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The iron and manganese status of seven upper montane tree species in Colorado, USA, following long-term waterlogging

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Summary

1 Seven tree species were investigated for possible Fe and Mn toxicity following prolonged exposure to enriched groundwater in Rocky Mountain National Park, Colorado, USA. The groundwater contained abnormally high levels of Fe and Mn following a catastrophic flood in July 1982. The tree species were *Pinus ponderosa*, *Pinus contorta*, *Pinus flexilis*, *Picea engelmannii*, *Abies lasiocarpa*, *Populus tremuloides*, and *Populus angustifolia*. A shrub, *Salix monticola*, was also studied. The Fe-enriched conifers and *Populus tremuloides* died, while *Populus angustifolia* and *Salix monticola* remained healthy.

2 Foliar Fe and Mn concentrations were determined for normal and flood-affected trees. All seven tree species accumulated abnormally large amounts of Fe, with mean Fe concentration ratios (enriched:normal plants) ranging from 2.0 to 7.7.

3 A possible threshold of Fe toxicity was observed in *Pinus ponderosa*, *Pinus contorta*, *Picea engelmannii*, *Abies lasiocarpa*, and *Populus tremuloides*. In these species, we found a small range of foliar Fe concentrations separating the highest Fe concentration of normal, healthy trees and the lowest concentration in dead trees.

4 The mean Fe:Mn ratios in the adversely affected species exhibited large changes from normal values. The ratios increased between 1.4 and 3.9 times, indicating that these trees experienced a shift towards proportionately higher foliar Fe before death.

5 In the case of *Pinus contorta*, we compared the dead flood-affected trees with the results of a dose–response experiment and found that both groups died after about a doubling of the mean Fe:Mn ratio. Also, an increase in the Fe:Mn ratio above about 1.0 might represent a threshold of Fe toxicity.

6 The healthy, flood-affected *Populus angustifolia* accumulated large amounts of Fe and Mn, but the Fe:Mn ratio exhibited relatively little variation over a 5-year period of observation. The ability to maintain a stable Fe:Mn ratio might be an important mechanism that permits the success of the species in riparian habitats.

Keywords: toxicity, Fe:Mn ratio, flood tolerance

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Introduction

Tolerance or sensitivity to excess Fe and Mn accumulation is an important ecological constraint on the distribution of wetland species and is possibly responsible for excluding some mesophytic species from waterlogged soils (Armstrong 1982; Hendry & Brocklebank 1985). Plants growing on wet soils must have mechanisms to exclude large doses of Fe and Mn from the rhizosphere, immobilize and store it in nonsensitive organs, or face adverse chemical reactions in the cells. The ecological role of Fe and Mn accumulation in waterlogged plants has been investigated for a wide variety of taxa, including herbs (Jones 1972; Hendry & Brocklebank 1985; Etherington & Thomas 1986), shrubs (Jones & Etherington 1970; Jones 1971; Talbot *et al.* 1987), and trees (Lafond & Laflamme 1970; Sanderson & Armstrong 1978; McKevlin *et al.* 1987). Fe and Mn toxicity is also recognized as a limiting factor for some agricultural plants (Ponnamperuma 1965; Benckiser *et al.* 1984).

Most studies of Fe and Mn toxicity in tree species are based on cultured seedlings and cuttings, so little is known about toxicity relationships in mature trees growing under natural conditions The aftermath of the Lawn Lake Flood (LLF) in Rocky Mountain National Park, USA, provided a rare opportunity to observe mature trees after prolonged exposure to Fe- and Mn-enriched groundwater Highly visible Fe deposits were common across the LLF-affected area, which led to the hypothesis that the death of exposed trees might be caused by Fe toxicity (or related stresses) In addition, we suspected that the LLF-affected groundwater contained high Mn concentrations, because Mn compounds are usually reduced before Fe (Armstrong 1982) The purpose of this research was to determine if the exposed tree species were capable of excluding Fe and Mn, or if high, possibly toxic, concentrations were accumulated in the foliage

We investigated seven tree species representing the upper montane and subalpine forests of the Colorado Front Range, including *Pinus ponderosa* Laws , *Pinus contorta* Dougl var *latifolia* Engelm , *Pinus flexilis* James, *Picea engelmannii* (Parry) Engelm , *Abies lasiocarpa* (Hook) Nutt , *Populus tremuloides* Michx , and *Populus angustifolia* James We also studied a common riparian shrub *Salix monticola* Bebb (All names follow Weber (1976)) To aid interpretation, we extended our investigation to include a dose–response experiment on *Pinus contorta* seedlings, chosen because the species is well represented in the LLF-affected area and because of its importance in ecology and forestry

Study area and postflood tree mortality

The study area is located in Rocky Mountain National Park, a 1067-km^2 UNESCO MAB International Biosphere Reserve in the Front Range of northern Colorado The Lawn Lake was a 0 22-km^2 irrigation reservoir located at an altitude of 3349 m a s l , which was contained by an 8-m-high earthfill dam (Blair 1987) On 15 July 1982 the lake was partially drained during a catastrophic dam failure, which released about 830 000 m^3 of water at a peak discharge rate of 340 m^3 s^{-1} (Jarrett & Costa 1985) The flood scoured forest soils along the steep Roaring River valley and deposited a 0 25-km^2 alluvial fan in Horseshoe Park at the confluence of the Roaring and Fall Rivers (2597 m a s l , 40°23′N, 105°38′W) Three phases of sedimentation resulted in distinct alluvial fan lobes (Blair 1987), which were eroded and reshaped after the flood by numerous distributory channels These small streams rerouted some of the Roaring River's discharge and continuously recharged the groundwater to within about 10 cm of the surface across much of the alluvial fan and environs The high water table lasted for about 4 years and during this time groundwater seeps were common

The exposure of the LLF-affected trees to Fe and Mn enrichment was due to the postflood groundwater and not the flood itself The first evidence of Fe-enriched groundwater was highly visible Yellow–orange ferric hydroxide precipitates stained the surface drainage channels and seepage points Although Fe^{3+} complexes predominated across the surface, the root zone below the shallow aerated layer was waterlogged The reducing conditions associated with the water saturated soil (Armstrong 1982) ensured that large amounts of plant available Fe^{2+} was being flushed through the root zone The actual Fe sources were not identified, but may have included (i) weathering of primary minerals from the exposed silicate rocks, (ii) removal from the eroded Roaring River soils, and (iii) release from buried soils or vegetation A detailed analysis of the LLF Fe geochemistry is provided by Litaor & Keigley (in press)

Many of the LLF-affected trees began to show signs of stress late in the 1982 growing season, while others survived for a year or two before dying Most of the conifers became chlorotic and died by the summer of 1983 Conifers located along the shore of Flood Lake, a small lake dammed by the LLF deposits, began to die in 1983, with the trend continuing into the summer of 1984 Individuals of *Pinus ponderosa*, *Pinus contorta*, and *Abies lasiocarpa* died quickly, with most succumbing within a year The deciduous trees appeared to be healthy until a large *Populus tremuloides* clone began to die shortly after producing leaves in the spring of 1984 The immature leaves became chlorotic and then turned black and curled By the summer of 1985, *Populus angustifolia* and *Salix monticola* were the only healthy woody plants in the LLF-affected area Both of these species remained healthy through 1990

Methods

FIELD SAMPLING

We collected foliage samples during the summer of 1984 from all the tree species, and from *Populus angustifolia* again in 1986 and 1987, and *Salix monticola* shrubs in 1986 All foliage samples were composite samples taken from the circumference of the plant We avoided mechanically damaged, diseased or insect damaged plants We selected dead LLF-affected trees from the perimeter of the alluvial fan where little or no sediment had accumulated, or in nearby backwater areas where the hydrology was altered We sampled a few chlorotic, dying trees The sample size of dead and dying trees was constrained by the limited size of the LLF-affected area and our avoidance of damaged plants Healthy, normal trees were selected within 1 km of the alluvial fan at sites that best represented the preflood habitat conditions of slope, aspect, soil moisture and elevation The sampled trees ranged in age as follows (means in parentheses) *Pinus ponderosa* 64–179 (117) years, *Pinus contorta* 23–69 (46), *Pinus flexilis* 17–71 (47), *Picea engelmannii* 27–80 (50), *Abies lasiocarpa* 29–49 (36), *Populus tremuloides* (75), and *Populus angustifolia* (73)

A few of the LLF-affected conifers died early in the growing season before the new needles matured The Fe concentration in young leaves is known to be much lower than in older leaves (Brown 1956) Therefore, in cases where death occurred before needle maturation, we sampled the needle set from the previous growth year In preliminary testing of LLF-affected trees, we confirmed that immature needles had consistently lower Fe concentrations than mature needles Because foliar Fe is not readily retranslocated between leaves (Brown 1956), our selection of mature needles provided a reasonably stable and representative estimate of foliar Fe accumulation

We collected LLF-affected groundwater samples on 18 August 1984 The groundwater samples were taken near dead or dying trees, and were drawn from soil pits excavated to a depth below the water table

Pinus contorta DOSE–RESPONSE EXPERIMENT

The trees used in the dose–response experiment were 2-year-old seedlings, grown in a nursery from Colorado montane seedstock In order to expose the seedlings to a chemical and physical environment as close to natural conditions as possible, we grew the seedlings in a native *Pinus contorta* forest soil rather than a nutrient solution (see Stone 1968) Bareroot seedlings having similar colour, height, circumference and needle length were individually transplanted into plastic pots containing a sandy loam growing medium taken from the A_1 horizon (pH 5 6) The potting medium was prepared by removing coarse fragments (6 35-mm sieve) and was thoroughly mixed After transplanting, the seedlings were placed in a Biotronette Mark III environmental chamber for the duration of the experiment, with temperature, humidity, and photoperiod maintained as close to natural conditions as possible The control trees remained healthy under the experimental conditions

Following a 4-week establishment period, 56 seedlings were assigned by random selection to four treatment groups containing 14 trees each Treatments began in August 1986 One group served as a control, the other three received Fe, Mn, and Fe + Mn treatments, respectively The Fe treatment consisted of an iron chelate solution, Fe-EDTA, and the Mn treatment consisted of Mn-EDTA The Fe + Mn group received equal amounts of Fe and Mn Because the lethal soil solution concentrations were unknown, the lethal doses were achieved stepwise in dose increments starting at 0 5 g l^{-1}, which was doubled to 1 g l^{-1} after 5 weeks and doubled again to 2 g l^{-1} after another 5 weeks The treatments consisted of 50 ml of solution applied every 3–4 days when the soil surface dried The treatments permeated the root zone, but care was taken to allow aerated soil conditions between applications Tree death in the treatment groups took 10–35 weeks Foliage samples were collected following the death of each tree A soil sample was taken from one pot selected randomly from each treatment group

LABORATORY PROCEDURE

Foliage

Within 8 h of collection the foliage samples were washed three times in deionized water to remove surface contamination, and then were dried in a forced-air oven at 40°C until friable (Allen *et al* 1967) The samples were ground and dry-ashed in a muffle furnace at 485°C The ash was dissolved in warm HCl, with HNO_3 added, then evaporated to dryness in a water bath and brought up to standard volume (Allen *et al* 1967) Foliar Fe and Mn concentrations were determined by flame atomic absorption spectrophotometry All concentrations are reported in ng g^{-1} dry weight

Based on US National Bureau of Standards pine needle reference material (NBS 1575), the recovery achieved during our 1984 foliar analysis was 58% for Fe and 98% Mn Fe recovery increased to 90% (based on NBS 1575) during the subsequent analyses of *Populus angustifolia* and *Salix monticola* To aid in making comparisons between years, we adjusted the 1984 Fe concentrations shown in Fig 2 to be consistent with the 90% recovery achieved in 1986 and 1987

Groundwater and soils

The total concentration of Fe and Mn in the groundwater was analysed by flame atomic absorption spectrophotometry The Fe and Mn concentration of the dose–response experiment soil was determined by spectrographic analysis on an NH_4HCO_3-DTPA extract (Workman *et al* 1988)

Results

LLF-AFFECTED GROUNDWATER

The Fe concentrations in the LLF-affected groundwater ranged from 3 2 to 26 6 mg l^{-1} in 1984, and Mn ranged from 0 08 to 5 71 mg l^{-1} In 1985, Litaor & Keigley (in press) reported 47 2 mg Fe l^{-1}

LLF-AFFECTED CONIFERS

All of the LLF-affected conifers accumulated Fe in the foliage (Fig 1) Expressed as a concentration

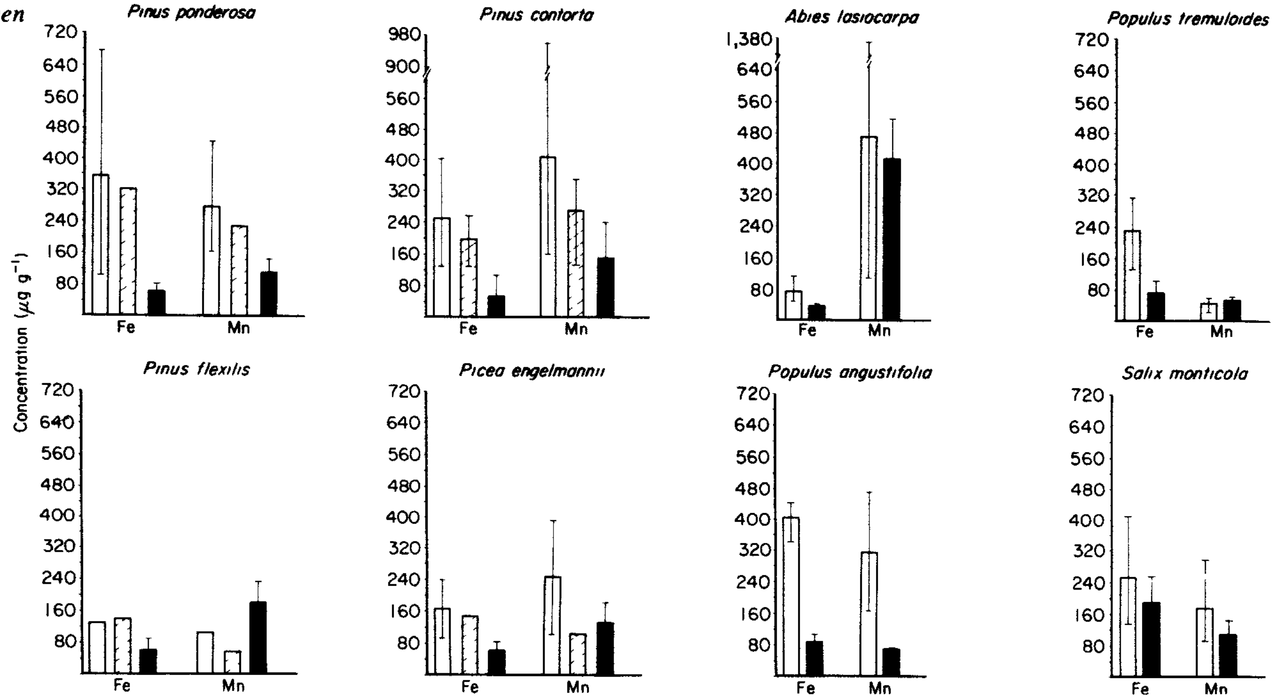

Fig 1 The mean (column) and range (bar) of foliar concentrations in LLF-affected plants (□) dying plants (▨), and healthy, normal plants (■)

Table 1 Mean foliar Fe Mn ratios and mean enriched normal concentration ratios (E N) in LLF-affected and normal foliage

			E N	
	n	Fe Mn	Fe	Mn
Pinus ponderosa				
LLF-affected dead	3	1 23	5 6	2 5
LLF-affected dying	1	1 40		
Normal	3	0 64		
Pinus contorta				
LLF-affected dead	5	1 03	4 6	2 7
LLF-affected dying	3	0 79		
Normal	5	0 41		
Pinus flexilis				
LLF-affected dead	1	1 23	2 1	0 6
LLF-affected dying	1	2 29		
Normal	3	0 33		
Picea engelmannii				
LLF-affected dead	2	0 82	2 8	1 9
LLF-affected dying	1	1 42		
Normal	3	0 57		
Abies lasiocarpa				
LLF-affected dead	6	0 26	2 0	1 1
Normal	3	0 11		
Populus tremuloides				
LLF-affected dead	4	5 42	3 2	0 8
Normal	3	1 39		
Populus angustifolia				
1984 LLF-affected healthy	3	2 43	4 7	4 5
1986 LLF-affected healthy	3	1 36	6 3	8 9
1987 LLF-affected healthy	3	1 85	7 7	7 8
1984 Normal	3	1 95		
Salix monticola				
LLF-affected healthy	5	1 61	1 3	1 6
Normal	4	1 81		

ratio (enriched foliage normal foliage), the mean Fe concentrations in the dead trees represented an increase of 5 6 times the normal level in *Pinus ponderosa*, 4 6 in *Pinus contorta*, and at least 2 0 in the other species (Table 1) Based on the Student's *t*-test (one-tailed *P*), the Fe increase was significant in *Pinus contorta* and *Abies lasiocarpa* ($P < 0\ 006$)

The Mn concentration ratio for *Pinus ponderosa*, *Pinus contorta*, and *Picea engelmannii* was about 2 0, which represented about half the increase of the respective Fe values (Table 1) The Mn increase was not significant among the conifers ($0\ 076 < P < 0\ 403$)

LLF-AFFECTED *Populus* SPECIES

The dead *Populus tremuloides* clone had a significant increase in foliar Fe ($P = 0\ 016$), which was three times higher than the normal concentration The change in Mn was not significant ($P = 0\ 198$) As a result, the clone experienced the largest change in the mean Fe Mn ratio, which increased from 1 39 to 5 42 before death (Table 1)

In 1984, after 2 years of exposure, *Populus angustifolia* accumulated large amounts of foliar Fe ($P = 0\ 003$) and Mn ($P = 0\ 053$), but remained healthy The Fe and Mn concentration ratios were 4 7 and 4 5, respectively (Table 1) The mean Fe Mn ratio increased from 1 95 (normal) to 2 43 Foliar Fe continued to increase in 1986 and 1987 (Fig 2), and by 1987 the Fe concentration ratio was 7 7 Foliar Mn continued to increase through 1986, but then

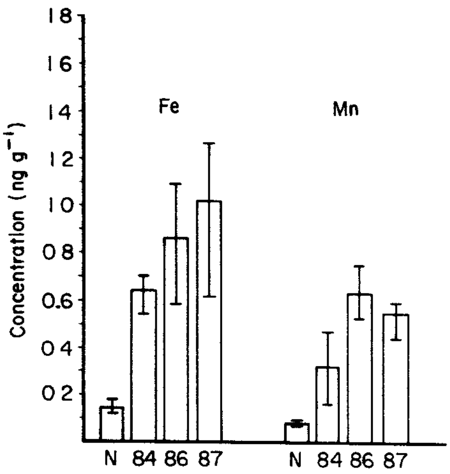

Fig 2 Mean (column) and range (bar) of foliar concentrations in LLF-affected *Populus angustifolia* in 1984 1986 and 1987 and normal trees (N) in 1984

decreased in 1987 (Fig 2) to a concentration ratio of 7 8 In 1987, after 5 years of exposure, the mean Fe Mn ratio was 1 85, which was near the normal value of 1 95

LLF-AFFECTED *Salix monticola*

The shrub *Salix monticola* remained healthy and numerous seedlings colonized the LLF deposits The species did not have a significant increase in foliar Fe ($P = 0\ 147$) or Mn ($P = 0\ 073$) It had the smallest Fe concentration ratio (1 3) among the species we investigated (Table 1)

Pinus contorta DOSE–RESPONSE EXPERIMENT

The Fe-treated trees had a significant increase in foliar Fe ($P = 0\ 040$), resulting in the highest mean foliar Fe concentration ratio (2 5) observed in the experiment (Fig 3) There was a significant increase in foliar Mn ($P = 0\ 033$), but the Mn concentration ratio (1 2) was the smallest of the experiment The large increase in foliar Fe relative to Mn resulted in the highest mean Fe Mn ratio (1 45) Both the Fe and Mn foliage soil concentration ratios were the highest obtained (Table 2)

In the Mn-treated group, there was a significant increase in foliar Mn ($P < 0\ 001$) (Fig 3), resulting in the largest mean Mn concentration ratio (6 9) (Table 2) The change in foliar Fe was not significant ($P = 0\ 345$) The mean Fe Mn ratio decreased to the lowest value of the experiment (0 15)

In the Fe + Mn treatment group, the change in foliar Fe was not significant ($P = 0\ 080$), but a significant increase in Mn ($P = 0\ 002$) resulted in a reduction of the Fe Mn ratio from 0 71 (control) to 0 29 (Table 2, Fig 3)

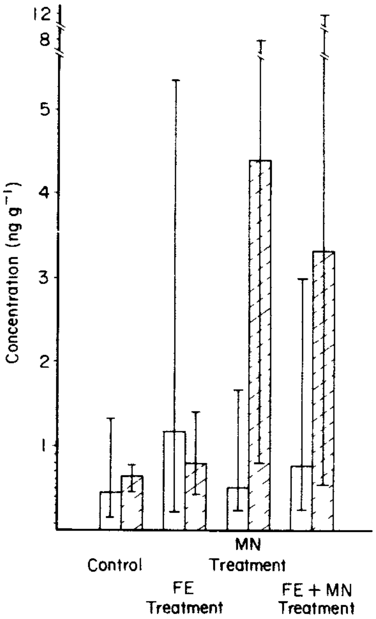

Fig 3 Mean (column) and range (bar) of foliar concentrations in *Pinus contorta* seedlings after the dose–response experiment Fe (☐) and Mn (▨)

Table 2 *Pinus contorta* dose–response experiment Mean Fe and Mn concentrations in the foliage and soil foliar Fe Mn ratios enriched normal foliar concentration ratios (E N) and foliage soil concentration ratios (F S)

		Concentration (µg g⁻¹)			E N		F S	
	n	Fe	Mn	Fe Mn	Fe	Mn	Fe	Mn
Fe group								
Foliage	14	1176 5	797 0	1 45	2 5	1 2	1 8	40 6
Soil	1	635 0	19 6					
Mn group								
Foliage	14	518 0	4418 6	0 15	1 1	6 9	1 4	6 0
Soil	1	358 0	730 0					
Fe + Mn group								
Foliage	14	773 7	3330 3	0 29	1 7	5 2	1 5	3 6
Soil	1	521 0	920 0					
Control group								
Foliage	14	462 0	641 8	0 71	–	–	1 0	20 8
Soil	1	448 0	30 9					

Discussion

Fe AND Mn IN LLF GROUNDWATER

The Fe and Mn concentrations found in the LLF-affected groundwater exhibited considerable spatio-temporal variation However, some of the Fe concentrations approached the maximum 50–100 mg l^{-1} that can exist in oxygen deficient waters (Hem 1970) The highest Fe concentrations in LLF-affected groundwater were about five times the normal concentration in culture nutrient solutions (Hewitt 1966), and twice the published toxicity thresholds (Fe, 12 0 $\mu g\ g^{-1}$, and Mn, 2 5 $\mu g\ g^{-1}$) for seedlings of *Agathis australis* Salisb (Peterson 1962)

Fe AND Mn ACCUMULATION IN LLF-AFFECTED TREES

All of the LLF-affected tree species accumulated abnormally high levels of Fe in the foliage, which suggests that they were unable to exclude Fe or accumulation was advantageous We cannot completely separate the potential for mineral toxicity from the host of other physiological and environmental impacts of prolonged waterlogging (discussed later), or the possibility that root damage allowed the Fe accumulation Although we are unable to prove the case for Fe toxicity, three observations support the hypothesis that nutrient imbalance(s) associated with Fe toxicity played a role in the death of the adversely affected tree species

1 There was a large increase in foliar Fe among the adversely affected species, with mean Fe concentration ratios ranging from 2 0 to 5 6 (Table 1) Moreover, the abnormally large uptake occurred rapidly Large and rapid increases in foliar Fe no doubt induce chemical stresses that require physiological mediation for survival—a response that might not be available in species normally relying on Fe exclusion mechanisms related to aerated soils

2 Possible toxicity thresholds are suggested by the small range separating the foliar Fe concentrations in healthy and dead trees In five of the LLF-affected species (*Pinus ponderosa*, *Pinus contorta*, *Picea engelmannii*, *Abies lasiocarpa*, and *Populus tremuloides*), the highest Fe concentration observed in healthy trees is close to, but does not overlap, the lowest concentration found among the dead trees (Fig 1) This zone might mark the approximate boundary separating luxury consumption and Fe toxicity The Fe levels in dying trees were consistent with these approximate thresholds Dying trees of *Pinus ponderosa*, *Pinus contorta* and *Picea engelmannii* all had foliar Fe concentrations above their suggested toxicity thresholds

3 The adversely affected species had abnormally high mean Fe Mn ratios, indicating proportionately more Fe in the foliage before death In the dead trees, the mean Fe Mn ratio was 1 4 to 3 9 times higher than the normal level In contrast, the healthy LLF-affected *Populus angustifolia* had a small change in the Fe Mn ratio despite accumulating large amounts of Fe and Mn

The ability of tree species to maintain a balanced Fe Mn ratio might be among the adaptations needed for success in waterlogged environments Somers & Shrive (1942) demonstrated the interactive effects of these metals in plants by observing that excess Mn oxidizes ferrous ions in cells, resulting in the precipitation of Fe in the form of organic complexes With some cellular Fe inactivated, Fe deficiency can result despite a high total Fe concentration Robb & Pierpoint (1983) suggested that free Fe molecules might turn into a kind of 'redox mill' in the cell, with oxidation and production of organic and oxygen radicals leading to the death of the cell if superoxide dismutase is insufficient to keep the oxygen level low This process might be the main cause of death in plants growing on waterlogged soils where the entrance of Fe^{2+} into the roots cannot be restricted (Robb & Pierpoint 1983)

Hendry & Brocklebank (1985) proposed a biochemical classification based on plant response to soils containing high concentrations of ferrous ion Our results show that foliar Fe concentrations increased in the LLF-affected trees To the extent that Fe toxicity caused the death of the conifers and *Populus tremuloides*, these species would appear to be type 2 (death response) accumulators The Fe-enriched, but healthy *Populus angustifolia* appears to be a Type 3 (healthy response) accumulator *Salix monticola* responded by limiting the amount of Fe reaching the foliage and appears to be a Type 1 (Fe uptake suppressed) excluder

LLF-AFFECTED *Populus angustifolia*

After 5 years of exposure, the LLF-affected *Populus angustifolia* had foliar Fe and Mn concentrations that were more than seven times higher than the normal level However, the mean Fe Mn ratio varied relatively little during the 5-year period, suggesting a plant regulated relationship The minor variations in foliar Fe Mn concentrations were consistent with a system seeking a steady-state equilibrium after large inputs of Fe and Mn Because foliar Fe increased throughout the observation period, the Mn decrease in 1987 appears to be the change that brought the ratio to a near normal level Thus, it is tempting to suggest that Mn regulation was involved in mediating the Fe Mn relationship Jones (1971) suggested that waterlogging might increase mobility of plant Mn resulting in translocation from roots to leaves If so, the increase in foliar Mn observed in the LLF-affected ramets might relate in part to Mn relocation from plant storage to balance increased Fe uptake from the soil

Examination of annual wood increments indicated that the growth of *Populus angustifolia* increased substantially after the flood Thus, the species appears to have been fertilized by the increased uptake of these micronutrients *Populus* species are known to be successful in floodplain environments because the roots resist undermining (Gill 1970) However, the ability of *Populus angustifolia* to maintain a near normal Fe Mn ratio while taking up massive doses of both Fe and Mn might also play a role in the success of the species in riparian habitats

Pinus contorta – LLF-AFFECTED AND EXPERIMENTAL TREES

The Fe concentrations of the dose–response and LLF-affected trees were so different that there would appear to be little hope of finding a common toxicity threshold The mean foliar Fe concentration in the experimental control group was more than eight times higher than in the normal, healthy trees from the LLF-affected area Following Fe-enrichment, the mean Fe concentration ratio of the dead LLF-affected trees (4 6) was nearly twice that of the Fe-treated seedlings (2 5) Despite these differences, the Fe Mn ratio might reveal a common relationship Both groups had abnormally high postenrichment Fe Mn ratios, representing about a doubling of Fe with respect to Mn In the Fe-treated group, the mean Fe Mn ratio doubled from 0 71 (control) to 1 45 (treated) In the LLF-affected trees, the Fe Mn ratio increased from 0 41 (normal) to 1 03 (enriched) Although more data are needed, these results suggest that it might be possible to express the Fe toxicity threshold as the magnitude of change from the normal Fe Mn ratio This is important because nutritional ecotypes differ in normal foliar concentrations and tolerances, so toxicity thresholds will be more generalizable if they can be expressed as proportions in multielement ratios instead of absolute concentrations

It is also interesting to note that both groups had postenrichment Fe Mn ratios that were greater than 1 0 Published Fe Mn ratios for the species (including all 4 subspecies) range between 0 14 and 0 86, with no natural populations having more Fe than Mn in the foliage However, mean Fe Mn ratios must be interpreted as approximate thresholds because some individual trees might have died with a ratio lower than 1 0 It is also possible that an increase in the Fe Mn ratio might be a symptom of the loss of ferrous ion exclusion control in the roots, caused by toxicity or other stresses, with Fe being more easily absorbed than Mn

The Mn treatment required higher dose concentrations and took longer to kill the trees, suggesting that at least some populations are rather Mn tolerant The lethal dose of Mn was quite high, resulting in a Mn concentration ratio of 6 9 The massive increase in Mn without a concomitant increase in Fe produced the lowest Fe Mn ratio (0 15) The resulting stress might include Fe deficiency induced by the 'redox mill' suggested by Robb & Pierpoint (1983) Because the lowest mean Fe Mn ratio we know of in a natural population is about 0 15 (Stark 1983), perhaps there is a minimum Fe Mn ratio threshold However, the mechanism for a minimum ratio of about 0 15 is less clear, unless it is induced Fe deficiency

In the case of the Fe+Mn treatment group, the decrease in the mean foliar Fe Mn ratio from 0 71 (control) to 0 29 was due to large increases in Mn The lack of a significant increase in foliar Fe despite the Fe-enrichment of the soil might be explained by Fe storage in the roots under conditions of excess Mn accumulation or Mn interference in the transport of Fe from the roots (Epstein & Stout 1951) It is interesting that the presence of excess amounts Fe and Mn in the soil resulted in a mean foliar Fe Mn ratio within the normal range for the species

The large range of foliar Fe and Mn concentrations resulting from the dose–response experiment were not unexpected (Fig 3) Similar experiments reported high Fe variability (Jones & Etherington 1970), and normal Mn concentrations are known to exhibit large variations (Stone 1968)

NUTRITIONAL ECOTYPES

The potential toxicity thresholds suggested for the LLF-affected trees must be interpreted with care when applied to other populations The nutrient status of populations might be under genetic control and ecotypic separation can occur (Clark 1983) The healthy trees in the LLF-affected area fall within the normal range of foliar Fe and Mn concentrations (see Beaton *et al* 1965, Verry & Timmons 1976, Stark 1983), but some of these trees do occupy the low end of the Fe and Mn continuum The healthy *Pinus ponderosa* and *Pinus contorta* from the LLF-affected area have relatively low Fe levels *Pinus ponderosa*, *Pinus contorta*, *Picea engelmannii*, and *Abies lasiocarpa* have relatively low Mn concentrations Thus, other populations might have normal Fe or Mn concentrations greater than the potentially toxic levels in the dead LLF-affected trees For example, *Pinus contorta* ssp *contorta* is adapted to the perennially wet Alaskan muskegs, and normally has high concentrations of Fe and Mn in the foliage However, the subspecies maintains a relatively low mean Fe Mn ratio of about 0 30 (Barrick and Schoettle, unpublished data)

OTHER IMPACTS OF WATERLOGGED SOILS

We concentrated on the role of Fe and Mn toxicity in the death of the LLF-affected trees, but other impacts might be involved The negative effects known to accompany waterlogged root systems include respiration failure, carbon-dioxide accumulation, changes in energy metabolism or storage and membrane biosynthesis, accumulation of toxic compounds like ethanol, acetaldehyde, or ethylene, postanoxic peroxide damage, and infections of pathogenic microorganisms or fungus (see Gill 1970, Kozlowski 1984, Crawford 1989) These effects cannot be ruled out in the case of the mature LLF-affected trees, but they are not likely to have been factors during the dose–response experiment

Several relationships deserve further consideration when separating the role of toxicity from other stresses Some populations of *Pinus contorta* are flood tolerant (Critchfield 1957, Boggie 1972, Taylor 1990) and respond with root adaptations to escape the worst vagaries of anaerobiosis These adaptations include upwardly growing primary roots, root regrowth in the form of shavers, lenticels on roots above the water table, and the ability to convey oxygen from shoot to root by gaseous diffusion in intercellular spaces (Armstrong & Read 1972, Coutts & Armstrong 1976, Coutts & Philipson 1978a,b, Coutts 1982) Root growth is most successful if there is an aerated zone at the soil surface (Boggie 1972) A shallow aerated layer existed over much of the LLF-affected area, but we found no evidence of root adaptation among the dead trees It appears that the roots of the LLF-affected trees did not have the ability to respond or stress rendered new growth impossible

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