longer than the maximum time between solution changes).

Solution oxidation−reduction potential and pH (initially 5-5) were measured in situ at 24-h intervals (08.00 hours). A 15−20-ml subsample was centrifuged at 2000 g for 5 min at 20°C, and iron was estimated in the supernatant (Pyte-Unicam SP190 atomic absorption spectrophotometer). Soluble reactive phosphorus concentration was also measured by a molybdenum-blue method (Stainton, Capel & Armstrong 1977), using an SP8−100 UV/VIS spectrophotometer at 710 nm. Additional pH and oxidation-reduction potential measurements were made after the first 12 h. There was little change in both iron concentration and oxidation−reduction potential over the 4 days, while soluble reactive phosphorus concentration fell rapidly (especially during the first 24h). pH also fell, to around 5-2 in the control, and to 4-2−4-5 in the treatment solutions (Cook 1990).

ASSESSMENT OF IRON TOLERANCE

Tolerance indices

Several tolerance indices were derived, using the various growth measurements made. Of these, relative growth rate was found to be the most appropriate tolerance indicator (Cook 1990) and a tolerance index (Σ%RGR) was calculated. For this, mean relative growth rate (RGR) measurements for each treatment were expressed as a percentage of the mean relative growth rate of the control treatment (RGRc) (i.e. %RGR). The standardized response was summed over all treatments, except the control, which was always 100% (Σ%RGR). From these values the species were ranked in a 'league table' of tolerance. For the four species not grown at all iron concentrations (Agrostis stolonifera, Carex echinata, Carex pulicaris and Lythrum sali-
caria), values of $RGR$ were extrapolated for the missing treatments.

**Multivariate analysis**

Comparison of the species' responses was also made using multivariate ordination and classification techniques (principal components analysis and Ward's method of hierarchical fusion (Ward 1963)). The species (cases) were clustered using as attributes the six standardized mean values of $RGR$ (%$RGR$). Because the attributes are sequential, the mean value for each cluster of the standardized relative growth rate could be plotted against iron concentration. This approach effectively uses response curve shape as a measure of tolerance.

This method was also used to identify trends in shoot:root dry weight ratios. In this case standardization was made against maximum mean shoot:root ratio for each species (which was not necessarily found in the control treatment).

**Alternative Methods of Assessing Iron Tolerance**

Two other approaches to the assessment of iron tolerance were tested on a selection of species, that of plant mortality with time (over a 10-week period), and that of germination. Neither was successful: percentage germination was little affected by iron concentration, and the subsequent growth of the newly germinated seedlings was greatly influenced by seed size. The 10-week mortality test was beset with difficulties, including those of determining the exact point of death, eventual contamination by micro-organisms, and practical considerations (Cook 1990).

**Field Measurements**

As part of a survey of British fen vegetation (Shaw & Wheeler 1991), species composition was recorded in 864 visually uniform 4-m$^2$ stands chosen to represent a particular fen community type (Wheeler 1984). Field measurements ($n = 5$) were made of oxidation–reduction potential, water pH and depth of the water table.

Soil samples ($n = 5$) were collected in the autumn, for chemical analysis, and for estimation of fertility using a phytometric method (Al-Farraj, Giller & Wheeler 1984) with *Phalaris arundinacea* as test species. After measuring the pH of the fresh peat in a 1:1 slurry, cations were extracted from a 38-ml fresh sample with 200 ml of 1-m ammonium acetate adjusted to the pH of the sample (see Gottoh & 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 2-M KCl and 0.5-M NaHCO$_3$ (pH 8.5), respectively. In each case, five replicate extracts were made and results were expressed volumetrically (mg l$^{-1}$ fresh peat). This permitted comparison with the work of Giller (1982), Al-Farraj (1983), Wheeler (1983), and Wheeler *et al.* (1985), but not with studies using dried soils. It should be noted that this survey was not undertaken specifically to examine iron availability in fens. Indeed, highest exchangeable iron concentrations may occur in summer (e.g. Jones 1973; Nazrul-Islam 1976) when microbial activity is at its peak.

The iron tolerance of each species, as assessed by the Standard Screening Method, was analysed in relation to field data and to concentrations of extractable iron and other elements measured in those sites from which the species was recorded.

**Results**

**Visible Effects of High Iron Concentrations on Plants Screened**

A number of visible effects associated with high iron concentrations were commonly observed, and some species showed several symptoms (Cook 1990; and unpublished data). These included: 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. Some species had an ochreous root precipitate, while in others it was yellow. There was a clear relationship between iron tolerance of a species ($\Sigma RGR$) and the nature of its root precipitate (Cook 1990; and unpublished data); the more tolerant species tended to form ochreous precipitates on their roots more readily than did the more sensitive ones.

**Measured Effects of High Iron Concentrations on Growth of Plants Screened**

**Iron Tolerance Ranking**

Histograms of mean value of standardized relative growth rate response ($%RGR$) against supplied iron concentration are given for selected species (Fig. 1). Figure 2 shows the species ranking based on the iron tolerance index $\Sigma RGR$. High iron concentrations significantly ($P < 0.05$) reduced the growth of all
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Fig. 1. Histograms of mean (±SE) relative growth rates as a percentage of control treatment against supplied ferrous iron concentration for a selection of species screened for iron tolerance.

species (compared with control) except *Eriophorum angustifolium* and *Juncus effusus*. Most monocotyledons were more tolerant of iron than were most dicotyledons, though there was some overlap. The mean (±SE) tolerance index for all dicotyledons screened (170.7 ± 16.8) was significantly ($P < 0.05$) less than that for all monocotyledons (316.6 ± 19.9).

**Multivariate analysis of plant growth response**

Ward's classification generated four main species clusters which agreed closely with $\Sigma \% RGR$ tolerance ranking (Fig. 2). Mean response of standardized relative growth rate to iron for each cluster is shown in Fig. 3. The 14 species showing least growth re-
Fig. 2. Iron tolerance 'league table'. Species are ranked by Σ% RGR in order of decreasing tolerance to iron, and clustering from Ward's method is also given. Monocotyledon species are indicated by bold type and shading.

Production with increasing iron concentration (cluster 1) were the most tolerant of iron, while species in clusters 2 and 3 were progressively less tolerant; the six species in cluster 4 were the most sensitive. In this last cluster, mean RGR of species is reduced by an average of 60% between the control and 10 mg FeL−1 treatments. There is the greatest differential response amongst the four groups of species at these low iron concentrations.

Species in cluster 1 are almost exclusively monocotyledons (exceptions are Pedicularis palustris and Parnassia palustris, both very slow-growing dicotyledons). Cluster 2 contains both monoloredonous and dicotyledonous species. The more sensitive species in cluster 3 are mainly dicotyledons (except Carex appropinquata), while species in cluster 4 (the most sensitive to iron), are exclusively dicotyledons. Figure 4 shows the four clusters displayed on a three-dimensional principal-components-analysis plot based on standardized RGR data (% RGR). Ninety per cent of the variance in these data was accounted for by axis 1 (axes 2 and 3 accounted for 6-6 and 1-5%, respectively). Axis 1 species scores correlated exactly with their tolerance indices (Σ% RGR) \( r = -0.51; \ P < 0.001 \) suggesting that axis 1 is effectively an axis of iron tolerance. There was no significant relationship between tolerance and axis 2 or 3.

**Relationship between iron tolerance and relative growth rate of control plants**

There was a highly significant negative correlation \( r = -0.51; \ P < 0.001 \) between tolerance index (Σ% RGR) and relative growth rate in the control treatment \( (RGR_c) \) when all species were considered. There was also a significant regression relationship, although the variation in tolerance which could be accounted for by \( RGR_c \) was only about 25% (Table 1).

When dicotyledons were considered alone there was no significant relationship, and the weak one for monocotyledons alone was strongly influenced by the abnormally high relative growth rate (see also Grime & Hunt 1975) and low iron tolerance of Holcus lanatus.
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![Graph showing % RGR vs supplied ferrous iron concentration](image)

**Fig. 3.** Cluster diagnostics (Ward's analysis) of mean relative growth rate (standardized against control mean relative growth rate (%RGR), with treatment: (+) cluster 1 (12 monocots, 2 dicots); (×) cluster 2 (9 monocots, 6 dicots); (○) cluster 3 (1 monocot, 9 dicots); (△) cluster 4 (6 dicots only).

![Graph showing plot of principal components analysis](image)

**Fig. 4.** Plot of principal components analysis axes 1, 2 and 3 based on standardized relative-growth-rate data (%RGR). Symbols indicate Ward's analysis clusters 1 (+), 2 (×), 3 (○), and 4 (△).

<table>
<thead>
<tr>
<th>Group</th>
<th>Regression equation</th>
<th>Significance</th>
<th>% fit (100r²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All species</td>
<td>y = 338.1 - 403.7x</td>
<td>P &lt; 0.001</td>
<td>26.09</td>
</tr>
<tr>
<td>Monocotyledons</td>
<td>y = 378.6 - 310.9x</td>
<td>P &lt; 0.05</td>
<td>21.58</td>
</tr>
<tr>
<td>Dicotyledons</td>
<td>y = 236.8 - 229.4x</td>
<td>NS</td>
<td>15.24</td>
</tr>
</tbody>
</table>

**Table 1.** Regression equations relating tolerance indices (y) to relative growth rate (x)

Iron Tolerance and Response of Shoot:Root Dry Weight Ratio

Ward's classification using standardized shoot:root dry weight data grouped the species into two main clusters (Fig. 5). In one group (A) increasing iron concentrations had little effect on shoot:root ratio. Species with this type of response were mainly very iron-tolerant and included all the monocotyledons tested (except one), and only six of the dicotyledons. These were *Parnassia palustris* and *Pedicularis palustris* (the two most tolerant dicotyledons, in which neither shoot nor root growth were appreciably affected) and four of the most iron-sensitive dicotyledons (*Rumex hydrolapathum*, *Epilobium hirsutum*, *Lychnis flos-cuculi*, and *Valeriana officinalis*), in which both shoot and root growth were severely affected.

The majority of the dicotyledonous species (16) plus one monocotyledon (*Briza media*) were found in the second group (B). In these, shoot:root ratio

![Graph showing cluster diagnostics](image)

**Fig. 5.** Cluster diagnostics (Ward's analysis) of mean shoot:root dry weight ratio (standardized against maximum mean shoot:root ratio), with treatment. Note, the four species with missing treatments are not included: (●) group A (17 monocots, 6 dicots); (●) group B (16 dicots, 1 monocot).
increased with iron concentration (i.e. roots of these plants were affected more severely than shoots). These species were generally less tolerant of iron than those in group A.

One reason why iron-tolerant species tend to show little change in shoot:root ratio in response to increased iron concentrations may be because accumulating ochreous deposits on the roots might increase root mass.

**RELATIONSHIP BETWEEN ASSESSED IRON TOLERANCE AND FIELD MEASUREMENTS OF SOIL IRON CONCENTRATION AND OTHER ENVIRONMENTAL VARIABLES**

In many (48%) of the fen sites examined, iron concentrations in ammonium acetate-extracted soil were low (<2 mg Fe l⁻¹). Higher concentrations were less common, and extremely high concentrations (maximum = 5272 mg Fe l⁻¹) were rare. The extracted iron concentration correlated positively with height of the summer water table \( r = 0.46, P < 0.01 \) and negatively with both substrate and water pH values \( r = -0.86 \) and \(-0.89\), respectively; \( P < 0.001 \).

A strong positive correlation was found between the species' iron tolerance index and the mean \( r = 0.57, P < 0.001 \) and maximum \( r = 0.51, P < 0.001 \) concentration of iron extracted from the soils which supported them (Table 2). This relationship occurs because iron-sensitive species were confined to soils with relatively low concentrations of extracted iron and not because iron-tolerant species were restricted to iron-rich soils (Fig. 6). This is further illustrated by examining the relationship between the iron tolerance index of species and the number of times they were each recorded in samples from different categories of extracted iron concentration. When only sites with <2 mg Fe l⁻¹ were considered, there was no significant relationship (i.e. iron-tolerant and sensitive species were similarly represented). However, in the higher categories of iron concentration, the relationship became increasingly strong as only the more iron-tolerant species tended to be found in the more iron-rich sites \( 2-5 \text{ mg Fe l}^{-1}, r = 0.31, P < 0.01; 5-50 \text{ mg Fe l}^{-1}, r = 0.51, P < 0.001; 50-500 \text{ mg Fe l}^{-1}, r = 0.53, P < 0.001; >500 \text{ mg Fe l}^{-1}, r = 0.58, P < 0.001 \).

There was also a positive relationship between species' iron tolerance and the mean concentrations of other potentially toxic metals (manganese and aluminium) in the sites where they grew, and with mean extractable nitrogen concentration. Similarly mean summer water level was positively related to mean iron tolerance index (Table 2).

Species' iron tolerance index correlated negatively with mean soil and water pH values of the samples in which the species grew, and with mean values of pH-related variables (titratable water alkalinity and extractable calcium concentration) (Table 2).

There was also a negative relationship between species' iron tolerance and both mean extracted phosphorus concentration and mean fertility (assessed phytometrically) of soils that supported them (Table 2). Similarly, a positive correlation was found between the relative growth rate of the screened species and both mean fertility \( r = 0.47, P < 0.01 \) and mean extractable phosphorus concentration \( r = 0.33, P < 0.05 \) of the soils upon which they grew.

It thus appears that iron-sensitive fen species tend to occupy wetland soils that are relatively base-rich and fertile and sometimes summer dry, as well as having only low concentrations of extractable iron, manganese and aluminium.

**Table 2.** Correlation of selected variables measured in fen substrata with the iron tolerance index of species growing on them

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation coefficient ( r )</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean extractable aluminium concentration</td>
<td>+0.58</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Mean extractable iron concentration</td>
<td>+0.57</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Maximum extractable iron concentration</td>
<td>+0.51</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Mean extractable nitrogen concentration</td>
<td>+0.56</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Mean extractable manganese concentration</td>
<td>+0.52</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Mean summer water table</td>
<td>+0.43</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>Mean fertility (assessed phytometrically)</td>
<td>-0.55</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Mean extractable phosphorus concentration</td>
<td>-0.48</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Mean titratable water alkalinity</td>
<td>-0.48</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Mean substrate pH</td>
<td>-0.46</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>Mean water pH</td>
<td>-0.44</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>Mean extractable calcium concentration</td>
<td>-0.42</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>Mean extractable magnesium concentration</td>
<td>-0.06</td>
<td>NS</td>
</tr>
<tr>
<td>Mean extractable potassium concentration</td>
<td>-0.13</td>
<td>NS</td>
</tr>
<tr>
<td>Mean extractable sodium concentration</td>
<td>-0.20</td>
<td>NS</td>
</tr>
<tr>
<td>Mean oxidation−reduction potential ( E_{o} )</td>
<td>-0.21</td>
<td>NS</td>
</tr>
</tbody>
</table>
Discussion

Assessment of Iron Tolerance

Screening of a large number of species for metal tolerance demands that the method used should be relatively rapid (Baker & Walker 1989), as well as reliable. The germination response of plants potentially provides rapid screening for metal tolerance, but has been found unsatisfactory (Wong & Bradshaw 1982). Similarly in the present study germination per se was generally unaffected by iron concentration, and assessed tolerance was largely dependent on seed size (Cook 1990).

Growth of plants in solution culture for relatively short periods, as in this study, has often been used for assessing metal tolerance (Kuraev 1966; Jones 1972b). This approach undoubtedly has limitations, as strikingly different rankings of species tolerance can be produced by using different formulations of the base solution (e.g. Tanaka et al. 1966; Wilkins 1978; McCain & Davies 1983; Shaw 1984). It may also be argued that 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 true test of tolerance); but in this study, longer-term screening was found to be less satisfactory than the relatively short-term approach that was ultimately adopted. Many studies of metal tolerance utilize root extension tests (e.g. Wilkins 1957, 1978; McCain & Davies 1983), though there is some evidence that these may be unsuitable in screening for iron tolerance (Al-Farraj 1983).

One of the main problems of assessing the efficacy of any screening methodology is the lack of an independent yardstick with which to validate the results. Here, the tolerance rankings derived from laboratory screening have been corroborated by field observations on iron availability and species occurrence, suggesting that the screening method used was realistic.

In this study, standardized relative growth rate was used as a measure of tolerance. This provided effective discrimination between a large number of plant species and facilitated inter-species comparisons (Wilson 1988). The tolerance index ($\% RGR$) derived from this was simple to calculate and involved little manipulation of the raw data, except standardization. Each species could be ranked by a unique index which was independent of the other species being assessed. By contrast, the multivariate classification of species grouped them into clusters of similar response to iron supply. The classification of any one species is, of course, dependent upon the other cases (species) considered, but provides a useful simplification of the species’ response to iron that complements, and is compatible with, the species tolerance rankings.

Mechanisms of Iron Tolerance

It is now well established that an important mechanism which excludes many dryland plants from the wetland environment is their inability to prevent uptake of reduced toxins such as Fe$^{2+}$ by oxidative precipitation (Bartlett 1961; Martin 1968; Jones & Etherington 1970; Jones 1972a). It seems likely that a similar mechanism may also help explain differential tolerance of wetland plants to available iron (Wheeler et al. 1985; Cook 1990; and unpublished data).

This study provides further evidence to support this proposition.

1. There was significant correlation between the iron tolerance of species, and both the mean depth of the summer water table, and mean extracted iron concentration, at sites where they grew (Table 2) – i.e. the more sensitive species tended to occur in the drier sites which were less reducing and had lower iron availability.
2. The more tolerant species tended to form ochreous precipitates on their roots more readily than did less tolerant ones (Cook 1990; and unpublished data). 3. Comparison of the tolerance index (Σ% RGR) with root porosity values available for 14 of the species screened here (Justin & Armstrong 1987) showed a significant correlation between tolerance and root porosity (measured both under drained ($r = 0.52, P < 0.05$) and flooded ($r = 0.51, P < 0.05$) conditions).

Variation in porosity may also help explain why, in this study, monocotyledonous species were usually more tolerant of iron than were dicotyledonous species. Monocotyledon shoots are frequently hollow, facilitating oxygen diffusion to the roots (Crawford 1978; Etherington 1983). Analysis of data presented by Justin & Armstrong (1987) indicates that monocotyledons (and particularly wetland ones) also have a more porous root system than do dicotyledons. Although dicotyledons (and particularly wetland ones) seem 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 (Smirnoff & Crawford 1983; Justin & Armstrong 1987).) Many wetland monocotyledons also 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 (Armstrong & Beckett 1987). Such adaptations have not been found in dicotyledons.

Development of adventitious roots is sometimes regarded as an important adaptation to flood tolerance (Armstrong 1968; Gill 1975; Keeley 1979; Etherington 1984; Drew 1987). Although a detailed study was not made of the role of adventitious roots, casual observations provided no evidence that they were more effective at rhizosphere oxygenation than the main root system (cf. Laan et al. 1989), in that ochreous deposits, which provide some measure of occurrence of oxidation, were no better developed on the adventitious roots. (Adventitious roots may, of course, have a role under field conditions by operating in the better-aerated surface layers in circumstances where deeper rooting structures are damaged by reduced toxins.)

It seems unlikely that variation in capacity for oxidative exclusion of iron is the only important mechanism of iron tolerance in wetland plants (Jayawardena, Watanabe & Tanaka 1977; Smirnoff 1981). There is evidence of elevated foliar iron content in some iron-tolerant wetland plants (Wheeler et al. 1985; Talbot et al. 1987; Talbot & Etherington 1987; Laan et al. 1989; Mansfield 1990) which suggests an important internal tolerance.

One problem in identifying mechanisms of internal iron tolerance is that the causes of iron toxicity are little understood. As well as apparent direct toxicity by excess iron, various workers (e.g. Howeler 1973; Tadano 1975; Ottow et al. 1983) have reported indirect toxicities, which are essentially nutritional disorders (e.g. in uptake of Mg, Mn, K or P) induced by high iron concentrations.

**Relationship between iron tolerance and relative growth rate (control treatment)**

In this study, in agreement with findings reported for other metal toxicities (e.g. Ernst 1976; Wilson 1988) and for salinity (Hannon & Bradshaw 1968), it is clear that tolerance is associated with slow growth. The adaptive significance of this is not known for certain. Ernst (1976) suggested that reduced growth might be due to the energy expenditure required to operate a tolerance mechanism but this is unlikely since growth rates were measured under control conditions requiring no such mechanism (also cadmium-tolerant and non-tolerant genotypes of Holcus lanatus can have the same relative growth rate (Walker 1990)). A low relative growth rate may alleviate direct toxicity by helping to ensure low rates of iron uptake, and indirect nutritional effects by accommodation of low rates of nutrient supply, since sites rich in heavy metals frequently have low fertilities (Clarkson 1967; Cox & Hutchinson 1981). In this study there was a significant correlation between a species' relative growth rate and the fertility and extractable phosphorus concentration of the sites in which it grew (see also Rorison 1968). Iron tolerance was also inversely correlated with site fertility, and with mean extractable phosphorus concentration. Thus low growth rates which adapt a plant to infertile conditions may fortuitously protect them from indirect effects of iron toxicity.

This may also help explain the greater iron tolerance of monocotyledons in this study, as those examined tended to have slower growth rates than did the dicotyledons. In addition, iron affected root growth of most dicotyledons more severely than shoot growth, whilst there was little effect in monocotyledons. Hence dicotyledons may suffer more severely from indirect iron toxicity supplementing the effects of an inferior oxidative detoxification system.

**Iron availability and the distribution of fen plant species**

*Relationship between assessed iron tolerance, field iron availability and other field conditions*

There are some clear limitations to an assessment of the relationship between the index of iron tolerance and field availability of iron. These are: 1. that measurements of pH-adjusted ammonium acetate-extracted iron may not adequately represent...
actual iron availability to plants;  
2. that extractable iron measurements were made from 4-m² vegetation samples and not specifically for individual species; there may be oxidized microsites in which iron-sensitive plants can grow in ostensibly iron-enriched soil; and  
3. environmental variables other than iron may strongly influence the distribution of plant species in wetlands.

There was, nonetheless, a strong positive correlation between values of the tolerance index of the screened species and the mean and maximum extractable-Fe concentrations of the soils supporting them (Table 2; Fig. 6). Although tolerant species did not necessarily occupy iron-rich sites, only the most tolerant (cluster 1) species were found there.

Iron solubility is largely determined by the pH and oxidation—reduction potential of the substratum (Gotoh & Patrick 1974; Armstrong 1982). It is therefore not surprising that the iron tolerance index was negatively correlated with soil and water pH and with pH-related variables (Ca in fen soil extracts and titratable alkalinity in the irrigating water). There was, however, no significant relationship between values of $E_I$ and iron tolerance. This may in part reflect the inherent difficulties in obtaining reliable readings of oxidation—reduction potential, as the occurrence of iron-tolerant species was positively correlated with height of summer water table.

As high concentrations of extractable manganese and aluminium were also frequently found in sites supporting iron-tolerant species, it is possible that some plants have co-tolerance to these metals (see Hodgson 1972). However, aluminium solubility is not redox-related, and whilst some wetland plants growing in low pH, aluminium-rich mires may have aluminium tolerance, this may not necessarily be associated with comparable tolerance to iron. Conversely, the aluminium-sensitive species Carex lepidocarpa (Clymo 1962) was relatively tolerant of iron.

The lack of correlation between mean extractable potassium and magnesium concentrations and iron tolerance is notable, because there is much evidence that plants insufficiently supplied with either of these elements are prone to iron toxicity (e.g. Tanaka et al. 1966; Howeler 1973; Nazrul-Islam 1976; Foy et al. 1978; Ottow et al. 1983; Benckiser et al. 1984).

Iron tolerance shows a negative relationship with extractable phosphorus concentration and with soil fertility (assessed phytometrically). The least tolerant species tend to grow in the more fertile sites and in sites with higher extractable phosphorus concentration. This may be partly because many iron-sensitive species have a high relative growth rate.

Relationship between assessed iron tolerance and the field distribution of fen plant species

It is likely that iron availability may affect the distribution of fen plant species in a variety of ways. These include direct toxic effects and indirect nutritional disorders induced by high iron concentrations, acting directly on individual plant species or mediated by species interactions within fen vegetation. The unqualified terms ‘iron tolerance’ and ‘iron toxicity’ are used here as collectives for this range of effects. The iron tolerance index ($\% RGR$) can be qualitatively related to the distribution, in British fens, of the screened species, although it should be recognized that some species may survive, but not flourish, in supra-optimal iron environments.

In general, the most iron-tolerant species were monocotyledons, the group of vascular plants that is naturally most prominent in many types of herbaceous fen vegetation. Thus, cluster 1 species were mainly monocotyledons. They were characterized by a high iron tolerance index and low relative growth rates and all of them were recorded from very wet situations. There is experimental evidence for internal iron tolerance in at least one cluster 1 species, Eriophorum angustifolium, which can have very high foliar iron concentrations ($>10\, \text{mg} \, \text{g}^{-1}$) (Mansfield 1990).

Tolerance cluster 2 contains a mixture of dicotyledon and monocotyledon species. Many of these are typically found in waterlogged situations, although some occur frequently in wet grassland (Juncus inflexus and Phalaris arundinacea) and even dry grassland (Briza media and Holcus lanatus).

Cluster 3 species are almost entirely forbs, some of which occur widely in wet grassland as well as in waterlogged fens. Several of them (most notably Thalictrum flavum) frequently grow in the drier parts of fens and one (Galium aparine) is atypical of waterlogged conditions and is most prominent in sites that are disturbed and partly drained. Several of the Cluster 4 species may also occupy fen sites with a relatively low summer water level, but they also grow in summer-waterlogged conditions (e.g. Filipendula ulmaria, Proctor 1974)). It is possible that in such circumstances these species are restricted to sites with relatively low concentrations of available iron. There is evidence for this in some sites supporting Epilobium hirsutum (Wheeler 1983; Wheeler et al. 1985). Similarly, the very wet sites in which Rumex hydrolapathum grows in the flood-plain fens of Broadland have only very low concentrations of extractable iron (Giller & Wheeler 1986).

Although cluster 4 species are intolerant of high iron concentrations compared to other wetland species, they may still be much more tolerant than many non-wetland plants. Thus Hodgson (1972) found Rumex acetosa and R. hydrolapathum, two of the most iron-sensitive species in this study,
to be relatively iron-tolerant compared with dry-land taxa. Similarly, *Epilobium hirsutum* is more waterlogging-tolerant than *Chamerion angustifolium* (Etherington 1984).

It is clear that the iron tolerance of the fen plant species screened in this study varies considerably and in a way consistent with their field distribution. Such evidence does not, of course, prove conclusively that iron does, directly or indirectly, determine the field distribution of the species concerned either generally or in specific situations. It does, however, provide experimental support for a multiple regression relationship given by Shaw & Wheeler 1991, which included iron concentration as one of the few chemical variables accounting (negatively) for vegetation species-richness in a general survey of British fen vegetation. And, when linked with evidence from specific sites that iron toxicity influences the differentiation of the vegetation cover (Wheeler et al. 1985) there is clearly reason to suspect that iron concentration may have an important role in helping to determine the species composition of herbaceous fen vegetation.

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References


Relation between root oxidising power and resistance to iron toxicity in rice. Report of the Society of Crop Science and Breeding in Kinki (Japan), 22, 38–47.


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