INVESTIGATION OF THE ZINC AND MANGANESE STATUS OF SOME STANDS OF *TSUGA HETEROPHYLLA* IN BRITISH COLUMBIA

by

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ABSTRACT

Western hemlock has lower foliar Zn and higher foliar Mn concentrations compared to some other conifers. Existing foliar diagnostic norms for conifers imply a Zn deficiency and possibly a Mn toxicity in many stands of western hemlock. This study was undertaken in order to determine the significance of these foliar levels in the nutrition of western hemlock. The nutrition of hemlock was studied using comparative nutrition and fertilizer screening trials. The screening trials consisted of treatments of Zn and Mn applied as foliar sprays and as soil treatments. Different methods of application were utilized to determine if factors of the plant such as uptake and/or translocation could account for the characteristic foliar zinc and manganese levels in hemlock. In addition, a treatment was applied consisting of a complete fertilizer without Zn and Mn. This "complete-Zn-Mn" treatment was included to investigate the possibility of additional nutrient deficiencies and/or toxicities.

In a comparison of total foliar concentrations, hemlock had lower Zn compared to Douglas-fir, amabilis fir and white pine. In contrast, hemlock had higher Mn compared to Douglas-fir, amabilis fir, white pine, red cedar and yellow cedar.

Analysis of cellular fractions of foliage produced two results. First, Zn accumulated in the mitochondrial fraction and Mn accumulated in the ribosomal and vacuolar fraction, irrespective of the level of the treatment or the species.

Accumulation in certain fractions may indicate a physiological need in that fraction or a tolerance mechanism. Second, comparing hemlock to Douglas-fir, total Zn levels tend to be consistent with levels in different fractions, indicating that total levels may be an adequate indication of physiologically active levels. But comparing hemlock to Douglas-fir, total Mn levels are not consistent with Mn levels in different fractions, indicating that total Mn levels may not be an adequate indication of physiological levels.

In the fertilizer screening trials, nutrient uptake and growth responses were dependent upon the site, the level of fertilizer application, and the time since application.

Nutrient uptake and positive growth responses were obtained with foliar treatments of Zn and soil treatments of Mn in both the first year and second year following fertilization. Nutrient and growth responses to soil Zn treatments were delayed until the second year following fertilization. Additional evidence supporting a Zn deficiency was indicated by a positive relationship between foliar Zn and height increment, evidence of retranslocation of Zn to new foliage in the second year following foliar Zn treatment, and the high ranking of Zn in the vector analysis from the "complete-Zn-Mn" treatment.

Positive growth responses to the "complete-Zn-Mn" treatment were obtained in the first and second years following treatment.

Ranking of nutrient response vectors using relative values indicated the existence of other nutrient deficiencies, besides Zn and Mn.

Growth response, as measured by shoot increment ratio, was obtained primarily in the second year after treatment with foliar applications of Zn. Shoot increment ratio response occurred to soil Mn treatments in the first year of treatment. For the "complete-Zn-Mn" treatment there was an increase in shoot increment ratio in both the first and second years following treatment.

Height increment ratio increased in response to foliar Zn applications in the second year, and to soil Mn treatments in the first year.

Foliar Zn and foliar N were positively correlated with each other. Foliar Zn concentrations increased as a result of soil applications of Mn, but applications of Zn had no effect on Mn uptake. Therefore, there was no evidence in this study to suggest that low foliar levels of Zn in hemlock are due to a Mn antagonism. The only interaction obtained with the "complete-Zn-Mn" treatment was a synergism: it caused an increase of foliar Zn.

Ingestad's nutrient ratios were calculated for the foliar levels from the control and the "complete-Zn-Mn" treatments.

Comparing these ratios to the optimum revealed that most of the nutrients were in balance except for Fe and Mn.

Existing diagnostic norms for Zn appear to adequately describe the Zn nutrition of hemlock. Response to fertilization occurred with control foliar Zn concentrations for hemlock being below the critical level of 15 $\mu g\ g^{-1}$. Diagnostic norms for Mn need to be revised. Response occurred even though control foliar Mn concentrations for hemlock were well above the critical level of 25 $\mu g\ g^{-1}$. Therefore, total foliar manganese may not be indicative of the physiological manganese status of hemlock.

These results for hemlock are discussed in light of existing knowledge from the literature regarding the nutrient strategy of metal tolerant plants and low nutrient adapted plants.

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CHAPTER 1. INTRODUCTION

The species *Tsuga heterophylla* (Raf.) Sarg. (western hemlock) characteristically has lower foliar zinc (Zn) and higher foliar manganese (Mn) concentrations relative to some other conifer species throughout British Columbia and the United States Pacific Northwest. Curiosity about this phenomena motivated this research.

The patterns of foliar Zn and Mn levels in hemlock are of practical significance in forestry. Attempts to increase the productivity of hemlock with nitrogen fertilizers have met with variable and inconsistent results. This has lead to interest in the status of other nutrients in hemlock such as Zn and Mn.

In comparison to suggested critical foliar levels for some B.C. conifers, hemlock often has foliar Zn levels which fall into the low to possibly deficient zone (being less than the critical foliar Zn level of 15 $\mu g\ g^{-1}$), and has foliar Mn levels which fall into the high to possibly toxic range (being higher then the critical foliar Mn level of 25 $\mu g\ g^{-1}$). Therefore, the foliar Zn and Mn concentrations characteristic of hemlock suggest that Zn is at possibly deficient and Mn is at possibly toxic levels.

Up to the present time the patterns of foliar Zn and Mn concentrations of hemlock have been described but no work has been done on their significance in the nutrition of hemlock.

Therefore, the objective of this study was to investigate the significance of the pattern of foliar Zn and Mn concentrations in the nutrition of hemlock. The specific questions asked were: Is Zn sometimes deficient and is Mn sometimes toxic for the growth of hemlock? Are the lower foliar Zn concentrations due to a Mn antagonism? Are the lower foliar Zn and higher foliar Mn concentrations of hemlock compared to some other conifers due to factors of the plant such as uptake and/or translocation or factors of the soil such as fertility? Are there additional nutrient deficiencies in hemlock?

A comprehensive literature review was made to learn what was already known about hemlock nutrition, Zn and Mn levels in plants, plant factors affecting nutrition, deficiency and toxicity levels for Zn and Mn, nutrient-growth relationships, and methods of nutrient diagnosis.

Two approaches were taken in this research. The first step involved a comparison of Zn and Mn nutrition among several conifer species, to check the premise for this study. This led to another question: although plants may have different total foliar Zn and Mn concentrations do they have similar physiological levels? A review of the literature suggests some possible mechanisms, and some experimental work (extractable and active nutrient levels, and foliar Zn and Mn distributions between cellular fractions) was directed to this question. The second step involved the use of fertilizer screening trials,

using foliar and soil treatments of Zn and Mn, and a soil application of a "complete-Zn-Mn" treatment. By measuring nutrient and growth responses, inferences could be made about deficiencies and toxicities. Nutrient retranslocation, nutrient interactions, and nutrient-growth relationships could also be examined using data from the fertilizer trials.

Since the patterns of Zn and Mn foliar concentrations are found over a wide area of hemlock's range, the sites selected for the fertilizer trials did not cover a wide range of ecosystem conditions. In addition, the enormity of collecting several hundred samples, each of which would be subjected to several chemical analyses, and the requirement of having sites easily accessible from the University of British Columbia to facilitate the work, limited the number of sites which could be investigated. These constraints obviated using data and conclusions to generalize about hemlock in the region as a whole. However, the site selection proved adequate for its purpose: it succeeded in finding hemlock stands low in Zn and high in Mn suitable for testing hypotheses about deficiency, toxicity and antagonism.

CHAPTER 2. LITERATURE REVIEW

A. Nutritional Characteristics of Western Hemlock

Western hemlock occurs in five biogeoclimatic zones of B.C. It grows as a climax species in the Coastal and Interior Western Hemlock zones. In the Coastal Douglas-Fir zone, it develops as a climax species only in subhydric habitats. In the subalpine Mountain Hemlock and Engelmann Spruce-Subalpine Fir zones, the short vegetative season is the major obstacle which limits the distribution and growth of western hemlock (Krajina *et al.* 1982).

Western hemlock is commonly associated with strongly podzolized soils and these soils characteristically have a mor humus layer which has a low rate of mineralization. This suggests that hemlock is tolerant of acidic soils and requires lower quantities of nutrients compared to some other trees (Krajina *et al.* 1982). The productivity of hemlock was found by Lowe and Klinka (1981) to have a lower negative correlation with the percent yield of soil lipids of the humus layer as compared to Douglas-fir. Lipids accumulate to high levels in soils where biological activity is inhibited (Lowe and Klinka 1981), which indicates a negative relationship between the abundance of soil lipids and mineralization. The lower correlation between the amount of soil lipids and the productivity of hemlock suggests that hemlock is less sensitive to low rates of decomposition,

mineralization and nutrient release compared to Douglas-fir (Lowe and Klinka 1981). The productivity of hemlock was also positively correlated to the pyrophosphate-extractable Fe+Al (PFeAl) and carbon content in the B horizon (TCB). Both the PFeAl and TCB are indices of the extent and intensity of podzol formation. This helps to explain the occurrence of hemlock on podzolic soils.

Hemlock regeneration flourishes on rotten logs, stumps or mineral soil exposed on trails or on mounds and pits created by windthrown trees (Christy *et al.* 1982; Stewart 1989). The organic layer is frequently a substratum for hemlock even above the more base-rich, high pH soil layers in which this tree does not regenerate (Krajina *et al.* 1982). Rotten wood is an important substrate for mycorrhizal associations of moist hemlock habitat types in the northern Rockies (Harvey *et al.* 1979). More than 95% of the active primary roots of hemlock have been found to be mycorrhizal (Gill and Lavender 1983). Since mycorrhizae have been found to enhance nutrient uptake in many species, their abundance on hemlock roots suggests that mycorrhizae play an important role in hemlock nutrition. During the first season, seedlings are able to survive on rotten wood without mycorrhizae; however, mycotrophy is advantageous for growth (Harvey *et al.* 1979). Two-year-old seedlings growing on mineral soil had greater growth than those growing on the rotten wood substrate. The effect of substrate on growth disappeared in the third year (Christy *et al.* 1982). Roots of seedlings which originally

established on rotten wood eventually extended their roots into mineral soil and the original substrate disappeared. The nutritional source then shifts from woody substrate to mineral soil (Christy *et al.* 1982).

Hemlock occurs on soils which characteristically have low pH and a pH-dependent cation exchange capacity (CEC); often low base saturation and abundant Al on the soil exchange complex; high organic matter contents; and the presence of sesquioxides and/or "andic" soil properties (Ryan 1983). This is evident when one compares soil properties for hemlock forests versus Douglas-fir forests (Table 1). Consequently, soluble Al and Mn are high (Foy 1984), and nitrogen is predominantly in the ammonium (NH_4^+) form (Haynes 1986). Anderson *et al.* (1982) found that nitrification in two coastal Washington soils under hemlock stands was negligible. This was also found for forest floor and mineral soils associated with hemlock trees in northwestern Washington (Turner and Franz 1985). Therefore, the nitrogen nutrition of hemlock appears to be adapted to the ammonium form. Nutrient studies by Krajina *et al.* (1973), and van den Driessche (1971, 1976) tend to support this observation.

Ryan *et al.* (1986a, 1986b) investigated the tolerance of seedlings of Douglas-fir, western hemlock, and western redcedar to solution acidity and Al concentration in solution cultures. Western hemlock survived and thrived in acid solution of pH 3 but the other species had greater mortality and reduced growth (Ryan

Table 1. Comparison of surface soil and forest floor properties under second growth Douglas-fir (A) and western hemlock (B) stands from coastal (W) versus Cascade (E) sites (from Zasoski *et al.* (1986)).

	Region	A	B
Surface Soil 0-15 cm			
pH	E	5.1	4.5
	W	4.9	4.4
Exchangeable Cations (meq per 100g)			
Ca	E	3.8	1.7
	W	3.0	1.2
Mg	E	0.8	0.36
	W	0.96	0.73
K	E	0.41	0.27
	W	0.38	0.32
CEC	E	26.0	35.1
	W	37.1	47.3
Base Saturation (%)			
	E	20.4	7.2
	W	11.4	4.8
Available P ($\mu g\ g^{-1}$)			
	E	97.0	44.0
	W	51.0	15.0
Total P ($\mu g\ g^{-1}$)			
	E	1070	809
	W	1370	954
Available S ($\mu g\ g^{-1}$)			
	E	7.7	9.9
	W	9.5	10.2
Total N (%)			
	E	0.16	0.31
	W	0.31	0.45

Table 1. (concluded).

Total C (%)			
	E	4.7	8.4
	W	8.3	10.9
C:N Ratio			
	E	30.3	28.5
	W	27.2	24.4
Forest Floor			
Total Mass (kg ha^{-1})			
	E	21700	29800
	W	18700	27000
Total N (%)			
	E	0.95	1.05
	W	1.05	1.02
Total C (%)			
	E	39.1	41.5
	W	39.7	43.4
C:N Ratio			
	E	38.7	38.0
	W	39.0	42.9

et al. 1986a). Both western hemlock and western red cedar were found to be especially tolerant of acid-Al conditions with a solution pH of 3.5 and at the highest Al treatment of 100 μg g^{-1} (Ryan *et al.* 1986b). Aluminium adversely affected the tissue concentrations of Ca and Mg. The ability of western hemlock to grow in acid-Al conditions is suggested to be related to this species' physiological tolerance of excess H-cations in solution and low tissue requirements of Ca and Mg (Ryan *et al.* 1986a, 1986b). The specific physiological or biochemical Al tolerance mechanisms for hemlock have not been investigated. In a mature mixed subalpine stand, *T. mertensiana* and *A. amabilis* had the highest concentration of Al in the fine root component relative to all tissues analyzed (Vogt *et al.* 1987). It was hypothesized that accumulation in the roots is an effective mechanism for avoiding Al toxicity. The large root biomasses of these subalpine stands allow for large amounts of Al to be taken up and immobilized in roots. The high root turnover in these stands may be a result of root senescence occurring in response to high Al accumulation. Root senescence would be an effective mechanism for removing Al from the biological component (Vogt *et al.* 1987b).

B. Zinc and Manganese Levels in Plants

The concentrations of Zn and Mn in the foliage of various tree species in the Pacific Northwest are listed in Tables 2 and 3. Hemlock tends to have lower foliar Zn and higher foliar Mn

Table 2. Tissue micronutrient levels (μg g^{-1}) in Pacific Northwest conifers.

Species	Age	Type	Location	Zn	Mn	Source
Douglas-fir						
	13-49	field	Van. Is. B.C.	17-35	452-758	1
	1	outdoors	Seattle, WA	11-28	125-785	2
	4	plant	Hoquiam, WA	21-32	390-580	3
	7	plant	Pack For. WA	21		4
	20	field	Puget Sound WA	14-31	350-2010	5
	30	field	Coastal B.C.	11-18	174-465	6
	30	field	Coastal B.C.	23	121	6
	5-32	plant	Coastal B.C.	13	292	7
	5-32	plant	Coastal B.C.	8	316	7
	60-73	field	Coastal B.C.	18	186	7
	plugs	nursery	Seattle, WA	35		8
Sitka spruce						
	1	outdoors	Seattle, WA	31-68	98-403	2
	plugs	nursery	Seattle, WA	59		8
Western red cedar						
	plugs	nursery	Seattle, WA	24		8
	27	field	Coast OR, WA, B.C.	13-26	69-383	9
	25	field	Interior OR, WA	21-48	102-368	9
Ponderosa pine						
	plugs	nursery	Seattle, WA	64		8
	7	plant	Pack For., WA	40		4

Table 2 (continued)

Western hemlock

1	outdoors	Seattle, WA	33-36	140-619	2
plugs	nursery	Seattle, WA	45		8
23-39	field	Cascades, OR	13-24	885-1213	10
23-39	cont.	Coastal OR	7-15	617-764	10
23-39	field	Cascades OR	15-23	736-1087	10
23-39	fert.	Coastal OR	9-15	434-586	10
60-150	field	Van. Is. B.C.	3-14	1580-198	1
4	plant	Hoquiam, WA	10-19	480-930	3
1-2	lath	Olympia, WA	0.1-3	249-696	11
20-30	field	Cascades WA	16-20	1200-190	12
20-30	field	Coastal WA	17-20	1000-110	12
6-10	field	Western WA	17-19	616-1922	13
13-15	plant	Ozett, WA	7-16		14
70	field	Gold River, B.C.	5	1804	15
70	field	Kaprino I, B.C.	3	1017	15
72	field	Kaprino II, B.C.	1	1263	15
52	field	Island Hwy, B.C.	5	1493	15
70	field	Beaver Lake, B.C.	6	1720	15
71	field	Rupert Main, B.C.	5	1626	15
30	field	Brittain River, B.C.	7	1187	15
			5.9	1114	15
			6.6	753	15
			6.4	880	15
			7.4	1043	15
			7.7	930	15
			8.8	1127	15
			7.8	811	15

Table 2 (concluded)

Western white pine					
	20	field	Puget Sound, WA	26-62	141-1800	5
Sub-alpine fir						
		field	Interior, B.C.	16-64	369-1175	16
Lodgepole pine						
		field	Interior, B.C.	33-71	245-747	16
White spruce						
		field	Interior, B.C.	37-76	154-1025	16
Douglas fir						
		field	Interior, B.C.	13-27	274-2244	16
Western red cedar						
		field	Interior, B.C.	8-12	92-384	16
Spruce hybrid						
		field	Interior, B.C.	33-66	225-637	16

Sources

1. Beaton *et al.* 1965
2. Rollwagen 1981
3. Porada 1987
4. Greenleaf-Jenkins 1985
5. Zasoski *et al.* 1977
6. Carter *et al.* 1984
7. Carter *et al.* 1986
8. Zasoski *et al.* 1984
9. Radwan and Harrington 1987
10. Gill and Lavender 1983
11. Radwan and DeBell 1980a
12. Radwan and DeBell 1980b
13. Zasoski *et al.* 1990
14. Zasoski *et al.* 1990
15. Carter unpublished
16. Ballard unpublished

Table 3. Foliar Zn/Mn levels ($\mu g\ g^{-1}$) for various tree species occurring in the same stands located in the interior of B.C. (from Ballard (personal communication)[1]).

Species	Plots				
Western hemlock	3/2225	1/825	2/850	5/1850	2/732
Western red cedar				11/358	11/100
Douglas fir	14/697	11/280	14/338		
Lodgepole pine	50/662				
White spruce					53/227
Spruce hybrid			61/474		

Species	Plots				
Western hemlock	3/1625	3/2425	3/1775	1/1125	5/975
Western red cedar	12/316		11/384		
Spruce hybrid					47/427

Species	Plots	
Western hemlock	1/1175	5/1275
Western red cedar	8/180	
Douglas-fir		18/379

1. T. M. Ballard. Professor, Faculty of Forestry/Department of Soil Science, University of British Columbia. Unpublished Data 1984.

concentrations compared to other species, not only in different stands (Table 2), but also in the same stands (Table 3). In a mixed subalpine stand of *A. amabilis* and *T. mertensiana*, the two species were found to have clear differences in their ability to accumulate specific elements from the soil (Vogt *et al.* 1987a).

Differential nutrient levels have also been found between other wild plant species on the same sites (Gerloff *et al.* 1966), and between agricultural plants of different species (Gladstones and Loneragan 1970; Collander 1941) and varieties when supplied with the same amounts of nutrients (Brown *et al.* 1972). This indicates that different species of plants have different tolerances and requirements for nutrients, and that factors of the plants themselves affect nutrient uptake.

C. Nutritional Differences between Plants

There are a number of reasons for differences in nutrition between species and genotypes which are summarized in Figure 1. These are related to uptake, transport and utilization in the plant (Marschner 1986). Both uptake and growth are assumed to be controlled by cytoplasmic pools through feedback and substrate supply, respectively. Consequently, species differences in uptake and growth might be related to cellular compartmentation of nutrients (Chapin 1988). Species adapted to different soil fertilities generally differ in the distribution of nutrients among various chemical fractions (Chapin 1988). Species may

(I) Nutrient Efficiency

(1)

a) Demand on cellular level

b) Utilization within the shoot (eg. retranslocation)

(2)

a) Root-shoot transport (long distance)

b) Transport within the root (short distance)

c) Compartmentation/binding-form within the roots

(II) Acquisition of Nutrients

(1) Root morphology

a) Roots themselves

I) Inherent

II) response to deficiency

b) Mycorrhizae

(2) Root physiology and biochemistry

a) Affinity of the uptake system (Km)

b) Threshold concentration (Cmin)

c) Modifications of the rhizosphere

I) Passive (eg. cation-anion uptake)

II) Active response to deficiency (eg. secretions of chelating, reducing compounds, protons)

Figure 1. Factors of the plant which affect plant nutrition (from Marschner (1986)).

differ in the compartmentation of nutrients into specific plant parts, chemical fractions, or cellular compartments (Chapin 1988). Foliar concentrations may reflect these differences (Bowen 1981), or some of the same fractions may have similar nutrient concentrations for the same physiological processes.

Hemlock tends to have lower nutrient requirements compared to other conifers (Krajina *et al.* 1982). Plants adapted to low nutrient conditions have a growth rate which is relatively insensitive to variation in the rate of nutrient supply (Chapin 1988). During periods of high nutrient availability there would be accumulation of vacuolar stores (luxury consumption). Luxury consumption of nutrients buffers the plant from variation in external nutrient supply (Chapin 1988). There is a tendency towards stable ionic composition of the cytoplasm (Glass and Siddiqi 1984; Leigh and Jones 1986).

Plants can affect nutrient uptake by affecting the pH of the rhizosphere. There are differences in the rhizosphere pH among plant species growing in the same soil (Marschner 1986). Hemlock has a higher ratio of H^+ release / NH_4^+ uptake than Douglas-fir. This suggests hemlock may not only tolerate acid conditions but would tend to create acidity in the rhizosphere (Rygiewicz *et al.* 1984). Hemlock had lower mean ammonium uptake rates for both mycorrhizal and nonmycorrhizal roots compared to Douglas-fir. However, mycorrhizal roots enhanced ammonium uptake rates in hemlock (Rygiewicz *et al.* 1984).

The form of nitrogen used by a plant can have an influence on the plant's overall nutrition. Plants with ammonium nutrition tend to absorb cations in excess of anions (N being the element often absorbed in the largest amounts), with a net efflux of H^+ into the rhizosphere. As a consequence of ammonium nutrition there is a decrease in the uptake of cations compared to that observed with nitrate nutrition. This may be attributed to ionic competition with ammonium or with the excreted H^+ ions (Haynes 1986). The reduction in cation uptake with ammonium nutrition may be a mechanism of plant tolerance to Al and Mn on acid soils. In general, ammonium inhibits the plant's uptake of Mn and Al (Haynes 1986). Ammonium nutrition has two other consequences. Firstly, binding of free ammonium must take place with organic compounds in order to tolerate the high ammonium levels (Kirkby and Hughes 1970). Secondly, ammonium plants must ensure a synthesis of organic anions independently of nitrate reduction as a means of compensating for the inadequate supply of anions. The smaller quantity of anions would hinder the transfer of cations to the shoots. It is through an active synthesis of organic acids that plants could have an equivalent or better growth when they depend on ammonium as a nitrogen source (Salsac *et al.* 1987).

Baker (1981), identified three nutrient models of plant-soil relations. These are the accumulator, the excluder and the indicator (Figure 2). Accumulators are plants where metals are concentrated in above-ground plant parts from low or high soil levels (Baker 1981). Excluders are plants where metal concentrations in the shoots are maintained constant and low over a wide range of soil concentrations, by differential uptake and transport, up to a critical soil value above which the mechanism breaks down and unrestricted transport results. Indicators are plants where uptake and transport of metals to the shoot are regulated so that internal concentration reflects external levels (Baker 1981).

In both the accumulators and the excluders, the mechanisms of tolerance are largely 'internal' in that there is active detoxification of metal ions. It is the sites of detoxification which differ, being largely within the root in excluders and in the shoots in accumulators (Baker 1981). Detoxification may result from cell-wall binding, active pumping of ions into vacuoles, complexing by organic acids and possibly by specific metal-binding protein, enzymatic adaptations and effects on membrane permeability (Baker 1987). Ectomycorrhizae may play a role in metal exclusion by restricting uptake or through accumulation.

Taylor (1987) made a distinction between internal tolerance and exclusion based upon the site of metal

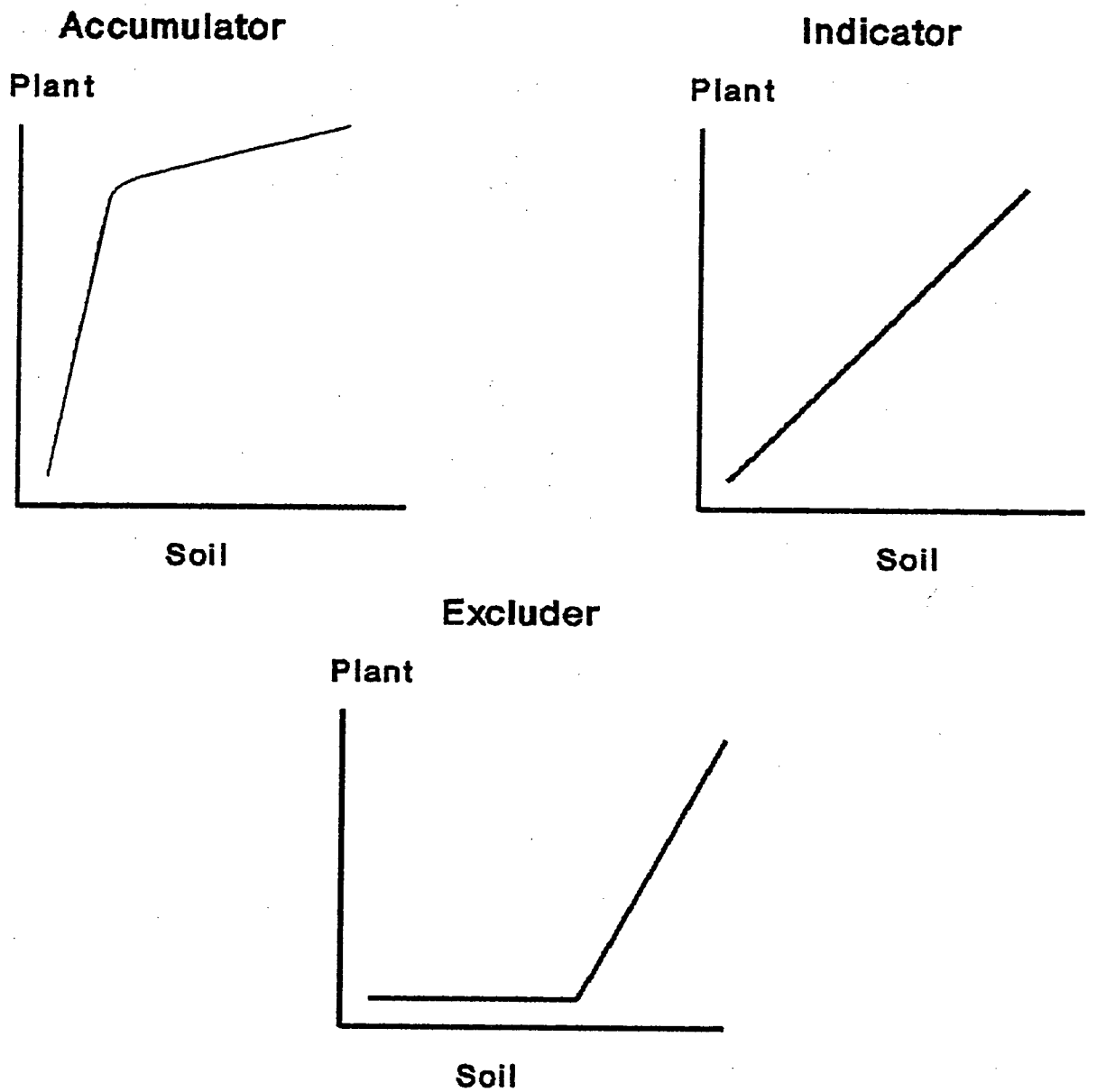


Figure 2. The three models of plant-soil relationships. The axes represent nutrient concentrations (from Baker (1981)).

detoxification or immobilization, either in the symplasm (internal) or apoplasm (exclusion) of roots. Exclusion of a metal may be by immobilization in the cell wall, exudation of chelates or organic acids from roots, a redox barrier at the plasma membrane, or a pH barrier at the plasma membrane (Taylor 1987).

D. Deficiency and Toxicity Levels for Zn and Mn

The foliar nutrient concentrations tend to be an integration of all the soil and plant factors which affect nutrient availability to the plant. Therefore, they serve as an indicator of plant nutrient availability.

The critical Mn deficiency level found in general for most plant foliage ranges from 15 to 25 $\mu g\ g^{-1}$ with the sufficient level being 20-300 $\mu g\ g^{-1}$ (Kabata-Pendias and Pendias 1984). Toxicity to Mn generally occurs at a concentration above about 500 $\mu g\ g^{-1}$ (Kabata-Pendias and Pendias 1984); toxicity to Mn at foliar concentrations of over 1,000 $\mu g\ g^{-1}$ is common (National Academy of Sciences 1973).

There is abundant evidence which suggests that Mn levels found in plants are not reflective of physiological requirements. Species as diverse as sugarbeets and wheat have a critical deficiency level in the range of 10-20 $\mu g\ g^{-1}$ (Clarkson 1988). In *Vaccinium vitis-idaea*, plants having foliar Mn levels of 6,800

to 12,300 μg g^{-1} had similar rates of photosynthesis, dry matter production and number of leaves as plants containing 18 to 1,500 μg g^{-1} of Mn (Miller 1987). In tree species from the temperate forests of Central Japan, Mn concentrations of the shoots vary between species by a factor of 180 (Marschner 1988). In addition, large differences have been found between barley genotypes in Mn efficiency. Therefore, differences in Mn concentrations between species growing on the same soil probably have little to do with differences in Mn requirements (Clarkson 1988) or utilization in the plants (Marschner 1988). They are probably due to differences in Mn acquisition from the soil (Marschner 1988). These differences may partially reflect the extent to which species are able to acidify the rhizosphere (Clarkson 1988) or produce Mn reducing organic root exudates.

Memon and Yatazawa (1982) extracted water-soluble Mn from Mn accumulator plants. More than 70% of the total Mn was water-soluble. Results of electron probe x-ray microanalysis revealed that the portion of Mn which was water-insoluble was contained in the cell walls.

The critical Zn deficiency level for plants is from 10-20 μg g^{-1}, with the level of sufficiency being from 27-150 μg g^{-1}, and the threshold of toxicity at 100-400 μg g^{-1} (Kabata-Pendias and Pendias 1984). There are some reports that only a portion of the total level of zinc is physiologically active. Water-soluble Zn has been examined as an indication of the Zn status of the

plant. Cakmak and Marschner (1987) found water-soluble zinc to be a suitable indicator of zinc nutritional status in general. This was because of the close correlation between water-soluble zinc and visual Zn deficiency symptoms, levels of chlorophyll, superoxide dismutase, and membrane permeability. Rahimi and Schropp (1984) found water-soluble zinc to be an indicator of the Zn nutrient status of the plant because of its relationship to the activity of carbonic anhydrase. When one tries to make an accounting of all the zinc, there is a discrepancy between total and identifiable zinc (Hewitt 1983). Up to 60% of the plant zinc has been accounted for in its identifiable forms in proteins (Hewitt 1983).

E. Nutrient-Growth Models

1. Classical Plant Growth Curve

The classical plant nutrient-growth model is an empirical relationship described by a curve of diminishing returns. The curve may represent a relationship between plant growth and tissue nutrient concentrations (Figure 3), between plant growth and either soil nutrient concentrations or fertilizer additions. The curve consists of four zones: deficient (A-B), sufficient (B-C), luxury (C-D) and toxicity (D-E). The deficient zone may be distinguished from the adequate zone by the critical nutrient

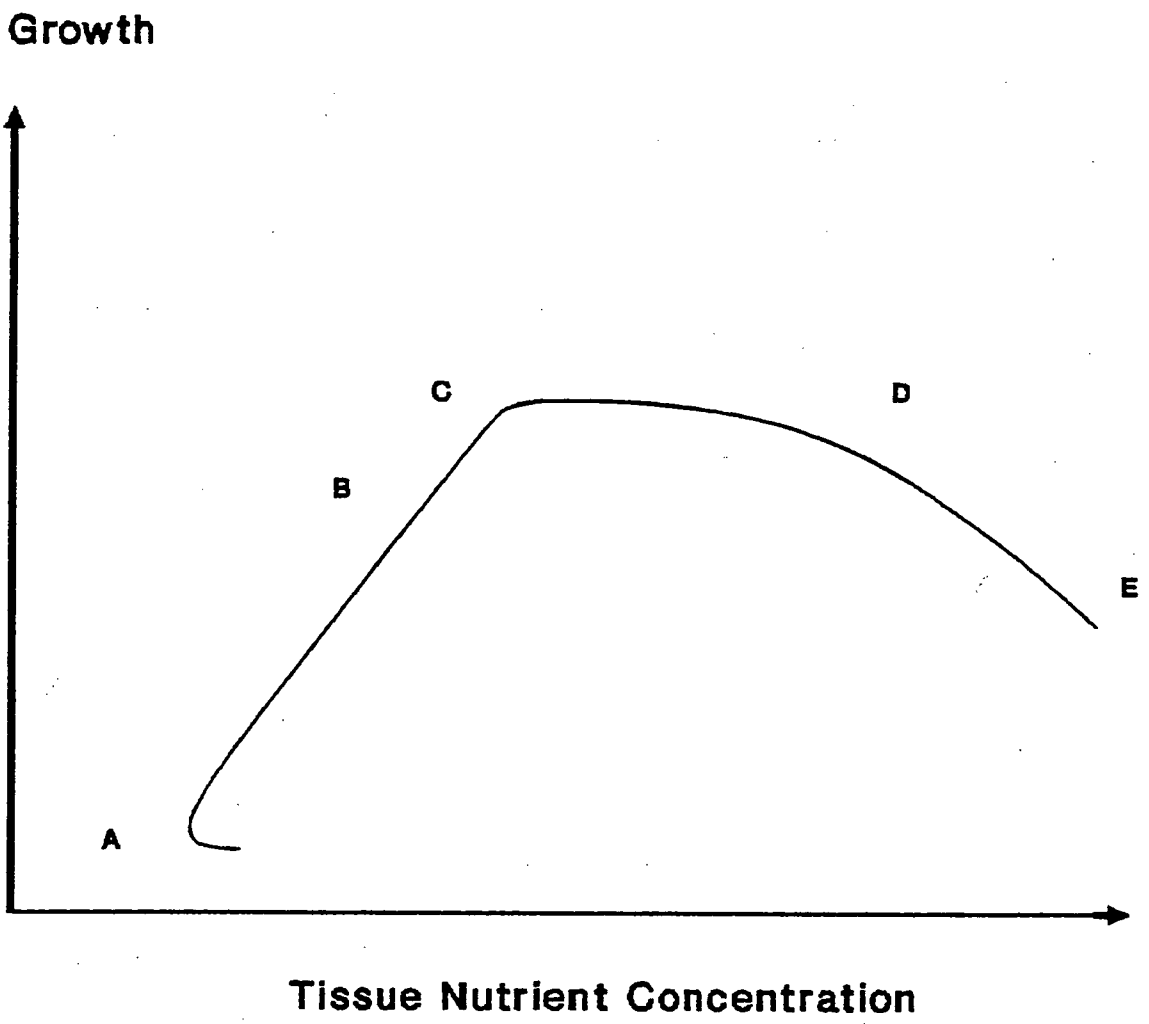


Figure 3. Relationship between plant growth and tissue nutrient concentrations.

concentration. This is the nutrient concentration that corresponds to 90% of maximum yield. Other modifications of the curve by Dow and Roberts (1982) have a critical nutrient range rather than a critical nutrient concentration (B-C). This relationship has been expressed mathematically using the Mitscherlich model, quadratic and exponential models, and inverse polynomials and hyperbolic models (Walworth and Sumner 1988).

This traditional model of plant growth-nutrient relationships has certain theoretical problems. Firstly, this type of relationship is empirical and does not lead to results of general application (Landsberg 1986). From the growth curve one attempts to define critical foliar nutrient concentrations by establishing relationships between final yield, or growth increment over a period, and nutrient concentration in the foliage, measured at the end of that period. These lead to a second problem in that the results are highly variable because the tissue nutrient status (x) at any time (t) affects the growth rate at that time (dy/dt) (Equation 1) (Landsberg 1986).

$$dy/dt = f(x) \qquad (1)$$

where: y = growth

t = time

x = nutrient concentration

2. Ingestad's Nutrient Flux Density Model

An alternative model of nutrition and growth, the nutrient flux density model has been formulated and demonstrated by Ingestad (Figure 4). The basic premise of the model is that it is the rate of nutrient supply to the roots or relative addition rate (R_A) (amount of nutrient added per unit of time and unit of nutrient present in the plant) which is the driving variable of growth within the sub-optimum range up to and including the optimum. Since plant growth is exponential with time in the sub-optimum range, a nutrient alone or nutrients in fixed proportions must be supplied in exponentially increasing amounts (relative addition rate R_A) corresponding to the exponential growth of the plant (R_G) (equation 2), and therefore the relative nutrient uptake rate (R_U) will be proportional to R_A and R_G (Ingestad and Lund 1986).

$$R_G = cR_A \qquad (2)$$

where: $R_G = 1/W(dW/dt)$

$R_A = 1/W(dM/dt)$

c = constant of proportionality

W = total plant mass

M = nutrient concentration

t = time

Under field conditions the nutrient flux density (amount of nutrients available per unit of time and unit of area) corresponds to R_A (Ingestad 1987). The nutrient flux density may be regarded as nutrient flow which enters the plant, similar to energy flow (Ingestad *et al.* 1981). A constant relative growth rate and constant internal nutrient concentration can only be maintained where the nutrient supply to the roots increases in proportion to the relative growth.

The curve (Figure 4) is not continuous like the classical response curve but consists of a sub-optimum range (the straight line below the saturation point) which is related to R_A, and a supra-optimum range (the curve to the right of the saturation point) (Ingestad 1982). In the classical response model where a constant amount of nutrients is added per unit of time the amount added becomes uptake restricting. This is due to the fact that the added amounts become less and less sufficient in relation to the requirements of both the internal concentration and relative growth. There are two consequences. Firstly, the deficiency level is overestimated because the classical response curve is the result of changing internal nutrient state. Therefore, plants can be grown having much lower tissue concentrations when steady state nutrition is maintained. Secondly, the potential maximum growth is underestimated using the classical response curve because of insufficient nutrient addition rates to maintain the growth rate. Deficiency symptoms occur during the lag phase when the growth rate of a plant is adjusting from a higher to a

lower R_A resulting in a decrease in the internal nutrient concentration. The symptoms disappear once a new steady state is formed between R_A and R_o resulting in a stable internal nutrient concentration (Ingestad 1982). At the optimum R_A the nutrient requirement is saturated and a further increase of R_A does not increase the R_o. The external nutrient concentration increases because the R_A is not matched by a corresponding increase in the R_u (Ingestad 1982). It is suggested that steady state nutrition is the characteristic situation under natural conditions because growth adjusts to the nutritional resources of the site (Ingestad 1982).

Agren (1985) has put forth the nutrient productivity model in which the relative growth rate is proportional to the amount of a nutrient in the plant with the nutrient productivity being the proportionality factor. The nutrient productivity is a constant for a given species under fixed environmental conditions (Agren 1985). Agren (1988) has produced a single formulation which relates growth to the content of several nutrients. The plant growth rate is proportional to the nutrient content minus a given minimum concentration of the nutrient (Equation 3). The proportionality factor, the nutrient productivity, and the minimum concentration are species-specific.

$$R_o = P_n\{\min(c_n, c_{n,opt}) - c_{n,min}\} \quad (3)$$

$$= P_n N$$

where: P_n is nutrient productivity

$$= (1/N)(dW/dt)$$

$= (1/N)(R_G)$

R_G is plant growth rate

$= dW/dt$

$c_{n,min}$ is the minimum concentration of a nutrient that must be present before any growth is realized.

$c_{n,opt}$ is the upper concentration of a nutrient above which no further growth response is obtained.

c_n is nutrient concentration

$N = \{\min(c_n, c_{n,opt}) - c_{n,min}\}$

N is the optimum concentration

1/N is nutrient efficiency

F. Diagnosis of Nutrient Status and Requirements

1. Plant and Soil Analysis

The diagnosis of the nutritional status of trees may be performed in several ways through plant analysis or soil analysis.

The aim of diagnostic plant analysis is to use some characteristic of the plant which is reflective of plant nutrient status. Plant analysis may involve examination of visual symptoms of deficiencies or toxicities. However, it would be desirable to make a diagnosis before the nutritional problem has manifested itself morphologically. Other methods of plant

analysis include chemical analysis of the foliage, buds, phloem, xylem sap, wood, bark, roots, and litter.

Diagnostic soil nutrient analysis commonly attempts to use chemical extractants to remove that fraction of nutrients which may be plant available. An advantage of soil analysis is that the nutrient status of the soil could be determined prior to plantation establishment (Mead 1984). However, this method requires the calibration of the extracted nutrient levels with plant nutrition and growth after determining the chemical extracting solution which can give a useful index of nutrient availability.

2. Foliar Analysis

a. Background

In this study foliar analysis has been used as a diagnostic tool. Several reviews of foliar analysis have been prepared explaining its theory, sampling, interpretation and limitations. These have been by van den Driessche (1974), Lavender (1970), Everard (1973), Walworth and Sumner (1988), Bates (1971), and Ballard and Carter (1986). Pioneering work in foliar analysis was done by Lundegardh (1951, 1947, 1943), Chapman (1941), Thomas (1937, 1945), Thomas and Mack (1941, 1944), Ulrich (1943), Moser (1940) and Scarseth (1943).

b. Physiological and Empirical Basis of Foliar Analysis

The aim of diagnostic foliar analysis calibration is to determine the relationship between plant performance and foliar composition, thus enabling the use of the latter as an index of the nutrient status of a plant. It does not reveal anything about why the plant may have a deficiency, sufficiency or toxicity of a nutrient. There is both a physiological and an empirical basis for foliar analysis. Figure 5 presents a somewhat mechanistic model of tree growth based on physical/physiological processes. It illustrates the relationship between plant nutrient status, leaf area and tree growth. The amount of foliage is an important factor in determining the amount of solar radiation intercepted by the canopy. The amount of solar radiation intercepted in turn is an important factor affecting tree growth. Fertilization with a growth-limiting nutrient in a non-closed coniferous stand can be expected to increase growth by increasing needle biomass (leaf size, area, number), thus increasing the photosynthetic capacity (Linder and Rook 1984). Increases in leaf area after fertilization are related to increases in rates of photosynthesis per leaf area and stem wood production (Linder and Rook 1984). This has been found for Scots pine, Douglas-fir and *Pinus nigra* (Linder and Rook 1984). Vose and Allen (1988) found that leaf area index (LAI) increased up to 60% following N fertilization on two N-deficient sites and that

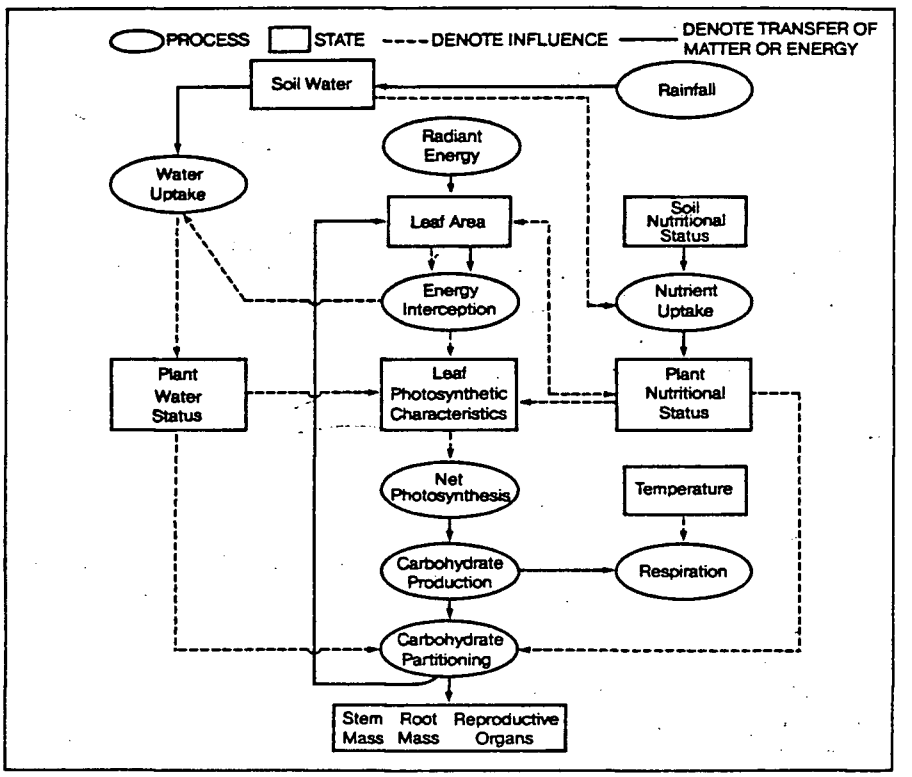


Figure 5. Schematic representation of a detailed mechanistic model of tree growth (from Landsberg (1986)).

stemwood production was positively related to LAI. Leyton (1956) detected an increase in the current leaf size which preceded an increase in height growth in the following growing season, induced by changes in soil fertility. Empirical relationships have also been found in terms of correlations between foliar parameters and subsequent tree growth (Table 4).

c. Plant Nutrient Diagnosis Using Foliar Analysis

i. Total Analysis with Single Nutrients

Foliar analysis involves evaluation of the foliage in terms of nutrient concentration, nutrient content, foliar mass, on a foliar area basis or using nutrient ratios. Using combinations of these parameters, several procedures have been used for nutrient diagnosis.

The relationship of growth to nutrition based on the classical plant growth curve is the first method which may be used for plant nutrient diagnosis. The first stage of diagnosis involves inferring the existence of one or more nutrient deficiencies or toxicities through the use of visual symptoms, by comparing foliar levels to those plants having superior growth, or by comparing foliar levels to critical levels for the species (applying an existing calibration) or for other species. Since the relationship in part of the deficient zone is quasi-linear, linear regression analysis has sometimes been applied for

Table 4. Linear correlations (r^2) between growth response and current needle N concentration (N%), N content (Nc) and unit dry weight (Wt) in the two years following fertilization. All stands were N deficient (from Timmer (1979)).

Response Parameter	Response Period (year)	Season	N%	Nc	Wt	Species
Leader Length	2	1	0.67	0.70	0.79	Balsam fir
		2	0.30	0.61	0.62	
Shoot Length	2	1	0.72	0.76	0.77	Balsam fir
		2	0.49	0.74	0.58	
Basal Area	2	1	0.69	0.76	0.76	Loblolly pine
		2	0.36	0.49	0.56	
Height	2	1	0.81	0.86	0.90	Loblolly pine
		2	0.82	0.87	0.88	
Basal Area	3	1	0.66	0.85	0.65	Jack pine
		2	0.31	0.36	0.69	
Volume	5	1	0.59	0.66	0.58	Douglas fir
		2	0.62	0.81	0.93	

diagnostic purposes. This has been extended to the use of multiple regression to infer whether one or more nutrients may be limiting growth. These regression procedures are applied to untreated stands. Those elements with the most significant positive correlation coefficients are considered to be deficient and those with zero and negative correlation coefficient are considered to be sufficient and toxic, respectively. Regression analysis can be problematic if a limiting nutrient is invariant, if the variability of a non-nutritional factor is unaccounted for, or if measured variables lie outside the range where the relationships are linear. Regression analysis has been used by Leyton and Armson (1955) in Scots pine, Leyton (1956, 1957) in Japanese larch, and Prusinkiewicz (1982) in Scots pine. White and Mead (1971) demonstrated the use of multivariate discriminant analysis of foliar nutrients to help distinguish between trees having green and yellow foliage.

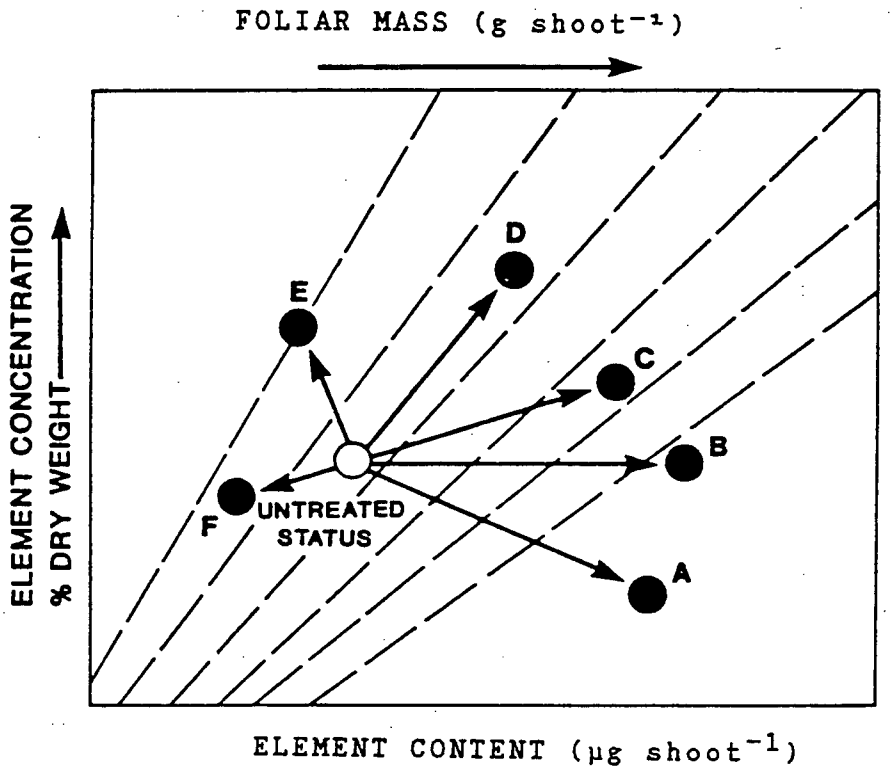
The second stage in nutrient diagnosis involves demonstration of the deficiency or toxicity. The demonstration of a deficiency or toxicity requires the application of the nutrients in question, through screening trials, and measurement of the subsequent nutrient uptake and growth. If a stand is in the deficient zone one would expect to obtain a response to a wide range of nutrient levels.

Regression (linear, multiple or curvilinear) can also be applied to analyse or interpret growth response to nutrient

treatments. Regression models have been produced in which foliar nutrient concentrations have been correlated with some function of growth such as height, site index, volume production, mean or periodic increment (Bevege 1984).

Interpretation of nutrient growth response data using foliar nutrient concentration and growth alone can be problematic due to the concentration and dilution effects. To overcome these problems a second method, vector analysis as described by Timmer and Stone (1978), simultaneously examines nutrient concentration, nutrient content and foliar mass. The vector analysis method and its interpretation are presented in Figure 6 with additional interpretations by Jarrell and Beverly presented in Table 5. Interpretation of growth response is based upon the direction and extent of the shift of the vector.

A third method, the boundary line model, uses data accumulated through field surveys to identify optimum foliar nutrient values (Figure 7). When a plant is close to its optimum nutrient value there often is very little relationship between nutrition and growth (Sumner 1978) which is represented by the boundary line model. In this situation other interacting factors cannot be controlled with the result being the data represented as an array of points. The scatter of points may be due to errors of measurement, variability of the biological material and the overall variation caused by other interacting factors (Webb



DIRECTION OF SHIFT	RESPONSE IN			CHANGE IN	
	NEEDLE WEIGHT	NUTRIENT		NUTRIENT STATUS	POSSIBLE DIAGNOSIS
		CONC.	CONTENT		
A	+	−	+	DILUTION	NON-LIMITING
B	+	0	+	UNCHANGED	NON-LIMITING
C	+	+	+	DEFICIENCY	LIMITING
D	0	+	+	LUXURY CONSUMPTION	NON-TOXIC
E	−	++	±	EXCESS	TOXIC
F	−	−	−	EXCESS	ANTAGONISTIC

Figure 6. Vector method for the interpretation of nutrient-growth response data using nutrient concentration, nutrient content and dry mass of needles (from Timmer and Stone 1978)).

Table 5. General representation of changes in total content, yield and concentration as affected by imposed treatments. An increase is represented by (I), a decrease by (D) and no change by (0) (From Jarrell and Beverly (1981).

Case	Change in			Comments
	Content	Yield	Concentration	
1	I	I	I	Synergism
2	I	I	0	
3	I	I	D	Dilution
4	I	0	I	Synergism
5	I	D	I	Concentration
6	0	0	0	No Response
7	D	I	D	Dilution
8	D	0	D	Antagonism
9	D	D	I	Concentration
10	D	D	0	
11	D	D	D	Antagonism

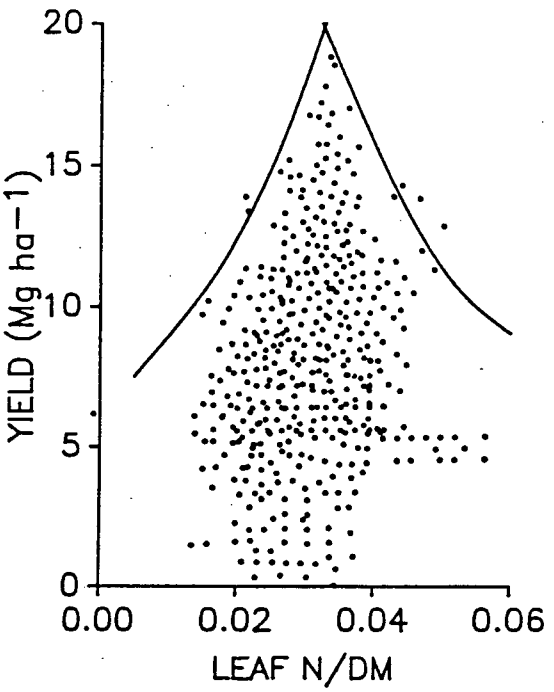


Figure 7. An example of a boundary line confining the data representing over 8,000 data points of maize yield versus leaf nitrogen:dry matter (N:DM)(g kg^{-1}) (from Walworth and Sumner (1988)).

1972). This method sets about consciously varying the controllable growth factors as much as possible by collecting a bank of observations that represent the variability encountered in the real world (Walworth *et al.* 1986). Therefore, the data actually represent different response curves from single-factor experiments as in Figure 8. A response curve from a single-factor experiement may follow any one of the curves depending on the degree of yield limitation exerted by other factors. Curve fitting may be used to fit a model which describes the boundary line response surface. A point on the boundary line represents the maximum attainable yield at a given foliar concentration under a given set of conditions (Walworth and Sumner 1988). This is not the same as the maximum yield attainable where all growth factors are optimal (Sumner 1978).

ii. Total Analysis using Nutrient Balances

A number of other methods take nutrient balance into account. The nutrient-element balance concept was introduced by Shear *et al.* (1946). Sumner (1978) used the boundary line model with nutrient ratios. He then considered a number of ratios simultaneously. By combining the information obtained from each ratio, the order in which the plant requires these nutrients is obtained.

Prevot's factorial method uses factorial experiments to

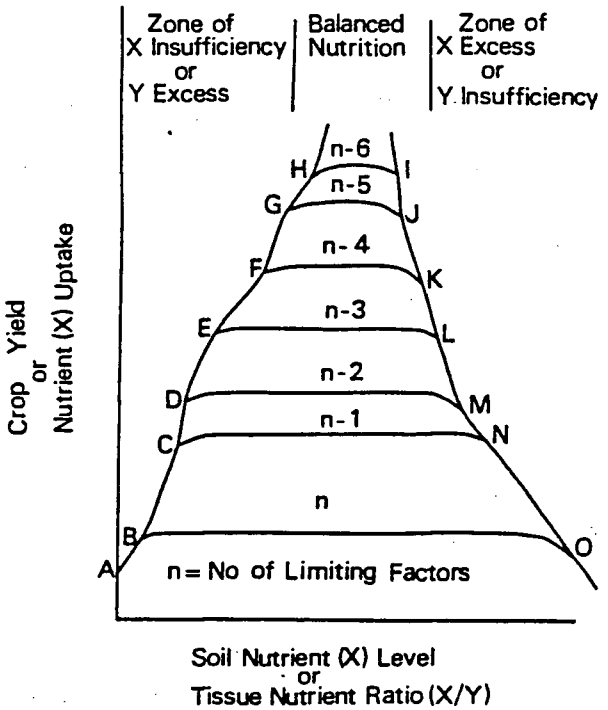


Figure 8. Diagrammatic representation of crop response to a number of limiting factors (from Walworth and Sumner (1988)).

study and calibrate increasing levels of one or more factors while all other conditions are kept constant. From these calibrations one is able to determine the relative proportions of interacting nutrients which would result in balanced nutrition.

Kenworthy's balance indices (Walworth and Sumner 1988) are based on foliar optima generated by averaging tissue values of healthy plants gathered from survey data. These values are specific to the stage of growth and position of the sampled foliage. The results from analysis of a sample are compared to the norms and also weighted using coefficients of variation which represent the normal variations of the standard values. This is done using nutrient indices which are calculated as follows.

$$\text{Balance Index} = (x/s)(100 + (1 - (x/s))(cv) \quad \text{when } x < s$$

$$\text{Balance Index} = (x/s)(100 - (1 - (x/s))(cv) \quad \text{when } x > s$$

where x = nutrient concentration
s = standard (optimum) value
cv = coefficient of variation (in percent) of s

Nutrients are then ranked in order of requirement with the nutrient having the lowest index being the most required.

The Moller-Nielsen Technique attempts to overcome problems of physiological age and nutrient interactions. Four steps are

involved. The first step is the production of response curves from nutrient response experiments. With the aid of these curves individual plant samples are corrected back to a standard plant mass. In the second step, standard nutrient values are developed from factorial experiments using the boundary line method to determine optimal concentrations. The plant samples are then compared to the boundary line values and the maximum attainable yield is determined. The most limiting nutrient is the one with the lowest maximum yield. In the third step, boundary line curves for plots of interacting nutrients are used to determine the optimum levels of other nutrients at the existing level of the most-limiting nutrient. The final predicted yield is determined by estimating the yield reduction due to each nutrient.

The DRIS method (Diagnostic and Recommendation Integrated System) (Walworth and Sumner 1988) attempts to overcome problems associated with plant age, nutrient interactions, and foliar optima determination. Foliar optima are calculated by averaging nutrient levels from healthy or high yielding plants. The deviations from the mean optimum value are estimated by the coefficients of variation of the high yielding plants. Indices are then calculated for each nutrient using nutrient ratios in the following equations.

$$A \text{ index} = (f(A/B) + f(A/C) + f(A/D) + \ldots + f(A/N))/z$$

where $f(A/B) = (((A/B)/(a/b))-1)(1000/cv)$

when $(A/B) > (a/b)$

where $f(A/B) = (1-((a/b)/(A/B)))(1000/cv)$

when $(A/B) < (a/b)$

A/B is the value of the ratio of the two elements in the tissue under diagnosis

a/b is the value of the corresponding norm

z is the number of functions

cv is the coefficient of variation of the norm

The magnitude of each nutrient index represents the relative excess (positive value) or deficiency (negative value) of the nutrient in the tissue.

Ingestad (1979) developed a method using nutrient balance. Nitrogen is expressed as 100 and all other nutrients are expressed relative to nitrogen. A set of ratios was developed for macronutrients in hemlock, and for micronutrients in some conifers of the family *Pinaceae* (Ingestad 1979).

iii. Analysis of Separate Fractions

Foliar concentrations in themselves do not necessarily indicate anything about the physiological requirements of a nutrient. With wild plants, nutrient levels may serve both a

physiological as well as an ecological function in the plant's strategy, as in accumulator plants. Total levels may not necessarily be reflective of the physiological nutrition of the plant because of compartmentation of nutrients or differences in the distribution of nutrients among chemical fractions. Therefore, it would be desirable to measure the physiologically active fraction of a nutrient in order to diagnose the nutrient status of a plant and correlate that nutrient concentration with biochemical and physiological activities. The "functional nutrient requirement" of a plant is "the minimal nutrient concentration which can sustain its metabolic function at rates which do not limit growth" (Ohki 1987).

These methods involve isolation and analysis of separate cellular components, analysis of different chemical fractions, enzyme analysis, the measurement of physiological processes, the use of extractants, *in situ* analysis, and the use of cell cultures. The methods which have been used for Zn and Mn will be discussed.

Memon and Yatazawa (1984) isolated different cellular components of Mn accumulator plants using differential centrifugation. The cell wall material, chloroplasts, mitochondria, ribosomes, and vacuolar contents were separated using this method.

Enzyme activity has been used as a method to diagnose nutrient status. The activity of the Mn isozyme of superoxide dismutase (Mn-SOD) in pea leaves was directly related to the Mn nutrient levels (del Rio *et al.* 1978). It was suggested that this isozyme could be an indicator of the biologically active Mn involved in cell metabolism (del Rio *et al.* 1978). The activity of IAA oxidase was found to increase in cotton with Mn toxicity (Morgan *et al.* 1966). In bean leaves, Mn toxicity increased the activities of isocitric dehydrogenase and malic enzyme (Anderson and Evans 1956). The activity of ribonuclease (RNAase) was found to be a sensitive, reliable and better index for detecting Zn deficiency in rice and maize than Zn concentration (Dwivedi and Takkar 1974). There was an inverse relationship between Zn supply and RNAase activity. Carbonic anhydrase activity is related to the Zn nutrient level. A shortage in Zn supply to spinach drastically reduced carbonic anhydrase levels (Randall and Bouma 1973). Carbonic anhydrase activity from foliage was used to detect Zn deficiencies in pecan (Snir 1983), and in maize (Gibson and Leece 1981). Rahimi and Schropp (1984) also used carbonic anhydrase activity as an indication of the Zn nutritional status of maize, millet, tobacco, sugar beet and grape. The activity of SOD which was a Cu-Zn isozyme in cotton leaves was used as an indicator of Zn status (Cakmak and Marschner 1987).

Water-soluble Zn from foliage has been found to be a suitable indicator of Zn nutritional status. Cakmak and

Marschner (1987) found the concentration of water-soluble Zn in leaves to be closely correlated with visual Zn deficiency symptoms and superoxide dismutase in cotton. In orange trees, visual Zn deficiency symptoms in leaves were closely related to the concentration of water-soluble Zn (Cakmak and Marschner 1987). This supports the work of Rahimi and Schropp (1984) who also found that water-soluble Zn was a better indicator of Zn nutritional status than was total Zn. This was due to the direct relationship between water-soluble Zn and the activity of carbonic anhydrase.

The possibility of quantifying the levels of nutrients in plants *in situ* and examining their distribution has been demonstrated using X-ray microanalysis. The first system which has been used is electron probe X-ray microanalysis (EPMA). In this system, electrons excite atoms in a sample, resulting in the production of characteristic X-ray patterns which can be measured. A scanning electron microscope is used in conjunction with the electron microprobe. A description of the instrument and its application in biology has been given by Hall (1979). EPMA has been used by Memon *et al.* (1980) and Memon and Yatazawa (1982) looking at Mn in Mn-accumulators, by Horiguchi and Morita (1987) who looked at Mn in leaves of barley, and for the study of Zn accumulation in the roots of *Betula* (Denny and Wilkins (1987), and in the roots of *Deschampsia caespitosa* (Van Steveninck *et al.* 1987).

A second instrument which has begun to be used in plant research for *in situ* analysis is the scanning proton microprobe (SPM). A description of the instrument and its use in biological research has been reviewed by Legge and Mazzolini (1980), Legge (1982), Enderer (1982), Legge *et al.* (1982), Legge (1980), and Legge *et al.* (1979). This combines a scanning mode with a proton microprobe which utilizes proton-induced X-ray emission (PIXE) (Legge *et al.* 1979). SPM has enhanced sensitivity compared to EPMA-SEM because the background radiation which is produced (Bremsstrahlung) is orders of magnitude lower (Mazzolini *et al.* 1985). Consequently, this allows one to detect elements in a sample down to 1 μg g^{-1} (Legge *et al.* 1979). The SPM instrument could also be used to detect and measure isotopes using nuclear scattering (Legge *et al.* 1979). The application of the PIXE in plant nutrition studies has been demonstrated by Mazzolini *et al.* (1985) using wheat seeds, and in the foliage of *Eucalyptus obliqua* (Mazzolini *et al.* 1982). The instrument allows one to produce a quantitative elemental analysis of different tissues, as well as producing elemental maps showing the distribution of the elements in the tissue.

G. Some Conclusions

Hemlock is a species which may be classified as a calcifuge plant because hemlock has in common with this group the following characteristics. These plants favor acid soils and they have ammonium-based nitrogen nutrition. Plants which

utilize ammonium-nitrogen can in fact promote acidification of the surrounding soil. Acid soil characteristically has high plant available levels of aluminium and manganese. Hemlock thrives under these acid soil conditions. Hemlock also favors organic rich media which may keep Mn in the reduced state.

Tissue nutrient concentrations in a plant are not necessarily reflective of the physiological requirements for that particular nutrient. This is evident for Mn where there is abundant evidence that Mn levels in plants are not reflective of physiological requirements. Nutrients not only have a physiological role but may also play a role in the ecological strategy of a plant. There are three types of ecological strategies of plant nutrition: the accumulator, the excluder and the indicator.

Western hemlock has lower foliar Zn levels and higher foliar Mn levels as compared to conifers in other genera in B.C. and the U.S. Pacific Northwest. Whether these differences represent actual different physiological requirements of species, or are an indication of a Zn deficiency and Mn toxicity in hemlock requires some investigation, due to the possibility of increasing productivity through alleviation of these stresses.

A review of the literature leads to some conclusions useful in selecting research methods. The classical method (a single application of a fertilizer dosage) was used due to the

greater ease in establishing and carrying out the treatments. Diagnosis of foliar data was performed using the vector method. Investigation of the physiological active fractions of Zn and Mn were made using analyses of extractions and of foliar cellular fractions.

CHAPTER 3. METHODS AND MATERIALS

A. Site Description

1. Location of Study Areas

Fertilizer screening trials were established in five different stands in the Vancouver Forest Region in Coastal British Columbia (Figure 9). Information on the location of the stands is given in Appendix A. Three of the stands are located at the University of British Columbia Research Forest, referred to as sites 1, 2 and 4. The remaining two stands are located at Chipmunk Creek (Chilliwack Provincial Forest) and Mission Tree Farm referred to as sites 3 and 5 respectively. Sites were selected on the basis of their proximity to Vancouver, supporting a uniform stand of trees and consisting of enough trees to support a fertilizer trial. Sites were not selected on the basis of any specific site characteristic.

Sites were classified according to Klinka *et al.* (1984). Site 1 is located in the Pacific Ranges Drier Maritime Coastal Western Hemlock biogeoclimatic subzone. According to Klinka *et al.* (1984), the climate is characterized by 57 mm of precipitation in the driest month, warm summers (17.6°C warmest month mean, 33.3°C absolute maximum), mild winters, no month with a mean minimum temperature below 0°C, a mean annual precipitation

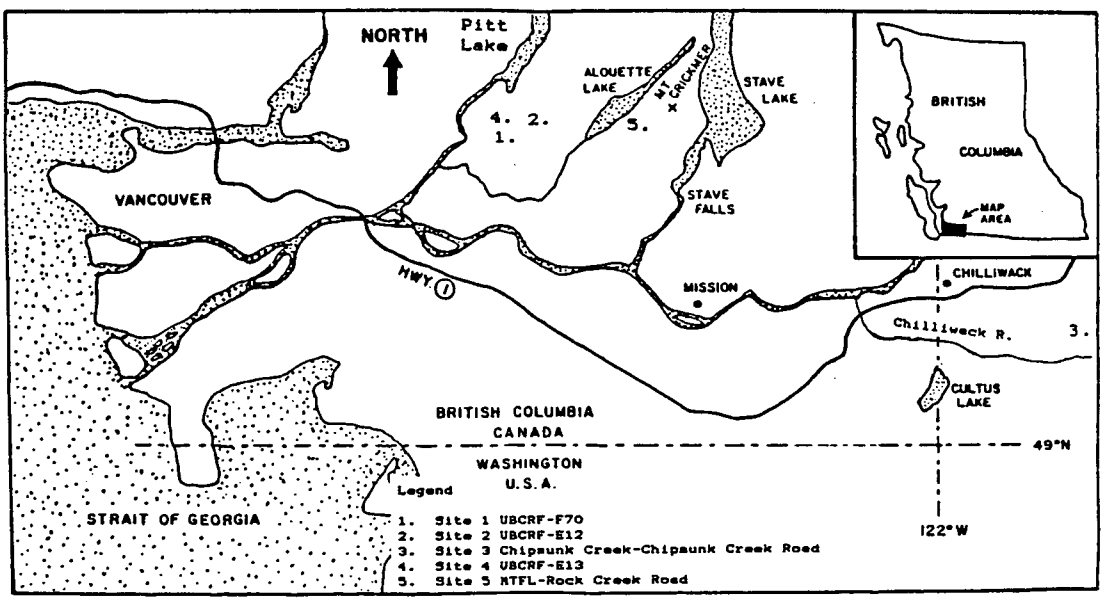


Figure 9. Location of the study plots in the Lower Mainland.

of 1860 mm, with 5% as snow, and a range between minimum winter and maximum summer means of 15.2°C. Sites 2, 3, 4, and 5 are located in the Windward Montane Maritime Wetter Coastal Western Hemlock biogeoclimatic subzone. According to Klinka *et al.* (1984), the characteristic climate in this zone is intermediate between the Windward Submontane Maritime Coastal Western Hemlock zone and the Maritime Forested Mountain Hemlock zone. A climate station is not located in this variant; therefore there are no climate data.

2. Stand Characteristics

Sites 1, 2, 3, 4, and 5 were composed of young uneven-aged western hemlock of natural origin. In 1987, the estimated age of the trees ranged from about 10 to 20 years old and they were "free-growing". These stands were characteristic of western hemlock, having clumps of trees with the spaces between clumps being filled in by individual trees. Crown closure had not yet occurred in these stands. Further descriptions on the species composition of these stands is presented in Appendix A.

3. Soil Characteristics

Soil profiles of the five sites were described according to the Canadian system of soil classification (Canada Soil Survey Committee 1978). The soils at sites 1 and 3 were classified as Orthic Humo-Ferric Podzols, at site 2 as a Rego Gleysol, at site

4 as a Duric Humo-Ferric Podzol, and at site 5 as an Orthic Ferro-Humic Podzol.

The chemical characteristics of the soil profiles are presented in Table 6 and other soil characteristics in Appendix A. The methods used for chemical analysis of the soils are described in section G.

B. Experimental Design

The experiment was carried out using single trees as plots (Viro 1967) in a randomized block design with the blocks acting as replicates. There were ten replicates per treatment at each of the sites. The number, types and year of treatments varied between sites (Table 7).

On each site, trees were selected according to the guidelines given by Ballard and Carter (1986). Dominant and codominant trees were selected which were devoid of deformities, insect and diseases, and cone crops. The minimum distance between selected trees was six meters. There was a compromise between having trees close enough to decrease the effect of site variability, but far enough to prevent contamination from adjacent treatments. The trees were identified with plastic tags.

Table 6. Chemical characteristics of the soil profiles.

SITE	HORIZON	DEPTH	Zn	Mn	K	Ca	Mg	P	Al
		-cm-	----------μg g^{-1}----------					μg g^{-1}	
1	1 LFH	3-0	11.8	67.2	221	718	57.0	33	1891
1	2 Bf	0-42	1.4	7.4	43	62	6.8	0	2084
1	3 C	42-52	0.9	2.0	12	48	7.3	0	2148
2	1 LFH	15-0	67.0	72.0	295	2566	528.0	131	814
2	2 Cg	0-18	7.8	5.7	129	619	77.0	52	991
3	1 L(f)	2-0	2.1	2.1	74	115	35.0	0	2052
3	2 Ae	0-5	1.1	9.3	105	65	16.0	0	2114
3	3 Bf	5-20	0.5	5.0	255	16	3.0	0	2102
3	4 Bf	20-35	0.3	3.0	51	8	1.4	0	2099
3	5 Bf	35-60	0.3	3.0	0	6	1.0	0	1894
3	6 Cg	60+	0.5	6.0	23	5	0.8	43	1710
4	1 LFH	20-0	39.0	129.0	404	1782	224.0	51	1049
4	2 Ae	0-10	0.8	3.2	37	96	3.0	0	2022
4	3 Bf	10-46	1.9	9.7	0	26	5.6	0	2079
4	4 BCc	46-72	2.2	20.0	0	47	6.4	2	2039
4	5 C	72+	0.5	0.5	48	24	2.2	0	2075
5	1 LFH	25-0	30.3	51.7	212	2471	234.0	26	1412
5	2 Bhf	3-6	3.6	2.4	86	369	52.0	6	1869
5	3 Bhf	6-18	0.6	0.4	0	45	8.3	0	2123
5	4 Bf	18-27	0.7	0.5	45	53	10.0	0	2141
5	5 Bf	27-35	1.1	1.2	78	80	17.0	0	2054
5	6 C	35+	0.3	0.4	57	25	2.3	0	2123

SITE	HORIZON	DEPTH	Fe	Cu	B	Extr. N	Total N	CEC	pH	pH
		---cm--	----------μg g^{-1}-------			--mg kg^{-1}-	-mg kg^{-1}-	meq 100 g^{-1}	H_2O	$CaCl_2$
1	1 LFH	3-0	249	1.5	0	0.006	0.062	6.0	3.9	3.4
1	2 Bf	0-42	48	1.1	0	0.003	0.195	12.1	4.9	4.4
1	3 C	42-52	204	0.9	0	0.005	0.213	21.7	4.5	4.1
2	1 LFH	15-0	174	1.6	0	0.015	0.907	16.0	3.6	3.2
2	2 Cg	0-18	157	1.0	0	0.003	0.218	14.0	3.7	3.1
3	1 L(f)	2-0	381	0.4	0.2	0.003	0.076	20.3	3.8	3.4
3	2 Ae	0-5	106	1.0	0.08	0.006	0.039	12.5	4.8	4.2
3	3 Bf	5-20	106	0.7	0.06	0.003	0.076	9.9	4.8	4.4
3	4 Bf	20-35	51	0.7	0.05	0.002	0.057	4.7	4.9	4.6
3	5 Bf	35-60	71	0.5	0.04	0.003	0.028	1.0	4.8	4.6
3	6 Cg	60+	40	0.4	0.03	0.004	0.016	0	4.8	4.8
4	1 LFH	20-0	238	2.2	0	0.016	0.709	12.1	3.6	3.3
4	2 Ae	0-10	59	1.1	0	0.002	0.076	5.1	5.0	4.6
4	3 Bf	10-46	125	1.1	0.08	0.002	0.04	0.8	4.7	4.4
4	4 BCc	46-72	130	0.8	0.07	0.004	0.256	9.5	4.4	3.9
4	5 C	72+	68	1.0	0.03	0.005	0.072	1.9	4.4	4.0
5	1 LFH	25-0	223	2.2	0	0.008	0.755	6.9	4.2	3.6
5	2 Bhf	3-6	135	2.2	0.08	0.007	0.074	24.0	4.3	3.7
5	3 Bhf	6-18	44	0.8	0.03	0.003	0.233	21.2	4.6	4.1
5	4 Bf	18-27	67	1.0	0.02	0.005	0.317	13.0	4.6	4.1
5	5 Bf	27-35	88	1.0	0.001	0.005	0.259	15.1	4.5	4.0
5	6 C	35+	20	1.2	0.1	0.001	0.09	5.6	4.9	4.5

Table 7. Treatment-levels used in the fertilizer trials.

Sites 1, 2, and 3

Application Method	Number		Rate
Soil			
	1	$ZnSO_4$	10 kg Zn ha^{-1}
	2	$ZnSO_4$	50 kg Zn ha^{-1}
	3	$MnSO_4$	200 kg Mn ha^{-1}
	9	Na_2SO_4	5 kg S ha^{-1}
	10	Na_2SO_4	25 kg S ha^{-1}
	12	Control	
Foliar			
	4	$ZnSO_4$	360 mg Zn L^{-1}
	5	$ZnSO_4$	3600 mg Zn L^{-1}
	6	$MnSO_4$	2730 mg Mn L^{-1}
	7	Na_2SO_4	177 mg S L^{-1}
	8	Na_2SO_4	1770 mg S L^{-1}
	11	Control	Demin H_2O + Surfactant

Table 7 (continued)

Sites 4 and 5

Soil			
	1	$ZnSO_4$	50 kg Zn ha^{-1}
	2	$ZnSO_4$	100 kg Zn ha^{-1}
	3	$ZnSO_4$	200 kg Zn ha^{-1}
	4	$MnSO_4$	200 kg Mn ha^{-1}
	5	$MnSO_4$	400 kg Mn ha^{-1}
	6	$MnSO_4$	600 kg Mn ha^{-1}
	7	S	25 kg S ha^{-1}
	8	S	49 kg S ha^{-1}
	9	S	98 kg S ha^{-1}
	10	S	118 kg S ha^{-1}
	11	S	235 kg S ha^{-1}
	12	S	352 kg S ha^{-1}
Foliar			
	13	$ZnSO_4$	360 mg Zn L^{-1}
	14	$ZnSO_4$	1800 mg Zn L^{-1}
	15	$ZnSO_4$	2700 mg Zn L^{-1}
	16	$MnSO_4$	2730 mg Mn L^{-1}
	17	$MnSO_4$	4095 mg Mn L^{-1}
	18	Na_2SO_4	177 mg S L^{-1}
	19	Na_2SO_4	883 mg S L^{-1}
	20	Na_2SO_4	1325 mg S L^{-1}
	21	Na_2SO_4	1605 mg S L^{-1}
	22	Na_2SO_4	2408 mg S L^{-1}
	24	Control	Demin H_2O + Surfactant

Table 7 (concluded)

Soil			
	23	"Complete -Zn -Mn"	
		Urea	100 kg N ha^{-1}
		Triple Super Phosphate	150 kg P ha^{-1}
		K_2SO_4	22 kg K ha^{-1}
		$MgSO_4$	18 kg Mg ha^{-1}
		$CaSO_4$	11 kg Ca ha^{-1}
		Degra-sul	22 kg S ha^{-1}
		$CuSO_4$	4.4 kg Cu ha^{-1}
		Solubor	0.9 kg B ha^{-1}
		$FeSO_4$	11 kg Fe ha^{-1}
	25	Control	

C. Fertilizer Treatments

Fertilization can be used as a tool to diagnose the nutrient status of a stand. The purpose is to increase the supply of the nutrient of interest. Then, using the dose-response curve and vector analysis, one can evaluate the nutrient status of the stand.

The fertilizers used were zinc sulphate, manganese sulphate, sodium sulphate, elemental sulphur (as Degra-sul), urea, triple super phosphate, potassium sulphate, calcium sulphate, magnesium sulphate, iron sulphate, copper sulphate, and boron (as Solubor). The specific treatments used for each site are outlined in Table 7. There were a total of 120 trees in the trials on each of sites 1, 2, and 3, and 250 trees on each of sites 4 and 5. Fertilizer was applied in solid form to the soil and in solution as a spray to the foliage. The soil and foliar treatments were applied at different times. The soil treatments were applied in May, and the foliar treatments were applied at the beginning of July when there was a large amount of new growth.

For sites 1, 2 and 3, the trials were established in 1985. For sites 4, and 5 the trials were established in 1986. On sites 1, 2, and 3, sodium sulphate was used as the sulphur source for the foliar and soil treatments. On sites 4 and 5, elemental sulphur was used as the sulphur source for the soil treatments

and sodium sulphate for the foliar treatments. The "complete - Zn-Mn" treatment was applied only to soils, and only at sites 4 and 5.

The fertilizer solutions were prepared using demineralized water. A commercial detergent (trade name 'Joy') at a concentration of 0.5% by volume was added to the fertilizer solution as a surfactant to enhance nutrient absorption by the foliage. The fertilizer solutions were applied using a backpack plastic sprayer. The trees were sprayed until the solution began to drip from the canopy (approximately one litre per tree). In this way, if there were canopy size differences between trees, each unit area of foliage of each tree will have had the same rate of nutrient application.

D. Field Sampling

The sampling procedure was carried out according to the guidelines given by Ballard and Carter (1986). Foliage and shoot samples were collected from September 15-December 15 from sites 1 and 2 in 1985, from sites 1, 2, 3, 4, and 5 in 1986, and from sites 4 and 5 in 1987. For sites 4 and 5 only replicates 1-5 were sampled in 1986. All replicates were sampled in 1987. For all sites and sampling years both the current and previous year's foliage and shoot samples were collected using hand and pole pruners. Foliage was collected from the upper one-half to one-quarter of the crown but below the third whorl. A shoot sample

refers to the tip growth of a branch formed in the current year. Three shoot samples per tree were taken from the third whorl for growth analysis. For site 3, shoot samples were taken for the current year (1986) and the previous two years. For sites 4 and 5 in 1987, branch samples were collected from the third and fourth whorls from the top consisting of the previous three years of growth for replicates 6-10. This allowed for analysis of shoot increment for 1986 at whorl three for all 10 replicates. Foliage and branch samples were placed in plastic bags, transported to the laboratory, and stored in a cold room until further processing. Height increment measurements were taken in the fall of 1986 on sites 1, 2, and 3 for the previous three years of growth, and in 1987 on sites 4 and 5 also for the previous three years of growth. Height increment measurements were made to the nearest 0.5 cm. At the time of sampling trees of other species were also sampled. One codominant or dominant tree of other species occurring adjacent to the control hemlock tree per block was sampled.

Soil samples were collected from sites 1, 3, 4, and 5 in the fall of the year in which the fertilizer trial was established. Samples were collected from around each control hemlock tree at a distance of 30 cm from the base of the stem. Up to three samples were collected at intervals of 120° both of the forest floor and of the mineral soil down to 15 cm. These are identified as (T) for forest floor and (B) for mineral soil. Mineral soils were composited in the field.

A soil pit was excavated at each of the sites for a description of the profiles. In addition, soil samples were collected from each horizon for chemical analysis. Data from the soil pit were used only for descriptive purposes; no statistical interpretations were made from the soil pit data.

Foliage samples were separated into current and previous-year's portions. Shoot lengths of the current and the previous year's growth were measured to the nearest mm. Foliage was oven-dried at 70°C for 24 hours in paper bags. Growth measurements were made on the samples used for the shoot increment measurements. These were foliar mass per shoot to the nearest milligram, and number of needles per shoot. From these values the mass per needle, the needle mass per cm of shoot and the number of needles per cm of shoot were calculated. In addition, for the samples from the control western hemlock trees and samples of trees of other species, the mass of needles per 100 needles was also determined to the nearest milligram. Dry foliage samples to be used for chemical analysis were ground in a Braun type KSM-2 coffee grinder and stored in air-tight plastic containers.

The shoot samples gave a data base of 2,580 samples for both first and second year. Approximately 490 samples were used to measure first year nutrient response, and 860 in the second

year. There were approximately 860 height increment values for each of three years for the entire experiment.

E. Chemical Analysis

The wet digestion method of Parkinson and Allen (1975), slightly modified by Ballard (1981) was used for the determination of total N, P, K, Ca, Mg, Mn, Zn, and Al (Ballard and Carter 1986). N and P were measured on the original digest by colorimetric analysis for N (phenol-hypochlorite method) and P (unreduced vanadomolybdate complex), by the Technicon Autoanalyzer II. The elements K, Ca, Mg, Mn, Zn, and Al were measured using atomic absorption spectrophotometry (AA) (Perkin Elmer 306). For foliage from 1986 and 1987, the elements P, K, Ca, Mg, Mn, Zn, and Al were measured using Inductively Coupled Plasma Emission Spectroscopy (ICP) (Jarrell Ash AtomComp Series 1100) on the original digests. Details of the method are described in Appendix B1. The elements Fe and Cu were determined using a nitric acid digestion followed by atomic absorption spectrophotometry (Ballard and Carter 1986) (Appendix B2). Although there was a good agreement between the standard foliage samples using AA and ICP for Fe analysis using the Parkinson and Allen digest, there was poor agreement for the hemlock samples (Appendix C). Copper was found to be below the detection limits of the ICP using the Parkinson and Allen digests. The standards were used to construct equations in order to convert Zn and Mn concentrations measured on the AA in the first year (1985) to

equivalent values for the ICP (Appendix D). The equations were used in the retranslocation study to compare first year values measured on the AA to foliar values from the second year (1986) measured on the ICP. A comparison was made between AA and ICP for the National Bureau of Standard samples in the percentage recovery of Ca, Mg, K, Mn, Zn, and Al from foliage. This information is presented in Appendix E.

Boron was determined by dry ashing, followed by colorimetric analysis by the azomethine H method of Gaines and Mitchell (1979).

Total sulphur was determined using a Fisher Model 475 Sulphur Analyzer using the procedure described by Guthrie and Lowe (1984). Sulphate-sulphur was extracted from the foliage according to the procedure outlined in Appendix B3 and determined according to the method described by Kowalenko and Lowe (1972) (Appendix B3).

The idea that only a portion of the total level of an element may be physiologically active led to the need to determine active iron, and water-extractable zinc and manganese. The concept of a metabolically active fraction of iron was investigated by Oserkowsky (1933). His procedure for the determination of active iron, slightly modified by Ballard (1981), is described in Appendix B4. The physiologically available zinc was determined using a modified procedure of

Cakmak and Marschner (1987) described in Appendix B5. Extractable manganese was determined using a modified water extraction method of Memon and Yatazawa (1982) (Appendix B6).

The cellular fractions in foliage were separated using the method of Memon and Yatazawa (1984) slightly modified by this author described in Appendix F. Total zinc and manganese were determined in the different fractions using the Parkinson and Allen digestion. Three replicates were done for each sample. One experiment compared zinc and manganese levels in different fractions from the high foliar zinc treatment and the high soil manganese treatment. These samples were from current year's foliage in the first season following fertilization (1986) from site 5. The second experiment compared zinc and manganese levels in the foliage from different species using the current year's foliage of 1987 from site 5.

F. Scanning Electron Microprobe

In addition to comparing the nutrition of species using total nutrient levels, extractable levels and cellular fractions, an attempt was made to measure the nutrients in the foliage of different species *in situ*. This was done using electron probe microanalyzer (EPMA) with scanning electron microscopy (SEM). The tissue was fixed and prepared partly in the field and the laboratory according to the method described in Appendix G. The SEM-EPMA in the Department of Metallurgy at the University of

British Columbia was used. However, the resolution of EPMA is not fine enough to measure micronutrients in the range of $\mu g\ g^{-1}$. Alternatively, an attempt was made to have the analysis done on a scanning proton microprobe (SPM); however, the price of the analysis was cost-prohibitive.

G. Soil Sample Preparation and Analysis

The mineral soil and forest floor samples were air-dried at room temperature (22°C). The soil samples were sieved through a 2.0-mm stainless steel mesh sieve. Forest floor samples were ground in a Waring blender to pass a 20-mesh sieve.

The samples were analyzed for pH, cation exchange capacity, total N (TN), extractable NH_4^+ (EXTN), P, K, Ca, Mg, Mn, Zn, Cu, and Fe. Soil pH was measured in water and 0.01 M $CaCl_2$, using a glass electrode pH meter. The soil to solution dilution ratios were 1:2 for mineral soils and 1:8 for organic soils. Cation exchange capacity was determined using the sodium chloride method. This is an unbuffered solution enabling evaluation of the cation exchange capacity of the soil at its inherent pH. Total N was determined using semi-micro Kjeldahl digestion followed by colorimetric determination using the Autoanalyzer. Available NH_4^+ was assayed using an extraction in 2% K_2SO_4 followed by colorimetric determination using the Autoanalyzer. Available P, K, Ca, Mg, Mn, Zn, Cu, and Fe were

extracted using the Mehlich 3 soil test extractant (Mehlich 1984) as modified by Ballard (Appendix H). The extractants were analyzed by ICP.

H. Measurement of Fertilizer Response

Response to fertilization was measured using various growth parameters: height increment ratio, shoot length increment ratio, foliar mass per shoot and foliar nutrient concentration. Where there was a significant difference in foliar mass: foliar nutrient concentration, nutrient content per shoot and foliar mass per shoot were evaluated together as a measure of growth response using the vector method of Timmer and Stone (1978) (Figure 6). Results of the "complete-Zn-Mn" treatment were presented using vector analysis on a relative basis. This permits comparison of various elements together on one graph. The ascending order of elements along a relative unit weight line indicates the degree of deficiency for these elements (Timmer and Morrow 1984). Otherwise nutrient concentration was used alone. Interpretation of foliar nutrient concentrations was done using the summary of Ballard and Carter (1986) presented in Appendix I. Growth and nutrient responses were evaluated in terms of the statistical significance of the treatments from their respective controls.

Relative shoot increment and height increment were also used to evaluate growth response using the pretreatment increment method of Ballard and Majid (1985). The use of pretreatment increment allows adjustment for site as well as stand structure differences. Shoot growth as well as height growth response were expressed as the ratio of post-fertilization to pre-fertilization shoot and height increment.

Af/Bf compared with Ac/Bc

where: A = increment after fertilization

B = increment before fertilization

f = fertilized

c = control

The ratio Af/Bf is an index of fertilizer response as well as environmental effects, whereas Ac/Bc is an index of only environmental influence. Their difference provides a measurement of solely the treatment effect (Ballard and Majid 1985). Shoot length increment may sometimes be an indicator of future volume growth. Barker (1978) found a correlation (r=0.95) of the difference in shoot increment (between the fertilized and control in the previous year) with the difference in volume increment (between the fertilized and the control) in the current year.

I. Statistical Analysis

All statistical tests for significance were performed using both parametric and non-parametric methods. There were three groups of data subjected to statistical tests.

Because each fertilizer treatment had its own control, the null hypothesis of no treatment effect could be evaluated by parametric two-sample t-tests. If the differences between the variances were more than three-fold, the results were checked using the non-parametric Mann-Whitney test.

For the experiment involving comparison of foliar nutrient concentrations between species, the null hypothesis which was tested was that there were no differences in foliar nutrient concentrations between species. The null hypotheses was tested by a one-way analysis of variance. Differences between means were analyzed using the parametric Tukey multiple comparison test. The Tukey test allows for the unbalanced condition and is generally preferred to the other multiple range tests (Wilkinson 1988). If the variances were not homogeneous, as determined by Bartlett's test, the significance of difference between means was checked using the Kruskal-Wallis test as a non-parametric analysis of variance, and the Mann-Whitney test as a multiple comparison test. The probability table used for the Mann-Whitney test was adjusted using the Bonferroni procedure (Wilkinson 1988) to take into account the comparison of more than one pair of

means. Without such an adjustment, there would be increased probability of making a type I error when using a two-sample test for more than two means for reasons described by Zar (1984).

The data examining retranslocation in the foliage were analyzed using the parametric paired t-test and the non-parametric equivalent test, the Wilcoxon signed rank test.

Relationships between nutrients, between nutrients and growth, and between growth parameters were investigated using curvilinear regression methods. All t-tests, ANOVAs and regressions were evaluated at the 5% level of significance.

CHAPTER 4. RESULTS

A. COMPARATIVE NUTRITION

A comparison of nutrition was made among species occurring in the same stands (Table 8) to determine whether the nutritional characteristics of hemlock are species- or site-related.

1. Total Levels

Hemlock tends to have lower foliar Zn concentrations compared with Douglas-fir, amabilis fir and white pine. But it has higher foliar manganese concentrations compared to all the other species examined.

2. Extractable Zn and Mn

Although not examined in this study, it is inferred that substantially different concentrations of extractable nutrients in different species on the same site may be indicative of different physiological requirements. The water-soluble fraction of Zn has been shown to represent the physiologically active component by Rahimi and Shropp (1984) and Cakmak and Marschner (1987). Water-soluble Mn has been shown by Memon *et al.* (1980) to exclude the fraction of Mn bound in the cell wall and the latter may be physiologically inactive.

Table 8. Total foliar Zn and Mn, water-soluble Mn (AMN), and active Zn (AZN) for different species in the same stands. Within a column means with different letters are significantly different at the 5% level using ANOVA. Values are nutrient mass/dry matter in $\mu g\ g^{-1}$.

SITE 1 1986
MEAN

SPECIES	ZN	MN	AZN	AMN
Douglas-fir	22.3a	473a	19.0a	755a
Amabilis fir	17.2a	571a	11.5b	842ab
Hemlock	9.7b	1139b	8.8b	1218b

SITE 2-1986
MEAN

SPECIES	ZN	MN	AZN	AMN
Red cedar	12.6a	246a	4.3a	117a
White pine	29.0b	375a	20.7b	439a
Hemlock	11.1a	1932b	12.1c	1948b

SITE 3-1986
MEAN

SPECIES	ZN	MN
Amabilis fir	18.3a	916a
Hemlock	11.0b	1505b

SITE 4-1986
MEAN

SPECIES	ZN	MN
Douglas-fir	20.0a	713a
Amabilis fir	19.1a	735a
Hemlock	8.7b	1428b

SITE 5-1986
MEAN

SPECIES	ZN	MN
Douglas-fir	18.0a	472a
Amabilis fir	10.3b	220b
Hemlock	11.4b	1308c

SITE 4-1987
MEAN

SPECIES	ZN	MN
Douglas-fir	17.2a	515a
Amabilis fir	16.0	675a
Hemlock	11.7b	1545b

Site 5-1987
MEAN

SPECIES	ZN	MN
Douglas-fir	14.9	265a
Amabilis fir	17.2a	415b
Yellow cedar	11.9b	23a
Hemlock	11.8b	1243c

It was hypothesized that although different species may have different total foliar levels of a nutrient, the physiologically active fraction may be similar. Therefore, to examine this hypothesis the active Zn and water-soluble levels of Mn, and total foliar levels of Zn and Mn were compared among species on sites 1 and 2 (Table 8). On site 1, Douglas-fir and amabilis fir have higher total Zn than hemlock. Douglas-fir has higher active Zn than hemlock, but amabilis fir has similar levels of active Zn to hemlock. On site 2, white pine had higher total and active Zn than hemlock, which in turn had higher levels than red cedar. On site 1, hemlock had higher total and water-soluble Mn levels as compared to Douglas-fir and amabilis fir. On site 2 hemlock had higher total and water-soluble Mn concentrations than white pine and red cedar.

3. Cellular Fractions of Foliage

Levels of Zn and Mn were compared between control and treated samples, and between species. The data appear in Appendix J. It was hypothesized that although different species may have different total foliar levels of Zn and Mn the levels in the different cellular fractions which represent different physiological processes may be similar. The fractions identified by Memon (1984) were A, corresponding to cell wall and debris; B, chloroplasts; C, mitochondria; D, ribosomes; and E, vacuoles. In the present study, fractions A and B corresponded to Memon's A and B, C contained chloroplasts, D corresponded to the

mitochondrial fraction, and E contained the ribosomal and vacuolar fractions.

For Zn, the cell fractions from the high Zn treatment had higher levels of Zn than the control fractions (Figure 10). The situation was similar for Mn in that for all fractions the Mn treatment had the higher level of Mn compared to the control (Figure 11). In comparing Zn and Mn treatments accumulation occurred in two different fractions. Zn tended to accumulate in fraction D and Mn tended to accumulate in fraction E.

In a comparison of unfertilized trees, there was a difference among species for Mn concentrations in cellular fractions (Figure 12). Hemlock had higher Mn levels in fractions A, B, C, D and E. Yellow cedar had the lowest Mn levels of any species in fractions A, B, C, D and E.

There was a predominance for Zn to accumulate in fraction D for all species (Figure 13). Douglas-fir had the highest Zn concentration in the D fraction compared to the other species.

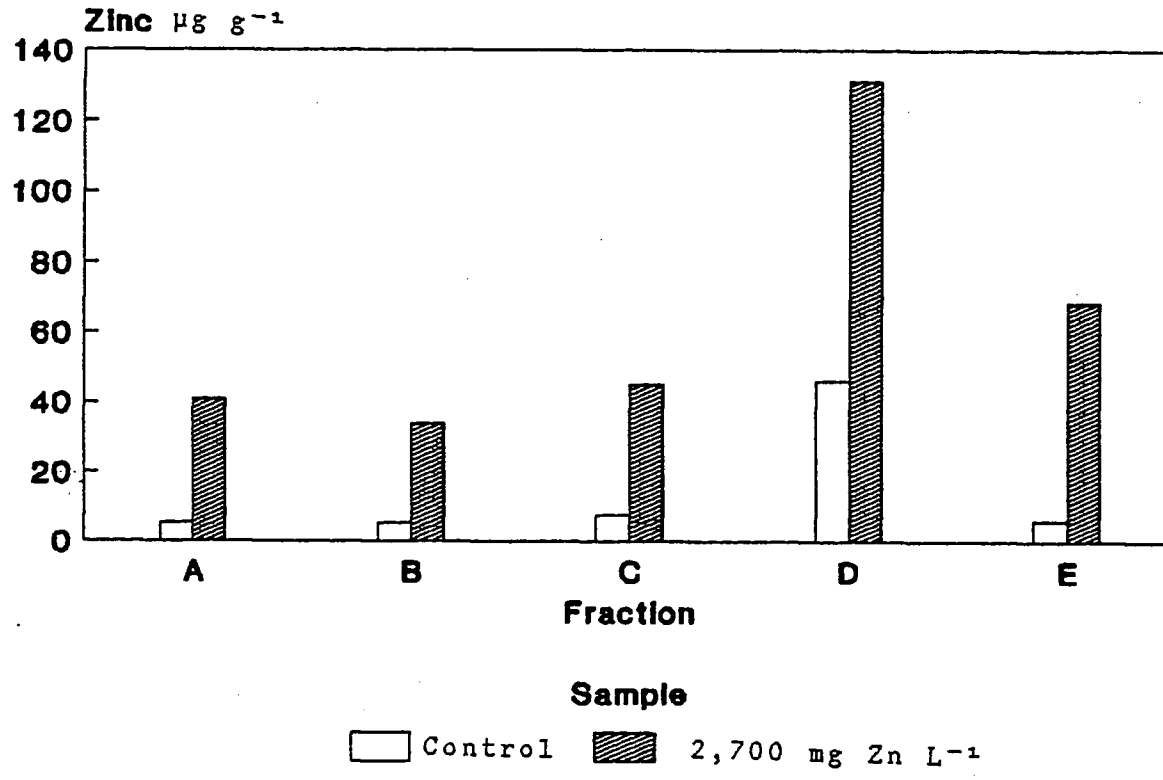


Figure 10. Zinc concentrations in different cellular fractions from the current year's foliage (1986) of the control treatment and high foliar Zn treatment from site 5 in the first year of treatment. Fraction A = cell wall and debris, B + C = chloroplasts, D = mitochondria, E = ribosomes and vacuolar contents.

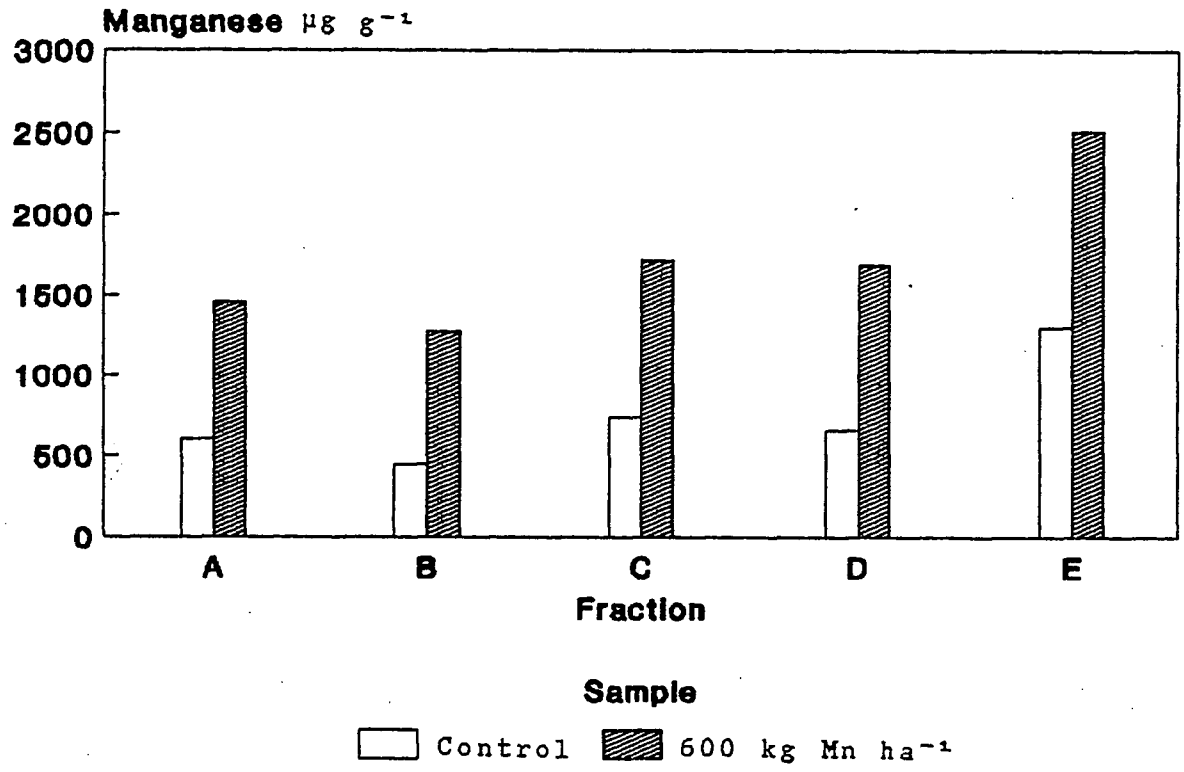


Figure 11. Manganese concentrations in different cellular fractions from the current year's foliage (1986) of the control treatment and high soil Mn treatment from site 5 in the first year of treatment. Fraction A = cell wall and debris, B + C = chloroplasts, D = mitochondria, E = ribosomes and vacuolar contents.

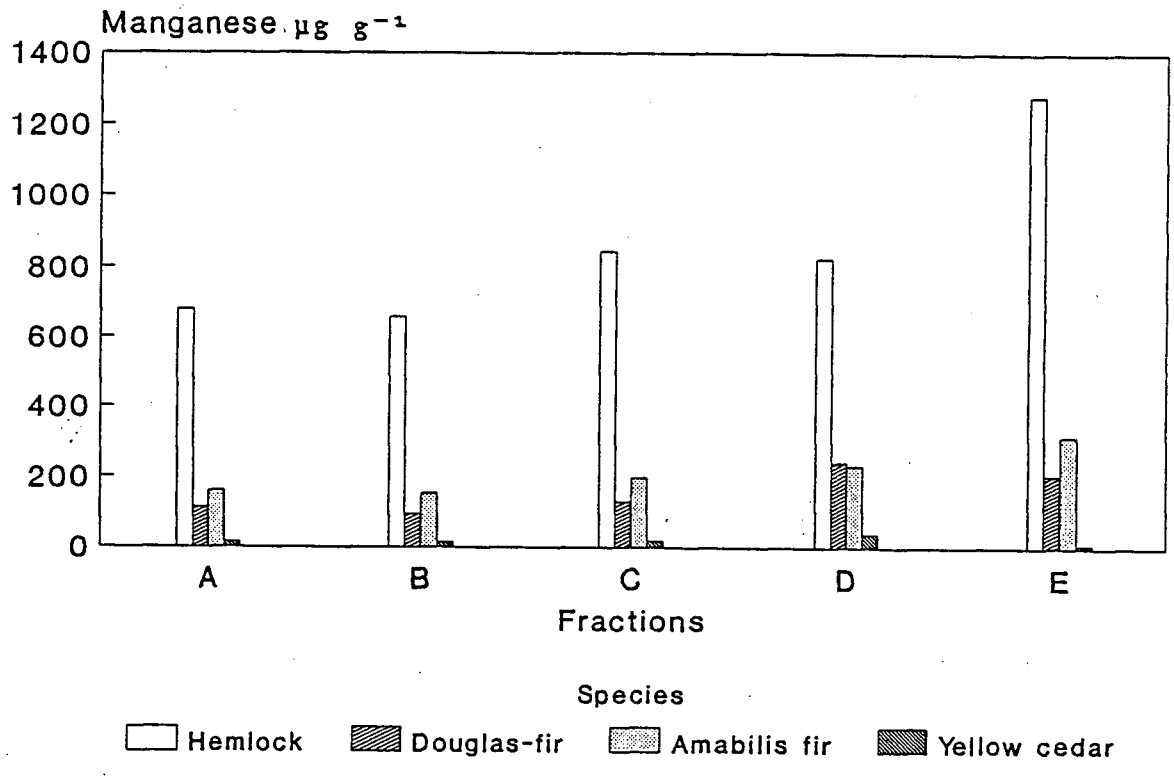


Figure 12. Manganese concentrations in different cellular fractions from the current year's foliage in 1987 of unfertilized trees of different species on site 5. Fraction A = cell wall and debris, B + C = chloroplasts, D = mitochondria, E = ribosomes and vacuolar contents.

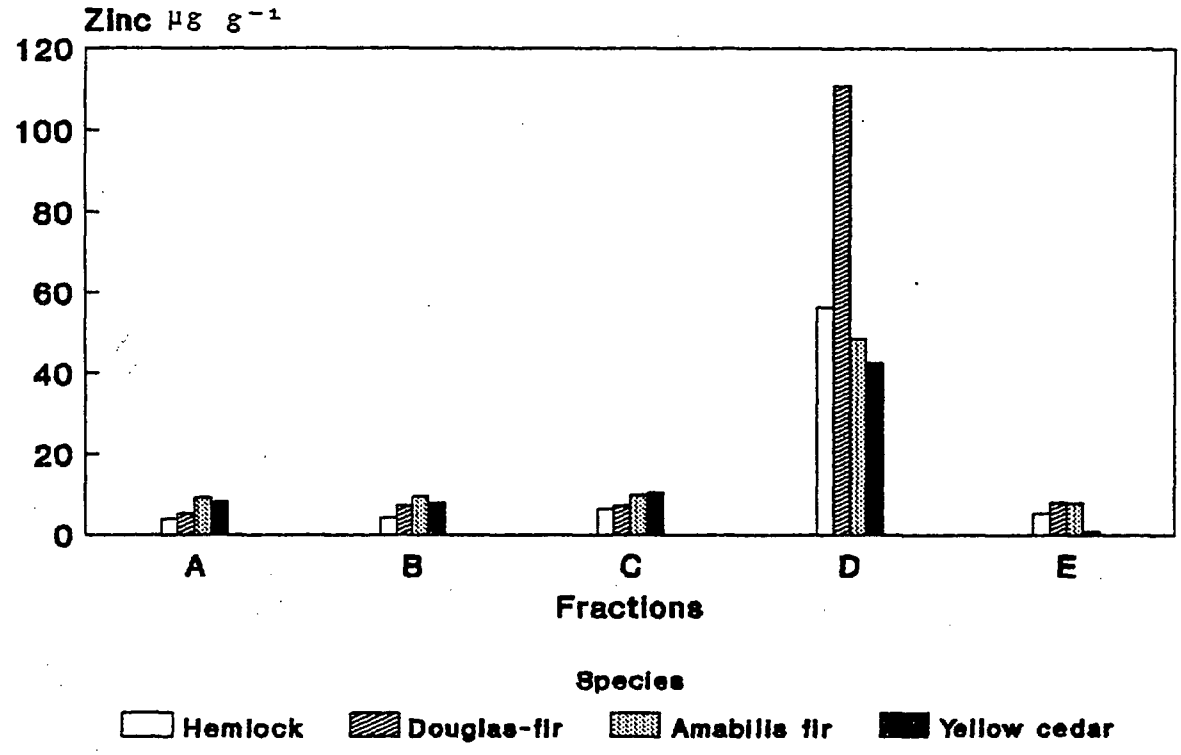


Figure 13. Zinc concentrations in different cellular fractions from the current year's foliage in 1987 of unfertilized trees of different species on site 5. Fraction A = cell wall and debris, B + C = chloroplasts, D = mitochondria, E = ribosomes and vacuolar contents.

In fractions A, B, and C hemlock tended to have lower levels than the other species. In fraction E, hemlock had lower Zn than Douglas-fir and amabilis fir. In all fractions Douglas-fir tended to have higher Zn concentrations than hemlock.

B. Fertilization Experiments

1. Nutrient and Growth Responses

Conventionally vector analysis has been applied to species with determinate shoot growth and has been expressed as mass per fixed number of needles. For hemlock, the question has arisen whether foliar mass should be expressed on the basis of a fixed number of needles or on a per shoot basis. Hemlock has both determinate and indeterminate shoot growth, hence its species name *heterophylla*, *hetero* meaning different, and *phylla* meaning leaves (Harlow and Harrar 1958). The distal needles on a 1-year-old shoot are shorter than the proximal needles (Owen and Molder 1973). The proximal needles are the result of determinate shoot growth. These needles were formed in the bud the previous year, and therefore their number would depend upon the environmental conditions of the previous year, but their mass would reflect the current year's environment. The needles formed at the distal part of the shoot are the result of indeterminate shoot growth which means that they were initiated and elongated in the same

year. Therefore, both their number and mass would reflect the current year's environmental conditions.

It appears that foliar mass per shoot would be an appropriate parameter in which to express growth for two reasons. Firstly, foliar mass per shoot is a function of both number of needles per shoot and mass per needle. Therefore, changes in either of the two components would be reflected in foliar mass per shoot. Secondly, from regression analysis there were significant relationships between current height increment and foliar mass per shoot (Figure 14 and Table 9). The relationship between foliar mass per shoot and current height increment was positive and linear, but in some cases the relationship was curvilinear. The curvilinear relationship indicates that a maximum point was reached beyond which an increase in foliar mass per shoot was associated with a reduction in height increment.

In cases where the change in foliar mass per shoot and foliar concentration were determined to be significantly different from the controls, the response was assessed using vector analysis. Otherwise, nutrient concentration was used alone. The nutrient and growth data for the fertilization trials for all sites and years are presented in Appendix L.

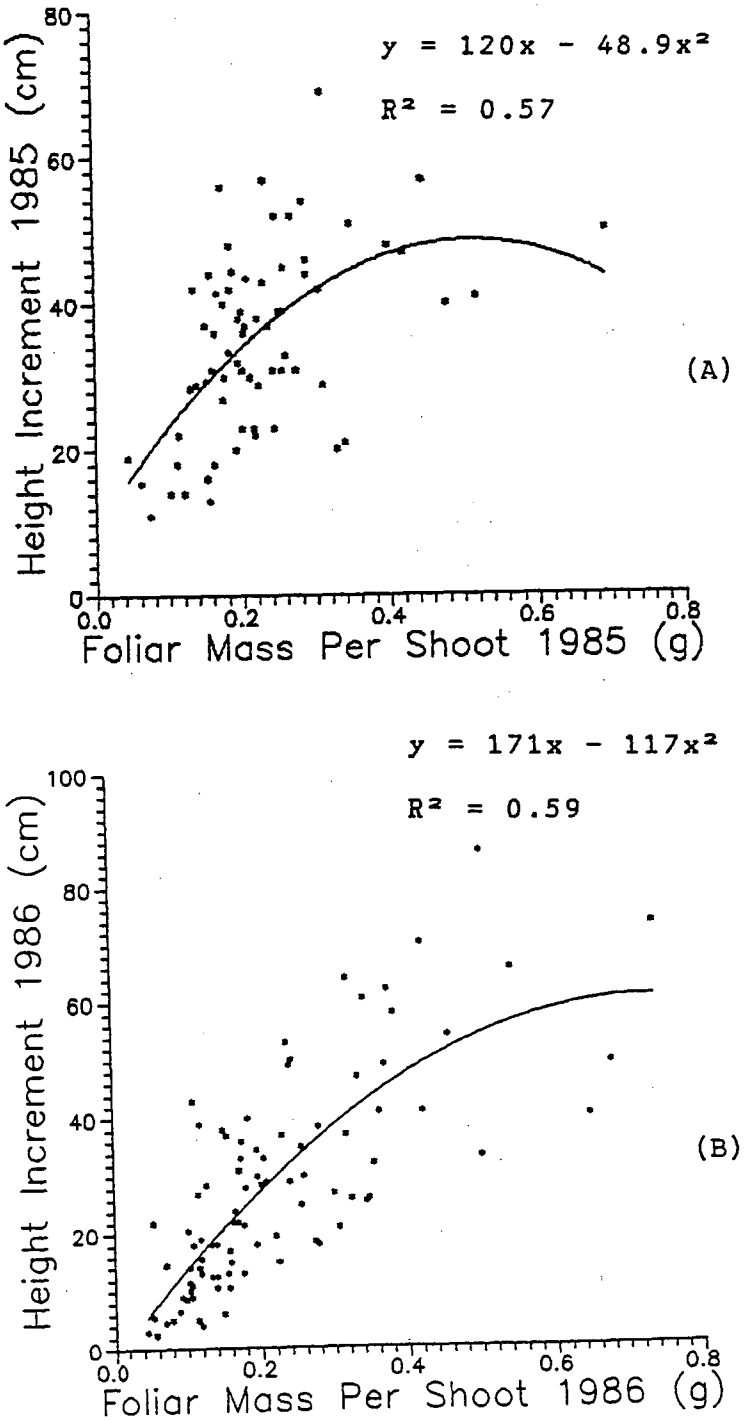


Figure 14. Scatter plots of the height increment versus the foliar mass per shoot for site 1 in the first (1985) (A) and second years (1986) (B).

Table 9. Equations and correlation coefficients (R^2) for the height increment (y) - foliar mass per shoot (x) relationships for each site. Refer to Appendix K for additional scatter plots.

Site	Year	Equation	R^2	Appendix
1	1985	$y = 120.0x - 48.9x^2$	0.57	
1	1986	$y = 171.0x - 117.0x^2$	0.59	
2	1986	$y = 291.0x - 440.0x^2$	0.38	K.1
3	1986	$y = 154.0x - 151.0x^2$	0.32	K.2
4	1986	$y = 160.0x - 71.0x^2$	0.55	K.3
4	1987	$y = 16.0 + 163.0x - 82.0x^2$	0.49	K.4
5	1986	$y = 204.0x - 143.0x^2$	0.43	K.5
5	1987	$y = 206.0x - 124.0x^2$	0.49	K.6

a. Zinc

i. Foliar Zinc Treatments

In the first growing season following foliar zinc applications, an increase in uptake of foliar zinc occurred. This was true for all sites and all levels of application (Table 10). In the second growing season following foliar zinc applications, foliar zinc levels were still elevated on sites 1 and 2 from treatment 5 (3600 mg Zn L^{-1}), on site 3 from treatment 4 (360 mg Zn L^{-1}), and on site 5 from treatment 15 (2700 mg Zn L^{-1}) (Table 10).

On site 2 in the first year there was an increase in foliar mass and foliar Zn from treatment 4 (360 mg Zn L^{-1}), which implies a growth response (Table 10 and Figure 15A) (shift in C direction). Conversely a reduction in mass and increase in foliar Zn from treatment 5 (3600 mg Zn L^{-1}) indicate a Zn toxicity (Figure 15A) (shift in E direction). On site 3 there was an increase in foliar mass and foliar Zn after the second year, from treatment 4 (360 mg Zn L^{-1}), indicating a growth response (Figure 15B) (shift in C direction). On site 5 in the first year, there was a decrease in foliar mass and increase in foliar Zn from treatment 13 (360 mg Zn L^{-1}), suggesting a Zn toxicity (Figure 15C) (shift in E direction).

Table 10. Foliar zinc (μg g^{-1}) concentration response and foliar mass per shoot (g) growth response to foliar applications of zinc in the first and second years following treatments. Values in parenthese are the standard deviation. An (*) indicates a significant difference and n.s.d. indicates no significant difference at the 5% level between a treatment and its respective sulfur control.

Site 1

	First Year		Second Year	
Treatment	Zn	Mass	Zn	Mass
	--μg g^{-1}--	--g--	--μg g^{-1}--	--g--
360 mg Zn L^{-1}				
	58.1	0.18	13.4	0.264
	(46.8)	(0.154)	(2.2)	(0.193)
177 mg S L^{-1}				
	10.5	0.202	11.5	0.202
	(3.4)	(0.064)	(3.5)	(0.117)
	*	n.s.d.	n.s.d.	n.s.d.
3600 mg Zn L^{-1}				
	149.7	0.241	15.2	0.206
	(46.8)	(0.154)	(5.2)	(0.149)
1770 mg S L^{-1}				
	7.9	0.208	11.1	0.140
	(2.1)	(0.076)	(3.5)	(0.056)
	*	n.s.d.	*	n.s.d.

Site 3

	Second Year	
Treatment	Zn	Mass
	--μg g^{-1}--	--g--
360 mg Zn L^{-1}		
	21.3	0.255
	(2.4)	(0.125)
177 mg S L^{-1}		
	14.4	0.171
	(3.7)	(0.089)
	*	*
3600 mg Zn L^{-1}		
	19.1	0.193
	(8.3)	(0.081)
1770 mg S L^{-1}		
	17.4	0.172
	(3.6)	(0.105)
	n.s.d.	n.s.d.

Table 10 (continued).

Site 2

	First Year		Second Year	
Treatment	Zn	Mass	Zn	Mass
	--μg g^{-1}--	--g--	--μg g^{-1}--	--g--
360 mg Zn L^{-1}				
	58.8	0.153	11.5	0.158
	(21.8)	(0.034)	(5.6)	(0.068)
177 mg S L^{-1}				
	6.7	0.107	8.8	0.123
	(2.3)	(0.019)	(3.4)	(0.039)
	*	*	n.s.d.	n.s.d.
3600 mg Zn L^{-1}				
	75.0	0.077	19.6	0.131
	(99.0)	(0.049)	(8.8)	(0.092)
1770 mg S L^{-1}				
	5.8	0.118	11.4	0.163
	(2.8)	(0.042)	(2.7)	(0.113)
	*	*	*	n.s.d.

Table 10 (concluded)

Site 4

Treatment	First Year Zn	First Year Mass	Second Year Zn	Second Year Mass
	--μg g^{-1}--	--g--	--μg g^{-1}--	--g--
360 mg Zn L^{-1}				
	18.3	0.263	10.2	0.421
	(6.9)	(0.156)	(2.6)	(0.340)
177 mg S L^{-1}				
	8.2	0.216	8.5	0.327
	(3.3)	(0.231)	(3.0)	(0.174)
	*	n.s.d.	n.s.d.	n.s.d.
1800 mg Zn L^{-1}				
	26.2	0.299	8.3	0.371
	(10.4)	(0.082)	(2.6)	(0.266)
883 mg S L^{-1}				
	9.4	0.267	8.5	0.459
	(3.1)	(0.071)	(3.1)	(0.277)
	*	n.s.d.	n.s.d.	n.s.d.
2700 mg Zn L^{-1}				
	48.6	0.171	9.4	0.368
	(25.1)	(0.136)	(3.0)	(0.293)
1325 mg S L^{-1}				
	6.4	0.136	9.7	0.515
	(2.1)	(0.063)	(3.5)	(0.335)
	*	n.s.d.	n.s.d.	n.s.d.

Site 5

Treatment	First Year Zn	First Year Mass	Second Year Zn	Second Year Mass
	--μg g^{-1}--	--g--	--μg g^{-1}--	--g--
360 mg Zn L^{-1}				
	79.3	0.184	11.9	0.306
	(30.1)	(0.078)	(2.9)	(0.152)
177 mg S L^{-1}				
	11.2	0.291	10.5	0.278
	(1.9)	(0.052)	(4.6)	(0.110)
	*	*	n.s.d.	n.s.d
1800 mg Zn L^{-1}				
	100.9	0.173	15.4	0.292
	(31.8)	(0.078)	(4.9)	(0.104)
883 mg S L^{-1}				
	10.1	0.198	12.5	0.301
	(3.3)	(0.071)	(5.1)	(0.151)
	*	n.s.d.	n.s.d.	n.s.d.
2700 mg Zn L^{-1}				
	132.3	0.171	16.5	0.314
	(30.5)	(0.093)	(4.5)	(0.170)
1325 mg S L^{-1}				
	10.5	0.174	12.4	0.308
	(1.9)	(0.052)	(3.5)	(0.165)
	*	n.s.d.	*	n.s.d.

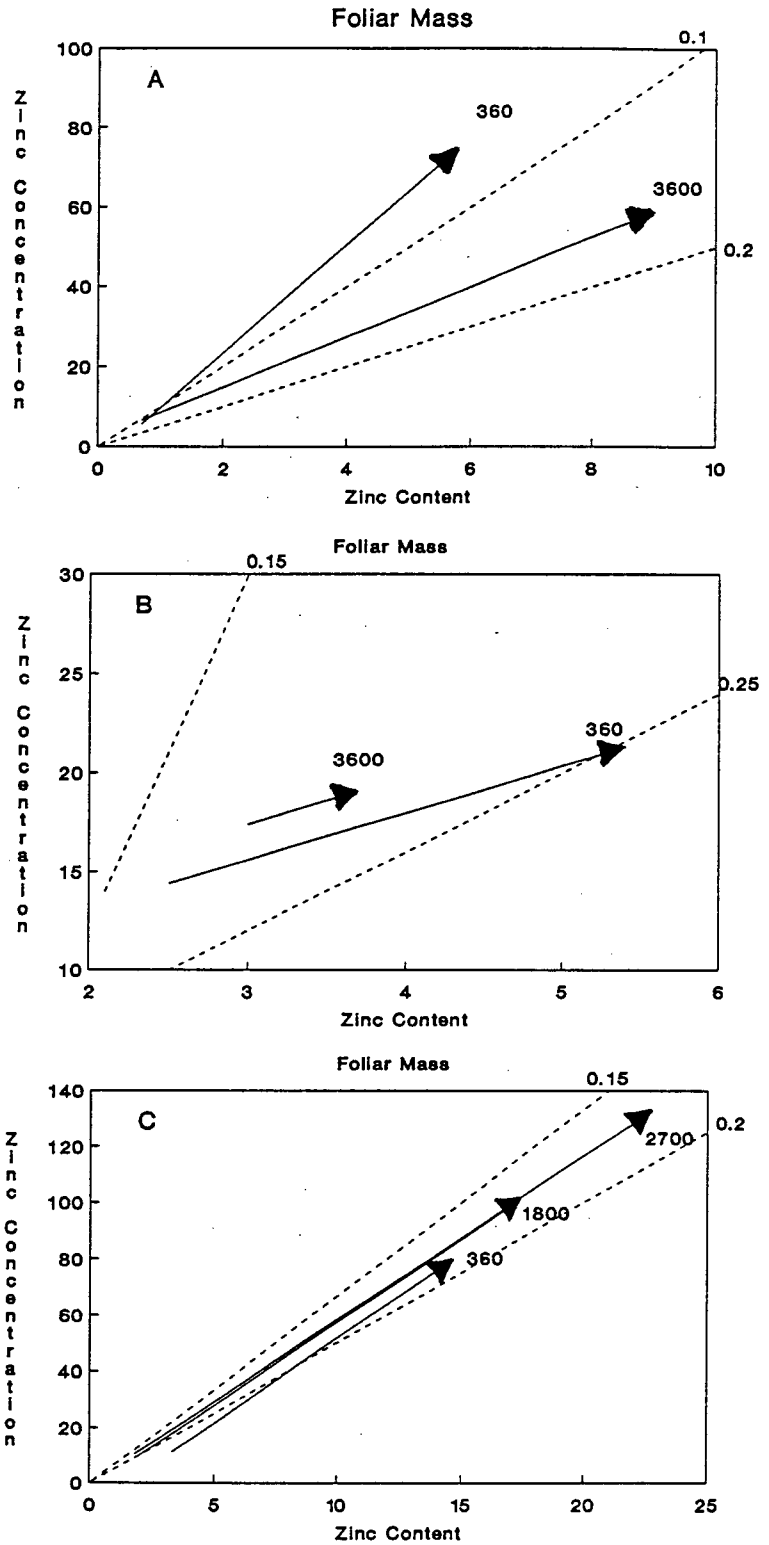


Figure 15. Vector diagrams of growth response to foliar applied zinc. (A) First year in 1985 on site 2, (B) second year in 1986 on site 3, and (C) first year in 1986 on site 5. The x-axis is µg Zn per shoot, the y-axis is µg Zn g^{-1} and the dashed line is foliar mass per shoot in grams. The arrow indicates the direction of the response. Treatments are in mg Zn L^{-1}.

There was no toxicity found with greater applications of foliar Zn. There were no significant effects of Zn application on foliar mass per shoot in the first year on site 1 from treatment 4 (360 mg Zn L^{-1}), on site 4 from treatments 13 (360 mg Zn L^{-1}), 14 (1800 mg Zn L^{-1}), and 15 (2700 mg Zn L^{-1}), and site 5 from treatments 14 (1800 mg Zn L^{-1}) and 15 (2700 mg Zn L^{-1}) but increased uptake of Zn occurred, indicating luxury consumption on those sites.

ii. Soil Zinc Treatments

A different pattern of response was found for the zinc soil treatments. No response occurred in the first growing season following fertilization on all sites. However, in the second growing season, nutrient concentration response occurred from treatments 1 (10 kg Zn ha^{-1}) and 2 (50 kg Zn ha^{-1}) on site 1, treatment 2 (50 kg Zn ha^{-1}) on site 3, and from treatment 3 (200 kg Zn ha-1) on site 5 (Table 11). Since there were no incidents of significant increases in foliar mass per shoot, these results indicate luxury consumption of Zn on these sites.

On sites 4 a positive growth response occurred in the second year from treatment 3 (200 kg Zn ha^{-1}) (Figure 16) (shift in C direction).

Table 11. Foliar zinc (μg g^{-1}) concentration response and foliar mass per shoot (g) growth response to soil applications of zinc in the second year following treatment. Values in parenthese are the standard deviation. An (*) indicates a significant difference and n.s.d. indicates no significant difference at the 5% level between a treatment and its respective sulfur control.

Site 1

Treatment	Zn	Mass
	--μg g^{-1}	--g--
10 kg Zn ha^{-1}		
	13.3	0.248
	(3.9)	(0.206)
5 kg S ha^{-1}		
	9.9	0.213
	(2.4)	(0.131)
	*	n.s.d.
50 kg Zn ha^{-1}		
	17.2	0.221
	(10.3)	(0.120)
25 kg S ha^{-1}		
	11.4	0.343
	(1.5)	(0.231)
	*	n.s.d.

Site 3

50 kg Zn ha^{-1}		
	17.7	0.303
	(10.0)	(0.248)
25 kg S ha^{-1}		
	12.8	0.303
	(4.3)	(0.360)
	*	n.s.d.

Site 4

Treatment	Zn	Mass
	-μg g^{-1}-	--g--
200 kg Zn ha^{-1}		
	11.2	0.364
	(2.7)	(0.195)
98 kg S ha^{-1}		
	6.4	0.199
	(2.3)	(0.084)
	*	*

Site 5

200 kg Zn ha^{-1}		
	15.2	0.258
	(5.7)	(0.212)
98 kg S ha^{-1}		
	8.8	0.272
	(1.8)	(0.108)
	*	n.s.d.

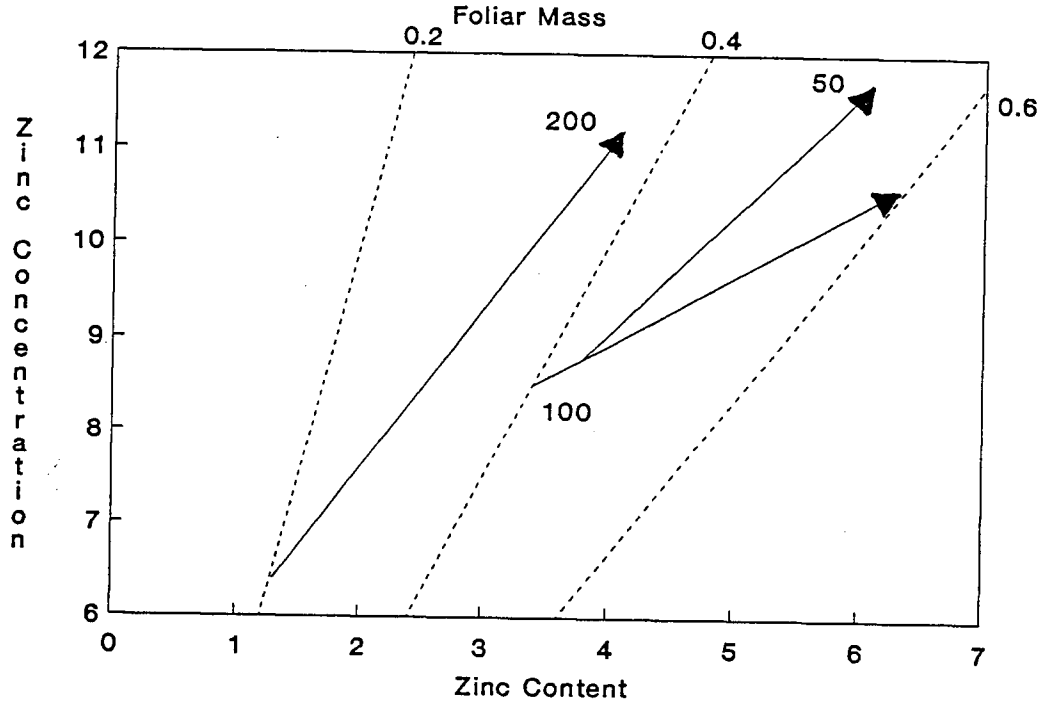


Figure 16. Vector diagram of the second year growth response in 1987 to soil applied zinc on site 4 for foliar zinc. The x-axis is µg Zn per shoot, the y-axis is µg Zn g^{-1} and the dashed line is foliar mass per shoot in grams. The arrow indicates the direction of the response. Treatments are in kg Zn ha^{-1}.

iii. Comparison of Foliar Versus Soil Treatments

Foliar Zn treatments from all sites were more efficient in supplying the plant with Zn (Figure 17). This was calculated as the change in foliar Zn concentration (of treated minus control values) per gram of Zn applied per tree. In addition, the increase in foliar Zn occurred in the first year with the foliar Zn applications whereas, it was delayed until the second year with the soil Zn applications. The lower foliar Zn treatment (360 mg Zn L^{-1}) was more efficient in supplying the plant with Zn than the higher foliar Zn treatments on all sites.

b. Manganese

i. Foliar Manganese Treatments

The nutrient response to Mn tended to follow an opposite trend as compared with the Zn response. Response to foliar-applied Mn occurred in the second year only on sites 4 and 5, both to treatment 17 (4095 mg Mn L^{-1}). On site 4 the increase in growth resulted in a manganese dilution (Figure 18A) (shift in A direction), and on site 5 the reduction in growth and Mn concentration is evidence of a manganese toxicity (concentration effect, shift in F direction) (Figure 18B).

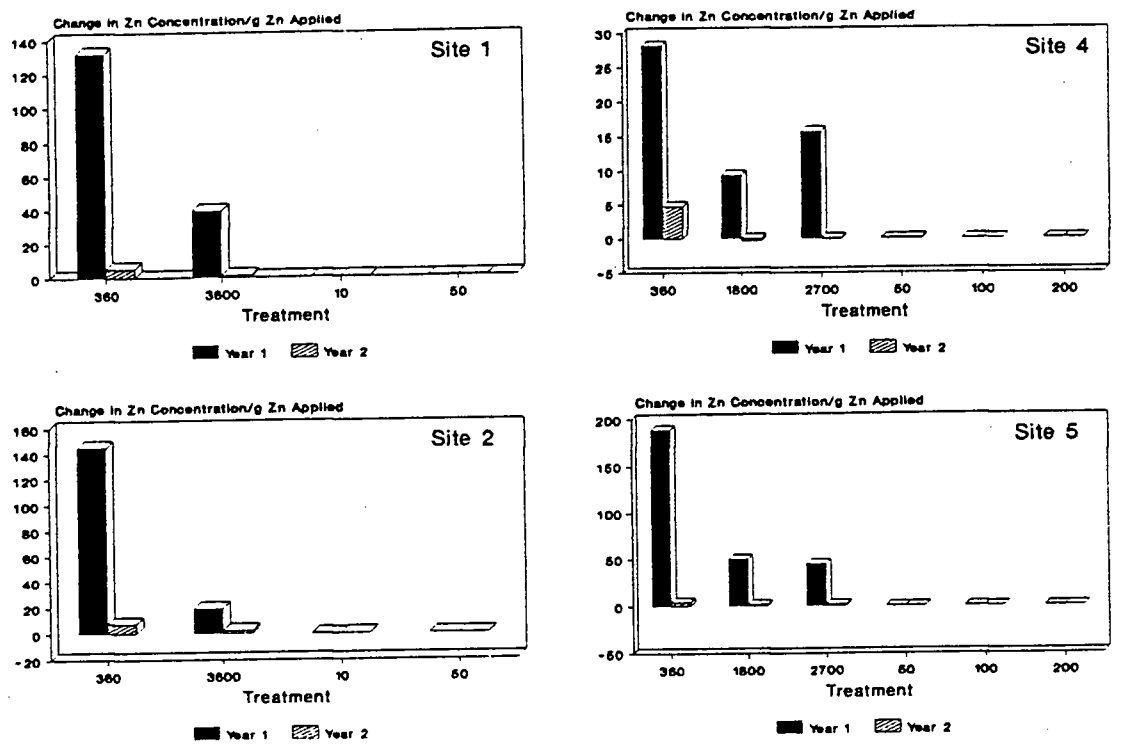
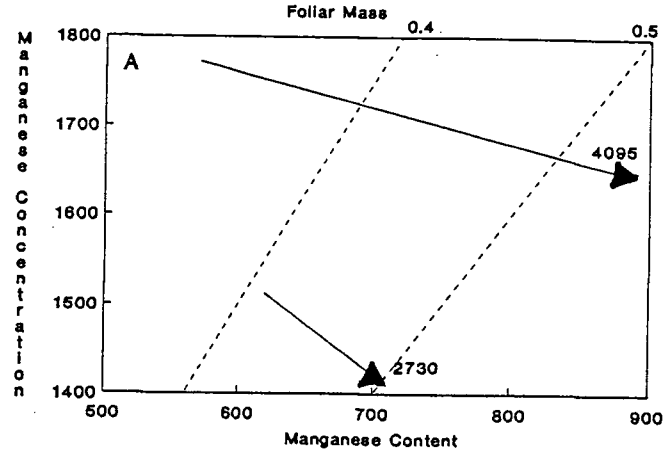


Figure 17. Nutrient efficiency of foliar Zn versus soil Zn treatments in supplying the plant with Zn. Treatments 360, 1800, 2700 and 3600 are in mg Zn L^{-1}, and treatments 10, 50, 100 and 200 are in kg Zn ha^{-1}. Nutrient efficiency is change in foliar Zn concentration (µg g^{-1}) per gram of Zn applied per tree.



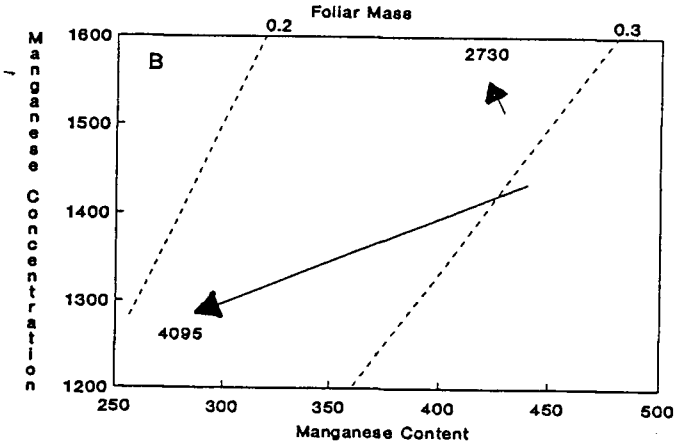


Figure 18. Vector diagrams of the second year growth response in 1987 to foliar applied Mn on site 4 (A) and on site 5 (B) for foliar Mn. The x-axis is µg Mn per shoot, the y-axis is µg Mn g^{-1} and the dashed line is foliar mass per shoot in grams. The arrow indicates the direction of the response. Treatments are mg Mn L^{-1}.

ii. Soil Manganese Treatments

In contrast, Mn nutrient concentration response to the soil Mn treatments occurred on all sites at all levels of treatments except for treatment 4 (200 kg Mn ha^{-1}) on site 4 (Table 12). This response was still evident in the second growing season following manganese fertilization for all manganese treatments and sites. This was luxury consumption on sites 1, and 2 from treatment 3 (200 kg Mn ha^{-1}) in the first and second years, from treatment 3 (200 kg Mn ha^{-1}) on site 3 in the second year, on site 4 from treatment 5 (400 kg Mn ha^{-1}) in the first and second years, from treatment 6 (600 kg Mn ha^{-1}) in the first year, and on site 5 from treatments 4 (200 kg Mn ha^{-1}), 5 (400 kg Mn ha^{-1}) and 6 (600 kg Mn ha^{-1}) in the second year (Table 12).

Growth responses to soil-applied manganese were obtained on sites 4 and 5. On site 4 a growth response was not detected until in the second year from treatment 6 (600 kg Mn ha^{-1}) (shift in C direction) (Figure 19A). With treatment 4 (200 kg Mn ha^{-1}), a Mn toxicity was obtained (shift in E direction), but at the highest Mn level (treatment 6 (600 kg Mn ha^{-1})), a positive growth response to manganese was obtained (shift in C direction). Response on site 5 was different in that a positive growth response to manganese was evident in the first season.

Table 12. Foliar manganese (μg g^{-1}) concentration and foliar mass per shoot (g) growth response to soil treatments of manganese. Values in parenthese are the standard deviation. An (*) indicates a significant difference and n.s.d. indicates no significant difference at the 5% level between a treatment and its respective sulfur control.

Site 1

	First Year		Second Year	
Treatment	Mn	Mass	Mn	Mass
	-μg g^{-1}-	--g--	-μg g^{-1}-	--g--
200 kg Mn ha^{-1}				
	3008	0.360	2971	0.348
	(1298)	(0.246)	(1278)	(0.192)
Control				
	1002	0.283	1139	0.195
	(306)	(0.127)	(395)	(0.131)
	*	n.s.d.	*	n.s.d.

Site 2

200 kg Mn ha^{-1}				
	3142	0.145	3875	0.181
	(1470)	(0.056)	(1429)	(0.096)
Control				
	1449	0.143	1932	0.186
	(655)	(0.049)	(589)	(0.096)
	*	n.s.d.	*	n.s.d.

Site 3

	Second Year	
Treatment	Mn	Mass
	-μg g^{-1}-	--g--
200 kg Mn ha^{-1}		
	2427	0.308
	(441)	(0.175)
Control		
	1505	0.262
	(287)	(0.085)
	*	n.s.d.

Table 12 (concluded)
Site 4

Treatment	First Year Mn	First Year Mass	Second Year Mn	Second Year Mass
	-µg g^{-1}-	--g--	--µg g^{-1}-	--g--
200 kg Mn ha^{-1}				
	2026	0.270	2980	0.449
	(727)	(0.096)	(918)	(0.219)
118 kg S ha^{-1}				
	1639	0.271	1598	0.665
	(597)	(0.178)	(412)	(0.427)
	n.s.d.	n.s.d.	*	*
400 kg Mn ha^{-1}				
	2802	0.352	3460	0.506
	(710)	(0.388)	(780)	(0.358)
235 kg S ha^{-1}				
	1647	0.275	1743	0.457
	(604)	(0.058)	(471)	(0.350)
	*	n.s.d.	*	n.s.d.
600 kg Mn ha^{-1}				
	3206	0.363	3737	0.646
	(1341)	(0.284)	(1237)	(0.459)
352 kg S ha^{-1}				
	1175	0.287	1593	0.248
	(214)	(0.239)	(365)	(0.316)
	*	n.s.d.	*	*

Site 5

Treatment	First Year Mn	First Year Mass	Second Year Mn	Second Year Mass
	-µg g^{-1}-	--g--	--µg g^{-1}-	--g--
200 kg Mn ha^{-1}				
	1832	0.302	2487	0.317
	(510)	(0.096)	(592)	(0.089)
118 kg S ha^{-1}				
	1083	0.193	1359	0.291
	(363)	(0.075)	(523)	(0.156)
	*	*	*	n.s.d.
400 kg Mn ha^{-1}				
	2172	0.250	2817	0.340
	(794)	(0.134)	(651)	(0.157)
235 kg S ha^{-1}				
	1268	0.168	1626	0.326
	(408)	(0.037)	(632)	(0.152)
	*	*	*	n.s.d.
600 kg Mn ha^{-1}				
	2933	0.367	3518	0.316
	(1021)	(0.187)	(881)	(0.103)
352 kg S ha^{-1}				
	1240	0.250	1301	0.314
	(526)	(0.210)	(454)	(0.180)
	*	*	*	n.s.d.

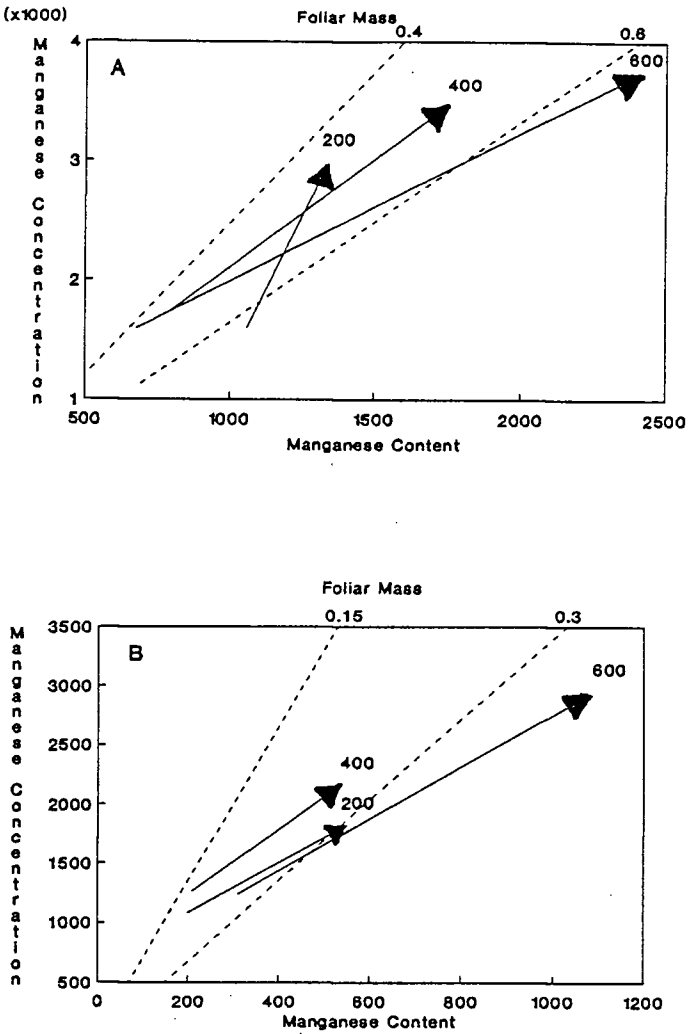


Figure 19. Vector diagrams of the growth response to soil applied Mn for foliar Mn. (A) In the second year (1987) on site 4 and (B) in the first year (1986) on site 5. The x-axis is µg Mn per shoot in, the y-axis is µg Mn g^{-1} and the dashed line is foliar mass per shoot in grams. The arrow indicates the direction of the response. Treatments are in kg Mn ha^{-1}.

A positive growth response to manganese occurred at all levels of treatment (shift in C direction) (Figure 19B).

c. Complete-Zn-Mn Treatment

i. Nutrient and Growth Responses

A complete treatment was carried out in order to assess whether other nutrient deficiencies were present. The results are summarized in Table 13. On both sites 4 and 5, growth and nutrient concentration responses were produced in both the first and second years to the "complete -Zn -Mn" treatment. There was a positive growth response from the "complete-Zn-Mn" treatment on sites 4 and 5 in the first and second years (Table 13 and Figures 20 and 21) (shift in C direction).

On site 4 in the first year (1986) the severity of nutrient deficiencies ranked from most to least deficient were in the order Zn, N, and B (Figure 20). On site 5 in the first year (1986) the severity of nutrient deficiencies ranked from most to least deficient were in the order B, Zn, AFe, Cu, Fe, and N (Figure 21A). In the second year the severity of nutrient deficiencies were in the order P, Zn and N (Figure 21B). Since there was a Zn response on sites 4 and 5 to the "complete-Zn-Mn" treatment, the Zn response was a synergism to the application of other nutrients (shift in C direction).

Table 13. Foliar nutrient concentrations and foliar mass per shoot growth response in unfertilized (control) trees and trees subjected to the "complete-Zn-Mn" treatment in the first and second years following treatment. An (*) indicates a significant difference at the 5% level of significance.

	N	P	K	Ca	Mg	Mn	Fe	Active Fe	Zn	Cu	B
	cg g^{-1}					μg g^{-1}					
Site 4 1986											
Complete -Zn-Mn	1.67	0.16	0.67	0.30	0.12	1343	38.4	37.2	17.2	3.5	33.2
	(0.26)	(0.014)	(0.108)	(0.017)	(0.022)	(400)	(1.8)	(2.4)	(6.2)	(0.6)	(4.1)
Control	1.0	0.20	0.68	0.28	0.14	1428	25.0	32.0	8.7	3.2	24.5
	(0.06)	(0.071)	(0.195)	(0.088)	(0.062)	(360)			(1.9)		(1.8)
	*								*		*
Site 4 1987											
Complete -Zn-Mn	1.03	0.18	0.56	0.21	0.10	1259	51.2		11.1	2.5	
	(0.15)	(0.024)	(0.094)	(0.053)	(0.029)	(305)	(10.6)		(3.5)	(0.6)	
Control	0.94	0.12	0.57	0.23	0.09	1545	48.8		11.7	2.9	
	(0.21)	(0.041)	(0.112)	(0.088)	(0.040)	(415)	(15.4)		(4.6)	(0.6)	
Site 5 1986											
Complete -Zn-Mn	1.67	0.21	0.81	0.30	0.136	1064	46.4	34.8	15.5	4.0	45.8
	(0.22)	(0.066)	(0.115)	(0.088)	(0.052	(370)	(9.1)	(6.4)	(4.2)	(0.6)	(10.9)
Control	1.11	0.18	0.78	0.32	0.14	1308	39.3	26.2	11.4	3.1	28.4
	(0.11)	(0.052)	(0.042)	(0.061)	(0.016	(531)	(6.7)	(5.3)	(1.9)	(0.6)	(6.0)
	*						*	*	*	*	*
Site 5 1987											
Complete -Zn -Mn	1.32	0.24	0.86	0.34	0.15	962	53.7		14.7	3.6	
	(0.15)	(0.037)	(0.225)	(0.085)	(0.040)	(387)	(7.0)		(2.3)	(0.7)	
Control	1.15	0.17	0.84	0.31	0.13	1243	52.7		11.8	3.9	
	(0.017)	(0.062)	(0.177)	(0.072)	(0.024)	(666)	(12.0)		(3.6)	(0.9)	
	*	*							*		

Table 13 (continued).

	N/P$_i$	N/P	P/Al	K/Ca	Ca/Mg
Site 4 1986					
Complete -Zn -Mn	10.4	10.3	4.3	2.2	2.6
Control	5.1	6.2	4.5	2.5	2.0
Site 4 1987					
Complete -Zn -Mn	5.9	7.6	4.6	2.2	2.2
Control	6.4	7.3	3.1	2.3	2.1
Site 5 1986					
Complete -Zn -Mn	7.8	10.3	4.9	2.7	2.2
Control	6.1	6.9	3.3	2.4	2.3
Site 5 1987					
Complete -Zn -Mn	5.5	8.2	4.4	2.6	2.3
Control	6.9	7.1	2.7	2.7	2.4

	S (cg g-1)	N/S
Site 4 1986		
Complete -Zn-Mn	0.173	9.65
	(0.029)	
Control	0.160	6.25
Site 5 1986		
Complete -Zn-Mn	0.114	14.65
	(0.048)	
Control	0.124	8.95
	(0.047)	

1. Critical value calculated according to the formula.

Table 13 (concluded).

	Foliar Mass Per Shoot	
	1986	1987
	----------g----------	
Site 4		
Complete -Zn -Mn	0.804	0.635
	(0.646)	(0.346)
Control	0.200	0.280
	(0.127)	(0.145)
	*	*
	-------	------
Site 5		
Complete -Zn -Mn	0.394	0.742
	(0.162)	(0.306)
Control	0.198	0.288
	(0.054)	(0.140)
	*	*
	-------	-------

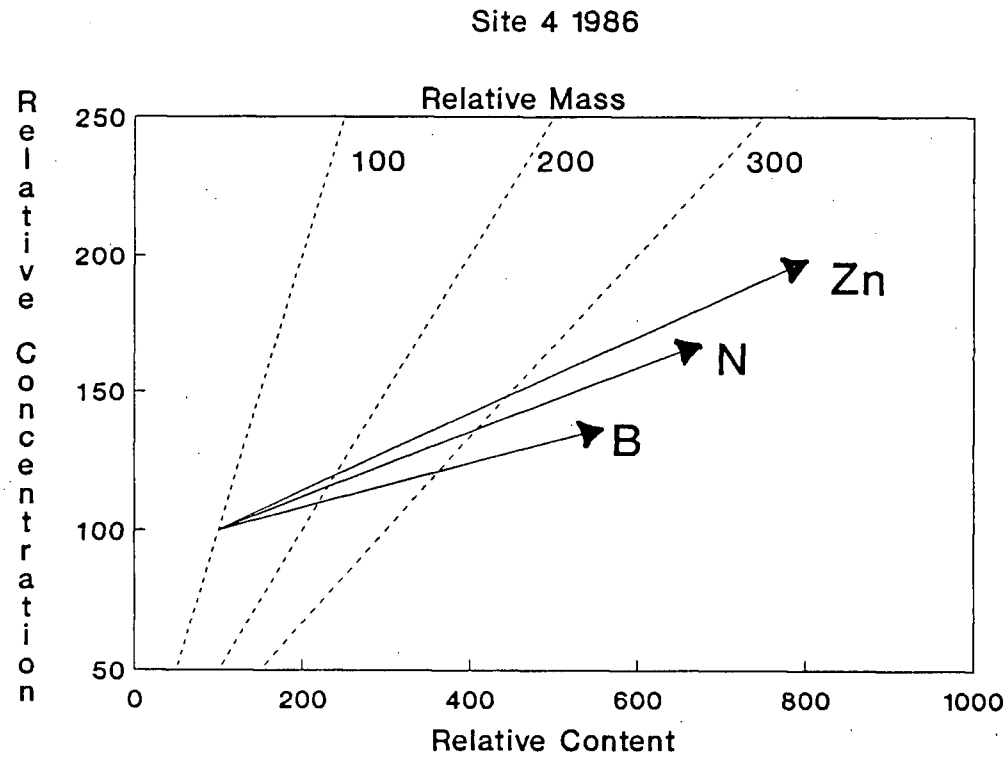


Figure 20. Vector diagram of the first year (in 1986) growth response to the "complete-Zn-Mn" treatment on site 4. The x-axis is relative nutrient content per shoot, the y-axis is relative nutrient concentration and the dashed line is relative foliar mass per shoot. The arrow indicates the direction of the response. The control = 100.

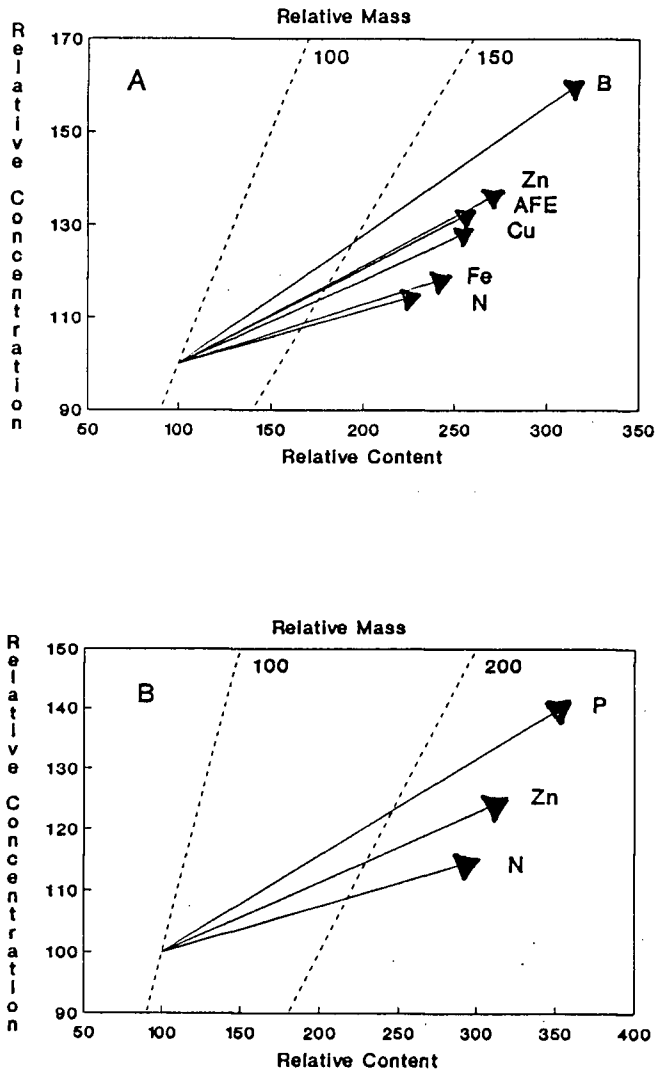


Figure 21. Vector diagrams of the first (in 1986) (A) and second year (in 1987) (B) growth responses to the "complete-Zn-Mn" treatment on site 5. The x-axis is relative nutrient content per shoot, the y-axis is relative nutrient concentration and the dashed line is relative foliar mass per shoot. The arrow indicates the direction of the response. The control = 100.

The increase in foliar Zn suggests that additional Zn was required with fertilization, and the site was able to meet these requirements. Referring to Table 13 in the first year on site 4, the control foliar Zn concentration was 8.7 μg g^{-1}, and was increased to 17.2 μg g^{-1} in response to the "complete-Zn-Mn" treatment. Following the second year it was 11.7 μg g^{-1} and the treated level was 11.1 μg g^{-1}. On site 5 in the first year the control foliar Zn concentration was 11.4 μg g^{-1} and increased to 15.5 μg g^{-1} with the "complete-Zn-Mn" treatment. In the second year the control level was 11.8 μg g^{-1} and the treatment increased foliar Zn to 14.7 μg g^{-1}.

It is interesting to see how the pattern of Zn response follows the pattern of nitrogen response. They both occurred only in the first year on site 4 and in years one and two on site 5. There was not a great difference in foliar N levels for the second year controls on sites 4 and 5, 1.17 and 1.15 cg g^{-1} respectively, yet there was a residual N response on site 5.

2. Shoot Increment Ratio

For all the soil Zn applications on all the sites, a growth response occurred only on site 2 to treatment 2 (50 kg Zn ha^{-1}) in the second year (Table 14).

Table 14. Shoot increment ratio growth response to zinc. Values in parenthese are the standard deviation. An (*) indicates a significant difference and n.s.d. indicates no significant difference at the 5% level between a treatment and its respective sulfur control.

Site	Year	Treatment	Shoot Increment Ratio	
2	2	50 kg Zn ha^{-1}	1.58	(0.53)
		25 kg S ha^{-1}	1.14 *	(0.53)
1	2	3600 mg Zn L^{-1}	1.61	(0.71)
		1770 mg S L^{-1}	0.87 *	(0.23)
4	2	360 mg Zn L^{-1}	1.86	(1.22)
		177 mg S L^{-1}	1.14 *	(0.39)
		1800 mg Zn L^{-1}	1.75	(0.70)
		883 mg S L^{-1}	1.46 *	(0.20)
		2700 mg Zn L^{-1}	1.43	(0.32)
		1325 mg S L^{-1}	1.30 n.s.d.	(0.34)
5	2	360 mg Zn L^{-1}	1.91	(0.89)
		177 mg S L^{-1}	1.20 *	(0.29)
		1800 mg Zn L^{-1}	1.60	(0.54)
		883 mg S L^{-1}	1.38 n.s.d.	(0.41)
		2700 mg Zn L^{-1}	1.77	(0.43)
		1325 mg S L^{-1}	1.29 *	(0.54)
3	1	360 mg Zn L^{-1}	1.18	(0.20)
		177 mg S L^{-1}	0.86 *	(0.27)
		3600 mg Zn L^{-1}	0.78	(0.38)
		1770 mg S L^{-1}	0.82 n.s.d.	(0.23)
	2	360 mg Zn L^{-1}	1.02	(0.49)
		177 mg S L^{-1}	0.86 n.s.d.	(0.37)
		3600 mg Zn L^{-1}	1.02	(0.32)
		1770 mg S L^{-1}	0.79 *	(0.30)

Positive growth response to foliar Zn applications occurred only in the second year. On site 1, it took place at the highest application rate (treatment 5 (3600 mg Zn L^{-1})) (Table 14). On site 4 it was detected with treatments 13 (360 mg Zn L^{-1}) and 14 (1800 mg Zn L^{-1}) (Table 14), and on site 5 with treatments 13 (360 mg Zn L^{-1}) and 15 (2700 mg Zn L^{-1})(Table 14). On site 3 it occurred in both years but to different levels of Zn (Table 14). In the first year, response took place to treatment 4 (360 mg Zn L^{-1}), and in the second year to treatment 5 (3600 mg Zn L^{-1}).

Positive growth response to soil-applied Mn occurred on sites 4 and 5 in the first year (Table 15). On site 4 response occurred to all Mn treatments. On site 5, growth response was significant only to the 400 kg ha^{-1} treatment.

Positve growth response occurred to the "complete-Zn-Mn" treatment on both sites 4 and 5 (Table 16) in the first year, and in the second year on site 5.

Table 15. Shoot increment ratio response to soil applications of manganese in the first year (1986) on sites 4 and 5. An (*) indicates a significant difference and n.s.d indicates no significant difference at the 5% level between a treatment and its respective sulfur control.

Treatment	Site 4 ----- Shoot Increment Ratio -----		Site 5 ----- Shoot Increment Ratio -----	
200 kg Mn ha^{-1}	1.03	(0.26)	1.25	(0.35)
118 kg S ha^{-1}	0.83	(0.27)	1.09	(0.35)
	*		n.s.d.	
400 kg Mn ha^{-1}	1.15	(0.38)	1.21	(0.52)
235 kg S ha^{-1}	0.85	(0.28)	0.98	(0.37)
	*		*	
600 kg Mn ha^{-1}	1.18	(0.37)	1.23	(0.39)
352 kg S ha^{-1}	0.90	(0.32)	1.08	(0.39)
	*		n.s.d.	

Table 16. Shoot increment ratio response to the "complete-Zn-Mn" treatment. An (*) indicates a significant difference and n.s.d indicates no significant difference at the 5% level between a treatment and its respective sulfur control.

Site	Year	Treatment	Shoot Increment Ratio	
4	1	Complete-Zn-Mn	1.23	(0.48)
		Control	0.76 *	(0.22)
5	1	Complete-Zn-Mn	1.58	(0.67)
		Control	0.96 *	(0.27)
5	2	Complete-Zn-Mn	1.94	(1.11)
		Control	1.39 *	(0.14)

3. Height Increment

Where growth response to Zn in terms of height growth increment occurred, it was not evident until in the second year (Table 17). On site 1 there was a significant response to treatment 5 (3600 mg Zn L^{-1}), and a trend to response on site 2. On site 5, the response occurred from treatment 13 (360 mg Zn L^{-1}), and on site 4 there was a trend towards a response. In the first year of treatment for these sites the Zn treatments tended to depress growth.

Height increment response to foliar Mn treatments did not occur until in the second year (Table 17). This was found on site 5 for treatment 17 (4095 mg Mn L^{-1}). On site 5 response to soil Mn treatments occurred in the first year to treatments 4 (200 kg Mn ha^{-1}) and 6 (600 kg Mn ha^{-1}). On sites 4 and 5 in the second year there was a trend towards a response to treatments 4 (200 kg Mn ha^{-1}) and 5 (600 kg Mn ha^{-1}) respectively.

C. Retranslocation

Zinc and manganese concentrations were compared separately between first year foliage from 1985 and 1986, and between one-year-old and two-year-old foliage from sites 1 and 2 (Appendix M). For foliar Zn from the low treatment (treatment 4) there was a decrease in the two-year-old foliage but levels in the foliage formed in the second year were not different from the control

Table 17. Height increment ratio response. An (*) indicates a significant difference and n.s.d. indicates no significant difference at the 5% level between a treatment and its respective sulfur control.

Site	Year	Treatment	Height Increment Ratio
1	2	360 mg Zn L^{-1}	1.15 (0.64)
		117 mg S L^{-1}	0.97 (0.73) n.s.d.
		3600 mg Zn L^{-1}	2.54 (1.14)
		1770 mg S L-1	0.83 (0.30) *
2	2	360 mg Zn L^{-1}	1.57 (0.85)
		117 mg S L^{-1}	1.42 (0.41) n.s.d.
		3600 mg Zn L^{-1}	1.85 (1.62)
		1770 mg S L-1	1.57 (0.20) n.s.d.
4	2	360 mg Zn L^{-1}	3.30 (3.02)
		117 mg S L^{-1}	2.35 (1.66) n.s.d.
5	2	360 mg Zn L^{-1}	2.36 (1.28)
		117 mg S L^{-1}	1.43 (0.46) *
5	1	200 kg Mn ha^{-1}	1.82 (0.76)
		118 kg S ha^{-1}	1.24 (0.46) *
		400 kg Mn ha^{-1}	1.52 (0.71)
		235 kg S ha^{-1}	1.69 (0.87) n.s.d.
		600 kg Mn ha^{-1}	1.74 (0.53)
		352 kg Mn ha^{-1}	1.27 (0.57) *
5	1	4095 mg Mn L^{-1}	2.22 (1.49)
		2408 mg Mn L^{-1}	1.26 (0.67) *

(Table 18). The foliar Zn levels for the high treatment (treatment 5) decreased for the two-year-old foliage, and were elevated for the foliage formed in the second year relative to the control (Table 19). The increased Zn concentration in the foliage formed in the second year and the decrease in the two-year-old foliage suggests that there was retranslocation in the second year, of Zn from the old to the new foliage. This depended upon the level of the foliar Zn treatment, with remobilization occurring for the high foliar Zn treatment. On site 1 the Zn retranslocation was associated with a growth response in terms of shoot and height increment ratio (Tables 14 and 17, respectively).

Manganese levels were higher in the two-year-old foliage than the one-year-old foliage (Table 20). The foliage formed in the second year also had elevated levels of Mn relative to the control (Table 20). Both these results indicate that the two-year-old foliage, as well as the current year's foliage formed in the second season following fertilization, continued to take up Mn. This suggests that increased uptake will occur if soil Mn is available.

In the study of nutrient retranslocation it was assummed that there was no change in unit foliar mass with foliage age. Therefore, foliar nutrient concentrations would no be changed from the dilution or concentrations effects. There is evidence which indicates that the decrease in foliar nutrient

Table 18. Change in foliar zinc (μg g^{-1}) of current year's foliage over time and with age of foliage following treatment for the low zinc foliar treatment on sites 1 and 2. Treatment 4 is 360 mg Zn L^{-1} and treatment 7 is 177 mg S L^{-1}.

Site 1

Time		Year since treatment			Age	Age since treatment		
Treatment		1	2	t-test	1	2	t-test	
Zn	4	51.2	13.6	*	51.2	48.7	n.s.d	
Control	7	5.1	11.5	*	5.1	19.8	*	
t-test		*	n.s.d		*	*		

Site 2

Time		Year since treatment			Age	Age since treatment		
Treatment		1	2	t-test	1	2	t-test	
Zn	4	53.0	11.5	*	53.0	35.2	*	
Control	7	2.0	8.8	n.s.d	2.0	15.6	n.s.d	
t-test		*	n.s.d		*	*		

Table 19. Change in foliar zinc ($\mu g\ g^{-1}$) of current year's foliage with time and with age of foliage following treatment for the high zinc foliar treatment on sites 1 and 2. Treatment 5 is 3600 mg Zn L^{-1} and treatment 8 is 1770 mg S L^{-1}.

Site 1

Time					Age			
		Years since treatment				Age since treatment		
Treatment		1	2	t-test		1	2	t-test
Zn	5	144.4	15.4	*		144.4	135.5	*
Control	8	2.5	11.0	*		2.5	17.9	*
t-test		*	*			*	*	

Site 2

Time					Age			
		Years since treatment				Age since treatment		
Treatment		1	2	t-test		1	2	t-test
Zn	5	139.6	19.6	*		139.6	78.7	*
Control	8	2.5	11.4	*		2.5	17.0	*
t-test		*	*			*	*	

Table 20. Change in foliar manganese (μg g^{-1}) of current year's foliage with time and with age of foliage following treatment for the manganese soil treatment (3) on sites 1 and 2. Treatment 3 is 200 kg Mn ha^{-1} and treatment 12 is the untreated control.

Site 1

Time		Years since treatment			Age	Age since treatment	
Treatment		1	2	t-test	1	2	t-test
Mn	3	3059	2971	n.s.d	3059	4610	*
Control	12	1019	1139	n.s.d	1019	1432	*
t-test		*	*		*	*	

Site 2

Time		Years since treatment			Age	Age since treatment	
Treatment		1	2	t-test	1	2	t-test
Mn	3	3196	3875	*	3196	5920	*
Control	12	1474	1932	*	1474	2365	*
t-test		*	*		*	*	

concentrations in the two-year-old foliage was not due to foliar leaching. With an increase in foliage age, foliar Zn concentrations for the controls increased (Tables 18 and 19) indicating no evidence of Zn leaching.

D. Nutrient-Growth Interactions

Data were fitted to the model $y = a + bx + cx^2$, where y is a measure of growth and x is foliar Zn concentration, to test the hypothesis that the relationship of foliar Zn concentration to growth follows the model of diminishing returns. Significant regressions were found between height increment and foliar Zn concentrations (Figure 22 and Table 21). These were found between current year's height increment and foliar Zn for 1986 and 1987 for sites 4 and 5. Comparing the foliar levels of the optimum height increment to the control Zn levels suggests that height increment benefited from the higher foliar Zn concentrations.

There were no relationships found between other growth parameters either measured or calculated and foliar nutrient concentrations.

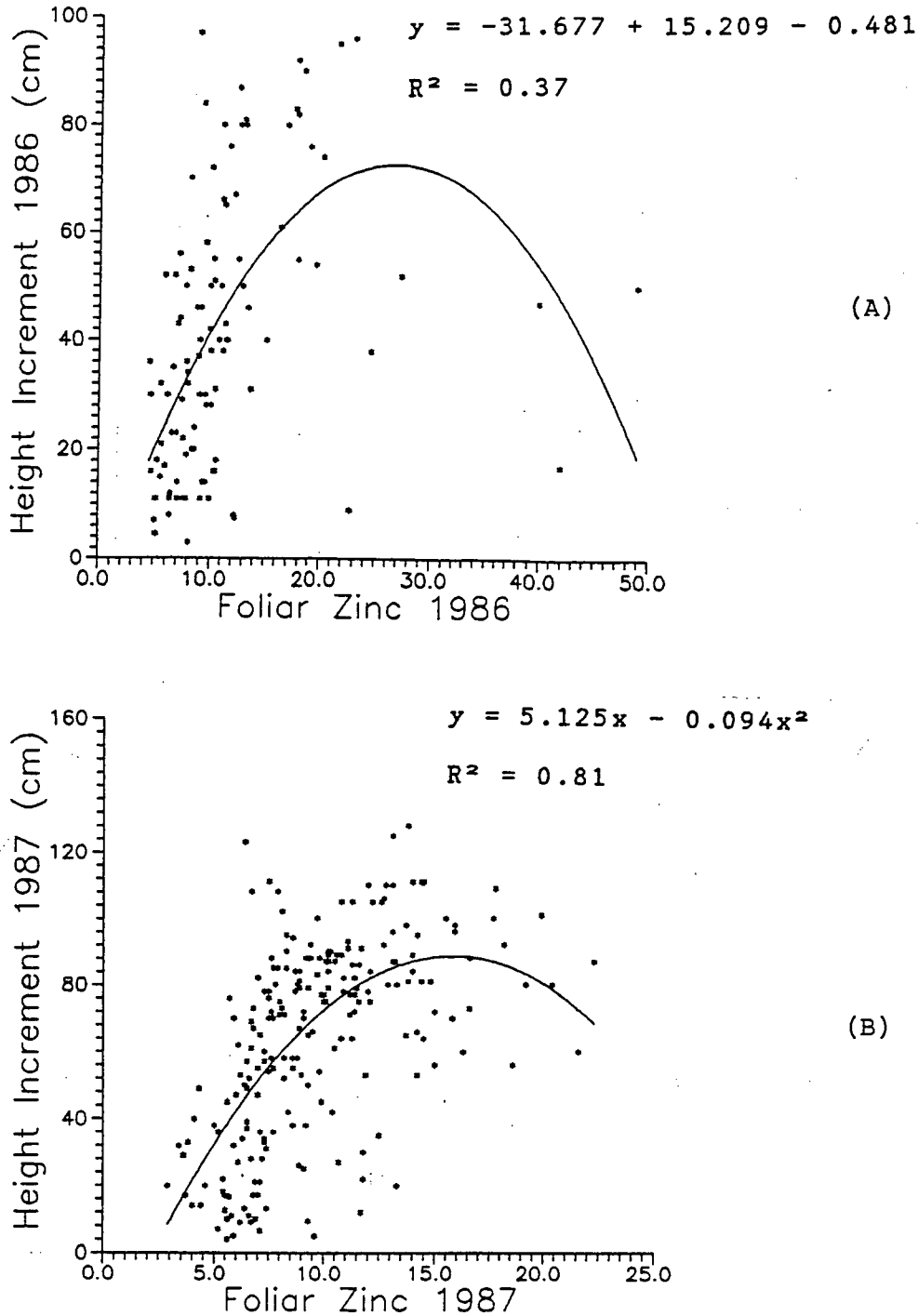


Figure 22. Scatter plots of height increment (cm) versus current year's foliar zinc levels (μg g^{-1}). (A) In the first year (1986) (for cases where Zn $\leq$50 μg g^{-1}) and (B) in the second year (1987) for site 4.

Table 21. Equations and correlation coefficients for the height increment (y) - foliar zinc (x) relationships for sites 4 and 5. Refer to Appendix N for scatter plots other than given in Figure 22.

Site	Year	Equation	R^2
4	1986	$y = 5.125x - 0.094x^2$	0.81
4	1987	$y = -31.677 + 15.209 - 0.481x^2$	0.37
5	1986	$y = 2.709x - 0.03x^2$	0.22
5	1987	$y = 8.577x - 0.234x^2$	0.18

E. Nutrient Interactions

1. Interactions with Zinc

a. Manganese

On site 5 in the first year, treatment 15 (2700 mg Zn L^{-1}) produced the only incident where Zn application reduced foliar Mn (Table 22). Treatment 1 (50 kg Mn ha^{-1}) actually increased foliar Mn.

b. Nitrogen

Scatter plots of foliar N versus foliar Zn revealed significant relationships between the two (Figure 23 and Table 23). In addition, a linear relationship was found to exist between extractable soil Zn and total soil N concentrations (Figure 24).

Table 22. Foliar manganese ($\mu g\ g^{-1}$) nutrient concentration response to soil and foliar applications of zinc in the first year (1986) on site 5.

Soil Treatments		Foliar Treatments	
Treatment	Mn	Treatment	Mn
50 kg Zn ha^{-1}		360 mg Zn L^{-1}	
	1262 (137)		1217 (235)
25 kg S ha^{-1}		117 mg S L^{-1}	
	1178 (416)		1140 (249)
	*		n.s.d.
100 kg Zn ha^{-1}		1800 mg Zn L^{-1}	
	1273 (209)		1066 (319)
49 kg S ha^{-1}		883 mg S L^{-1}	
	1525 (670)		1272 (751)
	n.s.d.		n.s.d.
200 kg Zn ha^{-1}		2700 mg Zn L^{-1}	
	1289 (548)		867 (487)
98 kg S ha^{-1}		1325 mg S L^{-1}	
	1327 (536)		1094 (261)
	n.s.d.		*

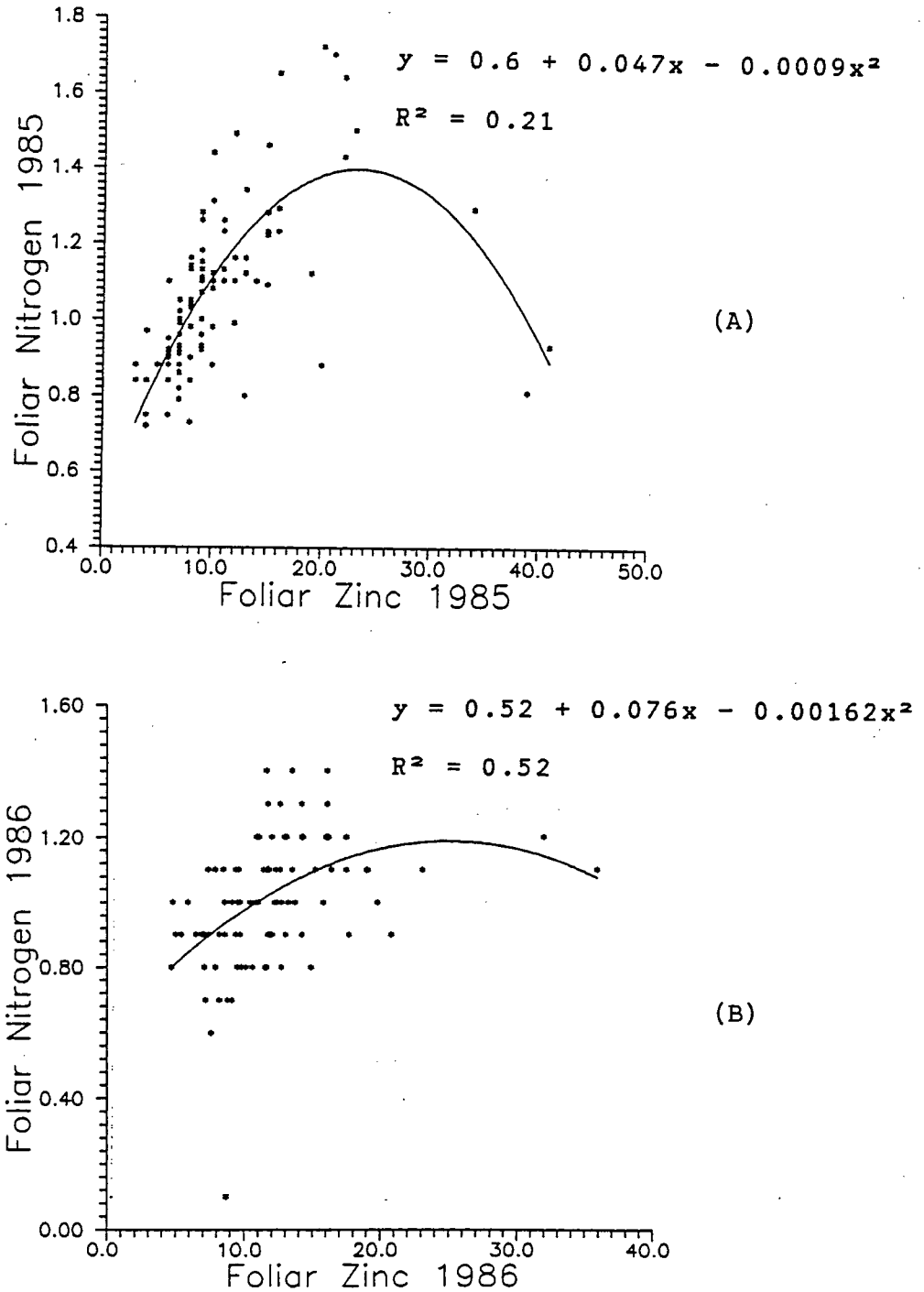


Figure 23. Scatter plots of foliar nitrogen (cg g^{-1}) versus foliar zinc (µg g^{-1}) of the current year's foliage (A) in the first year (1985) (for cases where Zn $\leq$50 µg g^{-1}) and (B) in 1986 (for cases where Zn $\leq$40 µg g^{-1}) from site 1. The correlation coefficient (R^2) is given.

Table 23. Equations and correlation coefficients (R^2) for foliar nitrogen (y) - foliar zinc (x) relationships according to site. Refer to Appendix O for scatter plots other than given in Figure 23.

Site	Year	Equation	R^2
1	1985	$y = 0.52 + 0.07x - 0.00162x^2$	0.52
1	1986	$y = 0.6 + 0.047x - 0.0009x^2$	0.21
2	1985	$y = 0.6 + 0.0997x - 0.00331x^2$	0.44
2	1986	$y = 0.8 + 0.038x - 0.0009x^2$	0.21
4	1986	$y = 0.137x - 0.00361x^2$	0.46
4	1987	$y = 0.55 + 0.074x - 0.00134x^2$	0.50
5	1986	$y = 0.306 + 0.0932x - 0.00176x^2$	0.65
5	1987	$y = 0.234 + 0.115x - 0.00293x^2$	0.54

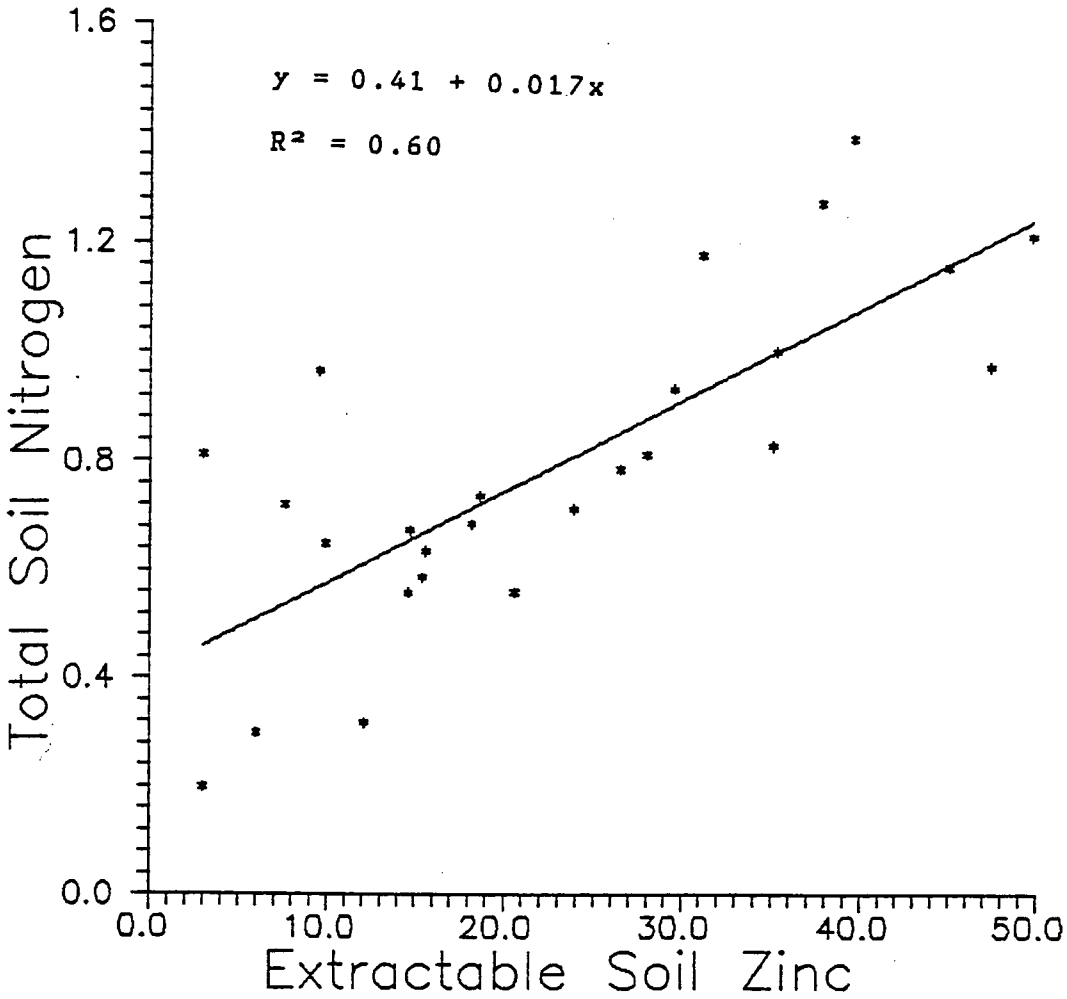


Figure 24. Scatter plot of total soil nitrogen (cg g^{-1}) vesus extractable soil zinc (µg g^{-1}) both from the forest floor for all sites.

2. Interactions with Manganese

a. Zinc

Manganese foliar and soil treatments affected foliar Zn levels on site 1 (Table 24). Foliar-applied Mn increased foliar Zn in both the first and second years. The Mn soil treatment (200 kg Mn ha^{-1}) increased foliar Zn in the first year. On site 5, foliar Zn was also increased by soil applications of Mn in the first year from treatments 4 (200 kg Mn ha^{-1}) and 6 (600 kg Mn ha^{-1}) (Table 25). As shown in Figure 25 this was a synergism of foliar Zn to Mn applications (shift in C direction).

There were no other relationships found between Mn treatments and foliar concentrations of other nutrients. Therefore, it is unlikely that the growth responses obtained with Mn soil treatments were due to the alleviation of toxicities of other nutrients or metallic elements.

Table 24. Foliar zinc ($\mu g\ g^{-1}$) nutrient concentration response to soil and foliar manganese applications on site 1.

Foliar Treatment

Treatment	1985		1986	
	Zn		Zn	
	$\mu g\ g^{-1}$			
2730 mg Mn L^{-1}	9.4	(2.5)	10.6	(1.5)
Control	7.2	(1.7)	8.8	(2.3)
	*		*	

Soil Treatment

Treatment	1985	
	Zn	
	$\mu g\ g^{-1}$	
200 kg Mn ha^{-1}	15.1	(5.8)
Control	7.6	(1.9)
	*	

Table 25. Foliar zinc (μg g^{-1}) nutrient response in the first year (1986) to soil applications of manganese on site 5.

Soil Treatments	
Treatment	Zn
	-μg g^{-1}--
200 kg Mn ha^{-1}	11.9 (2.3)
118 kg S ha^{-1}	8.3 (1.5)
	*
400 kg Mn ha^{-1}	12.1 (5.2)
235 kg S ha^{-1}	10.5 (2.7)
	n.s.d.
600 kg Mn ha^{-1}	15.7 (5.8)
352 kg S ha^{-1}	10.1 (1.4)
	*

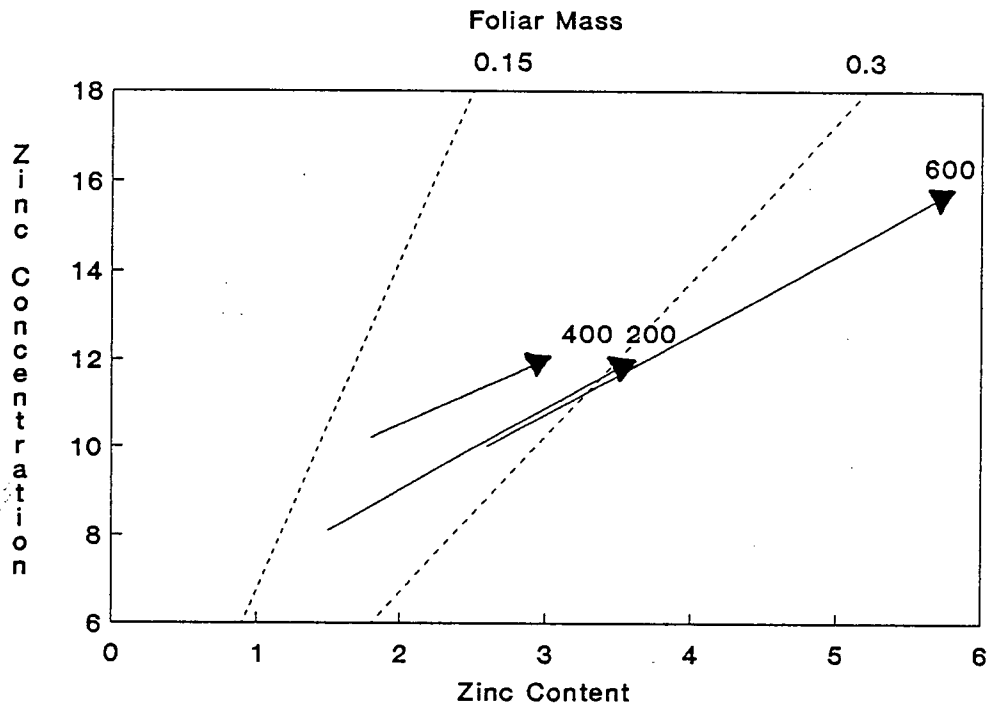


Figure 25. Vector diagram of first year growth response (in 1986) of Zn to soil treatments of Mn from site 5. The x-axis is µg Zn per shoot, the y-axis is µg Zn g^{-1} and the dashed line is foliar mass per shoot in grams. The arrow indicates the direction of the response. Treatments are in kg Mn ha^{-1}.

F. Relationship of Response to Site

A summary of nutrient and growth responses to foliar and soil Zn treatments are presented in Tables 26 and 27 respectively. There was no obvious relationship between response and site conditions. In similar manner, for the soil Mn treatments (Table 28) there was no relationship between response and site.

An interesting relationship was found between site conditions and response to the "complete-Zn-Mn" treatment on sites 4 and 5 (Table 29). There are distinct differences between sites 4 and 5. Site 4 is CWHb1, low elevation and has a humo-ferric podzol with an Ae horizon. In contrast site 5 is CWHb2, high elevation and has a ferro-humic podzol with an enriched organic B horizon. There was a response to N, Zn and B in the first year regardless of site (Figures 20 and 21A). On site 5 response to N and the Zn synergism continued into the second year (Figure 21B). In addition there were responses to Cu and Fe in the first year (Figure 21A) and a strong response to P in the second year (Figure 21B).

Table 26. Summary of nutrient and growth responses to foliar Zn treatments. A (*) indicates a significant increase, a (**) indicates a significant decrease and a (-) indicates no significant difference at the 5% level between a treatment and its control.

Site	Treatment (mg Zn L-1)	First Year Zn	First Year Mass	First Year Shoot Ratio	First Year Height Ratio	Second Year Zn	Second Year Mass	Second Year Shoot Ratio	Second Year Height Ratio
1	360	*	-	-	-	-	-	-	-
	3600	*	-	-	-	*	-	*	*
2	360	*	*	-	-	-	-	-	-
	3600	*	**	-	-	*	-	-	-
3	360			*	-	*	*	-	-
	3600			-	-	-	-	*	-
4	360	*	-	-	-	-	-	*	-
	1800	*	-	-	-	-	-	*	-
	2700	*	-	-	-	-	-	-	-
5	360	*	**	-	-	-	-	*	-
	1800	*	-	-	-	-	-	-	*
	2700	*	-	-	-	-	-	*	-

Table 27. Summary of nutrient and growth responses to soil Zn treatments. An (*) indicates a significant increase and a (-) indicates no significant difference at the 5% level between a treatment and its control.

Site	Treatment (kg Zn ha-1)	Second Year Zn	Mass	Shoot Ratio	Height Ratio
1	10	*	-	-	-
	50	*	-	-	-
2	10	-	-	-	-
	50	-	-	*	-
3	10	-	-	-	-
	50	*	-	-	-
4	50	-	-	-	-
	100	-	-	-	-
	200	*	-	-	-
5	50	-	-	-	-
	100	-	-	-	-
	200	*	-	-	-

Table 28. Summary of nutrient and growth responses to soil Zn treatments. An (*) indicates a significant increase, a (**) indicates a significant decrease and a (-) indicates no significant difference at the 5% level between a treatment and its control.

Site	Treatment (kg Mn ha-1)	First Year Mn	First Year Mass	First Year Shoot Ratio	First Year Height Ratio	Second Year Mn	Second Year Mass	Second Year Shoot Ratio	Second Year Height Ratio
1	200	*	-	-	-	*	-	-	-
2	200	*	-	-	-	*	-	-	-
3	200			-	-	*	-	-	-
4	200	-	-	*	-	*	**	-	-
	400	*	-	*	-	*	-	-	-
	600	*	-	*	-	*	*	-	-
5	200	*	*	-	*	*	-	-	-
	400	*	*	*	-	*	-	-	-
	600	*	*	-	*	*	-	-	-

Table 29. Summary of nutrient and growth responses to the "complete-Zn-Mn" treatment. An (*) indicates a significant increase and a (-) indicates no significant difference at the 5% level between a treatment and its control.

Site	First Year N	P	Zn	Fe	Active Fe	Cu	B	Mass	Shoot Ratio	Height Ratio
4	*	-	*	-	-	-	*	*	*	-
5	*	-	*	*	*	*	*	*	*	-

	Second Year N	P	Zn	Mass	Shoot Ratio	Height Ratio
4	-	-	-	*	-	-
5	*	*	*	*	*	-

CHAPTER 5. DISCUSSION

A. COMPARATIVE NUTRITION

1. Total Levels

Hemlock had lower foliar Zn concentrations and higher foliar Mn concentrations compared to Douglas-fir, amabilis fir and red cedar. Zasoski *et al.* (1990) also reported foliar Zn concentrations to be lower and foliar Mn concentrations to be higher than some other conifers in the Pacific Northwest. The fact that the comparison in this study was made among species on the same sites and in the same stands would indicate that the levels of Mn and Zn in hemlock are plant-related and not soil-related. In addition, it is unlikely different levels of foliar Zn and Mn between species are due to the exploitation of different soil horizons by the root systems. Root excavations done by Eis (1987) found that on similar soils, roots of Douglas-fir, cedar and hemlock trees penetrated to similar depth and trees of similar size extended their root system over similar areas. Since there were no significant differences in the rooting depth between species of 50-year-old trees, it may be safe to assume that the rooting depth of species of younger trees, as used in this study, would not be significantly different. Even if hemlock was exploiting the forest floor more than other species, this part of the soil horizon has the highest available Zn (Table 30).

Table 30. Soil data for all sites. 'T' refers to the top horizon which was forest floor and 'B' refers to the top 15 cm of the mineral soil horizon.

MEAN SITE	ZnT	ZnB	MnT	MnB	KT	KB	AlT	AlB	BT	BB	EXNT	EXTNB	TNT	TNB	pHT	pHB
	μg g^{-1}						μg g^{-1}				cg g^{-1}				H_2O	
1	22.1	2.9	49.6	5.1	196.1	55.4	1116	1707	0.47	0.16	0.016	0.004	0.60	0.19	3.8	4.1
3	15.2	1.8	226.0	13.6	431.0	188.6	1037	1758	0.40	0.11	0.063	0.016	0.67	0.24	4.5	4.6
4	29.2	5.8	44.4	21.0	198.4	166.0	874	1495	0.03	0.19	0.013	0.005	0.90	0.24	4.0	3.8
5	23.7	1.8	47.6	2.8	249.6	28.6	1176	1762	0.14	0.05	0.017	0.005	0.93	0.20	4.1	4.5
STANDARD DEVIATION																
1	17.6	1.6	40.2	6.5	121.4	41.8	438	289	0.78	0.06	0.021	0.002	0.38	0.10	0.3	0.3
3	6.7	1.3	51.3	3.1	190.5	45.2	364	343	0.03	0.04	0.044	0.010	0.07	0.11	0.3	0.3
4	10.6	3.6	21.8	35.9	48.1	95.5	325	210	0.01	0.11	0.012	0.001	0.26	0.14	0.6	0.7
5	13.4	0.7	27.6	2.3	204.0	14.5	425	541	0.09	0.06	0.020	0.003	0.24	0.11	0.3	0.4

MEAN SITE	CaT	CaB	MgT	MgB	PT	PB	CuT	CuB	FeT	FeB	pHT	pHB	CECT	CECB
	μg g^{-1}						μg g^{-1}				$CaCl_2$		meq $100g^{-1}$	
1	879	80	208	23.2	56.3	12.7	0.96	0.40	184	226	3.5	3.8	12.9	14.2
3	1175	210	350	48.7	58.0	6.9	0.64	0.56	261	285	3.9	4.1	6.7	19.6
4	1447	233	267	64.0	70.3	17.5	1.32	0.82	123	217	3.4	3.7	7.7	13.2
5	1268	137	288	22.1	72.6	7.2	1.22	0.96	179	177	3.5	3.7	9.4	12.4
STANDARD DEVIATION														
1	688	77	178	21.2	53.3	16.1	0.59	0.26	43	118	0.4	0.4	6.1	2.8
3	416	113	17	20.6	26.7	5.8	0.17	0.16	37	252	0.1	0.4	1.2	7.4
4	541	200	145	56.2	25.4	18.1	0.22	0.50	38	114	0.4	0.2	2.8	6.9
5	706	68	141	6.9	48.8	7.8	0.50	0.63	89	93	0.3	0.5	3.1	6.4

The differences among species may be attributed to differences in the regulation of nutrient uptake and/or differences in the regulation of nutrient transport between root and shoot. Among the possible reasons for unusual nutrient concentrations of Zn and Mn may be different physiological requirements and/or ecological strategies in hemlock.

A situation similar to hemlock has been found for black spruce and jack pine. Black spruce has higher foliar concentrations of manganese as compared with jack pine (Morrison and Armson 1968; Lafond and Laflamme 1968). This is an example of how the physiology of the plant is tied into its ecology. Morrison and Armson (1968) contend that the high Mn levels of the spruce foliage create Mn-rich surface layers which allow regeneration of spruce but inhibit the regeneration of jack pine.

2. Extractable Zn and Mn

Douglas-fir has higher water-soluble Zn than hemlock but amabilis fir has similar levels of water-soluble Zn to hemlock. This may suggest that Douglas-fir has a higher component of active zinc, which might indicate a higher requirement for Zn than in hemlock and amabilis fir. The higher total and water-soluble Zn in white pine than hemlock which in turn had a higher level than cedar suggests a relative physiological requirement in the order pine>hemlock>cedar.

The higher level of water-soluble Mn in hemlock than Douglas-fir, amabilis fir, pine and cedar may indicate a higher requirement for Mn by hemlock.

3. Cellular Fractions of Foliage

Fractions of foliage represent different cellular components which are involved in specific physiological processes. For example, fraction B and C represent the chloroplastic component which are involved in the process of photosynthesis.

Hemlock had lower Zn in most fractions compared to Douglas-fir. This relationship is consistent with total foliar levels. Therefore total foliar Zn concentrations may reflect physiological levels of Zn. The method used to separate fraction E is similar to the method used by Rahimi and Schropp (1984), in which they measured the physiologically active Zn associated with carbonic anhydrase activity. Therefore the Zn level in the E fraction may be the physiologically active Zn.

Hemlock had higher Mn levels compared to other species in all fractions. These results for Mn may have several interpretations. Higher levels may mean that hemlock has a greater physiological requirement for Mn, or alternatively may represent some Mn accumulation as a tolerance mechanism.

Therefore, total foliar Mn levels may not necessarily be an indication of physiologically active Mn.

The only other study which has examined Mn in cellular fractions of foliage was by Memon (1984). He examined the manganese concentration of different cell fractions in the foliage of a Mn accumulator. Manganese was highest in the supernatant fraction which in this study was part of fraction E.

B. Fertilization Experiments

1. Nutrient and Growth Responses

There were positive linear and curvilinear relationships between current height increment and foliar mass per shoot. There is evidence which supports a relationship between foliar mass and growth. For example, there is a curvilinear relationship between leaf area index and the net primary productivity. Net primary productivity reaches a maximum with a specific leaf area index but above this point yield is reduced because of high respiratory losses required to maintain a large volume of leaf and supporting tissues (Kramer and Kozlowski 1979). Ford (1984) reviews a study in which a positive correlation was found between annual branch-and-bole production

and foliage biomass for both conifers and deciduous trees. However, the study did not show as the authors claim that there is "no decrease in dry matter production even at the highest leaf biomass". To do this they should have demonstrated no evidence of a curvilinear relationship (Ford 1984). Therefore, it has not been proven that tree growth may continue to increase indefinitely as foliage amount increases (Ford 1984). Some equations fitted to data from this thesis suggest that there may be a decrease; however, unambiguous evidence has not been obtained.

a. Zinc

i. Comparison of Foliar Versus Soil Treatments

The foliar and soil methods of application were used in order to test whether some factor(s) [e.g. of the soil, the uptake mechanism, or translocation] was interfering with the movement of soil-applied zinc to the foliage. The foliar hemlock nutrient data indicate the greater efficiency of Zn uptake with foliar applications compared to soil applications. Foliar applications of Zn have been found to be up to 12 times more efficient than soil applications (Murphy and Walsh 1972). Stout *et al.* (1987), found that with alfalfa, Zn recovery in the plant was less than 2% of the soil-applied Zn. Since in hemlock there was a delay in zinc response to soil applications, and the changes in foliar concentration were not as dramatic as the

foliar treatments, one or more of the above mentioned factors may have accounted for the pattern of response observed for soil Zn applications.

In agriculture, zinc is applied to the soil for row crops and as a foliar spray for tree and vine crops (Traynor 1980). Chevis (1983) found that a 2.5 mg L^{-1} zinc sulphate foliar spray raised the foliar zinc concentrations in *Pinus radiata*, correcting a deficiency, while soil applications of zinc oxide did not correct the deficiency. Seedlings of *P. elliottii* grown in pots took up zinc in the foliage when zinc was applied to the soil in a solution form (van Lear and Smith 1972). McKee (1976) found that seedlings of *P. elliottii* responded to zinc applied in solution form to the soil in a pot experiment. Seedlings of *P. radiata* did not display symptoms of zinc deficiency when it was supplied in solution culture (Smith and Bayliss 1942). McGrath (1978) concluded that application of zinc as a foliar spray was a more effective and reliable method of supplying zinc to *P. radiata* than addition to the soil of zinc oxide with superphosphate. Symptoms of zinc deficiency were overcome when a 1% solution of zinc chloride was applied to the foliage (Kessel and Stoate 1936). Foliar applications of 0.6 mg L^{-1} zinc to seedlings of *P. radiata* in the nursery raised their foliar zinc concentrations and prevented visual symptoms of zinc deficiency (Knight 1975). A foliar application of 9,800 µg g^{-1} of zinc chelate corrected the growth disorders of 46-month-old trees of *P. caribaea* (Rance *et al.* 1982). Stoate (1950) found that foliar

applications of 2.5 mg L^{-1} zinc sulphate as a foliar spray or soil dressings of 0.12 grams to 1.8 kg zinc sulphate to individual trees prevented the continuation of growth disorders in *P. radiata*.

Firstly, availability may have been affected by factors of the soil. Availability of Zn from the soil may be a problem due to transport from the soil to the root. Autoradiographs of wheat roots using ^{65}Zn have indicated zones of depletion around the roots. This depletion indicates the creation of a concentration gradient, and that the ion moves in part by diffusion (Wilkinson *et al.* 1968). Since diffusion is important in the transport of the ion, root contact with the soil is important in the absorption of zinc (Boawn *et al.* 1957). This is demonstrated by the fact that availability of zinc is increased when it is mixed with the soil rather than applied in a band (Shaw *et al.* 1954; Murphy and Walsh 1972). Wider distribution would increase its contact with roots (Murphy and Walsh 1972).

The soil pH affects the availability of zinc through its solubility. Studies examining the influence of pH on zinc adsorption show a decrease in zinc solubility with increasing pH (Saed and Fox 1977; Bar-Yosef 1979; McBride and Blasiak 1979). The solubility of zinc decreases 100-fold for each unit increase in pH (Tisdale *et al.* 1985). With soil acidification, plant uptake of soil Zn or banded applications were increased (Viets *et al.* 1957).

In a study by Maclean (1974), corn, lettuce and alfalfa plants were grown with Zn levels similar to those found in the soils used in this study (Table 30). It is interesting to note that the soils had a DTPA-extractable Zn of 26.8 $\mu g\ g^{-1}$, and a pH of 4.9 while the corresponding foliar levels of the plants were 238 (corn), 523 (lettuce), and 321 (alfalfa) $\mu g\ g^{-1}$, which were toxic to growth. Since the soil Zn levels found by Maclean (1974) were toxic to plants, Zn availability in hemlock may be limited by uptake or some interaction but not by soil supply. Two additional sources of information suggest this. First, some other conifer species on the same sites have higher foliar Zn concentrations than hemlock. Second, increased uptake of Zn occurred with the "complete-Zn-Mn" treatment indicating additional soil Zn was available.

Therefore, with the soil pH levels of the study sites being relatively low (3-4), the high inherent supply of available Zn, the use of broadcast applications, and the use of the soluble $ZnSO_4$, it is unlikely that there was a problem of Zn fixation, insolubility or low soil Zn levels.

The second possible reason involves the uptake and translocation of zinc. This involves two aspects; firstly the physiological requirements of the plant for Zn, and secondly the ecological aspect of metal tolerance. Hemlock may accumulate Zn in the roots and restrict transport from the roots to the shoots,

or restrict uptake from the soil to the root. Work done with several species [soybean (White *et al.* 1979), bush beans (Ruano *et al.* 1988; Hawf and Smith 1967), maize and barley (Singh and Steenberg 1974), maize (Nair and Prabhat 1977), various species (Carroll and Loneragan 1968), and subterranean clover (Riceman and Jones 1958)] indicates that with Zn application, Zn accumulates in the roots relative to the shoot.

The delay in foliar response to soil applications is similar to the situation found by West (1979) with Cu in *P. radiata*. Roots tended to accumulate Cu, but over time there was a net decrease in root Cu levels and an increase in shoot Cu levels. It appears that some of the excess Cu, stored in roots which had been exposed to high Cu levels, was released to shoots when sufficient Cu was no longer available (West 1979).

The results of the foliar and soil Zn treatments suggest that it is factors of the plant related to uptake and/or translocation which account for the observed pattern of response to Zn, and hence the characteristic Zn nutrition of hemlock.

ii. Zinc Tolerance

Of the three types of plant-soil nutritional relationships (accumulator, excluder and indicator), hemlock may be an excluder with respect to Zn, judging from the pattern of response to soil Zn treatments. Observations of metal-resistant plants

from different ecological situations show that tolerance is not achieved by exclusion with respect to uptake (Ernst 1975). Restriction of transport from root to shoot is the likely mechanism of control (Baker 1981; Woolhouse 1983; Foy *et al.* 1978). Consequently, metal concentrations in the shoot are maintained constant and low over a wide range of soil concentrations, up to a critical soil value above which the mechanism breaks down and unrestricted transport results (Baker 1981). This phenomenon of Zn exclusion has been examined in several species. For example, different populations of *Silene maritima* from Zn-contaminated soils, when grown in solution culture, excluded Zn from their shoots (Thurman 1981). *Agrostis tenuis* is a species of grass which has zinc-tolerant clones in which tolerance is conferred on the plant through increased retention of Zn in the roots (Woolhouse 1983). Zinc-tolerant clones of the grasses *Deschampsia caespitosa* and *Anthoxanthum odoratum* concentrated Zn in their roots (Brookes *et al.* 1981). Seedlings of hemlock grown in solution culture were found to have higher zinc concentrations in the roots compared to the foliage (Table 31) (Zasoski *et al.* 1990).

In excluders, detoxification occurs largely within the roots (Baker 1981). The mechanisms by which Zn may be detoxified in the root are immobilization of Zn in cell walls and compartmentation as a soluble complex, free ion or insoluble complex (metallothionein) (Woolhouse 1983).

Table 31. Effect of solution pH on micronutrient concentrations (mg kg^{-1}) in Douglas-fir and western hemlock roots (R) and needles (L) (from Zasoski *et al.* 1990)).

		Solution pH				
		3	3.5	4	4.5	5
		Douglas-fir				
Fe	R	424	666	1180	1810	2240
	L	78	80	90	95	88
Mn	R	21	27	25	27	30
	L	87	97	100	102	97
Zn	R	90	87	78	80	74
	L	47	45	46	48	43
Cu	R	53	84	95	129	144
	L	28	32	30	34	31
		Western Hemlock				
Fe	R	301	779	1950	3290	3780
	L	104	112	116	141	142
Mn	R	34	37	37	39	42
	L	265	270	266	289	256
Zn	R	78	69	74	84	77
	L	35	41	33	39	34
Cu	R	54	63	78	110	117
	L	54	55	60	66	63

Denny and Wilkins (1987a), studying zinc tolerance in *Betula*, rejected the tolerance mechanisms of internal detoxification and cell wall binding. They found using microanalysis that at Zn concentrations above the lethal level, Zn accumulated intracellularly, at the endodermis, in the form of electron-dense granules. They also investigated the mechanism of ectomycorrhizal amelioration of zinc toxicity to *Betula*. As the fungal hyphae penetrate the soil, Zn is adsorbed to the surface of hyphae, thereby lowering the concentration of zinc in the soil solution surrounding the roots. The metal may also be adsorbed to electro-negative sites in the hyphal cell walls and to extra-hyphal, polysaccharide slime (Denny and Wilkins 1987b). Another method of detoxification has been investigated by van Steveninck *et al.* (1987). They found high levels of Zn in globules in the roots of zinc-tolerant ecotype of *Deschampsia caespitosa*. The globules appear to occur in small vacuoles within the cytoplasmic matrix of elongating cells in the cortex. An additional mechanism is that Zn may be concentrated by the mycorrhizae. Zinc has been found to be concentrated in the roots of mycorrhizal plants of *Pinus virginiana* (Miller and Rudolph 1986), *Betula* (Brown and Wilkins 1985), and *Ericaceous* plants (Bradley *et al.* 1982) compared to uninfected plants. Since hemlock roots are known to be highly mycorrhizal (Gill and Lavender 1983), it is possible that such a mechanism is operating in hemlock.

b. Manganese

i. Comparison of Foliar Versus Soil Treatments

Nutrient responses to soil applications of Mn were much more efficient than foliar applications. In addition, responses were consistent with soil applications.

The reasons for the different responses between Zn and Mn when applied as a foliar spray require an examination of the process of solute movement through the foliage as outlined in Figure 26. Foliar uptake of nutrients involves deposition, absorption through the cuticle, epidermal cells (Bukovac and Wittwar 1957), and the stomates (Swietlik and Faust 1984), adsorption on the surface of the plasma membranes, passage through the plasma membranes and movement into the cytoplasm (Swietlik and Faust 1984). Initial penetration of the cuticle occurs by diffusion, which depends upon temperature and a concentration gradient (Swietlik and Faust 1984). Manganese deficiency was corrected in *P. radiata* using foliar sprays with Mn at 8.5 and 8.75 mg L^{-1} compared to the foliar levels of 10 µg g^{-1} (Ruiter 1983). The concentration of manganese in the solution may have been too small relative to the concentration in the foliage for diffusion to occur. In agriculture, foliar application of Mn is one of the most efficient ways of correcting Mn deficiency (Murphy and Walsh 1972), and $MnSO_4$, which was used in this study, is considered to be the most effective inorganic carrier of Mn (Murphy and Walsh 1972).

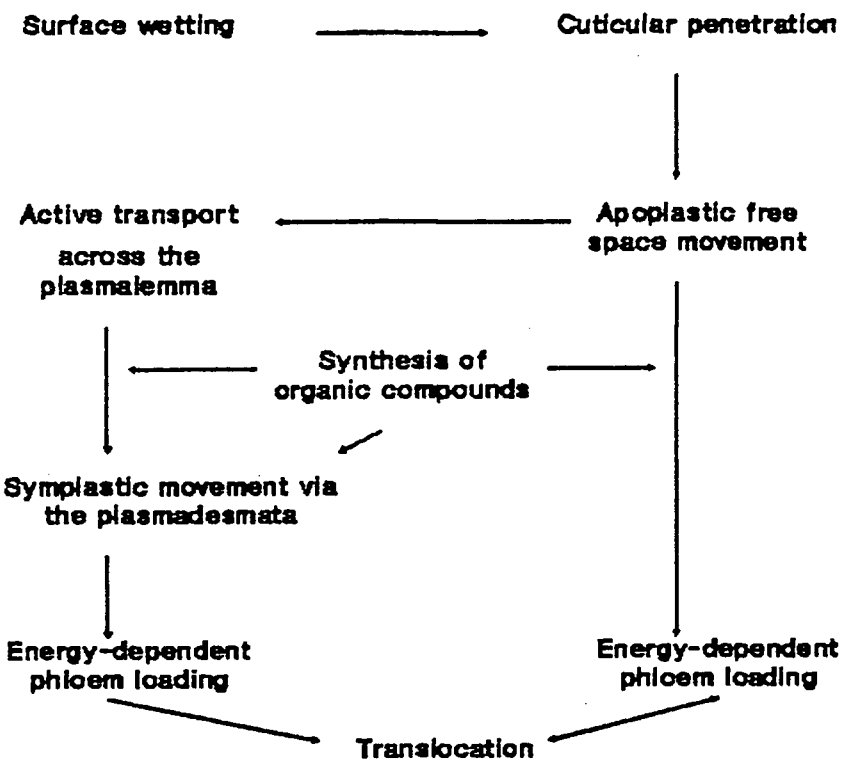


Figure 26. Possible pathways of solute movement through the leaf (from Haynes (1986)).

Therefore the lack of response to foliar applied Mn found in this thesis is probably not due to the application method *per se* or the type of carrier.

Differences in the foliar uptake and translocation of Zn and Mn have been demonstrated in several studies. Bukovac and Wittwar (1957) found that ^{65}Zn moved through the transpiration stream, whereas Mn tended to concentrate where it was applied and a greater percentage of the Zn was absorbed. Romey and Toth (1954) found ^{54}Mn to be absorbed through the foliage although there were differences between plant species in the amount absorbed. But, Mn was again found to concentrate in the interveinal tissues, forming small islands. Chamel (1985) found Mn to be poorly absorbed, since discs which were initially charged with ^{54}Mn lost almost all the applied Mn by washing, whereas 80% of the ^{65}Zn was lost. He concluded that cuticular retention is dependent upon the element considered. Manganese has been found to be readily leached from foliage (Tukey *et al.* 1958). However, in another study with pea plants there was 100% absorption of Mn into the treated area of the leaf (Ferrandon and Chamel 1988). In this study, the characteristics of the element, the concentration of the solution, and the characteristics of the foliage may have all contributed to the low Mn absorption by the hemlock foliage.

ii. Toxicity Levels

The results indicate a growth response to soil-applied Mn. This is interesting because the foliar levels for the control trees are higher than toxicity levels reported for agronomic crop plants. Reported toxicity levels are 1,000 μg g^{-1} in beans, 550 μg g^{-1} in peas, and 200 μg g^{-1} in barley (White 1970), 380 μg g^{-1} in *Medicago sativa*, 1,600 μg g^{-1} in *Centrosema pubescens*, > 2,600 μg g^{-1} in carrots, > 160 μg g^{-1} in Bragg soybeans, 450-500 μg g^{-1} in tomato (young leaves) (Foy *et al.* 1978), 120-600 μg g^{-1} in apple, 445-1400 μg g^{-1}, respectively, in upper and lower leaves of sweet sorghum, 500 μg g^{-1} in flax, 500-1,960 μg g^{-1} in cotton, 2,000 μg g^{-1} in Easter lily, 2,600 μg g^{-1} in carnation, 2,500-6,500 μg g^{-1} in maize (Foy 1983), 1,200-2,600 μg g^{-1} in coffee, and 120 μg g^{-1} in snap beans (Foy 1984).

There are several examples of Mn foliar levels and the occurrence of toxicity symptoms in forest trees. Dying European fir seedlings having blackened roots suggestive of a Mn toxicity had foliar levels of 1,300 to 2,500 μg g^{-1} compared to 120 to 500 for living seedlings (Stone 1967). A 60% reduction in growth of black spruce and jack pine seedlings grown in solution culture was noted when the culture solution contained 100 μg g^{-1} Mn, and the corresponding foliar Mn levels of the seedlings were 4,200 to 4,400 μg g^{-1} (Morrison and Armson 1966). Although hemlock had higher or similar foliar Mn levels for the Mn soil treatments compared to the toxicity levels reported in the literature for other plants, there was no evidence of a Mn toxicity in this study.

iii. Manganese Requirements

The foliar Mn levels are higher for hemlock than for other tree species in the same stands. In addition, there was a foliar nutrient concentration response to soil applications of Mn, without a reduction in growth. These facts suggest that hemlock is manganese-tolerant and has a greater total Mn requirement compared to some other B.C. conifers. This is consistent with other studies which have reported an increased need for metals in metal-tolerant ecotypes of many species. Examples of these are wheat (Mn) (Macfie 1989; Foy *et al.* 1973), copper moss (*Scopelophila cataractae*) (Shaw 1987), *Agrostis tenuis* (Cu, Pb, Zn), *Mimulus guttatus* (Cu), *Anthoxanthum odoratum* (Zn), *Holcus lanatus* (Zn), *Armeria maritima* (Zn), *Silene vulgaris* (Zn) (Antonovics 1971), *Succisa pratensis* (Mn) (Pegtel 1986), *Avenella flexuosa*, *Chamaenerion angustifolium*, *Rumex acetosella*, and *Senecio sylvaticus* (Mn) (Ernst and Nelissen 1979). This phenomenon has been explained by invoking zinc-tolerant plants. If a tolerant plant complexes the metal in order to detoxify it, one would expect a shortage in these plants on normal soils (Ernst 1975). The biomass production would be diminished and can be stimulated by Zn concentrations which are already toxic for non-tolerant plants. The activity of carbonic anhydrase was found to be stimulated at high amounts of zinc in tolerant populations, but not in non-tolerant populations (Ernst 1975). Therefore, because of the efficiency of the tolerance mechanism

in inactivating the metals, the external trace element requirement is higher (Antonovics *et al.* 1971).

vi. Manganese Tolerance

Hemlock is similar to other plants growing on acidic substrates in that it is a manganese accumulator which enables it to tolerate this type of environment. These types of plants are classified as calcifuges, which are plants adapted to low soil pH. *Ericaceae* is a family of plants which like hemlock are calcifuges. Species in the *Ericaceae* belonging to the genus *Vaccinium* also occur in stands of hemlock. This is of interest because some species of *Vaccinium* are of agronomic importance and work has been done on their nutrition.

Of the three types of plant-soil relationships, hemlock may be classified as an accumulator with respect to Mn. This is similar to the *Vaccinium* where internal tolerance especially in leaf tissue is the mechanism of tolerance (Korcak 1988). Foliar levels of Mn in *Vaccinium* species have been reported in excess of 2,000 to 4,000 $\mu g\ g^{-1}$ (Korcak 1988). There are several mechanisms by which the high levels of manganese in the foliage may be detoxified. One hypothesis is that of compartmentalization (Foy *et al.* 1978). This is where ions are excluded from the active parts of the cell and concentrated in vacuolar and other inert regions as complexes or ions (Ernst 1975). There is further support for compartmentation as a

detoxifying mechanism. Memon and Yatazawa (1984) identified a manganese-oxalate complex in the supernatant fraction of leaf cells of the manganese accumulator *Acanthopanax sciadophylloides*. The supernatant constituted the vacuolar fraction and had the highest Mn concentration of all the fractions. Similarly, hemlock may tolerate high Mn by isolating it in vacuoles since, from the cellular fraction study, this fraction had the highest Mn level. The Mn may be in the free ionic state since most of the foliar Mn was water-soluble, or it may be weakly complexed judging from Memon and Yatazawa's findings (1982, 1984).

C. Comparison and Consideration of Adequate Levels.

i. Individual Nutrients

According to vector analysis some of these hemlock stands had deficiencies of nutrients in addition to Zn and Mn (Figures 20 and 21). Although vector analysis indicates a strong Zn deficiency there were strong responses to other nutrients. It is interesting to consider these results in light of the laws of limiting factors. There are two laws which describe the relation of growth to limiting factors. Liebig's law of the minimum suggests growth is controlled by the most limiting factor. That is growth response to a nutrient will not occur unless deficiency of the most limiting nutreint is at first alleviated. This law is analogous to a barrel with staves of different sizes. The ability of the barrel to hold water is limited by the shortest

stave. This law may be limited to the situation in biology where the minimum factor is so low as to stop the process entirely (Daniel *et al.* 1979), perhaps where visual symptoms of a deficiency are evident. Mitscherlich's law of the minimum argues that increasing any factor that is below its optimum will improve growth, but increasing the factor furthest from its optimum will give the greatest increase in growth (Daniel *et al.* 1979). A response occurred to a "complete-Zn-Mn" treatment even though Zn was shown from vector analysis as being strongly limiting. This treatment was synergistic to Zn uptake. Therefore, it is difficult to say whether response was due to a nutrient in the "complete-Zn-Mn" treatment, the Zn, or an improvement in nutrient balance. According to the "barrel" analogy, a growth response should not have occurred in this study unless the Zn deficiency was first alleviated. This seems to demonstrate the limited applicability of the "barrel" approach in biological systems. It does not recognize that there are interactions between nutrients, such as synergisms or antagonisms, or a nutrient may improve growth creating a sink for another nutrient.

The response data from the "complete-Zn-Mn" treatment (Table 13) were compared to existing foliar nutrient interpretations (Appendix I), to assess if the interpretations from the guidelines were consistent with the observed results.

Ballard and Carter (1985) in their review (Appendix I) indicate an adequate foliar N threshold of 1.45 cg g^{-1}. Both

sites had foliar N levels below 1.0 cg g^{-1} and 1.11 cg g^{-1} in the year of fertilization and they responded to 100 kg ha^{-1} of N. This is in the severely deficient range. Fertilization brought foliar levels up to 1.67 cg g^{-1} for both sites, which is adequate. Hardie (1985) found that 100 kg ha^{-1} of N and 150 kg ha^{-1} of P did not affect foliar N concentrations but total seedling dry mass was increased over the control. The control and treated foliar concentrations were both above the adequate threshold. Weetman *et al.* (1989) have also identified deficiencies of N and P in young regeneration of coastal western hemlock using foliar analysis. Deficiencies of N and P were confirmed by subsequent 3-year height growth response. Data from Gill and Lavender (1983) showed a response to 224 kg N ha^{-1}, with control trees having foliar N levels of 1.33 to 1.53 cg g^{-1}. Seedlings in the field having foliar N levels of 0.96 cg g^{-1} (severely deficient) responded to 200, 300 and 400 kg N ha^{-1} (Germain 1984). Zasoski and Gessel (1982) found that seedlings having a foliar N level of 0.88 cg g^{-1} (very severely deficient) responded to application of 198 kg N ha^{-1}. In a study of 940 plots of immature stands of Douglas-fir and hemlock in coastal B.C. measured over 12 years, 50 - 70% of hemlock stands responded to N fertilization. Fertilization increased both total and merchantable volume but height was not significantly affected. Unthinned hemlock stands on fresh and moist sites responded to fertilization better than did those on the moderately dry sites (Omule 1990).

After the first year, site 4 had a control P level of 0.197 cg g^{-1} and site 5 had 0.182 cg g^{-1}. After the second year, site 4 had a foliar P level of 0.183 cg g^{-1} and site 5 had 0.167 cg g^{-1}. When foliar P dropped to 0.167 cg g^{-1} on site 5 after the second year, there was a nutrient response to P. When the foliar P level was higher there was no response. There have been reports of hemlock response to P fertilization. Hardie (1985) found increased P in seedlings fertilized with 150 kg P ha^{-1} with controls having a mean foliar P level of 0.124 cg g^{-1}. Ballard and Carter (1985) suggest 0.15-0.35 cg g^{-1} represents the range of slightly deficient to adequate. Data from Heilman and Ekuan (1980) indicate that a P response to 300 kg of P ha^{-1} occurred to seedlings with foliar P levels in the severely deficient range (<0.11 cg g^{-1}). Germain (1984) found that applications of P at either 50 kg ha^{-1} or 38 kg ha^{-1} increased foliar P levels of seedlings in the field which had control levels of 0.13 µg g^{-1}. Zasoski and Gessel (1982) found that seedlings which had a foliar level of 0.08 cg g^{-1}, which is considered deficient, responded to a P application of 447 kg ha^{-1}.

Adequate levels for foliar B are suggested to be in the range of 10-15 µg g^{-1} (Ballard and Carter 1985). An experiment by Majid (1984) with greenhouse grown lodgepole pine seedlings suggests a critical range for foliar B of 7 to 16 µg g^{-1}. Control foliar B levels for sites 4 and 5 were 24.5 and 28.4 µg g^{-1} respectively, and B fertilization increased them to 33.2 and 45.8 µg g^{-1} respectively. These are much greater than the

suggested critical range and the B response vectors (Figures 20 and 21) indicate a greater requirement for B.

A foliar concentration of 4 μg g^{-1} is proposed to be an adequate level for Cu. Field trials with lodgepole pine indicate a critical value for foliar Cu to be 4 μg g^{-1} (Majid 1984). Foliar Cu on site 4 was 3.2 μg g^{-1} and 3.1 for site 5. Fertilization increased foliar Cu on site 5 to 4 μg g^{-1} which is inferred to be just adequate with evidence of a deficiency from Figure 21A. Although Cu on site 4 may be classified as possibly inadequate there was no significant response.

Foliar S concentrations were 0.16 and 0.12 cg g^{-1} for sites 4 and 5 respectively, and the N/S ratio of control foliage on site 5 was 9.0. According to the diagnostic guidelines in Appendix I, site 4 is between a S deficiency unlikely and no S deficiency, there is no S deficiency, and a N induced deficiency is unlikely. The recommendations from the guidelines appear to be consistent with the results of the S treatments in which there was no increased uptake, suggesting that S is not limiting.

There was no response to Ca, K, or Mg. Foliar Ca was 0.28 and 0.32 cg g^{-1} for sites 4 and 5, respectively, which is greater than 0.2 cg g^{-1} critical value which is considered to be adequate. Foliar K was 0.68 and 0.78 cg g^{-1} for sites 4 and 5 respectively. An adequate value for hemlock is suggested to be 0.8 cg g^{-1}. However, since there was no increased nutrient

uptake, a lower value may perhaps be considered as adequate. Foliar Mg was 0.14 cg g^{-1} for both sites, which was sufficient compared to the level considered adequate of 0.12 cg g^{-1}. Since there was no response to Ca, K or Mg fertilization, these limits considered adequate may be applicable for hemlock. In the case of K this limit may be reduced.

The increase in foliar active Fe concentration on site 5 after the first year suggests that physiologically active iron is limiting. Foliar active Fe went from 26.2 µg g^{-1} for the control to 34.8 for the treatment. The guidelines suggest that 30 µg g^{-1} separates the "deficiency likely" category from the "deficiency unlikely" category. This seems to be a suitable recommendation for hemlock since an Fe nutrient concentration response occurred when foliar levels were below 30 µg g^{-1} on site 5, but there was no significant response where foliar levels where above 30 as on site 4. Foliar Fe levels were 25 and 39 µg g^{-1} for sites 4 and 5 respectively which are in the range of possible deficiency (25-50 µg g^{-1}).

Control foliar Zn concentrations (Table 8) are below the level considered to be adequate: 15 µg g^{-1}. Since positive growth responses occurred to Zn fertilization, the diagnostic norms for Zn appear to adequately describe Zn nutrition of hemlock. Further evidence of a Zn deficiency comes from the strong Zn response to the "complete-Zn-Mn" treatment (Figures 20 and 21)

The diagnostic norms for Mn need to be revised for hemlock. Although foliar Mn concentrations for the controls (Table 8) were higher than the "stated" adequate level of 25 μg g^{-1}, growth response to Mn fertilization occurred.

ii. Nutrient Balance

Ingestad's nutrient ratios were calculated for the foliar nutrients from the "complete -Zn -Mn" and the control treatments for sites 4 and 5. These data are presented in Table 32 along with Ingestad's ratios considered to be optimum for growth (Ingestad 1979). The nutrient ratios for both the "complete -Zn-Mn" and control treatments tend to be in balance relative to the optimum ratios. The exceptions are Mn and Fe which may be considered to be out of balance with respect to N, with Mn being too high and Fe being too low. This may be an indication of an insufficient supply of Fe. The "complete -Zn -Mn" treatment tended to maintain the foliar nutrient balance; however, after the first year of treatment, fertilization tended to decrease the ratios of K, P, and Cu from the optimum ratios. Since there had been an increase in foliar P and Cu concentrations, the decrease to suboptimal ratios suggests that these levels are insufficient and further additions are required.

Table 32. Ingestad's foliar nutrient ratios for the complete -Zn -Mn and control treatments on sites 4 and 5 for the years 1986 and 1987. The optimum ratios are from Ingestad (1979).

Zn	Mn	K	Ca	Mg	P	TS
Site 4 1986						
Complete						
0.1	8	39.8	18.1	7.1	9.6	10.4
Control						
0.087	14.3	68	27.7	14	19.7	16
Site 4 1987						
Complete						
0.09	10.3	57.5	26.1	11.7	17	
Control						
0.1	13.2	65.1	27.9	13.6	15.6	
Site 5 1986						
Complete						
0.09	6.4	48.4	18.1	8.2	12.8	
Control						
0.1	11.7	69.7	28.7	12.6	16.3	
Site 5 1987						
Complete						
0.11	7.3	65.2	25.5	11.3	18.3	
Control						
0.1	10.8	72.7	27.3	11.4	14.4	

Table 28 (concluded).

Fe	Cu	B	AFe	AZn	AMn
Site 4 1986					
Complete					
0.23	0.021	0.2	0.22	0.11	2.66
Control					
0.25	0.032	0.25	0.32	0.068	12.4
Site 4 1987					
Control					
0.42	0.031				
Complete					
0.42	0.031				
Site 5 1986					
Complete					
6.8	0.28	0.024	0.27	0.21	
Control					
11.1	0.35	0.028	0.26	0.24	
Site 5 1987					
Complete					
0.41	0.077				
Control					
0.46	0.034				

Ingestad's Ratio

Zn	Mn	K	Ca	Mg	P
0.03	0.4	70	8	5	16

TS	Fe	Cu	B
9	7	0.03	0.2

Since there had been no increase in the foliar concentration of K but uptake had been maintained and there was a growth response, these results could suggest that the true optimum ratio for K is actually not so high.

D. Retranslocation

The existence of nutrient deficiencies may be assessed by determining the occurrence and the extent of nutrient remobilization. Remobilization of mineral nutrients is important during ontogenesis in periods of insufficient supply of nutrients to the roots during vegetative growth (Kramer and Kozlowski 1979). If a plant is under a nutrient stress some nutrients will be remobilized from the old foliage and translocated to the new foliage. This retranslocation could then be used as a means of diagnosing the nutrient status of a plant.

In the second year following soil applications of Mn the current year's foliage and two-year-old foliage continued to accumulate Mn. Therefore, it could not be determined if retranslocation occurred. Retranslocation of Zn occurred in the second year following fertilization from the the high foliar Zn treatment. This result was in contrast to the situation in *P. radiata* found by McGrath and Robson (1984). In their seedlings, the Zn concentration of the older primary needles remained constant regardless of the Zn status of the seedlings. In contrast, the results from this research for Zn are consistent

with the finding that remobilization of Zn from old leaves depends upon the Zn status of the plant. Zn is more mobile when the concentration is high (Kramer and Kozlowski 1979). For example, subterranean clover plants given luxury supplies of Zn moved up to 25% of the Zn in their old leaves and petioles into developing fruits, whereas those given deficient or marginal supplies moved little or none at all (Loneragan 1976).

There is evidence that the decrease in foliar Zn concentrations in the two-year-old foliage was due to remobilization. Firstly, foliar Zn concentrations in the control foliage did not decrease with foliar age. Secondly, Zn has been found to be leached with difficulty. Less than 1% of the Zn was leached with water applied for 24 hours to young leaves of squash and beans (Tukey *et al.* 1958).

E. Nutrient Interactions

Applications of Zn had no effect on uptake of Mn and in some cases applications of Mn increased foliar Zn. In soybeans, Zn has been shown to increase the translocation of Mn to the tops of the plants, which can lead to Mn toxicity (Foy 1983). However, an inverse relationship was found between Zn application and Mn in soybean (White *et al.* 1979), maize (Singh and Steenberg 1974), and bush beans (Ruano *et al.* 1987).

Since there appeared to be no antagonism between foliar Zn concentrations and Mn applications or foliar Mn concentrations and Zn applications, the evidence from this study indicates that it is unlikely the lower foliar Zn levels in these hemlock stands are due to Mn antagonism. The evidence from the literature suggests an antagonism between Zn and Mn (Reddy *et al.* 1978; Nair *et al.* 1977; Malavolta *et al.* 1956; Hawf *et al.* 1967). However, in agreement with the results in this study, Kohno *et al.* (1984) found in bean plants and Shuman and Anderson (1976) found in soybeans that the foliar levels of Zn increased with increasing Mn concentrations in the solution.

It is interesting that soil applications of Zn did not increase foliar Zn after the first year but in some cases soil applications of Mn did. Since there was a positive growth response with Mn in some cases this may have created a sink for Zn. This may suggest an increased Zn requirement with Mn.

With similar soil application levels of Zn and Mn (200 kg ha^{-1}) a greater percentage of Mn was found in the foliage compared to the Zn treatment. This suggests different soil chemical reactions or plant uptake/translocation mechanisms for the two elements. Different retention and/or release mechanisms of the soil for Zn and Mn may be ruled out because hemlock had different foliar levels of Zn and Mn in comparison to other tree species in the same stands. This would indicate that the differences between foliar levels of the two elements is due to

plant factors such as differences in nutrient uptake or in translocation. Nair and Prabhat (1977), found that with identical application rates, relatively more Zn was root absorbed than Mn, but of the amount root absorbed, relatively more Mn was translocated to the shoots. This would suggest different mechanisms of translocation for Zn and Mn.

An interaction was found between foliar Zn and foliar N concentrations. An interaction between Zn and N in trees has been found in several other studies. Results of Zasoski and Porada (1986) indicate a strong relationship between N and Zn in hemlock growing on slashburnt sites. Foliar levels were <1.1 cg g^{-1} N and <12 μg g^{-1} Zn, which according to a DRIS analysis were strongly growth limiting. High applications of N did not stimulate growth nor increase foliar N levels, which suggested that Zn was inadequate for N. McGrath and Robson (1984) investigated the effect of N and P supply on the response of *P. radiata* to Zn. They found that a response to Zn depended on the requirements for N and P being provided.

Evidence from these empirical studies implies an interaction between Zn and N. There is evidence in the literature to support a functional relationship between Zn and N. Firstly, this interaction may occur because of the effect of one element on the transport of the other element in the soil or the plant. For example transition metals such as Zn have high affinities for -N-ligands (Robson and Pitman 1983).

The second way in which this interaction may occur is in protein synthesis. There are three mechanisms by which Zn affects protein synthesis. Nitrogen is known to be an essential component of proteins. Zinc deficiency causes a reduction in protein and ribosomal RNA contents (Kitagishi and Obata 1986, 1987; Obata and Umebayashi 1988; Prask and Plocke 1971; Wacker 1962; Sharam *et al.* 1981). Concurrently there is an accumulation of amino acids and amides (Kitagishi and Obata 1986; Wacker 1962; Possingham 1956). The first mechanism involves the transcription of DNA by RNA. Zinc forms chemical bonds with the amino acids cystine and histidine in such a way that the chain of amino acids becomes folded around the zinc to form a loop or 'zinc finger' (Figure 27). Proteins (RNA polymerase) that regulate the transcription of DNA to RNA do so through their zinc fingers. If the zinc is absent, the protein cannot bind to DNA and regulate its transcription (Parraga *et al.* 1988). Therefore, zinc is essential in terms of its structural role. The second way is through the occurrence of Zn in the total RNA fraction, and its effect on the ribosomes; Zn may be required to maintain the structural intergrity of the ribosomes (Prask and Plocke 1971).

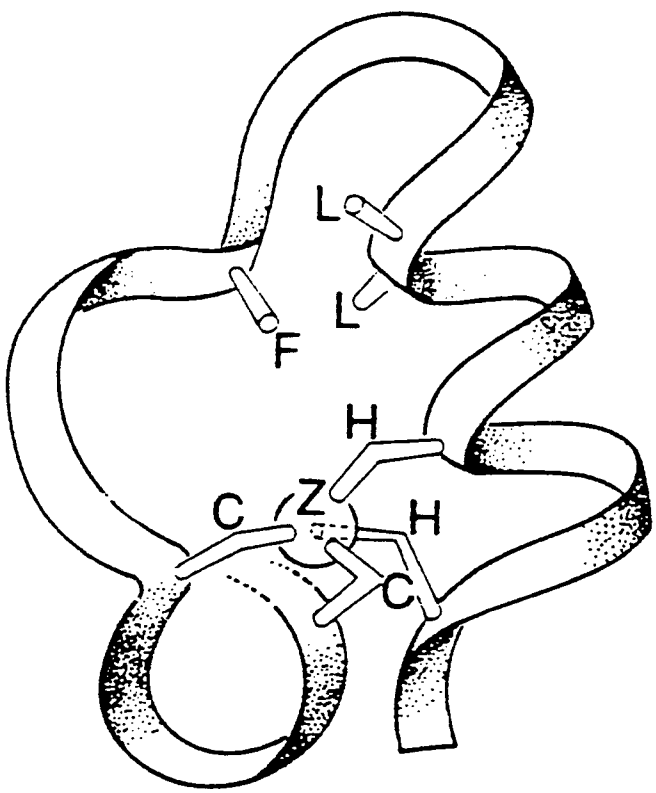


Figure 27. Ribbon model of a single zinc finger domain (ADR1a) incorporating tetrahedral coordination of zinc by cystine (C) and histidine (H) (from Parraga *et al.* (1988)).

The third mechanism involves the regulation of RNA degradation by Zn. Higher rates of RNAase activity are observed with Zn deficiency (Marschner 1986). This demonstates the importance of Zn for protein synthesis.

F. Foliar Application of Zn in Forestry

Foliar-applied sprays are the most efficient means of supplying hemlock trees with Zn. This method would appear to be of impractical use in larger fertilization experiments or in operational fertilization of stands. However, pesticides in solution form are routinely applied in operational forestry with fixed-winged aircraft and helicopters. This same technology could be applied to the foliar application of nutrients.

CHAPTER 6. CONCLUSIONS

This study was undertaken in order to investigate the the significance of the characteristic pattern of foliar Zn and Mn concentrations of hemlock in its nutrition. Two approaches to the study were used: comparative nutrition and the screening trial-growth response technique.

In a comparison of total foliar concentrations, hemlock had lower Zn compared to Douglas-fir, amabilis fir and white pine. In contrast, hemlock had higher Mn compared to Douglas-fir, amabilis fir, white pine, red cedar and yellow cedar.

Analysis of cellular fractions of foliage produced two results. First, zinc and manganese accumulated in two different fractions irrespective of the level of the treatment or the species. Accumulation in certain fractions may indicate a physiological need in that fraction or a tolerance mechanism. Second, comparing hemlock to Douglas-fir total Zn levels tend to be consistent with levels in different fractions, indicating total levels may be an adequate indication of physiological levels. Comparing hemlock to Douglas-fir, total Mn levels are consistent with Mn levels in different fractions, indicating hemlock may have a greater physiological Mn requirement or a Mn tolerance mechanism.

There were both a nutrient concentration response and a growth response to zinc applications. The timing of response was dependent upon the method of application. Response to foliar applications occurred in the first year following treatment, and response to soil applications occurred in the second year following treatment. Growth response as measured by shoot increment ratio was obtained primarily in the second year after treatment to foliar applications of zinc. Height increment ratio increased in response to foliar zinc applications in the second year.

The fact that there were cases of positive growth responses to Zn fertilization is evidence that the relatively lower foliar Zn concentrations of hemlock compared to some other conifers do indicate some deficiency of Zn in the stands used for this study. Additional evidence of a Zn deficiency are; the higher foliar Zn levels associated with optimum height increment compared to control foliar Zn levels, retranslocation of Zn in the second year following the high foliar Zn treatment and the high ranking of Zn in the vector graphs from the "complete-Zn-Mn" treatment.

In some cases, rather than a growth response to Zn, luxury consumption of Zn occurred. For plants having low nutrient requirements, luxury consumption is a physio-ecological mechanism which supplies the plant during periods of nutrient insufficiency between the pulses of luxury availability. A similar situation

may operate for hemlock, since this species has low nutrient requirements.

There were both positive nutrient and growth responses to soil Mn treatments. Positive nutrient concentration and growth responses occurred in the first year. Foliar Mn was still elevated in the second year in the one-year-old and two-year-old foliage. The elevated foliar Mn in the second year must have been from increased uptake from the soil or translocation from the roots, since there was no retranslocation from the two-year-old foliage. Hemlock has foliar Mn levels which are considered toxic in some plants, and the fact that hemlock continued to take up soil-applied Mn with positive growth responses indicate that hemlock is Mn-tolerant and also has increased requirements for Mn.

The foliar Mn levels may not adequately reflect the physiological requirements for Mn. There are both a physiological component and an ecological component of a plant's nutrition. For Mn, there may be two physiological pathways in hemlock. One pathway may be active in complexing the metal, operating as a tolerance mechanism, giving hemlock a competitive advantage in acid soil. The second pathway may channel the metal into the growth metabolism of the plant. How the allocation of manganese is regulated between the two competing paths is not known. In general this situation may apply to metal-tolerant plants where a metal on the one hand is being detoxified by the

tolerance mechanism, but on the other hand is required in growth metabolism. There must be a mechanism which regulates the allocation of the metal between the two pathways. This topic which involves both ecological and physiological plant nutrition requires greater investigation.

Determining the nutrient status of a micronutrient in a plant which is an accumulator of the micronutrient may be problematic. It is necessary to determine the physiologically active fraction of the nutrient. This may be done using extracts of the nutrient whose level is correlated to the rate of a physiological activity, or separation and analysis of cellular fractions which are involved in a physiological process.

Foliar application of Zn and soil application of Mn appeared to be the most efficient means of supplying the plant with these nutrients. Increased foliar levels of Zn did occur with soil Zn treatments but this was delayed until the second year after fertilization. It is hypothesized that this is due to inhibited uptake and/or translocation of Zn. Low foliar Zn concentrations in hemlock are not due to low inherent zinc fertility of the soil, since species which coexist with hemlock in the same stands tend to have higher foliar zinc.

A growth response (foliar mass per shoot and shoot increment ratio) was obtained with the "complete-Zn-Mn" treatment. Vector analysis which ranked the relative responses

revealed the existence of nutrient deficiencies other than Zn and Mn. Vector analysis also revealed evidence of a strong Zn deficiency. Foliar Zn was synergistic to the "complete-Zn-Mn" treatment in both the first and second years after treatment. Therefore, it was difficult to say whether the growth response was due to the applied nutrients, the Zn, or an improved balance of all nutrients. Conceivably, fertilization could lead to a Zn deficiency if the native supply in the soil cannot meet the increased demand by the plant. Such a situation is termed an induced deficiency. Induced Zn deficiency or other nutrient deficiencies may be additional reasons why growth response in hemlock to nitrogen fertilization has been inconsistent.

Ingestad's nutrient ratios were calculated for the foliar levels from the control and the "complete-Zn-Mn" treatments. Comparing these ratios to the optimum revealed that most of the nutrients were in balance except for iron and manganese.

Existing diagnostic norms for Zn appear to adequately describe the Zn nutrition of hemlock. Response to fertilization occurred with control foliar Zn concentrations for hemlock being below the critical level of 15 $\mu g\ g^{-1}$. Diagnostic norms for Mn need to be revised. Response occurred even though control foliar Mn concentrations for hemlock were well above the critical level of 25 $\mu g\ g^{-1}$. Therefore, total foliar Mn may not be indicative of the physiological Mn status of hemlock.

The empirical relationship found between foliar Zn and N suggests an interaction between the two elements. The response of the plant to the addition of one of these elements is dependent upon the adequate supply of the other element. This interaction requires further investigation to determine if variability in response to N fertilization may be affected by variability in the Zn status of the plant.

Foliar Zn was rather than being antagonistic was in some cases synergistic to soil applications of Mn . Therefore, there was no evidence in this study to suggest that low foliar levels of Zn in hemlock are due to a Mn antagonism. The only interaction obtained with the "complete-Zn -Mn" treatment was a synergism with foliar Zn.

There are several aspects of this research which require further investigation. For the information from these screening trials to be applied to the management of hemlock, several further steps need to be taken. The next step following the analysis of screening trials would be studies of correlation between site factors and responsiveness. This information would then be used for classifying operational stands into response categories. Another aspect which needs investigating is the interaction between nitrogen nutrition and zinc. Since there is evidence of deficiencies of Zn, Mn and other micronutrients optimum nutrition experiments need to include these. This may be done by supplying optimum dosages of macronutrients with

different dosages of individual micronutrients or different balances of micronutrients. Manganese nutrition of hemlock may be similar to that of other metal-accumulating plants in that there is a high requirement for the metal. This would involve investigation of the regulation of Mn between the two physiological pathways of metal-tolerance and growth. Luxury consumption may be a characteristic of plants having low nutrient requirements. Luxury uptake of nutrients would occur during periods of high nutrient availability and utilization during periods of low nutrient availability. A further study of the nutritional significance of luxury consumption of nutrients would be of interest. Some aspects which need to be resolved deal with the significance of luxury consumption to the plant's ecological strategy, physiological regulation of nutrients between accumulation and growth, and methodology to study nutrition of plants with low nutrient requirements.

LITERATURE CITED

Agren, G.I. 1985. Theory for growth of plants derived from the nitrogen productivity concept. Physiol. Plant. 64:17-28.

Anderson, S., R.J. Zasoski, and S.P. Gessel. 1982. Phosphorus and lime response of Sitka spruce, western hemlock seedlings and romaine lettuce on two coastal Washington soils. Can. J. For. Res. 12:985-991.

Antonovics, J., A.B. Bradshaw, and R.G. Turner. 1971. Heavy metal tolerance in plants. Adv. Ecol. Res. 7:1-85.

Baker, A.J.M. 1981. Accumulators and excluders- strategies in the response of plants to heavy metals. J. Plant Nutr. 3:643-654.

Baker, A.J.M. 1987. Metal tolerance. New Phytol. 106:93-111.

Ballard, T.M., and R.E. Carter. 1986. Evaluating forest stand nutrient status. B.C. Land Management Report No. 20. Victoria, B.C. 60 pp.

Ballard, T.M., and N. Majid. 1985. Use of pretreatment increment data in evaluating tree growth response to fertilization. Can. J. For. Res. 15:18-22

Bar-Akiva, A. 1971. Functional aspects of mineral nutrients in use for the evaluation of plant nutrient requirements. *In* Recent advances in plant nutrition, Vol. 1. Proc. of the 6th. Inter. Coll., March 1970. Tel-Aviv. *Edited by* R.M. Samish. pp. 115-139.

Bar-Yosef, B. 1979. pH-dependent zinc adsorption by soils. Soil Sci. Soc. Am. J. 43:1095-1099.

Bates, T.E. 1971. Factors affecting critical nutrient concentrations in plants and their evaluations: A review. Soil Sci. 112:116-130.

Bischof, W., M. Hofert, H.R. Wilde, B. Raith, and B. Gonsior. 1982. Trace element analysis of biological samples using a proton microprobe. Nuclear Instruments and Methods 197:201-207.

Boawn, L.C., F.G. Viets, Jr., and C.L. Crawford. 1957. Plant utilization of zinc from various types of zinc compounds and fertilizer materials. Soil Sci. 83:219-227.

Bowen, G.D., M.F. Skinner, and D.I. Bevege. 1974. Zinc uptake by mycorrhizal and uninfected roots of *Pinus radiata* and *Araucaria cunninghamii*. Soil Bio. Biochem. 6:141-144.

Bradley, R., A.J. Burt, and D.J. Read. 1982. The biology of mycorrhiza in the *Ericaceae*. VIII. The role of mycorrhizal infection in heavy metal resistance. New Phytol. 91:197-209.

Brown, M.T., and D.A. Wilkins. 1985. Zinc tolerance in *Betula*. New Phytol. 99:91-106.

Bukovac, M.J., and S.H. Wittwar. 1957. Absorption and mobility of foliar applied nutrients. Plant Physiol. 32:428-435.

Cakmak, I., and H. Marschner. 1987. Mechanism of phosphorus -induced zinc deficiency in cotton. III. Changes in physiological availability of zinc in plants. Physiol. Plant. 70:13-20

Campbell, I. 1989. Micro-PIXE analysis with the proton microprobe. Can. Res. May 18, 38-39.

Carroll, M.D., and J.F. Loneragan. 1968. Response of plant species to concentrations of zinc in solution. I. Growth and zinc content of plants. Aust. J. Agr. Res. 19:859-868.

Carter, R.E., A.M. Scagel, and K. Klinka. 1986. Nutritional aspects of distorted growth in immature forest stands of southwestern coastal British Columbia. Can. J. For. Res. 16:36-41.

Chamel, A. 1985. Survey of different approaches to determine the behavior of chemicals directly applied to aerial parts of plants. *In* Foliar fertilization. *Edited by* A. Alexander. Martinus Nijhoff. The Netherlands. pp. 66-86

Chamel, A.P., and B. Gambonnet. 1982. Study with isolated cuticles of the behavior of zinc applied to leaves. J. Plant Nutr. 5:153-171.

Chamel, A., R.D. Marcelle, and J.F. Elroy. 1982. Cuticular retention *in vitro* and localization of zinc after a foliar application of zinc-containing fungicides. J. Amer. Soc. Hort. Sci. 107:804-807.

Chapin, F.S. 1988. Ecological aspects of plant nutrition. Adv. Plant Nutr. 3:161-191.

Chapman, W. 1941. Leaf analysis and plant nutrition. Soil Sci. 52:63.

Chevis, H. 1983. The effect of two methods of zinc application on the growth of *Pinus radiata* D. Don on sands of the Swan coastal plain. Forests Dep. of West. Austr. Res Pap. 73. 7 pp.

Christy, E.J., P. Sollins, and J.M. Trappe. 1982. First-year survival of *Tsuga heterophylla* without mycorrhizae and subsequent ectomycorrhizal development on decaying logs and mineral soil. Can. J. Bot. 60:1601-1605.

Clarkson, D.T. 1988. The uptake and translocation of manganese by plant roots. *In* Manganese in soils and plants. *Edited by* R.D. Graham, R. J. Hannam, and N.C. Uren. Kluwer Academic Publishers. The Netherlands. pp. 101-111.

Collins, J.C. 1981. Zinc. *In* Effect of heavy metal pollution on plants, Vol. 1. *Edited by* N.W. Lepp. Applied Science Publishers, London. pp. 145-169.

Daniel, T.W., J.A. Helms, and F.S. Baker. 1979. Principles of silviculture. McGraw-Hill Book Company, New York. pp. 299-300.

DeBell, D.S., E.H. Mallonee, J.Y. Lin, and R.F. Strand. 1975. Fertilization of western hemlock; a summary of existing knowledge. Crown Zellerbach Corp. Centr. Res. Dep., For. Res. Note 5. 15 pp.

De Neeling, A.J., and W.H.O. Ernst. 1986. Manganese and aluminium tolerance of *Senecio sylvaticus* L. Acta OEcologica 7:43-56.

Del Rio, L.A., F. Sevilla, M. Gomez, J. Yanez, and J. Lopez. 1978. Superoxide dismutase: an enzyme system for the study of micronutrient interactions in plants. Planta 140:221-225.

Denny, H.J., and D.A. Wilkins. 1987a. Zinc tolerance in *Betula* spp. II. Microanalytical studies of zinc uptake into root tissues. New Phytol. 106:525-534.

Denny, H.J., and D.A. Wilkins. 1987b. Zinc tolerance in *Betula* spp. IV. The mechanism of ectomycorrhizal amelioration of zinc toxicity. New Phytol. 106:545-553.

Dill, B., and S.A. Wilson. 1985. Effect of zinc supply on growth of three species of *Eucalyptus* seedlings and wheat. Plant and Soil 88:377-384.

Dwivedi, R.S., and P.N. Takkar. 1974. Ribonuclease activity as an index of hidden hunger of zinc in crops. Plant and Soil 40:173-181.

Eis, S. 1987. Root systems of older immature hemlock, cedar, and Douglas-fir. Can. J. For. Res. 17:1348-1354.

Ernst, W.H.O. 1975. Physiology of heavy metal resistance in plants. *In* Inter. conf. on heavy metals in the environment symp. proc. Vol. II. Tor. Ont. Oct. 27-31. pp.121-135.

Everard, J. 1973. Foliar analysis, sampling methods, interpretation and application of the results. Q.J. For. 67:51-66.

Ferrandon, M., and A. R. Chamel. 1988. Cuticular retention, foliar absorption and translocation of Fe, Mn, and Zn supplied in organic and inorganic form. J. Plant Nutr. 11:247-263.

Ford, E.D. 1984. The dynamics of plantation growth. *In* Nutrition of plantation forests. *Edited by* G.D. Bowen, and E.K.S. Nambiar. Academic Press, London. pp. 17-52.

Foy, C.D. 1983. The physiology of plant adaptation to mineral stress. Iowa State J. Res. 57:355-391.

Foy, C.D. 1984. Physiological effects of hydrogen, aluminium, and manganese toxicities in acid soil. In Soil acidity and liming. Agronomy Monographs No. 12. Second Edition. ASA-CSSA-SSSA, Madison, WI. pp. 57-97.

Foy, C.D., A.L. Fleming, and J.W. Schwartz. 1973. Opposite Al and Mn tolerances of two wheat varieties. Agron. J. 65:123-126.

Foy, C.D., R.L. Chaney, and M.C. White. 1978. The physiology of metal toxicity in plants. Ann. Rev. Plant Physiol. 29:511-566.

Gibson, T.S., and D.R. Leece. 1981. Estimation of physiologically active zinc in maize by biochemical assay. Plant and Soil 63:395-406.

Glass, A.D., and M.Y. Siddiqi. 1984. The control of nutrient uptake rates in relation to the inorganic composition of plants. Adv. Plant Nutr. 1:103-208.

Godbold, D.L., W.J. Horst, J.C. Collins, D.A. Thurman, and H. Marschner. 1984. Accumulation of zinc and organic acids in roots of zinc tolerant and non-tolerant ecotypes of *Deschampsia caespitosa*. J. Plant Physiol. 116:59-69.

Graham, R.D., R.M. Welch, D.L. Grunes, E.E. Cary, and W.A. Norvell. 1987. Effect of zinc deficiency on the accumulation of boron and other mineral nutrients in Barley. Soil Sci. Soc. Am. J. 51:652-657.

Hall, T.A. 1979. Biological X-ray microanalysis. J. Microscopy 117:145-163.

Hawf, L.R., and W.E. Schmid. 1967. Uptake and translocation of zinc by intact plants. Plant and Soil 27:249-260.

Haynes, R.J. 1986. Uptake and assimilation of mineral nitrogen by plants. *In* Mineral nitrogen in the plant-soil system. *Edited by* R.J. Haynes. Academic Press Inc. USA. pp. 303-378.

Harlow, W.M., and E.S. Harrar. 1958. Textbook of dendrology. Fourth Edition. McGraw-Hill Book Company Inc. USA. 561 pp.

Heilman, P.E., and G. Ekuan. 1973. Response of Douglas-fir and western hemlock seedlings to lime. For. Sci. 19:220-224.

Hewitt, E.J. 1983. A perspective of mineral nutrition: essential and functional metals in plants. *In* Metals and micronutrients: uptake and utilization by plants. *Edited by* D.A. Robb and W.S. Pierpoint. Academic Press Inc. London. pp. 277-323.

Ingestad, T. 1979. Mineral nutrient requirements of *Pinus silvestris* and *Picea abies*. Physiol. Plant. 45:373-380.

Ingestad, T. 1982. Relative addition rate and external concentration; driving variables usedin plant nutrition research. Plant Cell Environ. 5:443-453.

Ingestad, T. 1987. New concepts on soil fertility and plant nutrition as illustrated by research on forest trees and stands. Geoderma 40:237-252.

Ingestad, T., and A-B. Lund. 1986. Theory and techniques for steady state mineral nutrition and growth of plants. Scand. J. For. Res. 1:439-453.

Ingestad, T., A. Aronsson, and G. I. Agren. 1981. Nutrient flux density model of mineral nutrition in conifer ecosystems. Studia Forestalia Suecica 160:61-71.

Institute of Forest Products. 1977. Biennial Report, Regional Forest Nutrition Research Project. Coll. Forest Resources, Univ. Washington, Seattle.

Jarrell, W.M., and R.B. Beverly. 1981. The dilution effect in plant nutrition studies. Adv. Agron. 34:197-224.

Kabata-Pendias, A., and Pendias, H. 1984. Trace elements in soils and plants. CRC Press, Inc. Boca Raton, Florida. 315 pp.

Kessel, S.L., and T.N. Stoate. 1936. Plant nutrients and pine growth. Aust. For. 1:4-13

Kitagishi, K., and H. Obata. 1986. Effects of zinc deficiency on the nitrogen metabolism of meristematic tissues of rice plants with reference to protein synthesis. Soil Sci. Plant Nutr. 32:397-404

Kitagishi, K., and H. Obata. 1987. Effect of zinc deficiency on 80S ribosomes content of meristematic tissues of rice plants. Soil Sci. Plant Nutr. 33:423-429.

Knight, P.J. 1975. Zinc deficiency in nursery-grown *Pinus radiata* seedlings. N.Z.J.For. Sci. 5:260-264.

Kohno, Y., C.D. Foy, A.L. Fleming, and D.T. Krizek. 1984. Effect of manganese on concentrations of Zn, K, Ca, and Mg in two bush bean cultivars grown in solution culture. J. Plant Nutr. 7:897-909.

Korcak, R.F. 1988. Nutrition of blueberry and other calcifuges. Hort. Rev. 10:183-227.

Krajina, V.J., K. Klinka, and J. Worrall. 1982. Distribution and ecological characteristics of trees and shrubs of British Columbia. The University of British Columbia. pp. 58-60.

Krajina, V.J., S. Madoc-Jones, and G. Mellor. 1973. Ammonium and nitrate in the nitrogen economy of some conifers growing in Douglas-fir communities of the Pacific North-West of America. Soil Biol. Biochem. 5:143-147.

Kramer, P.J. and T.T. Kozlowski. 1979. Physiology of woody plants. Academic Press, New York. pp. 65

Kropp, B.R. 1982. Fungi from decayed wood as ectomycorrhizal symbionts of western hemlock. Can. J. For. Res. 12:36-39.

Labanauskas, C.R., W.W. Jones, and T.W.Embleton. 1963. Effects of foliar applications of manganese, zinc, and urea on yield and fruit quality of valencia oranges, and nutrient concentrations in the leaves, peel and juice. Proc. Am. Soc. Hort. Sci. 82:142-153.

Labanauskas, C.R., and R.E. Puffer. 1964. Effects of foliar applications of manganese, zinc, and urea on valencia orange yield and foliage composition. Proc. Am. Soc. Hort. Sci. 84:158-164.

Labanauskas, C.K. 1969. Interactions of nutrients in valencia orange leaves as affected by the composition of manganese, zinc and urea sprays. Hilgardia 39:507-513.

Lafond, A., and Y. Laflamme. 1968. Relative concentrations of iron and manganese: a factor affecting Jack pine regeneration and Jack pine-black spruce succession. *In* Tree growth and forest soils. *Edited by* C.T. Youngberg, and C.B. Davey. Proc. of the 3rd North American Forest Soils Conf. Oregon State, Univ. Press. pp. 305-312.

Landsberg, J.J. 1986. Physiological ecology of forest production. Academic Press, Toronto. 198 pp.

Lavender, D.P. 1970. Foliar analysis and how it is used. A review. Oreg. State Univ. Res. Note 52. 8 pp.

Leece, D.R. 1978. Effects of boron on the physiological activity of zinc in maize. Aust. J. Agric. res. 29:739-747.

Legge, G.J.F. 1980. The scanning proton microprobe. *In* Microbeam analysis. *Edited by* D.B. Wittry. San Francisco Press, Inc. San Francisco, CA. pp. 70-76

Legge, G.J.F. 1982. Proton and nuclear microprobe developments. Nuclear Instruments and Methods 197:243-253.

Legge, G.J.F., and A.P. Mazzolini. 1980. Elemental microanalysis of biological and medical specimens with a scanning proton microprobe. Nuclear Instruments and Methods. 168:563-569

Legge, G.J.F. Legge, C.D. McKenzie, and A.P. Mazzolini. 1979. The Melbourne proton microprobe. J. of Microscopy 117:185-200.

Legge, G.J.F., A.P. Mazzolini, A.F. Roczniok, and P.M. O'Brien. 1982. Application of a scanning proton microprobe to biology and medicine. Nuclear Instruments and Methods 197:191-200.

Leidi, E.O., M. Gomez, and M.D. de la Guardia. 1986. Evaluation of catalase and peroxidase activity as indicators of Fe and Mn nutrition for soybean. J. Plant Nutr. 9:1239-1249.

Leigh, R.A., and R.G. Wyn Jones. 1986. Cellular compartmentation in plant nutrition: the selective cytoplasm and the promiscuous vacuole. Adv. Plant Nutr. 2:249-279.

Leyton, L. 1956. The relationship between growth and mineral composition of the foliage of the foliage of Japanese larch. Plant and Soil 7:167-177.

Leyton, L. 1957. The relationship between growth and mineral composition of Japanese larch. II. Evidence from manurial trials. Plant and Soil 9:31-48.

Leyton, L., and K.A. Armson. 1955. Mineral composition of the foliage in relation to the growth of Scots pine. For. Sci. 1:210-218.

Linder, S., and D.A. Rook. 1984. Effects of mineral nutrition on carbon dioxide exchange and partitioning of carbon in trees. *In* Nutrition of plantation forests. *Edited by* G.D. Bowen, and E.K.S. Nambiar. Academic Press, London. pp. 211-236.

Lowe, L.E., and K. Klinka. 1981. Forest humus in the CWH biogeoclimatic zone of British Columbia in relation to forest productivity and pedogenesis. Province of British Columbia, Ministry of Forests, Res. Note 89. 83 p.

Lundegardh, H. 1943. Leaf analysis as a guide to soil fertility. Nature 151:310.

Lundegardh, H. 1947. Mineral nutrition. Ann. Rev. Biochem. 16:503-523.

Lundegardh, H. 1951. Leaf analysis, an authorized translation by R. L. Mitchell. London: Hilger and Watts Ltd.

Macfie, S.M., G.J. Taylor, K.G. Briggs, and J. Hoddinott. 1989. Differential tolerance of manganese among cultivars of *Triticum aestivum*. Can. J. Bot. 67:1305-1308.

Maclean, A.J. 1974. Effects of soil properties and amendments on the availability of zinc in soils. Can. J. Soil Sci. 54:364-378.

Marschner, H. 1986. Mineral nutrition of higher plants. Academic Press, Orlando, Florida. 674 p.

Marschner, H. 1988. Mechanisms of manganese acquisition by roots from soils. *In* Manganese in soils and plants. *Edited by* R.D. Graham, R. J. Hannam, and N.C. Uren. Kluwer Academic Publishers. The Netherlands. pp. 191-204.

Mazzolini, A.P., C.K. Pallaghy, and G.J.F. Legge. 1985. Quantitative microanalysis of Mn, Zn, and other elements in mature wheat seeds. New Phytol. 100:483-509.

Mazzolini, A.P., C.A. Anderson, P.Y. Ladiges, and G.J.F. Legge. 1982. Distribution of mineral elements in green and chlorotic leaf tissue of *Eucalyptus obliqua* L'Herit., determined with a scanning proton microprobe. Aust. J. Plant Physiol. 9:261-269.

McBride, M.B., and J.J. Blasiak. 1979. Zinc and copper solubility as a function of pH in an acid soil. Soil Sci. Soc. Am. J. 43:866-870.

McGrath, J.F., and A.D. Robson. 1984a. The distribution of zinc and the diagnosis of zinc deficiency in seedlings of *Pinus radiata*, D. Don. Aust. For. Res. 14:175-186.

McGrath, J.F., and A.D. Robson. 1984b. Effect of nitrogen and phosphorus supply on the response of seedlings of *Pinus radiata*, D. Don to applied zinc. Aust. For. Res. 14:163-173.

Mead, D.J. 1984. Diagnosis of nutrient deficiencies in plantations. *In* Nutrition of plantation forests. *Edited by* G.D. Bowen, and E.K.S. Nambiar. Academic Press, London. pp. 259-291.

Memon, A.R., M. Chino, Y. Takeoka, K. Hara, and M. Yatazawa. 1980. Distribution of manganese in leaf tissues of manganese accumulator: *Acanthopanax sciadophylloides* as revealed by electonprobe x-ray microanalyzer. J. Plant Nutr. 2:457-476.

Memon, A.R., and M. Yatazawa. 1984. Nature of manganese complexes in manganese accumulator plant-*Acanthopanax sciadophylloides*. J. Plant Nutrition 7:961-974.

Memon, A.R., and M. Yatazawa. 1982. Chemical nature of manganese in the leaves of manganese accumulator plants. Soil Sci. Plant Nutr. 28:401-412.

Miller, F.A., and E.D. Rudolph. 1986. Uptake and distribution of manganese and zinc in *Pinus virginiana* seedlings infected with *Pisolithus tinctorius*. Ohio J. Sci. 86:22-25.

Miller, P.M. 1987. Physiological responses of *Vaccinium vitis -idaea* to high tissue concentrations of manganese. Can. J. Bot. 65:1643-1646.

Millikan, C.R. 1951. Radio-autographs of manganese in plants. Aust. J. Sci. Res. B4:28-41.

Morrison, I.K., and K.A. Armson. 1968. Influence of manganese on growth of Jack pine and black spruce seedlings. For. Chron. 44:32-35.

Moser, F. 1940. Plant composition as an index of soil fertility. Soil Sci. Soc. Am. Proc. 5:147.

Murphy, L.S., and L.M. Walsh. 1972. Correction of micronutrient deficiencies with fertilizers. *In* Micronutrients in agriculture. *Edited by* J.J. Mortvedt, P.M. Giordano, and W.L. Lindsay. Soil Sci. Soc. Am., Inc. Madison, WI. USA. pp. 347-387

National Academy of Sciences. 1973. Manganese. National Academy of Sciences. Washington, D.C. pp. 59-76.

Nair, K.P.P., and G. Prabhat. 1977. Differential reponse of tropical maize genotypes to zinc and manganese nutrition. Plant and Soil 47:149-159.

Neilsen, G.H., and E.J. Hogue. 1986. Some factors affecting leaf zinc concentration of apple seedlings grown in nutrient solution. Hort. Sci. 21:434-436.

Obata, H., and M. Umebayashi. 1988. Effect of zinc deficiency on protein synthesis in cultured tobacco plant cells. Soil Sci. Plant Nutr. 34:351-357.

Ohki, K. 1987. critical nutrient levels related to plant growth and some physiological processes. J. Plant Nutr. 10:1583-1590.

Omule, S.A.Y. 1990. Response of coastal Douglas-fir and western hemlock to fertilizing and thinning treatments. Proc. EP 703 Workshop. Nov. 7, 1990. Res. Br., B.C. Min of Forests.

Orphanos, P.I. 1982. Soil and spray applications of zinc to apples. J. Hort. Sci. 57:259-266.

Parraga, G., S.J. Horvath, A. Eisen, W.E. Taylor, L. Hood, E.T. Young, and R.E. Klevit. 1988. Zinc-dependent structure of a single-finger domain of yeast ADR1. Sci. 241:1489-1492.

Pegtel, D.M. 1986. Response of plants to Al, Mn, and Fe, with particular reference to *Succisa pratensis* Moench. Plant and Soil 93:43-55.

Peterson, P.J. 1983. Adaptation to toxic metals. *In* Metals and micronutrients: uptake and utilization by plants. *Edited by* D.A. Robb, and W.S. Pierpoint. Academic Press, London. pp. 51 -69.

Porada, H.J. 1987. The effect of aluminum on the growth and mineral composition of Douglas-fir and western hemlock. Ph. D. Thesis. Univ. of Wash. Seattle WA.

Prask, J.A., and D.L. Plocke. 1971. A role for zinc in the structural integrity of the cytoplasmic ribosomes of *Euglena gracilis*. Plant Physiol. 48:150-155.

Prusinkiewicz, Z. 1982. A new multivariate approach to foliar analysis for diagnostic purposes in mature Scots pine stands. For. Ecol. Manage. 4:301-315.

Radwan, M.A., and D.S. DeBell. 1980. Site index, growth and foliar chemical composition relationships in western hemlock. For. Sci. 26:283-290.

Rahimi, A., and A. Schropp. 1984. Carboanhydrase-Aktivitat und extrahierbares Zink als MaBstab fur die Zink-Versorgung von Pflanzen. Z. Pflanzenernahr. Bodenkd. 147:572-583.

Rance, S.J., D.M. Cameron, and E.R. Williams. 1982. Correction of crown disorders of *Pinus caribaea* var. *hondurensis* by application of zinc. Plant and Soil 65:293-296.

Randall, P.J., and D. Bouma. 1973. Zinc deficiency, carbonic anhydrase, and photosynthesis in leaves of spinach. Plant Physiol. 52:229-232.

Reddy, M.R., M.R. Tucker, and S.J. Dunn. 1987. Effect of manganese on concentrations of Zn, Fe, Cu, and B in different soybean genotypes. Plant and Soil 97:57-62.

Riceman, D.S., and G.B. Jones. 1958. Distribution of zinc in subterranean clover grown to maturity in a culture solution labelled with the radioactive isotope ^{65}Zn. Aust. J. Agric. Res. 9:730-744.

Romney. E.M., and S.J. Toth. 1954. Plant and soil studies with radioactive manganese. Soil Sci. 77:107-117.

Ruano, A., J. Barcelo, and Ch. Poschenrieder. 1987. Zinc toxicity-induced variation of mineral element composition in hydroponically grown bush bean plants. J. Plant Nutr. 10:373-384.

Ryan, P.J. 1983. The role of acid and aluminum-rich media in the growth and nutrition of Pacific Northwest conifers. Ph.D. thesis, Colledge of Forest Resources, Univ. of Wash., Seattle WA.

Ryan, P.J., S.P. Gessel, and R.J. Zasoski. 1986a. Acid tolerance of Pacific Northwest conifers in solution culture. I. Effect of high aluminium concentration and solution acidity. Plant and Soil 96:239-257.

Ryan, P.J., S.P. Gessel, and R.J. Zasoski. 1986b. Acid tolerance of Pacific Northwest conifers in solution culture. II. Effect of varying aluminium concentration at constant pH. Plant and Soil 96:259-272.

Rygiewicz, P.T., C.S. Bledsoe, and R.J. Zasoski. 1984. Effects of ectomycorrhizae and solution pH on [^{15}N]ammonium uptake by coniferous seedlings. Can. J. For. Res. 14:885-892.

Saed, M., and R.L. Fox. 1977. Relations between suspension pH and zinc solubility in acid and calcareous soils. Soil Sci. 124:199-204.

Salsac, L., S. Chaillou, J-F. Morot-Gaudry, C. Lesaint, and E. Jolivet. 1987. Nitrate and ammonium nutrition in plants. Plant Physiol. Biochem. 25:805-812.

Scarseth, G.D. 1943. Plant tissue testing in diagnosis of the nutritional status of growing plants. Soil Sci. 55:113.

Sevilla, F., J. Lopez-Gorge, M. Gomez, and L.A. Del Rio. Manganese superoxide dismutase from a higher plant. Planta 150:153-157.

Sharma, C.P., J.P. Gupta, and S.C. Agarwala. 1981. Metabollic changes in citrullus subjected to zinc stress. J. Plant Nutr. 3:337-344.

Shaw, E., R.G. Menzel, and L.A. Dean. 1954. Plant uptake of ^{65}Zn from soils and fertilizers in the greenhouse. Soil Sci. 77:205-214.

Shear, C.B, H.L. Crane, and A.T. Myers. 1946. Nutrient-element balance: A fundamental concept in plant nutrition. Proc. Amer. Soc. Hort. Sci. 47:239-248.

Shuman, L.M., and O.E. Anderson. 1976. Interactions of Mn with other ions in wheat and soybeans. Commun. Soil Sci. Plant Anal. 7:547-557.

Singh, B.R., and K. Steenberg. 1974. Plant response to micronutrients. III. Interaction between manganese and zinc in maize and barley plants. Plant and Soil 40:655-667.

Smith, M.E., and N.S. Bayliss. 1942. The necessity of zinc for *Pinus radiata*. Plant Physiol. 17:303-310.

Smith, R.B., R.H. Waring, and D.A. Perry. 1981. Interpreting foliar analyses from Douglas-fir as weight per unit of leaf area. Can. J. For. Res. 11:593-598.

Snir, I. 1983. Carbonic anhydrase activity as an indicator of zinc deficiency in pecan leaves. Plant and Soil 74:287-289.

Stoate, T.N. 1950. Nutrition of the pine. Western Austr. Forest Dept., Bull. 30. 61 pp.

Stone, E.L. 1967. Microelement nutrition of forest trees: a review. *In* Forest fertilization: theory and practice. TVA. pp. 132-175.

Stout, W.L., G.A. Jung, and J.A. Shaffer. 1987. Effect of foliar and soil applied zinc on soil test levels, herbage composition, and yields of alfalfa. Commun. Soil Sci. Plant Anal. 18:743-752.

Sumner, M.E. 1978. Interpretation of nutrient ratios in plant tissue. Commun. Soil Sci. Plant Anal. 9:335-345.

Swietlik, D., and M. Faust. 1984. Foliar nutrition of fruit crops. Hort. Rev. 6:287-355.

Taylor, G.J. 1987. Exclusion of metals from the symplasm: a possible mechanism of metal tolerance in higher plants. J. Plant Nutr. 10:1213-1222.

Thomas, W. 1937. Foliar diagnosis. Principle and practice. Plant Physiol. 12:571.

Thomas, W. 1945. Present status of diagnosis of mineral requirements of plants by means of leaf analysis. Soil Sci. 59:353.

Thomas, W., and W.B. Mack. 1941. Foliar diagnosis in relation to soil heterogeneity. Soil Sci. 52:455.

Thomas, W., and W.B. Mack. 1944. Misconceptions relative to the methods of foliar diagnosis. Proc. Amer. Soc. Hort. Sci. 44:355.

Thurman, D.A. 1981. Mechanism of metal tolerance in higher plants. *In* Effects of heavy metal pollution on plants. Vol. 2. Metals in the environment. *Edited by* N.W. Lepp. Applied Science Publishers, London. pp. 239-249.

Timmer, V.R. 1979. Foliar diagnosis of nutrient status and growth response in forest tress. Ph.D. thesis, Cornell Univ. 94 pp.

Timmer, V.R., and E.L. Stone. 1978. Comparative foliar analysis of young Balsam fir fertilized with nitrogen, phosphorus, potassium, and lime. Soil Sci. Soc. Am. J. 42:125-130.

Timmer, V.R., and L.D. Morrow. 1984. Predicting fertilizer growth response and ntrient status of jack pine by foliar diagnosis. p. 335-351. *In* Forest soils and treatment impacts. *Edited by* E. L. Stone. Proc. 6th North Am. For. Soils conf., Univ. of Tennessee, Knoxville. June 1983. Univ. of Tennessee, Knoxville.

Tisdale, S.L., W.L. Nelson, and J.D. Beaton. 1985. Soil fertility and fertilizers. Fourth Edition. MacMillan Publishing Company, Inc. New York, USA. 754 pp.

Tukey, H.B. Jr., H.B. Tukey, and S.H. Wittac. 1958. Loss of nutrients by foliar leaching as determined by radioisotopes. Am. Soc. Hort. Sci. 71:496-506.

Turner, D.P., and E.H. Franz. 1985. The influence of western hemlock and western redcedar on microbial numbers, nitrogen mineralization, and nitrification. Plant and Soil 88:259-267.

Ulrich, A. 1943. Plant analysis as a diagnostic procedure. Soil Sci. 55:101.

U.S. Dept. of Commerce. 1976. National Bureau of Standards Certificate of Analysis, Standard Reference Material, Tomato Leaves, No. 1573. Wash. D.C.

U.S. Dept. of Commerce. 1977. National Bureau of Standards Certificate of Analysis, Standard Reference Material, Orchard Leaves, No. 1571. Wash. D.C.

Van den Driessche, R. 1971. Response of conifer seedlings to nitrate and ammonium sources of nitrogen. Plant and Soil 34:421-439.

Van den Driessche, R. 1976. Mineral nutrition of western hemlock. *In* Proc. western hemlock management conf. *Edited by* W.A. Atkinson, and R.J. Zasoski. College of Forest Resources Univ. Wash., and Institute of Forest Products Contri. No. 34. pp.56-70

Van Steveninck, R.F.M., M.E. Van Steveninck, D.R. Fernando, W.J. Horst, and H. Marschner. 1987. Deposition of zinc phytate in globular bodies in roots of *Deschampsia caespitosa* ecotypes; a detoxification mechanism? J. Plant Physiol. 131:247-257.

Van Steveninck, R.F.M., M.E. Van Steveninck, D.R. Fernando, D.L. Godbold, W.J. Horst, and H. Marschner. 1987. Identification of zinc-containing globules in roots of a zinc-tolerant ecotype of *Deschampsia caespitosa*. J. Plant Nutr. 10:1239-1246.

Viets, F.G., Jr., L.C. Boawn, and C.L. Crawford. 1957. The effect of nitrogen and types of nitrogen carrier on plant uptake of indegenous and applied zinc. Soil Sci. Soc. Amer. Proc. 21:197-201.

Vose, J.M., and H.L. Allen. 1988. Leaf area, stemwood growth, and nutrition relationships in loblolly pine.

Wacker, W.E.C. 1962. Nucleic acids and metals. III. Changes in nucleic acid, protein, and metal content as a consequence of zinc deficiency in *Euglena gracilis*. Biochemistry 1:859-865.

Walworth, J.L., and M.E. Sumner. 1988. Foliar diagnosis: a review. Adv. Plant Nutr. 3:193-241.

Walworth, J.L, W.S. Letzsch, and M.E. Sumner. 1986. Use of boundary lines in establishing diagnostic norms. Soil Sci. Soc. Amer. J. 50:123-128.

Webb, R.A. 1972. Use of the boundary line in the analysis of biological data. J. Hort. Sci. 47:309-319.

Webster, S.R., D.S. DeBell, K.N. Wiley, and W.A. Atkinson. 1976. Fertilization of western hemlock. *In* Western hemlock management conf. proc. *Edited by* W.A. Atkinson and R.J. Zasoski. Univ. Washington, Seattle. pp. 247-252.

Weetman, G.F., R. Fournier, J. Barker, and E. Schnorbus-Panozzo. 1989. Foliar analysis and response of fertilized chlorotic western hemlock and western red cedar reproduction on salal-dominated cedar-hemlock cutovers on Vancouver Island. Can. J. For. Res. 19:1512-1520.

West, P.W. 1979. Release to shoots of copper stored in roots of *Pinus radiata* D. Don. Ann. Bot. 43:237-240.

White, E.H., and D.J. Mead. 1971. Discriminant analysis in tree nutrition research. For. Sci. 17:425-427.

White, M.C., R.L. Chaney, and A.M. Decker. 1979. Differential cultivar tolerance in soybean to phytotoxic levels of soil Zn. II. Range of Zn additions and the uptake and translocation of Zn, Mn, Fe, and P. Agron. J. 71:126-131.

Woolhouse, H.W. 1983. Toxicity and tolerance in response of plants to metals. *In* Encyclopedia of plant physiology. *Edited by* O.L. Lange, Vol. 12C. Springer-Verlag, New York. pp. 246-300.

Zar, J.H. 1984. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J. 620 pp.

Zasoski, R.J., and S.P. Gessel. 1982. Response of western hemlock seedlings to N, P, and S fertilization in a coastal Washington soil. Agron. Abstr. 273.

Zasoski, R.J., and H.J. Porada. 1986. Tissue nutrient status and DRIS as indicators of outplanted Douglas-fir and western hemlock growth. Agron. Abstr. 270.

Zasoski, R.J., S.G. Archie, W.C. Swain, and J.D. Stednick. 1977. The impact of sewage sludge on Douglas-fir stands near Port Gamble. Wash. Coll. For. Res. Univ. Wash.

Zasoski, R.J., H.J. Porada, P.J. Ryan, J. Greenleaf-Jenkins, and S.P. Gessel. 1990. Observations of copper, zinc, iron and manganese status in western Washington forests. For. Ecol. Manage. 37:7-25.

Zasoski, R.J., R.L. Edmonds, C.S. Bledsoe, C.L. Henery, D.J. Vogt, K.A. Vogt, and D.W. Cole. 1984. Municipal sewage sludge use in forests of the Pacific Northwest, U.S.A.: Environmental Concerns. Waste. Manage. Res., 2:227-246.

APPENDIX A.

SITE AND SOIL DESCRIPTION

APPENDIX A1. SITE 1

NTS Sheet: Port Coquitlam 92/G7
Location: UBC Research Forest
Road: F70
Long: 122° 34' 10"
Lat: 49° 17' 40"
Elevation: 360 m
Slope: Very gentle slopes 2-5%
Aspect: North-west
Landform: Till over bedrock
Parent Material: Till
Drainage Class: Well
Perviousness: Moderate
Depth of Pit: 52 cm
Rooting: 52 cm
Depth to Water Table: Never Present
Compact Till: 45 cm
Bedrock: 52 cm
Soil Classification: Orthic Humo-Ferric Podzol
Humus Form: Humimor
BGCZ: Windward Submontane Maritime Coastal Western Hemlock Wetter CWHb1
Major Vegetation: *Tsuga heterophylla, Abies amabilis, Pseudotsuga menziesii* (planted)
Lower Vegetation: *Vaccinium parvifolium, Hylocomium splendens, Vaccinium alaskaense, Blechnum spicant.*

Soil Profile

LFH 3-0 Black (10 YR 3/1, moist); fresh and partially decomposed organic matter; clear and irregualr boundary, 3-5 cm thick.

Bf 0-42 Dark yellowish brown (10 YR 4/4, moist); loamy sand; single grained; fine sub-angular blocky; slightly sticky, slightly plastic, very friable; few fine and coarse roots; about 40% coarse fragments; abrupt, irregular boundary;

C 42-52 Brown (10 YR 5/3, moist); loamy sand; singled grained; fine-coarse sub-angular blocky; slightly sticky, slightly plastic, very firm; very few medium and coarse roots; about 40% coarse fragment content; abrupt, irregular boundary;

APPENDIX A2. SITE 2

NTS Sheet: Port Coquitlam 92/G7
Location: UBC Research Forest
Road: E12
Long: 122° 34' 10"
Lat: 49° 18' 50"
Elevation: 440 m
Slope: Very gentle slopes 2-5%
Aspect: None
Landform: Till over bedrock
Parent Material: Till
Drainage Class: Imperfectly Drained
Perviousness: Slowly pervious
Depth of Pit: 43 cm
Rooting: 43 cm
Depth to Water Table: Never Present
Compact Till: None
Bedrock: 43 cm
Soil Classification: Rego Gleysol
Humus Form: Humimor
BGCZ: Windward Submontane Maritime Coastal Western Hemlock Wetter CWHb1
Major Vegetation: *Tsuga heterophylla, Thuja plicata, Pinus monticola*
Lower Vegetation: *Vaccinium parvifolium, Gaultheria shallon, Rhytidiadelphus loreus, Kindbergia oregana*

Soil Profile

LFH 15-0 Black (10 YR 3/1, moist); fresh and partially decomposed organic matter; abundant very fine and fine, and few medium roots; abrupy, wavy boundary; 15-20 cm thick.

Cg 0-18 Gray (10 YR 5/1, moist); loamy sand; sinle grained; moderatley, fine, subangular blocky; slightly sticky, slightly plastic, very friable, soft; plentiful very fine and fine roots; about 30% coarse fragments; abrupt and wavy boundary; 7-20 cm thick.

APPENDIX A3. SITE 3

NTS Sheet: Chilliwack 92/H4
Location: Chipmunk Creek
Road: Chipmunk creek Road
Long: 121° 41' 50"
Lat: 49° 8' 10"
Elevation: 880-960 m
Slope: Very strong slopes 31-45%
Aspect: South-east
Landform: Till over bedrock
Parent Material: Till
Drainage Class: Rapidly Drained
Perviousness: Rapidly pervious
Depth of Pit: 1.5 m
Rooting: 60 cm
Depth to Water Table: Never Present
Compact Till: 60 cm
Bedrock: None
Soil Classification: Orthic Humo-Ferric Podzol
Humus Form: Humimor
BGCZ: Windward Montane Maritime Coastal Western Hemlock Wetter CWHb2
Major Vegetation: *Tsuga heterophylla, Abies amabilis, Pseudotsuga menziesii* (planted)
Lower Vegetation: *Rhytidiopsis robusta, Vaccinium alaskaense, Blechnum spicant, Moneses uniflora*

Appendix A3 (continued)

Soil Profile

L 2-0 Black (10 YR 3/1, moist); fresh and partially decomposed organic material; abundant fine and very fine roots; abrupt, broken boundary.

Ae 0-5 Gray (10 YR 6/1, moist); loamy sand; single grained; weak, fine-coarse subangular blocky; nonsticky, nonplastic, loose, soft; abundant very fine-fine roots; about 5% coarse fragments; abrupt, broken boundary; 0-5 cm thick.

Bf 5-60 Yellowish Brown (10 YR 5/6, moist); loamy sand; single grained; moderately, fine-coarse subangular blocky; slightly sticky, slightly plastic, very friable, soft; abundant fine-medium, and very few coarse roots; about 30% coarse fragment content; gradual wavy boundary; 45-60 cm thick.

Cg 60+ Gray (10 YR 6/1, moist); coarse sand; single grained; stong, fine-coarse subangular blocky; slightly sticky, slightly plastic, very friable, soft; about 50% coarse fragment content.

APPENDIX A4. SITE 4

NTS Sheet: Port Coquitlam 92/G7
Location: UBC Research Forest
Road: E13
Long: 122° 34' 20"
Lat: 49° 18' 50"
Elevation: 520 m
Slope: Nearly level 0.5-2%
Aspect: North
Landform: Till over bedrock
Parent Material: Till
Drainage Class: Well Drained
Perviousness: Moderately pervious
Depth of Pit: 90 cm
Rooting: 70 cm
Depth to Water Table: Never Present
Compact Till: 80 cm
Bedrock: None
Soil Classification: Duric Humo-Ferric Podzol
Humus Form: Humimor
BGCZ: Windward Submontane Maritime Coastal Western Hemlock Wetter CWHb1
Major Vegetation: *Tsuga heterophylla, Abies amabilis, Pseudotsuga menziesii* (planted)
Lower Vegetation: *Vaccinium parvifolium, Gaultheria shallon, Rhytidiadelphus loreus, Kindbergia oregana*

Soil Profile

LFH 20-0 Black (10 YR 3/1, moist); freash, partially and well decomposed organic material; abundant fine and very fine, and few coarse roots; gradual smooth boundary; 5-20 cm thick.

Ae 0-10 Gray (10 YR 6/1, moist); loamy sand; single grained; moderately, medium, subangular blocky; slightly sticky, slightly plastic, very friable, soft; abundant fine and medium roots; about 30% coarse fragments; clear and irregular boundary; 0-10 cm thick.

Bf 10-46 Dark yellowish brown (10 YR 4/4, moist); loamy sand; single grained; moderately, medium, subangular blocky; slightly sticky, slightly plastic, very friable, soft; few fine and medium roots; about 30% coarse fragment content; clear and wavy boundary; 36 cm thick

BCc 46-72 Yellowish red (5 YR 5/8, moist); loamy sand; single grained; moderately, medium, subangular blocky; slightly sticky, slightly plastic, very friable, soft; about 30% coarse fragment content; clear and wavy boundary; 10-26 cm thick;

C 72+ Yellowish brown (10 YR 5/6, moist); coase sand; single grained; moderately, medium, subangular blocky to platy; slightly sticky, slightly plastic, very friable, soft; about 50% coarse fragment content.

APPENDIX A5. SITE 5

NTS Sheet: Stave Lake 93/G6
Location: Mission Tree Farm
Road: Rock Creek Road
Long: 122° 34' 20"
Lat: 49° 18' 50"
Elevation: 1,100-1,200 m
Slope: Very Strong Slopes 31-45%
Aspect: East
Landform: Till over bedrock
Parent Material: Till
Drainage Class: Well Drained
Perviousness: Moderately pervious
Depth of Pit: 80 cm
Rooting: 40 cm
Depth to Water Table: Never Present
Compact Till: 80 cm
Bedrock: None
Soil Classification: Orthic Ferro-Humic Podzol
Humus Form: Humimor
BGCZ: Windward Montane Maritime Coastal Western Hemlock Wetter CWHb2
Major Vegetation: *Tsuga heterophylla, Abies amabilis, Chamaecyparis nootkatensis, Pseudotsuga menziesii* (planted)
Lower Vegetation: *Vaccinium alaskaense, Dryopteris expansa, Blechnum spicant, Sphagnum girgensohnii, Tiarella unifoliata*

Appendix A5 (continud)

Soil Profile

LFH 25-0 Black (10 YR 3/1, moist); fresh, partially, and well decompose organic material; abundant very fine and fine, few coarse, and plentiful medium; abrupt and smooth boundary; 20-25 cm thick.

Bhf 0-18 Black (10 YR 3/1, moist); loamy sand; single particles; strong, fine-very coarse subangular blocky; sticky, plastic, very firm, soft; plentiful fine, and medium roots; about 20% coarse fragment content; abrupt, smooth boundary; 18-20 cm thick.

Bf 18-35 Dark yellowish brown (10 YR 4/4, moist); loamy sand; single particles; strong, fine-very coarse subangular blocky; sticky, plastic, very firm, soft; very few fine, and medium roots; about 40% coarse fragment content; abrupt, smooth boundary; 17-20 cm thick.

C 35+ Light gray (7.5 YR 7/0, moist); sand; single grained; red (2.5 YR 4/8, moist) mottles; nonsticky, nonplastic, very friable, soft; about 50% coarse fragment content.

APPENDIX B1.

MODIFIED PARKINSON AND ALLEN DIGESTION FOR PLANT TISSUE ANALYSIS

1) Weigh 1 g (to the nearest mg) subsample of oven-dried (70°C for 3 hours), ground foliage and place in 100 ml digestion tube. 45 tubes in each set can be prepared, with a reference sample and a blank in each set.

2) Add 5 ml of conc. H_2SO_4 (reagent grade) to each sample, and mix on a mechanical vibrator immediately.

3) Dispense 1 ml of Li_2SO_4 - H_2O_2 mixture (prepared by mixing 7.0 g Li_2SO_4, 0.21 g selenium powder in 175 ml 30% H_2O_2) into each tube. Wait until reaction (foaming and spattering) ceases before continuing.

4) Repeat step 3.

5) Heat the rack of tubes on the digestion block at 360°C. Use discontinuous heating to overcome initial foaming; that is, 20-40 seconds on block, cool for about 2 minutes, 40-50 seconds of heating and cool, 1-2 minutes on block and cool for 5-10 minutes.

6) Add another 1 ml Li_2SO_4 - H_2O_2 mixture to each tube. Wait till reaction ceases.

7) Repeat step 6.

8) Digest on block for 1 1/2 hours at 360°C.

9) After 1 1/2 hours, remove rack from block. Add 0.5 ml H_2O_2 to each tube, return rack to block and digest for another 30 minutes.

10) Repeat step 9. Total digestion time is 2 1/2 hours.

11) Remove rack from block and allow digests to cool (approximately 1 hour). Samples should be pale yellow to milky white in colour.

12) Add about 80 ml of demineralized water. Allow to cool to room temperature before making to a final volume (100 ml) with demineralized water.

13) Cover tubes with an inert stopper, invert 3-4 times to mix, and pour contents into a labelled 125 ml plastic bottle.

APPENDIX B2.

NITRIC ACID DIGESTION FOR ANALYSIS OF COPPER AND IRON IN FOLIAGE

1) Weigh 0.7 g (to the nearest mg) subsample of oven-dried (70°C for 3 hours), ground foliage and place sample into digestion tube. A set of 45 tubes can be prepared for one run, each set having a reference sample and a blank.

2) Add 5 ml conc. HNO_3 to sample, and mix on mechanical vibrator.

3) Cover the tubes with glass marbles and heat on digestion block at 40°C for 1 hour.

4) Increase heat up to 140°C and continue heating for 2 hours, couting from time the block reaches 140°C.

5) Remove tubes from block and allow to cool.

6) Add about 7 ml demineralized water to each tube and mix by swirling. Allow to cool. Pour sample into a 25 ml measuring cylinder. Rinse digestion tube with demineralized water and add rinsings to the cylinder. Make volume up to 25 ml with demineralized water. Cover cylinder with an inert rubber stopper and mix content by inverting 3-4 times. Transfer contents to a 60 ml plastic bottle.

7) Analyze the solutions for iron and copper by atomic absorption spectrophotometry.

APPENDIX B3.

PROCEDURE FOR THE DETERMINATION OF SULPHATE-SULPHUR IN FOLIAGE

Extraction from foliage

1) Weigh one gram of oven-dried foliage into an erlenmeyer flask. Add 20 ml of 1 N HCL and record weight.

2) Heat on a hot plate to boiling, then continue boiling for 10 minutes.

3) Add water to bring back to the original weight.

4) Filter through #42 filter paper.

Digestion

1) Use from 0.5 to 2 ml of sample.

2) Place 5 ml of 1 N NaOH into a 20 ml test tube and attach so the delivery tube reaches almost to the bottom of the test-tube.

3) Add 4 ml of reducing agent (mix 300 ml of hydriodic acid (57% and 1% preservative) with 75 ml of 50% hypophosphorous acid and 150 ml of 90% formic acid. Boil gently with a stream of N_2 flowing through the solution for 10 minutes after the temperature reaches 115°C. Cool with the N_2 still flowing) and sample up to 40 µg S in an aliquot to the boiling flask. Adjust the N_2 flow to 75 ml $minute^{-1}$ per flask.

4) Heat for 20 minutes so it is just barely boiling.

5) Remove, and add 2.5 ml bismuth reagent and mix. Read immediately at 400 mµ and compare against a standard.

APPENDIX B4.

DETERMINATION OF ACTIVE IRON IN FOLIAGE

1) Weigh 0.5 g subsample of oven-dried (70°C for 3 hours), ground foliage into a 60 ml screw-capped plastic bottle.

2) Add 10 ml 1 N HCL (reagent grade) in demineralized water to each sample. Tightly cap the bottle to prevent leakage. (70 samples can be prepared in one run).

3) Shake the bottle horizontally for 24 hours on a reciprocating shaker at room temperature. Have a blank and reference samples for each set.

4) Filter the extract through Whatman # 41 filter paper and collect the filtrate in a 60 ml plastic bottle.

5) Analyze for iron on atomic absorption spectrophotometer. Analysis should be done within 48 hours.

APPENDIX B5.

DETERMINATION OF EXTRACTABLE ZINC

1) Weight 0.5 grams of a subsample of oven-dried (70°C for 3 hours), ground sample into a 60 ml plastic bottle.

2) Add 20 ml of 1.0 mM MES (2-(N-morpholino)ethanesulfonic acid) (prepared in demineralized water) to each bottle.

3) Shake for 5 hours horizontally on a reciprocating shaker at room temperature. Include a blank and a reference sample with each set.

4) Filter through Whatman #41 filter paper.

5) Analyze for Zn on atomic absorption.

APPENDIX B6.

WATER EXTRACTABLE MANGANESE FROM FOLIAGE

1) Weigh out 0.1 g of a subsample of oven-dried (at 70°C for 3 hours), ground foliage into a 60 ml plastic bottle.

2) Add 50 ml of demineralized water.

3) Shake for 1 hour horizontally on a reciprocating shaker at room temperature. Have a blank and reference sample for each set.

4) Filter through Whatman #41 filter paper.

5) Determine Mn using atomic absorption on the extracts.

APPENDIX C.

COMPARISON OF FOLIAR Zn, Mn, AND Fe LEVELS USING AA VERSUS ICP

SAMPLE	ZnAA	ZnICP	MnAA	MnICP	FeAA	FeICP
			$\mu g\ g^{-1}$			
1	30	21.7	65	82.6	170	245
2	28	22.2	66	83.3	170	252
3	29	33.9	180	200	75	79.7
4	40	33.8	220	200	70	82.8
5	58	53.5	240	207	220	361
6	58	53.2	240	210	260	315
7	13	6.8	620	633	35	7.6
8	185	178.6	1220	1344	50	16.9
9	23	15.6	540	510	30	12.5
10	22	14.9	520	507	35	14.4
11	27	21.3	2400	2506	35	0.5
12	60	58	1420	1521	32	5.2
13	113	107.6	880	846	30	7.3
14	114	107.5	840	845	32	12
15	18	9.9	1200	1198	35	15.4
16	18	10.7	1100	1164	40	19.5
17	17	8.8	1080	1092	33	4.2
18	15	8.9	3880	3783	30	0.5
19	19	12.6	3120	3192	30	0.5
20	19	12.3	3100	3182	27	0.5
21	15	9.7	1040	1064	31	10.2
22	16	9.9	960	1076	32	14.2
23	11	5.6	600	583	32	9.9
24	23	16.3	900	937	30	7.2
25	24	16.4	860	946	30	6.1
26	28	21.2	64	79.4	160	227
27	38	30.5	200	184	75	81.7
28	55	50.3	20	201	255	37

Given

Sample	Zn	Mn	Fe
NBS	25	91	300
Tomato	62	238	690

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1. Sample Numbers

1, 2, 26 National Bureau of Standards (NBS) Orchard Leaves
3, 4, 27 Pine Reference 84-3
5, 6, 28 NBS Tomato Leaves
8 - 25 Hemlock Foliage Samples

2. (U.S. Dept. of Commerce 1977)
3. (U.S. Dept. of Commerce 1976)

APPENDIX D.

FORMULAS TO CONVERT AA VALUES TO THE CORRESPONDING ICP VALUES[1].

Equation for Zn

$$y = 1.005x - 6.075$$

$$R^2 = 0.996$$

$$SE = 2.53$$

Equation for Mn

$$y = 1.017x$$

$$R^2 = 0.998$$

$$SE = 63.57$$

Where

x = the concentration measured using the AA

y = the equivalent concentration on the ICP

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1. Data are from Appendix C.

APPENDIX E.

RECOVERY OF ELEMENTS IN NATIONAL BUREAU OF STANDARDS SAMPLES USING ICP AND AA

	CaICP	CaAA	MgICP	MgAA	KICP	KAA
			cg g^{-1}			
ORCHARD LEAVES						
	1.98	1.47	0.605	0.566	1.4	1.42
	1.88	1.37	0.616	0.566	1.42	1.36
	1.89	1.4	0.577	0.574	1.39	1.37
MEAN						
	1.92	1.41	0.599	0.569	1.4	1.38
GIVEN						
	2.09	2.09	0.62	0.62	1.47	1.47
% RECOVERY						
	91.9	67.5	96.6	91.8	95.2	93.9
TOMATO LEAVES						
	2.94	2.05	0.644	0.602	4.18	4.2
	2.87	2.18	0.653	0.61	4.18	3.92
	2.7	2.01	0.625	0.604	4.05	3.84
MEAN						
	2.84	2.08	0.641	0.605	4.14	3.99
GIVEN						
	3	3	0.7	0.7	4.46	4.46
% RECOVERY						
	94.7	94.7	91.6	86.4	92.8	89.5

Appendix E (concluded)

MnICP	MnAA	ZnICP	ZnAA	FeICP	FeAA	AlICP	AlAA
			$\mu g\ g^{-1}$				
ORCHARD LEAVES							
82.6	65	21.7	30	245	170	241	180
83.3	66	22.2	28	252	170	233	180
79.4	64	21.2	28	227	160	218	160
MEAN							
81.8	65	21.7	28.7	241	167	231	173
GIVEN							
91	91	25	25	300	300		
% RECOVERY							
89.9	71.4	86.8	114.8	80.3	55.7		
TOMATO LEAVES							
207	240	53.5	58	361	220	371	240
210	240	53.2	58	315	260	315	290
201	200	50.3	55	370	255	389	255
MEAN							
206	227	52.3	57	349	184	358	262
GIVEN							
238	238	62	62	690	690		
% RECOVERY							
86.6	95.4	84.4	91.9	50.6	26.7		

APPENDIX F.

PREPARATION AND ANALYSIS OF CELLULAR FRACTIONS FROM FOLIAGE

1) Weigh 20 g of oven-dried (70°C for 3 hours), ground sample of foliage into 500 ml plastic bottles for the centrifuge, and add 100 ml of a sucrose-buffer solution (0.5 M sucrose, 0.05 M Tris-HCL). Shake to wet the plant material thoroughly.

2) A progressive separation was done using centrifugation;

Fraction A	500 g for 5 minutes
Fraction B	3000 g for 10 minutes
Fraction C	Settled by gravity
Fraction D	15000 g for 30 minutes
Fraction E	Supernatant remaining

The fraction was the pellet formed in the bottom of the centrifuge tube following centrifugation of the supernatant. The remaining supernatant was decanted and recentrifuged at the next highest speed. Fraction C formed following decanting the supernatant from which fraction B had been formed and before centrifugation at 15000 g.

3) Fractions A to D were dried in an oven in porcelain crucibles to a constant weight. Fraction E was stored in 60 ml plastic bottles in the refrigerator.

4) Fractions A to D were digested using the Parkinson and Allen acid digestion. Because of the small amount of material from fraction D, the acid used for the digestion was added to the crucibles left overnight, and then transferred to the digestion tubes.

5) Zn and Mn were determined on the digests and on the fraction E supernatant using atomic absortion spectrophotometry.

APPENDIX G.

FIXATION AND EMBEDDING PROCEDURE

1) Place tissue in a small vial to completely cover it containing 2.5% glutarnaldehyde in 0.1 M Na-cacodylate (pH 7.2-7.4). Leave it for 3 hours.

2) The solution is prepared by combining 10 ml of 25% glutaraldehyde, 50 ml of 0.2 M Na-cacodylate and 40 ml of demineralized water.

3) The tissue is than rinsed three times at 10-minute intervals in 0.1 M Na-cacodylate (pH 7.2-7.4). This is prepared by adding 40 ml of 0.2 M Na-cacodylate to 40 ml of demineralized water.

4) The tissue is fixed for a second time for 1 hour in a solution of 1% osmium tetroxide in 0.1 M Na cacodylate (pH 7.2-7.4). This is prepared by adding a 1:1:2 volume of 2% aqua osmium tetroxide, demineralized water and Na cacodylate.

5) The tissue is rinsed in demineralized water three times at 10 minute intervals.

6) The tissue is then taken through a dehydration series using ethanol at 10-minute intervals in the following concentrations of ethanol; 30%, 50%, 70%, 85%, 95%, 100%, 100%.

7) The tissue is then embedded in the following series; for 10 minutes in propylene oxide, twice, for 3 hours in 3:1 mixture of propylene oxide and plastic, for 7-12 hours in 1:1 propylene oxide and plastic, for 7-12 hours in 1:3 propylene oxide and plastic, and then 7-12 hours in 100% plastic. The tissue is then polymerized.

8) The plastic is prepared by mixing 34.58 g of Epon Ep812 WPE #190, 13.0 g of DDSA (dodecenyl succinic anhydride, 13.0 g of NMA nadic methyl anhydride, and 0.75 g of DMP -30.

APPENDIX H.

THE MEHLICH 3 SOIL EXTRACTION METHOD

1) Weigh 5.0 g of soil samples into 125 ml plastic bottles. Include 2 blanks and 2 references soils per run.

2) Have extracting solution (M3) in 4 L carboy with outlet connected to teflon tubing closed with clip. Set carboy on stool on work bench.

3) Calibrate plastic graduated cylinder "to deliver" 50 ml.

4) Set up required number of 60 ml plastic bottles with funnels and Whatman #541 X 15.0 cm filter paper.

5) Since extraction time of samples should be precise use a stopwatch on the bench.

6) Fill graduated cylinder with M3 solution to the "to deliver" mark.

7) Pour into 1st 125 ml bottle, cap and put onto shaker at slow speed, starting the stopwatch.

8) Refill the graduated cylinder and, when stopwatch gets to 50 seconds, pour M3 solution into a 2nd 125 ml bottle, cap and put onto shaker.

9) Continue this at every 50 second reading (i.e. at 60 second intervals) of the stopwatch until the 6th bottle is put on the shaker, then immediately remove the 1st bottle (which will have shaken for 5 minutes) and pour through filter. Then transfer the label to the 60 ml collection bottle.

10) Continue adding 50 ml M3 solution to samples in 125 ml bottles at 50 second readings on the stopwatch, putting the bottle immediately onto the shaker, and then immediately removing and filtering the sample which has shaken for 5 minutes. Thus there should always be 5 bottles shaking after the initial start up, until the last 5 samples, which should be removed from the shaker at 1 minute intervals, starting at the 58 second reading on the stopwatch.

11) Measure elements on ICP.

12) Extracts may be kept for a week, however dilutions must be read within 3 days.

Appendix H (concluded).

Extracting Solution

Reagents: All should be ACS grade.

R1 Ammonium nitrate
R2 Ammonium fluoride
R3 Acetic acid, glacial 99.5%, 17.4 N
R3D Dilute 200 ml glacial acetic acid to 1000 ml with demineralized water (DMH_2O).
R4 Nitric acid (HNO_3) 68-70%, 15.5 N
R4D Dilute 20 ml conc. HNO_3 to 1000 ml with DMH_2O
R5 Ethylenediaminetetraacetic acid (EDTA), fw 292.24

Stock Mehlich 3:

Put about 120 ml DMH_2O in a 200 ml volumetric flask. Add 27.78 g R2 and mix, then add 14.61 g EDTA, dissolve and make to 200 ml. Mix well and immediately transfer to a plastic bottle. It is necessary to dissolve the R2 first and then add the EDTA to get the EDTA into solution.

Extractant:

Use a plastic carboy with outlet, calibrated to 4 L, and add about 3 L of DMH_2O.
Add 80.0 g R1 and dissolve.
Add 16 ml Stock Mechlich 3 and mix. Measure with a plastic graduated cylinder.
Add 230 ml R3D.
Add 164 ml R4D.
Make to 4 L with DMH_2O and mix thoroughly. pH should be 2.5 ± 0.1.

APPENDIX I.

FOLIAR NUTRIENT GUIDELINES FOR THE INTERPRETATION OF NUTRITIONAL STATUS FOR HEMLOCK (FROM BALLARD AND CARTER (1986).

Interpretation	N%	P%	K%
	0	0	0
Very severely deficient			
	1.05	0.08	0.35
Severely deficient			
	1.3	0.1	0.45
Slight moderate deficiency			
	1.45	0.15	0.75
Adequate			

	Ca%	Mg%
	0	0
Severely deficient		
	0.1	0.06
Moderate-severely deficient		
	0.15	0.08
Possible slight-moderate deficiency		
	0.2	0.1
Little, if any deficiency		
	0.25	0.12
No deficiency		

Appendix I (continued)	µg g^{-1}
Mn	
Severe deficiency	0
Probable deficiency	4
Possible deficiency or near deficiency	15
No deficiency	25
Fe	
Deficiency likely	0
Possible deficiency	25
Low to zero probability of deficiency	50
Active Fe	
Deficiency likely	0
Deficiency unlikely	30
Zn	
Probable deficiency	0
Possible deficiency	10
No deficiency	15
Cu	
Probable deficiency	0
Possible moderate deficiency	1
Possibly somewhat deficient	2
Slight possibility of deficiency	2.6
No deficiency	4

Appendix I (continued)

	μg g^{-1}
B	
Deficiency likely	0
B possibly deficient; Possible NID	10
B probably not deficient If N<1.5%, then NID possible If N>1.5%, then NID unlikely	15
No deficiency	

Appendix I (continued)

N/P	
No P deficiency; NID[a] unlikely	0
No P deficiency; NID unlikely	6.111N + 0.11[b]
Possible P deficiency; NID or NAD[a] possible	12
P deficiency	16
P/Al	
P/Al suggests P deficiency, unless P> 0.13%	0
No interpretation	3
K/Ca	
Possible K deficiency	0
No interpretation	0.5
High K/Ca suggests desirability of checking for possible Fe deficiency	3.5
Ca/Mg	
Ca/Mg is unusually low and may impair growth. If soil parent material is of ultramafic origin, consider possibilities of Mo deficiency and Ni and/or Cr toxicity.	0
No interpretation	0.8

---

a. NID = Deficiency inducible by N fertilization; NAD = deficiency may be aggravated by N fertilization.

b. N = N percent, dry mass basis.

Appendix I (concluded)

S	
	0.00 %
If sulfate-S is not evaluated: data suggests actual or inducible S deficiency. If sulfate-S < 0.01%, possible S deficiency and possible NAD[a]. If sulfate-S >0.01%, no S deficiency but NID[a] possible.	
	0.12 %
Possible S deficiency and If sulfate-S < 0.01%, NID[a] possible. If sulfate-S > 0.01%, NID unlikely.	
	0.14 %
S deficiency and NID are both unlikely.	
	0.16 %
No S deficiency; NID unlikely.	
N/S (Not interpreted where total S exceeds 0.14%)	
	0
No S deficiency; NID unlikely.	
	4.2N + 4.94[b]
No S deficiency; NID possible.	
	13.6
Possible S deficiency.	
	14.6
S deficiency.	
Sulfate-S (The following applies only where total S has not been evaluated)	
	0.000 %
Actual or inducible S deficiency; NAD or NID likely.	
	0.008 %
No S deficiency; but NID possible.	
	0.020 %
No S deficiency; but NID unlikely.	
	0.040 %
Very high; possibly N deficient.	

---

a. NID = Deficiency inducible by N fertilization; NAD = deficiency may be aggravated by N fertilization.

b. N = N percent, dry mass basis.

APPENDIX J.

CONCENTRATIONS OF ZINC AND MANGANESE IN THE CELLULAR FRACTIONS OF HEMLOCK FOLIAGE.

SAMPLE	ZNA	MNA	ZNB	MNB	ZNC
	----------	μg g^{-1}	----------		
MEANS					
1	5.3	603	5.3	447	7.7
2	6.3	1460	7.3	1277	8.0
3	41.0	528	34.0	430	45.3
4	4.0	677	4.3	657	6.4
5	5.3	114	7.3	95	7.3
6	9.3	160	9.7	153	9.9
7	8.3	15	8.0	15	10.6
STANDARD DEVIATION					
1	1.5	62	1.5	36	1.2
2	1.2	32	0.6	56	2.0
3	14.8	82	11.3	75	15.9
4	0.0	49	0.6	125	0.7
5	1.5	3	2.1	10	0.6
6	1.2	31	0.6	18	0.8
7	1.2	3	2.0	4	1.6
MINIMUM					
1	4.0	545	4.0	407	7.0
2	5.0	1437	7.0	1227	6.0
3	24.0	452	21.0	387	27.0
4	4.0	622	4.0	537	5.7
5	4.0	111	5.0	83	7.0
6	8.0	127	9.0	137	9.0
7	7.0	13	6.0	12	8.8
MAXIMUM					
1	7.0	668	7.0	477	9.0
2	7.0	1497	8.0	1337	10.0
3	51.0	615	41.0	517	55.0
4	4.0	717	5.0	787	7.0
5	7.0	117	9.0	102	8.0
6	10.0	187	10.0	172	10.4
7	9.0	18	10.0	19	11.7

Appendix J (concluded)

SAMPLE	MNC	ZND	MND	ZNE	MNE
	----------µg g^{-1}----------				
1	740	46.4	661	6.1	1300
2	1717	74.4	1692	7.4	2513
3	602	131.3	668	68.7	
4	844	56.4	824	5.4	1280
5	130	110.9	242	8.1	205
6	198	48.5	232	7.9	315
7	19	42.6	39	1.0	9

	MNC	ZND	MND	ZNE	MNE
1	91	35.0	123	0.5	96
2	17	35.7	141	0.4	153
3	90	26.6	113	20.5	
4	64	25.3	126	0.3	46
5	6	20.9	35	0.1	15
6	12	21.2	58	0.4	28
7	10	44.2	34	0.1	1

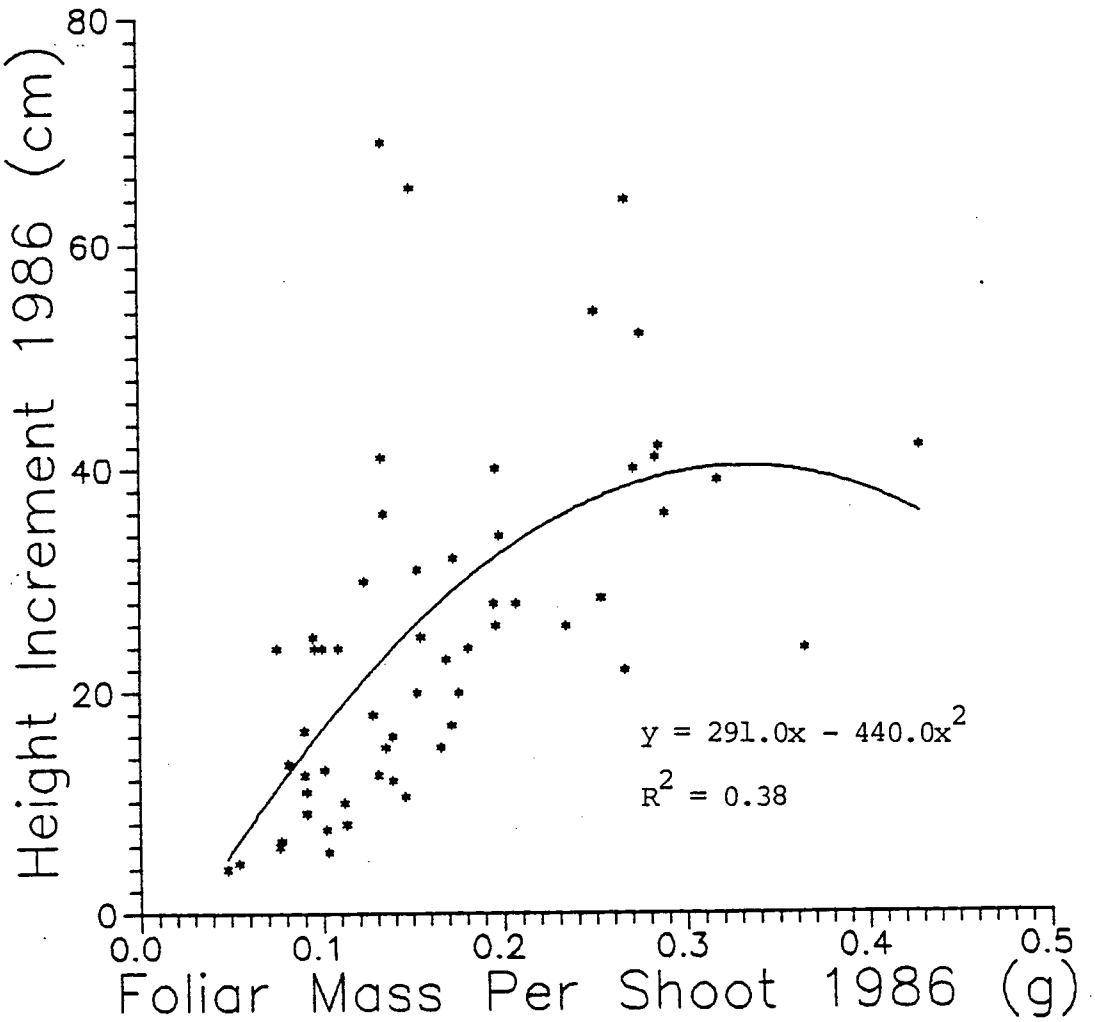
	MNC	ZND	MND	ZNE	MNE
1	657	16.9	525	5.8	1230
2	1707	50.6	1532	7.1	2380
3	527	108.4	580	45.0	
4	776	31.2	687	5.0	1240
5	127	87.7	217	8.0	190
6	185	26.1	183	7.6	285
7	8.8	0.0	0	0.9	9

	MNC	ZND	MND	ZNE	MNE
1	837	85.1	766	6.7	1410
2	1737	115.4	1800	7.9	2680
3	702	160.5	795	81.5	
4	902	81.8	936	5.6	1330
5	137	128.2	282	8.3	220
6	207	68.2	295	8.4	340
7	28.1	88.2	59	1.0	10

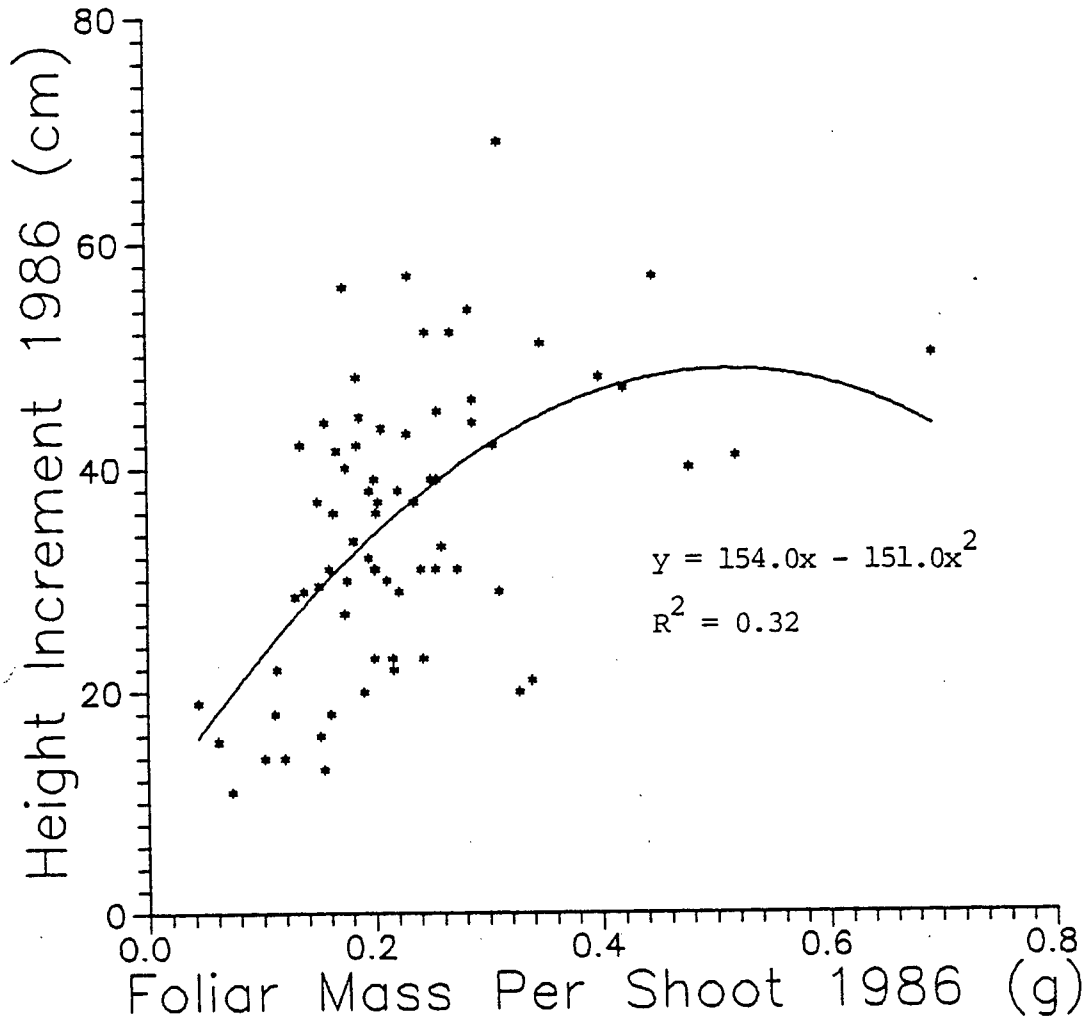
Sample
1. Hemlock, site 5, control, 1986.
2. Hemlock, site 5, treatment 6, 1986.
3. Hemlock, site 5, treatment 15, 1986.
4. Hemlock, site 5, 1987.
5. Douglas-fir, site 5, 1987.
6. Amabilis fir, site 5, 1987.
7. Yellow cedar, site 5, 1987.

APPENDIX K.

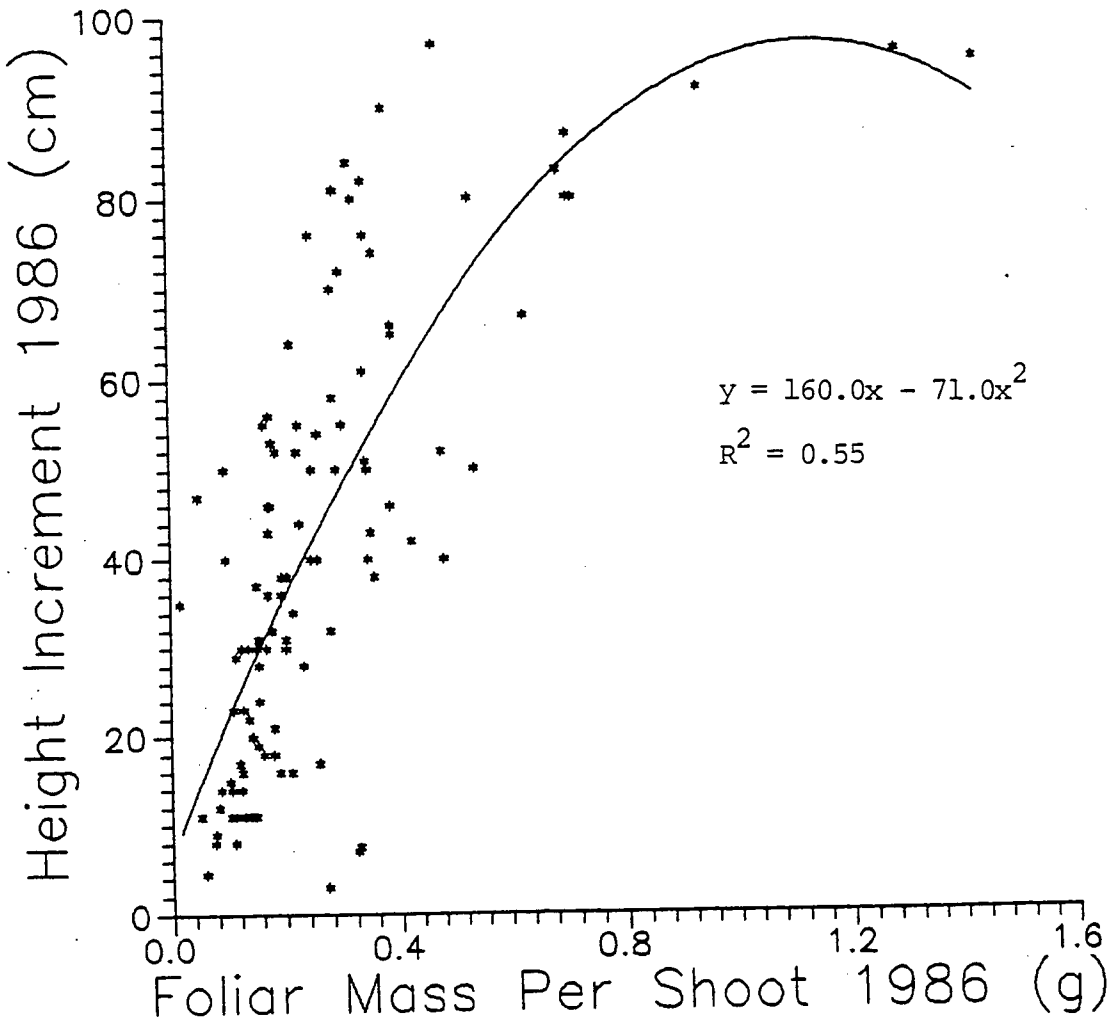
SCATTER PLOTS OF HEIGHT INCREMENT VERSUS FOLIAR MASS PER SHOOT.



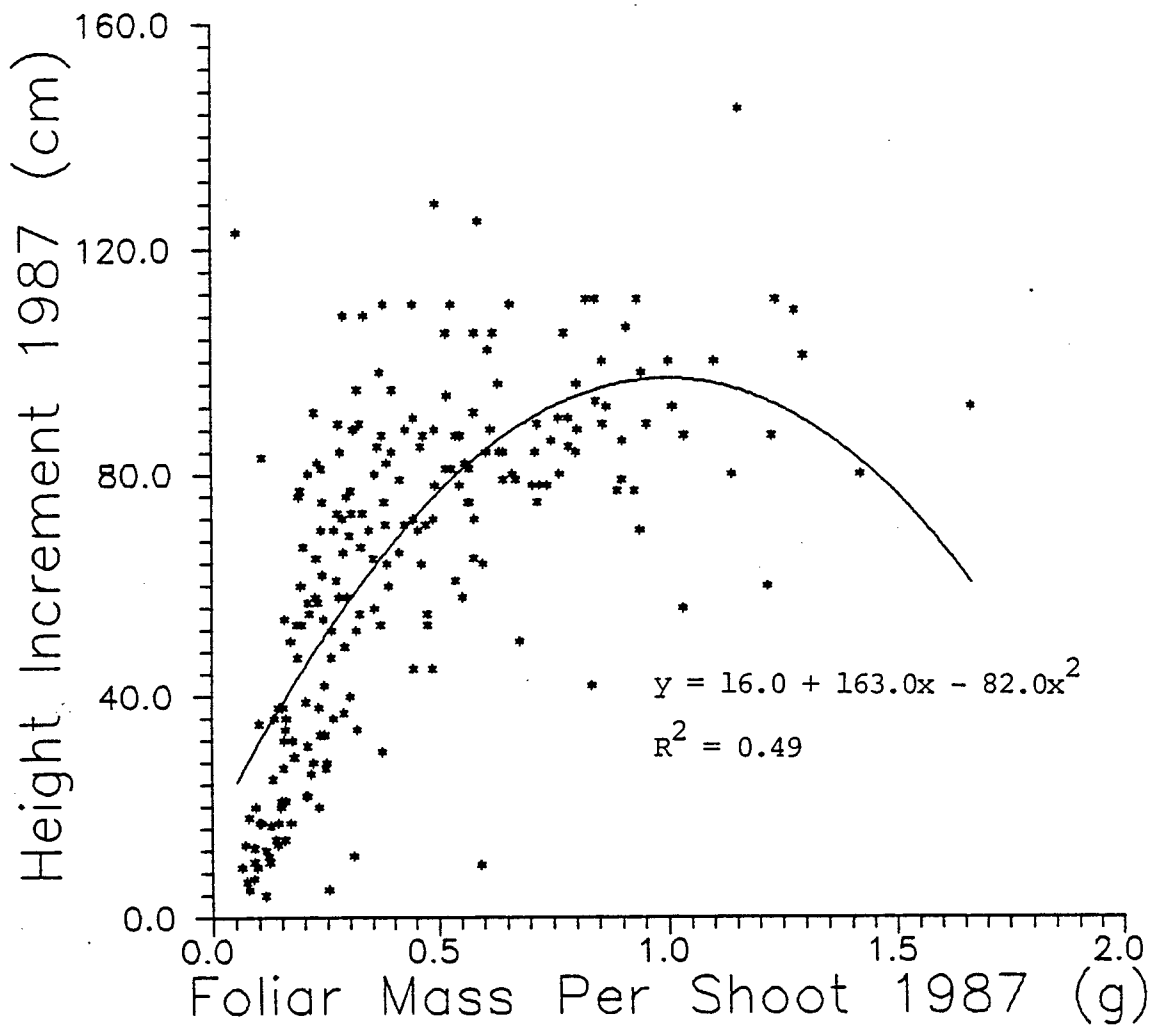
Appendix K.1. Scatter plot of the 1986 height increment versus the 1986 foliar mass per shoot for site 2.



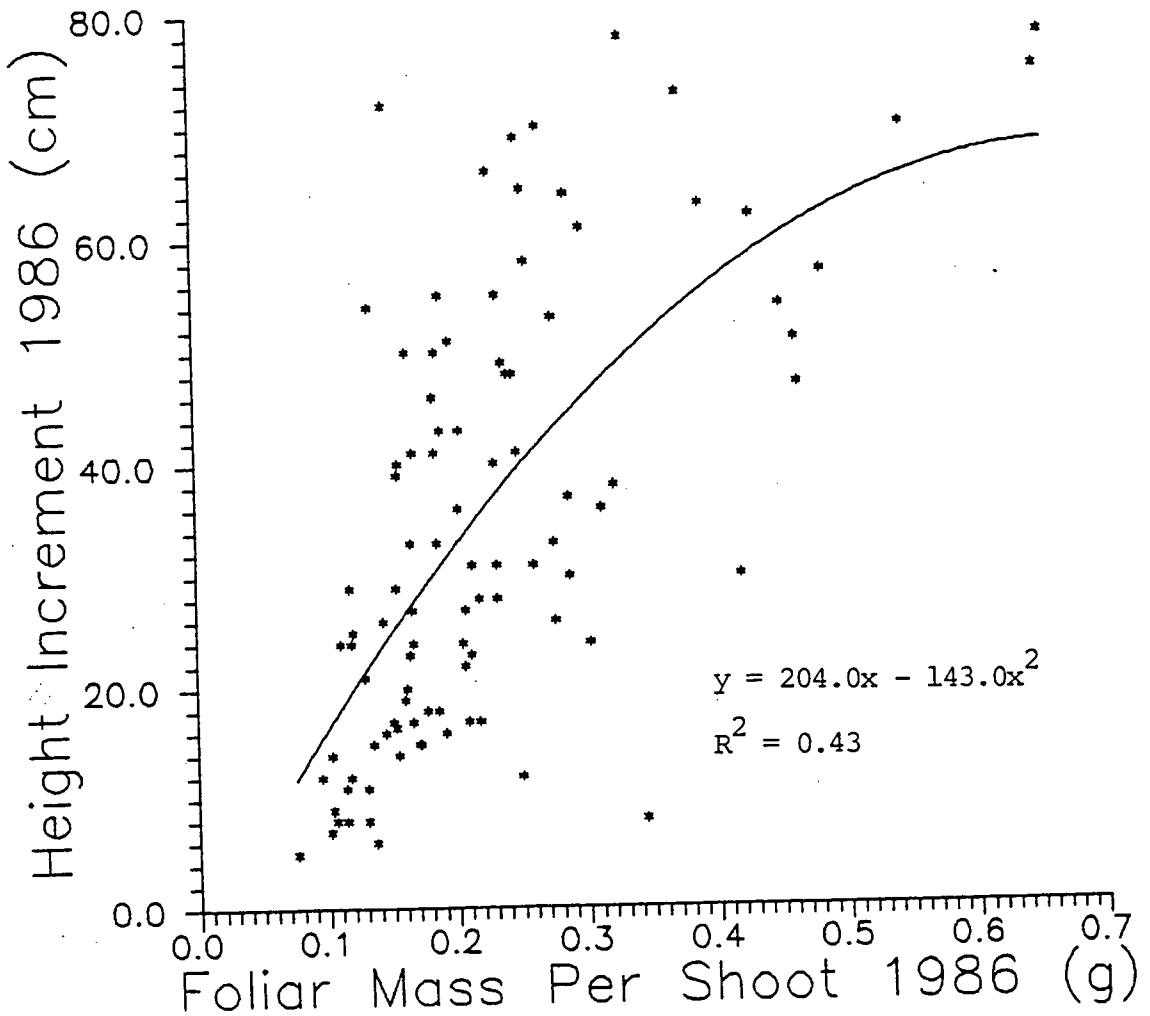
Appendix K.2. Scatter plot of the 1986 height increment versus the 1986 foliar mass per shoot for site 3.



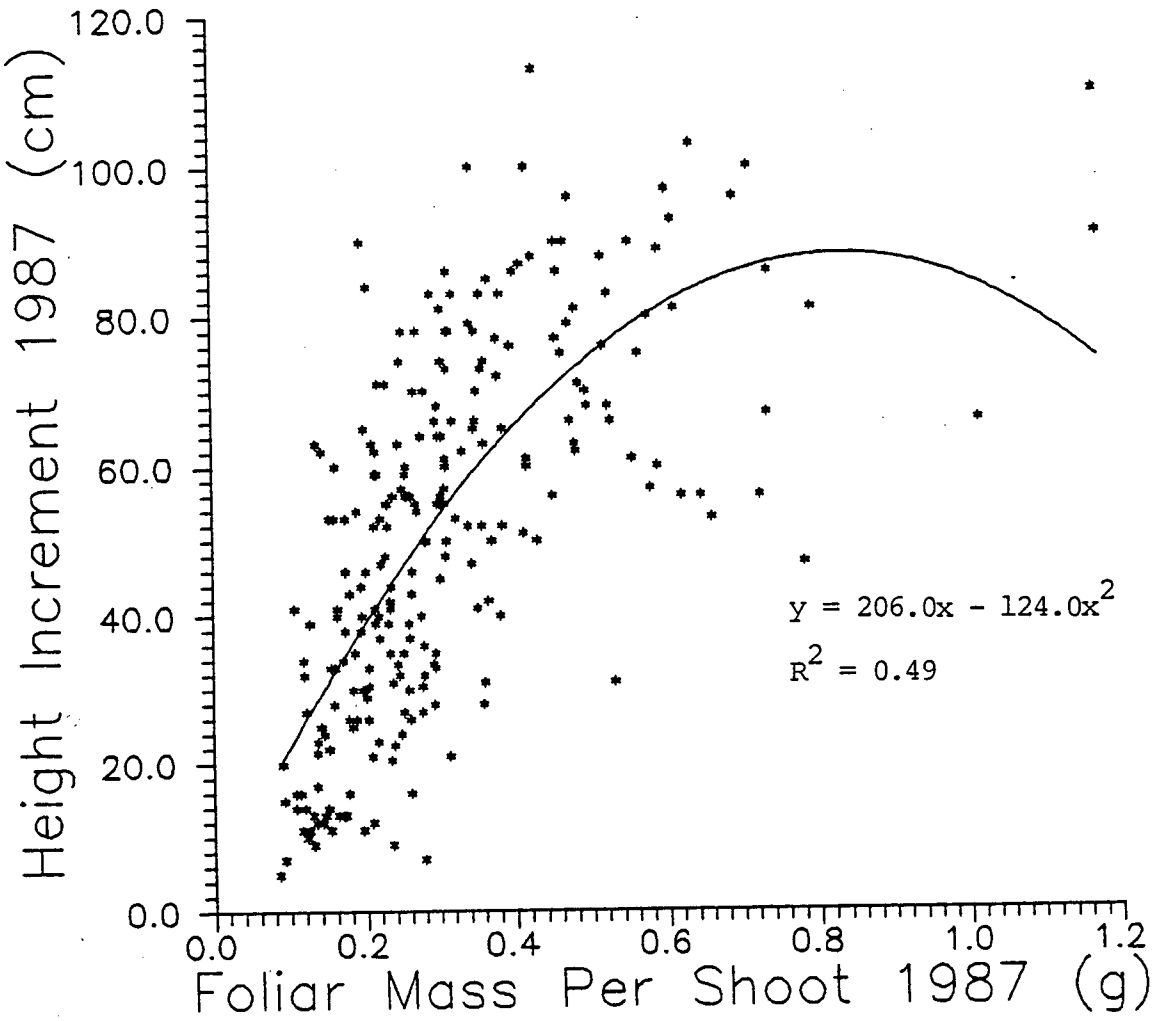
Appendix K.3. Scatter plot of the 1986 height increment versus the 1986 foliar mass per shoot for site 4.



Appendix K.4. Scatter plot of the 1987 height increment versus the 1987 foliar mass per shoot for site 4.



Appendix K.5. Scatter plot of the 1986 height increment versus the 1986 foliar mass per shoot for site 5.



Appendix K.6. Scatter plot of the 1987 height increment versus the 1987 foliar mass per shoot for site 5.

APPENDIX L.

FOLIAR NUTRIENT DATA.

MEANS 1-85[1]-85[2]-85[3]

TRT	ZN	MN	P	FE	CU	B
	--μg g^{-1}-----		cg g^{-1}	---------μg g^{-1}-------		
1	10.9	1284	0.199	37.8	2.1	13.6
2	14.2	1131	0.225	40.1	2.4	13.3
3	15.1	3008	0.181	41.5	2.9	14.4
4	58.1	1133	0.177	40.2	2.5	14.5
5	149.7	1180	0.174	41.6	2.1	13.5
6	9.4	1354	0.192	40.9	2.4	15.4
7	10.5	1175	0.176	43.3	2.4	13.3
8	7.9	1129	0.185	42.2	2.2	13.3
9	9.6	1008	0.191	41.4	2.2	16.5
10	9.2	1082	0.187	39.5	2.3	13.6
11	7.2	1022	0.168	40.3	2.2	13.8
12	7.6	1002	0.185	38.9	2.4	14.2

STANDARD DEVIATION

TRT	ZN	MN	P	FE	CU	B
1	5.6	330	0.043	5.4	0.6	3.6
2	9.5	367	0.057	5.3	0.6	4.4
3	5.8	1298	0.060	5.4	0.7	5.3
4	15.1	292	0.020	3.7	0.4	3.5
5	46.8	327	0.041	4.9	0.3	2.9
6	2.5	416	0.032	6.2	0.6	4.4
7	3.4	325	0.025	3.5	0.7	2.6
8	2.1	352	0.034	11.3	0.5	4.3
9	4.0	388	0.038	5.9	0.6	9.0
10	2.4	426	0.019	4.2	0.6	3.2
11	1.7	321	0.024	4.1	0.8	4.8
12	1.9	306	0.034	5.2	0.7	6.3

---

1. Year of fertilizer treatment.
2. Year of foliage collection.
3. Year in which foliage was formed.

Appendix L (continued).

NUMBER OF CASES

TRT	ZN	MN	P	FE	CU	B
1	10	10	10	10	10	10
2	9	9	9	9	9	9
3	8	8	8	8	8	8
4	8	8	8	8	8	8
5	7	7	7	7	7	7
6	10	10	10	10	10	10
7	8	8	8	8	8	8
8	9	9	9	9	9	9
9	10	10	10	10	10	9
10	9	9	9	9	9	9
11	10	10	10	10	10	10
12	10	10	10	10	10	10

MEAN

TRT	SOS	TOTS	FLWTBR	BI1	HTI1	HTI2
	$\mu g\ g^{-1}$	$cg\ g^{-1}$	g			
1			0.236	0.80	0.73	0.91
2			0.221	0.64	0.60	1.18
3			0.360	0.84	0.78	2.02
4			0.180	0.59	0.65	1.15
5			0.241	0.80	0.51	2.54
6			0.175	0.63	0.69	1.26
7	342	0.146	0.202	0.77	0.69	0.97
8	509	0.160	0.208	0.76	0.73	0.83
9	671	0.165	0.239	0.74	0.72	1.08
10	851	0.190	0.219	0.75	0.61	0.89
11	460	0.131	0.188	0.71	0.56	0.77
12	401	0.124	0.283	0.81	0.62	0.79

Appendix L (continued).

STANDARD DEVIATION

TRT	SOS	TOTS	FLWTBR	BI1	HTI1	HTI2
1			0.121	0.29	0.31	0.38
2			0.099	0.15	0.23	0.47
3			0.246	0.32	0.35	1.32
4			0.042	0.14	0.23	0.64
5			0.154	0.28	0.19	1.14
6			0.059	0.17	0.21	0.41
7	204	0.024	0.062	0.17	0.20	0.73
8	182	0.012	0.076	0.16	0.27	0.30
9	264	0.019	0.133	0.22	0.57	0.56
10	112	0.044	0.163	0.25	0.24	0.35
11	130	0.007	0.050	0.18	0.21	0.18
12	81	0.013	0.127	0.17	0.22	0.50

NUMBER OF CASES

TRT	SOS	TOTS	FLWTBR	BI1	HTI1	HTI2
1	0	0	10	10	7	7
2	0	0	9	9	8	8
3	0	0	8	8	8	8
4	0	0	8	8	7	7
5	0	0	7	7	7	7
6	0	0	10	10	9	9
7	8	8	8	8	8	8
8	8	8	9	9	9	9
9	10	10	10	10	10	10
10	9	9	9	9	8	8
11	10	10	10	10	9	9
12	10	10	10	10	10	10

Appendix L (continued).

1-85-86-86

MEAN

TRT	ZN	MN	P	FE	CU
	---µg g^{-1}----		cg g^{-1}	---µg g^{-1}----	
1	13.3	1083	0.189	61.2	3.0
2	17.2	915	0.228	67.8	3.6
3	11.0	2971	0.169	62.9	4.3
4	13.4	1247	0.203	75.5	3.6
5	15.2	1142	0.192	70.6	3.3
6	10.6	1177	0.200	71.0	3.7
7	11.5	1231	0.184	59.4	2.7
8	11.1	1160	0.184	70.2	2.3
9	9.9	856	0.184	53.6	2.2
10	11.4	914	0.193	58.7	2.4
11	8.8	992	0.197	70.8	3.1
12	9.7	1139	0.207	77.8	3.2

STANDARD DEVIATION

TRT	ZN	MN	P	FE	CU
1	3.9	446	0.041	20.7	1.0
2	10.3	285	0.041	33.8	0.7
3	4.6	1278	0.039	18.4	2.1
4	2.2	656	0.029	14.0	0.7
5	5.2	382	0.071	29.8	0.8
6	1.8	488	0.028	32.5	0.9
7	3.5	352	0.024	15.8	0.6
8	3.5	477	0.050	28.1	0.7
9	2.4	407	0.035	11.4	0.7
10	1.5	378	0.034	17.6	1.2
11	2.3	200	0.040	19.4	0.6
12	3.2	395	0.045	16.2	0.9

Appendix L (continued).

NUMBER OF CASES

TRT	ZN	MN	P	FE	CU
1	7	7	7	7	7
2	9	9	9	9	9
3	8	8	8	8	8
4	7	7	7	7	7
5	9	9	9	9	9
6	8	8	8	8	8
7	8	8	8	8	8
8	9	9	9	9	9
9	9	9	9	9	9
10	7	7	7	7	7
11	9	9	9	8	8
12	9	9	9	9	9

MEAN

TRT	B	TS	FLWTBR	BI2
	$\mu g\ g^{-1}$	$cg\ g^{-1}$	g	
1	21.8		0.248	1.26
2	23.8		0.221	1.59
3	19.6		0.348	1.54
4	24.5		0.264	1.22
5	22.9		0.206	1.61
6	22.5		0.193	1.06
7	20.8	0.151	0.202	1.10
8	22.5	0.153	0.140	0.87
9	22.8	0.156	0.213	0.99
10	22.3	0.157	0.343	1.36
11	23.7	0.143	0.159	0.92
12	23.2	0.150	0.195	0.84

Appendix L (continued).

STANDARD DEVIATION

TRT	B	TS	FLWTBR	BI2
1	6.2		0.206	1.02
2	4.1		0.120	1.92
3	6.1		0.192	0.69
4	6.4		0.193	0.51
5	3.6		0.149	0.71
6	6.5		0.141	0.32
7	4.0	0.020	0.117	0.52
8	7.2	0.021	0.056	0.23
9	10.4	0.018	0.131	0.33
10	4.0	0.020	0.231	0.88
11	6.6	0.015	0.061	0.24
12	8.3	0.031	0.131	0.31

NUMBER OF CASES

TRT	B	TS	FLWTBR	BI2
1	7	0	24	24
2	9	0	27	27
3	8	0	24	24
4	7	0	21	21
5	9	0	27	27
6	8	0	24	24
7	8	8	24	24
8	9	9	18	18
9	9	9	27	27
10	7	7	18	18
11	8	7	27	27
12	9	8	30	30

Appendix L (continued).

2-85-85-85

MEAN

TRT	ZN	MN	P	FE	CU
	---µg g^{-1}----		cg g^{-1}	---µg g^{-1}----	
1	6.9	903	0.181	35.7	2.8
2	7.9	1403	0.126	37.7	2.9
3	7.2	3142	0.111	36.5	3.1
4	58.8	1648	0.188	40.0	3.2
5	75.0	1230	0.124	35.7	3.6
6	9.0	1380	0.163	44.6	3.3
7	6.7	1167	0.141	35.7	3.0
8	5.8	1140	0.163	37.1	2.8
9	8.3	1343	0.136	35.7	2.7
10	8.5	1268	0.167	40.8	3.0
11	7.0	1030	0.136	40.5	3.1
12	6.9	1449	0.126	38.3	3.1

STANDARD DEVIATION

TRT	ZN	MN	P	FE	CU
1	2.8	393	0.050	4.6	0.5
2	1.6	489	0.044	6.5	0.8
3	3.3	1470	0.021	4.6	0.4
4	21.8	548	0.056	3.0	0.4
5	99.0	99	0.042		
6	3.0	328	0.053	7.4	0.9
7	2.3	794	0.037	7.1	0.4
8	2.8	667	0.046	5.4	0.8
9	6.2	401	0.036	12.9	0.5
10	2.3	572	0.060	6.1	0.3
11	2.4	172	0.027	2.1	1.1
12	2.5	655	0.040	4.0	0.8

Appendix L (continued).

NUMBER OF CASES

TRT	ZN	MN	P	FE	CU
1	7	7	7	7	7
2	8	8	8	7	7
3	9	9	9	9	9
4	5	5	5	5	5
5	2	2	2	1	1
6	6	6	6	4	4
7	3	3	3	3	3
8	5	5	5	5	5
9	8	8	8	7	7
10	8	8	8	7	7
11	4	4	4	3	3
12	9	9	9	7	7

MEAN

B $\mu g\ g^{-1}$	TOTS $cg\ g^{-1}$	SOS $\mu g\ g^{-1}$	FLWTBR g	BI1	HTI2	HTI1
13.9			0.143	0.76	1.29	1.05
17.5			0.129	0.76	1.92	0.77
17.3			0.145	0.82	1.82	0.90
16.6			0.153	0.86	1.57	0.72
18.2			0.077	0.59	1.85	0.55
17.3			0.136	0.79	1.32	0.83
15.7	0.119	407	0.107	0.74	1.42	0.75
18.6	0.151	635	0.118	0.75	1.57	0.91
19.6	0.141	585	0.144	0.87	1.50	0.85
17.1	0.160	837	0.168	0.72	1.35	0.77
17.1	0.624	505	0.136	0.73	1.38	0.63
18.9	0.273	621	0.143	0.71	1.20	0.94

Appendix L (continued).

STANDARD DEVIATION

B	TOTS	SOS	FLWTBR	BI1	HTI2	HTI1
3.7			0.048	0.16	0.58	0.35
5.4			0.040	0.14	0.96	0.35
4.0			0.056	0.15	0.51	0.12
3.2			0.034	0.22	0.85	0.32
3.9			0.041		1.62	0.16
8.7			0.048	0.18	0.66	0.33
2.4	0.027	51	0.019	0.01	0.41	0.28
3.4	0.023	305	0.042	0.21	0.20	0.54
2.7	0.012	149	0.037	0.28	0.39	0.18
5.7	0.019	109	0.059	0.11	0.68	0.39
8.1	0.889	370	0.054	0.08	0.73	0.14
4.2	0.395	135	0.049	0.15	0.45	0.34

NUMBER OF CASES

B	TOTS	SOS	FLWTBR	BI1	HTI2	HTI1
7	0	0	7	7	6	6
7	0	0	8	8	7	7
9	0	0	9	9	7	7
5	0	0	5	5	7	7
2	0	0	2	1	5	6
6	0	0	6	6	4	4
3	2	2	3	3	3	3
5	5	5	5	5	5	5
7	8	8	8	8	6	6
8	8	7	8	8	4	5
4	3	3	4	4	4	4
8	8	8	9	9	7	7

Appendix L (continued).

2-85-86-86

MEAN

TRT	ZN	MN	P	FE	CU
	---µg g^{-1}----		cg g^{-1}	---µg g^{-1}----	
1	13.3	1323.8	0.145	53.7	2.4
2	13.6	1399.3	0.148	42.9	2.3
3	12.4	3875.1	0.128	39.3	2.5
4	11.5	1526.7	0.153	55.6	3.1
5	19.6	1140.8	0.130	52.8	2.7
6	12.7	1327.8	0.118	51.4	2.8
7	8.8	1727.5	0.140	31.4	2.6
8	11.4	1180.8	0.152	35.1	2.7
9	11.4	1401.4	0.136	33.8	2.6
10	13.0	2088.0	0.132	32.5	2.9
11	10.1	1249.3	0.125	57.1	2.9
12	11.1	1931.8	0.126	41.4	2.8

STANDARD DEVIATION

TRT	ZN	MN	P	FE	CU
1	3.8	289.3	0.064	18.0	0.4
2	2.7	433.9	0.042	14.4	0.9
3	5.6	1428.5	0.042	13.2	0.6
4	5.6	1031.0	0.035	10.3	1.0
5	8.8	433.6	0.055	9.6	0.7
6	3.7	370.9	0.024	23.3	0.5
7	3.4	1025.1	0.028	7.7	0.9
8	2.7	601.0	0.042	5.4	0.6
9	6.8	359.5	0.033	8.0	0.7
10	5.5	985.4	0.027	7.1	0.7
11	4.2	357.4	0.029	14.3	0.9
12	4.8	589.0	0.029	7.8	0.4

Appendix L (continued).

NUMBER OF CASES

TRT	ZN	MN	P	FE	CU
1	6	6	6	6	6
2	6	6	6	6	6
3	9	9	9	9	9
4	7	7	7	7	7
5	5	5	5	5	5
6	5	5	5	5	5
7	4	4	4	4	4
8	6	6	6	6	6
9	7	7	7	7	7
10	5	5	5	5	5
11	4	4	4	4	4
12	8	8	8	8	8

MEAN

TRT	B	TOTS	FLWTBR	BI2
	$\mu g\ g^{-1}$	$cg\ g^{-1}$	g	
1	28.8		0.140	1.31
2	25.8		0.196	1.58
3	33.0		0.181	1.42
4	28.7		0.158	1.58
5	26.5		0.131	1.34
6	30.8		0.190	1.06
7	37.1	0.147	0.123	1.23
8	22.9	0.156	0.163	1.08
9	30.0	0.153	0.140	1.28
10	31.3	0.162	0.156	1.14
11	31.0	0.140	0.163	1.47
12	30.0	0.148	0.186	1.02

Appendix L (continued).

STANDARD DEVIATION

TRT	B	TOTS	FLWTBR	BI2
1	7.5		0.047	0.48
2	7.6		0.119	0.53
3	9.4		0.096	0.55
4	5.0		0.068	0.68
5	6.7		0.092	0.51
6	4.1		0.104	0.25
7	13.1	0.012	0.039	0.45
8	4.9	0.019	0.113	0.51
9	3.8	0.008	0.057	0.40
10	6.1	0.019	0.147	0.53
11	10.2	0.008	0.074	0.76
12	5.0	0.012	0.076	0.33

NUMBER OF CASES

TRT	B	TOTS	FLWTBR	BI2
1	6	0	18	18
2	6	0	18	18
3	9	0	27	27
4	7	0	18	18
5	5	0	15	15
6	5	0	14	14
7	4	3	12	12
8	6	5	18	15
9	7	6	24	24
10	5	5	15	15
11	4	4	12	12
12	8	6	24	24

Appendix L (continued).

3-85-86-86

MEAN

TRT	ZN	MN	P	B	TOTS
	---µg g^{-1}----		cg g^{-1}	µg g^{-1}	cg g^{-1}
1	11.1	1502	0.174	16.2	
2	17.7	1638	0.193	25.7	
3	15.5	2427	0.187	22.5	
4	21.3	2019	0.220	27.8	
5	19.1	1793	0.178	21.7	
6	15.4	1899	0.173	27.8	
7	14.4	2197	0.230	28.1	0.158
8	17.4	2268	0.170	28.7	0.140
9	13.1	1245	0.185	28.2	0.145
10	12.8	1212	0.173	26.6	0.140
11	17.9	1853	0.180	22.7	0.143
12	11.7	1505	0.188	23.6	0.140

STANDARD DEVIATION

TRT	ZN	MN	P	B	TOTS
1	1.9	455	0.026	3.2	
2	10.0	648	0.028	5.4	
3	2.4	441	0.010	4.6	
4	2.4	994	0.036	8.7	
5	8.3	775	0.049	8.7	
6	6.7	839	0.039	10.2	
7	3.7	331	0.047	5.7	0.023
8	3.4	1037	0.038	12.2	0.029
9	3.5	280	0.021	4.5	0.021
10	4.3	795	0.022	9.1	0.024
11	5.5	547	0.026	4.8	0.047
12	1.7	287	0.033	9.2	0.023

Appendix L (continued)

NUMBER OF CASES

TRT	ZN	MN	P	B	TOTS
1	7	7	7	7	0
2	7	7	7	6	0
3	7	7	7	7	0
4	3	3	3	3	0
5	8	8	8	8	0
6	8	8	8	8	0
7	8	8	8	8	8
8	8	8	8	8	8
9	4	4	4	4	4
10	4	4	4	4	4
11	8	8	8	8	8
12	6	6	6	6	6

MEAN

TRT	FLWTBR g	BI2	BI1	HTI2	HTI1
1	0.241	0.90	0.94	1.04	1.01
2	0.303	0.87	1.06	1.16	1.00
3	0.308	0.99	1.09	1.05	1.07
4	0.255	1.02	1.18	0.99	1.15
5	0.193	1.02	0.78	1.10	0.92
6	0.214	1.00	0.93	1.07	1.07
7	0.171	0.86	0.86	0.79	1.00
8	0.172	0.79	0.82	1.09	0.84
9	0.252	1.02	0.93	0.97	0.80
10	0.303	0.92	1.09	1.17	1.18
11	0.315	1.05	0.90	1.20	0.94
12	0.262	0.95	0.88	1.28	0.83

Appendix L (continued).

STANDARD DEVIATION

TRT	FLWTBR	BI2	BI1	HTI2	HTI1
1	0.106	0.28	0.35	0.58	0.28
2	0.248	0.23	0.33	0.48	0.29
3	0.175	0.23	0.40	0.27	0.36
4	0.125	0.49	0.20	0.21	0.41
5	0.081	0.32	0.38	0.37	0.44
6	0.087	0.50	0.34	0.54	0.22
7	0.089	0.37	0.27	0.22	0.45
8	0.105	0.30	0.23	0.30	0.45
9	0.072	0.57	0.24	0.07	0.23
10	0.360	0.26	0.37	0.47	0.37
11	0.210	0.35	0.42	0.46	0.36
12	0.085	0.26	0.26	0.53	0.38

NUMBER OF CASES

TRT	FLWTBR	BI2	BI1	HTI2	HTI1
1	21	21	21	8	8
2	21	21	21	6	6
3	21	21	21	8	8
4	12	12	12	3	3
5	23	23	23	8	8
6	22	22	22	6	6
7	27	27	24	9	9
8	26	26	26	9	9
9	12	12	12	4	4
10	15	15	15	5	5
11	27	27	24	9	9
12	24	24	24	8	8

Appendix L (continued).

4-86-86-86
MEAN

TRT	ZN	MN	N	P	MG
	---μg g^{-1}----		----------cg g^{-1}------		
1	10.9	1469	1.07	0.170	0.136
2	8.7	1112	0.99	0.175	0.125
3	11.8	1098	0.97	0.153	0.118
4	9.2	2026	1.18	0.120	0.140
5	11.4	2802	1.30	0.125	0.140
6	9.1	3206	1.33	0.138	0.118
7	8.8	1012	1.05	0.160	0.135
8	9.6	1360	1.04	0.178	0.140
9	8.1	1168	0.94	0.150	0.128
10	11.3	1639	1.09	0.184	0.136
11	10.7	1647	0.98	0.178	0.158
12	11.8	1175	1.16	0.134	0.126
13	18.3	1448	1.10	0.163	0.130
14	26.2	1384	1.04	0.155	0.123
15	48.6	1536	1.06	0.178	0.148
16	9.1	1208	0.99	0.160	0.126
17	7.7	1169	1.01	0.148	0.120
18	8.2	1464	0.97	0.155	0.143
19	9.4	1389	1.13	0.148	0.123
20	6.4	986	0.91	0.118	0.110
21	10.4	1411	1.09	0.162	0.124
22	7.8	1433	0.97	0.135	0.138
23	17.2	1343	1.67	0.160	0.118
24	9.2	1432	1.12	0.136	0.130
25	8.7	1428	1.00	0.197	0.140

Appendix L (continued)

STANDARD DEVIATION

TRT	ZN	MN	N	P	MG
	---μg g^{-1}----		----------cg g^{-1}------		
1	4.5	445	0.28	0.010	0.025
2	2.3	378	0.18	0.053	0.024
3	4.6	264	0.12	0.051	0.022
4	1.7	727	0.07	0.023	0.016
5	4.5	710	0.13	0.035	0.014
6	1.5	1341	0.18	0.015	0.029
7	2.9	257	0.35	0.042	0.029
8	2.9	432	0.29	0.036	0.008
9	2.6	456	0.13	0.042	0.011
10	4.3	597	0.23	0.040	0.034
11	6.0	604	0.25	0.050	0.017
12	3.6	214	0.25	0.038	0.034
13	6.9	247	0.20	0.057	0.029
14	10.4	283	0.09	0.047	0.015
15	25.1	724	0.32	0.073	0.051
16	2.0	415	0.20	0.034	0.023
17	3.3	287	0.24	0.031	0.021
18	3.3	550	0.35	0.013	0.010
19	3.1	112	0.17	0.039	0.017
20	2.1	239	0.17	0.025	0.014
21	4.3	613	0.23	0.040	0.015
22	2.1	310	0.13	0.006	0.022
23	6.2	400	0.26	0.014	0.022
24	2.6	410	0.19	0.030	0.025
25	1.9	360	0.06	0.071	0.062

Appendix L (continued)

NUMBER OF CASES

TRT	ZN	MN	N	P	MG
1	5	5	5	5	5
2	4	4	4	4	4
3	4	4	4	4	4
4	5	5	5	5	5
5	4	4	4	4	4
6	4	4	4	4	4
7	4	4	4	4	4
8	4	4	4	4	4
9	5	5	5	5	5
10	5	5	5	5	5
11	4	4	4	4	4
12	5	5	5	5	5
13	4	4	4	4	4
14	4	4	4	4	4
15	4	4	4	4	4
16	5	5	5	5	5
17	5	5	5	5	5
18	4	4	4	4	4
19	4	4	4	4	4
20	4	4	4	4	4
21	5	5	5	5	5
22	4	4	4	4	4
23	4	4	4	4	4
24	5	5	5	5	5
25	3	3	3	3	3

Appendix L (continued)

MEAN

FE	CU	B	AFE	FLWTBR	BI2
	μg g^{-1}			g	
30.7	3.1	26.2	37.0	0.193	0.91
24.1	3.0	23.1	32.3	0.253	0.91
38.4	3.1	24.1	28.8	0.225	1.18
25.0	3.6	22.5	31.8	0.270	1.03
24.1	3.7	23.4	31.9	0.352	1.15
33.9	3.7	23.0	36.3	0.363	1.18
30.9	2.5	36.7	30.8	0.229	0.92
27.4	2.7	36.9	30.8	0.308	0.92
35.7	2.3	38.8	31.8	0.191	0.85
32.1	2.9	42.2	32.5	0.271	0.83
32.1	2.5	89.5	32.6	0.197	0.85
33.6	2.6	79.0	32.0	0.287	0.90
29.5	3.7	24.3	34.9	0.263	0.98
25.9	3.8	24.0	35.0	0.299	0.84
31.2	3.1	25.1	33.0	0.171	0.79
25.0	3.9	25.7	30.2	0.286	0.83
32.1	4.0	22.8	35.2	0.211	0.82
34.8	2.6	23.8	32.0	0.216	0.95
35.7	2.7	21.8	30.8	0.267	0.80
28.6	2.3	22.7	27.5	0.136	1.04
34.3	2.9	26.8	30.6	0.317	0.94
33.0	2.9	22.9	30.8	0.243	0.94
38.4	3.5	33.2	37.2	0.804	1.23
32.1	2.6	26.0	30.2	0.198	0.78
25.0	3.2	24.5	32.0	0.200	0.76

Appendix L (continued)

STANDARD DEVIATION

FE	CU	B	AFE	FLWTBR	BI2
		µg g^{-1}		g	
12.8	1.0	4.1	9.3	0.100	0.39
8.9	0.6	7.0	3.9	0.170	0.21
29.9	0.4	6.1	5.8	0.083	1.13
5.0	0.4	6.3	3.8	0.096	0.26
8.4	0.9	2.8	5.5	0.388	0.38
8.5	0.4	2.5	3.3	0.284	0.37
4.1	0.6	6.7	4.2	0.112	0.27
2.1	0.7	15.2	1.7	0.276	0.31
11.6	0.5	7.5	2.0	0.080	0.34
5.6	0.3	11.0	4.4	0.178	0.27
12.4	0.8	30.7	6.0	0.058	0.28
6.0	0.6	21.6	4.1	0.239	0.32
10.3	0.7	2.4	7.1	0.156	0.47
5.4	0.6	4.9	5.7	0.082	0.39
4.5	0.8	5.1	5.4	0.136	0.35
5.6	0.9	3.7	4.0	0.231	0.29
9.2	1.2	9.6	8.4	0.073	0.24
3.4	0.5	6.8	1.6	0.231	0.46
9.2	0.5	2.5	5.5	0.071	0.17
5.8	0.8	3.2	5.8	0.063	0.37
7.4	0.5	2.7	6.2	0.239	0.29
6.1	0.6	3.7	3.5	0.062	0.46
1.8	0.6	4.1	2.4	0.646	0.48
5.6	0.7	2.9	1.8	0.120	0.28
		1.8		0.127	0.22

Appendix L (continued)

NUMBER OF CASES

FE	CU	B	AFE	FLWTBR	BI2
5	5	5	5	5	30
4	4	4	4	4	27
4	4	4	4	4	27
5	5	5	5	5	30
4	4	4	4	4	24
4	4	4	4	4	26
3	3	3	3	4	27
3	3	3	3	4	21
5	5	5	5	5	26
5	5	5	5	5	30
4	4	4	4	4	30
5	5	5	5	5	24
4	4	4	4	4	30
4	4	4	4	4	21
4	4	4	4	4	25
5	5	5	5	5	27
4	4	4	4	5	28
4	4	4	4	4	27
4	4	4	4	4	30
4	4	4	4	4	27
5	5	5	5	5	30
4	4	4	4	4	27
4	4	4	4	4	30
5	5	5	5	5	30
1	1	3	1	3	24

Appendix L (continued)

4-86-87-87
MEAN

TRT	ZN	MN	N	P	MG
	---μg g^{-1}----		----------cg g^{-1}------		
1	11.7	1719	1.15	0.193	0.140
2	8.5	1302	1.03	0.198	0.149
3	11.2	1465	1.01	0.184	0.141
4	10.1	2980	1.12	0.157	0.150
5	9.2	3460	1.12	0.163	0.130
6	9.7	3737	1.19	0.167	0.116
7	8.8	1525	1.10	0.184	0.147
8	10.6	1804	1.19	0.207	0.143
9	6.4	1452	0.96	0.169	0.149
10	13.8	1598	1.20	0.197	0.145
11	11.6	1743	1.16	0.198	0.152
12	10.2	1593	1.18	0.159	0.154
13	10.2	1412	1.12	0.176	0.148
14	8.3	1407	0.96	0.169	0.146
15	9.4	1481	1.04	0.160	0.167
16	9.0	1409	1.16	0.164	0.154
17	9.4	1644	1.28	0.166	0.135
18	8.5	1577	1.06	0.165	0.143
19	9.0	1586	1.14	0.172	0.151
20	9.7	1635	1.25	0.168	0.137
21	8.5	1512	0.99	0.161	0.137
22	8.5	1772	1.11	0.178	0.170
23	11.1	1259	1.22	0.208	0.143
24	9.7	1587	1.16	0.157	0.163
25	11.7	1545	1.17	0.183	0.159

Appendix L (continued)

STANDARD DEVIATION

TRT	ZN	MN	N	P	MG
	---µg g^{-1}----		----------cg g^{-1}------		
1	5.8	511	0.27	0.040	0.030
2	1.8	432	0.20	0.045	0.031
3	2.7	447	0.20	0.055	0.033
4	4.2	918	0.17	0.025	0.021
5	2.5	780	0.19	0.035	0.022
6	3.8	1237	0.21	0.034	0.023
7	3.2	747	0.31	0.064	0.025
8	4.2	689	0.27	0.043	0.015
9	2.3	428	0.22	0.034	0.021
10	8.9	412	0.25	0.026	0.029
11	6.0	471	0.21	0.042	0.024
12	3.5	365	0.29	0.047	0.012
13	2.6	454	0.12	0.026	0.027
14	2.6	263	0.20	0.038	0.027
15	3.0	352	0.27	0.051	0.031
16	3.1	542	0.12	0.029	0.033
17	3.5	544	0.25	0.029	0.028
18	3.0	332	0.27	0.024	0.021
19	3.1	270	0.22	0.049	0.034
20	3.5	375	0.22	0.048	0.031
21	3.9	281	0.22	0.039	0.022
22	2.0	343	0.20	0.035	0.026
23	3.5	305	0.15	0.024	0.029
24	2.7	363	0.27	0.031	0.022
25	4.6	415	0.21	0.041	0.040

Appendix L (continued)

NUMBER OF CASES

TRT	ZN	MN	N	P	MG
1	9	9	9	9	9
2	9	9	9	9	9
3	9	9	9	9	9
4	10	10	9	10	10
5	9	9	9	9	9
6	9	9	9	9	9
7	10	10	10	10	10
8	7	7	7	7	7
9	9	9	9	9	9
10	10	10	10	10	10
11	9	9	9	9	9
12	8	8	8	8	8
13	9	9	9	9	9
14	8	8	8	8	8
15	8	8	8	8	8
16	7	7	7	7	7
17	10	10	10	10	10
18	8	8	8	8	8
19	9	9	9	9	9
20	10	10	10	10	10
21	9	9	9	9	9
22	9	9	9	9	9
23	9	9	9	9	9
24	10	10	10	10	10
25	9	9	9	9	9

Appendix L (continued)

MEAN

FE	CU	FLWTBR	BI3	HTI3	HTI2
---µg g^{-1}----		g			
50.4	3.4	0.523	1.42	1.86	1.31
46.4	3.1	0.407	1.15	1.59	1.15
52.0	3.2	0.364	1.14	1.59	1.40
47.5	3.4	0.449	1.91	3.14	1.21
50.4	3.3	0.506	1.77	2.65	1.32
55.9	3.6	0.646	1.33	2.27	1.20
46.4	3.4	0.431	1.43	1.76	1.18
50.5	3.5	0.592	1.80	1.98	1.04
41.7	3.1	0.199	1.24	1.82	0.87
49.3	3.3	0.665	1.62	2.57	1.11
48.0	3.8	0.457	1.62	2.26	1.24
46.4	3.7	0.428	1.35	1.98	1.26
42.8	3.8	0.421	1.86	3.30	0.98
43.3	3.4	0.371	1.75	2.39	0.78
37.9	3.6	0.368	1.43	1.85	0.99
47.4	3.8	0.505	1.51	1.66	1.04
54.3	3.6	0.540	1.66	1.75	1.19
46.0	3.6	0.327	1.14	2.35	1.01
41.7	3.9	0.459	1.46	2.11	0.98
43.6	4.0	0.515	1.30	1.60	1.25
44.8	3.3	0.410	1.46	2.00	0.83
46.8	3.7	0.321	2.27	1.95	1.14
51.2	3.8	0.635	1.87	2.60	1.45
47.1	3.6	0.344	1.51	3.17	1.19
48.8	3.6	0.280	1.77	1.93	1.31

Appendix L (continued)

STANDARD DEVIATION

FE	CU	FLWTBR	BI3	HTI3	HTI2
---µg g^{-1}----		g			
10.9	1.2	0.318	0.33	0.79	0.61
9.1	1.1	0.285	0.36	0.50	0.60
12.5	0.9	0.195	0.51	1.01	0.89
18.4	0.7	0.219	0.65	2.29	0.77
20.2	0.7	0.358	0.59	1.55	0.77
9.8	0.8	0.459	0.50	1.57	0.56
12.0	1.1	0.295	0.45	0.61	0.40
10.6	1.0	0.411	0.51	0.65	0.49
8.9	0.7	0.084	0.35	0.84	0.54
10.6	0.7	0.427	0.72	1.99	0.89
19.4	0.8	0.350	0.46	1.34	0.61
8.5	0.9	0.316	0.70	1.23	0.52
16.2	0.9	0.340	1.22	3.02	0.67
13.3	0.9	0.226	0.70	3.13	0.59
7.6	0.8	0.293	0.32	1.02	0.50
7.9	0.6	0.288	0.62	1.00	0.27
12.7	1.0	0.266	0.37	0.63	0.48
12.7	1.1	0.174	0.39	1.66	0.31
8.4	0.7	0.277	0.20	1.25	0.48
6.3	0.9	0.335	0.34	0.41	0.59
12.1	0.7	0.329	0.40	1.00	0.42
12.3	0.9	0.166	2.37	1.04	0.83
10.6	0.6	0.346	1.25	2.50	0.67
12.7	0.9	0.233	0.30	3.18	0.80
15.4	0.6	0.145	0.59	0.57	0.62

Appendix L (continued)

NUMBER OF CASES

FE	CU	FLWTBR	BI3	HTI3	HTI2
9	9	9	9	9	9
9	9	9	9	9	9
9	9	9	9	9	9
10	10	10	10	9	10
9	9	9	9	9	9
9	9	9	9	9	9
10	10	10	10	10	10
7	7	7	7	7	7
9	9	9	9	9	9
10	10	10	10	10	10
9	9	9	9	9	9
8	8	8	8	8	8
9	9	9	9	9	9
8	8	8	8	7	8
8	8	8	8	8	8
7	7	7	7	7	7
10	10	10	10	10	10
8	8	8	8	8	8
9	9	9	9	9	9
10	10	10	10	10	10
9	9	9	9	9	9
9	9	9	9	9	9
9	9	9	9	9	9
10	10	10	10	10	10
9	9	9	9	8	9

Appendix L (continued)

5-86-86-86
MEAN

TRT	ZN	MN	N	P	MG
	---µg g^{-1}----		----------%g g^{-1}------		
1	10.7	1262	1.02	0.155	0.123
2	11.6	1273	1.02	0.193	0.140
3	14.3	1289	1.12	0.183	0.137
4	11.9	1832	1.21	0.153	0.105
5	12.1	2172	1.14	0.176	0.100
6	15.7	2933	1.35	0.164	0.124
7	10.1	1178	1.06	0.158	0.125
8	12.0	1525	1.10	0.204	0.134
9	8.8	1327	0.98	0.167	0.150
10	8.3	1083	0.92	0.178	0.140
11	10.5	1268	1.05	0.195	0.140
12	10.1	1240	0.99	0.213	0.143
13	79.3	1217	1.26	0.188	0.140
14	100.9	1066	1.13	0.195	0.108
15	132.3	867	1.04	0.188	0.138
16	14.1	1251	1.08	0.182	0.106
17	10.1	1425	1.09	0.207	0.137
18	11.2	1140	1.21	0.192	0.118
19	10.1	1272	1.04	0.182	0.116
20	10.5	1094	1.02	0.178	0.133
21	11.2	1008	1.16	0.176	0.124
22	10.1	1270	1.01	0.150	0.165
23	15.5	1064	1.67	0.214	0.136
24	8.6	1091	1.02	0.138	0.134
25	11.4	1308	1.11	0.182	0.140

Appendix L (continued)

STANDARD DEVIATION

TRT	ZN	MN	N	P	MG
	---µg g^{-1}----		----------cg g^{-1}------		
1	5.8	137	0.27	0.042	0.013
2	4.9	209	0.17	0.017	0.050
3	7.1	548	0.26	0.025	0.012
4	2.3	510	0.08	0.041	0.019
5	5.2	794	0.24	0.036	0.012
6	5.8	1021	0.12	0.025	0.040
7	2.7	416	0.34	0.032	0.019
8	2.9	670	0.14	0.073	0.026
9	1.6	536	0.05	0.055	0.044
10	1.5	363	0.12	0.056	0.026
11	2.7	408	0.19	0.048	0.034
12	1.4	526	0.22	0.049	0.033
13	30.1	235	0.16	0.028	0.018
14	75.8	319	0.27	0.031	0.022
15	30.5	487	0.24	0.043	0.019
16	4.4	509	0.14	0.047	0.011
17	3.7	535	0.15	0.055	0.023
18	4.8	249	0.24	0.054	0.022
19	3.3	751	0.13	0.015	0.015
20	1.9	261	0.06	0.043	0.026
21	3.3	174	0.19	0.017	0.026
22	2.1	249	0.17	0.024	0.040
23	4.2	370	0.22	0.066	0.052
24	1.9	672	0.15	0.030	0.038
25	1.9	531	0.11	0.052	0.016

Appendix L (continued)

NUMBER OF CASES

TRT	ZN	MN	N	P	MG
1	4	4	4	4	4
2	4	4	4	4	4
3	3	3	3	3	3
4	4	4	4	4	4
5	5	5	5	5	5
6	5	5	5	5	5
7	4	4	4	4	4
8	5	5	5	5	5
9	3	3	3	3	3
10	4	4	4	4	4
11	4	4	4	4	4
12	4	4	4	4	4
13	4	4	4	4	4
14	4	4	4	4	4
15	5	5	5	5	5
16	5	5	5	5	5
17	3	3	3	3	3
18	5	5	5	5	5
19	5	5	5	5	5
20	4	4	4	4	4
21	5	5	5	5	5
22	4	4	4	4	4
23	5	5	5	5	5
24	5	5	5	5	5
25	5	5	5	5	5

Appendix L (continued)

MEAN

FE	CU	B	AFE	FLWTBR	BI2
	μg g^{-1}			g	
40.2	2.9	22.3	26.7	0.169	1.01
41.9	2.9	22.1	27.8	0.201	1.03
40.5	3.3	23.1	25.9	0.171	0.87
45.5	3.6	20.1	30.2	0.302	1.25
40.0	3.1	24.2	29.2	0.250	1.21
40.0	3.6	23.9	28.2	0.367	1.23
32.1	3.5	35.7	28.5	0.256	1.08
32.1	3.0	38.5	25.8	0.175	0.99
44.0	2.7	47.1	27.7	0.189	0.86
32.1	3.0	40.9	24.2	0.193	1.09
36.6	2.9	51.9	25.6	0.168	0.98
36.6	3.1	52.6	27.3	0.250	1.08
46.4	4.9	22.1	30.0	0.184	0.97
36.6	3.0	22.4	30.3	0.173	0.87
40.7	2.8	26.8	32.6	0.171	0.83
39.3	3.1	21.1	31.3	0.277	1.10
45.2	3.8	24.5	33.9	0.160	0.95
36.4	3.8	23.3	32.1	0.291	0.98
32.1	3.5	24.9	28.9	0.198	0.93
32.1	2.9	25.5	29.7	0.174	0.94
32.1	3.4	24.8	26.4	0.200	0.98
30.3	3.2	23.5	29.6	0.181	0.93
46.4	4.0	45.8	34.8	0.394	1.58
51.4	3.6	23.2	32.7	0.146	0.88
39.3	3.1	28.4	26.2	0.198	0.96

Appendix L (continued)

STANDARD DEVIATION

FE	CU	B	AFE	FLWTBR	BI2
	μg g^{-1}			g	
12.2	0.7	1.3	7.4	0.045	0.26
6.1	0.6	7.6	6.2	0.081	0.38
8.2	0.2	3.5	3.0	0.028	0.32
11.8	0.7	5.2	3.2	0.096	0.35
8.9	0.7	4.8	7.7	0.134	0.52
6.4	0.7	5.3	4.2	0.187	0.39
13.0	1.6	12.2	3.1	0.107	0.36
7.1	0.6	17.8	6.3	0.078	0.31
7.4	0.5	11.3	4.1	0.021	0.21
14.9	0.4	13.3	9.0	0.075	0.35
3.4	0.3	10.1	5.0	0.037	0.37
5.4	0.3	15.2	4.4	0.210	0.39
15.4	1.3	3.0	5.9	0.078	0.22
8.4	1.1	2.3	4.6	0.052	0.31
15.5	1.3	4.5	10.9	0.093	0.37
11.6	0.4	1.7	5.5	0.149	0.36
10.3	0.5	4.2	9.0	0.045	0.27
8.5	1.0	6.3	6.8	0.072	0.35
12.6	0.5	5.7	7.6	0.071	0.26
12.7	0.7	9.2	2.4	0.052	0.35
7.1	0.6	5.5	7.3	0.100	0.35
7.4	0.4	1.9	7.0	0.049	0.23
9.1	0.6	10.9	6.4	0.162	0.67
12.5	0.6	2.4	3.5	0.048	0.21
6.7	0.6	6.0	5.3	0.054	0.27

Appendix L (continued)

NUMBER OF CASES

FE	CU	B	AFE	FLWTBR	BI2
4	4	4	4	4	24
4	4	4	4	4	27
3	3	3	3	3	27
4	4	4	4	4	27
5	5	5	5	5	25
5	5	5	5	5	27
4	4	4	4	4	24
5	5	5	5	5	30
3	3	3	3	3	23
4	4	4	4	4	24
4	4	4	4	4	27
4	4	4	4	4	24
4	4	4	4	4	24
4	4	4	4	4	24
5	5	5	5	5	26
5	5	5	5	5	30
3	3	3	3	3	21
5	5	5	5	5	27
5	5	5	5	5	21
4	4	4	4	4	29
5	5	5	5	5	30
4	4	4	4	4	24
5	5	5	5	5	24
5	5	5	5	5	30
5	5	5	5	5	27

Appendix L (continued)

5-86-87-87
MEAN

TRT	ZN	MN	N	P	MG
	---μg g^{-1}----		----------cg g^{-1}--------		
1	11.9	1190	1.04	0.163	0.139
2	12.6	1296	1.12	0.185	0.139
3	15.2	1342	1.04	0.173	0.148
4	11.2	2487	1.16	0.157	0.114
5	12.6	2817	1.12	0.171	0.113
6	12.5	3518	1.16	0.159	0.117
7	11.3	1603	1.20	0.164	0.133
8	11.5	1322	1.15	0.177	0.147
9	8.8	1439	1.03	0.143	0.140
10	9.9	1359	1.09	0.159	0.159
11	12.0	1626	1.23	0.175	0.139
12	11.6	1301	1.12	0.181	0.160
13	11.9	1581	1.12	0.165	0.147
14	15.4	1540	1.19	0.174	0.124
15	16.5	1533	1.22	0.215	0.154
16	11.5	1515	1.10	0.173	0.133
17	9.9	1285	1.11	0.162	0.139
18	10.5	1231	1.17	0.192	0.139
19	12.5	1463	1.18	0.170	0.130
20	12.4	1127	1.16	0.173	0.143
21	12.2	1551	1.21	0.179	0.145
22	12.0	1434	1.20	0.172	0.146
23	14.7	962	1.32	0.242	0.149
24	9.9	1142	1.07	0.148	0.141
25	11.8	1243	1.15	0.166	0.131

Appendix L (continued)

STANDARD DEVIATION

TRT	ZN	MN	N	P	MG
	---µg g^{-1}----		----------cg g^{-1}-------		
1	5.2	282	0.28	0.037	0.026
2	4.7	217	0.25	0.028	0.036
3	5.7	573	0.23	0.044	0.021
4	3.1	592	0.16	0.029	0.015
5	4.2	651	0.20	0.038	0.018
6	4.4	881	0.12	0.035	0.027
7	3.5	574	0.26	0.030	0.037
8	2.8	618	0.18	0.066	0.026
9	1.8	233	0.16	0.037	0.016
10	2.5	523	0.23	0.051	0.031
11	3.5	632	0.28	0.053	0.028
12	3.2	454	0.22	0.047	0.025
13	2.9	683	0.17	0.041	0.026
14	4.9	550	0.24	0.059	0.018
15	4.5	656	0.19	0.049	0.029
16	2.6	344	0.19	0.047	0.031
17	3.9	382	0.19	0.053	0.026
18	4.6	568	0.28	0.043	0.026
19	5.1	577	0.22	0.027	0.024
20	3.5	231	0.24	0.054	0.022
21	2.7	462	0.12	0.037	0.027
22	2.9	418	0.27	0.048	0.040
23	2.3	383	0.15	0.037	0.040
24	3.6	571	0.22	0.032	0.028
25	3.6	666	0.17	0.062	0.024

Appendix L (continued)

NUMBER OF CASES

TRT	ZN	MN	N	P	MG
1	10	10	10	10	10
2	8	8	8	8	8
3	9	9	9	9	9
4	10	10	10	10	10
5	10	10	10	10	10
6	10	10	10	10	10
7	10	10	10	10	10
8	10	10	10	10	10
9	10	10	10	10	10
10	9	9	9	9	9
11	10	10	10	10	10
12	10	10	10	10	10
13	10	10	10	10	10
14	9	10	10	10	10
15	10	10	10	10	10
16	10	10	10	10	10
17	10	10	10	10	10
18	10	10	10	10	10
19	10	10	10	10	10
20	9	9	9	9	9
21	10	10	10	10	10
22	10	10	10	10	10
23	9	9	9	9	9
24	10	10	10	10	10
25	10	10	10	10	10

Appendix L (continued)

MEAN

FE	CU	FLWTBR	BI3	HTI3	HTI2
---μg g^{-1}----		g			
42.1	3.1	0.268	1.30	1.76	1.08
44.6	3.4	0.393	1.40	1.78	1.38
41.7	3.2	0.258	1.15	1.63	1.17
43.2	3.5	0.317	1.22	1.74	1.82
40.7	3.4	0.340	1.17	2.03	1.52
42.5	3.6	0.316	1.11	1.50	1.74
38.9	3.6	0.366	1.35	1.31	1.58
38.9	3.6	0.257	1.59	2.09	1.35
37.1	3.1	0.272	1.23	1.64	1.12
38.9	3.3	0.291	1.14	1.63	1.24
44.6	3.7	0.326	1.34	1.43	1.69
41.4	3.2	0.314	1.42	1.46	1.27
38.6	3.4	0.306	1.91	2.36	1.02
42.8	3.4	0.292	1.60	2.37	0.98
43.2	3.5	0.314	1.77	2.68	0.64
41.8	3.0	0.283	1.60	1.73	1.70
38.9	3.3	0.223	1.37	2.22	0.78
42.5	3.5	0.278	1.20	1.43	1.09
45.0	3.5	0.301	1.38	1.75	1.16
40.9	3.3	0.308	1.29	2.06	1.03
44.3	3.5	0.272	1.41	1.94	1.28
42.1	3.2	0.307	1.25	1.26	1.03
53.7	3.6	0.742	1.94	1.82	1.49
38.9	3.0	0.233	1.53	2.06	1.08
52.7	3.9	0.288	1.39	1.69	1.25

Appendix L (continued)

STANDARD DEVIATION

FE	CU	FLWTBR	BI3	HTI3	HTI2
---µg g^{-1}----		g			
7.3	0.7	0.181	0.47	0.88	0.58
8.3	0.8	0.222	0.44	1.04	0.91
3.6	0.4	0.121	0.42	1.41	0.98
7.0	0.6	0.089	0.30	0.44	0.76
20.6	0.9	0.157	0.38	1.26	0.71
12.0	0.8	0.103	0.22	0.41	0.53
9.3	1.0	0.217	0.36	0.46	0.89
9.4	1.2	0.136	0.95	1.33	0.64
7.7	0.9	0.108	0.35	0.69	0.43
5.5	0.9	0.156	0.42	0.59	0.46
6.8	1.3	0.152	0.20	0.28	0.87
7.9	1.2	0.180	0.69	0.86	0.57
5.0	1.1	0.152	0.89	1.28	0.56
9.7	0.5	0.104	0.54	1.20	0.26
8.3	0.8	0.170	0.43	1.56	0.30
11.7	0.8	0.126	1.14	0.48	0.94
11.8	0.9	0.083	0.45	1.49	0.32
8.5	1.3	0.110	0.29	0.46	0.46
8.4	1.0	0.151	0.41	0.56	0.67
6.0	0.6	0.165	0.54	1.09	0.41
10.3	0.8	0.081	0.42	0.98	0.47
6.0	0.7	0.193	0.27	0.67	0.36
7.0	0.7	0.306	1.11	0.83	0.48
7.8	0.7	0.116	0.79	1.14	0.43
12.0	0.9	0.140	0.14	0.56	0.61

Appendix L (concluded)

NUMBER OF CASES

FE	CU	FLWTBR	BI3	HTI3	HTI2
10	10	10	10	10	10
8	8	8	8	8	8
9	9	9	9	9	9
10	10	10	10	10	10
10	10	10	10	10	10
10	10	10	10	10	10
10	10	10	10	10	10
10	10	10	10	10	10
10	10	10	10	10	10
9	9	9	9	9	9
10	10	10	10	10	10
10	10	10	10	10	10
10	10	10	10	10	10
10	10	10	10	10	10
10	10	10	10	10	10
10	10	10	10	10	10
10	10	10	10	10	10
10	10	10	10	10	10
10	10	10	10	10	10
9	9	9	9	9	9
10	10	10	10	10	10
10	10	10	10	10	10
9	9	9	9	9	9
10	10	10	10	10	10
10	10	10	10	10	10

Symbols

SOS = sulphate-sulphur
TOTS = total sulphur
FLWTBR = foliar mass (g) per shoot of the current year's growth
BI = shoot increment ratio
HTI = height increment ratio
1 = growth increment in 1985/growth increment in 1984
2 = growth increment in 1986/growth increment in 1985
3 = growth increment in 1987/growth increment in 1986

APPENDIX M.

FOLIAR NUTRIENT DATA FOR ZINC AND MANGANESE (µg g-1) WITH TIME AND AGE.

Appendix M1. Site 1.

MEAN

TRT	ZN1	MN1	ZN2	ZN3	MN2	MN3
3	11.0	2971	24.4	10.4	4610	3059
4	13.6	1352	48.7	51.2	1481	1146
5	15.4	1143	135.5	144.4	1851	1200
7	11.5	1231	19.8	5.1	1733	1195
8	11.0	1228	17.9	2.5	1464	1203
11	8.8	992	13.0	2.1	1382	1067
12	9.7	1139	18.5	2.2	1432	1019

STANDARD DEVIATION

TRT	ZN1	MN1	ZN2	ZN3	MN2	MN3
3	4.6	1278	5.7	4.9	2001	1320
4	2.3	650	20.2	17.0	850	289
5	5.7	291	40.7	47.0	722	333
7	3.5	352	6.6	3.1	474	331
8	3.7	460	4.5	1.1	630	340
11	2.3	200	2.0	0.8	395	334
12	3.2	395	11.8	1.4	594	311

STANDARD ERROR

TRT	ZN1	MN1	ZN2	ZN3	MN2	MN3
3	1.6	452	2.1	1.8	756	467
4	1.0	265	7.6	6.9	321	118
5	2.2	110	15.4	17.8	273	126
7	1.2	124	2.5	1.2	179	117
8	1.3	163	1.6	0.4	223	120
11	0.8	67	0.7	0.3	132	111
12	1.1	132	3.7	0.5	188	98

NUMBER OF CASES

TRT	ZN1	MN1	ZN2	ZN3	MN2	MN3
3	8	8	7	7	7	8
4	6	6	7	6	7	6
5	7	7	7	7	7	7
7	8	8	7	7	7	8
8	8	8	8	7	8	8
11	9	9	9	6	9	9
12	9	9	10	8	10	10

Appendix M2. Site 2.

MEAN

TRT	ZN1	MN1	ZN2	ZN3	MN2	MN3
3	12.4	3875	15.5	3.2	5920	3196
4	11.5	1527	35.2	53.0	1704	1676
5	19.6	1141	78.7	139.6	1687	1251
7	8.8	1728	15.6	2.0	1082	1633
8	11.4	1181	17.0	2.5	1617	1037
11	10.1	1249	9.5	2.0	1726	1048
12	11.1	1932	14.8	3.0	2365	1474

STANDARD DEVIATION

TRT	ZN1	MN1	ZN2	ZN3	MN2	MN3
3	5.6	1429	5.7	3.0	2804	1495
4	5.6	1031	18.8	21.9	1322	558
5	8.8	434	34.2		746	101
7	3.4	1025	3.0	0.0	1030	482
8	2.7	601	7.0	2.1	737	677
11	4.2	357	3.5	1.7	206	175
12	4.8	589	8.2	2.2	956	666

STANDARD ERROR

TRT	ZN1	MN1	ZN2	ZN3	MN2	MN3
3	1.9	476	2.0	1.3	991	498
4	2.1	390	8.4	9.8	591	249
5	3.9	194	15.3		334	71
7	1.7	513	2.1	0.0	728	278
8	1.1	245	3.1	1.5	330	276
11	2.1	179	2.0	1.0	119	87
12	1.7	208	2.9	1.1	338	222

NUMBER OF CASES

TRT	ZN1	MN1	ZN2	ZN3	MN2	MN3
3	9	9	8	5	8	9
4	7	7	5	5	5	5
5	5	5	5	1	5	2
7	4	4	2	2	2	3
8	6	6	5	2	5	6
11	4	4	3	3	3	4
12	8	8	8	4	8	9

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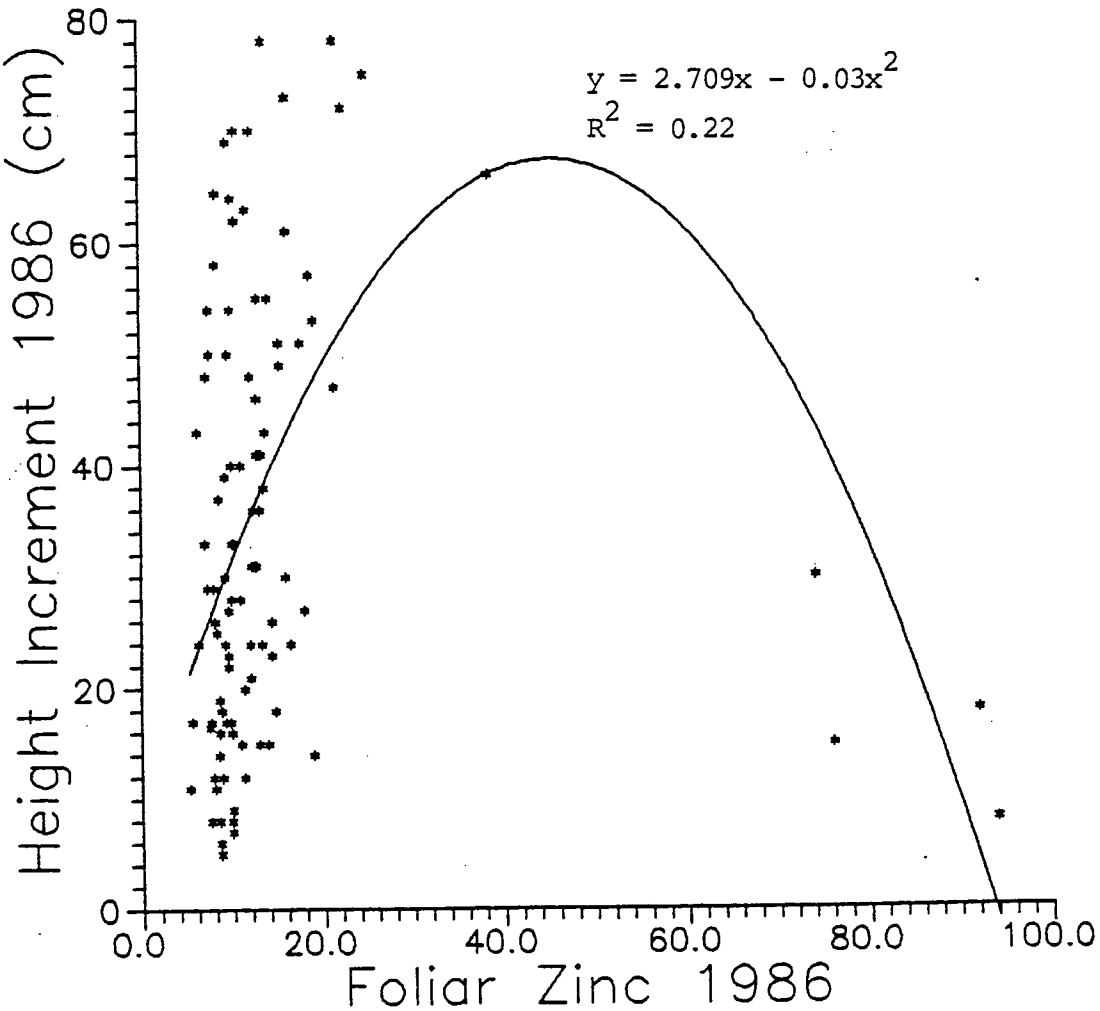
1 = one-year old foliage formed and collected in 1986, two years after treatment.

2 = two-year-old foliage formed in 1985 and collected in 1986, two years after treatment.

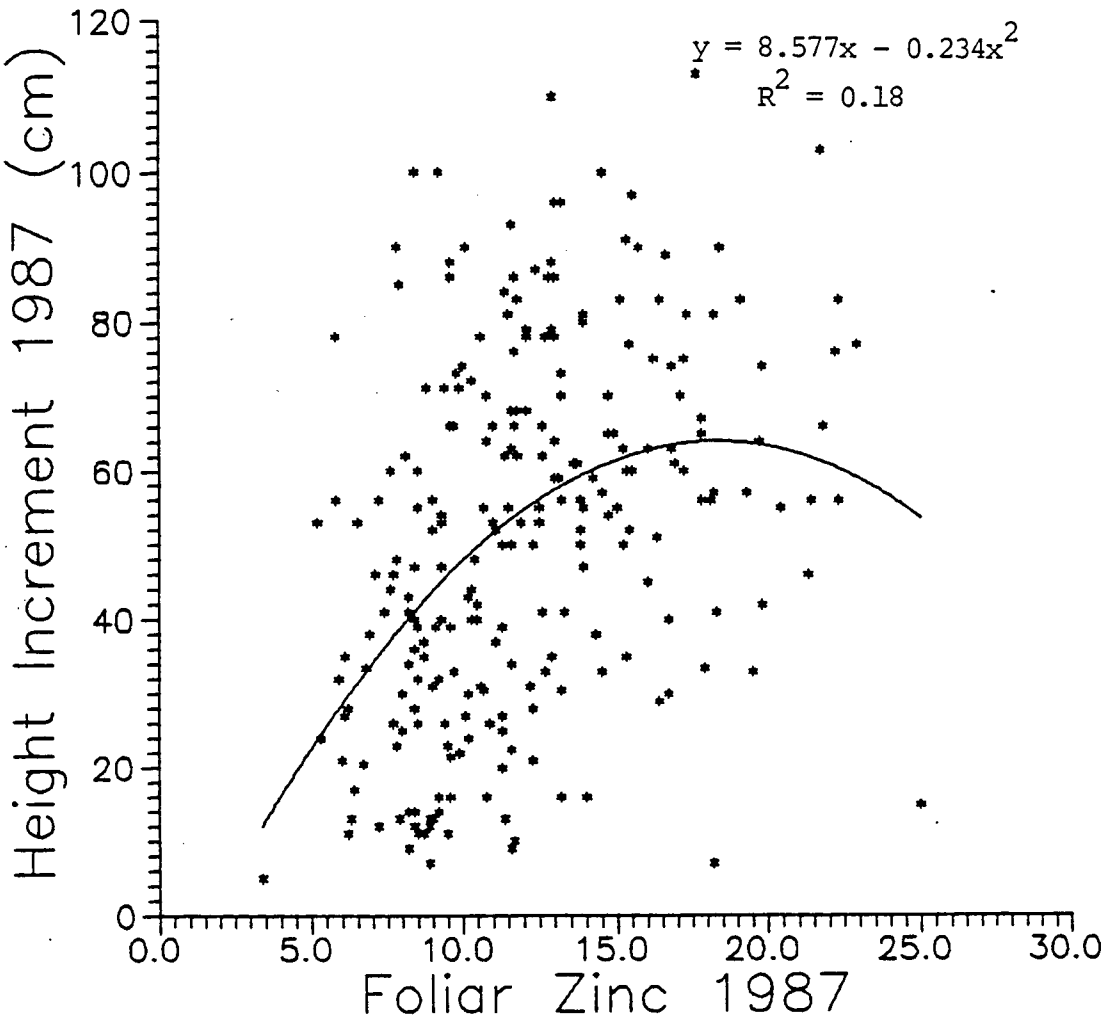
3 = one-year-old foliage formed and collected in 1985, one year after treatment. Equations from Appendix D were used to convert the original AA values to their equivalent values for the ICP which are presented here.

APPENDIX N.

SCATTER PLOTS OF HEIGHT INCREMENT VERSUS FOLIAR ZINC.



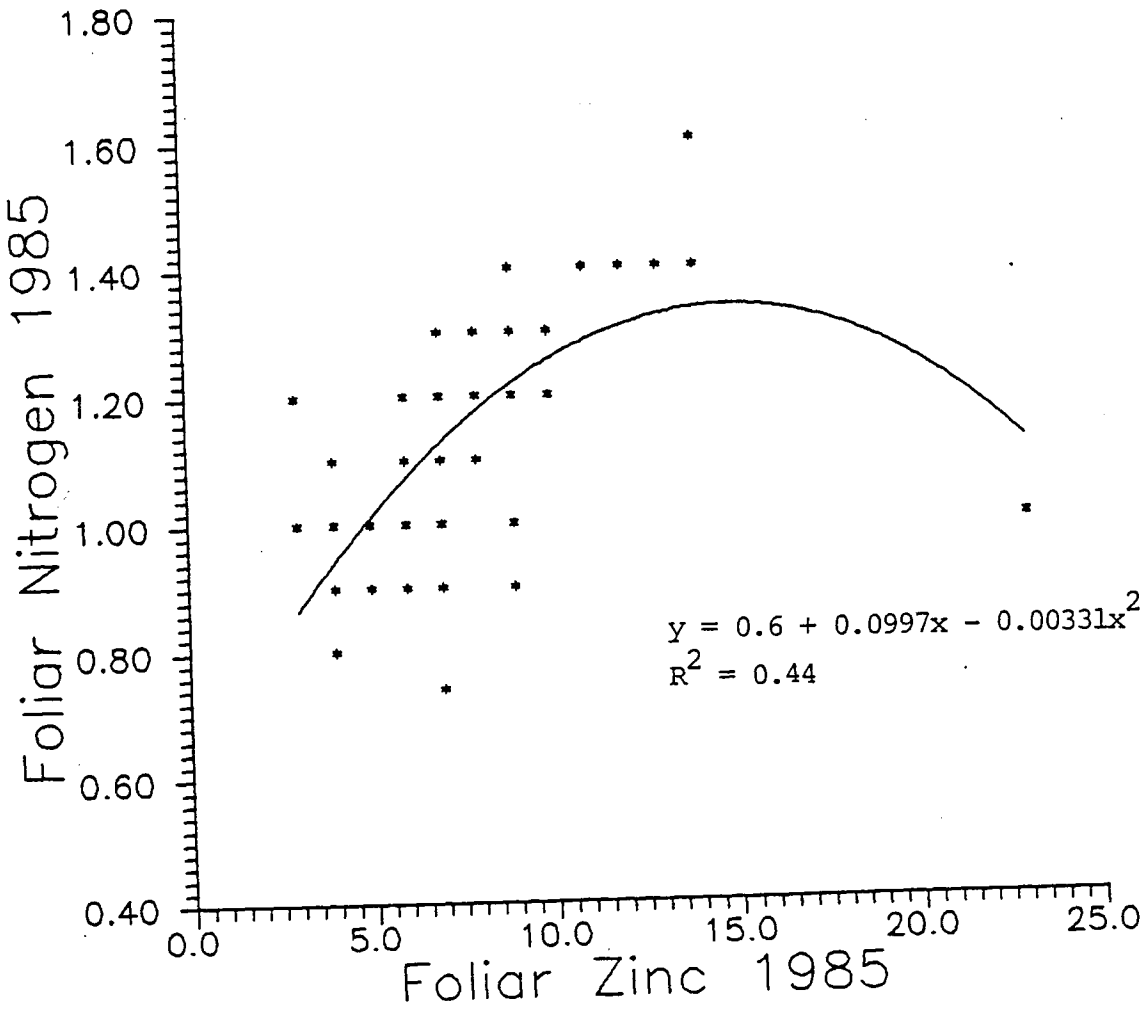
Appendix N.1. Scatter plot of first year total height increment (cm) (in 1986) versus first year foliar zinc levels (µg g^{-1}) (for cases where Zn $\leq$100 µg g^{-1}) on site 5.



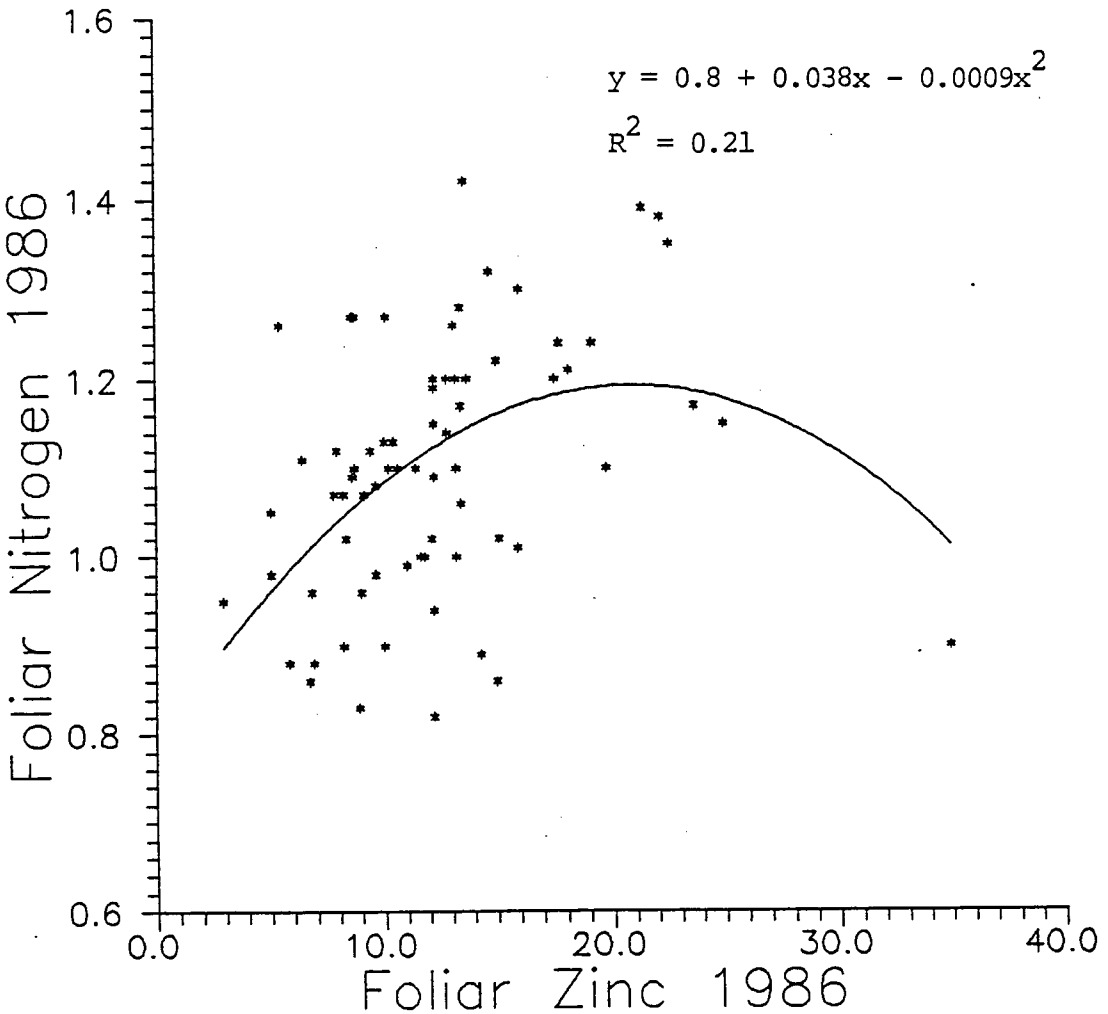
Appendix N.2. Scatter plot of second year total height increment (cm) (in 1987) versus second year foliar zinc levels (µg g^{-1}) on site 5.

APPENDIX O.

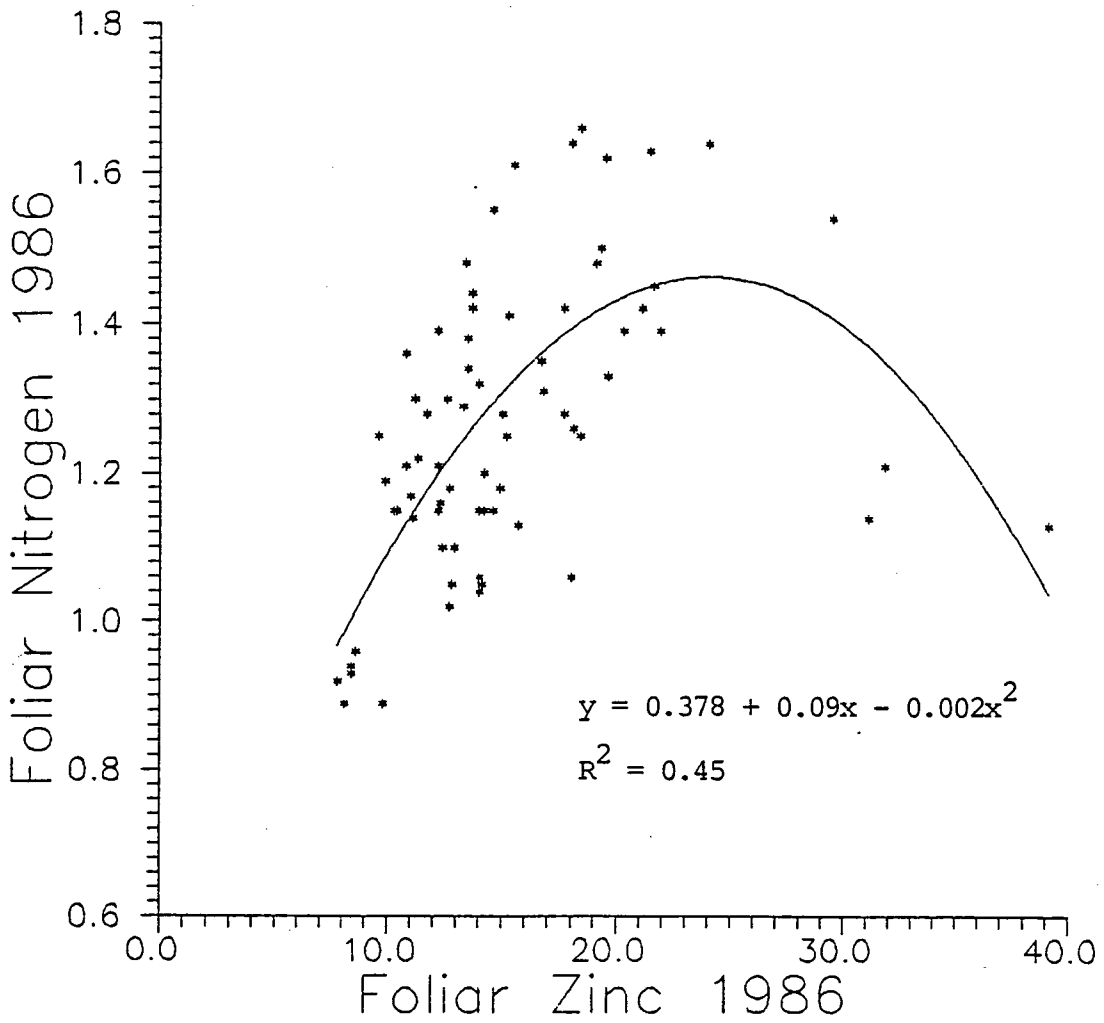
SCATTER PLOTS OF FOLIAR NITROGEN VERSUS FOLIAR ZINC.



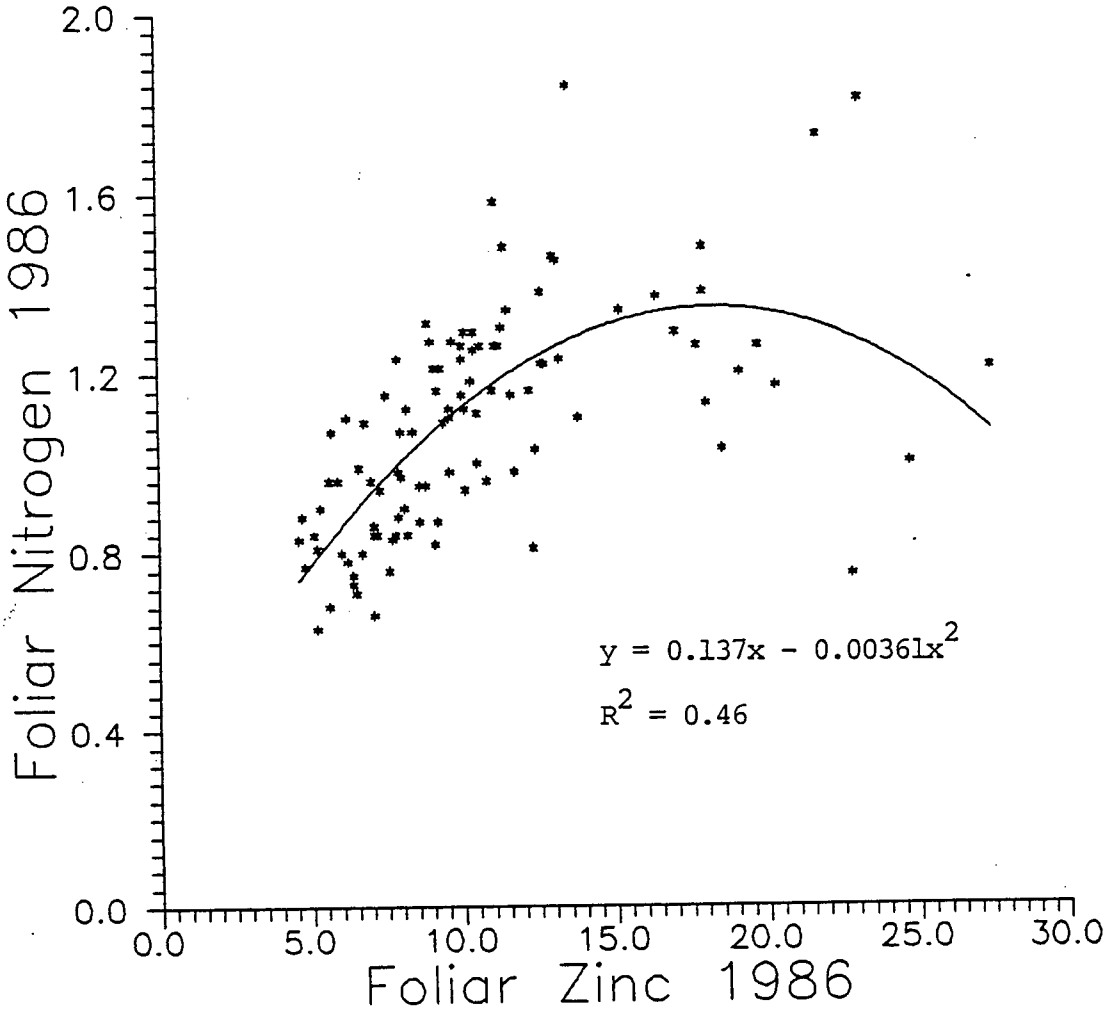
Appendix O.1. Scatter plot of foliar nitrogen (cg g^{-1}) versus foliar zinc (µg g^{-1}) (for cases where Zn $\leq$30 µg g^{-1}) for current year's foliage (in 1985) from site 2.



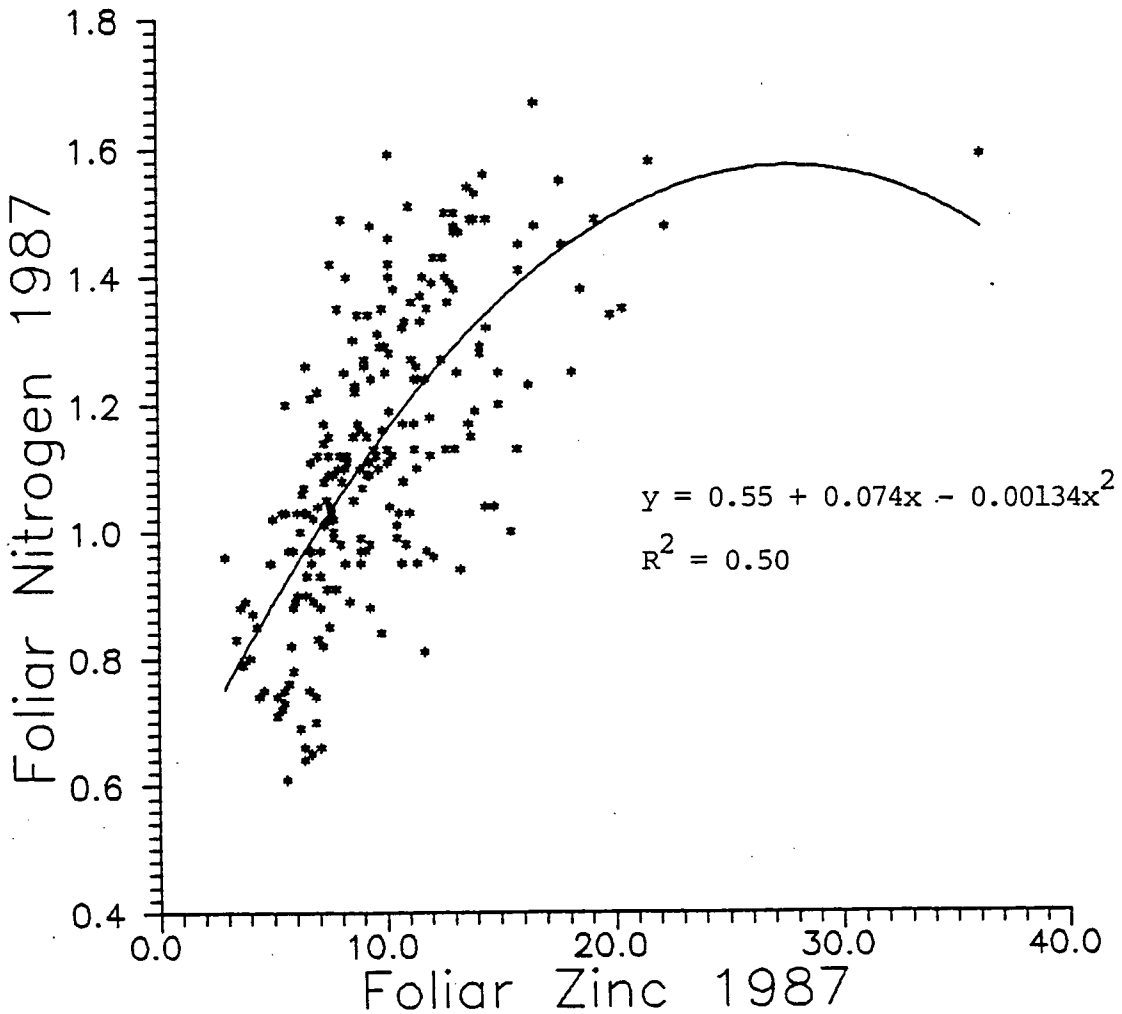
Appendix 0.2. Scatter plot of foliar nitrogen (cg g^{-1}) versus foliar zinc (µg g^{-1}) using all treatments (for cases where Zn $\leq$30 µg g^{-1}) for current year's foliage (in 1986) from site 2.



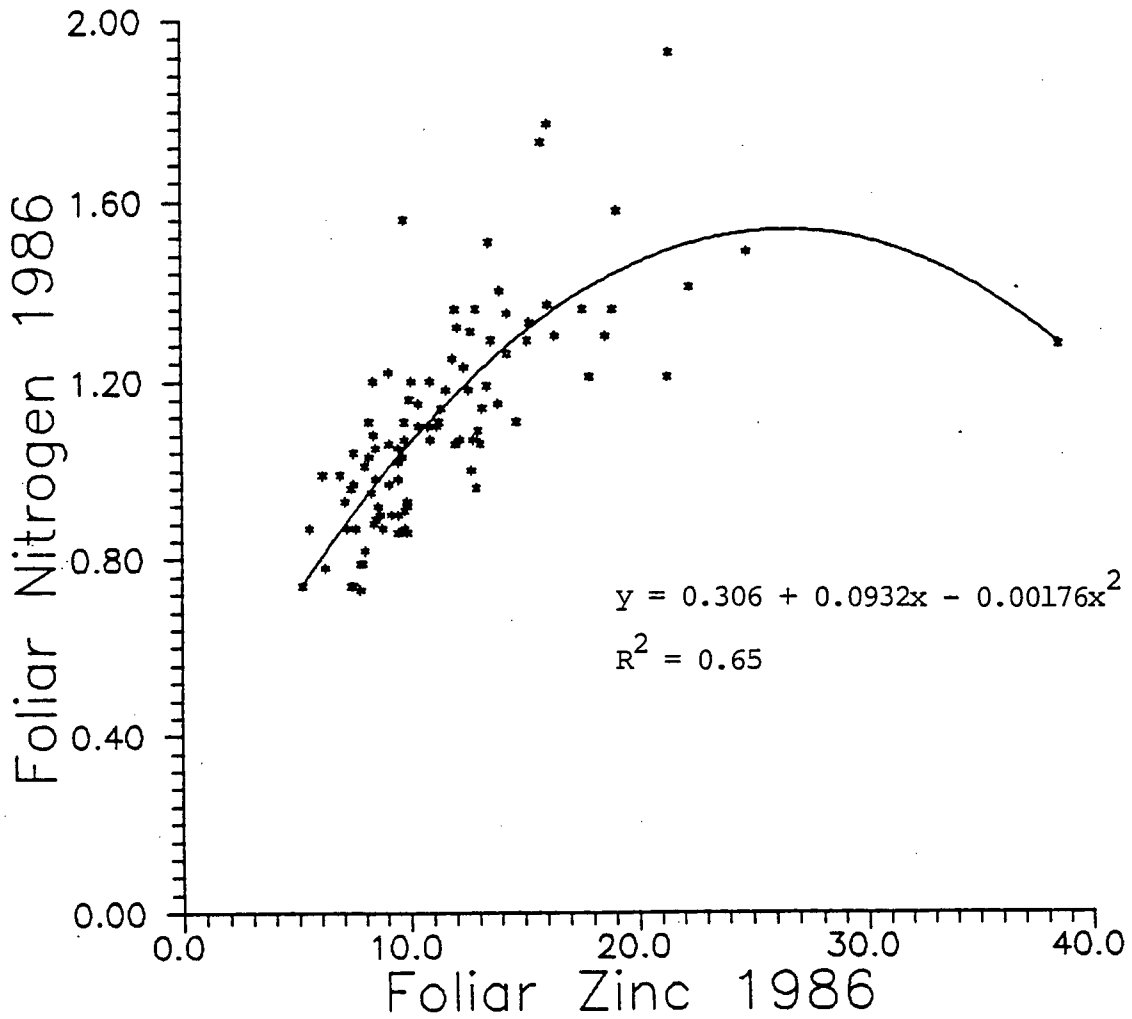
Appendix 0.3. Scatter plot of foliar nitrogen (cg g^{-1}) versus foliar zinc (µg g^{-1}) using all treatments for current year's foliage (in 1986) from site 3.



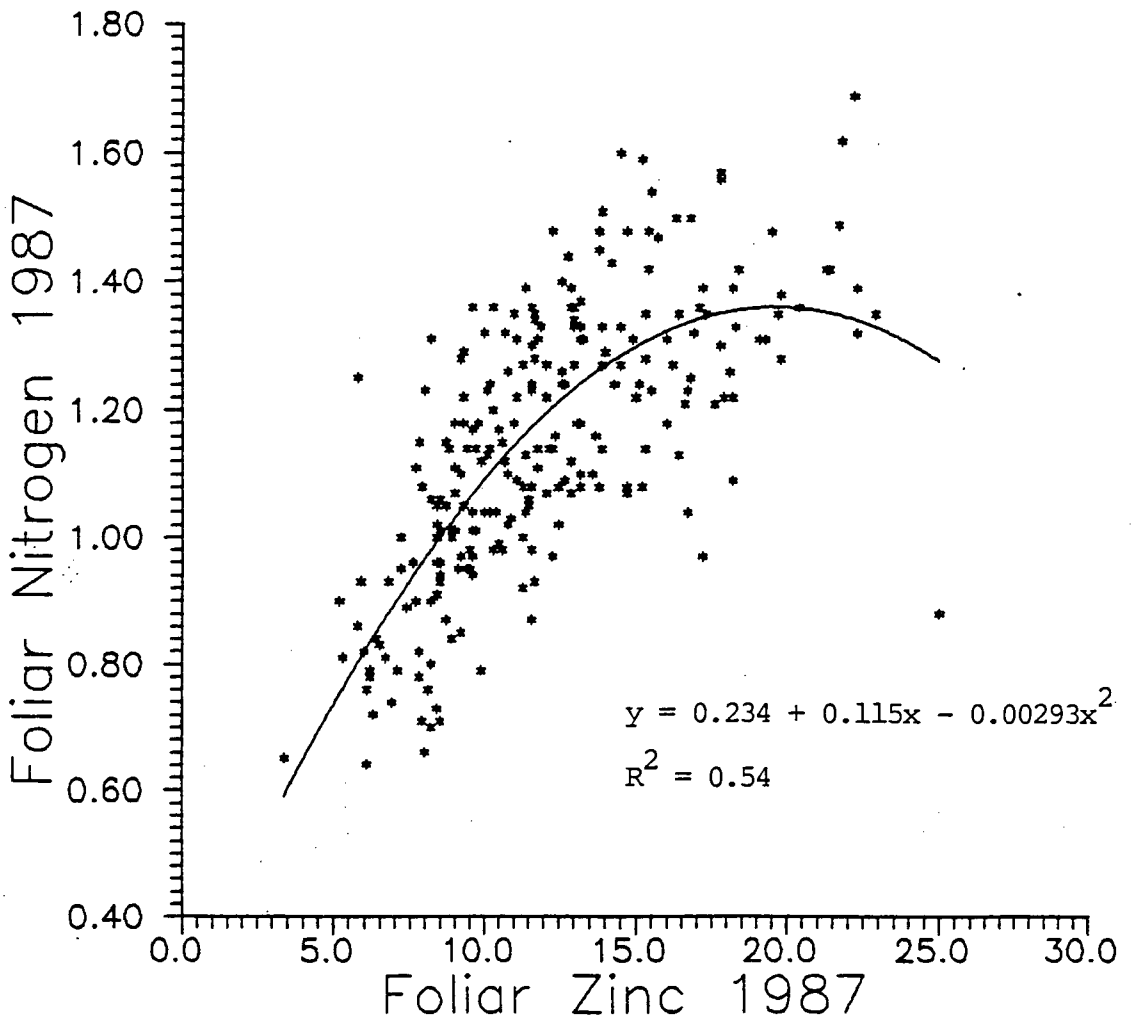
Appendix 0.4. Scatter plot of foliar nitrogen (cg g^{-1}) versus foliar zinc (µg g^{-1}) (for cases where Zn $\leq$30 µg g^{-1}) for current year's foliage (in 1986) from site 4.



Appendix 0.5. Scatter plot of foliar nitrogen (cg g^{-1}) versus foliar zinc (µg g^{-1}) using all treatments of current year's foliage (in 1987) from site 4.



Appendix 0.6. Scatter plot of foliar nitrogen (cg g^{-1}) versus foliar zinc (µg g^{-1}) (for cases where Zn $\leq$40 µg g^{-1}) of current year's foliage (in 1986) from site 5.



Appendix 0.7. Scatter plot of foliar nitrogen (cg g^{-1}) versus foliar zinc (µg g^{-1}) using all treatments of current year's foliage (in 1987) from site 5.