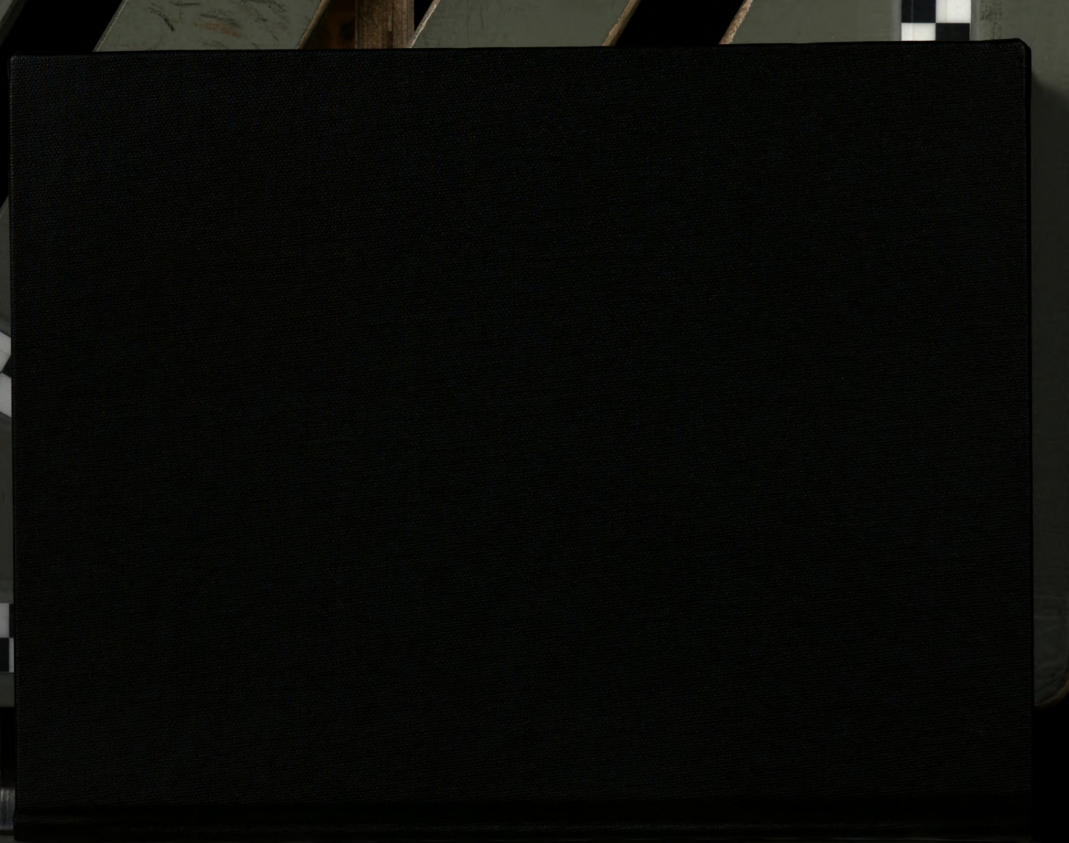

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PREDISPOSING FACTORS CONTRIBUTING TO RED PINE POCKET MORTALITY

By

Carolyn J. Randall

A DISSERTATION

Submitted to Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Forestry

1997

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ABSTRACT

PREDISPOSING FACTORS CONTRIBUTING TO RED PINE POCKET MORTALITY

By

Carolyn Joyce Randall

A disease known as "red pine pocket mortality" (RPPM) has been reported relatively recently affecting older plantation-grown red pines in the Lake States. A common symptom of RPPM is a large, often circular, area of dead trees (pocket), surrounded by trees showing reduced diameter and height growth, thinning crowns and browning foliage. The causes of this disease are as yet unknown. This study investigated the potential causes of the disease particularly those that are "abiotic" in origin. The condition of red pines at mortality sites was tracked over a five-year period (1991-1995) and the element contents of soil, needle, and root samples from disease versus non-disease locations were determined.

Plots were established at pocket decline sites within the Huron-Manistee National Forest, Newago County, Michigan for comparison with check plots of healthy plantation red pines within and outside of the Huron-Manistee National Forest. Analysis of variance was used to identify

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significant differences at the 0.05 level between element contents from areas within disease plots, including the center of the pocket, the edge of the decline, and the outer region where trees appear healthy; and between healthy check-plot stands.

Results of disease progress studies indicated that drought may be a contributing factor to the decline; measures of disease intensity tended to increase during drought years and decrease during non-drought years.

Increasing gradients in live fine-root (roots < 3 mm in diameter) were found from the center to the edge, to the outside region of disease plots, and finally to the healthy plantation check plots. These increasing gradients suggested that fine roots are dying first before symptoms are seen in tree crowns.

Statistical analyses of nutrient contents indicated that manganese (Mn) followed by magnesium (Mg) in soil, needle, and root samples were the nutrient factors most strongly associated with areas of decline and were significantly lower in disease plots versus non-disease plots. Further, increasing gradients in Mn and Mg and corresponding decreasing gradients of metals, particularly aluminum (Al), from diseased to healthy areas, suggested that the pattern of disease spread is associated with nutrient stress.

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The author thanks, Dr. John H. Hart, my advisor, for his support and guidance throughout the course of this study, without which this project could not have been completed. Special thanks go to the other members of my committee: Dr. Boyd Ellis, Dr. Andrew Jarosz, Dr. J. James Kielbaso, Dr. Phu Nguyen and to the memory of Dr. Robert P. Scheffer. Each member of the committee made a unique contribution to the study from their area of expertise.

Thanks also to numerous undergraduate and graduate students who have assisted me in field and laboratory work. Finally, thanks to my family and friends, especially my husband Timothy Grotjohn, for your love, patience, and support.

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INTRODUCTION

There are about 600,000 hectares of red pine (*Pinus resinosa*) forest and plantations in the Lake States, approximately one-third of which are in Michigan's northern Lower Peninsula (Smith and Hahn, 1986; Hahn and Smith, 1987; Spencer et al., 1988). A substantial proportion of these stands are in plantations. Traditionally, red pine has been managed for revenue from pulp wood and sawtimber. In the last decade a sustained market for red pine utility poles has increased the value of this resource in the northern Lower Peninsula (Grossman and Potter-Witter, 1990).

Red pine exhibits little genetic variation and has relatively few insect and disease problems. It is noted for its good growth characteristics and high productivity (Bassett, 1984; Schone et al., 1984). Because of these attributes, it is a desirable timber species.

When pest problems do occur on red pine they are usually associated with younger plantations but some occur in older plantations as well. These include pine root collar weevil (*Hylobius radicis*), redheaded pine sawfly (*Neodiprion lecontei*), European pine shoot moth (*Rhyacionia buoliana*), Saratoga spittlebug (*Aphrophora saratogensis*), white grubs (*Phyllophaga*, spp.), diplodia tip blight

(*Sphaeropsis sapinea*), Sirococcus shoot blight (*Sirococcus conigenus*), Armillaria root rot (*Armillaria,* spp.), and schleroderris canker (*Ascocalyx abietina*) (Bassett, 1984; Schone, et al., 1984).

Adding to this list, a new disease has been identified affecting older plantation-grown red pines. The disease is known as "red pine pocket mortality" (RPPM) and was first reported in 1975 (Klepzig and Carlson, 1988; Raffa and Hall, 1988). The causes of the disease are unknown and it occurs on a variety of soil types in Wisconsin, Michigan, and Illinois. The disease is restricted to mature plantation-grown red pines on marginal farmland. A common symptom of red pine decline is a large, often circular, area of dead trees (pocket), ringed by trees showing reduced diameter and height growth. Dead trees and greatly increased growth of understory plants can be found in the center of the pocket (Klepzig and Carlson, 1988).

The rate at which RPPM is spreading and the degree of crop loss has not yet been assessed in Michigan or in other Lake States. Aerial surveys during the summers of 1988 and 1989 suggested that the amount of mortality had increased significantly. An aerial survey by the U.S. Forest Service of the Huron-Manistee National Forest found 440 pockets of dying red pine (USDA Forest Service, 1989). Many of these pockets were likely caused by bark beetles (*Ips pini*) rather than RPPM, but it was not possible to distinguish between the two without follow-up ground surveys. Since the drought

years of 1988 and 1989, however, the number and size of red pine pockets in Michigan have not increased appreciably.

RPPM may be viewed as a disease of complex origin, i.e., one that is caused by both primary and secondary factors. This study considers the possibility that the primary stress-inducing factor is abiotic in origin, i.e., one that relates to site conditions such as the soil nutrient status. Therefore, the primary hypothesis of this study is that predisposing, abiotic factors present in some red pine plantations are the primary cause of RPPM.

The predominant factor under consideration is soil acidification as it affects soil nutrient content, but other possible causes such as deficiencies/toxicities of certain nutrients have been considered as well. The main focus of this study, however, has been on the effect that aluminum, which becomes more soluble in soils of low pH, is having on the uptake and availability of other nutrients, especially calcium, magnesium, and potassium. This study compares site conditions and nutritional factors at pocket decline sites versus healthy red pine plantations. The purpose is to examine the abiotic factors associated with RPPM. Hopefully, this information will allow plantation managers to establish guidelines and management practices for avoiding or preventing the disease.

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CHAPTER I

LITERATURE REVIEW

Abiotic Factors

Acidification of Forest Ecosystems

Acidification from anthropogenic inputs has been associated with forest decline in Europe (Godbold et al., 1988a; Ulrich, 1989; Ulrich, 1980). In central Europe, acid precipitation is thought to have contributed to soil acidity, which in turn may have adverse effects on plant health and microbial activity (Ulrich et al., 1980). Some of the most severely affected areas occur in the mountains of Czechoslovakia where forests appear devastated. This severe decline or "Waldsterben" as it is known in Germany has been dramatic in selected regions on silver fir (*Abies alba*), Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*), and European beech (*Fagus sylvatica*) (Smith, 1990).

In the northeastern United States, the possibility that air pollution is a contributing factor leading to red spruce (*Picea rubens*) decline is being considered among other possible causes (McLaughlin and Kohut, 1992; Smith, 1990). It has been shown that acid precipitation in contact with the foliage of red spruce trees can cause damage depending upon factors such as sulfate concentration and length/

intensity of exposure (Schier and Jensen, 1992). Another potential source of injury from acidic precipitation results from the increase in soil acidity. With increasing soil acidity, aluminum (Al) becomes more soluble and available for plant uptake. Increased acidity/Al toxicity and calcium (Ca) or magnesium (Mg) deficiency due to Al inhibition of uptake are also being considered as causes of red spruce decline (Joslin and Wolfe, 1988). Investigations of red spruce decline suggest that an imbalance of Al and Ca in the fine root environment leads to reduced growth rates and renders mature trees vulnerable to extant secondary diseases and insect pests (Shortle and Smith, 1988).

The results of red spruce and other forest decline studies in northeastern America, however, have been inconclusive (Hertel et al., 1993; Johnson and Fernandez, 1992; Cronan et al., 1989). In one study, 48 Adirondack soil profiles that had been sampled in 1930-32, were resampled in 1984. The researchers found changes in pH and dilute-acid extractable Ca during the 50-year plus interval; the change differed in soil horizons. Moderately acidic organic horizons (pH>4.0) showed a significant reduction in Ca without a pH reduction. The E horizons appeared to lose extra Ca while the B and C horizons showed no evidence of acidification. A partial Ca budget in a mixed hardwood-softwood forest in the study showed that Ca uptake was approximately equal to the loss of Ca from the soil. This suggested that uptake by vegetation rather than acid

deposition may be the major cause of Ca loss. While acid rain has increased hydrogen-ion loading and base-cation leaching in the region, acid consuming processes are apparently balanced by acid additions in the B and C horizons so that no acidification was observed. The authors concluded that there was no "compelling" evidence that acid deposition has had a major adverse influence on Adirondack soil chemistry through the mid-1980's (Johnson, et al., 1994).

Acid precipitation has the potential to acidify soils through wet deposition of sulfates and nitrates that come from fuel-burning emissions. Dry deposition in the form of gases and fine particles also contributes to soil acidification and may damage forest canopy layers (Mohnen, 1992). Not all forest declines, however, can be explained by the presence of pollutants. Forest declines occur in areas throughout the world where acid deposition does not appear to be a significant factor (Johnson et al., 1991). Forest declines may also be attributed to natural, internal acidification processes. In western North America, for example, excessive nitrogen fixation by red alder (*Alnus oregona*) stands increased the rate at which soils underneath them were acidified (Johnson et al., 1991). Harvesting practices have also been shown to increase the rate of base-cation depletion on managed forest lands versus natural forests (Johnson and Taylor, 1989). Brand et al. (1986) found that the pH of soils under white spruce (*Picea glauca*) and red

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pine (*Pinus resinosa*) plantations in central Ontario decreased as a result of afforestation. The pH change could be attributed to uptake and immobilization of exchangeable cations from the soil nutrient pool by incorporation into the stand biomass and accumulating forest floor and by the production of acidic decomposition products under spruce and pine litter.

Soil acidification, whether from anthropogenic deposition or through natural processes, mobilizes aluminum which reaches levels toxic to fine roots (Ulrich et al., 1980). Death of fine roots then leads to development of symptoms associated with reduced uptake of water and nutrients. In the case of Norway spruce stands in Germany, it was observed that the fine-root system had shifted to the topsoil as a consequence of increased acidity in the subsoil. This shift led to chronic water stress and eventual crown thinning in the affected Norway spruce stands (Ulrich, 1989). Drought may interact as a predisposing stressor to fine roots magnifying the effects of Al toxicity (Cronan et al., 1989; Ulrich, 1985).

Lawrence et al. (1995) report that during the past six decades, concentrations of root-available Ca (exchangeable and acid extractable forms) in forest floor soils of the northeastern United States have decreased. The depletion of Ca from the root zone can result in acidification of soil and surface water and possibly growth decline and dieback of red spruce. Their research does not support the notion that

acid deposition or net forest growth (i.e., uptake by vegetation) is the cause of Ca depletion. Instead they propose that Al mobilized in the soil by acid deposition is transported into the forest floor in a reactive form that competitively reduces storage of Ca and its availability for root uptake. If acid deposition alone were the cause of decreases in adsorbed Ca ions then increases in hydrogen (H) ions should be observed. But no significant correlations between Ca and H ions were identified in the study. Instead, inverse correlations between exchangeable Ca and reactive forms of Al were observed in the forest floor. The researchers propose that Al, mobilized in the mineral soil by acid deposition, is transported into the forest floor where it accumulates in a reactive, mostly organically complexed form. The mechanism by which Al is transported up to the forest floor is through biocycling (including Al transported from roots in the mineral soil to roots in the forest floor) and through water movement (i.e. upward movement of water through evapotranspiration, rising water tables, etc.).

Aside from natural and anthropogenic causes of soil acidification, tree species vary in their sensitivity to Al. Aluminum toxicity may be present in almost any inorganic soil with a pH less than 5.0-5.5 depending upon the sensitivity of the plant species and the amount of organic material in the soil (Foy, 1974). Steiner et al. (1984), reported that some clones of hybrid poplars (*Populus*, spp.)

were relatively sensitive to Al in solution. Honeylocust (*Gleditzia triacanthos*) and Norway spruce (*Picea abies*) also are considered Al-sensitive species (Sucoff et al., 1990 and Godbold et al., 1988b, respectively).

Studies of pines, however, have shown that they are relatively tolerant to Al stress compared to other tree species including other conifers (Cronan et al., 1989; Schaedle et al., 1989; Hutchinson et al., 1986). A study of red spruce (*Picea rubens*) and loblolly pine (*Pinus taeda*) revealed that surprisingly low concentrations of Al were phytotoxic to both species affecting their growth, but loblolly pine was less sensitive to Al than red spruce (Raynal, et al., 1990). Controlled experiments and field data in the study indicated that reductions of Ca and Mg in tissues may occur at Al concentrations that are well below those causing direct injury. Thus, it was postulated that Al may be involved in reductions in growth of pine and spruce through interference with nutrient uptake and translocation (Raynal, et al., 1990).

Many authorities have found that a more reliable indicator of acid stress in forests were the ratios of Al to other nutrients, primarily Ca and Mg and sometimes potassium (K). These ratios have been found to correlate better with symptoms of forest decline, rather than the total concentrations of Al in soil solution (Ulrich, 1989, 1985; Godbold et al., 1988a; Murach and Ulrich, 1988; Shortle and Smith, 1988).

Ca/Al Ratios as Indicators of Stress

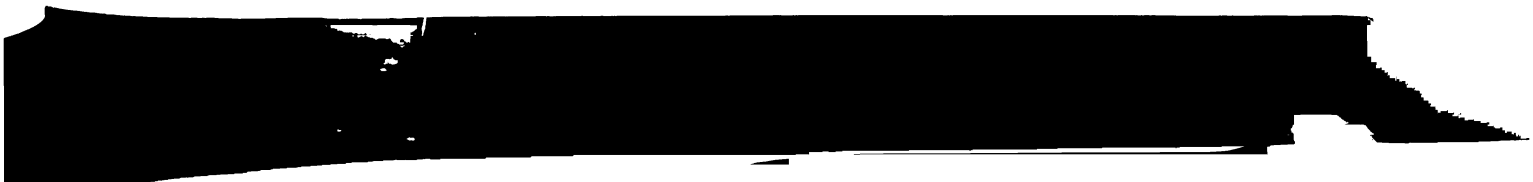
The soil Ca/Al ratio has been proposed as one of the key indicators of nutritional problems associated with acid conditions at a site. It has been suggested that a soil molar Ca/Al ratio below one in forested areas indicates a potentially Al-toxic condition (Johnson and Fernandez, 1992; Shortle and Smith, 1988; Ulrich, 1983). Low soil solution Ca/Al ratios have been associated with the decline of beech-spruce forests in Germany (Cronan et al., 1989; Ulrich, 1989). By contrast, Johnson et al. (1994), in a study of declining red spruce in the Adirondacks, found that soil solution Ca/Al ratios were well above the threshold (1:1 molar ratio) proposed as detrimental to Ca uptake, and soil-solution Al concentrations were well below levels thought to inhibit spruce root growth. The results suggest that Al toxicity was not a factor in the decline of high elevation red spruce in the Adirondacks. In sum, there was no evidence that exchangeable Al, Ca, or Mg played a role in red spruce decline on the area studied.

Correlations have been found between soil and solution culture Ca/Al ratios and root growth. Godbold et al., (1988b) found that root elongation in seedlings of *Picea abies* was severely inhibited by Al in nutrient solutions. Their study revealed that the concentration of Al itself was not critical, but that the molar ratio of Ca/Al was critical. Aluminum disturbed the mineral nutrition of the tree seedlings, displacing Mg and Ca in the root cortex in

solution culture. Additions of Mg and Ca ameliorated the effects of Al inhibition of root growth. A German study of declining spruce showed that fine-root biomass was highest in the upper soil layers with higher amounts of organic matter and higher Ca/Al ratios in the soil solution. In the lower soil mineral layers, the decrease in fine-root biomass was correlated with decreasing Ca/Al ratios within the fine roots and the soil solution (Murach and Ulrich, 1988).

Ratios of Ca/Al have been cited as key indicators of forest stress in soil and in plant tissues. Dewald et al. (1990) grew red oak (*Quercus rubra*) seedlings in soils that had been amended to achieve different levels of Al in the soil solution. Increasing Al levels in soil reduced concentrations of Ca and Mg in foliar tissues. Molar ratios of Ca/Al and Mg/Al were reduced significantly in the foliage even though tissue Al concentrations did not vary significantly across soil-solution Al treatment levels. Although there was not a strong correlation between soil Al concentration and shoot growth, the study did find that 40 percent of the variation in root growth was related to soil solution Al, with the Al treatments significantly reducing total root weight.

Murach and Ulrich (1988) report that fine roots (< 2 mm in diameter) distinctly reflect variations in soil chemistry. There was a strong correlation between the Ca/Al ratio gradients in fine roots on beech-spruce stands in Germany and the gradients in soil solution. Ulrich (1989)



reported that the Ca/Al ratios of dead fine roots approaches that of the soil solution. Although correlations have been found between foliar and soil solution Ca/Al ratios, it appears that the correlation between fine roots and soil Ca/Al ratios is a more reliable indicator of Al-stress in forest ecosystems. Other authorities have examined wood chemistry in an attempt to link divalent cation trends in stemwood to acidic deposition (Bondietti, et al., 1990; Bondietti, et al., 1989). There are criticisms of using Ca/Al as indicators of Al-stress in wood; the same cation trends have been identified in unpolluted environments. Concentrations of elements in sapwood are not stable indicators of wood chemistry over time (Cronan, 1995). Thus it appears that the most reliable indications of Al-stress in forests are in soil and fine-root tissues.

Cronan and Grigal (1995) reviewed the literature on Ca/Al ratios as indicators of stress in forest ecosystems. They identified four measurement end points to use as ecological indicators for identifying thresholds beyond which the risk of forest damage from Al stress and nutrient imbalances increases. The four measurement end points were as follows: 1) A soil base saturation < 15% of effective CEC; 2) A soil solution molar Ca/Al ratio $\leq$ 1.0 (for a 50% risk); 3) A fine root tissue Ca/Al ratio $\leq$ 0.2 (for a 50% risk); and 4) A foliar tissue Ca/Al molar ratio $\leq$ 12.5 (for a 50% risk). These threshold values are recommended as guidelines to identify sites where Al stress is likely to

adversely affect tree growth. The authors recognized that these threshold values must be used with certain precautions. Variability arises, for example, from differences in species tolerances, sampling techniques, and chemical analyses. The authors concluded from the literature reviewed that there is a strong relationship between tree growth and Ca/Al ratios in soil solutions and in plant tissues. Many research studies have substantiated the relationship between Ca/Al ratios and forest health; but the question is "what are the biological and chemical mechanisms whereby Al interferes with nutrient uptake?"

Inhibition Mechanisms

Many studies have indicated that monomeric inorganic forms of aluminum, mainly Al^{3+}, are more toxic to plants than are polymeric and organic forms (Balsberg-Pahlsson, 1990; Nakos, 1989; Ulrich, 1989; Joslin and Wolfe, 1988; Schaedle et al., 1989; Lathwell and Grove, 1986). In organic soils, even with low pH values, Al is complexed with organic matter and is unavailable for uptake. In low pH (below 5.0) mineral soils, however, monomeric forms predominate.

As previously discussed, the data show that Al may be directly toxic to plant cells, especially those of sensitive species. There is also an indirect toxic effect in an antagonistic/competitive relationship with other cations (Cronan, 1995; Schaedle et al., 1989). Aluminum, because of

its higher bonding strength, displaces Ca within the soil and root zone. Once inside the root, Al displaces Ca primarily in the apoplast; also there is some interference in the symplast (Raynal et al., 1990; Ulrich, 1989; Schaedle et al., 1989; Godbold et al., 1988a; Murach and Ulrich, 1988).

Visual symptoms of Al toxicity on roots include decreased root elongation, decreased number and length of lateral roots, browning of roots, increases in diameter, and necrosis (McQuattie and Schier, 1992; Schaedle et al., 1989). At the cellular level, Al has been shown to inhibit mitosis. Dividing and elongating cells in the region of the root tip are especially affected. Aluminum also causes premature cell maturation and senescence. Vacuolization of root cortical cells closer to the meristem and vacuolization of meristematic cells have been observed after exposure to toxic levels of Al (Schaedle et al., 1989).

Aluminum is taken up by roots in a non-metabolic process. Once taken up, Al^{3+} may bind to apoplast surface adsorption sites and interfere with plant uptake or selectivity for nutrient ions such as Ca^{2+} and Mg^{2+} (Cronan and Grigal, 1995; Arp and Ouimet, 1986). Bengtsson et al., (1988) found that Al^{3+} ions can reduce the concentration of Ca, Mg, and P in plants and increase concentrations of K in both nutrient and soil solution. The effect of Al^{3+} on Ca^{2+} uptake was immediate and primarily of a competitive nature, preventing Ca^{2+} from being adsorbed. The immediate effect of

Al^{3+} exposure on Ca^{2+} uptake in roots indicates that most Al^{3+} effects are primarily extracellular; the cation balance at the root surface is disrupted. Al^{3+} ions were found to compete especially with Ca^{2+} and Mg^{2+} for binding sites in the free space of roots, leading to a shortage of these ions at key sites in the plasma membrane. This is proposed as a reason why Al toxicity sometimes gives symptoms similar to Ca^{2+} deficiency.

It is not clearly understood how Al disrupts plant cells once it breaches the exclusion mechanism and interacts with components of the symplasm. One or more of several possible disturbances may occur. Aluminum may inhibit cell division by binding to nucleotides and nucleic acids, and/or it may interfere with energy transfer by binding to ATP, ADP, or membrane-bound ATP phases. Aluminum may also disrupt enzyme systems such as acid phosphates (Cronan, 1995).

Haug et al. (1994) found that Al ions disrupt elements of signal transduction pathways within the cell. They indicated that Al interfered with the action of guanine nucleotide-binding proteins (G proteins) and with an interrelated Ca^{2+}/phosphinositide signalling pathway. Other studies have found the Al may interfere with the activities of calmodulin, a Ca-dependent regulatory protein (Sucoff et al., 1990; Haug and Caldwell, 1985). Aluminum binds to calmodulin, inhibiting calmodulin-stimulated, membrane-bound ATPase activity (Haug and Caldwell, 1985).

Aluminum has also interferes with the structure and function of the plasma membrane by disrupting the movement of lipids, thus altering membrane permeability (Haug and Caldwell, 1985). The various pathways of interference by Al are not completely understood and require further study. The end result, however, is that Al toxicity will stress plants, affecting energy transformations, cell division, membrane transport, nutrient accumulation, and various activities regulated by calmodulin.

There is also evidence that soil acidification and aluminum toxicity may be detrimental to mycorrhizae. Holopainen (1989) studied the distribution of mycorrhizae in Scots pine (*Pinus sylvestris*) growing near a pulp mill. There were few mycorrhizal types and dominance of one mycorrhizal species was observed in this severely polluted, acidic site. The mycorrhizae also had important structural changes, which included accumulation of tannin in the cortical and pericycle cells, intercellular hyphae in the cortical cells, and increase of electron dense vacuolar accumulations in the fungal cells. These observed ecological and ultrastructural changes suggest that severe disturbance in the composition and function of pine mycorrhizae can occur in certain acidic environments.

McQuattie and Schier (1992) exposed pitch pine (*Pinus rigida*) seedlings to various levels of Al and ozone. Ozone alone reduced the percentage of mycorrhizal colonization. Aluminum, by contrast, increased colonization at low

concentrations but reduced it only at the highest level. However, the combination of ozone and Al on the anatomy of mycorrhizae was greater than either alone; apparently, there was a synergistic effect. Ozone had less effects on root anatomy than did Al, which caused deterioration of the root cortex. Greater colonization by mycorrhizae at low Al concentrations than at high Al levels may result from more inhibitory effects of Al on root growth than on fungal growth. With less root system to infect, a greater percentage of the seedlings' roots became colonized.

Cumming and Weinstein (1990) grew pitch pine seedlings with or without the ectomycorrhizal fungus *Pisolithus tinctorius* and exposed the roots to low levels of Al in sand culture. The nonmycorrhizal seedlings were affected by Al, resulting in decreased growth, increased transpiration rates, decreased efficiency in use of water, increased levels of foliar Al and Na, and reduced concentrations of P in foliage. By comparison, seedlings with *P. tinctorius* mycorrhizae, after exposure to Al, were not altered in growth, physiological function, and ionic relations. The fungal symbiont evidently modulated ionic relations in the rhizosphere, reducing Al uptake and Al-P precipitation reactions. This suggests that mycorrhizae may be able to protect pine roots, at least in part, from the adverse effects of Al.

Biotic Factors

Trees stressed by nutrient deficiencies/toxicities, weather extremes, and/or other adverse site factors are more susceptible to insect and disease problems. Toxic effects of Al, whether direct or indirect, may weaken trees predisposing them to attack by insects and fungi. Abebe and Hart (1990) found that the incidence of *Cytospora* canker on hybrid poplar increases with increases in Al levels in the soil.

There are only a few articles written specifically about the role of insects and fungi in red pine pocket mortality. One was a comparison of declining and of healthy red pine plantations in Wisconsin (Klepzig, et al., 1991). There was a significant difference in the abundance of five insect species in declining stands compared to healthy stands of *Pinus resinosa*. Insects involved were the root collar weevil (*Hylobius radicis*), the pales weevil (*Hylobius pales*), the pitch-eating weevil (*Pachylobius picivorus*), and the turpentine beetle (*Dendroctonus valens* and *Hylastes porculus*). These root- and lower stem-infesting insects consistently carried two fungal species--*Leptographium terebrantis* and *Leptographium procerum*. The researchers proposed that *Leptographium* is transmitted to red pine roots by the five insect vectors; root infections lead to expression of above-ground symptoms. *Leptographium*, spp., which may spread via root graphs or contacts and/or through soil, are proposed to cause below-ground root mortality

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which precedes the above-ground symptoms of reduced radial growth, thin crown structure, and infestation by the pine engraver (*Ips pini*). As trees die they become suitable habitats for *H. pales* and *P. picivorus* and other members of the root- and lower-stem-feeding insect group. These insects increase in number and introduce additional inoculum; the result is expansion of the site of infection i.e. pocket dieback. Final tree mortality is attributed to *Ips pini* and its fungal associate *Ophiostoma ips* (Klepzig, Raffa, and Smalley, 1991).

In further analysis of the above scenario, Klepzig et al. (1995) examined the possibility that *D. valens* and *H. porculus* can vector the associated fungi to red pine. In this study, *D. valens* and *H. porculus* were caged with wounded red pine roots. *D. valens* collected from the field transmitted *L. terebrantis, L. procerum* and *O. ips*. with 45%, 30% and 5% success, respectively. *H. porculus* collected from the field transmitted *L. terebrantis, L. procerum* and *O. ips* with 55%, 40% and 5% success, respectively. Only two of the control roots, which were mechanically wounded and not exposed to insect vectors, contained *Leptographium, spp.* The researchers concluded that *D. valens* and *H. porculus* can vector *Leptographium* fungi to red pine trees and that these organisms probably are involved in red pine decline disease.

Leptographium terebrantis, vectored by black turpentine beetles (*Dendroctonus terebrans*), has been

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associated with mortality of Japanese black pine (*Pinus thunbergiana*) and Scots pines (*P. sylvestris*) on Cape Cod, Massachusetts (Highley and Tattar, 1985). At this site, blue stain caused by *L. terebrantis* developed beneath larval galleries of the beetle in the lower bole and buttress roots; this was followed by foliar symptoms and attack by bark beetles (*Ips* spp.) and other staining fungi.

The pathogenicity of *Leptographium* spp. and their *Ophiostoma* teleomorphs are questionable. Alexander, et al. (1988) reported that *L. procerum*, the causal agent of procerum root disease of eastern and western white pine (*Pinus strobus and P. monticola*), affects mainly young trees (< 20 years old) in plantations and is associated with poor site conditions especially poorly drained soils. Harrington (1993) reports that many *Ophiostoma/Leptographium* species appear to be weak pathogens, perhaps contributing in a minor way to the disease syndrome, whether of abiotic or biotic origin. Harrington also states that the role of *Leptographium* in beetle-attacked trees remains unclear and that only two species, *O. ulmi* and *L. wageneri* have been studied extensively and found to be causal agents of major plant diseases: Dutch elm disease and black stain root rot on conifers.

Summary

It is evident from the literature that acidification of forest ecosystems may occur as a result of both natural and

anthropogenic processes. One of the primary concerns with soil acidification is the increase in soluble/exchangeable forms of Al (mainly Al^{3+}) which can be toxic to plants either directly or indirectly. The biochemical pathways and mechanisms of Al toxicity to plants are not completely understood. The end result, however, is that the plant/tree may become weakened and prone to secondary insect and disease problems. The following chapters of this dissertation will examine whether or not aluminum toxicity is the primary cause of red pine pocket mortality (RPPM). Emphasis will be placed on the indirect toxic effects of aluminum as a deterent to the uptake of other nutrients since this is a more likely scenario with Al-tolerant red pines. Ca/Al ratios and other measurement end points will be the main parameters used to identify unfavorable site conditions in pockets of red pine mortality.

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CHAPTER II

Root Biomass Volumes in Red Pine (*Pinus resinosa*) Mortality Pockets Versus Healthy Red Pine Stands in Michigan

ABSTRACT

Roots were collected from red pine mortality pockets by two different methods: pit-sampling and core-sampling. The pit-sampling method revealed that percentages and actual volumes of live roots for all root-size classes increase starting from the center to the edge of pockets, and finally to the outside "healthy-looking" regions of disease plots. Pocket centers and edges were characterized by relatively low root volumes and high proportions of dead roots. Relatively high percentages of symptomatic root volumes were also found at pocket edges, where trees were declining. Root volumes within disease plots were largest and healthiest in the area outside the pockets where trees appeared healthy. The core-sampling method enabled quantification of very fine roots (< 1 mm) in diameter that were not discernable using the pit-sampling method. However, the core-sampling method failed to reveal the same trends as the pit-sampling method, probably owing to the fact that roots from competing vegetation interfered with sampling only red pine roots.

Root volumes collected by the pit-sampling method revealed that disease centers and edges, where red pines were dead or dying, had significantly lower volumes of root biomass than did areas of healthy red pines. These healthy-looking regions included areas within the Huron-Manistee disease plots but outside the edge of decline, healthy plantation plots within the Huron-Manistee National forest, and healthy plantation plots at the Houghton Lake State Forest in Roscommon County, Michigan. There were no statistically significant differences between volumes of roots from outer "healthy" regions of disease plots and the check plots at Huron-Manistee and Roscommon. However, smaller volumes of live roots, and more symptomatic and dead roots in the outside healthy-looking regions of disease plots compared to non-disease plantations suggests that the disease is spreading below ground and that trees are dying from the bottom up rather than from the top down. Roots, especially in the fine, $\leq$0.3 cm in diameter category, die first, eventually leading to symptoms of red pine pocket mortality (RPPM) in the crown. However, our attempts at isolating a fungal agent which may be responsible for root mortality in red pine pockets were unsuccessful.

INTRODUCTION

Red Pine Pocket Mortality (RPPM) is a relatively recent disease affecting 30- to 50-year-old plantation-grown red pine in the Lake States. The disease is characterized by

declining trees surrounding a large, often-circular, area of dead trees (Klepzig and Carlson, 1988; Raffa and Hall, 1988). Symptoms of the declining red pine include thinning crowns, browning foliage, stunted growth in needles, and reduced diameter and height growth. Many insects and fungi have been found associated with the disease in Michigan but none have been consistently present in all pockets.

The causes of this disease are uncertain. A study in Wisconsin identified a complex vector and pathogenic fungi association that may lead to the decline (Klepzig, Raffa, and Smalley, 1991). Investigators in the study found a significant difference in the abundance of five insect species in declining stands compared to healthy *Pinus resinosa* stands. These root- and lower stem-infesting insects consistently carried two fungal species--*Leptographium terebrantis* and *Leptographium procerum*. It was suggested that *Leptographium* is transmitted to the red pine root system through the five insect vectors resulting in expression of above-ground symptoms. *Leptographium*, spp., which may spread via root graphs or contacts and/or through soil, are proposed to cause below-ground root mortality which precedes the above-ground symptoms of reduced radial growth, thin crown structure, and infestation by the pine engraver beetle (*Ips pini*).

Our investigations of RPPM in Michigan have focused on the possible abiotic factors involved in the decline rather than on the role of biotic agents. Of particular interest

has been the possibility that aluminum (Al) toxicity may be the primary cause of fine-root death. This theory of Al-induced root mortality was derived mainly from the literature pertaining to pollution, soil acidification, and forest decline. Soil acidification has been cited as a cause of forest growth decline by creating nutrient deficiencies through leaching of base cations (especially Ca^{2+}) and also by increasing the concentration of aluminum (Al^{3+}) in solution to toxic levels (Johnson and Taylor, 1989). In the case of RPPM, the soils under red pine may have acidified as a result of pine litter accumulating on the forest floor as the trees mature. Pine needle accumulation over time could eventually affect soil pH as needles decompose and release organic acids into the soil (Johnson and Fernandez, 1992). This time-dependent factor may explain why symptoms of RPPM are not seen until the trees are mature.

Whether the causes are biotic or abiotic in origin, it has been presumed that root mortality leads to the expression of above-ground symptoms of RPPM. This study compares volumes of root biomass from declining stands and healthy stands in terms of the proportions of live and dead roots within a plot.

METHODS

Study Locations

Five RPPM sites as identified by the U.S. Forest Service were selected for this study. Research plots were

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established within the area of decline and for an area outside the pocket. Three disease plots (ML1, ML2, and ML3) are located at Minnie Lake at a latitude and longitude of 43°38' and 85°54' (township and range: T 14 N, R 13 W) and two disease plots (TG4 and TG5) are located at an area known as Thin-A-Gain at a latitude and longitude of 43°35' and 83°41' (township and range: T 14 N, R 12 W) (Figures 2-1 and 2-2).

In addition to disease plots there are two plantation check plots of healthy red pine located near the disease plots: one at Minnie Lake (MLC) and one at Thin-A-Gain (TGC). For comparison with plantation-grown red pine located in a forest other than Huron-Manistee, two additional check plots of healthy red pine were established at the Houghton Lake State Forest in Roscommon County, Michigan (R1 and R2) at a latitude and longitude of 44°15' and 84°40' (township and range: T 21 N, R 3 W). The Roscommon plots are about 115 km northeast of the disease plots. In sum, there were nine plots used in this study: five disease plots; two plantation check plots located in the same forest as the disease plots; and two plantation check plots located in another forest. Plantation check plots are similar to the disease plots in that they consist of mature, even-aged red pines that had been densely planted.

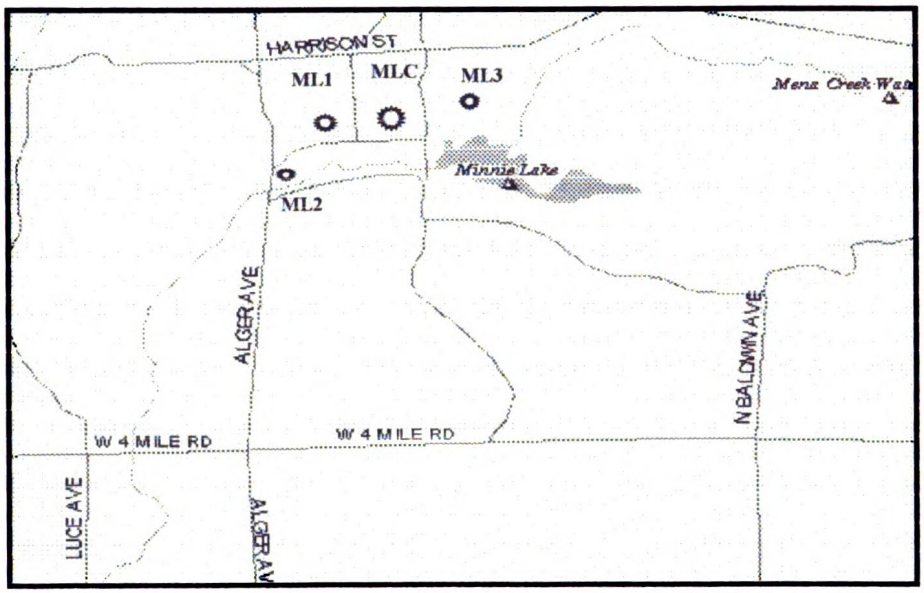

Figure 2-1. Location of disease plots (ML1, ML2 and ML3) and one check plot (MLC) at the Minnie Lake site, Huron-Manistee National Forest, Newago County, Michigan.

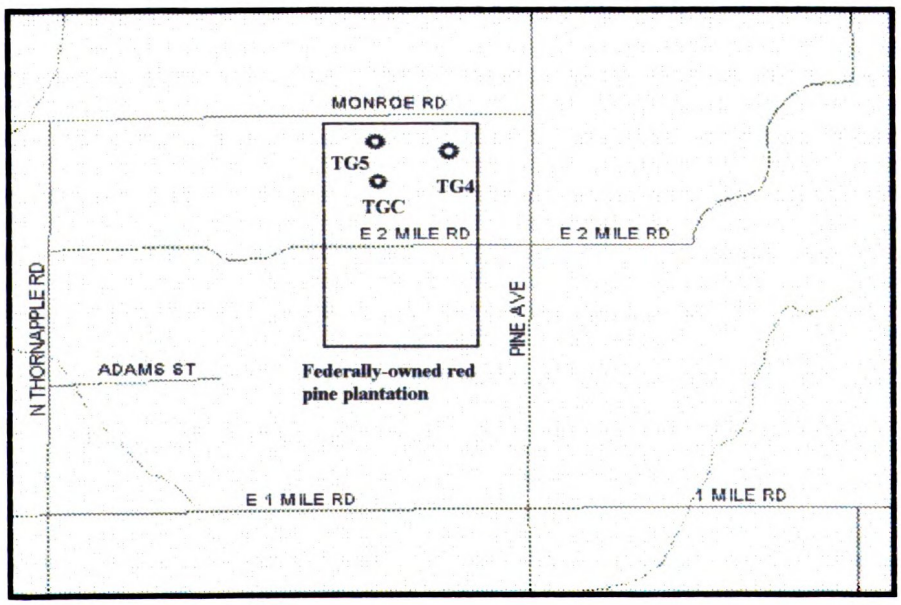

Figure 2-2. Location of disease plots (TG4 and TG5) and one check plot (TGC) at the Thin-A-Gain site, Huron-Manistee National Forest, Newago County, Michigan

Pit-Sampling Method

Roots were collected in August, 1992 by digging 1-m^2 pits in disease plots at three locations within the plot: in the center of the pocket where there were many dead standing trees; at the edge of the pocket where trees were declining; and outside of the decline where the trees appeared healthy. The 1-m^2 pits were divided into four 0.5 x 0.5 m sections and samples were taken from two depths: 0 to 30 cm deep, and 30 to 60 cm deep. Thus eight bags of root samples were collected from one 1-m^2 pit (4 sections x 2 depths).

In total, fifteen pits were dug (three at each of the five plots) in disease plots. In addition, two 1-m^2 pits were taken at each of the two check plots at Huron-Manistee. Samples were taken in August, 1994 from the two check plots at Roscommon County. At that time, only 0.5-m^2 pits were dug and bagged-samples removed from the two depths (two bags of roots per pit); four 0.5-m^2 pits were dug at each of the two check plots so that the area removed was the same size as a 1-m^2 pit. The pits were dug randomly at locations throughout the check plots because there were no features of center, edge, or outside area of a pocket within these plots.

The roots collected at both check plots and disease plots were extracted by sifting the soil through a mesh screen and separating the red pine roots from the roots of other plants growing in the area. Once collected, the roots

were washed by rinsing with water for 30 minutes and dipped in a 10% Chlorox solution for 2 minutes. The length and diameter of each root was recorded and they were classified as to live, dead, or symptomatic. Roots were classified as "symptomatic" if they were partially dead or dying, and/or if there was any root staining below the epidermis.

Core-Sampling Method

Core-sampled roots were collected at three disease plots located in the Minnie Lake region of the Huron-Manistee National Forest and from the two healthy plots at Roscommon in August, 1994 (ML1, ML2, ML3, R1, and R2). In disease plots, the root systems of red pines were sampled along transects. Three trees were sampled per disease plot (9 trees total). The first tree sampled was located at the edge of the pocket. From the edge, the transect extended out into the healthier regions of the disease plots from where the second and third trees were sampled. At Roscommon, because there were no features of center, edge, or outside area of a pocket, the samples were collected randomly from three different trees within each plot for a total of 6 trees. At both Huron-Manistee and Roscommon, five cores, at a distance of 60 cm from the main stem, were taken around each tree. The sampling depth was 30 cm and the cylindrical cores were 10 cm wide.

The soil was rinsed from the samples using the hydropneumatic elutriation method (Srivastava, et al.,

1981). The roots were placed in Whorl-pack^{tm} bags with 15-20% methanol solution and stored at 4°C. The roots were stained blue with 1.0 cc of Malachite-blue dye. Fine-root (< 1.95 mm in diameter) biomass was determined using the robotic camera method (Smucker, 1989). In this method, images of washed roots are stored on standard 1/2 inch VHS format video tapes. Each tape contains images from numerous root samples. Images from the tape were then processed by an image-processing computer to quantify the root length and width. Roots greater than 1.95 mm in diameter were too large to be measured accurately by the image-processing system. These roots had to be pulled out of the sample and measured and recorded by hand.

Statistical Analyses

Analysis of variance was used to test the hypothesis that root volumes from disease and healthy locations are significantly different from each other. The model used to test hypotheses of root volumes (i.e., $V=L\pi R^2$) differing by locations and soil depths (Table 2-1) is as follows:

$$Y_{ijk}=\mu+\alpha_i+\beta_j+\alpha\beta_{ij}+\epsilon_{ijk}$$

where variable Y is the root volume concentration per pit (k) associated with the ith level of factor α (location) and j^{th} level of factor β (soil depth), μ is the overall mean of all observations, α_i is the effect of ith level of the location factor, β_j is the effect of the jth level of the soil depth factor, $\alpha\beta_{ij}$ is the effect of the interaction

between the location and soil depth factors and $\epsilon_{(ij)k}$ is the experimental error associated with the kth experimental unit (1-m^2 pit) for the levels of location and soil depth factors.

Four different root-size classes were tested in separate ANOVA trials. These four variables belong to root-size categories: $\leq$ 0.3 cm in diameter; >0.3 and $\leq$0.6 cm; >0.6 and $\leq$1.0 cm; and, >1.0 cm. There were five levels included in the location factor: the center of Huron-Manistee disease plots; the edge of disease plots; outside the edge of the decline within disease plots; healthy plots at Huron-Manistee; and healthy plots at Roscommon County. Two levels are included in the soil depth factor from which roots were collected: the top 0-30 cm, and bottom 30 to 60 cm.

ANOVA was also used to test differences between root volumes by location and condition (i.e. live, dead, or symptomatic) (Tables 2-2 through 2-5). The model used to test the hypothesis that root volumes differ by location and condition is the same except that β_j is the effect of the jth condition factor, and $\alpha\beta_{ij}$ is the interaction between location and condition (i.e. volumes of live, dead or symptomatic roots) factors.

If the soil depth factor or the condition factor was identified as significant, additional ANOVA's were run by selecting the subpopulation of interest. For example, root volumes in the top level of soil depth were compared to each

other and in separate ANOVA trials, root volumes in the bottom level were compared. Similarly, for the condition factor, all root volumes in the live category were compared to each other, dead volumes were compared to other dead root volumes, and symptomatic root volumes to other symptomatic root volumes. These ANOVA's follow the model $Y_{ik}=\mu+\alpha_i+\epsilon_{ik}$ where α_i is the effect of the location factor.

The effect of the location, soil depth and/or condition factors are considered fixed because they are the only levels of interest in this investigation. If the experiment were to be repeated the same treatments would be included and randomness would be inherent in the replications (n) i.e., the number of soil pits sampled. If, however, the ANOVA had been run using plots (i.e. ML1, ML2, ML3, etc.) as the levels of a factor the effect would be considered random because the disease pockets and healthy plantation plots are considered subsamples of the larger population of all potential disease pockets and healthy plantations. Under the random effects model the objective is to make inferences to the larger population (i.e. disease pockets and healthy red pine plantations in other locations). However in this investigation the effects are considered fixed and any inferences made apply only to the disease and healthy areas included in this study.

Analysis of core-sampled roots also follow the model, $Y_{ik}=\mu+\alpha_i+\epsilon_{ik}$ except that α_i, the location factor, consists of four levels: three locations along transects within Minnie

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Lake disease plots which start from trees at the edge of pockets, to trees mid-range from the edge to outer region of the plots, to trees located at outer "border" regions of the plots. The fourth location is Roscommon, where trees were sampled randomly within the plots. In this analysis, the sampling unit k, is the 30 cm deep x 10 cm wide cylindrical core rather than the 1-m^2 pit. The effect of the location factor in this model is also considered fixed because statistical tests were run between samples from within plot locations rather than between individual plots. If the experiment were to be repeated the same levels of the factor would be included. There were no factors of soil depth or condition included in core-sampled roots.

All sample distributions for root volumes were transformed by taking the square root in order to normalize the distribution. However, for clarity, means and other descriptive statistics reported are non-transformed values. Means were separated at the 0.05 significance level using the Tukey's highly significant difference (HSD) procedure (Systat for Windows, Version 5, 1992).

Identification of Pathogenic Fungi

The live or symptomatic roots collected by the pit-sampling method were cultured using selective and non-selective media for *Leptographium,* spp. Potato dextrose agar (PDA) was used as non-selective media which was acidified with lactic acid. Selective media consisted of PDA with 100

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ppm cycloheximide and acidified with lactic acid (Harrington, 1981). Roughly 1000 roots were cultured; half on selective and half on non-selective media.

RESULTS

Pit-Sampled Roots

Table 2-1 lists the average volume per 1-m^2 pit in cm^3 of roots collected from locations within disease plots (center, edge, and outside) and from healthy areas (Huron-Manistee and Roscommon) from two levels of soil depth (0 to 30 cm, and 30 to 60 cm). In the pit-sampling method, it was difficult to distinguish red pine roots less than 1 mm in diameter. Therefore, the smallest size category represented is between 1 and 3 mm in diameter. Table 2-1 breaks down the volumes of roots collected according to four diameter size categories with the largest size category being greater than 1 cm in diameter.

The largest volume of red pine roots was found in the top 30 cm of soil for all locations (disease and non-disease) and for all root-size categories. Probabilities that there were no significant differences between root volumes by soil depth were $p<0.001$ for the ≤ 0.3 cm and >.6 to ≤ 1.0 categories; $p=0.003$ for the >0.3 to ≤ 0.6 cm category; and $p=0.055$ for the >1.0 cm category. The probabilities that there were no significant differences between root volumes by location were $p<0.001$ for the ≤ 0.3 cm category; $p=0.003$ for the >0.3 to ≤ 0.6 cm category;

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Table 2-1. Average root volumes per 1-m^2 pit in the top (0 to 30 cm) and bottom (30 to 60 cm) layers of soil as separated by diameter classes and locations (disease center, n=5; disease edge, n=5; disease outside, n=5; Huron-Manistee check, n=4; and Roscommon check, n=2).

Plot	Top Avg.			Bottom Avg.			Soil depth
Disease Center	**Volume cm^3**	**Std. Dev.**	**%**	**Volume cm^3**	**Std. Dev.**	**%**	**%top/%bot**
≤.3 cm	10.0	10.0	3.2	5.3	6.3	3.0	65.5/34.5
.3> ≤.6	20.8	22.4	6.6	18.1	17.1	10.1	53.5/46.5
.6> ≤1.0	41.0	32.7	13.1	25.3	26.6	14.2	61.8/38.2
>1.0	241.8	236.6	77.1	129.6	98.0	72.7	65.1/34.9
Totals	313.6		100	178.3		100	63.8/36.2
Disease Edge	**cm^3**	**Std. Dev.**	**%**	**cm^3**	**Std. Dev.**	**%**	**%top/%bot**
≤.3 cm	29.8	13.7	5.7	19.7	11.7	9.3	60.1/39.9
.3> ≤.6	60.5	37.0	11.5	36.6	21.0	17.3	62.3/37.7
.6> ≤1.0	120.2	90.1	22.8	46.8	20.9	22.1	72.0/28.0
>1.0	316.5	172.5	60.0	108.5	98.5	51.3	74.5/25.5
Totals	527.0		100	211.6		100	71.4/28.6
Disease Outside	**cm^3**	**Std. Dev.**	**%**	**cm^3**	**Std. Dev.**	**%**	**%top/%bot**
≤.3 cm	67.5	20.6	6.7	25.2	8.9	5.5	72.8/27.2
.3> ≤.6	109.0	53.6	10.9	28.9	21.3	6.3	79.1/20.9
.6> ≤1.0	133.6	70.7	13.3	36.8	16.8	8.1	78.4/21.6
>1.0	691.0	535.1	69.0	365.7	399.1	80.1	65.4/34.6
Totals	1001.1		100	456.6		100	68.7/31.3

Table 2-1. (Cont'd)

Plot	Top Avg.			Bottom Avg.			Soil depth
HM Check	**cm^3**	**Std. Dev.**	**%**	**cm^3**	**Std. Dev.**	**%**	**%top/%bot**
≤.3 cm	69.1	26.1	8.8	31.7	11.5	7.0	68.6/31.4
.3> ≤.6	67.7	18.8	8.6	34.4	6.5	7.6	66.3/33.7
.6> ≤1.0	122.9	74.7	15.7	51.1	10.5	11.2	70.6/29.4
>1.0	525.4	117.1	66.9	337.4	294.2	74.2	60.9/39.1
Totals	785.1		100	454.6		100	63.3/36.7
Rosc. Check	**cm^3**	**Std. Dev.**	**%**	**cm^3**	**Std. Dev.**	**%**	**%top/%bot**
≤.3 cm	132.4	67.7	20.1	39.7	14.0	15.5	76.9/23.1
.3> ≤.6	98.2	54.9	14.9	54.4	47.3	20.9	64.4/35.6
.6> ≤1.0	194.2	24.9	29.5	55.8	11.0	21.7	77.7/22.3
>1.0	234.1	226.6	35.5	108.4	65.2	41.9	68.4/31.6
Totals	658.9		100	258.3		100	71.8/28.2

$p=0.009$ for the >0.6 to $\leq$1.0 cm category; and $p=0.169$ for the >1.0 cm category. Thus, the only root-size category which did not differ significantly at the 0.05 level by location or depth was the >1.0 cm category. There were no significant interaction effects of location X depth for all root-size categories at the 0.05 level. In most cases, 60 to 80% of the root volume was in the top level of soil depth regardless of location (Table 2-1).

Roscommon plots, in Table 2-1, had the largest proportion of fine-root mass (roots $\leq$0.3 cm in diameter) in both levels of soil depth followed by the check plots at Huron-Manistee. Tukey's HSD procedure revealed that fine-root volumes at Roscommon (132.4 cm^3) differed significantly at the 0.05 level from disease centers (10.0 cm^3) and edges (29.8 cm^3) in the top level (0-30 cm) of soil depth. Also in the top soil depth, fine-root volumes from disease centers (10.0 cm^3) differed significantly at the 0.05 level from fine-root volumes at disease outer regions (67.5 cm^3) and Huron-Manistee healthy locations (69.1 cm^3).

In the bottom level (30 to 60 cm) of soil depth, fine-root volumes from Roscommon (39.7 cm^3) differed significantly only from the fine-root volumes from disease centers (5.3 cm^3) at the 0.05 level. Fine-root volumes from disease centers (5.3 cm^3) also differed at the 0.05 level from fine-root volumes at disease edges (19.7 cm^3), disease outer regions (25.2 cm^3), and Huron-Manistee healthy locations (31.7 cm^3) in the bottom soil depth.

Fewer significant differences were found within the larger-diameter root categories in Table 2-1 than for the fine-root category using Tukey's HSD procedure. In the >0.3 to $\leq$0.6 cm category, significance at 0.05 was found only between volumes between center (20.8 cm^3) and outside regions (109.0 cm^3) of disease plots in the top depth. In the >0.6 to $\leq$1.0 cm category, a significant difference was found only between volumes from the top depth of disease centers (41.0 cm^3) and Roscommon (194.2 cm^3). No significance at either soil depth was found in the >1.0 cm category. In short, there was no consistent or easily interpreted pattern associated with larger-diameter roots.

Table 2-2 lists the average volumes of fine roots, defined as roots $\leq$0.3 cm in diameter, per 1 m^2 X 60 cm deep pit, by location and condition class (i.e. live, dead, or symptomatic). In the fine-root category, ANOVA revealed significant differences between volumes by location, condition class, and for the interaction between location and condition ($p<0.001$ for all cases). A significant interaction effect indicates that volumes of live, dead, or symptomatic fine roots are partially dependent upon the location from where the roots were sampled (i.e. disease vs. non-disease areas). In disease centers, for example, where there were many tall dead standing trees, there was little expectation that many live fine roots would be recovered compared to healthier-looking locations. These location

Table 2-2. Average root volumes per 1m x 1m x 60 cm deep pit and proportions of live (L), dead (D), and symptomatic (S) roots for roots ≤0.3 cm in diameter by location within Huron-Manistee disease plots (center, edge, and outside) and check plots (Huron-Manistee and Roscommon).

	Huron-Manistee Disease Locations:						Non-Disease Locations:			
	Center		Edge		Outside		Huron-Manistee		Roscommon	
Condition:	cm^3	%	cm^3	%	cm^3	%	cm^3	%	cm^3	%
L	3.5	23.0	13.7	27.8	54.1	57.6	79.0	78.4	117.1	68.0
D	8.6	56.6	19.8	40.2	8.4	8.9	2.7	2.7	28.2	16.4
S	3.1	20.4	15.8	32.0	31.5	33.5	19.1	18.9	26.9	15.6
Totals	15.2	100	49.3	100	94.0	100	100.8	100	172.2	100

factors had a strong influence on the volumes of live and dead roots recovered.

Tukey's HSD procedure, in ANOVA trials on the subpopulation of live roots in Table 2-2, revealed that significant differences in fine-root volumes were found between disease centers (3.5 cm^3) and edges (13.7 cm^3), compared to the fine-root volumes in outside regions of disease plots (54.1 cm^3), Huron-Manistee non-disease plots (79.0 cm^3), and Roscommon non-disease plots (117.1 cm^3). Thus, diseased areas differed significantly from regions where trees appeared healthy, even the outside regions of disease plots. None of the healthy-looking regions (disease outside, Huron-Manistee check, and Roscommon check) differed significantly from each other.

The population of dead fine roots in Table 2-2, did not differ significantly from each other by location ($p=0.146$). This can be explained by the fact that dead-root volumes were small in healthy areas (disease outside 8.4 cm^3, Huron-Manistee 2.7 cm^3, Roscommon 28.2 cm^3) and comparable to the dead-root volumes found at the center (8.6 cm^3) and edges (19.8 cm^3) of disease plots. However, dead fine-root volumes constituted a relatively small proportion of the total fine-root volumes from healthy-looking areas (disease outside 8.9%, Huron-Manistee 2.7%, Roscommon 16.4%) compared to the disease centers (56.6%) and edges (40.2%).

For symptomatic fine-root volumes (i.e., roots that were partially dead or dying, and/or stained below the

epidermis) in Table 2-2, significant differences at the 0.05 level were found between disease centers (3.1 cm^3) compared to the fine-root volumes at disease edges (15.8 cm^3), outer regions (31.5 cm^3), healthy plots at Huron-Manistee (19.1 cm^3), and Roscommon (26.9 cm^3). This can be explained by the fact that the total volume of fine roots removed from disease centers was much less than at other locations, including the subpopulation of symptomatic roots.

Tables 2-3, 2-4, and 2-5 list the average root volumes per 1m x 1m x 60 cm deep pit by location and condition class for the larger root-size categories (i.e. >0.3 and $\leq$0.6 cm; >0.6 and $\leq$1.0 cm; and >1.0 cm in diameter, respectively). For all three large-root size categories, live-root volumes from disease centers were significantly lower at the 0.05 level compared to live-root volumes from healthy regions: disease outside, Huron-Manistee and Roscommon. These same relationships were found for the live fine-root ($\leq$0.3 cm) category.

Significant differences at the 0.05 level were also found between disease edges and healthy regions for the larger root-size categories. In the >0.3 and $\leq$0.6 cm live-root category, root volumes from disease edges (27.7 cm^3) were significantly lower than the root volumes from disease outside regions (89.4 cm^3), Huron-Manistee (81.8 cm^3) and Roscommon (100.2 cm^3) (Table 2-3). These same relationships were identified in the live fine-root category. In the >0.6 and $\leq$1.0 cm diameter category, disease edges (35.3 cm^3)

Table 2-3. Average root volumes per 1m x 1m x 60 cm deep pit and proportions of live (L), dead (D), and symptomatic (S) roots for roots >0.3 and ≤0.6 cm in diameter by location within Huron-Manistee disease plots (center, edge, and outside) and check plots (Huron-Manistee and Roscommon).

	Huron-Manistee Disease Locations:						Non-Disease Locations:			
	Center		Edge		Outside		Huron-Manistee		Roscommon	
Condition:	cm^3	%	cm^3	%	cm^3	%	cm^3	%	cm^3	%
L	6.4	16.4	27.7	28.5	89.4	64.8	81.8	80.0	100.2	71.1
D	20.6	52.8	30.5	31.4	14.3	10.4	0.0	0.0	38.4	27.2
S	12.0	30.8	38.9	40.1	34.2	24.8	20.4	20.0	2.4	1.7
Totals	39.0	100	97.1	100	137.9	100	102.2	100	141.0	100

Table 2-4. Average root volumes per 1m x 1m x 60 cm deep pit and proportions of live (L), dead (D), and symptomatic (S) roots for roots >0.6 and ≤1.0 cm in diameter by location within Huron-Manistee disease plots (center, edge, and outside) and check plots (Huron-Manistee and Roscommon).

	Huron-Manistee Disease Locations:						**Non-Disease Locations:**			
	Center		**Edge**		**Outside**		**Huron-Manistee**		**Roscommon**	
Condition:	**cm^3**	**%**	**cm^3**	**%**	**cm^3**	**%**	**cm^3**	**%**	**cm^3**	**%**
L	4.4	6.3	35.3	20.8	106.8	65.7	142.0	81.6	233.7	93.4
D	58.5	84.4	79.1	46.6	14.7	9.1	3.5	2.0	6.1	2.4
S	6.4	9.2	55.4	32.6	40.9	25.2	28.5	16.4	10.3	4.1
Totals	69.3	100	169.8	100	162.4	100	174.0	100	250.1	100

Table 2-5. Average root volumes per 1m x 1m x 60 cm deep pit and proportions of live (L), dead (D), and symptomatic (S) roots for roots > 1.0 cm in diameter by location within Huron-Manistee disease plots (center, edge, and outside) and check plots (Huron-Manistee and Roscommon).

	Huron-Manistee Disease Locations:						Non-Disease Locations:			
	Center		Edge		Outside		Huron-Manistee		Roscommon	
Condition:	cm^3	%	cm^3	%	cm^3	%	cm^3	%	cm^3	%
L	2.2	0.6	117.2	27.6	695.3	65.8	596.7	69.2	326.8	95.4
D	314.2	84.6	206.1	48.5	35.8	3.4	11.4	1.3	15.7	4.6
S	55.1	14.8	101.7	23.9	325.6	30.8	254.7	29.5	0.0	0.0
Totals	371.5	100	425.0	100	1057	100	862.8	100	342.5	100

differed significantly from Roscommon (233.7 cm^3) only (Table 2-4); and in the >1.0 cm category, disease edges (117.2 cm^3) differed from the disease outside areas (695.3 cm^3) and Huron-Manistee check plots (596.7 cm^3) but not from Roscommon (326.8 cm^3) (Table 2-5).

Unlike the fine-root category, some significant differences were found between dead-root volumes for the larger root-size categories. In the >0.3 and $\leq$0.6 diameter category, significant differences were found for dead-root volumes from disease edges (30.5 cm^3) and Roscommon healthy plots (38.4 cm^3) compared to the healthy plots at Huron-Manistee (0.0 cm^3) (Table 2-3) ($p=0.023$ and $p=0.050$). In the >0.6 and $\leq$1.0 cm category, significant differences were found between disease edges (79.1 cm^3) and Huron-Manistee healthy plots (3.5 cm^3) (Table 2-4) ($p=0.050$). In these cases, disease edges tended to have significantly higher volumes of dead roots than non-disease locations, with the exception of Roscommon in Table 2-3. Of healthy regions, Roscommon had the highest proportions of dead fine roots. No significant differences in dead-root volumes were identified for the >1.0 cm category (Table 2-5). For symptomatic root volumes, significant differences by location were not identified in any of the larger root-size categories.

Core-Sampled Roots

Table 2-6 illustrates the results of core-sampled roots. The cores were taken along transects around trees starting from the edge of the pockets to the outside regions of disease plots where trees appeared healthy and from the non-disease plots at Roscommon. The center regions of the pockets were not sampled since they consisted mainly of dead standing trees and a diverse range of plant species taking advantage of the pocket openings. It would not have been possible to select only red pine roots from other types of vegetation growing in the center areas.

It was expected that core-sampling would show increasing volumes of fine-root biomass starting with the lowest volumes at the pocket edges and increasing towards the outside healthy regions of the plot. The edge trees (Tree A from each plot) were always declining trees with obvious symptoms of RPPM. The trees midway between the transect (Tree B from each plot) appeared healthier compared to edge trees but many were starting to show symptoms of decline. The "C" trees, in the outer regions of the disease plots, were asymptomatic, healthy-looking trees. However, unlike pit-sampled roots, the data in Table 2-6 do not reveal any trend in increasing fine-root biomass from the declining to healthy looking trees within a plot.

These data are separated according to seven root-diameter categories ranging from .15 mm to > 3.00 mm in diameter. Roots that were 2.5 mm or larger were pulled out

of the sample and measured and recorded separately because they were too large to be processed by the robotic-camera method. ANOVA was used to test differences of root volumes within the seven diameter categories by location.

There were no significant differences found between root volumes located along transects within disease plots. But some significant differences were found between disease locations and Roscommon. These differences were only in the .15 and .4 mm diameter categories. Roscommon had significantly higher volumes of fine roots compared to all three disease locations within these categories ($P<0.001$ for the check compared to Tree A, Tree B, and Tree C locations in the .15 mm category, and Tree A and Tree B locations in the .4 mm category; and $P=0.002$ for the check compared to Tree C locations in the .4 mm category). There were no other significant differences identified for any of the other root-size categories except for the total volume of roots at each location which was significantly higher at Roscommon compared to the Tree B locations of disease plots ($P=0.050$).

The significant increase in very fine-root volumes (.15 and .4 mm categories) at Roscommon, suggests that these healthy locations may have higher production or perhaps reduced mortality of very fine roots compared to the disease locations. The core-sampling method failed to reveal the same increasing gradients in root volumes within disease plots revealed by the pit-sampling method. In the core-sampling method it was not possible to distinguish red pine

roots from roots of other plant species. In collecting core samples we attempted to avoid areas within the plot where competing vegetation might interfere with the results. However, roots of many plant species other than red pine, such as grasses, (*Poa*, spp.), raspberries (*Rubus*, spp.) and bracken fern *(Pteridium aquilinum*), especially near the more open canopy of pocket edges, may be increasing the volumes of fine roots collected. The Roscommon trees sampled were under a closed canopy with little interfering vegetation, so it is believed that the significant differences identified can largely be attributed to increased volumes of fine red pine roots.

Table 2-6. Average volumes (cm^3) per core (10 cm wide X 30 cm deep) of roots from the edge to outer regions of Minnie Lake disease plots, Huron-Manistee National Forest and in check plots at Roscommon County.

Minnie Lake Disease Plots		**Root Diameter (mm)**							
Transect Location	**n**	**.15**	**.4**	**.75**	**1.25**	**1.95**	**2.50**	**>3.0**	**Total Avg.**
Tree A-edge	15	0.20	1.21	2.35	0.43	0.03	1.54	0.00	5.75
Tree B-middle	15	0.16	1.50	2.56	0.43	0.02	0.81	0.22	5.70
Tree C-outer	15	0.17	1.63	2.75	0.37	0.02	0.48	0.28	5.78
Roscommon	30	0.37	2.31	2.86	0.37	0.02	1.24	0.09	7.39

Isolation of Pathogenic Fungi

No pathogenic fungi were recovered from root isolates on non-selective media. Of 489 isolates on selective media, only three cultures of *Leptographium*, spp. were identified. Two of the cultures were *Leptographium truncatum* and one was *Leptographium procerum*. All three *Leptographium* cultures came from symptomatic roots from disease plots (a subpopulation of 42 root cultures). Black staining was visible beneath the epidermis of the roots from which the three cultures were isolated. Two of the *Leptographium* isolates were from TG4 and one was from ML1. No *Leptographium* fungi were recovered from roots from non-disease check plots and black-staining was not observed in check plots and rarely in disease plots. The only other potential pathogenic fungi recovered were two isolates of *Ophiostoma*, spp., also from symptomatic roots in disease plots (TG4). Most of the isolations came from TG4, the only plot in the study with poorly drained soil (Chapter 3). Perhaps greater availability of moisture within the TG4 plot facilitated the growth of these fungi.

It was thought that there were not enough isolations done on symptomatic roots from disease plots, so in 1993 the process was repeated; this time 111 symptomatic roots from disease plots were cultured and no *Leptographium* nor any other noteworthy root pathogens were recovered. Overall it does not appear that *Leptographium*, spp. are a significant fungal pathogen in these plots.

DISCUSSION

The pit-sampled roots collected revealed that the highest concentration of roots were located in the top 30 cm of soil versus the bottom 30-60 cm. This was true for all root-size categories (Table 2-1). In our sampling of soils in disease plots, the top layers of soil, particularly the O and E horizons, were also the most acidic, with pH ranging from 4.1 to 4.8 at the Minnie Lake and Thin-A-Gain sites (Chapter 4). These pH's are within the range for considering the possibility of aluminum-toxic effects which may be involved in fine-root mortality at these sites.

Red pines at Roscommon appear to be more proficient at producing roots in the fine-root category ($\leq$0.3 cm in diameter) and/or the fine-root mortality rates are lower versus the red pines at Huron-Manistee healthy locations and diseased areas; although significance was found only in comparison with the diseased areas (Table 2-1). Conversely, red pines from healthy locations at Huron-Manistee appear to be more proficient at producing roots in the largest size category (>1.0 cm in diameter) compared to Roscommon; but again these differences were not statistically significant.

These observations suggest that fine-root turnover rates are higher at Roscommon than at Huron-Manistee. Not only were the fine-root volumes per pit the highest at Roscommon (172 cm^3), but so were the proportions of dead roots (16.4%) in comparison with the check plots at Huron-Manistee (2.7%) and the outside regions of disease plots

(9.1%) (Table 2-2). Significant differences were few, however, because of the limited number of replications (disease center, n=5; disease edge, n=5; disease outside, n=5; Huron-Manistee check, n=4; and Roscommon check, n=2). More pits would need to be dug in order to confirm the differences between these sites.

Tables 2-2 through 2-5 reveal that the same trends in volumes of live, dead, and symptomatic roots in the center, edges, and outside regions of pockets existed for all root-size categories. The greatest percentage of dead roots was located at the center, followed by the edge. Symptomatic roots tended to be found at the pocket edges but also extending into the outside "healthy-looking" regions.

Significant differences in fine-root volumes of pit-sampled roots were found mainly between disease centers and edges and regions where red pines appeared healthy. This was not a surprising result since trees were visibly declining in disease regions. One of the questions raised while observing RPPM was whether above-ground symptoms precedes below-ground root mortality or whether root mortality occurs before symptoms are seen in tree crowns? The fact that there was an increasing gradient in fine-root volumes from the center of pockets to the outside regions of disease plots, to the healthy plantations at Huron-Manistee and Roscommon suggests that roots are dying first before symptoms are expressed in tree crowns. The outside area of disease plots also tended to have higher volumes of

symptomatic roots than healthy plantations (Roscommon and Huron-Manistee), suggesting that the disease may be spreading beyond the pocket boundary into the healthy-looking tree population. However, the differences between fine-root volumes in the outside regions of disease plots compared to healthy plantations were not statistically significant.

Fine-root volumes were the root-size category of primary interest in this study because of the importance of fine roots in absorption and exchange of soil nutrients. If Al-toxicity is a cause of root mortality in red pine pockets, its effect would be primarily on the fine-root system. Joslin and Wolfe (1988) found that the primary effect of Al-toxicity to red spruce seedlings was upon the root system. Their greenhouse-pot study points to toxic levels of Al as the major cause of root and foliar biomass reductions in seedlings. Soil solution levels of inorganic monomeric Al and total Al were superior predictors of root biomass in the study. It may be that aluminum, or more importantly, the ratios of aluminum to other nutrients, especially calcium, are the primary factors controlling fine-root biomass within disease plots.

In trying to characterize the disease in terms of biotic agents, (i.e root pathogens), we were not able to corroborate the studies done in Wisconsin. No significant root pathogen populations were identified. It may be that whatever agents are responsible for the decline in Wisconsin

are not significant factors to Michigan pockets. The role of biotic agents should be looked into further because the often circular pattern of red pine mortality pockets suggests a strong biotic aspect to the disease. We did observe bark beetle (*Ips pini*), pine root collar weevil (*Hylobius radicis*), Armillaria root rot (*Armillaria*, spp.), and Diplodia tip blight (*Sphaeropsis sapinea*) activity in pockets. However these agents were not consistently present in all pockets and we do not have quantified information on them.

The significant interaction effects for all root-size categories between location and condition indicate that proportions of live, dead, and symptomatic roots will vary dependent upon the location from where the roots were sampled (i.e. areas within disease plots and from non-disease plots). Although there were no statistically significant differences in root volumes between outer, healthy regions of disease plots and non-disease plots, the increasing gradient in live fine-root percentages and corresponding decreasing gradient in dead fine-root percentages from pocket centers to the non-disease stands at Huron-Manistee suggest that fine-root mortality occurs before symptoms are seen in tree crowns.

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CHAPTER III

Condition and Disease Progress of Red Pine Pocket Mortality Stands in Michigan

ABSTRACT

Red Pine Pocket Mortality (RPPM) is a disease of unknown causes. This study tracked the condition of red pines (*Pinus resinosa*) in pockets in Michigan during the period 1991 through 1995. Disease progress rates indicated that the pattern of disease increase or decrease may coincide with drought and non-drought years. Declining trees within pockets are more likely to die or decline further during drought years than non-drought years. Studies of tree rings indicated that, as a result of the 1988 drought, red pines in all areas exhibited reduced growth, regardless of disease status. After 1988, trees in diseased plots continued to decline, while healthy trees exhibited a rebound in growth. Compound stresses, including drought, may eventually lead to expression of crown symptoms in previously healthy-looking trees and to the expansion of the pocket beyond the boundaries of the declining red pine.

Spatial autocorrelation analyses revealed that the pattern of disease progress is not random. Trees within proximity to each other are more likely to show symptoms of the disease than trees spaced further apart. This pattern

suggests movement of an infectious agent from one tree to another. *Leptographium* fungi were found to be associated with mortality pockets in Wisconsin; however, we were not able to verify this association in Michigan (Chapter 2).

Temporal analyses of disease spread revealed that the disease spreads slowly, suggesting that whatever agent(s) are involved, they are not very aggressive and/or virulent. We believe the disease to be one of complex origin involving interactions between biotic and environmental factors. Our further studies of RPPM focus on the abiotic factors associated with this disease.

INTRODUCTION

This study was conducted to assess the condition and disease progress of red pine (*Pinus resinosa*) mortality pockets in Michigan. Red pine pocket mortality (RPPM) is a relatively new disease that was first detected in 1975 (Klepzig and Carlson, 1988; Raffa and Hall, 1988). It has been reported with increasing frequency throughout the Lake States. The disease affects mature plantation-grown red pine in stands that are about 30- to 50-years old. Symptoms of RPPM include the presence of a large, often circular pocket opening within a stand; dead trees and greatly increased growth of understory plants in the center of the pocket; trees towards the edge of the pocket exhibit reduced diameter and height growth; and browning and stunted growth in needles. Trees further from the margin of decline appear

healthy and vigorous in comparison (Klepzig and Carlson, 1988).

In this study, five red pine pocket mortality sites identified by the U.S. Forest Service were selected for observation. The plots are all located in the Huron-Manistee National Forest in Newago County, Michigan. The stands were monitored over a five-year period (1991-1995) in order to determine the overall condition of the stands (i.e., site factors and general health of the trees) and rate of disease spread.

The causes of RPPM are under investigation. Studies in Wisconsin have identified a complex insect vector and pathogenic fungi association that may lead to the decline. In Michigan, several species of insects and fungi are associated with the decline but none are consistently present in all pockets (Dr. John Hart, unpublished). In Wisconsin, significant differences in the abundance of five insect species were found in declining stands compared to healthy *P. resinosa* stands. These root- and lower stem-infesting insects consistently carried two fungal species--*Leptographium terebrantis* and *L. procerum*. These fungal species are thought to be transmitted to the red pine root system through five insect vectors resulting in expression of above-ground symptoms. They may also spread via root grafts or contacts and/or through soil. *Leptographium* infection can cause below-ground root mortality which precedes the above-ground symptoms of reduced radial growth,

thin crown structure, and infestation by the pine engraver (*Ips pini*) (Klepzig, Raffa, and Smalley, 1991).

The rate at which RPPM is spreading and the degree of crop loss has not yet been assessed in Michigan or in other Lake States. An aerial survey by the U.S. Forest Service of the Huron-Manistee National Forest found 440 pockets of dying red pine (USDA Forest Service, 1988-1989). However, many of these pockets were most likely caused by bark beetles (*I. pini*) rather than RPPM. Ground surveys would be needed to distinguish between the two pocket types. Since the drought years of 1988-89, the number and size of red pine pockets in Michigan have not increased appreciably.

This study attempts to determine the rate of disease spread and the spatial patterns associated with the disease. This information may be useful in assessing how RPPM is affecting red pine as a marketable resource. Traditionally the red pine resource has been managed for revenue from pulpwood and sawtimber. In the last decade a sustained market for red pine utility poles has increased the value of this resource in the northern Lower Peninsula (Grossman and Potter-Witter, 1990). Under the right management scenario, increased production of red pine utility poles is projected to pay substantial stumpage premiums (Grossman and Potter-Witter, 1991). Presently there is no information on how RPPM is affecting red pine pulpwood, sawtimber, or utility pole markets.

METHODS

Study Locations

Five plots of RPPM at two different locations in the Huron-Manistee National Forest were identified. These areas have been mapped, and data collected on the diameter and condition of the trees within the area of decline and for an area outside the pocket. These plots are all located within the Huron-Manistee National Forest in Newago County, Michigan. Three disease plots (ML1, ML2, and ML3) are located at an area known as Minnie Lake and two disease plots (TG4 and TG5) are located about 17 km east of Minnie Lake in an area known as Thin-A-Gain (Figures 2-1 and 2-2).

The red pine at the disease and healthy sites examined are growing under similar conditions of soil, climate, and topography. The plots in Newago County are located in plant hardiness zone 5a (average annual minimum temperature -26.2 to -28.8° C). The two healthy plots in Roscommon County are located in plant hardiness zone 4b (average annual minimum temperature -28.9 to -31.6° C) near the boundary of plant hardiness zone 5a (USDA, 1990). The total annual precipitation in Newago County is 85.27 cm, the average annual temperature is 7.6° C; and the growing season is 120-150 days (USDA, 1995). The two check plots located in Roscommon county are about 115 km northeast of Newago county and are further inland from Lake Michigan. Therefore, the average annual temperature is slightly cooler and the growing season slightly shorter. The soils under red pine in both counties

are sandy Spodosols of low pH. These are characterized by a layer of pine needle litter; an O horizon (2.5 to 5 cm) of a black to dark grey humus and roots layer; an E (albic) horizon (2.5 to 10 cm) consisting of a grey leached mineral layer; followed by the spodic B horizon which consists of a yellow-brown sand. In this study sampling depth was 46 cm so no samples were taken below the spodic B horizon. The pH range, in both disease plots and healthy plantations, was 3.8 to 4.7 for the O horizon, 4.2 to 5.1 for the E horizon, and 4.8 to 5.4 for the B horizon.

Data Collection

During 1991, the first year of study, the disease plots were mapped, trees numbered, and the diameter and condition of each tree within the plot recorded. The condition of each tree was tracked over the 5-year period. Information recorded on each tree included its condition (i.e., its apparent health and/or any visual symptoms of RPPM), its location inside the plot (row number, and distance within row), crown class (dominant, codominant, intermediate or suppressed) and dbh (diameter-at-breast height). Observation of RPPM symptoms and tree decline allowed development of a numerical rating system that facilitated tracking of disease progress through time. The rating system used to evaluate tree condition is listed in Table 3-1. Other observations made in each plot include the flora, soil type and drainage, and the pH of soil horizons.

Information collected from healthy red pine plots included the number of trees within the plot, soil type and drainage, soil horizon pH, dbh, crown class, and number of dead stems. The average dbh, crown class, and number of dead stems were estimated from a 10% selected sample of the plot versus the complete inventory done in the disease plots. The sample was selected by first counting the number of trees in the plot, then randomly choosing a section of the plot and recording the information row-by-row until 10% of the total sample had been observed.

Table 3-1. Numerical rating scale for evaluating disease progress of red pine pocket mortality.

Code	Descriptions
0	No apparent symptoms in foliage.
1	Browning or stunted growth in needles, thinning crowns, and/or poor terminal growth (30% of crown or less).
2	Browning or stunted growth in needles, thinning crown, and/or poor terminal growth (30% to 60% of crown).
3	Browning or stunted growth in needles, thinning crown, and/or poor terminal growth (greater than 60% of crown).
4	Dead standing tree.

Tree Ring Analysis

Twenty-three stem cross-sectional areas were taken at 30 cm above the ground from declining and healthy trees at Minnie Lake and Thin-A-Gain in late July, 1993. The narrowest and widest radii from the top side of each disc was determined and the average radius calculated. Annual ring widths were measured along the average radius back to the pith. The average diameter growth for declining red pine was determined and compared to the average diameter growth for healthy red pine at both locations (Minnie Lake and Thin-A-Gain).

Evaluation of Disease Progress

To evaluate disease progress, plots were made of annual disease intensity. Intensity is defined as the quantity of disease present (Campbell and Madden, 1990). In this study, intensity was calculated by averaging the rating scale (Table 3-1) for each disease plot. Disease assessment was made once a year in mid to late June. This time interval was frequent enough to observe and record changes in tree health and survival. Intensity, or the average of the rating scale, was calculated with regard to cumulative mortality i.e., all dead (Rating 4) trees observed in previous years were maintained in the data set and used to calculate the average.

Also plotted, for each disease plot, was the change in disease intensity over time ($\Delta Y/\Delta T$). The $\Delta Y/\Delta T$ represents

an absolute growth rate of disease (Campbell and Madden, 1991).

The location of each tree was determined by measuring the distance between and within rows. This allowed display of the spatial relationships between trees in different rating classes and the spread of the disease over time. The maps were done for the Minnie Lake sites only because of the limited data available at Thin-A-Gain after thinning at this site in 1993.

Statistical Analyses

Durbin-Watson Test Statistic

The Durbin-Watson test statistic (*d*) was used to determine whether the pattern of disease progress within each of the plots is a factor dependent upon time. The test was applied to the data on disease intensity. The Durbin-Watson statistic measures whether residuals versus time are similar, (i.e., positively serially correlated) or dissimilar, (i.e, negatively serially correlated) (Durbin and Watson, 1951). The formula is:

$$d = \frac{\Sigma^{n-1} (\epsilon_{t+1} - \epsilon_t)^2}{\Sigma \epsilon^2_t}$$

where ϵ_t denotes the residual at time *t*, and *n* the total number of time points. Residuals (ϵ_i) were calculated by subtracting the mean disease rating averaged over the five-year period from the disease rating for each year.

When there is no serial correlation, the expected value of the Durbin-Watson statistic *d* is approximately 2.0. In general, values of *d* less than approximately 1.5 are considered a positive serial correlation and values of *d* greater than approximately 2.5 are considered a negative serial correlation (Ott, 1988).

Spatial Autocorrelation

Spatial autocorrelation analyses were used to detect spatial patterns of disease progress. Moran's I was the spatial autocorrelation method applied to maps showing the spatial distribution of trees in different rating classes as measured in 1991. The first step in calculating Moran's I was to determine distance classes (*k*) for establishing linear distance criteria for the distance (*d*) between trees. Distance classes chosen were a) <5 m b) 5 to 10 m; and c) >10 m apart. Trees were spaced in rows less than five meters apart so it was expected that this category would reflect the similarities between trees most accurately.

The linear distance (*d*) between all X_i and Y_i coordinates on the map was calculated by taking the square root of $(X_i-X_j)^2 + (Y_i-Y_j)^2$. The Moran's I formula is:

$$I_k = \frac{n\Sigma w^{(k)}_{ij}(x_i-\bar{x})(x_j-\bar{x})}{\Sigma w^{(k)}_{ij}\Sigma(x_i-\bar{x})^2}$$

where *n* is the number of locations sampled, $\bar{x}$ is the average value of x_j, *k* is the distance class, and $w^{(k)}_{ij}$ is equal to 1

if *i* and *j* are both within distance class *k* and equal to 0 if *i* and *j* are not (Slatkin and Arter, 1991; Cliff and Ord, 1981). MatLab^{tm} (1992) was used to calculate distances and Moran's I values.

To test the null hypothesis that the pattern has been generated in a random manner, it was necessary to compare observed to expected values. The expected value for I was calculated with the equation $E(I)=-(n-1)^{-1}$ (Cliff, et al., 1975). Variances of I_k were also calculated according to procedures detailed by Cliff, et al., 1975.

RESULTS

Site Conditions

Within disease plots, the vegetation present in pocket openings indicated similar site conditions (Table 3-2). Differences in the composition of flora may be partially explained by soil drainage factors. With the possible exceptions of ML3 and TG4, all of the disease plots had well-drained, dry soils with no evidence of a saturated water table. In ML3, which is located near a marshland, there was some evidence of a high water table. The soil appeared to be saturated at a depth of 1.37 m, yet the yellow color of the sand indicated that the soil was not permanently saturated. Water hemlock, *Cicuta maculata*, was found growing only at ML3, supporting the observation that this site is wetter than most other disease plots (Table 3-2). The soil profiles examined at TG4 indicated that it was a wetter site

Table 3-2. Flora present in the pocket openings of red pine mortality plots.

Common Name	**Species**	**Plots found in**
red maple	*Acer rubrum*	ML2, ML3, TG5
brown knapweed	*Centaurea jacea*	ML3
water hemlock	*Cicuta maculata*	ML3
bull thistle	*Cirsium vulgare*	TG4, TG5
sweet fern	*Comptonia peregrina*	ML1
beech	*Fagus grandifolia*	ML1
common St. John's wort	*Hypericum perforatum*	ML1, ML3, TG4
red pine	*Pinus resinosa*	ML1*, ML2†, ML3†, TG4‡, TG5†
grasses	*Poa,* spp.	ML1, ML2, ML3, TG4, TG5
pin cherry	*Prunus pensylvanica*	ML2, ML3
bracken fern	*Pteridium aquilinum*	ML1, ML2, TG4,
white oak	*Quercus alba*	ML1, ML3, TG5
swamp white oak	*Quercus bicolor*	ML2
red oak	*Quercus rubra*	ML2, ML3, TG5
black oak	*Quercus velutina*	ML1, TG5
currant	*Ribes, spp.*	TG5
raspberry	*Rubus,* spp.	ML1, ML2, ML3, TG4, TG5
mullein	*Verbascum thapsus*	ML3, TG5

*Abundant red pine regeneration
†Moderate red pine regeneration
‡Little red pine regeneration

than ML3. A water table close to the surface at 46 cm was identified. The grey sand and thick organic layer indicated that drainage may be a factor in the decline at this site (B. Ellis, personal conversation). Within this same plot on higher ground, however, soils were better drained. The poorly-drained area of TG4 was located in the center of the mortality pocket. Red pine was not regenerating as well within the TG4 pocket as it was in other disease plots (Table 3-2).

Oak was frequently found regenerating in all of the pockets with the exception of the wet site, TG4. From the annual growth (number of nodes on the main stem) of regenerating oak and red pine, it was estimated that the pockets opened up in the early 1980's. Much of the regenerating red pine at Minnie Lake plots ML1 and ML2 when first observed in 1991 were infected with *Diplodia* tip blight (*Sphaeropsis sapinea*). In more recent observations in 1994 and 1995, however, the young red pine had apparently grown out of this disease and appeared quite healthy and vigorous. It is not known why conditions at these sites seemed to favor the growth of young red pine while at the same time mature red pine surrounding the pockets were in a state of decline.

Tree Health and Annual Growth

The red pine stands examined were all even-aged, densely-planted, mature plantation red pine that ranged in age from 40- to 60-years old. The initial planting density

appeared to have ranged from 1.5 x 1.5 to 1.8 x 2.4 m. Row-thinnings in each plot (with the exception of R1) have since reduced stand density. Because of the variation in the size of red pine mortality pockets, the plots vary in size. Most of the disease plots were close to 46 x 46 m and all of the check plots were made this size. The exception is ML1, which contains the largest pocket and is 61 x 81 m.

The stand density and basal area information in Table 3-3 has been placed on a per hectare basis for comparison. The percent of trees in each crown class category is also listed. Dominant and codominant trees were those with crowns in the upper forest canopy and receiving full light from above. Codominant trees had moderately sized crowns compared to trees in the dominant size class and also tended to have a slightly smaller diameter-at-breast height (dbh). Intermediate trees had crowns in the middle to upper canopy and were shorter and had smaller crowns than in the dominant and codominant categories. Suppressed trees were in the lower canopy and were receiving little or no direct light from above. The dbh's were the smallest for suppressed trees.

In August of 1993, plots TG4 and TG5 were thinned in a timber sale in which over 50% of the trees in each plot were removed. The information given in Table 3-3 for TG4 and TG5 lists the stand density and basal area information prior to this thinning.

Table 3-3. Average diameter-at-breast height (dbh), basal area, and number and percent in each crown class (dominant, codominant, intermediate or suppressed) of red pines from disease plots and healthy plots.

Plot ID	Stand density stems/ ha	Avg. dbh (cm)	Basal Area m^2/ha	Crown Position Category (%)			
Disease Plots				**DOM**	**CO**	**INT**	**SUP**
ML1	714	21.3	26.4	56.0	30.1	9.4	4.5
ML2	442	24.6	22.0	78.2	17.6	2.5	1.7
ML3	590	23.1	25.5	73.5	22.0	1.5	3.0
TG4	1169	18.5	34.4	62.7	13.4	11.9	11.9
TG5	1290	17.5	34.4	48.6	19.0	21.0	11.4
Check Plots*							
MLC	736	22.9	31.0	68.2	27.3	4.5	0.0
TGC	1166	22.9	50.0	66.8	26.6	3.3	3.3
R1	2602	13.2	42.5	14.9	22.2	27.8	35.1
R2	1149	25.4	61.8	54.2	33.3	12.5	0.0

*Estimates of basal area, avg. dbh, and proportions of each crown position category come from a 10% subsample of the plot versus complete inventory done in disease plots.

From the stand density, average dbh, and basal area information in Table 3-3, it is apparent that stand structure was the most similar for plots located at the same site. Roscommon plots were an exception. The R1 plot had a much lower dbh and much greater stand density than R2. This was an apparently unthinned plot in which trees in the intermediate and suppressed crown position categories dominated. By contrast R2 was characterized by a number of vigorously growing, large-diameter trees.

Using 1991 as a base-line, the yearly mortality rate was determined by dividing the number of dead trees during the year of observation by the number of trees alive the previous year. The total or cumulative mortality rate for each plot was determined by summing the mortality rates for years 1992 - 1995. ML3 had the highest mortality rate (.190) followed closely by ML1 (.188) from the 1992 though 1995 study period.

Mortality at the Thin-A-Gain plots was substantially lower than Minnie Lake in 1992-1993. The thinning operation at Thin-A-Gain plots in late summer 1993, drastically changed their stand structure. The Thin-A-Gain mortality rates for 1994-1995 are based on the number of trees still standing in the plot; TG4 was reduced from 212 trees to 67 trees and TG5 was reduced from 243 trees to 105 trees. It was noted that some trees at Thin-A-Gain were damaged by the logging activities; yet mortality rates are low in 1994-95 compared to Minnie Lake plots. In terms of yearly

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mortality, 1992 was the most severe; a total of 38 trees died in all five plots (Table 3-4).

Table 3-4. Mortality in red pine pockets, 1991-1995.

Plot ID	Trees in Plot	No. Of Dead Standing Trees	No. of Dead Trees & Yearly Mortality Rate				Total
	(n)	1991	1992	1993	1994	1995	92-95
ML1	352	62	15 .052	20 .073	10 .039	6 .024	51 .188
ML2	119	12	8 .075	0 .000	4 .040	0 .000	12 .115
ML3	132	27	12 .114	1 .011	5 .054	1 .011	19 .190
TG4*	212 (67)	34	2 .011	1 .006	0 .000	3 .045	3 .062
TG5*	242 (105)	48	1 .005	0 .000	0 .000	0 .000	1 .005

*Mortality rates for 1994 and 1995 were based on 67 (TG4) and 105 (TG5) trees remaining in plot after thinning operation.

The only mortality found in check plots, based on a 10% subsample, was at TGC and R1. These were all suppressed trees that were dying due to the effects of natural thinning within the stand. They were found scattered throughout the plot and did not comprise a significant proportion of the stand. R1 had the largest number of dead and suppressed trees because of the high stand density at this site. It was estimated that 7.5% percent of the red pine at R1, and 3.3% at TGC, were dead and suppressed. Trees in the intermediate, codominant, and dominant categories appeared

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healthy in all of the check plots. Mortality data for check plots are not shown in Table 3-4 because they were only evaluated once. Thus, it is not possible to compare mortality increases in check plots to disease plots. Check plots were visited during each year of the study, however, and no significant disease or insect problems were found.

Trees were felled at Minnie Lake (11 trees) and Thin-A-Gain (12 trees) in order to ascertain the growth patterns of declining and healthy trees at these sites. The tree ring analysis revealed that the red pine at Minnie Lake are about 10 years older than at Thin-A-Gain. Rings were counted as far back in some of the discs to a year of origin of 1938 for Minnie Lake and 1949 for Thin-A-Gain. Compartment records from the USDA Forest Service reveal the year of origin to be 1937 for Minnie Lake, and 1947 for Thin-A-Gain. Some tree rings were missing in the analysis and may have been lost below the height at which the discs were taken. It was also difficult to distinguish some highly compressed rings, especially in declining trees.

At Thin-A-Gain and Minnie Lake the average annual ring width was determined for trees within disease plots and compared to nearby healthy trees growing outside of the plot. Some of the trees selected from within the disease plots appeared healthy, while others were clearly declining. Tree ring analysis suggests that non-disease plot red pine at Minnie Lake (MLC) grew more vigorously than the disease-plot trees as far back as 30 years ago (Figure 3-1). At

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Thin-A-Gain, however, red pine in disease plot TG5 grew more vigorously than the non-disease trees examined from 1963 until 1979. After 1979, growth for non-disease trees exceeded the growth of disease-plot trees at Thin-A-Gain (Figure 3-2).

At Minnie Lake and Thin-A-Gain, growth for both the non-disease and the disease-plot red pines declined in 1988, a severe drought year. Annual growth for non-disease red pines, however, increased after the 1988 drought period but continued to decline for the red pines located within disease plots (Figures 3-1 and 3-2). A notable growth spike is apparent at Thin-A-Gain during the early 1980's (Figure 3-2). There is evidence from the cutover stumps in these stands that a thinning occurred around this time, releasing the trees from suppression.

Disease Progress

Figure 3-3 shows disease progress annually in terms of intensity and cumulative mortality for all five disease plots. The plot shows that pockets are expanding each year as more dead and declining trees appear in the plot. One possible exception is TG5 which remains nearly unchanged during 1991-1993.

To determine the rate of disease increases or decreases each year it is necessary to plot the change in disease intensity between years. Figure 3-4 shows that the disease rate may increase, decrease, or remain constant with time.

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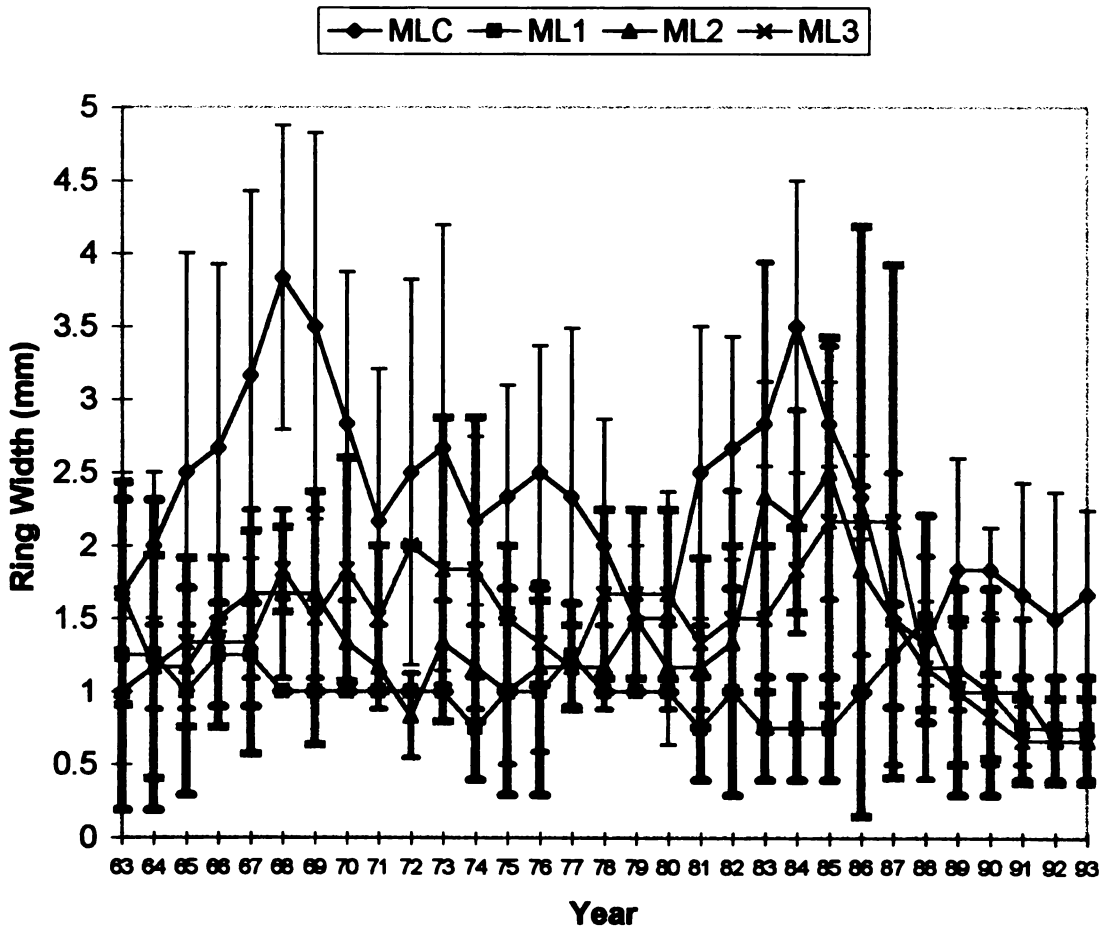
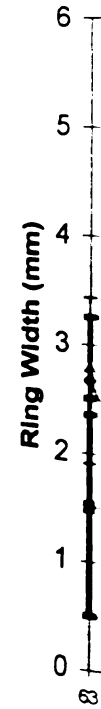


Figure 3-1. Average annual growth for disease-plot (ML1, n=2; ML2, n=3; and ML3, n=3) and non-disease trees (MLC, n=3) at Minnie Lake.



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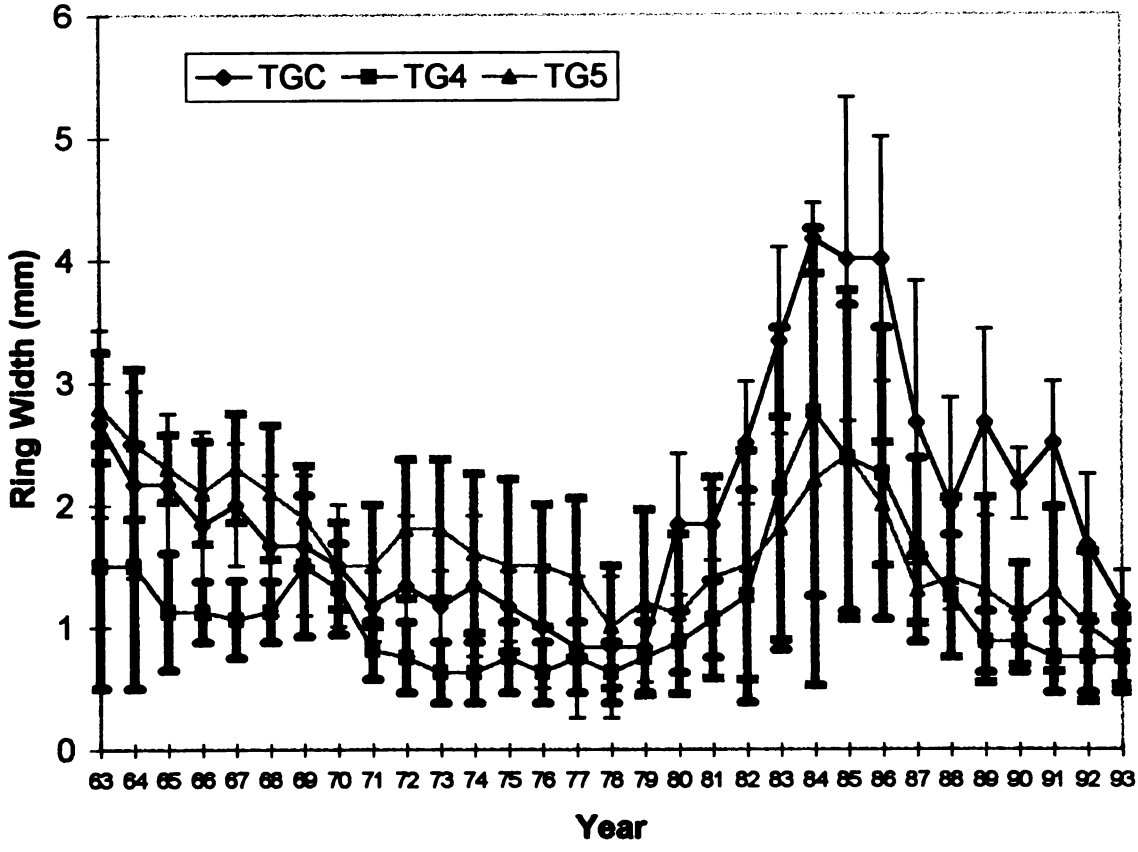

Figure 3-2. Average annual growth for disease-plot (TG4, n=4; TG5, n=5) and non-disease trees (TGC; n=3) at Thin-A-Gain.

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Thus, although pockets appear to be expanding in Figure 3-3, this does not mean that the disease rate is increasing each year. For example, little change in disease rate occurred between 1991 and 1992 for TG5, TG4, or ML1 and the disease rate actually decreased, i.e., some trees improved in condition, for most plots between 1992 and 1993 (Figure 3-4). Even though mortality was the highest in 1992 and the lowest in 1995 (Table 3-4), the rate of disease change was the highest between 1994 and 1995. This can be explained by the fact that several trees declined further i.e., entered higher rating classes in 1995.

Disease intensity was the highest at ML1 from 1991-1994 (Figure 3-3). This was the largest mortality pocket observed. ML3 exceeded the disease intensity of ML1 in 1995 (Figure 3-3) and also had the highest rate of change between 1994-1995 (Figure 3-4). ML2 had the lowest intensity ratings in 1991-1993 (Figure 3-3) but also had a higher rate of change than ML1 between 1994-1995 (Figure 3-4).

The Durbin-Watson statistic (d) was used to determine serial correlation for disease intensity in Figure 3-3. For all plots d was < 1.5 indicating positive serial correlations (ML1, d=0.84; ML2, d=0.58; ML3, d=0.76; TG4, d=1.01; TG5, d=1.00). Positive serial correlations suggest that there are specific patterns to disease progress which are time-dependent factors. However, with only three years of data available for Thin-A-Gain plots the d statistic is less reliable than for the Minnie Lake plots. More years of

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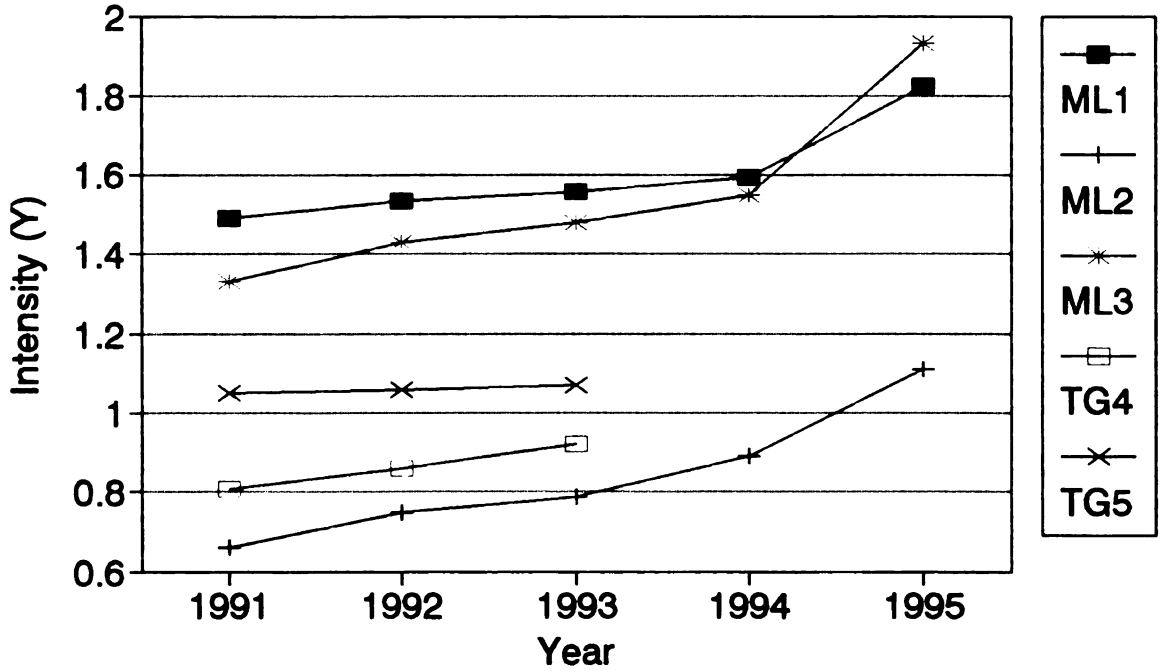

Figure 3-3. Disease intensity in terms of the average of the rating scale.

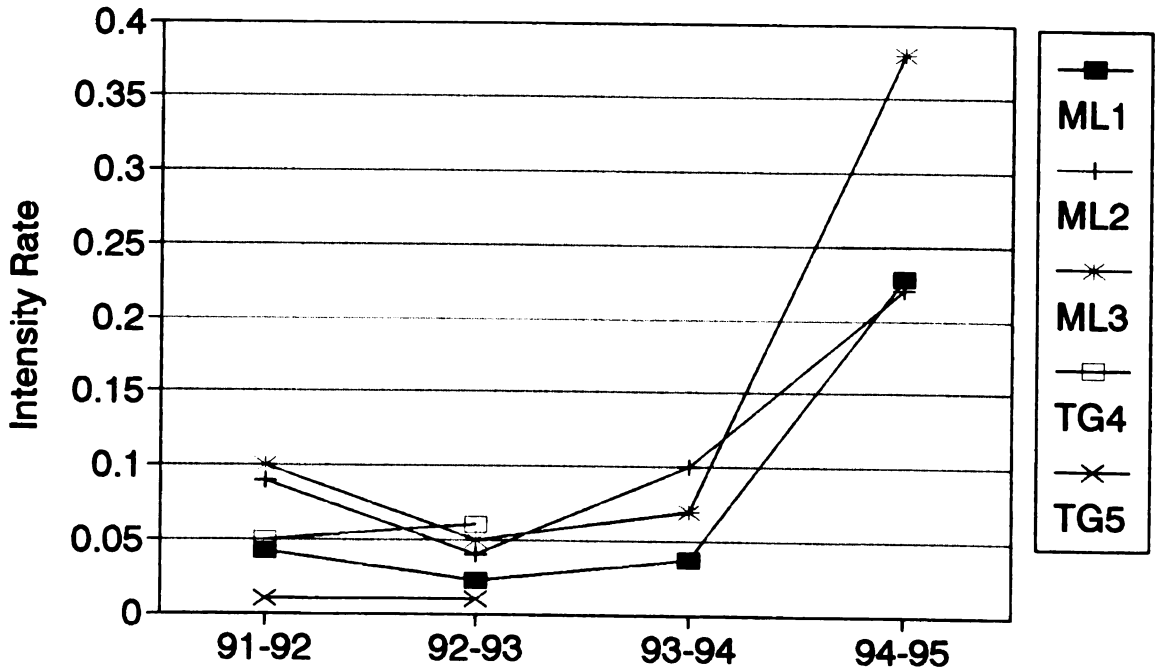

Figure 3-4. The rate of change in disease intensity ($\Delta Y/\Delta T$).

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study would be needed for both Minnie Lake and Thin-A-Gain plots to increase the reliability of the *d* statistic (Bowerman and O'Connell, 1987). However, the data currently available for these plots does indicate a trend toward positive serial correlation.

Spatial Analyses of Disease Progress

Maps were generated of disease ratings for each year of the study. Figures 3-5, 3-6, and 3-7 are maps of disease ratings from Table 3-1 for the Minnie Lake plots between 1991 and 1995. The increase in the size of pockets is not as obvious in maps between subsequent years and Thin-A-Gain maps show no obvious difference in disease progress. Minnie Lake maps, however, do reveal changes especially over the five-year period.

Figure 3-8 shows the results of spatial autocorrelation analyses. Of the three distance classes, the < 5 m category had the highest Moran's I value, i.e. the most strongly correlated. As distance increases, from 5 to 10 m and greater than 10 m, spatial autocorrelation values decrease. This suggests that trees nearer to each other are more closely related in terms of disease rating and that the patterns are not random. This result was evident in all plots examined.

The variances in Table 3-5 correspond to each correlation coefficient for each plot and distance category in Figure 3-8. These variances indicate that, for the most

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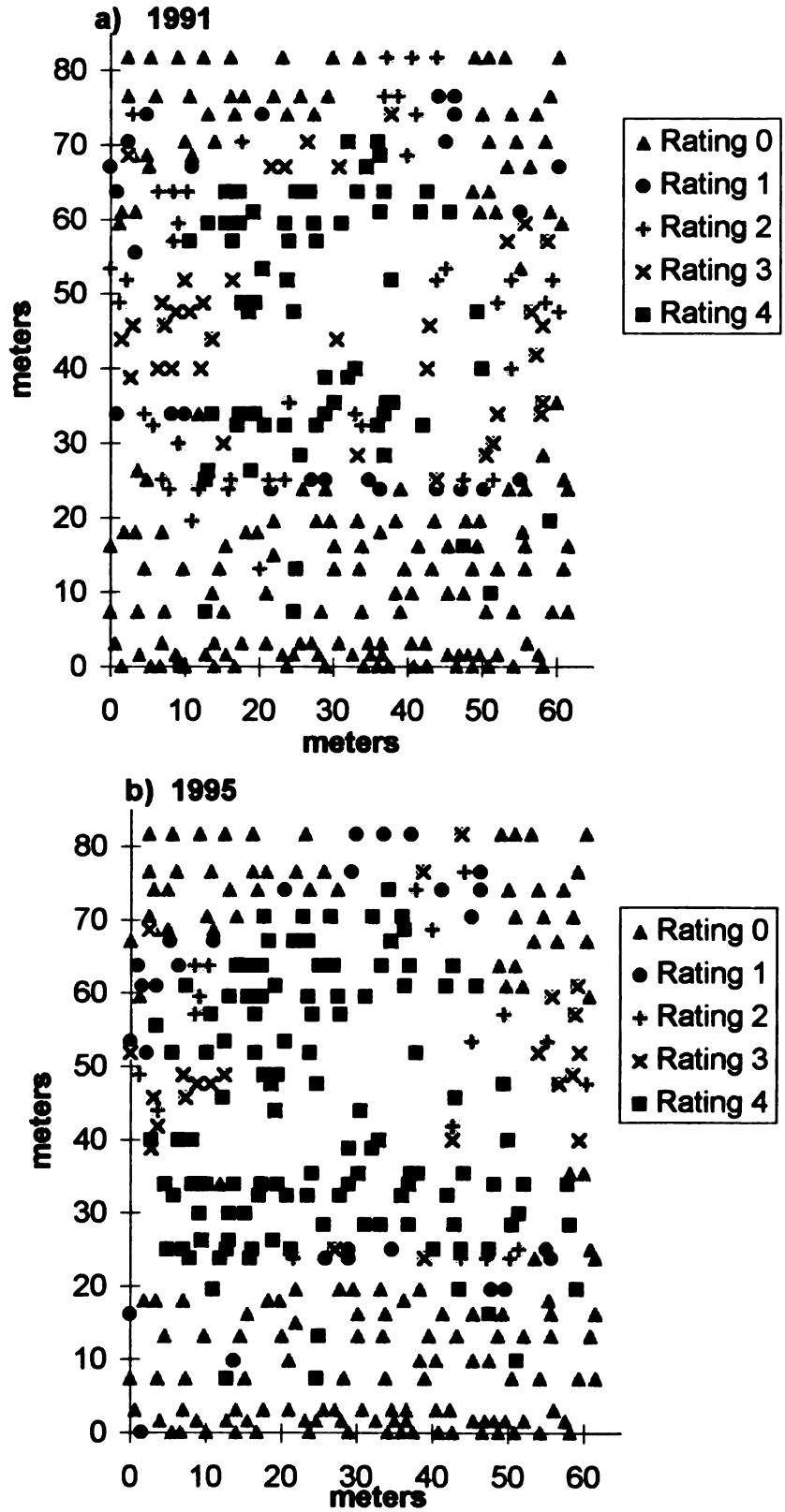

Figure 3-5. Disease ratings at ML1 in a) 1991 versus b) 1995.

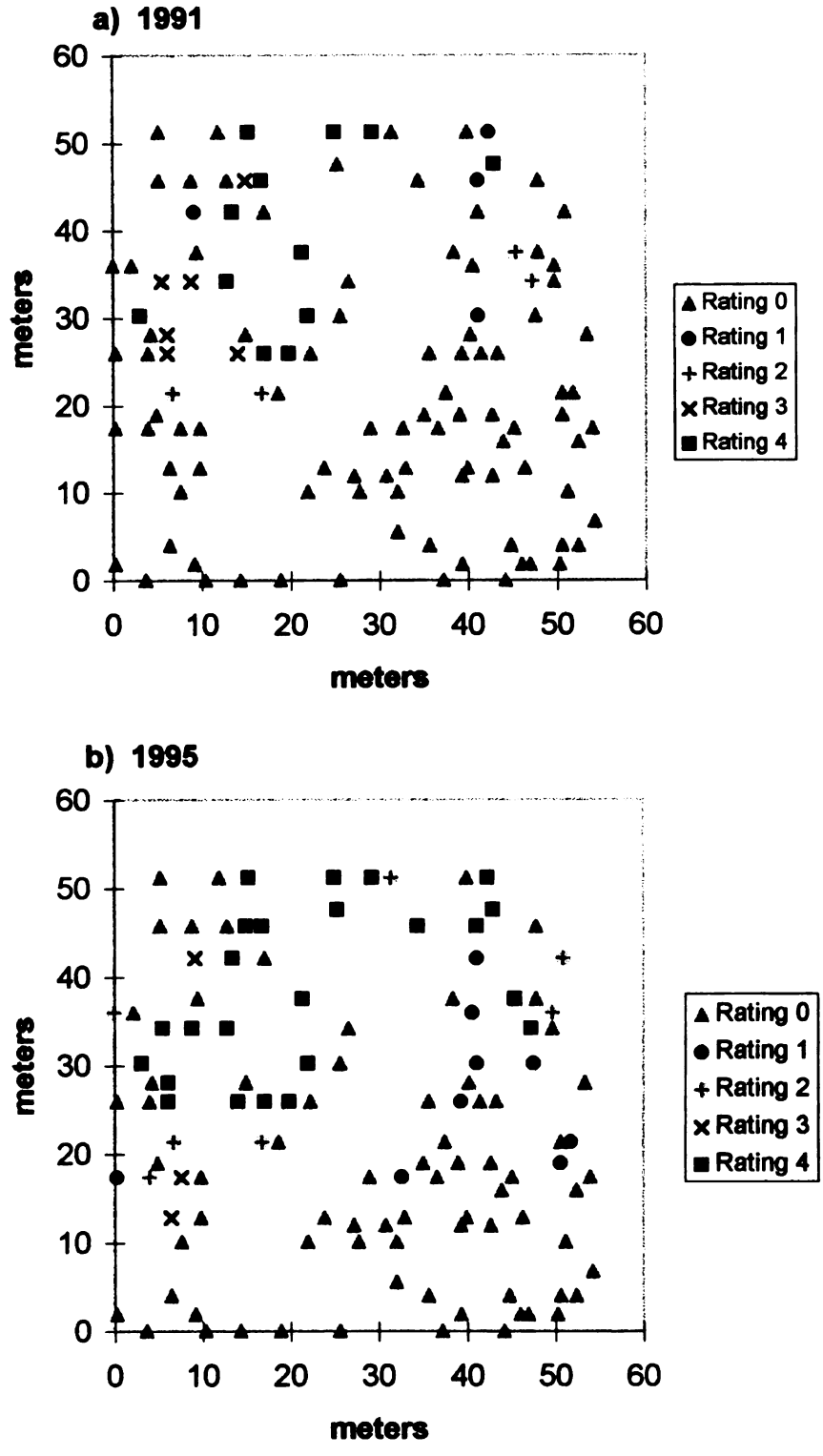

Figure 3-6. Disease ratings at ML2 in a) 1991 versus b) 1995.

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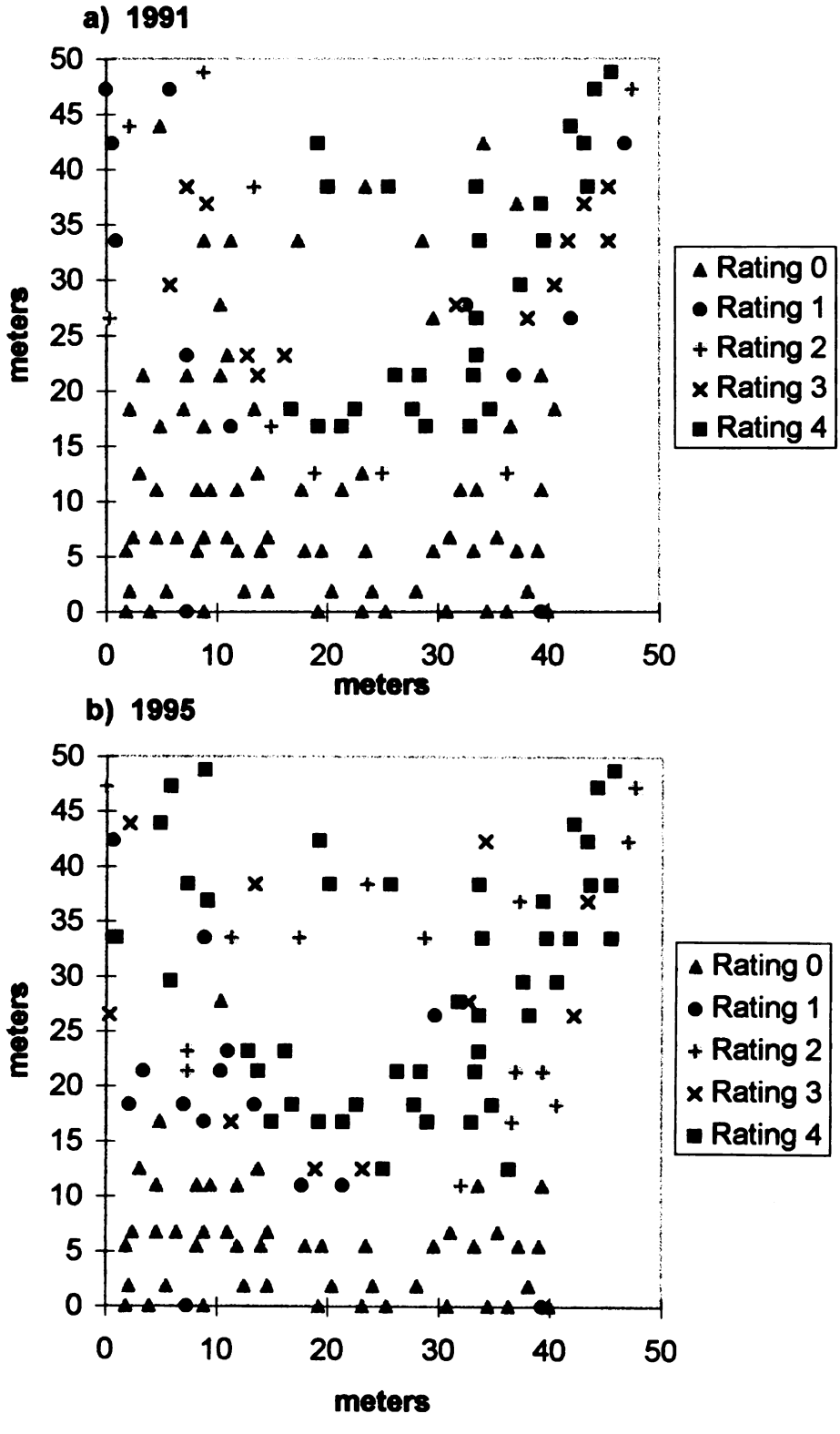

Figure 3-7. Disease ratings at ML3 in a) 1991 versus b) 1995.

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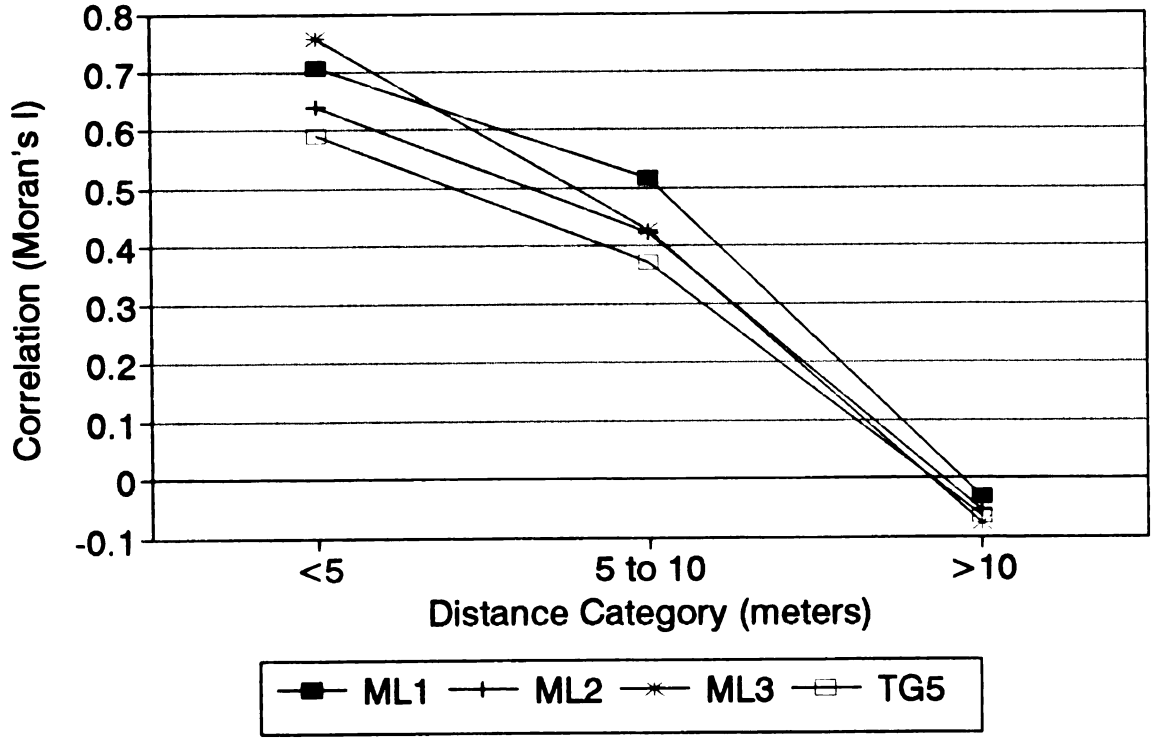

Figure 3-8. Moran's I values for three distance categories for disease plots (ML1, ML2, ML3, and TG5) in 1991.

Table 3-5. Expected I values and variances for three distance categories at disease plots in 1991.

	ML1 n=352 trees Expected I= -0.0028	**ML2** n=119 trees Expected I= -0.0085	**ML3** n=132 trees Expected I= -0.0076	**TG5** n=243 trees Expected I= -0.0041
Distance category (k)	**Variance S^2**	**Variance S^2**	**Variance S^2**	**Variance S^2**
<5 m	0.00076	0.0032	0.0021	0.00054
5 to 10 m	0.00024	0.0012	0.00077	0.00022
>10 m	0.00029	0.00022	0.00017	0.000053

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part, observed and expected I values do not overlap and the differences are significant. Moran's I values for the < 5 m and 5 to 10 m categories were statistically higher than the expected I values. However, in Figure 3-8, it can be seen that in the > 10 m category Moran's I values become negative and much lower than the Expected I values in Table 3-5. This indicates that the > 10 m category was too far of a distance to detect spatial patterns and was not significant.

DISCUSSION

Tree ring analyses suggest that growth of red pines at both Newago County locations declined as a result of the 1988 drought. This drought had an effect on both disease plot and non-disease plot trees. However, the main difference is that the disease-plot trees did not manage to recover from the drought but continued to decline further.

The largest pocket decline site was ML1. This site had the greatest number of dead standing trees and the highest stand density of Minnie Lake disease plots. Figure 3-3, indicates that ML1 has the highest level of disease intensity between 1991 and 1994, and is exceeded by ML3 in 1995. However, the graph of disease intensity rates (Figure 3-4 indicate that the smaller pockets, ML2 and ML3, are expanding at a faster rate than ML1.

Even though disease levels vary between plots, the trend, or the increase or decrease in disease each year, is very similar for plots located at the same site. This sug-

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gests that some factor is controlling the pattern of disease at a particular site. Thin-A-Gain followed a trend which differs from Minnie Lake. These pockets had apparently stopped expanding to any significant degree during the years studied (1991 to 1993). Soil pH's, on average, were slightly higher at Thin-A-Gain than at Minnie Lake (Figure 5-3). It may be that whatever threshold values of factors such as pH, nutrient contents, insect and pathogen populations, adverse climatic factors, etc. are needed to tip the scale in favor of disease, no longer existed at Thin-A-Gain. TG4 was the only plot in which poor soil drainage may have been a factor that initially lead to the decline. It may be that at TG4 the pocket stopped expanding once the red pine in the poorly-drained area had died.

The Durbin-Watson test revealed that the pattern of disease progress within a plot is a time-dependent factor. However, there are many approaches to time series data and further years of study are needed to characterize the disease adequately. Linear regression is one approach to the study of time series data that may be useful in analyzing the spread of RPPM. Regression techniques could be used to develop an equation for predicting the spread of the disease that could be applied to the entire population of red pine mortality pockets. In order to do this, the equation would have to based on randomly selected mortality pockets and on data collected over several years. This equation may be in the form of a multiple regression equation which would

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include other factors such as weather patterns and pest populations. In our limited study of mortality pockets, linear regression equations could be developed from the time series data but they could only be applied to each individual pocket studied and not generalized to the entire population of mortality pockets.

The Minnie Lake maps of disease ratings show that pockets do expand over time. This expansion is not very apparent in year-to-year comparisons as it is in the five-year comparisons because the disease spreads slowly. The pocket margin is often confined to trees that are already declining and there is little expansion into the healthy tree population. In 1992, most of the increase in disease was explained by increased mortality of trees that were already sick in 1991. It was not until 1995 that the disease appeared to spread significantly into the healthy tree population. The high rate of disease intensity in 1995 is better explained by increasing symptoms of RPPM rather than by increased mortality. The maps show that already sick trees tend to decline further but that healthy-looking trees near pocket edges may not show symptoms for several years.

The fact that there is a significant spatial autocorrelation of disease ratings in red pine mortality pockets indicates that the disease spread is not random. Individuals closer to one another are more likely to be affected by the disease than those spaced further apart.

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This pattern suggests activity by a biotic agent or agents which cause infection from one tree to another. Root grafting is reported to be common in red pine plantations and infection may be spreading via the root system. However, RPPM spreads slowly suggesting that the disease agent(s) are not very aggressive and/or virulent. In our studies or RPPM, we have not been able to identify any single causal agent that has been consistently present in all pockets.

Another possibility is that RPPM results from the interactions of environmental stresses and biotic agents. RPPM in Michigan has only been found in even-aged stands that were originally planted on marginal farmland sites. The soils are all sandy Spodosols of low pH. It may be that soil nutrient factors and acidification over time has stressed the trees, predisposing them to attack by insects and fungi. Significant amounts of fine-root mortality have been observed in mortality pockets versus healthy stands (Chapter 2). Whether this mortality is the result of nutrient factors, biotic agents, or a combination of the two is unknown.

Climate variables, such as precipitation, may offer a partial explanation for the pattern of disease at Minnie Lake. The Palmer Drought Severity Index or PDSI is a measure of drought severity and is reported on a monthly basis (National Climatic Data Center, NCDC). Positive values of PDSI indicate wet conditions and negative values indicate

dry conditions. The PDSI indices for the Newago County region of Michigan reveal that both 1991 and 1993 were non-drought years (i.e. positive monthly values). Disease intensity rates (Figure 3-4) decreased in 1993 because trees were improving in condition and because mortality rates decreased for some plots (Table 3-4).

The PDSI indices also reveal that a mild drought occurred in 1992 from May through August (PDSI between -1.0 and -2.0). An incipient drought occurred in 1994 in which the PDSI indices were between -0.5 to -1.0 from March though June and September though December. The "incipient" drought in 1994 contributed to even drier conditions in 1995 in which the PDSI was in the mild (-1.0 to -2.0) and sometimes reached the moderate (-2.0 to -3.0) drought severity range from January though September (NCDC).

Mortality was highest during the drought year 1992 (Table 3-4). After a decline in disease rates for most plots in 1993, rates increased somewhat in 1994, a slightly dry year. Disease rates increased substantially in 1995, an even drier year (Figure 3-4). Thin-A-Gain stays nearly level from 1991-1993 but it is suggested that the disease is no longer having an adverse effect on these plots.

These observations suggests that during drought years, declining trees are likely to be pushed over the mortality threshold or to show greater symptoms of decline. Seemingly healthy trees near the pocket edges may not show visible symptoms of decline but perhaps the disease syndrome is

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affecting non-visible parts of the trees such as the root system. After years of compound stresses, including drought, symptoms may show up in the crowns of previously healthy-looking trees as was apparent in 1995.

Plotting disease progress by looking only at crown symptoms may be misleading because it is not known how the disease is affecting non-visible parts of the tree. Factors such as fine-root mortality may be a better indicator of how the disease progresses. It will require more data points (more years of study) and a more accurate and invasive observation of symptoms to characterize this disease adequately.

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CHAPTER IV

Analyses of Soil, Needle, and Root Samples from Red Pine (*Pinus resinosa*) Mortality Pockets for Determination of Nutrient Stress

ABSTRACT

Soil, needle, and root samples from red pine mortality pockets within the Huron-Manistee National Forest, Newago County, Michigan were analyzed for nutrient content and compared to those from healthy red pine plantations at Huron-Manistee; and between soil and root samples from healthy plantations at the Houghton Lake State Forest, Roscommon County, Michigan, and natural stands at Osmun Lake, in Cheboygan County, Michigan.

Our initial hypothesis was that aluminum (Al) stress in the acid soils (pH < 5.0) may be a predisposing factor leading to the decline. The parameters of primary interest were the calcium (Ca)/Al ratios of soil, needle, and root samples for evaluating the risk of Al stress to the forest ecosystems. According to the criteria established by Cronan and Grigal (1995), Ca/Al ratios of soil and needle samples were within the threshold values identifying the red pine stands as being at risk for Al stress. However, this was true for both declining and healthy red pine stands.

Statistical analyses indicated that manganese (Mn), followed by magnesium (Mg) concentrations in soil, root and needle samples were the nutrient factors most strongly associated with areas of decline. Manganese was significantly lower in the needles and roots and Mg significantly lower in the soil and roots of diseased versus healthy plantation plots. Increasing gradients in Mg and Mn concentrations were observed from disease edges to the outer "healthy-looking" regions of disease plots to plantation check plots nearby.

Nutrient studies of fine roots ($\leq$ 3 mm in diameter) from the Huron-Manistee forest alone also revealed significant differences and increasing gradients in ratios of Ca/Al, Mg/Al, potassium (K)/Al, and Mn/Iron (Fe). In addition to Mg and Mn, K concentrations were found to be significantly lower and metals Al, Fe, and Zinc (Zn) were significantly higher in fine roots from Huron-Manistee disease plots. Fine roots may be reflecting unfavorable nutrient conditions within disease plots. Pine litter accumulation in mature even-aged red pine plantations and subsequent soil acidification may have increased leaching and loss of soil nutrients. Manganese, in particular, was consistently lower in both needles and roots of declining red pines. The gradients in element concentrations and ratios identified in soil and root tissues from declining to healthy regions of disease plots suggest that the pattern of disease spread is associated with nutrient problems.

INTRODUCTION

Soil, needle, and root samples from red pine (*Pinus resinosa*) mortality pockets were analyzed for nutrient content and compared to the nutrient contents of soil, needle, and root samples from healthy red pine plantations in order to identify potential predisposing stressors contributing to a decline disease known as Red Pine Pocket Mortality (RPPM). RPPM affects mature plantation-grown red pine in the Lake States (Wisconsin, Illinois, and Michigan). Symptoms of the decline include reduced diameter and height growth, thinning crowns, and browning and stunted growth in needles. Red pine mortality pockets are characterized by an open area of dead standing trees ringed by declining trees. Increased growth of understory vegetation can be found in the center of the pocket (Klepzig and Carlson, 1988).

The causes of this disease are unknown. This study examines the role of abiotic factors in the decline including the role that Al may have as a predisposing stressor causing mortality of fine roots. The possibility of deficiencies/toxicities of other elements including some micronutrients in soil and plant tissues is also examined.

Aluminum is being considered because in acid, inorganic soils below a pH of 5.2, monomeric and trivalent exchangeable forms of Al predominate (Ulrich, 1989; Arp and Ouimet, 1986; Ulrich, 1986; Foy, 1974). In these forms, Al may become water soluble and available for plant uptake. Therefore, in acidic soils, Al^{3+} ions taken up by plants may

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have a direct toxic effect to the roots, particularly in plant species that are intolerant of aluminum.

However, pines are adapted to growing on low pH soils and are considered to be Al-tolerant (Schaedle, 1989; Hutchinson et al., 1986). If red pines are affected by high levels of exchangeable Al, then they are more likely affected by the indirect toxic effects rather than the direct toxic effects. The indirect toxic effects relate to the competitive nature of trivalent Al^{3+} ions which displace divalent Ca^{2+} and Mg^{2+} ions and perhaps other nutrients within the root zone. Thus an "aluminum-induced calcium deficiency", for example, is of primary concern when considering toxic effects of aluminum in acid soils (Shortle and Smith, 1988). For these reasons, the parameters of primary interest when studying the possibility of Al-toxicity in a pine plantation are the ratios of Al to other nutrients, especially Ca, rather than the actual concentrations of Al in soil and plant tissues.

Cronan and Grigal (1995), by reviewing the literature of Ca/Al ratios as indicators of stress in forest ecosystems, ascertained four measurement end points to be used as ecological indicators for identifying threshold values beyond which the risk of forest damage from Al stress and nutrient imbalances increases. The four measurement end points identified were: 1) a soil base saturation < 15% of effective CEC (cation exchange capacity); 2) a soil solution molar Ca/Al ratio $\leq$ 1.0 (for a 50% risk of forest stress);

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3) a fine root tissue Ca/Al molar ratio $\leq$ 0.2 (for a 50% risk); and 4) a foliar tissue Ca/Al molar ratio $\leq$ 12.5 (for a 50% risk). These threshold values are recommended as guidelines to identify sites where Al stress is likely to affect tree growth adversely. In this study, soil, needle and root samples collected from red pine mortality pockets and also from healthy non-disease plantations are compared to Cronan and Grigal's four threshold values to determine whether there is a significant risk of forest stress at these sites.

Concentrations of Al, Ca, Fe, K, Mg, and Mn in soil, needle, and root samples were also examined to determine whether deficiencies/toxicities of these elements are associated with the decline. Soil P and needle and root Zn were also examined.

There is sparse literature available on micronutrients and the growth of pines. Manganese deficiency has been shown to limit the growth of *Pinus radiata* in South Africa (Grey, 1988). The deficiency was associated with strongly podzolized soils and good drainage; similar to the Spodosols examined in this study. In North America, young slash pines (*P. elliotii*) growing on coastal plain soils in northeastern Florida were fertilized with micronutrients (Cu, Zn, Mn, Fe, B and Mo). Manganese was the only micronutrient which elicited a significant growth response in the slash pine stands. The Mn stress was described as subacute (i.e. there were no visible symptoms in the foliage) and the stress was

found to be associated primarily with Spodosols (Jokela, et al., 1991).

Reported symptoms of Mn deficiency in the South African Radiata pine stands are similar to the symptoms observed in red pine mortality pockets. The symptoms included stunted growth, yellow-brown or bronze needle color, and very sparse or short needles (de Ronde, et al., 1988; Lange, 1969). Although "pockets" of *P. radiata* decline where not reported in the South African studies, the Mn deficiency symptoms were reported to occur in "patches" at various sites in the southern and western Cape (Grey and de Ronde, 1988).

Manganese is known to interact with iron oxides forming co-precipitates and application of Fe fertilizers may result in Mn deficiencies (Grey, 1988; Knezek and Greinert, 1971). Therefore, to discern whether a competitive relationship might exist between Fe and Mn, the Mn:Fe ratio at healthy and unhealthy red pine sites has also been determined in our study.

METHODS

Study Locations

Five red pine decline sites as identified by the U.S. Forest Service were selected for this study. Research plots were established within the area of decline and for an area outside the pocket. These plots are all located within the Huron-Manistee National Forest in Newago County, Michigan. Three disease plots are located at an area known as Minnie

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Lake (ML) and two other disease plots are located about 17 km east of Minnie Lake in an area known as Thin-A-Gain (TG) (Figures 2-1 and 2-2).

In addition to disease plots there are also two plantation check plots of healthy red pine located near the disease plots: one at Minnie Lake and one at Thin-A-Gain. To allow comparison of plantation-grown red pine to natural stand red pine, three check plots of healthy, non-plantation red pine were established near Osmun Lake at the Pigeon River Country State Forest in Cheboygan County, Michigan. The Osmun Lake stands are about are about 225 km northeast of the disease plots.

For comparison with plantation-grown red pine located in a forest other than Huron-Manistee, two additional check plots of healthy red pine were established at the Houghton Lake State Forest in Roscommon County, Michigan. The Roscommon plots are about 115 km northeast of the disease plots. In sum there were twelve plots used in this study: 5 disease plots; 2 plantation check plots located in the same forest; 2 plantation check plots located in another forest; and 3 check plots in natural red pines stands. Similarities between plantation check plots were that they consisted of mature, even-aged red pine that had been initially densely planted. The natural stand check plots, however, were widely spaced and uneven-aged. All stands selected were growing on sandy Spodosol soils of low pH.

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Soil Sampling

Soils were sampled in check plots (MLC and TGC) and at the Osmun Lake natural stands (O1, O2, and O3) to a depth of 46 cm. Each plot was sampled randomly, 30 times throughout, and soil samples were separated by horizon (i.e. soil color). The 30-composite soil samples consisted of three different horizons. Thus, 15 soil samples were collected at Huron-Manistee check plots and at the Osmun Lake natural stands (5 plots X 3 horizons).

Huron-Manistee disease plots (ML1, ML2, ML3, TG4 and TG5) were sampled by the same method except that 15-composite soil samples were taken at three different locations within the plot: at the center, edge, and outside regions of pockets. Thus, 45 additional soil samples were collected at Huron-Manistee disease plots (5 plots x 3 horizons X 3 locations within the plot). The following year, 1994, three 15-composite soil samples were taken randomly at the Roscommon plots for a total of 18 samples (2 plots X 3 horizons x 3 samples). All soil samples collected were dried at 35°C for 48 hours prior to analyses.

Soil Analyses

Soil pH, calcium, magnesium, phosphorus, and potassium content were determined according to the procedures utilized by Michigan State University's Soil and Plant Nutrient Laboratory. Potassium (K), Ca, and Mg were extracted with ammonium acetate (NH_4OAc) and pH was determined in distilled

water. Phosphorus (P) determination was done using Bray-Kurtz-P1. Levels of exchangeable aluminum were determined in a separate laboratory facility using 1N KCL as the extractant; five grams of soil were added to 50 ml of 1N KCl, shaken for 30 minutes, and filtered using Whatman no. 5 filter paper. Aluminum levels were determined using plasma emission spectroscopy. In 1995, soils were also analyzed for manganese (Mn) and iron (Fe) content using 0.1 N HCl as the extractant. Complete results for all elements are listed in the Appendix, Table 1.

Needle Sampling

In late July and early August of 1993, symptomatic and asymptomatic trees were felled in Huron-Manistee disease plots and four branch ends were collected from the top, middle, and bottom sections of the crown for a total of 12 samples per tree. Some symptomatic tree crowns, because of their small size, were divided into only two sections (top and bottom) and eight branch ends were collected. In total 23 trees were felled; 12 trees at the Thin-A-Gain site and 11 trees at the Minnie Lake site. One-year-old needles were separated from two-year-old needles in the laboratory and two samples belonging to the same age class from each crown section were combined. Thus, in most cases, 12 samples came from each tree felled (3 crown sections X 2 combined samples per section X 2 age classes). The needle samples were dried

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at 42°C for 48 hours then ground. In total 229 needle samples were analyzed for elemental concentration.

Needle Analyses

Analysis of needle samples was accomplished by using the perchloric acid digest method (MSU Forestry Dept. Procedures, unpublished). Each 150 ml digestion tube contained 0.5 g of dried, ground, plant tissue. Samples were partially digested by placing them in 5.0 ml of nitric acid (HNO_3) overnight before starting the acid digestion block. One-hour into the digestion process 2 ml of perchloric acid ($HClO_4$) was added to each tube. Forty tubes were digested at one time. For quality control, random sample replications, blanks, and NBS standards were included in the digests. Element concentrations of Zn, Cd, Mn, B, Fe, Pb, Al, Mg, Cu, Ca, K, Ni, and Cr were determined using plasma emission spectroscopy (compete results in Appendix, Table 2). Elemental standards used for calculating regression equations were 0, .20, .40, .60, .80 and 1.0 of the total standard.

Root Sampling

Roots were collected in 1992 by digging 1-m^2 pits in disease plots at three locations within the plot: in the center of the pocket where there were many dead standing trees; at the edge of the pocket where trees were starting to decline; and outside the edge of the decline where trees appeared healthy. The 1-m^2 pits were divided into four 0.5

x 0.5 m sections and samples were taken from two levels: 0 to 30 cm deep, and 30 to 60 cm deep. Thus eight bags of root samples were collected from each 1-m^2 pit (4 sections x 2 levels).

In total fifteen pits were dug, three at each of the five disease plots. In addition, two 1-m^2 pits were excavated at each of the two check plots at Huron-Manistee and one pit from each of the three natural stand plots at Osmun Lake. Three of the disease plots were partially resampled in 1993 by digging 0.5 m x 0.5 m x 60 cm deep square pits (four 0.5-m^2 square pits at ML3, three pits at TG4; and two pits at TG5); only outside and edge samples were collected at this time.

Samples were taken from the two check plots at Roscommon County in 1994 using 0.5-m^2 pits. Samples were removed from two levels (two bags of roots per pit); four 1-m^2 square pits were dug at each of the two check plots. Because there were no features of center, edge, or outside area of a pocket within check plots, the pits were dug randomly at locations throughout the plots.

The roots collected at both check plots and disease plots were extracted by sifting the soil through a mesh screen and separating the red pine roots from the roots of other plants growing in the area. Once collected the roots were brought back to the laboratory, washed by rinsing with water for 30 minutes, and sterilized by dipping in a 10% Chlorox solution for 2 minutes. The lengths and widths of

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each root were recorded and they were also classified into live, dead, or symptomatic categories. Roots were called symptomatic if they were partially dead or dying and/or if there was any fungal staining below the epidermis. Live and symptomatic roots were cultured in attempting to identify the pathogenic fungi associated with the disease (Chapter 2). The roots were kept frozen until thawed for drying and grinding. Roots were re-washed by rinsing for 30-minutes after removing from the freezer. Roots were separated by diameter classes (1 to 3 mm and 3 to 5 mm) and oven-dried for 48 hours at 42°C. Only live and symptomatic roots were included in the analysis; dead roots were discarded. Thus, no samples from the center areas of disease plots are included in the analyses, since they consisted mainly of dead roots.

Root Analyses

Analyses of root elemental content was accomplished by the same perchloric-acid digest method used for needle analyses. The only change was in the standard used to establish the regression equation. Root samples contained higher concentrations of Al and Fe, and lower concentrations of Ca and K than needle samples and the standard was adjusted accordingly. Elemental concentrations of Zn, Cd, Mn, B, Fe, Al, Mg, Cu, Ca, K, Ni, and Cr are listed in the Appendix, Table 3.

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Statistical Analyses

Analysis of variance (ANOVA) was used to test hypotheses that soil, needle, and root element concentrations and ratios from disease and healthy locations are significantly different from each other. Other soil factors tested were pH, CEC (i.e. cation exchange capacity or the sum total of exchangeable cations that a soil can adsorb), and percent base saturation. For soil, the model used to test hypotheses that element concentrations and ratios differ by locations and soil horizons (Table 4-1) is as follows:

$$Y_{ijk}=\mu+\alpha_i+\beta_j+\alpha\beta_{ij}+\epsilon_{ijk}$$

where variable Y is the element concentration/ratio or soil factor (pH, CEC, and % base saturation) per sample (k) associated with the ith level of factor α (location) and j^{th} level of factor β (soil horizon), μ=overall mean of all observations, α_i is the effect of ith level of the location factor, β_j is the effect of the jth level of the horizon factor, $\alpha\beta_{ij}$ is the effect of the interaction between the location and soil horizon factors and $\epsilon_{(ij)k}$ is the experimental error associated with the kth experimental unit (soil sample) for the levels of the location and horizon factors.

There were six levels included in the location factor: the center of Huron-Manistee disease plots, the edge of disease plots, outside the edge of the decline within disease plots; healthy plantation plots at Huron-Manistee;

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healthy plots at Roscommon County, and healthy non-plantation plots at Osmun Lake. Three levels are included in the soil horizon factor: an O horizon of a black to dark grey humus and roots layer; an E (albic) horizon consisting of a grey leached mineral layer; followed by a spodic B horizon which consists of a yellow-brown sand.

Needle samples had to be treated somewhat differently from soil samples in order to test the differences between nutrient concentrations and ratios in healthy and unhealthy red pines. The 229 needles samples consisted of two samples in each age category (one-year and two-year), with one missing case, from each crown level position (top, middle, and bottom) of the tree (Appendix, Table 2). The element concentrations and ratios from the two like-samples from each tree were averaged together for a total of 115 cases for statistical analyses. Averaging the two like-samples increased sample independence because only one sample from each crown position of the tree was used in the analysis.

Bimodal distributions and analysis of variance of the elements studied revealed that 1-year-old and 2-year-old needles should be treated as separate populations. Therefore separate ANOVA trials were run on samples from each age category. All samples came from the two locations within the Huron-Manistee National Forest (Minnie Lake and Thin-A-Gain) and samples were collected from unhealthy trees inside of disease plots and healthy trees outside of disease plots. The model used to test differences among needles

samples was the same as that used to test soil differences except that there are three levels to α_i, the effect of the ith level of the crown level factor (i.e., top, middle and bottom), and two levels to β_j, the effect of the jth level of the location or site factor (i.e., Minnie Lake or Thin-A-Gain); $\alpha\beta_{ij}$ is the interaction between crown level position and site.

The red pines at Thin-A-Gain were ten years younger than the red pines at Minnie Lake and analysis of variance was used to test the differences between nutrient concentrations and ratios of healthy and unhealthy trees at each site. Separate ANOVA trials were run on red pines at Minnie Lake and Thin-A-Gain which included the crown level factor (α_i) and two levels of the health factor β_j (healthy or unhealthy), where $\alpha\beta_{ij}$ is the interaction between the crown level and health factors. At Minnie Lake there were three levels to the crown level factors: top, middle, and bottom. However, at Thin-A-Gain, only two levels to the crown level factor were included: top and bottom, because there were no unhealthy trees sampled at Thin-A-Gain which included a middle crown level.

The analysis of variance model used to test differences between root nutrient concentrations and ratios is $Y_{ik}=\mu+\alpha_i+\epsilon_{ik}$, where α_i is the effect of the location factor. Separate ANOVA trials were run on two different root-size categories: $\leq$ 3 mm in diameter, and > 3 to $\leq$ 5 mm in diameter. In this model, α_i the location factor, consists

of five levels: Huron-Manistee disease edges, outside the edge of the decline, Huron-Manistee healthy plantation plots, Roscommon healthy plantation plots, and healthy natural stand plots at Osmun Lake. Unlike soil analyses, disease centers were not tested in this model because they consisted mainly of dead roots and only live roots were analyzed for nutrient content. Analysis of variance was also used to test the difference between root nutrient concentrations and ratios of disease versus non-disease plots within the Huron-Manistee forest only. In this model α_i is the effect of the disease status of a plot: i.e., healthy or unhealthy. These models do not include testing of any interaction effects on root samples.

The effects of the location and health factors studied in soil, needles, and roots are considered fixed because they are the only levels of interest in this investigation. If the experiment were to be repeated the same treatments would be included and randomness would be inherent in the replications (n) i.e. the number of root, needle, or soil samples collected. Analyses of variance were run using plots (i.e. ML1, ML2, ML3, etc.) as the levels of a factor. This effect is considered random because the disease pockets and healthy plantation plots are considered subsamples of the larger population of all potential disease pockets and healthy plantations. Under the random effects model, unlike the fixed effects model, inferences can be made to the

larger population (i.e., disease pockets and healthy red pine plantations in other locations).

All sample distributions for both ratios and element concentrations were normalized by placing them on a log scale. Log-transformation of data strengthened statistical testing. However, for clarity, means and other descriptive statistics reported are back-transformed nonlog values. Means were separated at the 0.05 significance level using the Tukey's highly significant difference (HSD) procedure (Systat for Windows, Version 5, 1992).

RESULTS

Soil Nutrient Status

Tables 4-1 and 4-2 show the results of soil analyses as separated by horizon and location within disease plots. Table 4-1 lists the pH, CEC, and % base saturation for locations within disease plots (center, edge, and outer) and for non-disease plots at Huron-Manistee, Roscommon, and Osmun Lake. The mean concentrations in mg/kg of six different soil nutrients: Al, Ca, Fe, K, Mg, Mn, and P are also listed in Table 4-1 and Table 4-2 lists the ratios of Ca/Al, Mg/Al, K/Al, and Mn/Fe.

Analysis of variance revealed that significant differences at the 0.05 level could be found for the soil horizon factor for all variables in Tables 4-1 and 4-2 except for Mg ($p=0.375$). Magnesium concentrations were fairly constant regardless of soil horizon. The soil pH and

percent base saturation tended to increase from the O horizon to the B horizon (soil sampling depth was 46 cm) and the CEC tended to decrease. The organic material present in the O horizon explains the higher CEC's. The O horizon was also the most acidic and contained the highest concentrations of extractable Al. Soil nutrients tended to decrease from the O horizon to the B horizon except for P, which tended to increase (Table 4-1). Ratios (Table 4-2) also tended to increase from the O horizon to the B horizon. In many cases, however, the grey leached E horizon had the lowest nutrient concentrations and ratios (Tables 4-1 and 4-2).

Significant differences at the .05 level were also found by location. The only variable which did not differ significantly by location was the soil Ca concentration ($p=0.170$). There were also few significant interaction effects between location and horizon except for soil Fe ($p<0.001$) and K ($p=0.022$). This indicates that the soil horizon concentrations of Fe or K depend on the location from where the sample was drawn. Iron (along with Al) tended to be highest at the healthy plantations at Roscommon. Potassium concentrations were also higher at healthy locations at Roscommon, Osmun Lake, and Huron-Manistee versus non-healthy locations (disease centers and edges).

Tukey's HSD procedure was used to separate means at the 0.05 level in Tables 4-1 and 4-2. Tables 4-1 and 4-2 show the significant differences found within the same soil

Table 4-1. Mean pH, C.E.C., percent base saturation and concentrations in mg/kg of Al, Ca, Fe, K, Mg, Mn and P from three different soil horizons at disease plots (center, edge, and outer), healthy plantations (HM and Rosc.), and natural stands (Osmun).*

Location:		Horizon:			Horizon:			Horizon:			Horizon:		
	n	O	E	B	O	E	B	O	E	B	O	E	B
		pH			CEC			% Base Saturation			Al		
Center	5	4.34*ab*	4.62*a*	5.06*a*	11.3*a*	5.9*ab*	5.7*a*	9.3*a*	10.9*ab*	28.5*a*	137*a*	121*a*	72*a*
Edge	5	4.28*ab*	4.54*a*	5.02*a*	10.5*a*	6.6*a*	5.9*a*	8.9*a*	9.5*a*	10.3*a*	121*a*	121*ab*	81*ab*
Outer	5	4.36*ab*	4.54*a*	4.96*a*	10.6*a*	7.5*a*	5.4*a*	9.7*a*	7.8*a*	11.6*a*	152*ab*	148*ab*	86*ab*
HM	2	4.65*b*	4.95*a*	5.10*a*	9.4*a*	6.8*ab*	4.8*a*	10.8*a*	15.5*ab*	13.5*a*	197*ab*	153*ab*	122*ab*
Osmun	3	4.00*a*	4.47*a*	5.20*a*	8.6*a*	2.2*b*	3.2*a*	12.1*a*	47.3*b*	46.2*a*	178*ab*	126*ab*	78*ab*
Rosc	6	3.98*a*	4.50*a*	4.98*a*	18.9*a*	11.2*a*	4.5*a*	7.8*a*	8.7*a*	15.5*a*	302*b*	261*b*	140*b*
		Ca			Fe			K			Mg		
Center	5	177*a*	104*a*	112*a*	65*ab*	50*ab*	51*a*	18.1*a*	10.3*a*	8.6*a*	8.6*a*	8.6*a*	8.6*a*
Edge	5	156*a*	104*a*	98*a*	61*a*	47*ab*	42*a*	19.0*a*	10.4*ab*	9.7*a*	8.6*a*	8.6*a*	8.4*a*
Outer	5	179*a*	97*a*	105*a*	76*ab*	56*bc*	40*a*	20.8*ab*	11.4*ab*	8.8*a*	8.4*a*	8.4*a*	8.4*a*
HM	2	160*a*	120*a*	100*a*	72*ab*	39*ab*	31*a*	43.8*bc*	21.8*ab*	14.5*ab*	14.0*b*	12.0*b*	12.0*b*
Osmun	3	160*a*	93*a*	120*a*	39*a*	28*a*	38*a*	41.2*bc*	19.5*b*	26.7*b*	11.3*b*	11.3*b*	11.0*b*
Rosc	6	240*a*	154*a*	109*a*	115*b*	97*c*	32*a*	43.0*c*	17.7*b*	9.7*a*	18.0*c*	18.6*c*	16.5*c*

Table 4-1 (Cont'd).

Osmun Lake Natural	n	Horizon: O	E	B	Horizon: O	E	B
		Mn			P		
Center	5	20.1*ab*	7.3*a*	2.9*a*	57.2*a*	82.2*a*	116.0*a*
Edge	5	23.0*ab*	6.3*a*	1.7*a*	38.1*a*	55.5*a*	60.5*a*
Outer	5	34.0*a*	17.7*a*	5.9*a*	58.2*a*	66.4*a*	91.2*a*
HM	2	58.6*a*	20.9*a*	9.6*a*	52.8*a*	59.5*a*	78.5*a*
Osmun	3	5.4*b*	3.5*a*	1.3*a*	5.2*a*	8.5*a*	28.7*a*
Rosc	6	54.6*a*	19.2*a*	4.0*a*	9.3*a*	19.1*a*	44.2*a*

*Means within the same column followed by the same letter did not differ at the 0.05 significance level using Tukey's honestly significant difference procedure.

Table 4-2. Mean molar Ca/Al, Mg/Al, K/Al, and Mn/Fe ratios from three different soil horizons at disease plots (center, edge, and outer), healthy plantations (HM and Rosc.), and natural stands (Osmun).

Location:		Horizon:			Horizon:			Horizon:			Horizon:		
	n	O	E	B	O	E	B	O	E	B	O	E	B
		Ca/Al			Mg/Al			K/Al			Mn/Fe		
Center	5	0.94*a*	0.70*a*	2.31*a*	0.07*a*	0.09*a*	0.21*a*	0.10*a*	0.06*a*	0.13*ab*	0.31*a*	0.14*a*	0.07*a*
Edge	5	0.91*a*	0.63*a*	0.98*a*	0.08*a*	0.08*a*	0.12*a*	0.11*a*	0.06*a*	0.09*a*	0.37*a*	0.15*a*	0.04*a*
Outer	5	0.83*a*	0.45*a*	0.84*a*	0.06*a*	0.06*a*	0.11*a*	0.10*a*	0.05*a*	0.07*a*	0.45*a*	0.30*a*	0.17*a*
HM	2	0.55*a*	0.67*a*	0.62*a*	0.08*a*	0.11*a*	0.12*a*	0.15*a*	0.11*a*	0.09*ab*	0.84*a*	0.48*a*	0.31*a*
Osmun	3	0.66*a*	0.51*a*	1.60*a*	0.74*a*	0.11*a*	0.25*a*	0.17*a*	0.12*a*	0.37*b*	0.15*a*	0.13*a*	0.04*a*
Rosc	6	0.55*a*	0.40*a*	0.54*a*	0.07*a*	0.08*a*	0.13*a*	0.10*a*	0.05*a*	0.05*a*	0.49*a*	0.20*a*	0.13*a*

*Means within the same column followed by the same letter did not differ at the 0.05 significance level using Tukey's honestly significant difference procedure.

horizon. In some cases (soil P, Ca/Al, Mg/Al, and Mn/Fe, for example) the table shows no significant differences even though analysis of variance indicated that the location factor was significant. This is because the significant differences at the 0.05 level were not found within the same soil horizon but across horizons.

Tukey's HSD revealed that K and Mg concentrations were significantly higher at healthy locations (Roscommon, Osmun Lake, and Huron-Manistee) than diseased areas (centers, edges, and outer regions). Significant differences within the same soil horizon were also identified for metals Al and Fe which were higher at Roscommon than at disease locations but no significant differences were found between metals for locations within the Huron-Manistee National forest. Ratios of Ca, Mg, and K to Al also tended to be the lowest at Roscommon although the differences are not significant. For the most part, it appears that soil elemental concentrations (especially Mg and K, Table 4-1) are better indicators of differences between diseased and non-diseased areas than the soil ratios (Table 4-2).

Soil samples were separated in disease plots according to location within the plot in order to determine if there were any gradients in concentrations of soil nutrients from within the Huron-Manistee National forest. There was an increasing gradient in soil concentrations of Mn, K and Mn/Fe ratios from the center of pockets to the healthy plantation plots at Huron-Manistee. However, for Mn and

Mn/Fe the differences were not significant (Tables 4-1 and 4-2).

The fact that red pines growing at the same site (Huron-Manistee, Roscommon, or Osmun Lake) were the most similar in terms of soil nutrient contents, raises questions about the selection of appropriate control groups for comparison with RPPM-affected red pines. The plots at Osmun Lake were all natural stands. These stands, unlike plantations, were not densely-planted nor even-aged. Thus it seems that natural stands may not be an adequate control group for comparing to plantation stands.

Roscommon stands, on the other hand, were similar to the Huron-Manistee stands in terms of being mature, even-aged, plantations. Variability still existed between Huron Manistee and Roscommon, however, in factors such as location, climate, stand density (Table 3-3), age, soil nutrient status, etc. It seems more appropriate to compare trees growing within the same forest that have adapted to similar site conditions over time. These stands would be more likely to respond in comparable ways to changes within the environment. Therefore, Huron-Manistee healthy plantations are considered to be the most appropriate control group for Huron-Manistee disease plots. Although nutrient data from Roscommon and Osmun Lake red pines are included in subsequent tables, the focus will be on the significant differences that occur between disease and non-disease areas within the Huron-Manistee National forest.

Needle Nutrient Status

Needle samples were collected from disease plots and healthy regions at two locations within the Huron-Manistee National Forest (Minnie Lake and Thin-A-Gain). Unlike soil and root samples, no needle samples were collected from a forest other than Huron-Manistee. Tree crowns were separated into top, middle, and bottom sections and crown symptoms were used to separate "healthy" trees from "unhealthy" trees. The number (n) of needle samples taken from unhealthy trees in the "mid" section of the crown is less than the number collected from healthy trees because of their smaller crowns that were separated into only "top" and "bottom" categories.

Table 4-3 shows the results of analysis of variance which tested differences between element concentrations and ratios from the three crown-position categories at two sites (Minnie Lake and Thin-A-Gain) for 1-year-old and 2-year-old red pine needles. Site (Minnie Lake or Thin-A-Gain), apparently, was a strong factor accounting for differences in needle elemental concentrations. For example, in 1-year-old needles significant differences were found for Al, Fe, K, Mg, Ca/Al, K/Al, Mg/Al, and Mn/Fe by site. In two year-old needles, only Mg, Zn, and K/Al differed significantly by site (Table 4-3).

Table 4-3 also shows significant differences by crown level. In 1-year-old needles Mn, Ca/Al, and Mn/Fe differed significantly by crown level. In 2-year-old needles Ca, Mg,

Table 4-3. Analysis of variance[1] of element concentrations and ratios by site (Minnie Lake or Thin-A-Gain) and by crown level (top, middle, or bottom) for 1-year-old (n=59) and 2-year-old needles (n=56).

	1-year-old needles				2-year-old-needles			
	Site		Level		Site		Level	
Element	F-ratio	P	F-ratio	P	F-ratio	P	F-ratio	P
Al	8.28**	0.006	1.44ns	0.245	2.63ns	0.111	0.93ns	0.402
Ca	0.001ns	0.981	2.49ns	0.092	0.36ns	0.551	7.13**	0.002
Fe	5.43*	0.024	0.99ns	0.377	1.09ns	0.301	0.77ns	0.470
K	11.97**	0.001	0.81ns	0.452	2.96ns	0.092	0.36ns	0.699
Mg	4.41*	0.040	1.84ns	0.169	12.98**	0.001	4.80*	0.012
Mn	2.31ns	0.135	3.54*	0.036	0.90ns	0.348	5.05**	0.010
Zn	0.96ns	0.331	0.75ns	0.476	7.11**	0.010	2.07ns	0.137
Ca/Al	11.60**	0.001	8.66**	0.001	0.49ns	0.487	13.80**	<.000
K/Al	16.20**	<.000	1.89ns	0.161	5.35*	0.025	1.19ns	0.314
Mg/Al	4.98*	0.030	2.51ns	0.091	0.27ns	0.605	4.34*	0.018
Mn/Fe	15.10**	<.000	4.59*	0.015	3.54ns	0.066	8.03**	0.001

[1]Significance:*=significant at 0.05;**=significant at 0.01; ns=not significant; there were no significant site X level interactions.

Mn, Ca/Al, Mg/Al, and Mn/Fe differed significantly by crown level. Analysis of variance indicated that there were no significant site X crown level interaction effects (results not listed in Table 4-3).

The significance of the site factor indicated that separate ANOVA trials should be run comparing needle element concentrations within a site. Table 4-4 lists the results of analysis of variance on the crown level and health factors for each site and age class. The corresponding means for the needle element concentrations and ratios tested are presented in Tables 4-5 and 4-6. Fewer significant differences were found for the crown level factor when sites were tested separately. Only Ca/Al ratios appeared to be strongly influenced by the crown level factor in both 1-year-old and 2-year-old needles at a site (Table 4-4).

Tables 4-5 and 4-6 show the actual mean element concentrations and ratios for healthy and unhealthy trees by site and crown level position for both age categories. Crown level position differences, for example, are illustrated by Ca/Al ratios which tended to increase from the top level of the crown to the bottom level in both 1-year-old and 2-year-old needles (Table 4-6). Differences between age categories can also be observed in these tables. For example, more physiologically active 1-year-old needles have higher K concentrations and K/Al ratios than 2-year-old needles. On the other hand, 2-year-old needles have higher

Table 4-4. Analysis of variance[1] of element concentrations and ratios by crown level[2] and by health (healthy or unhealthy) for 1-year-old and 2-year-old needles at Minnie Lake[3] and Thin-A-Gain[4] pocket decline sites, Huron-Manistee National Forest.

	Minnie Lake 1-year-old needles				Minnie Lake 2-year-old-needles			
	Level		Health		Level		Health	
Element	F-ratio	p	F-ratio	P	F-ratio	P	F-ratio	P
Al	0.43ns	0.654	4.88*	0.038	0.80ns	0.462	6.51*	0.019
Ca	0.74ns	0.490	2.02ns	0.170	2.59ns	0.099	0.56ns	0.461
Fe	0.53ns	0.599	1.02ns	0.324	2.77ns	0.086	0.06ns	0.636
K	0.42ns	0.664	0.21ns	0.653	0.15ns	0.862	1.54ns	0.228
Mg	0.55ns	0.585	1.00ns	0.328	3.17ns	0.063	2.27ns	0.147
Mn	1.24ns	0.311	9.59**	0.005	1.96ns	0.167	4.98*	0.037
Zn	0.58ns	0.568	7.07*	0.015	1.30ns	0.295	6.23*	0.021
Ca/Al	2.31ns	0.124	0.39ns	0.540	6.23**	0.008	0.74ns	0.399
K/Al	0.58ns	0.569	2.50ns	0.129	0.72ns	0.498	6.52*	0.018
Mg/Al	0.79ns	0.466	3.89ns	0.062	3.32ns	0.056	2.05ns	0.167
Mn/Fe	1.62ns	0.222	1.86ns	0.187	2.99ns	0.072	3.84ns	0.064
	Thin-A-Gain 1-year-old needles				Thin-A-Gain 2-year-old needles			
Al	0.78ns	0.389	8.37**	0.009	0.07ns	0.802	0.87ns	0.363
Ca	1.06ns	0.316	1.52ns	0.233	5.86*	0.026	2.87ns	0.107
Fe	0.46ns	0.504	9.91**	0.005	0.08ns	0.778	4.03ns	0.060
K	0.48ns	0.496	0.03ns	0.875	0.09ns	0.762	<0.00ns	0.967
Mg	2.49ns	0.130	0.02ns	0.892	4.23ns	0.055	1.23ns	0.281
Mn	2.00ns	0.173	11.96**	0.002	4.22ns	0.055	16.14**	0.001
Zn	0.00ns	0.987	1.19ns	0.288	0.99ns	0.334	1.43ns	0.248
Ca/Al	7.16*	0.015	1.42ns	0.248	12.44**	0.002	1.60ns	0.222
K/Al	1.58ns	0.223	9.27**	0.006	0.15ns	0.706	0.49ns	0.493
Mg/Al	2.77ns	0.112	9.95**	0.005	2.04ns	0.170	0.04ns	0.836
Mn/Fe	3.76ns	0.067	0.58ns	0.454	7.21*	0.015	5.29*	0.034

[1]Significance:*=significant at 0.05;**=significant at 0.01; ns=not significant; there were no significant level X health interactions.
[2]Crown level = top, middle, or bottom at Minnie Lake; and top and bottom only at Thin-A-Gain
[3]Minnie Lake: n=27 for 1-year-old and 2-year-old needles
[4]Thin-A-Gain: n=24 for 1-year-old needles; n=22 for 2-year-old needles

Table 4-5. Number of cases (n) and average concentrations (mg/kg) of Al, Ca, K, Mg, Fe, Mn, and Zn in one- and two-year-old needles of healthy (H) and unhealthy (U) red pine at Minnie Lake and Thin-A-Gain, Huron-Manistee National Forest.

Crown Level	n		Al		Ca		K		Mg		Fe		Mn		Zn	
	H	U	H	U	H	U	H	U	H	U	H	U	H	U	H	U
Minnie Lake Site: 1-year-old Needles																
TOP	3	8	270	183	1270	957	4499	4278	773	776	51	47	355	207	27	20
MID	3	2	256	164	1603	1247	4225	4354	853	772	61	39	426	291	30	24
BOT	3	8	216	163	1548	1316	4910	4662	882	802	40	41	449	252	32	23
Thin-A-Gain Site: 1-year-old Needles																
TOP	8	4	180	102	1186	1229	5169	5101	707	720	44	23	365	196	25	20
BOT	8	4	131	106	1518	1063	5473	5491	789	774	36	20	450	249	24	20
Minnie Lake Site: 2-year-old Needles																
TOP	3	8	366	252	1846	1740	2576	2938	849	883	76	81	641	408	28	23
MID	3	2	329	264	2625	2482	2644	2984	962	808	64	58	818	688	40	26
BOT	3	8	280	255	2915	2640	2727	3135	1100	958	71	82	1025	565	43	28
Thin-A-Gain Site: 2-year-old Needles																
TOP	7	4	272	222	1837	1478	3347	3125	731	706	85	54	649	343	21	15
BOT	7	4	239	236	2949	1925	3107	3396	911	776	68	58	1066	442	27	18

Table 4-6. Number of cases (n) and average molar Ca/Al, K/Al, Mg/Al, and Mn/Fe ratios in 1-year-old and 2-year-old needles of healthy and unhealthy red pine, Huron Manistee National Forest.

Crown Level	n		Ca/Al		Mg/Al		K/Al		Mn/Fe	
	H	U	H	U	H	U	H	U	H	U
Minnie Lake Site: 1-year-old Needles										
TOP	3	8	3.31	3.63	3.36	5.80	12.03	20.17	7.86	5.12
MID	3	2	4.32	5.58	3.85	5.67	11.77	20.07	10.01	9.51
BOT	3	8	4.89	6.26	4.66	7.62	16.28	28.24	11.88	8.01
Thin-A-Gain Site: 1-year-old Needles										
TOP	8	4	4.44	6.27	4.97	8.27	22.76	36.18	9.47	12.16
BOT	8	4	7.50	6.86	6.80	8.72	29.37	37.88	13.33	17.78
Minnie Lake Site: 2-year-old Needles										
TOP	3	8	3.39	4.69	2.60	4.16	4.91	8.46	8.96	5.33
MID	3	2	5.35	6.61	3.26	3.66	5.58	8.53	14.34	14.48
BOT	3	8	7.13	7.04	4.45	4.45	6.85	9.32	14.76	7.71
Thin-A-Gain Site: 2-year-old Needles										
TOP	7	4	4.47	4.79	3.09	3.93	8.92	11.24	8.02	8.21
BOT	7	4	8.26	6.03	4.41	4.20	9.48	11.01	16.78	8.83

concentrations of Ca in reserve. These tables also allow comparison of elemental concentrations and ratios by site (Minnie Lake and Thin-A-Gain). For example, Al concentrations tended to be higher in the older red pines at Minnie Lake and K concentrations tended to be higher in the younger red pines at Thin-A-Gain (Table 4-5).

Significant differences can be identified for the health factor for elements Al, Fe, Mn, and Zn and for ratios K/Al and Mn/Fe (Table 4-4). Contrary to expectations, metals Al and Zn were significantly higher in healthy 1- and 2-year-old needles at Minnie Lake, and Al and Fe were significantly higher in healthy 1-year-old needles at Thin-A-Gain versus their unhealthy counterparts (Tables 4-4 and 4-5). This may indicate, however, that healthy trees are more efficient at assimilating and storing metals than unhealthy trees. Also contrary to expectations, K/Al ratios were significantly lower in healthy 2-year-old needles at Minnie Lake, and K/Al and Mg/Al ratios were significantly lower in healthy 1-year-needles at Thin-A-Gain (Tables 4-4 and 4-6). This may be explained by the higher Al concentrations present in healthy red pines. As expected, Mn/Fe ratios were significantly higher but only in 2-year-old needles of healthy red pines at Thin-A-Gain (Tables 4-4 and 4-6). There were no significant interaction effects between the crown level and health factors (results not shown in Table 4-4).

The one nutrient which was consistently lower in unhealthy red pines was Mn. Manganese was significantly lower in the needles of unhealthy red pines at both sites (Minnie Lake and Thin-A-Gain) and for both age categories and also had the highest F values associated with it (Tables 4-4 and 4-5). These data suggest that Mn may play a significant role in the decline.

Root Nutrient Status

Statistical analysis (ANOVA) of red pine root tissues revealed that significant differences could be found between two root-size categories ($\leq$ 3 mm in diameter; and > 3 and $\leq$ 5 mm in diameter); and by plot location i.e. edge and outer regions of Huron-Manistee disease plots, Huron-Manistee healthy plantations, Osmun Lake, and Roscommon plots. The largest F values were for the root-size factor. Thus, these two populations are treated separately. No significant differences could be found by the soil depth from which the root sample was selected (0 to 30 cm; or 30 to 60 cm). Therefore, this factor was ignored in further analyses.

Table 4-7 displays the element concentrations of both fine roots and large roots from Huron-Manistee, Osmun Lake, and Roscommon forests. The fine-root category ($\leq$ 3 mm in diameter) is of primary interest because fine roots are expected to reflect soil chemistry and associated nutritional problems more distinctly than large roots (Ulrich, 1989; Murach and Ulrich, 1988). Analysis of variance

Table 4-7. Mean element concentrations (mg/kg) for disease plots and healthy plantations for both fine (≤ 3 mm in diameter) and large (>3 and ≤ 5 mm in diameter) red pine roots.*

Fine Roots ≤ 3 mm		Element Concentrations (mg/kg)						
Location	**n**	**Al**	**Ca**	**Mg**	**K**	**Mn**	**Fe**	**Zn**
Disease Edge	30	1943*ac*	2662*ab*	889*a*	1627*ab*	115*a*	429*ab*	56*a*
Disease Outer	42	1736*a*	2690*ab*	1067*b*	1388*a*	261*b*	331*ab*	50*ab*
HM Check	26	1090*b*	2850*ab*	1273*c*	1869*bc*	478*c*	247*ac*	38*b*
Osmun Lake	16	1034*b*	2313*a*	958*ab*	1863*bc*	296*bc*	220*c*	37*b*
Roscommon	16	2535*c*	3062*b*	1087*bc*	1924*c*	361*bc*	416*b*	50*ab*
Large Roots 3><5 mm		**Element Concentrations (mg/kg)**						
Location	**n**	**Al**	**Ca**	**Mg**	**K**	**Mn**	**Fe**	**Zn**
Disease Edge	28	506*a*	1852*a*	775*a*	1646*a*	137*a*	144*ab*	40*a*
Disease Outer	35	530*a*	2038*a*	981*bc*	1301*a*	257*ac*	175*a*	38*a*
HM Check	23	567*ab*	2272*ab*	1042*c*	1584*a*	456*b*	141*a*	33*ab*
Osmun Lake	20	544*ab*	2670*b*	773*a*	1475*a*	272*bc*	90*b*	26*b*
Roscommon	16	776*b*	2084*ab*	845*ab*	1696*a*	367*b*	162*a*	33*ab*

*Means within the same column and size category followed by the same letter did not differ at the 0.05 significance level using Tukey's honestly significant difference procedure.

revealed that significant differences could be found for element concentrations by location in both root-size categories with the exception of K concentrations in large roots at the .05 level.

Tukey's HSD procedure was used to separate means at the 0.05 level in Table 4-7. Within the Huron-Manistee National Forest, increasing gradients in Mg and Mn for both fine and large roots can be seen from the edge of the disease plots, to the outside region where trees appear healthy, and finally to the healthy check plots nearby. There is also corresponding decreasing gradients of metals Al, Fe, and Zn in fine roots at Huron-Manistee. These observations suggest that nutrient imbalances may be occurring within the fine root system. Tukey's HSD procedure does show a significant increasing gradient at the 0.05 level in Mg and Mn concentrations as well as a significant decreasing gradient in Al concentrations between diseased and healthy regions of Huron-Manistee in the fine- root category. The differences between fine-root Fe concentrations at Huron-Manistee, however, were not significant (Table 4-7).

In terms of comparing the Huron-Manistee National forest to the other forests included in the study (Osmun Lake natural stands and Roscommon plantations), root samples from Osmun Lake had comparable concentrations of Ca and Mg to Huron-Manistee disease plots. Mn in fine roots was significantly higher at Osmun Lake and Roscommon compared to Huron-Manistee disease edges. Osmun Lake also had

significantly lower concentrations of Al and Fe in fine roots compared to both the edge and outside regions of Huron-Manistee disease plots. Roscommon plots, on the other hand, had the highest root concentrations of Al compared to all other forests even though decline is absent there. However, Roscommon also has the highest fine-root Ca and K concentrations, which may be compensating for the high Al.

Table 4-8 lists the Ca/Al, Mg/Al, K/Al, and Mn/Fe ratios identified at Huron-Manistee disease plots (edge and outer regions), Huron-Manistee healthy plantations, Osmun Lake, and Roscommon plots. The table shows increasing gradients within the Huron-Manistee forest in the ratios of Ca/Al, Mg/Al, K/Al, and Mn/Fe starting from the edge of disease plots to the healthy check plots nearby. These increasing gradients suggest that conditions are improving for fine-root growth outside the diseased areas of Huron-Manistee.

Tukey's HSD procedure reveals that Ca/Al, Mg/Al, K/Al, and Mn/Fe ratios are significantly different at the 0.05 level between Huron-Manistee disease and check plots for fine roots. The increasing gradient is most apparent within the Huron-Manistee National Forest for Mn/Fe ratios, in which disease edges have a significantly lower ratio than the outer regions of disease plots, which were significantly lower than the plantation check plots nearby (HM check). The Mn/Fe gradient was also apparent for the large-diameter root category at Huron-Manistee. This relationship is not

Table 4-8. Molar ratios of Ca/Al, Mg/Al, K/Al, and Mn/Fe for disease plots and healthy plantations for fine (≤ 3 mm in diameter) and large (>3 and ≤ 5 mm in diameter) red pine roots.

Fine Roots ≤ 3 mm		**Molar Ratios**			
Location	**n**	**Ca/Al**	**Mg/Al**	**K/Al**	**Mn/Fe**
Disease Edge	30	1.10 a	0.62 a	0.69 a	0.44 a
Disease Outer	42	1.37 a	0.90 a	0.78 a	1.07 b
HM Check	26	2.18 b	1.53 b	1.43 b	2.27 c
Osmun Lake	16	2.24 b	1.63 b	1.79 b	2.22 cd
Roscommon	16	0.87 a	0.51 a	0.56 a	1.00 bd
Large Roots 3><5 mm		**Molar Ratios**			
Location	**n**	**Ca/Al**	**Mg/Al**	**K/Al**	**Mn/Fe**
Disease Edge	28	3.26 ab	2.38 ab	3.19 a	1.50 a
Disease Outer	35	3.18 ab	2.50 a	2.38 a	2.50 a
HM Check	23	3.06 ab	2.39 a	2.31 a	4.15 b
Osmun Lake	20	4.10 b	1.95 ab	2.34 a	3.89 b
Roscommon	16	2.09 a	1.39 b	1.72 a	2.89 ab

*Means within the same column and size category followed by the same letter did not differ at the 0.05 significance level using Tukey's honestly significant difference procedure.

apparent for Ca/Al, Mg/Al, or K/Al ratios in the large-diameter root category.

In comparing Huron-Manistee to Osmun Lake and Roscommon, it appears that the Osmun Lake natural stands are in fairly good condition in terms of fine-root ratios. Roscommon, on the other hand, has the lowest Ca/Al, Mg/Al, and K/Al ratios even though the red pines appear healthy. Roscommon stands are significantly lower at the 0.05 level than Huron-Manistee check plots for all four fine-root ratios. Fine root Mn/Fe ratios, however, are significantly lower at disease edges than at Roscommon.

Other than location or region from which root samples were taken, significant differences were also found between plots. Table 4-9 shows differences in element concentrations and ratios of fine roots ($\leq$ 3 mm in diameter) from both disease (ML1, ML2, ML3, TG4, and TG5) and non-disease plots (MLC and TGC) within the Huron-Manistee National Forest. Significant differences were found between fine root element concentrations and ratios by plot. These differences occurred from within disease plots and between disease plots and healthy plots. However, there were no significant differences between the two healthy plots (MLC and TGC) which tended to have lower concentrations of Al and higher Mn and elemental ratios than disease plots.

Not all disease plots differed significantly from check plots. The disease plot averages were calculated from root samples collected at both the edge and outer region of the

Table 4-9. Average element concentrations (mg/kg) and molar ratios of roots ≤ 3 mm in diameter for disease (ML1, ML2, ML3, TG4, TG5) and non-disease plots (MLC̄, TGC) within the Huron-Manistee National Forest.*

Plot	n	Al	Ca	Mg	K	Mn	Fe	Zn	Ca/Al	Mg/Al	K/Al	Mn/Fe
ML1	13	2430*a*	2702*a*	889*ac*	1142*a*	104*ab*	568*a*	46*ab*	0.83*a*	0.46*a*	0.36*a*	0.26*a*
ML2	15	1399*bc*	3082*a*	1011*ab*	1721*bc*	82*a*	252*b*	56*ab*	2.11*bc*	1.15*bcd*	1.18*bde*	0.33*ab*
ML3	16	1798*ab*	2721*a*	1214*bd*	1320*ab*	259*c*	393*ab*	59*a*	1.71*abd*	0.87*bc*	0.61*ac*	0.78*bc*
TG4	13	1785*abc*	2481*a*	837*a*	1919*bc*	230*bc*	401*ab*	55*a*	1.07*a*	0.61*ab*	0.83*bcd*	0.99*c*
TG5	15	1777*abc*	2380*a*	964*ab*	1357*ab*	314*cd*	274*b*	45*ab*	1.02*a*	0.76*ab*	0.70*ac*	1.64*c*
MLC	13	1102*c*	2881*a*	1136*bcd*	1610*abc*	372*cd*	285*b*	42*ab*	2.40*c*	1.48*cd*	1.36*de*	1.50*cd*
TGC	13	1077*bc*	2820*a*	1410*d*	2128*c*	585*c*	210*b*	34*b*	1.96*cd*	1.57*d*	1.50*e*	3.05*d*

*Means within the same column followed by the same letter did not differ at the 0.05 significance level using Tukey's honestly significant difference procedure.

plots. If these regions are calculated separately as in Tables 4-7 and 4-8, the nutritional problems associated with disease plot edges are apparent. The results of analysis of variance using plots as the levels of factor (considered a random effect) allows an inference to be made that element concentrations and ratios will vary between disease plots (i.e., other mortality pockets) within the Huron-Manistee National Forest, with the exception of fine root Ca concentrations which did not vary at the 0.05 level (Table 4-9).

To simplify understanding of differences between disease and non-disease plots, Table 4-10 lists the F-ratios and probabilities of comparisons between the mean element concentrations and ratios of fine roots. The F ratios listed in Table 4-10 test the average of fine-root samples from disease plots (ML1, ML2, ML3, TG4 and TG5) and non-disease plots (MLC and TGC) listed in Table 4-9. Table 4-10 clearly shows that, on average, fine-root samples from non-disease plots have significantly higher concentrations of nutrients K, Mg, and Mn and significantly lower concentrations of metals Al, Fe, and Zn. Fine root Ca concentrations were the only non-significant variable identified. Ca/Al ratios, however, along with Mg/Al, K/Al, and Mn/Fe ratios were significantly higher in non-disease plots than in disease plots.

Table 4-10. Analysis of variance[1] of element concentrations and ratios in fine roots samples (≤ 3 mm in diameter) from Huron-Manistee disease plots (n=72) and Huron-Manistee non-disease (check) plots (n=26).

Element	F-ratio	Probability	Significance Non>Dis., Non<Dis., or ns
Al	18.19**	< 0.001	<
Ca	1.05ns	0.309	ns
Fe	5.33*	0.023	<
K	11.59**	0.001	>
Mg	21.56**	< 0.001	>
Mn	28.99**	< 0.001	>
Zn	11.07**	0.001	<
Ratio	**F-ratio**	**Probability**	**Significance Non>Dis., Non<Dis., or ns**
Ca/Al	20.45**	< 0.001	>
Mg/Al	37.12**	< 0.001	>
K/Al	27.51**	< 0.001	>
Mn/Fe	29.34**	< 0.001	>

[1]Significance:*=significant at 0.05;**=significant at 0.01; ns=not significant.

DISCUSSION

Ca/Al Ratios and Risk of Forest Stress

Cronan and Grigal (1995) defined two soil-related measurement end points to aid in identification of forests at risk for Al stress. Disease plots at Huron-Manistee were consistent with these two risk rating criteria: i.e., base saturation percentages below 15%, and soil molar Ca/Al ratios below 1.0 (Tables 4-1 and 4-2). However, soils from healthy plantations at Huron-Manistee and Roscommon also fell within these threshold values. Soils at Osmun Lake natural stands, for the most part, were above 15% base saturation and were above the Ca/Al molar ratio of 1.0 in the B horizon only (Tables 4-1 and 4-2). These observations suggest, with the possible exception of Osmun Lake stands, that all forests studied, whether disease or non-disease, are at a 50% risk for Al stress.

These measurement endpoints were defined, however, by reviewing the literature on several different tree species. There are no known studies that identify soil solution Ca/Al threshold values specifically for red pines. Pines, in general, are considered to be Al-tolerant (Schaedle et al., 1989; Ulrich, 1985). Cronan, et al. (1989) reported a threshold Ca/Al soil solution ratio as low as 0.5 for Loblolly pine. Conversely, McCormick and Steiner, (1978) reported a threshold Ca/Al molar ratio of 1.35 for both Scotch pine and Virginia pine. Cronan and Grigal (1995) estimate that the overall uncertainty of the Ca/Al ratio

with a given probability estimate is ±50% depending on the Al sensitivity of the plant species. Thus further studies are needed in order to better characterize the threshold values specific to red pine plantations. Given this uncertainly, there is no strong indication that soil Al or Ca/Al ratios are a significant factor in RPPM.

The needle samples analyzed from the Huron-Manistee National forest included both healthy (symptomatic for RPPM) and unhealthy (asymptomatic for RPPM) red pines. Whether classified as healthy or unhealthy, the molar Ca/Al of needle tissues all averaged below Cronan and Grigal's threshold value of 12.5 (Table 4-5). Again this threshold value is based on studies which include Al-sensitive and insensitive tree species. Cronan et al. (1989) identified a threshold value of 2.3 for a foliar Ca/Al molar ratio in Loblolly pine. A more specific foliar threshold value is needed to identify Al tolerances within red pine.

In fine root tissues, Cronan and Grigal identified a Ca/Al molar ratio of 0.2 as the measurement end point beyond which the risk of forest stress increases. Fine roots are the primary organs involved in nutrient absorption, and are critical indicators of nutrient stress. The Ca/Al molar ratios of the "fine" roots included in this study were above the critical value of 0.2 (Table 4-8). However a larger diameter size class was used (between 1 mm and 3 mm in diameter) rather than the less than 1 mm diameter category recommended by Cronan and Grigal. Therefore, adequate

comparisons could not be made. Overall it appears that the Ca/Al ratios examined in this study are not reliable indicators of forest stress and are not a likely cause of RPPM.

Results of Soil, Needle, and Root Analyses in Red Pine Mortality Pockets

Soil analyses in Huron-Manistee forests as well as the Roscommon and Osmun Lake forests revealed that red pines were growing on acidic, nutrient-poor soils. For the most part, these soils were similar to each other in terms of pH, and element concentrations and ratios. However, significant differences were found between disease plots and healthy plots in soil K and Mg concentrations (Table 4-1). There were also increasing gradients in Mn and Mn/Fe from pocket edges to the Huron-Manistee check plots, but the differences were not significant. Metals, Al and Fe were highest at the healthy plantations at Roscommon and their role in the decline may not be as critical as first hypothesized. Low Mg was the nutrient factor which seemed to have the strongest association with disease-plot soils.

The needle nutrient status was assessed between healthy and unhealthy trees at each respective site, Minnie Lake and Thin-A-Gain within the Huron-Manistee National Forest. The goal was to determine differences between nutrient contents of symptomatic and asymptomatic red pine by controlling for all factors as much as possible and thereby isolating the variation attributed to the health factor. Some significant

differences were found between healthy and unhealthy red pines in needle Al, Mn, Zn, Fe, K/Al, Mg/Al and Mn/Fe. However, with the exception of Mn, these factors were not significantly different in all needle age-categories (1- and 2-year-old) and at both sites.

When needle concentrations of metals Al, Fe, and Zn were significantly different, they tended to be higher in healthy versus unhealthy red pines. It was expected that metals, especially Al, would be higher in unhealthy trees. However, the higher concentrations of Al and other metals in the red pines with healthy-looking crowns may merely be an indication of more efficient nutrient assimilation capabilities compared to the unhealthy red pine with their thinning crowns and browning foliage. In addition, mature pines do have the capacity to detoxify and immobilize Al within foliar cells enabling them to tolerate relatively high concentrations (McQuattie and Schier, 1992). Fine root mortality was much higher in disease plots compared to healthy plantations (Table 2-2). Uptake of Al^{3+} ions and other elements to the needles may have been prevented by the death of fine roots in the unhealthy red pines studied.

Manganese was the only nutrient which was significantly lower in the needles of red pines symptomatic for RPPM in both needle age categories (1- and 2-year) and at both sites (Minnie Lake and Thin-A-Gain) (Tables 4-4 and 4-6). The browning and stunted growth in needles observed in the unhealthy red pines is consistent with the Mn deficiency

symptoms reported in South African Radiata pine plantations (Grey and de Ronde, 1988; de Ronde, et al. 1988; Lange, 1969). However, the critical foliar level for *P. radiata* Mn deficiency was reported to be below 10 mg/kg, (Grey, 1988; Lange, 1969) much lower than the needle Mn levels observed in the symptomatic red pines at Huron-Manistee which averaged 232 mg/kg in 1-year-old needles (n=26) (Table 4-4).

Stone (1968) reports an intermediate range in foliar Mn concentrations for red pines to be between 200 and 900 mg/kg. Most of our unhealthy 1-year-old needle samples are at the lower end of this range but still within the normal limits, although eleven (42%) of the cases are below 200 mg/kg (range 116 to 418 mg/kg, n=26). However the red pines sampled in Stone's report were sampled in the fall (October-November) rather than summer (late July) as in our study. Red pine foliar Mn concentrations are reported to fluctuate widely throughout the year (Stone, 1968). It is also not clear whether Stone was referring to young red pines or to more mature red pines, similar to the ones examined in our study. The specific Mn requirements for mature red pines growing on spodzolic soils should be determined for a more specific comparison.

Fine roots are the main absorption and exchange sites for soil nutrients and are expected to reflect soil chemistry very distinctly (Murach and Ulrich, 1988). Soil nutrient factors whether related to Al or Mn stress are likely to be indicated more effectively by root elemental

concentrations rather than by needle concentrations. Fine roots did reflect increasing gradients in Mg, Mn, Ca/Al, Mg/Al, K/Al, and Mn/Fe within the Huron-Manistee forest from the edge of the disease plots to the healthy plantation check plots which differed significantly at the 0.05 level (Tables 4-7 and 4-8). There was also a corresponding decreasing gradient in Al concentrations (Table 4-7).

The Osmun Lake natural stands fell in line with improving fine root ratios of Ca/Al, Mg/Al, K/Al and Mn/Fe. The healthy plantations at Roscommon, however, had the lowest fine-root ratios of all areas studied (Table 4-8). The amount of root mortality was also relatively high in Roscommon plots as it was in disease plots (Table 2-2). However, among the dying roots at Roscommon, unlike the Huron-Manistee disease plots, there was also abundant new root growth. It is suggested that fine root turnover rates are much higher at Roscommon compared to Huron-Manistee disease plots. Soil conditions may be causing root mortality within Roscommon, but these roots are also being replaced more efficiently. Roscommon plots also had the highest fine root Ca and K concentrations which may be compensating for any Al stress present there (Table 4-7). Mn concentrations were also significantly higher in Roscommon fine roots compared to disease edges and outer regions (Table 4-7).

Focusing primarily on the Huron-Manistee National forest, disease plots had significantly higher fine-root

concentrations of metals Al, Fe, and Zn and significantly lower concentrations of nutrients Mg, K, and Mn than non-disease plots (Table 4-10). Fine-root Ca/Al, Mg/Al, K/Al, and Mn/Fe ratios were also significantly lower in disease plots compared to Huron-Manistee check plots. Fine-root Ca concentrations were not significantly different between disease plots and check plots, so it is possible that the higher Al concentrations within disease plots are a limiting factor of Ca absorption as well as Mg and K absorption.

Manganese may also be inhibited by Fe concentrations, but overall it appears that elemental Mn concentrations were the most indicative factors of diseased areas. Manganese was significantly lower in roots (both fine and large), and needles of red pine in RPPM plots and had the highest F values associated with it (Tables 4-6 and 4-10). Although Mn-deficiency is usually a problem associated with high pH soils (Kielbaso and Ottman, 1976), Ulrich (1989) reports that after a prolonged period of soil acidification, Mn will also be leached from soils. The may explain why RPPM does not appear until trees have matured. The stands were densely planted on highly disturbed, marginal farmland sites. Over time, the concentrated accumulation of pine litter in the even-aged stands may have been a major factor in the soil acidification process.

Magnesium concentrations also appear to be strongly associated with the decline. Increasing gradients in soil and root Mg concentrations were apparent from disease plots

to the healthy plots at Huron-Manistee (Tables 4-1 and 4-7). Lange (1969) found that Mn-deficiency symptoms in *P. radiata* could be corrected on a long-term basis with both foliar spray, and soil applications of $MnSO_4$. The treated trees also gave a favorable growth response. In addition, symptomatic *P. radiata* treated with soil applications of $MgSO_4$ responded in a similar manner to the $MnSO_4$-treated trees for about three years, after which growth rates fell and foliar symptoms returned. Lange postulated that the role of Mn in the metabolism of the trees was replaced by Mg, but only temporarily.

In the case of RPPM, soil deficiencies in Mn and/or Mg in addition to higher concentrations of metals, especially Al and Fe, in acid soils may tip the scale in favor of disease. The trees respond with decreased diameter and height growth rates, thinning crowns, and browning and stunted growth in the needles. The trees are then predisposed to attack by insect and disease pests, which may explain the often circular-pattern to the disease. The trees eventually die, but spread of the disease is slow (Chapter 3) and probably limited by adequate soil nutrient conditions in other locations. There is some indication that in pocket centers, soil conditions are improving with reduced acid loading by pine litter, allowing the regeneration of young red pines.

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CHAPTER V

Relationships Between Drought Indices, Disease Intensity, Nutrient Studies, and Fine Root Mortality in Red Pine *(Pinus resinosa)* Mortality Pockets in Michigan

ABSTRACT

To determine the factors associated with a decline of red pines know as Red Pine Pocket Mortality (RPPM) correlations were performed between 1) disease intensity and the Palmer Drought Severity Index (PDSI) at disease plots; 2) soil pH, soil/root molar nutrient concentrations/ratios and volume percentages of live, dead, and symptomatic roots recovered from disease and non-disease locations; and 3) soil pH, soil, and root molar nutrient concentrations between disease plots and non-disease plots.

Volumes of live and dead roots correlated well with root manganese (Mn), soil Mn, and root magnesium (Mg) concentrations, suggesting that these nutrients play a significant role in the decline. These nutrients were negatively correlated with dead root volumes which were highest in disease areas, and positively correlated with live root volumes which were highest in non-disease locations.

Correlations of nutrient contents by plot suggest that soil and root concentrations of Mn and Mg may be the

variables to investigate when identifying red pine plantations susceptible to decline. For example, soil Mg, was lower in disease plots compared to non-disease plots and was positively correlated with root Mg and root Mn.

Low soil and root potassium (K), ratios K/aluminum (Al), and Mn/iron (Fe), and higher concentrations of metals Al, Fe, and Zinc (Zn) were also implicated as being associated with disease plots. Soil pH's which tended to be lower in disease plots at Huron-Manistee were negatively correlated with root Mn, root Mg, root Mn/Fe and soil K/Al. In addition, root concentrations of Al and Fe were positively correlated and were higher in disease plots versus non-disease plots. Linear regressions of these correlations were significant at $p \leq 0.05$.

These observations were consistent with our prior studies using analysis of variance which indicated that disease plots had significantly lower fine-root (roots $\leq$ 3mm in diameter) concentrations of Mn, Mg, and K and significantly higher concentrations of metals Al, Fe, and Zn compared to non-disease plots (Chapter 4). However, the variable which has been the most consistently associated with disease plots and fine root mortality is Mn concentration. Multiple linear regression was used to develop a prediction equation for root Mn concentrations from soil pH and Al content that may facilitate identification of sites where fine root mortality and symptoms of RPPM are likely to occur.

INTRODUCTION

Abiotic factors associated with a disease of mature red pine (*Pinus resinosa*) stands in the Lake States known as Red Pine Pocket Mortality (RPPM) were studied. The disease is characterized by declining trees with thinning crowns, browning foliage, stunted growth in needles, and reduced diameter and height growth; surrounding a large, often circular area of dead trees (Klepzig and Carlson, 1988; Raffa and Hall, 1988).

The red pine mortality pockets studied in Michigan were all growing on sandy, low-pH soils. It was proposed that nutrient imbalances associated with acidic soils may be the initial stress-inducing factors leading to decline. Of particular interest was the effect that increased mobility and levels of exchangeable species of aluminum (primarily Al^{3+}) in acidic soils (below a pH of 5.2) may have on interfering with the uptake of other essential nutrients especially Ca^{2+}, Mg^{2+}, and K^{+} ions (Arp, et al., 1989; Balsberg-Pahlsson, 1990; Dewald et al., 1990; Foy, 1974). Therefore, the variables of primary interest in this study were ratios of extractable Al^{3+} to Ca^{2+}, Mg^{2+} and K^{+} in soil, needles, and roots of declining and healthy red pines.

In particular, aluminum-induced calcium deficiencies and ratios of Ca/Al, have been studied globally in relation to soil acidification and forest declines resulting from acid rain and/or other soil acidifying processes (Johnson et al, 1991; Ulrich, 1989; Murach and Ulrich, 1988; Shortle and

Smith, 1988; Ulrich, 1985). In the case of red pine pocket mortality, pine litter accumulation through time in densely planted, even-aged plantations and the subsequent release of humic acids through decomposition is proposed as the cause of soil acidification.

In addition to studying macronutrients and the possibility of competition with Al^{3+} ions, levels of micro-elements such as Mn, Fe, and Zn were determined in our study for identifying other possible nutrient deficiencies/ toxicities. Manganese/iron ratios are also included because of studies that have shown that high concentrations of Fe may cause Mn-deficiency (Grey, 1988; Knezek and Greinert, 1971). There are few studies of micronutrient contents in pine. Manganese deficiency, however, has been associated with stands of *Pinus radiata* in South Africa (Grey, 1988; de Ronde, et al., 1988; Lange, 1969). The Mn-deficiency symptoms on South African pines included stunted growth, yellow-brown or bronze needle color, and very sparse or short needles (de Ronde, et al., 1988; Lange, 1969). These symptoms are similar to those observed on red pines at the edge of mortality pockets. In addition, the Mn-deficiency observed in South Africa was associated with strongly podzolized soils and good drainage; similar to the Spodosols examined in this study.

The circular pattern to many red pine mortality pockets suggests activity by a biotic agent or agents. Prior studies of biotic factors associated with the disease in

Wisconsin revealed a complex association of five root- and lower-stem infesting insects vectoring two species of *Leptographium* fungi (*L. terebrantis* and *L. procerum*) in red pine mortality pockets versus healthy red pine stands (Klepzig, Raffa, and Smalley, 1991).

In our investigations, red pine roots from mortality pockets in Michigan were cultured on both selective and non-selective media for *Leptographium*, spp. but no significant root pathogens were identified (Chapter 2). Our investigation into RPPM did not include a formal study of the insects or pathogens involved beyond attempting to identify potential root pathogens. Our casual observations of biotic agents in Michigan pockets, however, have identified many associated insects and fungi but none that have been consistently present in all pockets. For this reason, we consider the various insects and fungi found within pockets to be secondary expressions of disease and not the primary cause of decline.

Our investigations of potential abiotic factors involved in the decline, have indicated that drought, in addition to nutrient stress, may be a contributing factor (Chapter 3). Symptoms of RPPM in the crowns of declining red pines were the principal indicators of increases in disease intensity. Disease intensity, as measured from a rating scale, was correlated with the Palmer Drought Severity Index (PDSI) to determine if disease symptoms increase during dry years.

Whether the causes are biotic or abiotic in origin, it has been presumed that root mortality leads to the expression of above-ground symptoms of RPPM. The results of our nutrient studies from red pine mortality pockets were compared with healthy stands to determine relationships between element concentrations and ratios in soil and fine-root samples. Soil and root nutrient levels were also compared to proportions of live, dead, and symptomatic roots removed from healthy and diseased stands. It is anticipated that identifying significant nutrient relationships at these sites will lend evidence to support the probable cause or causes of this disease. Prediction equations can be determined from significant relationships which will assist forest managers in identifying sites that may be subject to the disease.

METHODS

The Palmer Drought Severity Index (PDSI) was correlated with disease intensity (D.I.) for three disease plots (ML1, ML2, ML3) at the Minnie Lake site in the Huron-Manistee National Forest, Newago County, Michigan. Two disease plots (TG4 and TG5), in an area known as Thin-A-Gain, are not included because over 50% of the trees were removed in a thinning operation in 1993 making it difficult to continue tracking disease progress. The value for the PDSI was determined by averaging the monthly values from January to June for each year of the study (1991 through 1995). The

correlations were between PDSI and D.I. on a year-to-year basis for a total of 5 observations. The PDSI values for the Newago County region of Michigan (Zone 5) were obtained from the National Climatic Data Service through the Internet (http://www.ncd.noaa.gov/onlineprod/drought/xmgrg2.html).

Disease intensity was determined by averaging the disease rating for each tree within the plot. The trees were evaluated according to a rating scale in mid to late June of each year (1991 through 1995). The rating scale evaluated trees based on their diameter and percent of the crown showing symptoms of RPPM and was based on a 0 to 4 scale of decline with rating 4 being a dead standing tree (Table 3-1). Crown symptoms included browning or stunted growth in needles, thinning crowns, and/or poor terminal growth.

A correlation matrix has been used to examine relationships between root and soil element concentrations and ratios and volume percentages of live, dead, and symptomatic roots by location within disease and non-disease plots. The locations within disease plots (ML1, ML2, ML3, TG4 and TG5) at the Huron-Manistee National Forest, include the edge of pockets, consisting of declining trees and the outer "healthy-looking" regions of disease plots (Figure 2-2). Check plots of healthy red pine stands included in this comparison are plantation stands at the Huron-Manistee National Forest (TGC and MLC) and at the Houghton Lake State Forest in Roscommon County, Michigan (R1 and R2). Thus

correlations with live, dead, and symptomatic root volume percentages and element concentrations were derived from the average of all plots at four locations: Huron-Manistee disease edges; Huron-Manistee disease outer regions, Huron-Manistee check plots, and Roscommon County check plots.

Roots were removed from each of the locations, and recorded as live, dead, or symptomatic (Chapter 2). Roots were called symptomatic if they were partially dead or dying and/or if there was any staining below the epidermis. Soil and fine-root nutrient levels have been correlated with proportions of dead, live, and symptomatic roots to determine which nutrient factors may be associated with them.

Relationships between root and soil samples were examined by correlating molar ratios of Ca/Al, Mg/Al, K/Al, and Mn/Fe and molar element concentrations of Al, Ca, Mg, Mn, K, Fe, and Zn by plots within the Huron-Manistee National Forest. This includes both the disease plots (ML1, ML2, ML3, TG4, and TG5) and the healthy check plots nearby (MLC and TGC). Red pine stands within the Huron-Manistee National Forest were the most similar to one another in terms of site factors (climate, topography, soil type, etc). Thus, these stands are likely to be the best comparison group to one another rather than stands adapted to growing in a different forest (for example, Roscommon County plantations). Correlations between plots located within the Huron-Manistee forest may indicate which nutrient factors

are associated with disease versus non-disease stands. Correlations with significant linear regressions were also determined so that prediction equations could be developed.

Stepwise multiple linear regression analyses were used to determine the best-fit predictive models for the nutrient factors of interest. The model used assumes the effects of the independent variables are additive (i.e., no interaction effects) and follows the form:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k + \epsilon$$

All computations were done with Systat^{tm}.

RESULTS

Disease Intensity and the Palmer Drought Severity Index

Table 5-1 illustrates the relationship between the average Palmer Drought Severity Index (PDSI) and disease intensity (D.I.), per plot and as an average, measured on an annual basis (1991-1995). Average disease intensity is the mean D.I. value for the three Minnie Lake plots. The 6-month-averaged PDSI values are negatively correlated with disease intensity at plots ML1, ML2, and ML3 and with the average disease intensity (R=-0.738, R=-0.776, R=-0.764, and R=-0.765, respectively). A negative correlation is expected since the PDSI values become more negative with increasing drought severity and disease intensity (i.e., greater symptoms of RPPM in tree crowns).

With only five observations, one for each year of the study, the linear regressions for the correlations

between PDSI and D.I. are not significant. Further data collection and observations of disease intensity patterns in mortality pockets over time are needed to investigate the relationship between drought and disease intensity. The correlations do suggest, however, that symptoms of RPPM increase in tree crowns during drier years. In addition other factors such as pest problems and/or soil nutrients may contribute to changes in disease intensity. Thus, drought alone can not account for the entire disease intensity rating.

Table 5-1. Correlations between Average Palmer Drought Severity Index (PDSI), average disease intensity (D.I.) and disease intensity by plot (ML1, ML2, and ML3) (observations=5).

	Avg. PDSI	Avg. D.I.	D.I. ML1	D.I. ML2
Avg. D.I.	-0.765			
D.I. ML1	-0.738	*0.995		
D.I. ML2	-0.776	*0.993	*0.976	
D.I. ML3	-0.764	*0.999	*0.998	*0.988

*Correlation with significant linear regression ($p \leq 0.05$).

Strong positive correlations with significant linear regressions ($p \leq 0.05$) can be found with disease intensity between plots and also with the average disease intensity for all three plots. This indicates similar patterns of

disease progress were occurring in all Minnie Lake plots during each year. The plots consisted of mature, even-aged stands of red pine within the same region of the Huron-Manistee National Forest, and were likely being affected in similar ways by environmental factors such as drought, and biotic factors such as the population of insect pests present in the area.

Soil and Root Nutrient Contents and Root Vitality

Table 5-2 presents the correlations between soil pH and root and soil element concentrations and ratios in terms of proportions of live, dead, and symptomatic roots. The correlations are between the averages for each variable by location: the edge regions of disease plots (DE); the outer healthy-looking regions of disease plots (DO), the healthy plantation check plots at Huron-Manistee (HM), and the healthy plantations at Roscommon (ROS). Thus only four data points are available for linear regression.

Strong positive correlations were found between root Mg, root Mn, and soil Mn concentrations and the proportions of live roots (R=0.951, R=0.979, and R=0.988). These correlations had significant linear regressions as illustrated in Figure 5-1. Conversely, strong negative correlations can be found between root Mg, root Mn, and soil Mn concentrations and the proportion of dead roots (R=-0.916, R=-0.868, R=-0.862, respectively); although, with only four data points it was not possible to reject the null

Table 5-2. Correlations between soil pH, root and soil element concentrations/ratios and proportions of live, dead, and symptomatic roots by location (observations = 4).

	DEAD	LIVE	SYMP
LIVE	-0.926		
SYMP	0.417	-0.730	
pH	-0.487	0.415	-0.118
Root Al	0.461	-0.292	-0.132
Root Ca	-0.355	0.656	-0.935
Root Ca/Al	-0.624	0.526	-0.137
Root Fe	0.818	-0.682	0.160
Root K	-0.143	0.499	-0.942
Root K/Al	-0.591	0.544	-0.240
Root Mg	-0.916	*0.951	-0.628
Root Mg/Al	-0.678	0.582	-0.172
Root Mn	-0.868	*0.979	-0.783
Root Mn/Fe	-0.837	0.850	-0.528
Soil Al	-0.350	0.623	-0.866
Soil Ca	-0.198	0.454	-0.733
Soil Ca/Al	0.635	-0.845	0.883
Soil Fe	-0.039	0.252	-0.535
Soil K	-0.617	0.854	-0.938
Soil K/AL	-0.222	0.217	-0.119
Soil Mg	-0.181	0.533	*-0.954
Soil Mg/Al	0.148	0.126	-0.572
Soil Mn	-0.862	*0.988	-0.817
Soil Mn/Fe	-0.821	0.815	-0.476

*Correlation with significant linear regression ($p \leq 0.05$).

hypothesis of $\beta_1=0$ at a 0.05 significance level. However, these correlations do suggest that there is a relationship between proportions of dead roots and root Mg and Mn concentrations.

Of metals, root Fe was more negatively correlated with the proportion of live roots (R=-0.682) than root Al (R=-0.292). Root Fe was also more positively correlated with the proportion of dead roots (R=0.818) than root Al (R=0.461). This suggests that Fe has the stronger association with root mortality than does Al. Iron has been implicated as an inhibitor of Mn uptake (Grey 1988; Knezek and Greinert, 1971) and may be having a greater effect on Mn nutrition than the proposed effect of Al on Ca uptake and nutrition. However, the linear regressions for the correlations between Fe and Al to live and dead roots were not significant.

Of ratios, root and soil Mn/Fe had the strongest correlations with live and dead root volumes. Higher root and soil Mn/Fe ratios were associated with greater proportions of live roots (R=0.850 and R=0.815) and were negatively correlated with the proportion of dead roots (R=-0.837 and R=-0.821). Root ratios of Ca/Al, Mg/Al, and K/Al also correlate positively with live root ratios and negatively with dead root ratios but not as strongly as root Mn/Fe ratios. In addition, soil Ca/Al, Mg/Al, and K/Al, unlike soil Mn/Fe ratios, did not correlate as

expected in terms of positive/negative relationships and/or were only weakly correlated with the root proportions.

Again, significant linear relationships could not be established for soil and root ratios with only four observations. The only factors which provided correlations with significant linear regressions were root Mg, root Mn, and soil Mn to the proportion of live roots, and soil Mg to the proportion of symptomatic roots (R=-0.956). Therefore, it is suggested, that root and soil element concentrations of Mn and Mg are the most important factors in RPPM compared to other element concentrations or ratios.

Figure 5-1 shows the linear regressions for root molar Mg and Mn concentrations, soil molar Mn concentrations, and the percentage of live roots removed from each of the four locations. In each case, the percentage of live root volume increases with increasing Mn or Mg concentrations; and a gradient is apparent starting from the edge of the disease plots where trees are declining (DE), to the healthy-looking outside regions of disease plots (DO), to the Roscommon healthy plantations (ROS), and finally to the Huron-Manistee check plots (HM). Huron-Manistee check plots were the healthiest in terms of percentages of live and dead fine root volumes (Table 2-2) and also had the highest soil Mn, root Mn, and root Mg concentrations (Tables 4-1 and 4-7).

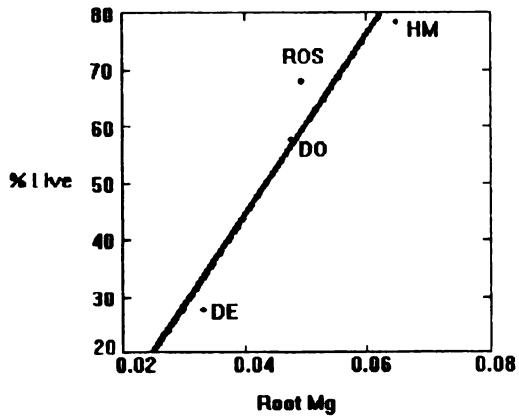


Figure 1a.
R^2=0.904
F=18.756
p=0.049
%LIVE=-84.6+3211RMG

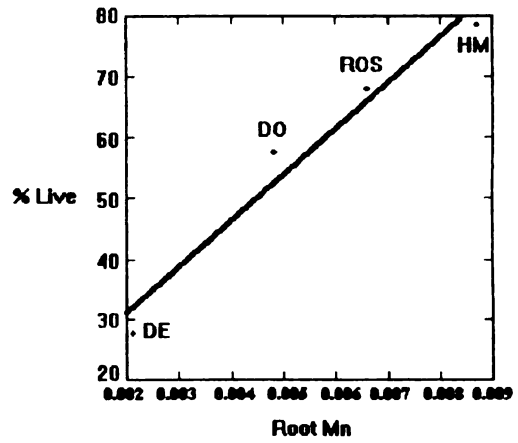


Figure 1b.
R^2=0.958
F=54.768
p=0.021
%LIVE=15.6+7631RMN

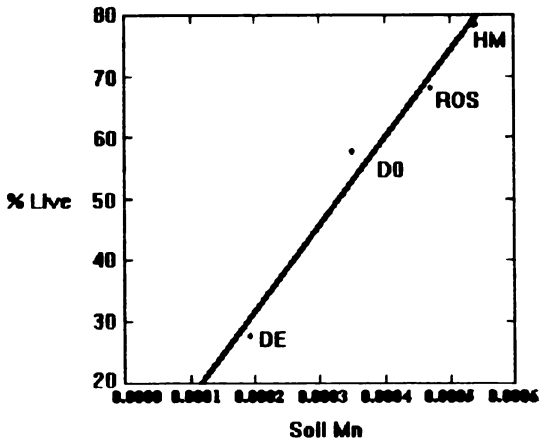


Figure 1c.
R^2=0.977
F=83.823
p=0.012
%LIVE=3.431+140695SMN

Figure 5-1. Linear regression for percentage of live root volume (LIVE) and concentrations of a. root molar Mg (RMG), b. root molar Mn (RMN) and c. soil molar Mn (SMN) by location: disease edge (DE), disease outer (DO), Roscommon non-disease plantations (ROS) and Huron-Manistee non-disease plantations (HM).

Soil and Root Nutrient Factors By Plot

Correlations between soil pH, and molar root and soil concentrations and ratios are presented in Table 5-3. Analysis of variance had indicated that significant differences in element concentrations and ratios could be identified according to plot (Table 4-9). Therefore, the correlations presented are between plots at the Huron-Manistee National forest. This includes both disease plots (ML1, ML2, ML3, TG4, and TG5) and non-disease plots (MLC and TGC). With seven observations (7 plots), significance of linear regressions at the 0.05 level was easier to demonstrate than with only the four observations available for Table 5-2.

As expected, linear regression equations predicting root Ca/Al (R=-0.863), Mg/Al (R=-0.949), and K/Al (R=-0.967) ratios can be derived from root Al concentrations. They could also be derived from their respective root Ca (R=0.788), root Mg (R=0.797), and root K (R=0.799) concentrations. Soil and root Mn/Fe ratios could also be predicted from their respective Mn concentrations (R=0.982 and R=0.975).

Some surprising results are that root Fe concentrations can also be used to predict root Mg/Al (R=-0.831) and K/Al (R=-0.802) ratios (but not root Ca/Al ratios, R=-0.665). This is explained by the fact that root Fe concentrations increase along with root Al concentrations (R=0.893). This relationship is illustrated in Figure 5-2. Root Al and root

Table 5-3. Correlations between soil pH, and root (R) and soil (S) element concentrations and ratios by Huron-Manistee disease plots and check plots (observations =7).

	pH	RAL	RCA	RCAAL	RFE	RK	RKAL
RAL	-0.558						
RCA	-0.123	-0.483					
RCAAL	0.257	*-.863	*0.788				
RFE	-0.585	*0.893	-0.289	-0.665			
RK	0.463	-0.723	0.225	0.429	-0.631		
RKAL	0.520	*-.967	0.549	*0.820	*-.831	*0.799	
RMG	*0.791	-0.666	0.414	0.655	-0.570	0.391	0.613
RMGAL	0.608	*-.949	0.614	*0.899	*-.802	0.601	*0.939
RMN	*0.966	-0.656	-0.087	0.355	-0.589	0.558	0.624
RMNFE	*0.968	-0.646	-0.117	0.282	-0.650	0.614	0.640
RZN	-0.726	0.385	-0.005	-0.147	0.321	-0.293	-0.478
SAL	0.024	-0.580	0.465	0.729	-0.228	0.169	0.548
SCA	0.129	0.003	-0.002	0.243	0.085	-0.425	-0.209
SCAAL	0.210	0.390	-0.690	-0.567	-0.004	-0.449	-0.514
SFE	-0.326	0.583	-0.515	-0.406	0.586	-0.744	-0.743
SK	0.474	-0.672	0.329	0.694	-0.362	0.251	0.634
SKAL	*0.855	-0.353	-0.179	0.204	-0.332	0.009	0.269
SMG	0.690	-0.722	0.486	0.649	-0.490	0.542	*0.794
SMGAL	0.649	0.063	-0.443	-0.394	-0.241	0.077	-0.043
SMN	0.169	-0.421	0.153	0.524	-0.092	-0.007	0.337
SMNFE	0.263	-0.545	0.238	0.608	-0.209	0.132	0.490

*Correlation with significant linear regression ($p \leq 0.05$).

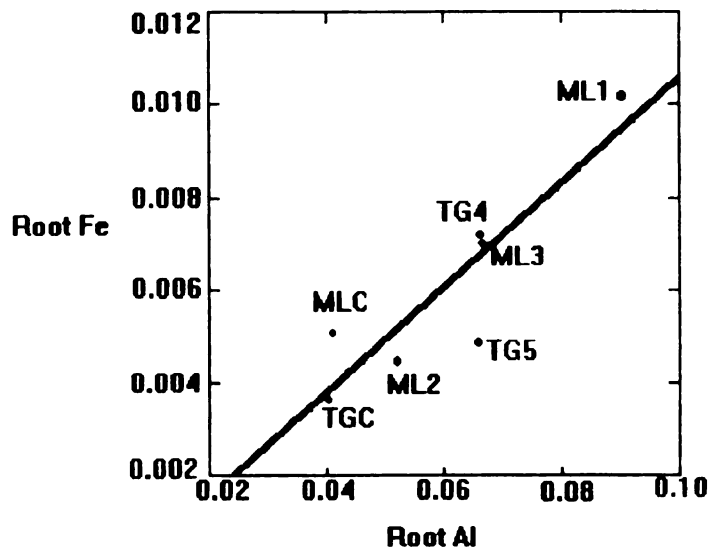
Table 5-3. (Cont'd)

	RMG	RMGAL	RMN	RMNFE	RZN	SAL	SCA
RMG							
RMGAL	*0.797						
RMN	*0.766	0.680					
RMNFE	0.699	0.652	*0.975				
RZN	-0.461	-0.519	-0.736	*-.776			
SAL	0.257	0.598	0.236	0.101	-0.188		
SCA	0.410	0.066	0.121	-0.062	0.286	0.257	
SCAAL	-0.123	-0.427	0.022	0.091	0.043	-0.672	0.189
SFE	-0.336	-0.599	-0.321	-0.454	0.475	-0.004	0.684
SK	0.579	0.751	0.635	0.508	-0.550	*0.877	0.316
SKAL	0.690	0.477	*0.831	*0.768	-0.722	0.208	0.410
SMG	*0.773	*0.863	*0.764	0.730	*-.789	0.522	-0.052
SMGAL	0.255	-0.015	0.468	0.595	-0.538	-0.665	-0.231
SMN	0.280	0.450	0.363	0.193	-0.243	*0.917	0.475
SMNFE	0.364	0.582	0.460	0.311	-0.372	*0.942	0.359

	SCAAL	SFE	SK	SKAL	SMG	SMGAL	SMN
SCAAL							
SFE	0.395						
SK	-0.469	-0.092					
SKAL	0.294	0.056	0.630				
SMG	-0.485	-0.596	*0.783	0.605			
SMGAL	0.689	-0.258	-0.259	0.502	0.130		
SMN	-0.420	0.285	*0.902	0.452	0.451	-0.511	
SMNFE	-0.491	0.104	*0.959	0.487	0.594	-0.450	*0.982

*Correlation with significant linear regression ($p \leq 0.05$).

Fe concentrations tend to be higher in disease plots (ML1, ML2, ML3, TG4, and TG5) compared to non-disease plots (MLC and TGC).



R^2=0.797
F=19.590
p=0.007
RFE=-001+0.113RAL

Figure 5-2. Linear regression between root Al (RAL) and root Fe (RFE) molar concentrations.

It was expected that the soil pH would correlate well with root Al and soil Al concentrations. However, the strongest correlations were found between soil pH and root Mn concentrations (R=0.966), root Mn/Fe ratios (R=0.968), root Mg concentrations (R=0.791), and soil K/Al ratios (R=0.855).

Figure 5-3 presents the relationship of soil pH to these factors. As soil pH increases, molar concentrations of root Mg, Mn, Mn/Fe, and soil K/Al increase. The check plot at Thin-A-Gain had the highest pH and therefore had the highest concentrations of root Mg and Mn. Soil K/Al ratios were only slightly higher at check plots. These linear

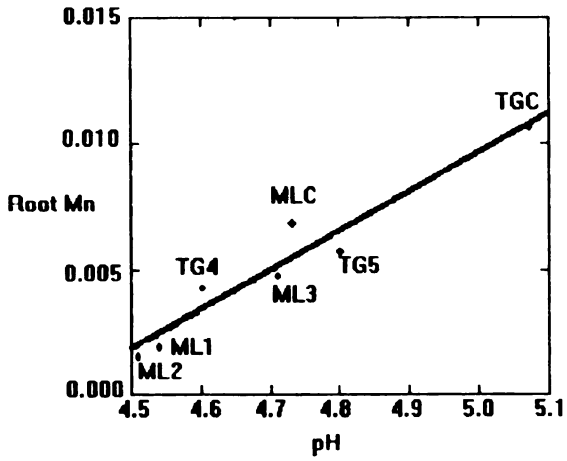


Figure 3a.
R^2=0.933
F=69.995
p<0.001
RMN=-0.069+0.016pH

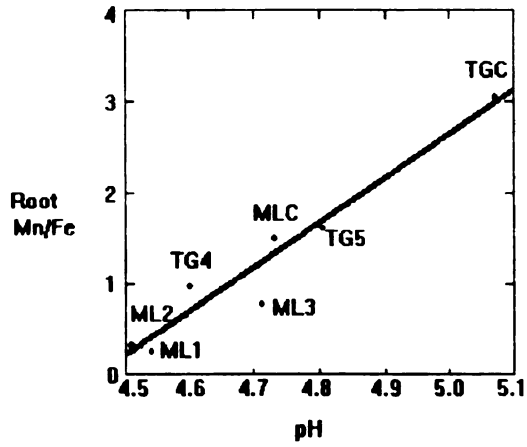


Figure 3b.
R^2=0.937
F=74.342
p<0.001
RMNFE=-21.747+4.878pH

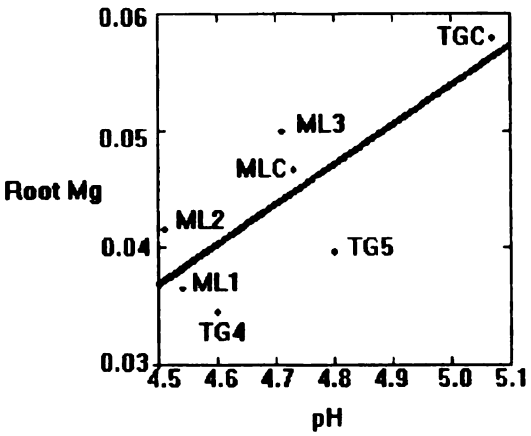


Figure 3c.
R^2=0.626
F=8.357
p=0.034
RMG=-0.117+0.034pH

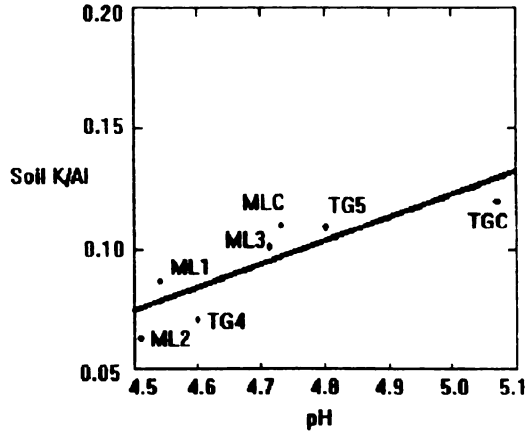


Figure 3d.
R^2=0.730
F=13.550
p=0.014
SKAL=-0.356+0.096pH

Figure 5-3. Linear regression of soil pH to molar concentrations of a) root Mn (RMN), b) root Mn/Fe (RMNFE), c) root Mg (RMG), and d) soil K/Al (SKAL).

regressions suggest that higher soil pH will favor higher root Mn, Mg, Mn/Fe, and soil K/Al.

One of the purposes of Table 5-3, was to determine if soil concentrations of nutrients would correlate well with root concentrations. This would make it possible to predict root concentrations of certain nutrients from soil analyses. There were few strong correlations between soil and root concentrations except in the case of soil Mg. Soil Mg correlated strongly with root Mg and with root Mg/Al concentrations. Surprisingly soil Mg also correlated well with root Mn, root Zn, and root K/Al. These relationships are presented in Figure 5-4. In all cases, soil Mg was highest in check plots compared to disease plots as were root Mg, Mn, and K/Al ratios. Root Zn was negatively correlated with soil Mg and decreased in check plots compared to disease plots.

It should be pointed out, however, that even though there is a significant linear relationship between soil Mg and the nutrient factors in Figure 5-4, the predictive power of soil Mg for these factors is somewhat limited; the coefficient of determination (R^2) ranged from only 0.597 to 0.630 (Figure 5-4). A multiple regression analysis would be needed to improve prediction of root nutrient factors from soil factors.

There are many strong correlations, in Table 5-3, between Mn, Mg, and K (or K/Al) concentrations. For example, soil K increases with soil Mn and with soil Mg

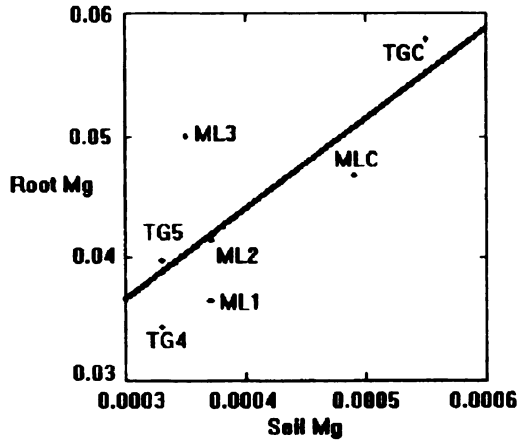


Figure 4a.
R^2=0.597
F=7.408
p=0.042
RMG=0.014+74.041SMG

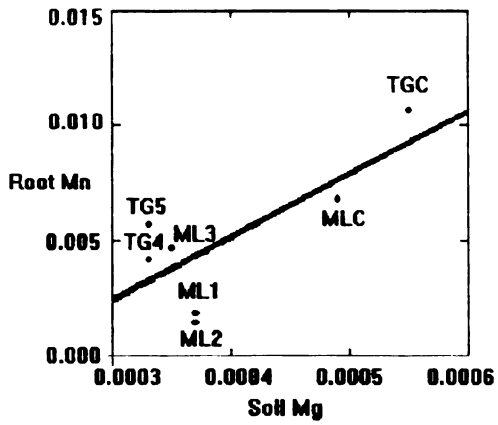


Figure 4b.
R^2=0.583
F=6.999
p=0.046
RMN=-0.006+27.698SMG

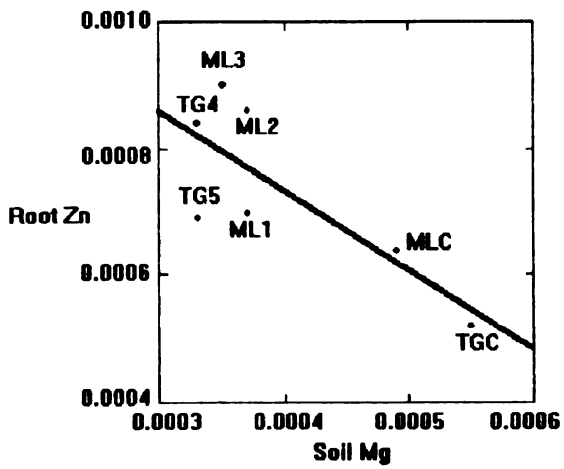


Figure 4c.
R^2=0.623
F=8.272
p=0.035
RZN=0.001+-1.252SMG

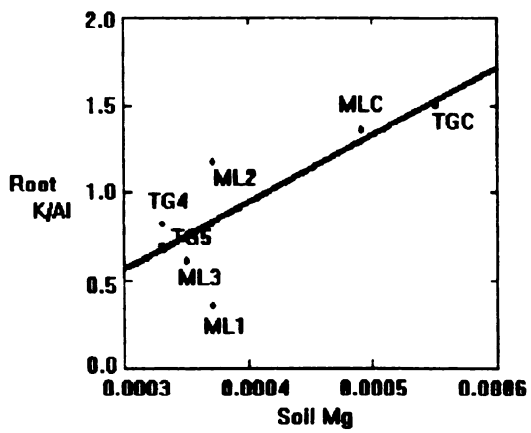


Figure 4d.
R^2=0.630
F=8.515
p=0.033
RKAL=-0.608+3860.205SMG

Figure 5-4. Linear regression between molar concentrations of soil Mg and a) root Mg (RMG) b) root Mn (RMN), c) root Zn (RZN), and d) root K/Al by plot (RKAL).

(R=0.902 and R=0.783, respectively), and root Mn also increases with root Mg (R=0.766). This suggests that these three nutrients are interacting in some way and, for the most part, are lower in disease plots compared to check plots.

Multiple Regression Analyses

Correlations between soil and root nutrient factors and volume percentages of live and dead fine roots, have indicated that low root and soil Mn and low root Mg concentrations are associated with disease areas and lower live fine-root volumes (Figure 5-1). Stepwise regression analyses were used to determine which soil or root nutrient factors should be included in multiple regression equations for predicting the concentrations of these nutrients. Soil factors, pH and element concentrations, were tested in each prediction model separate from root nutrient factors. No soil or root elemental ratios were included in the model. Stepwise regression analyses eliminated factors which did not contribute to improving the fit of the prediction equation by increasing the R^2. Using this method, the best-fit models for predicting concentrations of root Mn, soil Mn and root Mg were determined and are listed in Table 5-4.

These equations allow prediction of root Mn and soil Mn concentrations from soil nutrient factors which are easier to determine in field studies than root nutrient factors. In turn, root Mn and soil Mn were strongly correlated with

concentrations of live and/or dead fine roots (Table 5-2). Therefore, the equations could be used to identify sites from soil factors where root mortality and disease are likely to occur.

Table 5-4. Multiple regression equations for predicting root (R) Mn, soil (S) Mn, and root Mg concentrations.

Dep. Var. (Y)	Ind. Var. (X)	Std. Err.	Tolerance	Model	R^2	F-ratio	P
RMN	pH	0.001	0.999	RMN=-0.072+0.016pH +0.608SAL	0.979	92.04	<.001
	SAL	0.208	0.999				
SMN	SAL	0.023	0.224	SMN=-0.001+0.090SAL +0.557SFE+0.671SK	0.992	127.1	<.001
	SFE	0.086	0.966				
	SK	0.131	0.223				
RMG	RCA	0.297	0.992	RMG=-.013+0.674RCA +2.135RMN	0.820	9.09	0.033
	RMN	0.563	0.992				

The best prediction model for root Mg concentrations was provided by root nutrient factors (root Ca and Mn concentrations, Table 5-4). However stepwise regression analysis revealed that, of soil factors, pH could also be used to predict root Mg concentrations. No other soil factors were accepted for inclusion in the prediction equation for root Mg concentrations. Therefore, according to the stepwise analysis, the simple linear regression model of RMG=-0.117+0.034pH could be used to predict root Mg

concentrations but with an R^2 of 0.626 (p=0.034) the prediction capability of the equation is somewhat limited.

Root nutrient factors could also be used to predict root Mn concentrations. According to the stepwise regression analysis the model would be:

RMN=0.021-0.087RAL-0.321RCA+0.262RMG

with an R^2 of 0.905 (p=0.048). In this model, root Al increased with decreasing root Mn concentrations (Table 5-3). However this relationship was not true for the soil Al variables used to predict root Mn and soil Mn concentrations in Table 5-4. In these models soil Al increased with increasing root and soil Mn concentrations (Table 5-3). This indicates that, in soil, there is no apparent competitive effect of Al on Mn levels. If fine roots are examined, however, there does appear to be a competitive relationship between the levels of Al and Mn.

The stepwise procedure did not accept any of the root nutrient factors for prediction of soil Mn. But a good fit was provided for soil Mn prediction from the soil nutrient factors in Table 5-4 (R^2=0.992).

Figure 5-4 illustrated that soil Mg was a suitable predictor of root Mn and Mg. However, stepwise regression analysis did not select soil Mg as a variable for inclusion in the root Mn and Mg prediction equations in Table 5-4. This indicates problems with multicollinearity. There are too many variables that are strongly intercorrelated to allow for inclusion of all variables in the prediction equations.

DISCUSSION

Our observations of red pine pocket mortality have indicated that drought is a contributing stressor to the disease. Symptoms of RPPM increased in tree crowns during drought years compared to non-drought years (Chapter 3). The PDSI was averaged for 6-months (January though June) and correlated with the average disease intensity for that year. Attempts were made to correlate disease intensity with the previous year's average PDSI as averaged from May to November. Studies of red pine have shown that bud formation and shoot elongation are controlled more by the previous year's rainfall than the current year's (Clements, 1970, Motley, 1949). However, our measures of disease intensity did not correlate as well with the previous year's rainfall.

Other studies of red pine have found that needle elongation is more responsive to the current year's water supply (Garret and Zahner, 1973). One of the primary symptoms of RPPM observed when evaluating disease stands was browning and stunted growth in needles. Thus, our measures of disease intensity correlated better with the current year's water supply as it affects needle growth than with the previous year's effect on bud formation and shoot elongation.

Strong correlations were also found by location between root and soil nutrient concentrations with the volume percentages of live, dead, and symptomatic roots. In particular molar root and soil Mn and root Mg concentrations

were positively correlated with the proportion of live roots and negatively correlated with the proportion of dead roots. Disease edges had significantly higher volumes of dead fine roots than did Huron-Manistee check plots (Table 2-2). This suggests that root and soil Mn and root Mg concentrations play a role in determining volumes of live and dead fine roots (i.e. roots $\leq$3 mm in diameter). Prediction equations could be developed from the Mn and Mg concentrations to estimate percentages of live and dead roots.

Ulrich (1989) reports that mobile cations, K and Mn, are leached from dead roots and that the Al content of dead roots in acid soils approaches that of the soil solution. Beyond these differences, contents of other nutrients should be similar between live and dead roots. Only live and symptomatic roots were analyzed for element content in our investigations, so the difference in Mn concentrations between disease and non-disease areas can not be attributed to dead roots. Symptomatic roots, however, were defined as partially dead or dying and they do have a strong negative correlation with root K concentrations but also to soil Mg, soil K, and root Ca concentrations.

Red pine at the edge of the disease pockets were in a state of decline; whereas, the red pines examined in all other areas, even in the outer regions of disease plots, appeared healthy. However root mortality was higher in the outer regions of disease plots compared to healthy plantations even though tree crowns appeared healthy. This

suggests that the roots are dying first, leading to expression of symptoms in the crowns, rather than tree crowns declining and causing root mortality. The average molar concentrations of root Mg, root Mn, and soil Mn at the outer regions of disease plots were 4.4×10^{-2}, 4.8×10^{-3}, and 3.5×10^{-4}, respectively. These are suggested as threshold values below which the risk of decline in red pines plantations of low pH (<5.0) increases.

Further correlations between soil and root element contents in disease and non-disease plots at Huron-Manistee suggest a link between low pH and low root Mn and Mg concentrations and Mn/Fe ratios. This may be due to competition from increased solution concentrations of metals Al and Fe, which tend to increase in acid soils. Both root Al and Fe concentrations tended to be higher in disease plots compared to check plots (Figure 5-2).

Of soil variables, soil molar Mg concentrations correlated well with root concentrations of Mg, Mn, Zn and K/Al. Disease plots at Huron-Manistee had lower soil molar Mg concentrations than check plots which correlated with lower concentrations of root Mg, Mn, and K/Al and higher concentrations of Zn. It appears that a soil molar Mg concentration below $4X10^{-4}$ may indicate conditions where red pines begin to decline (Figure 5-4). This is suggested as another threshold value indicating conditions favorable to development of RPPM and would apply only to red pine

plantations growing in a similar soil type (sandy Spodosols) of low pH and in a similar climatic zone (Zone 4 or 5).

The variables of primary interest when we initiated this study were the ratios of Ca, Mg, and K to Al concentrations. It was expected that low Ca/Al ratios, in particular, would indicate conditions favoring root mortality and the decline of red pines. Ca/Al along with Mg/Al, and K/Al ratios did correlate negatively with dead root volumes and positively to live root volumes. However, root and soil Mn, and root Mg concentrations had stronger correlations with dead and live root volumes which allowed development of linear regression equations for predicting proportions of live roots from root Mn, root Mg, and soil Mn (Figure 5-1). In addition, stepwise multiple regression provided equations for predicting root Mn and Mg from soil variables which, in turn, could be used to identify sites prone to disease.

In analysis of variance studies, Mn was the only nutrient found to be significantly lower, not only in fine root tissues, but in large roots and needles from disease plots compared to non-disease plots and also had the largest F-ratios associated with it (Chapter 4). I propose that Mn deficiency is the primary cause of fine root mortality in red pine mortality pockets. Although Mn deficiency in trees is usually associated with high pH soils (Kielbaso and Ottman, 1976), Ulrich (1989) reports that after a prolonged period of soil acidification even Mn may be leached through

acid soils. The Mn-deficiency found on *P. radiata* stands in South Africa was associated with acid, highly-podzolized soils similar to the soils included in our study. The South African pines also exhibited similar symptoms of browning, and stunted growth in needles observed in RPPM-affected red pines (Grey, 1988; de Ronde, et al., 1988; Lange, 1969).

In the proposed etiology of RPPM, localized conditions of low soil Mn (along with Mg and perhaps K concentrations) and high concentrations of metals Al, Fe, and Zn may be enough to tip the scale in favor of fine-root death. Fine-root mortality inhibits absorption and translocation of essential nutrients to tree crowns, inhibiting growth. Trees become weakened and predisposed to attacks from insect and disease pests. The role of biotic agents in the disease may explain the often circular pattern of RPPM pockets. In addition, low nutrient and water absorption by fine roots increases the susceptibility of red pines to drought. The combination of these stresses results in the observed symptoms of RPPM, i.e. thinning crowns, browning and stunted growth in needles, and reduced diameter and height growth; and eventually in tree mortality.

Conclusions of Red Pine Pocket Mortality Studies

The following ten points summarize our observations and conclusions regarding the nature of red pine pocket mortality and about the primary cause of root mortality.

1. The total volume of fine roots (roots $\leq$ 3.0 mm in

diameter) were lower and the volume percentages of dead fine roots were higher in RPPM-affected areas versus healthy locations (Table 2-2). Within the Huron-Manistee National Forest, a gradient could be seen in the volumes of live fine roots which increased from the center of mortality pockets where there were many tall, dead-standing trees; to the edge of pockets where trees were declining; to the outer, healthy-looking regions of disease plots; and finally to the healthy plantations nearby (Table 2-2). These observations suggest that fine roots are dying first before symptoms of RPPM are seen in the crown.

2. Red Pine Pocket Mortality does not appear to be spreading quickly--a once-a-year evaluation was frequent enough to observe disease progress. Evaluation of disease progress indicated that the disease may increase, decrease, or remain constant during any given year (Chapter 3).

3. Spatial autocorrelation revealed that the pattern of disease progress is not random. Red pines near diseased trees are more likely to develop symptoms of the disease than those further away, suggesting movement of an infectious agent from one tree to another (Chapter 3).

4. Drought is likely to be a contributing factor to the disease. Increased mortality and further decline in tree crowns was observed in drought years versus non-drought years (Chapter 3).

5. Soil concentrations of Mg and K were significantly lower in disease areas compared to healthy locations and may be

associated with the decline (Table 4-1).

6. Of needle nutrient contents and ratios, Mn was the only factor that was consistently significantly lower in both one- and two-year old needles of red pines symptomatic for RPPM compared to asymptomatic red pines within the Huron-Manistee National Forest (Table 4-4).

7. An increasing gradient in fine root Mn and Mg concentrations was found at the edge of disease plots, to the outer healthy-looking region of disease plots, to the plantation check plots nearby. There was also a corresponding decreasing gradient in fine root Al concentrations. Significant differences in root elemental concentrations were found within these gradients at the 0.05 level (Table 4-7).

8. Analysis of variance revealed that nutrients Mn, Mg, and K and ratios Ca/Al, Mg/Al, K/Al, and Mn/Fe were significantly lower, and metals Al, Fe, and Zn were significantly higher in fine roots from disease plots versus non-disease plots (Table 4-10).

9. Despite significant differences identified for fine root Ca/Al, Mg/Al, and K/Al ratios, the theory of Al toxicity in red pine mortality pockets is not as strongly supported as Mn-deficiency. Manganese was consistently lower in disease plots, not only in fine roots, but in large diameter roots (3-5 mm in diameter), and needles. Manganese also had the highest F-ratios associated with it in statistical tests (Chapter 4). Root and soil Mn concentrations along with

root Mg were highly correlated with proportions of live and dead roots (Table 5-2, Figure 5-1).

10. Suggested threshold values for identifying susceptible-to-decline red pine plantations are a molar root Mg concentration of $4.4X10^{-2}$, a molar root Mn concentration of $4.8X10^{-3}$, a soil molar Mn concentration of $3.5X10^{-4}$ and a soil molar Mg concentration of $4X10^{-4}$. Levels of root and soil Mn and Mg which fall below these values in mature red pine plantations with sandy, low-pH soils are at risk for decline.

Implications for Further Study

Further investigations of red pine pocket mortality should focus on establishing cause and effect. Our studies have indicated low soil and fine-root Mn and Mg concentrations in acid soils may be the cause of fine-root mortality. It order to establish cause, it would be necessary to fulfill experimental requirements as outlined by Koch's postulates. One strategy would be to apply lime to mortality stands and to observe whether increasing soil pH results in increased soil and root Mn and Mg concentrations and in improving the health of declining trees. A magnesium-form of limestone would be recommended to compensate for Mg deficiencies in mortality pockets. Mn fertilization alone might also be tried to determine how much effect this nutrient is having on RPPM symptoms. Lange (1969) found that Mn-deficiency symptoms in *P. radiata* could

be corrected on a long-term basis with both foliar spray, and soil applications of $MnSO_4$.

Another step would be to acidify soils around healthy mature red pines and observe whether RPPM symptoms develop. Both Mn and Mg are involved in chlorophyll production and there may be a link to deficiencies of these nutrients to the symptoms of browning and stunted growth in needles.

Further investigations should also focus on the biotic factors associated with the disease. The often circular pattern of disease pockets suggests a strong biotic link. The only biotic factor examined in our study was in attempting to isolate pathogenic fungi from red pine roots. Unlike the Wisconsin studies, we were not able to isolate any significant pathogenic fungi even when using selective media for *Leptographium*. Although casual observations were made of bark beetle (*Ips pini*), pine root collar weevil *(Hylobius radicus*), Armillaria root rot *(Armillaria,* spp.), and Diplodia tip blight (*Sphaeropsis sapinea*) activity in some pockets, we did not quantify any of this information. In addition, the roots removed from disease as well as healthy plantations did not appear to have mycorrhizal infection. The role of mycorrhizae as a factor in the health of red pine stands should be examined further with regard to red pine pocket mortality.

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APPENDIX

Original Data
Soil, Needle, and Root Elemental Concentrations

Table 1. Element concentrations (ppm) of spodzolic soils from plantation stands at Huron-Manistee disease plots: Minnie Lake (ML1, ML2, and ML3) and Thin-A-Gain (TG4 and TG5); Huron-Manistee check plots (MLC and TGC); Roscommon check plots (R1 and R2); and Osmun Lake natural stand check plots (O1, O2, and O3); late July to early August 1992, 1993, and 1994.

Plot	Location*	Horizon+	pH	Elements (ppm) P	K	CA	MG	AL	FE	MN
ML1	C	O	4.5	17.0	17.0	182	9	95	54	24.0
ML2	C	O	4.1	6.5	17.0	182	9	211	55	2.9
ML3	C	O	4.3	38.0	29.5	218	9	123	84	28.0
TG4	C	O	4.3	24.5	13.5	153	8	121	62	33.0
TG5	C	O	4.5	200	13.5	153	8	135	70	12.4
	MEAN		4.34	57.20	18.10	177.40	8.60	137.07	65.00	20.06
	STD DEV.		0.15	72.13	5.91	24.21	0.49	39.08	11.10	10.95
ML1	E	O	4.1	7.5	17.0	146	9	81	52	5.7
ML2	E	O	4.2	6.0	17.0	146	9	137	49	16.2
ML3	E	O	4.4	40.0	17.0	182	9	107	65	24.0
TG4	E	O	4.3	34.0	22.0	153	8	140	69	34.0
TG5	E	O	4.4	103	22.0	153	8	140	71	35.0
	MEAN		4.28	38.10	19.00	155.60	8.60	120.99	61.20	22.98
	STD DEV.		0.12	35.21	2.45	13.57	0.49	23.77	9.00	11.06
ML1	O	O	4.4	41.5	21.0	218	9	142	82	39.0
ML2	O	O	4.4	7.0	17.0	182	9	111	49	21.0
ML3	O	O	4.5	45.5	22.0	191	8	162	86	67.8
TG4	O	O	4.3	87.5	22.0	191	8	159	83	29.0
TG5	O	O	4.2	110	22.0	115	8	185	82	13.3
	MEAN		4.36	58.20	20.80	179.10	8.40	151.72	76.40	34.02
	STD DEV.		0.10	36.20	1.94	34.51	0.49	24.60	13.78	18.92

Table 1. (Cont'd)

Plot	Location*	Horizon+	pH	Elements (ppm) P	K	CA	MG	AL	FE	MN
ML1	C	E	4.5	38.0	12.5	109	9	100	47	11.4
ML2	C	E	4.4	6.0	8.5	73	9	114	22	1.0
ML3	C	E	4.5	63.5	17.0	109	9	198	77	6.7
TG4	C	E	4.6	17.0	4.5	76	8	126	45	6.7
TG5	C	E	5.1	287	9.0	153	8	64	60	10.5
	MEAN		4.62	82.20	10.30	103.80	8.60	120.55	50.20	7.26
	STD DEV.		0.25	104.02	4.20	28.91	0.49	43.76	13.15	3.67
ML1	E	E	4.3	21.5	8.5	73	9	146	55	1.9
ML2	E	E	4.4	6.0	8.5	109	9	93	18	4.8
ML3	E	E	4.8	69.0	12.5	146	9	93	63	13.3
TG4	E	E	4.4	39.0	13.5	115	8	175	56	5.7
TG5	E	E	4.8	142	9.0	76	8	101	41	5.7
	MEAN		4.54	55.50	10.40	103.50	8.60	121.35	46.60	6.28
	STD DEV.		0.22	48.05	2.15	26.95	0.49	33.04	15.98	3.78
ML1	O	E	4.4	32.0	12.5	109	9	181	63	20.0
ML2	O	E	4.5	16.5	8.5	109	9	122	47	5.7
ML3	O	E	4.6	60.0	13.5	115	8	173	67	37.0
TG4	O	E	4.6	100	13.5	76	8	143	58	15.2
TG5	O	E	4.6	124	9.0	76	8	124	46	10.5
	MEAN		4.54	66.40	11.40	96.90	8.40	148.49	56.20	17.68
	STD DEV.		0.08	40.26	2.20	17.18	0.49	24.38	8.42	10.77

Table 1. (Cont'd)

Plot	Location*	Horizon+	pH	Elements (ppm) P	K	CA	MG	AL	FE	MN
ML1	C	B	5.1	71.0	8.5	109	9	52	54	2.9
ML2	C	B	4.8	20.0	8.5	73	9	122	50	1.0
ML3	C	B	4.8	124	12.5	73	9	124	43	2.9
TG4	C	B	4.9	18.5	4.5	76	8	43	31	5.7
TG5	C	B	5.7	349	9.0	229	8	19	77	1.9
	MEAN		5.06	116.30	8.60	111.70	8.60	71.99	51.00	2.88
	STD DEV.		0.34	122.36	2.54	60.00	0.49	42.97	15.17	1.58
ML1	E	B	4.8	37.0	8.5	73	9	93	51	
ML2	E	B	4.9	25.0	8.5	73	9	97	43	1.0
ML3	E	B	5.4	87.5	13.5	191	8	49	45	3.8
TG4	E	B	5.0	47.0	9.0	76	8	99	39	1.9
TG5	E	B	5.0	106	9.0	76	8	67	30	1.0
	MEAN		5.02	60.50	9.70	97.50	8.40	80.95	41.60	1.74
	STD DEV.		0.20	30.96	1.91	46.53	0.49	19.73	6.97	1.09
ML1	O	B	4.8	38.0	8.5	73	9	97	40	4.8
ML2	O	B	4.9	56.5	8.5	109	9	112	60	1.0
ML3	O	B	5.1	95.5	9.0	153	8	80	36	10.5
TG4	O	B	5.0	120	9.0	115	8	79	37	6.7
TG5	O	B	5.0	146	9.0	76	8	64	28	6.7
	MEAN		4.96	91.20	8.80	104.90	8.40	86.49	40.20	5.94
	STD DEV.		0.10	39.71	0.24	29.19	0.49	16.54	10.67	3.09

Table 1. (Cont'd)

Plot	Sample #	Horizon+	pH	Elements (ppm) P	K	CA	MG	AL	FE	MN
MLC	1	O	4.6	33.0	51.0	200	12	203	71	86.1
TGC	1	O	4.7	72.5	36.5	120	16	190	72	31.0
	MEAN		4.65	52.75	43.75	160.00	14.00	196.55	71.50	58.55
	STD DEV.		0.05	19.75	7.25	40.00	2.00	6.55	0.50	27.55
MLC	1	E	4.7	39.0	29.0	120	12	223	53	35.0
TGC	1	E	5.2	80.0	14.5	120	12	83	24	6.7
	MEAN		4.95	59.50	21.75	120.00	12.00	153.02	38.50	20.85
	STD DEV.		0.25	20.50	7.25	0.00	0.00	69.60	14.50	14.15
MLC	1	B	4.9	82.5	14.5	80	12	152	36	12.4
TGC	1	B	5.3	74.5	14.5	120	12	92	25	6.7
	MEAN		5.10	78.50	14.50	100.00	12.00	121.88	30.50	9.55
	STD DEV.		0.20	4.00	0.00	20.00	0.00	29.74	5.50	2.85
R1	1	O	4.1	11.0	35.5	320	13.5	283	116	55.2
R1	2	O	3.8	6.5	40.0	200	13.5	229	143	48.6
R1	3	O	3.9	7.5	35.5	120	18	339	105	31.0
R2	1	O	4.0	8.5	53.5	305	21	333	105	51.4
R2	2	O	4.0	8.5	44.5	229	21	322	116	65.6
R2	3	O	4.1	14.0	49.0	267	21	306	105	75.6
	MEAN		3.98	9.33	43.00	240.00	18.00	301.92	115.00	54.57
	STD DEV.		0.11	2.49	6.71	67.65	3.35	37.67	13.45	13.94

Table 1. (Cont'd)

Plot	Sample #	Horizon+	pH	Elements (ppm) P	K	CA	MG	AL	FE	MN
R1	1	E	4.6	18.5	18.0	160	18	306	89	21.0
R1	2	E	4.5	17.0	18.0	80	13.5	154	104	11.4
R1	3	E	4.3	12.5	12.0	191	21	284	104	7.6
R2	1	E	4.6	23.0	18.0	153	17	287	95	24.0
R2	2	E	4.6	21.5	18.0	153	21	248	94	25.0
R2	3	E	4.4	22.0	22.0	191	21	287	97	26.0
	MEAN		4.50	19.08	17.67	154.33	18.58	261.18	97.17	19.17
	STD DEV.		0.12	3.60	2.92	36.92	2.78	50.96	5.40	7.09
R1	1	B	5.0	49.0	13.5	80	13.5	173	28	4.8
R1	2	B	4.9	39.0	9.0	40	13.5	128	33	2.9
R1	3	B	5.0	41.0	9.0	191	21	132	31	2.9
R2	1	B	5.0	56.5	9.0	115	17	154	31	4.8
R2	2	B	5.1	31.0	9.0	115	17	111	32	3.8
R2	3	B	4.9	49.0	9.0	115	17	143	35	4.8
	MEAN		4.98	44.25	9.75	109.00	16.50	140.10	31.67	4.00
	STD DEV.		0.07	8.26	1.68	45.34	2.55	19.79	2.13	0.85
O1	1	O	3.7	6.0	43.5	120	12	240	40	1.0
O2	1	O	4.1	4.5	43.5	160	11	144	40	3.8
O3	1	O	4.2	5.0	36.5	200	11	151	37	11.4
	MEAN		4.00	5.17	41.17	160.00	11.33	178.36	39.00	5.40
	STD DEV.		0.22	0.62	3.30	32.66	0.47	43.40	1.41	4.39

Table 1. (Cont'd)

Plot	Sample #	Horizon+	pH	Elements (ppm) P	K	CA	MG	AL	FE	MN
O1	1	E	4.1	4.5	14.5	80	12	115	20	1.0
O2	1	E	4.4	10.0	22.0	120	11	177	42	4.8
O3	1	E	4.9	11.0	22.0	80	11	88	23	4.8
	MEAN		4.47	8.50	19.50	93.33	11.33	126.47	28.33	3.53
	STD DEV.		0.33	2.86	3.54	18.86	0.47	37.30	9.74	1.79
O1	1	B	4.9	26.5	22.0	120	11	159	44	1.0
O2	1	B	5.3	30.5	14.5	120	11	33	31	1.9
O3	1	B	5.4	29.0	43.5	120	11	42	40	1.0
	MEAN		5.20	28.67	26.67	120.00	11.00	77.82	38.33	1.30
	STD DEV.		0.22	1.65	12.29	0.00	0.00	57.27	5.44	0.42

*Location = center of pocket (C), edge of the decline (E), and outside the edge of the decline (O), within Huron-Manistee disease plots.
+Horizon = organic horizon (O), albic horizon (E), and spodic (B) horizon.

Table 2. Needle element data (ppm) for healthy (asymptomatic for RPPM) and unhealthy (symptomatic for RPPM) red pines for 1- and 2-year-old needles at Minnie Lake (ML1, ML2 and ML3) and Thin-A-Gain (TG4, TG5, TGC), Huron-Manistee National Forest, July-August 1993.

Plot	Crown Level*	Tree #	Element (ppm) ZN	CD	MN	B	FE	PB	AL	MG	CU	CA	K	NI	CR
Minnie Lake Site, 1-year-needles, Unhealthy red pines															
ML3	TOP	21	23.3	-0.52	269	15.0	28.7	0.44	215	676	4.05	1273	4700	4.24	1.01
ML3	TOP	21	19.0	-1.96	96	18.8	17.4	6.36	81	837	2.48	828	4132	3.47	1.05
ML3	BOT	21	11.5	0.78	113	7.0	14.2	4.76	41	762	3.81	796	4849	-0.05	-5.62
ML3	BOT	21	22.4	0.71	119	13.0	17.4	6.21	51	864	3.68	889	4574	4.31	0.66
ML3	TOP	31	23.8	1.59	292	20.4	35.9	4.06	199	823	3.42	883	4155	9.02	0.82
ML3	TOP	31	20.2	-0.44	311	12.8	33.6	-2.62	235	768	2.97	1069	4021	4.01	-0.64
ML3	MID	31	16.0	-2.43	289	10.8	24.5	-2.87	219	648	2.40	1040	3860	2.77	-2.16
ML3	MID	31	20.4	-1.97	349	9.5	21.7	-0.33	192	807	2.93	1248	3863	3.53	-1.21
ML3	BOT	31	31.9	-3.65	346	8.7	17.9	-11.5	214	755	3.35	1256	3978	1.89	-2.84
ML3	BOT	31	21.2	-2.80	199	9.3	21.6	-0.89	202	618	3.73	1159	4003	4.67	-0.75
ML2	TOP	91	15.9	-4.51	164	15.6	44.9	-13.3	206	636	2.91	797	4133	0.54	-4.63
ML2	TOP	91	28.6	3.07	225	29.0	53.8	17.7	162	836	4.01	863	4895	5.13	3.91
ML2	MID	91	27.9	2.08	266	18.3	53.7	12.3	127	827	6.34	1407	5116	3.69	3.20
ML2	MID	91	30.6	2.44	261	24.4	57.8	18.2	119	805	4.80	1293	4575	5.41	3.91
ML2	BOT	91	29.6	4.12	338	19.9	51.6	13.2	133	882	5.67	1694	5921	4.53	3.69
ML2	BOT	91	21.5	-2.49	264	15.7	29.7	5.46	220	688	4.82	1411	7181	0.39	-0.68
ML2	TOP	101	23.7	-0.88	319	21.1	82.2	0.82	340	1078	2.33	2280	1485	0.44	-0.75
ML2	TOP	101	20.4	-3.77	326	22.7	72.9	-1.92	363	961	1.73	2270	2922	-0.78	-1.97
ML2	BOT	101	28.3	-1.83	436	21.1	109	6.67	351	1186	2.26	3061	2742	0.28	-0.15
ML2	BOT	101	27.6	-4.79	401	22.3	84.2	1.34	391	1114	2.38	2758	3284	0.24	-3.01
ML2	TOP	111	21.4	-0.10	123	33.1	49.4	0.50	253	668	6.71	854	6758	5.62	0.03
ML2	TOP	111	26.0	1.76	150	32.9	61.7	7.57	230	773	5.25	1098	6277	5.72	1.93
ML2	BOT	111	40.0	1.79	239	25.0	49.9	13.0	188	845	5.68	1730	6006	4.33	3.21
ML2	BOT	111	32.1	4.45	225	30.3	58.2	19.2	153	888	4.80	1842	6842	6.94	3.92

Table 2. (Cont'd)

Plot	Crown Level*	Tree #	Element (ppm) ZN	CD	MN	B	FE	PB	AL	MG	CU	CA	K	NI	CR
ML3	TOP	11	19.9	-0.92	249	10.5	30.6	2.43	88	843	2.32	788	4784	3.91	-3.01
ML3	TOP	11	22.9	-0.31	257	9.9	29.6	5.06	108	827	2.97	776	4313	3.21	-5.98
ML3	BOT	11	20.4	-0.39	351	7.4	18.9	9.28	103	748	3.69	1091	4909	1.80	-3.27
ML3	BOT	11	24.2	0.06	382	10.3	26.2	7.56	110	786	3.24	1318	4388	1.83	-5.55
ML1	TOP	238	21.2	0.43	165	14.4	54.7	17.4	96	777	2.73	602	3418	3.56	2.44
ML1	TOP	238	16.0	1.94	122	16.6	54.2	18.5	81	672	6.90	343	3939	4.04	4.80
ML1	BOT	238	19.4	5.24	185	15.1	52.8	15.5	77	755	4.69	672	3571	3.46	7.28
ML1	BOT	238	17.8	3.54	189	13.9	32.5	16.3	82	774	4.50	662	3994	2.60	2.76
ML1	TOP	197	12.3	-0.05	120	13.4	60.9	0.83	130	628	2.31	269	4343	6.21	6.81
ML1	TOP	197	13.4	-2.94	120	11.0	38.9	3.11	146	619	2.10	323	4176	1.16	-3.16
ML1	BOT	197	11.5	-4.59	118	8.0	33.6	-7.14	148	605	2.42	333	4417	0.37	-3.53
ML1	BOT	197	10.3	-3.43	128	7.8	32.9	0.87	150	567	1.78	374	3941	-0.13	-4.71
	MEAN		22.0	-0.30	236	16.5	43.3	5.39	172	787	3.70	1149	4457	3.26	-0.61
	STD DEV.		6.59	2.69	95	7.35	21.6	8.33	88	139	1.39	659	1163	2.45	3.53
Minnie Lake Site, 1-year-needles, Healthy red pines															
ML1	TOP	42	28.0	2.20	251	11.5	64.5	0.29	245	788	4.76	1073	4389	5.23	0.11
ML1	TOP	42	27.1	2.56	251	9.6	71.6	3.59	260	829	5.56	1211	4632	4.88	1.02
ML1	MID	42	31.6	4.10	316	8.6	63.7	-0.97	201	961	6.24	1587	4423	2.84	1.14
ML1	MID	42	41.8	10.2	354	16.6	146	16.9	227	992	7.38	1673	4903	5.09	6.81
ML1	BOT	42	35.6	-1.06	409	8.5	37.3	4.24	267	1074	5.40	2327	5265	3.75	0.90
ML1	BOT	42	28.6	-1.36	506	5.6	46.3	3.84	237	816	6.03	1571	4360	2.72	-0.81
ML1	TOP	52	32.0	-0.72	340	11.2	32.5	2.12	309	771	5.70	1541	4147	3.21	-0.86
ML1	TOP	52	21.1	-1.47	230	9.5	44.4	-1.81	344	610	4.36	1065	4492	2.18	-2.29
ML1	MID	52	27.4	-2.94	304	6.6	33.5	-0.71	332	684	4.42	1531	3793	1.58	-3.23
ML1	MID	52	35.4	-1.30	414	12.1	29.4	-1.40	272	903	4.81	1807	4493	4.09	-0.88
ML1	BOT	52	34.5	0.26	366	15.2	23.7	11.0	228	853	4.86	1541	4824	5.35	2.54
ML1	BOT	52	36.9	1.64	483	15.7	34.8	15.7	194	1058	4.74	1829	5059	5.46	3.33

Table 2. (Cont'd)

Plot	Crown Level*	Tree #	Element (ppm) ZN	CD	MN	B	FE	PB	AL	MG	CU	CA	K	NI	CR
ML1	TOP	62	28.6	3.30	540	21.2	39.9	16.5	182	859	4.24	1377	4762	9.72	3.43
ML1	TOP	62	25.0	-1.15	516	11.8	52.6	-3.95	282	782	3.91	1350	4570	7.11	1.72
ML1	MID	62	24.5	-2.86	556	7.8	28.8	-1.38	242	786	4.10	1421	3880	2.71	-1.84
ML1	MID	62	22.0	-3.51	609	6.0	66.4	-8.88	262	795	3.41	1601	3860	2.50	-2.10
ML1	BOT	62	15.3	-4.38	359	11.8	36.8	-14.8	221	665	4.73	689	4751	1.80	-3.94
ML1	BOT	62	41.8	1.82	570	15.6	62.5	16.4	150	824	5.86	1331	5200	5.82	2.30
	MEAN		29.8	0.30	410	11.4	50.8	3.15	247	836	5.06	1474	4545	4.22	0.41
	STD DEV.		7.08	3.47	120	4.17	28.0	9.04	50	126	1.05	353	436	2.08	2.71
Thin-A-Gain Site, 1-year-needles, Unhealthy red pines															
TG5	TOP	134	23.6	1.96	220	16.7	34.5	3.60	103	747	3.13	1254	4945	1.24	-1.29
TG5	TOP	134	24.7	1.39	263	16.8	25.5	0.61	93	821	3.20	1204	5273	2.28	-0.37
TG5	BOT	134	28.5	1.96	358	15.8	30.1	6.44	117	868	3.44	1691	5465	2.40	0.03
TG5	BOT	134	26.0	2.13	249	16.2	16.4	1.63	88	780	2.32	1158	5785	1.90	-0.20
TG5	TOP	88	16.5	0.40	150	21.7	-1.6	6.21	116	737	3.66	878	5477	0.87	-5.05
TG5	TOP	88	15.0	0.06	194	24.2	7.6	5.94	134	671	3.83	844	5458	1.61	-4.77
TG5	BOT	88	12.9	-11.0	150	16.2	-10.1	-1.79	129	752	2.71	959	5712	1.46	-1.79
TG5	BOT	88	17.8	-0.60	187	19.9	5.1	3.32	128	787	4.21	1077	6298	-0.64	-2.50
TG5	TOP	135	19.5	0.98	193	8.9	24.8	5.23	111	675	3.27	951	5256	2.66	2.07
TG5	TOP	135	20.4	2.66	131	7.5	19.7	3.38	120	721	3.26	883	4685	2.70	0.91
TG5	BOT	135	20.6	1.03	179	2.1	23.4	3.47	121	587	4.17	1254	5600	2.43	0.73
TG5	BOT	135	19.5	-0.16	389	-11.4	26.3	5.63	126	768	3.60	965	4730	1.42	0.78
TG4	TOP	178	23.4	-0.67	199	8.0	30.4	-0.70	68	668	2.81	687	4662	-0.03	0.46
TG4	TOP	178	19.2	-0.15	214	15.2	30.1	4.37	70	721	3.99	669	5051	1.43	-1.19
TG4	BOT	178	17.5	0.69	287	10.2	28.2	2.63	74	879	3.19	796	4655	0.41	-3.05
TG4	BOT	178	18.9	-1.31	196	14.0	28.9	5.02	66	773	3.27	605	5678	1.40	-2.67

Table 2. (Cont'd)

Plot	Crown Level*	Tree #	Element (ppm) ZN	CD	MN	B	FE	PB	AL	MG	CU	CA	K	NI	CR
	MEAN		20.2	-0.04	222	12.6	20.0	3.44	104	747	3.38	992	5296	1.47	-1.12
	STD DEV.		4.13	3.14	72	8.6	13.0	2.46	24	76	0.52	276	479	0.96	2.06
Thin-A-Gain Site, 1-year-needles, Healthy red pines															
TG4	TOP	63	19.7	-0.18	317	28.1	34.2	1.49	120	784	2.26	803	4184	3.36	2.63
TG4	TOP	63	20.0	-0.15	198	17.9	49.7	1.73	115	671	2.11	457	5081	4.90	-0.66
TG4	MID	63	21.3	2.38	293	17.1	22.9	2.38	98	788	1.79	676	5176	3.13	-0.75
TG4	MID	63	19.1	-2.21	305	16.5	24.2	2.18	94	806	2.13	724	5101	3.91	-0.94
TG4	BOT	63	24.5	-1.04	303	20.6	24.5	2.46	116	755	2.39	703	5199	3.60	-0.18
TG4	BOT	63	26.1	-0.17	306	17.1	34.5	0.72	107	720	1.64	834	5137	2.70	-0.63
TG5	TOP	109	42.9	1.40	392	11.7	47.1	0.55	174	948	3.21	1319	5173	5.04	-0.62
TG5	TOP	109	31.4	-0.56	477	10.3	53.0	3.84	196	968	3.80	1635	4458	5.07	0.68
TG5	MID	109	21.8	1.66	363	13.9	21.2	-0.93	118	797	2.57	982	4448	2.88	-0.91
TG5	BOT	109	26.0	2.70	422	15.8	27.5	1.53	127	893	2.75	1237	4781	2.12	-0.64
TG5	BOT	109	18.1	0.30	310	12.5	37.8	0.95	111	766	3.27	881	4679	2.86	-0.14
TG5	TOP	48	26.9	0.56	446	17.0	50.5	0.79	205	731	6.29	1532	5687	11.3	0.42
TG5	TOP	48	19.7	0.66	745	26.1	76.9	-0.75	426	609	2.95	2348	3792	4.10	-0.52
TG5	MID	48	26.5	0.65	611	12.9	49.5	2.55	185	770	4.88	2020	6802	9.38	1.08
TG5	MID	48	26.6	-0.21	455	15.2	61.4	0.32	145	690	5.12	1767	6397	8.64	2.55
TG5	BOT	48	36.6	0.03	765	17.1	39.3	0.63	184	961	4.76	2265	7258	5.77	0.47
TG5	BOT	48	29.0	0.51	801	17.2	42.5	0.09	163	874	5.30	2505	7622	7.76	1.32
TG4	BOT	207	8.2	-10.3	431	18.2	1.2	5.29	104	673	1.31	950	2390	-3.19	-1.52
TG4	TOP	207	10.1	-11.2	146	18.6	5.3	-1.14	114	507	2.03	460	4250	0.25	-1.84
TG4	MID	207	12.6	2.62	347	17.4	31.3	3.56	78	635	2.29	852	4005	1.48	4.61
TG4	MID	207	11.9	0.08	182	15.3	18.9	1.07	56	571	2.89	564	5926	1.64	4.71
TG4	BOT	207	15.7	2.25	568	23.9	61.0	5.76	160	764	4.66	1747	3758	1.60	5.28
TG4	TOP	207	12.3	0.19	123	13.2	21.7	4.06	88	547	2.33	423	4617	3.18	-5.57
TG4	TOP	146	27.5	3.51	448	22.6	57.7	7.43	190	822	6.33	1608	6258	11.3	6.44
TG4	TOP	146	43.4	4.67	397	32.7	61.3	13.9	202	765	7.22	1414	7270	14.7	7.00

Table 2. (Cont'd)

Plot	Crown Level*	Tree #	Element (ppm) ZN	CD	MN	B	FE	PB	AL	MG	CU	CA	K	NI	CR
TG4	MID	146	24.5	0.65	456	14.9	43.5	9.15	158	825	5.76	1554	6265	9.58	5.91
TG4	MID	146	24.9	0.01	703	19.6	11.8	8.39	206	913	4.17	2220	4599	0.82	-4.78
TG4	BOT	146	22.1	0.07	331	21.0	-3.3	9.03	137	835	7.05	1345	6669	-0.38	-4.88
TG4	BOT	146	25.9	-0.66	323	15.1	32.7	6.23	130	909	6.03	2066	7231	9.36	5.66
TGC	TOP	32	25.4	0.68	310	21.1	46.8	2.18	221	657	4.97	1195	6400	12.8	-0.97
TGC	TOP	32	22.2	0.44	436	16.9	46.5	4.86	192	606	3.87	1478	5384	7.63	-0.93
TGC	MID	32	36.3	-0.09	414	19.1	36.3	4.03	135	665	3.90	1351	5577	7.55	-0.08
TGC	MID	32	30.4	1.51	521	15.3	28.0	1.84	134	823	3.79	1816	5776	6.52	0.18
TGC	BOT	32	27.3	1.96	720	18.9	48.1	3.71	143	847	2.42	2321	4986	5.29	0.44
TGC	BOT	32	25.8	1.93	498	17.1	25.1	4.53	134	853	3.65	2018	5996	4.99	-0.55
TGC	TOP	12	26.9	0.61	432	18.3	46.5	0.59	182	735	3.62	1457	5444	6.18	-1.13
TGC	TOP	12	30.4	3.35	378	26.7	43.6	3.95	241	683	4.59	1276	4972	9.09	0.50
TGC	MID	12	24.2	2.25	454	13.5	45.2	2.58	183	680	3.81	1557	4850	5.75	-0.55
TGC	MID	12	25.6	1.74	535	15.2	46.2	2.70	199	718	3.21	1785	4487	4.90	0.04
TGC	BOT	12	31.1	0.82	385	16.1	26.6	1.37	154	753	9.48	1878	5671	4.80	-1.35
TGC	BOT	12	30.2	2.02	534	17.3	25.8	2.92	143	813	3.19	2153	5303	4.65	-0.44
TGC	TOP	22	17.0	-3.74	308	20.5	35.5	5.67	130	638	3.00	760	4778	6.33	5.62
TGC	TOP	22	17.2	-0.94	284	19.6	28.8		87	637		815	4960	0.90	4.97
TGC	MID	22	34.9	-1.47	150	20.9	30.8	5.20	184	655	5.43	1452	5295	7.28	9.45
TGC	MID	22	20.8	-3.99	366	17.4	44.5	6.83	205	668	4.14	1278	5179	7.82	6.10
TGC	BOT	22	18.8	-5.55	253	18.7	28.0	7.58	99	663	3.63	784	5454	6.33	6.23
TGC	BOT	22	21.4	0.23	249	22.3	26.8	5.91	87	550	3.21	600	5435	2.80	6.06
	MEAN		24.3	0.00	408	18.2	36.1	3.47	152	743	3.90	1359	5323	5.29	1.23
	STD DEV.		7.63	2.96	161	4.32	16.2	3.10	60	113	1.69	580	1028	3.59	3.39
Minnie Lake Site, 2-year-needles, Unhealthy red pines															
ML3	TOP	21	18.8	-1.00	240	19.9	88.1	5.78	264	910	2.25	1782	2581	2.39	1.45
ML3	TOP	21	24.4	1.13	208	24.5	60.2	11.5	174	1085	2.05	1717	2875	3.20	1.53

Table 2. (Cont'd)

Plot	Crown Level*	Tree #	Element (ppm) ZN	CD	MN	B	FE	PB	AL	MG	CU	CA	K	NI	CR
ML3	BOT	21	11.9	-2.58	221	12.5	45.2	7.57	119	899	2.49	1547	3547	-0.50	-5.37
ML3	BOT	21	20.5	0.71	183	16.6	66.0	8.63	168	887	2.52	1660	3830	3.38	1.09
ML3	TOP	31	21.3	0.60	655	34.6	73.0	7.74	286	840	2.58	1799	2126	4.13	1.12
ML3	TOP	31	20.6	-0.74	734	17.4	62.9	4.54	312	794	2.41	1963	2279	1.42	-0.97
ML3	MID	31	24.1	-3.89	1015	11.3	37.2	-5.32	316	783	1.81	3374	2382	-0.03	-2.02
ML3	MID	31	20.0	-2.98	687	11.6	44.9	-1.38	315	652	1.73	2223	2479	1.10	-0.78
ML3	BOT	31	26.3	-2.38	1057	15.0	42.7	-1.86	312	874	2.04	2653	2224	0.23	-2.09
ML3	BOT	31	27.9	2.37	1001	22.2	74.0	6.72	274	882	2.68	2837	2790	2.07	0.91
ML2	TOP	91	28.8	0.54	308	27.5	98.5	6.73	225	760	2.99	1285	2663	1.51	1.12
ML2	TOP	91	23.3	2.25	291	40.0	82.5	15.5	213	841	3.30	1147	3393	3.35	4.78
ML2	MID	91	31.7	3.53	564	34.9	67.7	22.3	188	931	3.14	2362	3693	3.18	4.44
ML2	MID	91	27.7	1.62	485	30.7	80.8	14.7	234	865	3.19	1971	3383	1.94	2.77
ML2	BOT	91	35.4	3.17	736	28.0	102	15.4	243	1133	3.53	3287	3837	3.14	4.74
ML2	BOT	91	27.6	-1.48	542	23.7	86.2	8.13	308	931	3.64	2586	4930	0.74	-0.38
ML2	TOP	101	25.9	-3.93	410	15.6	87.3	0.04	342	1040	1.65	3210	2542	0.89	-1.38
ML2	TOP	101	34.4	-2.65	329	21.1	75.5	1.97	379	952	1.82	2465	2808	-0.30	-1.65
ML2	BOT	101	29.5	-4.86	460	15.3	142	7.34	401	1148	2.24	3929	2795	-0.09	-0.44
ML2	BOT	101	31.9	-1.76	430	20.0	101	3.16	329	1204	2.23	3660	2569	-0.34	-0.86
ML2	TOP	111	23.4	-0.33	274	21.7	74.4	-1.75	307	773	3.06	2061	4382	-0.10	-0.36
ML2	TOP	111	31.0	0.48	282	32.6	77.8	10.3	275	825	3.25	2001	4366	1.15	0.81
ML2	BOT	111	46.9	1.99	557	29.6	95.9	19.7	239	1247	3.29	4328	3983	1.98	3.50
ML2	BOT	111	48.4	4.35	610	29.0	113	20.0	233	1251	3.57	4858	3987	2.00	3.63
ML3	TOP	11	22.9	1.60	761	10.2	66.4	8.95	219	997	2.68	2411		1.77	-2.36
ML3	TOP	11	18.7	-0.01	598	6.8	89.7	7.32	211	916	2.71	1924	2985	0.49	-5.32
ML3	BOT	11	51.1	-0.55	1077	21.3	77.0	5.06	319	1040	3.95	3706	2890	1.90	1.39
ML3	BOT	11	39.7	-0.20	880	8.6	75.8	12.3	229	909	2.40	2843	3275	-0.72	-5.10
ML1	TOP	238	21.0	2.73	382	23.5	86.6	20.4	164	964	2.29	1263	2157	2.86	3.65
ML1	TOP	238	14.0	3.28	353	21.2	87.3	15.9	168	879	3.70	1031	2483	1.98	3.85
ML1	BOT	238	17.7	3.05	374	20.0	76.5	16.0	187	863	3.14	1322	2308	2.19	4.11
ML1	BOT	238	8.8	-0.01	306	12.3	81.1	4.92	243	630	2.97	1126	2131	1.80	3.75

Table 2. (Cont'd)

Plot	Crown Level*	Tree #	Element (ppm) ZN	CD	MN	B	FE	PB	AL	MG	CU	CA	K	NI	CR
ML1	TOP	197	29.0	-0.46	305	17.3	90.1	1.66	213	716	2.13	755	2744	1.56	0.34
ML1	TOP	197	18.3	-3.16	397	17.9	99.4	-1.62	272	833	2.50	1025	3647	0.15	-2.69
ML1	BOT	197	14.5	-3.90	381	10.9	65.5	-3.82	240	771	1.35	1091	2532	-1.13	-4.21
ML1	BOT	197	11.6	-3.22	223	7.0	75.2	-4.40	233	661	1.58	801	2528	-0.84	-3.84
	MEAN		25.8	-0.19	509	20.3	79.1	7.50	254	908	2.64	2222	3032	1.35	0.25
	STD DEV.		9.9	2.48	259	8.4	20.3	7.56	64	157	0.67	1044	736	1.39	2.98
Minnie Lake Site, 2-year-needles, Healthy red pines															
ML1	TOP	42	32.3	1.89	438	13.5	95.9	2.34	320	886	3.63	1483	2956	2.08	0.71
ML1	TOP	42	31.6	3.58	427	15.8	81.5	3.49	336	896	3.81	1670	2603	1.42	1.35
ML1	MID	42	35.9	7.06	487	16.7	92.0	9.54	286	978	4.33	2152	2761	1.48	3.79
ML1	MID	42	58.4	11.2	694	26.9	87.2	26.7	339	1291	6.79	2913	3356	1.59	6.77
ML1	BOT	42	46.5	-1.28	721	12.2	55.4	9.54	294	1176	3.00	3561	2149	0.43	-0.21
ML1	BOT	42	53.8	-1.17	971	11.4	90.5	5.84	336	1078	3.87	3064	2406	1.25	-0.61
ML1	TOP	52	25.6	-1.47	552	15.7	59.5	3.76	388	773	3.23	2051	2422	-0.72	-1.54
ML1	TOP	52	31.8	-2.36	578	15.2	80.6	0.62	422	870	2.87	2060	2620	0.29	-2.31
ML1	MID	52	53.7	1.13	718	21.0	52.6	7.50	349	1004	2.65	3129	2555	1.67	0.75
ML1	MID	52	34.5	-0.03	654	20.8	46.7	9.25	315	848	2.78	2249	2615	1.96	1.98
ML1	BOT	52	36.9	0.72	619	19.9	63.4	15.9	283	1000	2.52	2214	3018	3.91	4.01
ML1	BOT	52	50.2	3.43	1035	23.2	59.8	18.8	232	1318	2.19	3575	2604	4.61	4.76
ML1	TOP	62	24.7	-0.89	906	17.8	70.4	-1.66	354	815	2.58	1984	2413	0.74	-0.32
ML1	TOP	62	22.9	-1.67	945	22.6	68.6	0.21	378	853	2.56	1796	2445	0.60	-1.02
ML1	MID	62	26.7	-2.56	1141	11.8	53.9	-7.65	335	773	2.32	2563	2212	-0.32	-2.34
ML1	MID	62	28.9	-3.93	1217	13.5	53.9	-10.7	353	878	1.98	2748	2365	-0.46	-3.71
ML1	BOT	62	36.2	1.49	1391	24.1	84.1	20.5	257	1049	2.96	2517	2890	2.66	4.76
ML1	BOT	62	33.4	1.98	1416	26.7	71.8	21.6	275	979	3.15	2556	3292	2.72	3.40

Table 2. (Cont'd)

Plot	Crown Level*	Tree #	ZN	CD	MN	B	FE	PB	AL	MG	CU	CA	K	NI	CR
			Element (ppm)												
	MEAN		36.9	0.95	828	18.3	70.4	7.5	325	970	3.18	2462	2649	1.44	1.12
	STD DEV.		10.9	3.69	314	5.05	15.6	10.1	48	162	1.10	609	340	1.43	2.93
Thin-A-Gain Site, 2-year-needles, Unhealthy red pines															
TG5	TOP	134	21.4	3.38	489	20.5	62.6	4.15	252	868	1.58	1637	2868	-2.10	-0.82
TG5	TOP	134	20.8	2.92	370	19.2	85.2	1.87	238	753	2.31	1524	3227	-0.09	3.46
TG5	BOT	134	23.3	2.22	447	17.1	83.4	3.02	246	795	1.56	2147	2622	-2.03	-0.25
TG5	BOT	134	15.5	2.18	371	20.1	93.5	0.84	259	670	1.91	1625	2773	-0.37	1.41
TG5	TOP	88	6.35	-1.31	195	24.8	9.1	4.21	235	628	1.89	1370	3558	-3.98	-4.99
TG5	TOP	88	13.3	0.66	201	22.6	28.5	9.94	218	647	2.02	1250	63	-2.48	-4.81
TG5	BOT	88	7.6	-9.22	282	19.9	30.8	1.35	290	736	1.25	1752	3248	-3.19	-1.71
TG5	BOT	88	12.4	-0.01	260	22.3	17.1	5.28	254	635	2.13	1613	3709	-4.25	-5.18
TG5	TOP	135	19.5	3.70	364	13.1	50.4	5.26	291	687	2.35	1845	3584	0.59	1.13
TG5	TOP	135	16.5	2.10	279	7.4	57.6	3.59	303	664	2.50	1736	3772	0.33	0.89
TG5	BOT	135	29.6	1.72	360	4.6	52.2	4.67	312	727	2.84	2382	4173	0.08	0.93
TG5	BOT	135	23.3	-1.46	387	-11.4	46.3	3.98	255	731	2.22	1935	3498	0.55	0.73
TG4	TOP	178	8.2	2.19	462	6.7	70.4	4.86	115	708	2.50	1360	3902	-0.21	0.73
TG4	TOP	178	10.8	-0.11	386	7.7	72.2	3.98	129	691	2.50	1100	4024	0.36	-2.53
TG4	BOT	178	13.4	-0.61	793	19.9	79.1	10.5	146	939	2.49	2168	3248	0.91	-2.38
TG4	BOT	178	15.0	-1.13	634	10.0	60.0	6.43	126	977	2.43	1778	389	0.06	-2.64
	MEAN		16.0	0.45	392	14.0	56.1	4.62	229	741	2.16	1701	3261	-0.99	-1.00
	STD DEV.		6.5	3.09	154	9.4	24.9	2.64	65	105	0.43	347	965	1.73	2.58
Thin-A-Gain Site, 2-year-needles, Healthy red pines															
TG5	TOP	109	23.9	-0.70	664	22.6	73.4	1.74	252	992	1.73	1968	2760	2.02	0.23
TG5	TOP	109	24.2	-1.58	744	28.0	94.4	4.31	306	1064	2.21	2373	2439	2.45	1.74

Table 2. (Cont'd)

Plot	Crown Level*	Tree #	Element (ppm) ZN	CD	MN	B	FE	PB	AL	MG	CU	CA	K	NI	CR
TG5	MID	109	18.1	1.86	691	18.0	68.3	2.73	259	903	1.640	1934	2440	0.62	0.04
TG5	MID	109	14.8	2.11	506	20.1	60.2	2.19	253	729	1.54	1269	2835	-0.60	-0.63
TG5	BOT	109	18.4	3.23	746	23.0	91.0	4.24	232	920	1.75	1964	2611	-0.26	-0.32
TG5	BOT	109	20.4	-0.33	669	21.3	80.9	2.27	246	878	1.71	1817	3018	1.52	0.18
TG5	TOP	48	25.1	-0.42	457	16.1	56.1	-1.06	225	700	7.10	1638	5997	11.6	-1.07
TG5	TOP	48	27.8	0.65	937	17.5	202	0.17	310	757	2.80	2507	4088	43.0	43.2
TG5	MID	48	28.6	0.31	1292	26.5	81.7	1.82	380	819	2.40	3610	3621	3.03	1.57
TG5	MID	48	27.2	3.00	1077	22.8	45.2	2.82	271	771	2.04	2847	3488	0.11	0.09
TG5	BOT	48	43.1	0.09	1945	29.8	79.8	5.96	344	1016	2.48	4947	3281	0.71	2.10
TG5	BOT	48	39.1	0.49	1543	24.6	88.8	1.40	377	997	2.51	3874	3712	1.39	1.50
TG4	TOP	207	6.45	-8.15	219	23.5	46.6	-0.21	171	567	1.34	792	2769	-3.19	0.63
TG4	TOP	207	0.26	-11.4	181	18.3	31.6	1.27	157	443	0.97	630	2257	-3.32	-1.89
TG4	MID	207	4.82	-2.41	361	21.0	45.1	5.10	146	590	1.73	1046	2559	-3.00	1.49
TG4	MID	207	9.88	0.83	452	15.1	60.4	5.52	129	577	2.99	1350	3087	0.16	4.83
TG4	BOT	207	13.2	3.31	859	18.6	93.3	11.1	196	763	2.79	2632	2758	0.75	6.37
TG4	BOT	207	7.38	-9.76	547	20.1	26.0	2.52	156	758	0.86	1762	2588	-3.90	-1.71
TG4	TOP	146	28.2	2.69	884	26.7	81.0	15.5	334	929	3.47	2403	3722	5.07	6.78
TG4	TOP	146	28.1	5.88	763	26.2	87.1	16.6	324	762	3.49	1833	4318	6.42	6.81
TG4	MID	146	37.5	-0.76	1026	16.8	58.2	11.7	323	1153	2.68	3641	3306	3.29	6.30
TG4	MID	146	28.1	-0.20	865	21.9	66.8	12.1	290	877	3.22	2401	3569	4.03	6.20
TG4	BOT	146	29.7	-0.17	1144	27.3	11.2	16.1	232	1144	2.60	3039	3633	-3.58	-4.53
TG4	BOT	146	34.3	0.87	1135	20.6	49.1	13.9	231	1161	2.93	4000	4272	3.19	5.79
TGC	TOP	32	24.8	1.45	1034	25.2	107	4.22	318	776	2.58	2938	3518	5.10	-0.43
TGC	TOP	32	19.4	-2.06	707	21.3	101	3.12	360	600	2.14	2052	3572	0.91	-0.80
TGC	MID	32	25.4	1.88	717	21.7	61.8	3.61	203	677	2.12	1750	3247	1.69	0.11
TGC	MID	32	36.8	1.67	1360	21.1	110	2.43	326	864	2.08	3461	3141	1.97	0.58
TGC	BOT	32	31.9	1.25	1320	18.7	69.3	3.71	229	921	1.94	3422	2722	1.16	0.07
TGC	BOT	32	31.0	1.11	1143	20.3	85.6	3.92	218	917	3.63	3024	3514	1.03	0.11
TGC	TOP	12	37.2	1.69	708	28.8	74.5	0.28	323	679	2.14	2041	2936	2.02	-0.40
TGC	TOP	12	25.3	-0.14	724	22.2	68.0	2.73	273	729	2.25	2025	3090	2.73	-0.15

Table 2. (Cont'd)

Plot	Crown Level*	Tree #	Element (ppm) ZN	CD	MN	B	FE	PB	AL	MG	CU	CA	K	NI	CR
TGC	MID	12	31.1	0.13	886	19.1	89.0		258	742	5.00	2707	2760	80.6	0.18
TGC	MID	12	43.5	2.23	1041	15.6	67.9	6.20	290	785	1.77	2912	2960	1.30	0.09
TGC	BOT	12	35.7	0.71	1201	20.4	80.6	3.37	290	897	1.72	3954	2648	0.80	-0.52
TGC	BOT	12	38.4	0.88	1185	22.5	63.9	2.42	258	961	1.76	3693	2660	0.23	-0.38
TGC	TOP	22	9.06	-3.16	555	15.2	110	10.4	289	619	1.84	1311	2660	3.45	6.31
TGC	TOP	22	13.4	0.39	512	17.8	57.8	8.67	165	611	2.91	1204	2736	1.52	5.14
TGC	MID	22	27.9	-3.06	601	25.4	42.1	9.16	277	661	2.76	2198	2722	3.78	6.20
TGC	MID	22	23.5	0.60	994	33.2	85.4	19.8	287	733	2.74	2100	2898	5.69	7.93
TGC	BOT	22	14.8	-4.02	678	15.9	56.9	8.63	184	681	1.79	1514	3106	2.84	5.89
TGC	BOT	22	18.7	-2.03	813	20.6	43.8	9.42	146	741	2.32	1643	2975	0.96	4.22
	MEAN		24.4	-0.26	854	21.7	72.7	5.90	258	806	2.44	2386	3167	4.51	2.85
	STD DEV.		10.7	3.29	354	4.2	30.4	5.20	66	169	1.06	979	670	13.9	7.08

*Crown Level = Top (TOP), middle (MID), or bottom (BOT) one-third of the tree crown. In some cases only the top and bottom one-half of the tree crown was sampled.

Table 3. Root element concentrations for fine roots (< 3 mm in diameter) and large roots (3 to 5 mm in diameter) from plantation stands at Huron-Manistee disease plots: Minnie Lake (ML1, ML2, and ML3) and Thin-A-Gain (TG4 and TG5); Huron-Manistee check plots (MLC and TGC); Roscommon check plots (R1, R2, R3); and Osmun Lake natural stand check plots (O1, O2, O3), August, 1992, 1993, and 1994.

	Elements (ppm)											
Plot	**ZN**	**CD**	**MN**	**B**	**FE**	**AL**	**MG**	**CU**	**CA**	**K**	**NI**	**CR**
Huron-Manistee Disease Plots, Edge Location, Fine Roots < 3 mm												
TG5	45.7	-6.05	231	3.89	330	2888	1053	14.68	4107	687	-1.70	1.66
TG5	39.9	-2.88	138	3.08	344	2230	653	7.73	2159	772	0.06	-14.97
TG4	70.9	4.14	244	7.91	713	2207	977	8.18	3084	3167	1.35	3.05
TG5	56.7	-1.44	168	7.07	206	2415	659	13.44	2080	853	2.30	10.54
TG4	79.9	2.08	173	11.44	261	2951	1236	38.81	5736	5377	-0.63	-5.72
TG4	69.6	8.21	139	12.96	167	626	813	10.51	2406	1615	0.92	9.44
ML2	35.1	0.92	57	12.29	370	1839	1008	20.08	2952	1905	0.22	-11.40
ML2	42.3	1.67	53	7.25	250	2913	1010	24.78	3305	2036	-0.56	-0.11
TG5	48.4	6.36	180	10.96	168	1335	609	8.54	2111	559	3.94	16.13
ML3	72.3	8.61	158	14.76	160	1181	790	9.97	1852	621	5.02	23.97
TG5	55.9	-0.20	93	8.49	206	1178	799	10.33	2315	1829	-0.84	-33.71
ML2	46.7	2.73	84	9.44	293	2885	896	24.44	3235	1698	-0.54	4.19
ML2	27.6	-6.92	99	4.47	160	1101	1137	9.12	2876	1673	-1.74	-23.51
ML3	48.1	3.02	102	9.51	296	1302	834	9.71	1791	1819	1.38	-4.56
TG4	40.8	3.32	73	8.27	227	1706	898	16.43	2405	2602	4.22	-6.81
TG5	50.3	-1.49	95	5.76	256	1917	899	10.90	2448	970	0.87	-8.19
ML1	50.3	0.58	20	7.37	291	1170	895	13.24	2025	1059	4.14	7.48
ML3	127	-3.11	122	8.77	205	871	875	8.27	2375	1757	1.15	-24.07
ML3	76.5	-0.61	49	9.34	811	2383	908	17.71	3060	1712	2.48	5.64
TG4	64.9	0.01	183	10.64	1414	1553	1140	20.54	4003	1200	1.51	9.25
ML2	54.2	-2.98	16	8.64	121	706	770	19.18	2444	1136	1.10	17.54
ML3	53.6		147		148	1089	970	6.20	1997	2044		
ML1	76.1		59		993	2999	1088	7.88	2612	1907		
ML1	66.9		120		834	1892	1017	11.71	2291	1351		

Table 3. (Cont'd)

Plot	Elements (ppm) ZN	CD	MN	B	FE	AL	MG	CU	CA	K	NI	CR
ML1	34.0		29		1183	4048	619	3.97	1660	842		
TG5	45.4		138		543	2222	550	2.15	1970	1239		
ML2	45.3		96		303	1413	1080	11.60	336	2374		
ML2	43.7		104		155	1540	1026	14.29	3028	1968		
ML1	50.7		56		1206	3263	706	2.93	1926	1261		
ML3	62.9		230		257	2472	769	6.85	2202	789		
MEAN	56.1	0.76	115	8.68	429	1943	889	12.81	2662	1627	1.17	-1.15
S.D.	18.8	4.02	61	2.88	356	837	172	7.48	840	929	1.90	14.35
Huron Manistee Disease Plots, Outside Location, Fine Roots < 3 mm												
TG4	63.0	1.39	418	10.87	213	1354	644	20.34	1557	1230	0.14	7.67
TG4	47.2		312		199	1409	505	13.78	1809	872		
ML2	61.8		103		254	621	1229	8.92	3602	1590		
ML3	222		337		813	2685	1406	13.46	2962	1867		
TG4	21.5		114	2.20	600	3053	902	5.47	2265	1278	-2.54	
ML2	28.0	-5.67	11	7.47	187	599	871	5.62	2850	1534	0.20	
ML3	27.0	-4.05	411	9.03	285	1142	1800	2.50	2228	1920	-0.60	
TG5	54.4	1.25	246	11.01	393	1494	1015	11.94	2964	1148	1.11	
TG4	47.9	-3.72	307	7.63	728	3047	1036	4.63	2230	1210	-1.71	7.15
ML3	56.1	-5.77	480	2.84	570	2496	1410	3.96	2887	1865	-0.84	-16.24
TG5	40.6	0.08	430	2.65	270	1884	1102	4.86	2026	1811	-0.10	0.54
ML1	44.7	0.27	122	10.39	358	2018	783	3.04	2248	1451	-0.71	-9.11
TG4	59.1	3.59	266	12.10	184	1670	763	23.00	1767	1368	-0.01	8.05
ML2	37.1	-0.39	28	7.48	184	1456	801	17.92	3016	1352	-0.95	-1.60
ML2	44.2	-0.05	16	7.63	213	1569	819	30.92	3185	1540	-0.50	21.33
TG5	34.3	-2.13	596	9.68	73	508	1238	1.87	1634	2153	0.67	-1.03
ML1	58.3	-1.77	253	9.25	524	1995	939	9.70	2961	1161	4.59	2.32
ML3	104	3.84	122	15.14	557	925	1365	8.09	3109	873	2.67	19.56

Table 3. (Cont'd)

Plot	Elements (ppm)											
	ZN	CD	MN	B	FE	AL	MG	CU	CA	K	NI	CR
TG5	40.5	1.72	463	11.82	216	1692	1139	8.72	2552	2296	2.40	-35.56
ML3	20.8	0.98	196	6.87	326	2631	1260	16.89	2333	1336	0.61	1.36
ML3	25.5	0.87	196	8.27	546	1337	987	6.98	2374	949	2.26	3.32
ML1	28.4	-2.59	69	7.77	296	2220	922	6.28	3045	872	1.04	5.93
ML3	54.6	-0.63	420	9.94	487	2222	1390	16.43	3372	1123	2.12	5.66
ML3	51.2	-0.04	651	9.94	409	2388	1917	8.61	2877	1020	1.93	6.61
ML3	36.4	-0.47	166	6.97	486	3332	1019	13.81	3254	1143	0.76	-1.30
ML1	37.1	-3.8	203	6.97	284	1506	980	8.05	2814	901	0.51	-4.96
ML1	33.4	0.77	81	8.26	375	2967	1050	9.35	3712	864	1.02	2.06
ML1	30.5	1.43	56	10.05	272	2332	752	7.85	3550	840	1.23	-3.67
TG5	35.6	1.37	406	7.47	247	1605	1175	22.27	2560	1344	3.55	1.84
ML1	40.0	-2.39	103	7.07	336	2988	774	7.90	3162	1168	1.81	-14.00
ML3	81.1	-1.75	201	8.57	432	1334	1548	20.98	4265	988	-0.18	-16.66
TG4	56.7	-1.65	215	8.34	190	1220	832	34.11	1626	2345	2.44	6.87
ML3	48.3	2.90	496	12.52	309	1671	1589	5.45	3557	1165	0.65	2.13
TG5	64.3		408		394	2748	1213	8.30	2641	1509		
TG4	56.2		341		203	1750	658	21.01	2093	1785		
TG4	34.2		211		110	660	476	11.71	1275	890		
ML2	56.1		99		203	487	1127	7.47	2569	1906		
ML2	60.7		64		156	540	997	14.81	3726	1559		
ML2	41.4		64		119	625	995	7.68	3065	1678		
ML1	50.6		183		430	2187	1039	1.90	3117	1175		
TG5	34.6		531		194	1019	1173	0.52	2016	1589		
TG5	34.7		585		271	1523	1176	1.48	2123	1602		
MEAN	50.1	-0.57	261	8.54	331	1736	1067	10.92	2690	1383	0.71	-0.07
S.D.	31.2	2.47	175	2.76	166	788	310	7.69	674	402	1.56	11.32

Table 3. (Cont'd)

Plot	Elements (ppm) ZN	CD	MN	B	FE	AL	MG	CU	CA	K	NI	CR
Huron-Manistee Check Plots, Fine Roots < 3 mm												
TGC	40.8	-0.71	378	10.90	244	1403	1291	18.45	2162	1966	-2.38	
TGC	25.4	-3.55	290	10.46	338	1254	1413	7.47	5961	2161	-0.05	
TGC	31.0		795	9.58	259	1124	1658	11.45	2755	2021	0.98	
MLC	43.3	1.06	729	10.46	316	1116	1452	11.65	2490	2196	3.95	
MLC	47.6	1.85	674	12.32	357	1833	1496	9.96	3268	2026	1.16	
MLC	39.7	2.07	116	9.03	229	735	1068	4.52	2924	1742	1.51	
TGC	28.5	2.21	350	12.29	120	530	1071	10.69	1565	1574	3.51	10.03
MLC	60.8	2.78	755	15.52	234	1463	1186	16.26	2728	1453	0.01	5.14
MLC	35.2	0.55	262	11.44	407	1385	957	35.04	2660	1166	-1.07	-2.12
TGC	38.1	1.22	421	8.49	208	856	1353	35.12	3172	1818	-1.34	6.01
MLC	50.8	0.77	292	10.11	256	1502	1109	2.64	2700	1546	-1.38	-1.12
TGC	40.2	2.37	891	10.30	206	1113	1274	10.86	1818	2853	2.49	-6.37
TGC	39.6	-2.33	737	13.34	121	842	1245	3.22	2096	2621	2.72	-5.45
MLC	39.4	2.67	360	22.89	185	640	1229	15.78	2740	2023	2.98	20.26
TGC	18.8	-5.03	348	4.46	142	578	1389	10.71	4510	2169	-0.55	-18.62
MLC	40.2	3.77	184	8.57	155	400	1090	11.98	2791	1157	0.17	-9.83
MLC	45.6	-1.94	347	7.32	434	1936	1052	6.26	3393	1122	-0.18	-7.59
TGC	28.7	-3.98	574	5.49	218	1716	1548	27.97	2813	2012	0.15	-9.52
TGC	28.5	-3.54	475	5.03	166	1178	1450	24.28	2095	1874	3.75	-10.31
MLC	31.5	1.33	119	7.14	466	1086	1066	3.04	2899	1531	0.38	-1.47
MLC	46.1	1.57	367	11.35	112	416	975	8.14	3460	2021	0.96	-1.49
MLC	39.1	-1.67	530	12.04	403	1415	1229	22.29	2603	1686	1.12	5.07
TGC	50.2	0.03	973	9.46	283	1314	1806	16.06	2367	2197	5.21	-1.77
TGC	38.6	-1.44	657	8.21	162	924	1438	5.92	2832	2241	-0.25	-9.58
MLC	24.9		101		148	399	863	9.85	2793	1253		
TGC	34.9		709		258	1174	1397	6.83	2462	2167		

Table 3. (Cont'd)

Plot	Elements (ppm) ZN	CD	MN	B	FE	AL	MG	CU	CA	K	NI	CR
MEAN	38.0	0.00	478	10.26	247	1090	1273	13.32	2850	1869	0.99	-2.15
S.D.	9.2	2.43	244	3.69	100	430	224	8.92	841	427	1.89	8.79
Roscommon Check Plots, Fine Roots < 3 mm												
R2	62.7	0.60	309	8.84	522	3745	998	8.46	2211	1803	1.26	1.47
R1	24.3	1.27	173	8.34	453	2110	876	11.37	2760	1841	1.85	4.35
R2	30.0	-1.39	127	7.71	380	2094	944	7.04	2629	1833	0.97	5.53
R1	89.4	4.24	394	11.67	534	1903	1131	12.13	3891	1314	2.38	
R2	48.7	4.16	528	9.28	445	1637	1436	8.25	3052	1626	0.79	
R1	30.4		342		323	3088	874	13.26	4103	1695		
R1	44.9		339		458	2893	908	8.71	2388	1832		
R2	40.7		67		264	1747	870	15.74	3107	2353		
R2	117		120		723	2248	1112	37.97	4090	2565		
R1	36.2		681		197	1972	1083	5.56	2449	1861		
R1	33.2		330		264	4251	1009	7.88	2973	2196		
R2	45.8		183		382	2513	1208	8.17	3970	2080		
R1	72.5		419		416	3234	1060	10.31	3250	1496		
R2	33.1		498		341	2305	1353	6.39	2149	2209		
R2	42.5		541		506	2323	1258	8.66	2328	2547		
R1	56.0		732		454	2490	1278	8.04	3644	1531		
MEAN	50.4	1.78	361	9.17	416	2535	1087	11.12	3062	1924	1.45	3.78
S.D.	23.8	2.16	193	1.36	123	709	173	7.39	673	358	0.59	1.70
Osmun Lake Check Plots, Fine Roots < 3 mm												
O1	28.1	2.59	294	10.68	261	984	949	3.17	1874	1680	3.33	
O2	28.7	2.37	395	7.05	122	918	963	11.75	1514	2113	1.99	
O3	32.8	2.28	162	10.68	184	1225	843	7.72	3075	1811	1.80	

Table 3. (Cont'd)

Plot	Elements (ppm) ZN	CD	MN	B	FE	AL	MG	CU	CA	K	NI	CR
O3	41.7	0.92	237	9.53	56	420	887	1.82	2431	2503	-1.54	-22.01
O3	37.8	-0.10	174	8.96	277	1418	782	1.42	2456	1994	0.21	-7.50
O3	39.4	-5.91	424	6.99	164	611	976	1.56	1925	1732	-0.18	5.12
O1	34.3	4.63	285	10.15	94	500	1155	8.62	1896	1483	2.28	15.02
O1	24.4	-2.45	428	7.77	185	1106	1117	1.30	3049	1732	-0.99	-10.25
O1	29.7	-0.70	244	7.82	43	142	1013	1.67	2030	1250	0.73	8.76
O1	29.3		227		84	1002	1046	7.99	2002	1393		
O3	53.4		410		307	1512	1073	4.71	2357	2230		
O1	43.2		311		620	2015	1000	22.04	2931	1674		
O2	36.2		387		141	828	906	13.98	1485	2501		
O3	46.2		402		778	2455	979	13.05	2547	2291		
O1	43.2		200		116	635	791	15.22	2334	1610		
O2	42.4		159		90	777	851	2.42	3107	1809		
MEAN	36.9	0.40	296	8.85	220	1034	958	7.40	2313	1863	0.85	-1.81
S.D.	7.6	2.98	96	1.41	198	577	107	6.09	515	365	1.53	12.61
Huron-Manistee Disease Plots, Edge Location, Large Roots 3-5 mm												
TG5	39.0	1.66	159	8.26	109	369	664	-0.21	1785	1071	-1.80	
TG5	24.8	0.60	89	6.27	104	296	467	1.78	1322	847	-0.06	
TG5	17.8	-7.94	201	1.52	248	640	634	5.60	1909	367	-2.08	-1.46
TG4	39.9	0.04	177	10.01	328	623	744	4.57	1600	2281	2.31	-0.67
TG4	46.0	-3.47	130	9.34	58	327	663	7.28	1828	2558	-0.56	14.19
TG4	32.7	-5.39	56	4.95	105	298	563	12.47	1142	3460	-0.96	13.39
TG5	31.9	-0.44	185	6.02	61	265	519	0.89	1769	1839	-2.90	-9.85
TG4	49.1	0.92	122	5.16	81	766	775	13.35	2249	2486	-2.89	2.89
TG4	40.6	3.24	284	9.25	365	1426	942	17.13	1966	3432	-0.35	-6.13
ML3	49.6	7.76	163	11.44	132	251	929	5.89	1830	669	-0.51	10.76
ML2	30.2	0.76	34	8.87	150	676	786	16.17	2134	1360	20.15	4.90

Table 3. (Cont'd)

Plot	Elements (ppm) ZN	CD	MN	B	FE	AL	MG	CU	CA	K	NI	CR
ML2	25.6	0.95	32	8.01	131	1087	827	16.43	2630	1448	71.89	3.64
TG4	66.1	3.47	243	14.48	75	576	744	8.09	2433	1317	0.56	11.08
TG5	46.4	6.78	194	11.91	86	367	663	6.49	1954	731	5.39	16.46
ML3	49.8	-0.00	236	9.25	126	1118	886	4.68	2105	600	-1.57	-2.11
ML3	41.2	-0.27	121	10.11	155	615	903	7.91	1911	3523	-2.23	-29.88
TG5	44.6	-0.30	114	11.06	145	405	637	7.19	1719	1767	1.73	-34.63
ML1	55.9	-2.11	100	10.10	33	134	1002	1.63	1392	3082	-2.84	-12.16
ML2	36.9	0.14	141	8.49	60	378	962	3.85	2600	2531	-1.55	-35.44
ML1	21.2	-12.89	61	0.20	47	211	661	1.69	1161	1320	-2.74	5.02
ML2	30.7	0.74	106	5.87	29	151	779	6.07	1813	1375	-0.20	-2.13
ML3	4.1	-13.81	234	-4.35	76	368	1066	5.03	1717	546	-1.93	5.97
ML3	23.8	-5.07	60	5.32	108	521	585	4.34	1485	1200	1.30	2.59
TG5	33.1	-0.37	82	4.35	99	356	626	9.42	1681	1005	0.74	-10.37
ML3	80.5	-0.92	141	8.44	59	179	999	3.49	2031	1425	5.39	4.65
ML3	61.1	-0.40	114	8.84	517	779	1051	8.40	2091	1319	1.31	7.00
TG4	54.4	-0.88	141	8.44	423	479	931	10.49	1975	1572	1.26	8.24
ML3	37.5	0.42	124	7.18	114	510	702	8.83	1531	972	1.79	-0.42
MEAN	39.8	-0.96	137	7.46	144	506	775	7.11	1852	1646	3.17	-1.33
S.D.	15.6	4.64	64	3.76	120	304	165	4.60	370	910	13.92	13.65
Huron-Manistee Disease Plots, Outside Location, Large Roots 3-5 mm												
ML2	26.3	-5.70	21	7.71	330	537	659	53.54	1971	1145	1.81	136.60
ML3	20.9	-4.62	242	6.92	358	669	1076	4.44	1931	1481	0.40	
ML2	50.3	3.31	60	8.15	161	382	967	4.96	1929	1651	1.15	
TG5	31.2	3.08	527	9.58	165	516	1134	1.86	1766	1451	-0.91	
ML2	24.4	-0.03	17	9.13	180	906	760	14.17	1906	1217	-1.28	-17.97
TG5	32.1	-1.38	185	9.91	117	452	618	6.07	1911	1012	0.23	-17.39
ML3	31.4	-2.79	417	1.07	323	650	1463	2.68	1939	1274	1.87	-1.08

Table 3. (Cont'd)

Plot	Elements (ppm)											
	ZN	CD	MN	B	FE	AL	MG	CU	CA	K	NI	CR
TG5	30.7	3.85	483	2.99	51	371	921	7.07	1652	1332	1.63	-5.85
ML1	45.2	1.22	185	11.15	174	474	884	3.13	1660	1079	0.34	1.09
TG4	43.7	3.71	339	12.01	106	633	746	9.88	1206	1922	1.89	5.38
ML2	22.1	-1.56	10	7.09	228	740	679	21.46	2285	1604	-0.66	17.68
TG4	34.4	5.52	193	8.93	91	220	688	10.18	1607	1863	0.08	4.43
TG5	42.0	-8.16	347	6.13	32	141	787	-1.06	1304	1680	-2.78	6.87
ML1	52.2	-1.31	259	7.82	137	441	770	1.69	1873	889	12.46	0.94
TG4	37.7	-5.05	385	4.54	49	218	681	5.29	862	3763	0.59	2.06
ML3	55.5	4.10	71	10.58	229	360	993	6.22	2248	957	3.05	17.68
ML3	59.1	7.08	453	15.14	205	426	1302	6.14	2461	541	6.73	20.12
ML2	35.1	-0.64	74	10.87	57	247	874	4.16	3336	2113	-1.89	-28.14
TG5	23.6	-0.40	260	9.15	150	558	912	5.90	1817	1304	3.50	4.55
ML2	31.4	-4.54	59	4.87	70	199	836	7.26	2566	1250	-0.78	-15.88
ML3	39.8	2.52	313	7.27	751	966	1120	5.22	2143	850	1.80	0.17
TG5	34.1	1.08	466	6.62	64	317	976	6.07	1843	1221	1.01	-0.04
ML1	51.6	-1.35	129	6.54	129	599	996	3.27	2310	851	0.52	
ML3	37.9	-0.64	456	9.54	44	1115	1526	8.99	2567	987	2.70	
ML3	32.9	-0.41	580	8.54	139	481	1810	3.69	2140	1111	1.05	
ML3	19.9	-1.69	100	6.07	184	541	906	7.79	2321	1154	-0.36	-4.54
ML1	55.4	1.16	184	6.58	201	983	780	4.69	2301	844	3.13	0.72
ML1	44.5	-1.69	115	8.06	109	581	933	4.60	2587	919	0.38	-2.17
ML1	28.2	0.96	117	4.68	280	802	590	16.89	2156	770	1.81	6.89
ML1	49.2	-1.90	218	8.07	170	929	1114	8.21	2457	1181	0.25	-20.76
ML3	53.8	-4.36	190	8.37	170	376	1504	10.71	3184	1050	-1.14	-17.59
TG4	43.9	-1.49	334	8.39	96	420	929	22.54	1097	1616	1.43	8.04
TG5	29.2	1.15	323	7.39	60	253	940	5.13	1796	1028	0.19	1.86
ML3	37.0	0.67	509	5.22	124	691	1570	10.32	2389	1282	1.74	-11.37
TG5	26.7		361		108	358	890	1.38	1761	1149		

Table 3. (Cont'd)

Plot	Elements (ppm) ZN	CD	MN	B	FE	AL	MG	CU	CA	K	NI	CR
MEAN	37.5	-0.30	257	7.80	175	530	981	8.42	2038	1301	1.23	3.30
S.D.	10.9	3.30	162	2.62	131	243	291	9.26	509	545	2.62	28.17
Huron-Manistee Check Plots, Large Roots 3-5 mm												
TGC	30.2	-4.93	649	8.37	175	827	1265	7.61	2293	1927	-0.97	
MLC	26.7	2.57	469	10.9	100	343	939	4.58	2032	1595	0.25	
MLC	32.8	2.34	702	8.98	271	1552	1352	8.08	4063	1504	5.11	
TGC	33.0	2.49	606	12.86	94	298	1009	8.45	1347	1236	1.35	9.51
MLC	43.0	4.01	486	13.62	134	734	807	8.18	1868	1213	-0.34	5.91
MLC	22.2	0.24	317	8.49	234	637	818	11.13	2037	1101	-1.73	1.14
TGC	33.4	0.63	565	7.91	113	506	1197	19.82	1738	1374	3.98	23.42
MLC	36.2	1.30	170	10.11	149	480	1003	3.99	2409	1268	0.36	-2.03
TGC	34.8	-0.62	725	10.49	98	478	938	7.19	1159	2892	3.72	-14.40
TGC	21.2	-5.34	481	8.87	77	331	878	3.22	1947	2894	2.77	-11.75
MLC	41.8	2.92	426	11.23	153	484	975	13.05	1927	2110	4.38	18.41
TGC	25.4	-5.96	471	3.73	84	243	1297	5.25	2614	1833	-0.72	-21.17
MLC	35.6	-1.65	440	6.97	136	408	1192	4.34	2515	1024	0.09	-5.00
MLC	32.2	-2.24	361	6.37	209	881	886	3.71	2622	1060	-0.17	-8.25
TGC	31.7	-3.96	553	8.07	151	688	1181	18.16	2303	1757	-1.08	-10.34
TGC	26.4	-3.52	436	4.10	134	648	972	17.34	1630	1860	0.39	-11.58
MLC	18.0	1.12	71	6.66	164	433	828	7.29	4276	927	1.10	-0.98
MLC	45.6	1.14	170	7.51	190	538	980	6.16	2822	1287	0.30	-1.77
MLC	36.9	0.30	157	5.87	119	511	893	6.59	2510	877	0.39	1.08
MLC	31.0	-0.65	276	6.99	192	619	955	8.60	1966	1140	5.22	3.18
MLC	41.6	0.47	578	9.45	137	591	1116	27.07	2166	1517	5.89	-0.71
TGC	40.4	0.25	670		57	401	1233	3.77	1489	1817	0.02	-8.41
TGC	32.1	-0.31	704	8.67	61	419	1252	7.10	2524	2220	0.52	-18.75

Table 3. (Cont'd)

Plot	ZN	CD	MN	B	FE	AL	MG	CU	CA	K	NI	CR
	Elements (ppm)											
MEAN	32.7	0.41	456	8.46	141	567	1042	9.16	2722	1584	1.34	-2.62
S.D.	7.0	2.71	187	2.43	53	264	166	5.95	722	548	2.22	11.0
Roscommon Check Plots, Large Roots 3-5 mm												
R2	33.3	1.11	334	8.07	170	718	883	3.73	1734	1795	0.82	2.25
R1	15.8	-1.02	177	10.04	166	504	621	6.00	2304	1189	2.75	6.79
R2	23.3	-1.04	135	6.6	288	907	839	4.96	2632	1593	2.08	5.79
R1	47.8	4.47	364	9.85	209	511	741	6.81	2231	1345	1.03	
R2	30.7	5.34	470	7.56	133	347	1036	1.62	2087	1749	3.17	
R1	35.5		435		169	896	656	3.76	2728	1232		
R1	37.5		324		169	1209	713	2.83	1742	1379		
R2	15.4		234		249	524	641	5.98	1786	1532		
R2	45.5		217		179	786	873	5.81	2807	1926		
R1	30.5		444		86	554	739	6.72	1466	2100		
R1	31.0		394		99	754	757	4.92	1811	1356		
R2	33.3		348		103	609	1052	4.14	2546	1851		
R1	42.4		409		64	554	797	10.36	1789	1833		
R2	26.1		349		225	1722	776	3.65	1546	2164		
R2	42.9		570		178	915	1212	2.36	1916	2628		
R1	40.9		664		104	904	1187	3.41	2213	1471		
MEAN	33.2	1.77	37	8.42	162	776	845	4.82	2084	1696	1.97	4.94
S.D.	9.4	2.69	134	1.33	60	323	179	2.07	413	376	0.92	1.95
Osmun Lake Check Plots, Large Roots 3-5 mm												
O1	16.6	1.81	215	9.25	80	244	706	0.53	1590	1328	0.97	
O2	27.7	3.35	92	9.03	91	412	698	5.42	2514	1559	1.99	
O1	17.0	1.81	247	7.08	22	271	691	5.4	5119	1430	1.26	

Table 3. (Cont'd)

Plot	Elements (ppm)											
	ZN	CD	MN	B	FE	AL	MG	CU	CA	K	NI	CR
01	17.3	4.07	284	9.47	182	779	796	5.96	1769	1314	-0.47	
01	27.2	1.43	248	10.57	59	495	768	4.36	3661	1277	-2.22	
02	14.2	2.79	163	9.8	140	1039	813	5.45	2870	1189	-0.94	
03	37.5	2.39	179	9.58	139	746	756	2.11	3232	1361	-2.47	-16.70
03	25.2	-3.13	414	5.66	174	1250	827	4.15	1941	1492	-0.68	7.49
02	45.7	0.04	238	10.68	70	637	816	-0.02	4053	2074	-2.32	-25.45
03	28.4	1.03	453	9.06	124	686	779	1.65	2259	1852	-0.83	-5.10
03	36.2	1.31	378	9.34	116	466	911	2.05	2394	2079	-2.20	-14.40
03	30.6	1.49	238	8.11	40	345	801	0.47	4022	2095	-3.44	-25.60
03	20.8	-1.45	180	8.30	79	824	681	0.03	3105	1529	-1.69	0.26
03	23.6	-7.34	378	7.17	95	369	799	-0.97	1596	1530	-1.59	6.39
02	12.1	-12.34	484	-2.38	74	433	731	-0.68	1820	829	-3.55	2.13
01	17.7	-0.92	177	4.19	71	244	623	5.47	2155	1078	0.57	-8.12
01	16.9	-3.63	252	4.34	53	353	797	2.15	2041	1204	0.22	-11.96
02	37.8	-5.86	380	6.07	56	476	895	1.12	2315	1644	-0.09	-22.54
01	25.6	-1.242	144	7.16	27	187	656	1.49	1516	1312	0.75	10.86
03	38.3	4.06	304	8.74	110	618	911	6.63	3418	1315	1.09	6.63
MEAN	25.8	-0.52	272	7.56	90	544	773	2.64	2670	1475	-0.78	-6.86
S.D.	9.2	4.09	107	2.92	44	272	79	2.41	964	330	1.58	12.35

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