ABSTRACT

EFFECTS OF NURSERY SOIL FUMIGATION ON GROWTH AND PHOSPHORUS NUTRITION OF PINE AND SPRUCE SEEDLINGS

By

Jean-Paul Campagna

In a large scale nursery seedling production soil fumigation is an important and necessary technique to control parasitic organisms. In Quebec nurseries some adverse side effects of fumigation treatments have been observed including what appears to be severe P deficiency. This study seeks to define the influence of forest nursery soil fumigation on the N and P nutrition of spruce and pine.

Greenhouse and field studies showed that soil fumigation with vapam (sodium methyl dithiocarbamate) or methyl bromide (bromomethane), or soil heat sterilization (greenhouse), applied without supplemental P was associated with a decrease in growth and a deficiency of P in red pine (Pinus resinosa, Ait.) and white spruce (Picea glauca

(Moench) Voss) seedlings, while the addition of P (336 or 505 kg P/ha) significantly increased the total biomass and the shoot P concentration. Ammonium-N was a better source of N than NO_3-N for the development of conifer seedlings. Soil fumigation, NH_4 sulfate and superphosphate appeared to be the best combination for raising red pine and white spruce seedlings.

After two growth seasons much larger seedlings were still obtained following soil fumigation with applications of superphosphate and NH_4 sulfate than with any other treatment.

Soil fumigation and P addition were related to a significant soil pH increase. Nitrification was hindered by soil fumigation and NH_4 accumulated in the soil for a portion of the season. The level of P in soil was significantly increased only after addition of superphosphate or bone meal.

Inoculation of vapam or methyl bromide fumigated soil with mycelium from mycorrhizal fungi pure cultures failed. However, red pine and white spruce seedlings grown in the same soil inoculated with forest soil showed a healthy growth and excellent plant development, without the addition of P. Numerous dichotomously branched short roots were observed and appeared to be ectotrophic mycorrhizae.

Hand-made cross-sections of these short roots revealed the presence of intra and intercellular hyphae in an irregular pattern. These are believed to be true, but young ectotrophic mycorrhizae.

In the greenhouse, an addition of 6.0 and 9.0 g of forest soil to a fumigated nursery soil did not significantly alter the seedling biomass when compared to the 3.0 g addition. Furthermore there was not a significant interaction of forest soil and P additions on the total biomass of red pine and white spruce seedlings.

In the same experiment, application of 224 to 896 kg P/ha significantly increased the total biomass and shoot P concentration of red pine and white spruce seedlings. The increase may be best characterized by a logarithmic equation of form $y = a + b \log x$. Seedlings grown in pots without any supplemental P showed P deficiency symptoms and revealed a deficient P level in their shoot tissue at analysis.

In nursery seedbeds, application of 336 to 1680 kg P/ha significantly enhanced the growth and the shoot P concentration of red pine seedlings. The seedling growth and shoot P concentration are shown to be

characterized by a logarithmic equation ($y = a + b \log x$). This curve shape indicates a sharp increase due to the first P additions. An application of 672 kg P/ha to fumigated nursery soil appeared to be an optimum level of P fertilization beyond which there was no significant response. At this level, the seedlings were healthy, large and well balanced.

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TABLE OF CONTENTS

CHAPTER Page

LIST OF TABLES

TABLE Page

LIST OF FIGURES

FIGURE Page

FIGURE Page

CHAPTER I

INTRODUCTION

The success of tree seedling production in forest nurseries depends largely on how effectively soil-borne diseases and parasitic organisms are kept under control. Fumigants such as methyl bromide, vapam (sodium methyl dithiocarbamate), and vorlex (mixture of methyl isothiocyanate and chlorinated hydrocarbons) are now effectively used in forest nurseries to control these disease organisms. The fumigants are also very effective in destroying most weed seeds. Therefore these broad spectrum fumigants are a tool of every nurseryman and have gained wide acceptance as dual-purpose soil treatments (White and Potter, 1963).

Early forest tree nursery research in chemical damping-off and weed control dates back to at least the 1920's when Toumey and Korstian (1942) were experimenting with sulfuric acid, copper and zinc sulfates. The use of soil fumigants, a relatively new science, has really been developed in the last 15 or 20 years. The fumigants were most primarily used in horticultural and vegetable crops (Kock, 1951), and they were brought into the forest nurseries only in the early 1950's to solve the problem of soil-borne diseases (Howe and Clifford, 1962).

Seedling diseases not only reduce germination, but they also affect seedling vigor and consequently their ability to survive other unfavorable environmental conditions. Soil-borne diseases are devastating in a forest tree nursery. Damping-off, one of the worst, can completely destroy entire seedbeds of young coniferous seedlings. The intensity of this disease varies with the pH of the soil, the weather, method and time of sowing and the seedling species.

Formaldehyde is known as one of the first soil disinfectants used in forest nurseries. In the early 1950's fumigants of the bromide and methyl bromide type appeared and they are still largely used to-day even if others such as vapam, vorlex, mylone, etc. are also applied in forest nurseries against soil-borne diseases and weeds. Their selectivity, fungicidal and herbicidal properties make them indispensable in large nursery operations. However, in many cases these fumigants do show a residual effect on the growth of young coniferous seedlings. This seems to be related to their action on the balance of microbiological life in soil.

Iyer and Wilde (1965), studying the effects of many biocides and fumigants on the nutrient status of seedlings, found that unbalanced or large top/root ratio of conifer seedlings was related to soil treated with vapam. Others such as Henderson and Stone (1970) reported coniferous

seedlings deficient in P following a soil fumigation. Moreover these seedlings grew poorly and had a red purple discoloration of their needles. This poor growth was related to an absence of mycorrhizal development. Meanwhile Howe and Clifford (1962) reported very good growth or a "fertilizer effect" after soil fumigation with methyl bromide and attributed it to a reduction in weed competition and a soil free of pathogens.

It is now accepted that this decreased growth and purple discoloration of coniferous seedlings represents a deficiency of P in conifer seedlings caused by a decrease in P uptake in the absence of mycorrhizal development. Supplying a very high amount of P to the nursery soil allows adequate P nutrition even without mycorrhizae and results in good growth (Henderson and Stone, 1970).

In several Quebec nurseries, it was observed that adverse residual effects from fumigation interfered with the production of healthy seedlings.

The objectives of this study were: (1) to examine the possible sources of available phosphorus and suitable application rates which can be used to stimulate the growth of coniferous seedlings after soil fumigation and (2) to assess the ameliorative influence of inoculation with forest

soil or pure cultures of mycorrhizal fungi after soil fumigation in greenhouse and field conditions.

Red pine (*Pinus resinosa* Ait.) and white spruce (*Picea glauca* (Moench) Voss) were used as the indicator species.

CHAPTER II

LITERATURE REVIEW

Background

The gardener, nurseryman, greenhouse operator has long been fighting a battle with plant diseases caused by soil-borne microorganisms. These organisms which live in the soil cause a variety of diseases which trouble those who work in this area. It has been extremely difficult in the past to gain control over these microorganisms through the use of cultural practices. The operators of many large greenhouses have sterilized their soil by steam, but this method is costly and not always completely effective. Furthermore this method is hardly performed in nursery seedbeds. Then the nurserymen were at the mercy of the fungi until the development of soil fumigants.

The first fumigants used extensively in forest tree nurseries, horticultural and vegetable crops were of bromide or methyl bromide types. For many years methyl bromide has been the standard by which most wide-spectrum soil fumigants have been compared.

Methyl bromide is a colorless and nearly odorless liquid or gas. It is applied in this form under a polyethylene covering. This biocide is effective in repressing

soil-borne diseases and parasites that attack germinating seeds and young seedlings. It is also efficient in controlling weeds. Methyl bromide can also be mixed with other fumigants such as chloropicrin, propargyl bromide to give several new compounds: Methyl bromide MC-2 (98% methyl bromide, 2% chloripicrin), Trizone (61% methyl bromide, 30% chloropicrin, and 9% propargyl bromide), and Dowfume MC-33 (67% methyl bromide, 33% chloropicrin). One of the most notable attributes of these compounds is their broad spectrum of biological activity.

The high biological activity of these fumigants has aroused much interest and they gained wide acceptance amongst nurserymen. Since the early 60's several other products of which vapam is one, have been used. Vapam is a water-soluble liquid containing 32.7% sodium methyl dithiocarbamate. Applied to the soil as a preplanting treatment it is converted into a gaseous fumigant (methyl isothiocyanate). Vapam is widely used for the control of weeds and soil-borne pests that attack ornamental, food and fibre crops, and tobacco seedlings.

Waksman (1966) stated, "The soil is not a mass of rocks and residues; it is not a dead organic-inorganic system, but a living system teeming with numerous forms of life". Of the several forms of life mentioned roots of

higher plants and certain fungi are involved in intriguing natural phenomena. Specific fungi grow upon and vigorously invade portions of root systems that are primarily responsible for nutrient absorption by higher plants. The term "mycorrhyza" meaning fungus-root, designates these particular invasions of roots by fungi. Without mycorrhizae, many plants including our most important timber species, could not survive in the dynamic, fiercely competitive biological communities that abound in natural soil habitats. In nurseries, the addition of any potentially toxic molecule constitutes a serious threat to the presence of mycorrhizal fungi and their association with seedling roots.

Fumigants

Fumigants can be applied to the soil as a liquid or in gaseous form. As a gaseous chemical they are applied under a covering at the soil surface and move into the soil. If the chemical is in a liquid form it is injected into the soil at a depth of 4 to 6 inches, and covered. The injected fumigant evaporates and diffuses throughout the soil.

To be effective a fumigant must dissolve in soil water, because it is only in this form that it can kill nematodes, fungi and weed seeds. If the soil is dry, the fumigant is not absorbed or dissolved in water and it

diffuses rapidly throughout the soil. Then an optimum concentration of the fumigant is not retained in the soil moisture resulting in little effect. Under moist conditions, the diffusion is not so rapid, but the greatest part of the fumigant is in the soil moisture where it is needed (Howe, 1965; White, 1965). The ideal type of soil for fumigants is a sand or a sandy loam which permits a much better diffusion and distribution of fumigants than clay soils.

If soil temperature is too low (below 4.5° C), it stops the evaporation of the fumigants and thus their diffusion. The most common temperature of application of fumigants is about 16° C, with a range of 10° C to 30° C.

Other factors in the effectiveness of fumigants are the length of exposure, the amount applied and the covering. Methyl bromide must be applied under a tarp set up before the application in surface of the soil, or injected into the soil which is covered as soon as possible after application. Vapam can be injected into a soil which has to be sealed with water or covered with a tarp, or it can also be drenched into the soil. When only drenched into the soil vapam is not so effective.

In forest nurseries, fumigants have principally been used until now in a three-way action. Indeed they are used to kill fungi and control most of the common soil-borne

plant parasitic diseases such as damping-off and root rot of seedlings. They are also utilised in order to control most of the usual soil-borne plant parasitic species of nematodes causing severe damages to root seedlings. Furthermore they are used to kill weed seeds (Delong, 1960; Harrison, 1966).

Effect of Fumigants on Microbial Populations

The soil is a very complex and dynamic system; its microscopic inhabitants such as fungi, bacteria and actinomycetes are essential to plant growth and to soil fertility. By their enzymes they can decompose roots and crop residues and make nitrogen available to plants. They also form NO_3 from NH_4 fertilizers and transform P to compounds assimilable by plants. Moreover the soil microorganisms perform many other processes useful or harmful to plant (Alexander, 1958).

The soil fertility often depends on the delicate balance existing between these various types of microorganisms whose activities determine the efficiencies of the carbon, nitrogen, mineral, etc. cycles which they regulate. It is obvious that the addition of any potentially toxic molecule constitutes a serious threat to this equilibrium and to its fertility.

The methyl bromide fumigants control fungi such as Rhizoctonia solani Kuchn, Phytophtora spp. and Pithyum spp

which cause damping-off and heavy losses of forest tree seedlings at emergence. They also decrease considerably the mortality due to root rot disease by killing the fungus Macrophomina phaseoli (Maubl) (Smith and Bega, 1966; Turner, 1960).

Fumigants also have an effect on fungi related to mycorrhizal infections of tree roots. It seems that fumigants at least kill the vegetative stage of these fungi in soil; indeed quite a few authors reported that conifer seedlings growing in fumigated soil do not show any mycorrhizal infection the first year of growth (Howe and Clifford, 1962). Iyer and Wilde (1965) reported that a vapam treatment to a nursery soil in Wisconsin nearly eliminated mycorrhizal short roots and fibrous laterals with resulting 75 percent decrease in the absorbing capacity (titration value) of red pine roots. The same phenomenon was observed on Ponderosa pine and Douglas fir (Wright, 1964). After a second year of growth, roots of seedlings from fumigated beds are most of the time comparable in mycorrhizal development to those from unfumigated beds. Then it appears that mycorrhizae, even though drastically reduced by soil fumigation, regenerate rather quickly and within two years reach levels comparable to those in untreated plots. The absence of mycorrhizal development is most of the time related to a

red purple discoloration and a poor development of the seedlings. This effect can be alleviated by a high amount of P in the soil solution (Henderson and Stone, 1970).

The bacteria, an important group of soil flora, are essential for the maintenance of its fertility. Wensley (1953) studied the effect of soil fumigation with Dowfume MC-2 on 4 physiological groups of bacteria. He noted that the nitrifiers (Nitrosomonas, Nitrobacter) and certain cellulose decomposing bacteria are more susceptible to methyl bromide than the denitrifier or ammonifier bacteria. While nitrification is suppressed by methyl bromide, ammonification is slightly reduced. The resulting lag in nitrification may last eight to ten weeks or more (Good and Carter, 1965). Hence fumigation produced an unbalanced ratio in NO_3-N relative to that of ammonia N in the soil. But this fact seems to be slightly favorable to conifer seedlings which seem to prefer NH_4 to NO_3 as N source (McFee and Stone, 1968).

In general microbial numbers are initially decreased by fumigation, but most of the time many organisms will quickly reinvade the soil, including beneficial fungi and soil bacteria. Sometimes their numbers may exceed those in untreated soils. Several factors may contribute to this fact. The cell material of the organisms killed may offer

a ready source of energy and carbon material for the living organisms. But the most important factor seems to be the reduced competition.

Usually Trichoderma viride is the first fungus species to reinhabit fumigated soils. It often survives fungicide treatment and quickly develops in an environment which is less competitive. If annihilated by the treatment, it may reinvade the soil in which a sufficient amount of the chemical is still present to prevent the development of other species. Since there is no competition, it may grow faster than other organisms and become dominant. "The changes in the microbial population of the soil following treatment with fumigants may exert a biological control effect on root parasites. Trichoderma viride, which most commonly becomes dominant following partial soil sterilization with chemicals, is a well known antagonistic species. It has been shown to exert an antagonistic influence on Phytophtora, Pythium, Armillaria, Rhizoctonia, and other parasitic forms" (Martin and Pratt, 1958).

Effect of Fumigants on Seedling Growth

The effects of microbiological decomposition, chemical absorption, adsorption on soil colloids and losses by leaching upon the breakdown of these fumigants in the soil are not fully understood. However it is generally agreed

that the major disappearance is by volatility. Indeed we must allow a period of aeration before sowing in order to permit the volatile portion of these fumigants to dissipate into the air.

The breakdown of methyl bromide fumigants involved a lag period in the build up of responsive organisms, and an induction period for adaptive enzymes to metabolize the chemicals (Hollis, 1964). Soils fumigated with these chemicals hold residues containing bromide ions which are troublesome to a few species of plants (Worsham, 1964). A soil treated with Trizone shows an increase in soluble chlorides which may be harmful to plants if they are planted too soon following fumigation (Wright, 1964).

Soluble residues, such as those cited above, are reported capable of causing plant damage often located at the root level. Red and white pine 1-0 seedlings show little or no mycorrhizae when growing in a soil treated with Dowfume MC-2 or Trizone while untreated seedlings show numerous mycorrhizae. However at 2-0 there is no difference in roots of seedlings from treated or untreated seedbeds (Howe and Clifford, 1962). Therefore it is obvious that the mycorrhizae fungi have reinvaded the treated area. Wright (1964) also reported that Trizone significantly decreases the mycorrhizal formation during the first growth year of Ponderosa pine and Douglas fir.

For several years, at the Berthierville nursery, Quebec, severely stunted 1-0 pine and spruce seedlings in spots throughout the seedbeds have been observed following an application of fumigants. The stunted seedlings also showed a reddish purple coloration resembling P deficiency, and a lack of mycorrhizal development. Henderson and Stone (1970) observed that both growth and P content of non-mycorrhizal coniferous seedlings grown in fumigated soil without P fertilization were less than any other treatments; they attributed this phenomenon to the absence of mycorrhizae. In these New York experiments, inoculation with either mycorrhizal roots or untreated soil, or treatment with high application of inorganic P fertilizer, corrected the reduced P uptake and poor growth caused by fumigation alone. Iyer, Chesters and Wilde (1968) in Wisconsin report: "Some of the recently introduced organic biocides abnormally stimulate the growth of crowns of nursery stock, but depress the development of root systems. In consequence, reforestation material exhibits an extreme degree of succulence, low specific gravity abnormally high top: root ratio, greatly reduced titration value of roots, and impeded development of mycorrhizae. The latter deficiency hinders the uptake of P even when this element is abundant in the soil in available form". It seems that, following the experiments of these authors,

nursery plants lose their capacity for a normal uptake of P when we have either a growth stimulation or reduction following a soil fumigation. As these coniferous seedlings are characterized by an absence of mycorrhizal root development, this helps to point out the role of mycorrhizae in normal P and nutrient uptake which leads to production of balanced seedlings.

Morphological Characteristics and Formation of Mycorrhizae

On the basis of interrelation between the fungus hyphae and the root cells, mycorrhizae are classed in two main groups, ectotrophic and endotrophic. The kind is usually specific for the genus of higher plant in which it occurs, and mycorrhizae have been found on most genera of seed plants that have been examined. Ectotrophic mycorrhizae are common on the pine (Pinaceae) family among gymnosperms and on the birch (Betulaceae), beech and oak (Fagaceae) and a few other families among angiosperms. Endotrophic mycorrhizae are present on the roots of most shrubs and certain trees as maple, yellow poplar, sweet gum, redwood and apple among others (Hacskaylo, 1967).

Typical ectotrophic mycorrhizae are caused by invasion of actively growing absorbing roots usually by hymenomycetous, but sometimes by ascomycetous, fungi

(Hacskaylo, 1957). Mycorrhizal association is usually confined to those roots in the top few inches of soil. The smallest of the secondary roots are invaded by fungi during periods of active growth. With few exceptions, the fungi involved (nearly all basidiomycetes) produce mushrooms as fruiting bodies. Attachment of hyphae to tree roots apparently is requisite to fruiting under natural conditions.

Even to-day the principles governing the entrance of the hyphae of the fungal symbiont into the roots are far from clear and we can synthesize only a partial explanation of the mechanisms of the association.

First there is a contact between actively growing roots and compatible fungi. The contact may come from spores germinating in the vicinity of the roots or by extension through the soil of hyphae from either residual mycelium or established mycorrhizae.

Bjorkman (1942) states that the mycorrhizal fungi seeking soluble carbohydrates enter the roots only if a surplus of such sugars is present in the roots. According to this theory a surplus of soluble sugars becomes a main factor governing the initiation and the establishment of the symbiotic relationship. Meanwhile growth of mycorrhizal fungi on the surface of the roots is greatly

stimulated by exudates from the roots. These exudates contain at least one growth promoting metabolite that Melin (1954) designated as "M" factor. This substance has not been identified and the dependency on the "M" factor varies with the species of mycorrhizal fungi.

Entrance of ectotrophic mycorrhizal fungi into the roots requires secretion of pectolytic enzymes, which dissolve the middle lamellae and thus permit the hyphae to grow through the intercellular region of the cortex. The pattern formed by hyphae in the cortex is called a "Hartig net". The meristematic tips and the vascular cylinder are not invaded. The fungus and root cells retain their vital characteristics and show no pathological symptoms (Hacskaylo, 1957).

The invading organisms in endotrophic mycorrhizae are primarily inconspicuous phycomycetous fungi that produce subterranean, nearly microscopic fruiting bodies (Hacskaylo, 1967). Endotrophic fungi are present on surface of the mycorrhizal rootlets as individual threads or loose hyphae wefts. The fungi secrete cellulolytic enzymes that dissolve a minute portion of the cell wall. This allows the hyphae to penetrate root hairs and other epidermal cells.

Beside the two types of mycorrhizae described, there occasionally appears on tree roots, primarily in

nurseries, the typical intercellular organization of the ectotrophic mycorrhizae plus intracellular penetration by hyphae. These ectendotrophic mycorrhizae are sometimes thought to represent a transitional stage between the ectotrophic and the endotrophic type.

Conversion of roots into mycorrhizae is accompanied by considerable changes in the physiology of these roots. Generally these physiological changes are demonstrated by renewed meristematic activity in old short roots, swellings caused by growth of new cortical cells, sometimes dichotomous branching of roots, and inhibition of root hair development on the swollen parts (Slankis, 1961). Then it seems logical to presume that a profound change in the physiology of these roots is a prerequisite for the establishment of the symbiotic relationship.

Several factors are considered important for a normal mycorrhizal root development of trees. Mycorrhizae of trees develop most extensively in acidic soils; indeed mycorrhizal fungi of trees studied for pH requirements are acidophilic. These fungi also require certain vitamins and amino acids, available in sufficient amounts in the soil for maximum growth of the fungi. Glucose and other sugars seem to be the main source of carbon of these fungi (Melin, 1953).

Abundance of new mycorrhyzae is also correlated with higher soil moisture levels during spring and autumn as compared with those in summer. The soil should be well aerated for good development of mycorrhizae. Low light intensities are a limiting factor in the formation of mycorrhizae on both natural and long days (Hacskaylo and Snow, 1959).

The results of several investigators consistently show that formation of ectotrophic mycorrhizae varies inversely with soil fertility. Fowells and Krauss (1959) confirmed the findings of Hatch (1937) that mycorrhizae are generally more abundant with low levels of N and P. Experiments of Hacskaylo and Snow (1959) further support these conclusions. Daft and Nicholson (1966), who studied three Endogone endophytes on plants grown in sand and watered with a nutrient solution, obtained the largest increases in growth of mycorrhizal plants under conditions of low P availability.

Role of Mycorrhizal Development in Tree Nutrition

The significance of mycorrhizal fungi as a persistent component of the forest soil microflora and the importance of these fungi in tree growth can no longer be denied. In 1917 Professor Elias Melin, at Uppsala, Sweden, initiated new and significant approaches to studies on mycorrhizae of pine, spruce and other Scandinavian trees. He observed that

in drained peat bogs those trees whose roots become mycorrhizal grew normally. Thereafter he directed his efforts toward research on the physiology of tree mycorrhizae under carefully controlled laboratory conditions. At that time he hypothesized that organic N compounds might be absorbed by the hyphae and translocated into the root tissues.

Hatch (1937), by analyzing tissues of mycorrhizal versus non-mycorrhizal seedlings, found increases of 86 percent N, 234 percent P, and 75 percent K over the non-mycorrhizal seedlings. He theorized that mycorrhizae were considerably more effective in nutrient uptake than non-mycorrhizal roots because of the increased surface area provided through root proliferation and of large absorbing surfaces of hyphae in the mantle and surrounding soil. His data showed that mycorrhizae were most effective in soil moderately deficient in N, P and K. Other worker findings supported these theories. Kramer and Wilbur (1949) observed that mycorrhizae of pine accumulated radio-active P to a greater degree than non-mycorrhizal pine roots. Melin and Nilsson (1957) clearly demonstrated that mycorrhizal fungi transported radio-active inorganic and organic N, P, Ca, and Na from the substrate into roots of Scotch pine. The efficiency of the

mycorrhizal system was far greater than in the non-mycorrhizal root. Harley (1959) indicated that there is a certain difference in metabolism between mycorrhizal and non-mycorrhizal roots.

Gray and Gerdemann (1967) also demonstrated that mycorrhizal sweetgum (Liquidambar styraciflua) and tulip-tree (Liriodendron tulipifera) accumulated larger quantities of P^{32} than did non-mycorrhizal plants. The use of P^{32} brings evidence that ectotrophic mycorrhizal fungi play a very active role in nutrient uptake by trees and the enhanced ability of mycorrhizal plants to absorb P. Baylis (1967) concluded that, in New Zealand forest soils which are normally low in available P, mycorrhizae are essential for the uptake of enough P to bring about normal growth.

Soils in many parts of the world have been found devoid of fungi that form ectotrophic mycorrhizae. Attemps to establish trees that normally possess ectotrophic mycorrhizae in some of those areas have failed several times. Jorgensen and Shoulders (1967) reported that the presence of visible mycorrhizae on roots of seedlings of slash pine (Pinus elliottii Engelm) had a significant and important beneficial effect on the survival of the seedlings, when planted on deep sandy soils in northwest Louisiana and on sandy loams in the central part of the

United States. Wilde (1968) reported that in prairie and other grassland soils devoid of certain symbiotic fungi, the growth of trees is arrested at the stage of the first whorl of leaves; during the first growing season in such soils they exhibit symptoms of malnutrition and die. An introduction of fungal symbionts of trees into grassland soils, accomplished by an addition of a fraction of 1% of a forest soil or a few crushed roots of mycorrhizal seedlings invariably leads to a rapid initiation of a vigorous growth of tree seedlings.

Mycorrhizae are not only generally recognized to aid tree growth and be necessary for tree survival on many sites, but Zak (1964) suggested that ectotrophic mycorrhizal roots may be less susceptible than non-mycorrhizal roots to infection by root pathogens. He postulated that mycorrhizal fungi may protect absorbing roots of trees by: (i) utilizing root carbohydrates and other chemicals, thereby reducing the attractiveness of the root to pathogens; (ii) providing a mechanical barrier to the pathogens in the form of a fungus mantle; (iii) secreting antibiotics which may inhibit or kill potential pathogens; and (iv) attracting, while in mycorrhizal association with the host root, a protective rhizosphere population of other microorganisms.

Mycorrhizal fungi may afford protection also by stimulating host root cells to elaborate inhibitors that may maintain the symbiotic state and that also serve to inhibit infection by pathogens.

Marx and Davey (1969a, 1969b) showed that ectotrophic mycorrhizae formed aseptically by several symbiotic fungi on the roots of shortleaf (Pinus echinata Mill.) and loblolly (P. taeda L.) pine seedlings were resistant to infection by zoospores of Phytophthora cinnamoni Rands. The findings confirmed Zak's (1964) theory that ectotrophic mycorrhizal fungi function as biological controls against pathogenic root infections.

It is apparent that the use of biocides in nurseries can reduce the mycorrhizal development of tree seedlings. This effect should not be overlooked and the damages caused to the absorptive systems of the seedlings should not exceed the initial benefits from control of disease and reduction of competition. Indeed ectotrophic mycorrhizae seem to function not only as physiological entities beneficial to plant health, as reported by many researchers, but also as biological controls against pathogenic root infections.

CHAPTER III

METHODS OF INVESTIGATIONS

Greenhouse (Experiment 1)

The influence of soil sterilization (fumigation or autoclaving) and different N and P sources on the growth of red pine and white spruce seedlings was measured in this first greenhouse study. The seedlings were grown in 500 ml plastic containers containing 0.45 kg of sandy loam nursery soil from the Berthierville nursery.[1] A sample of this soil was analyzed for NO_3-N by the Brucine method and NH_4-N by the Nessler reagent method (Greweling and Peech, 1965). Available P was determined by the Bray P_1 technique. Exchangeable cations were determined in ammonium acetate extracts: K by flame photometer, Ca and Mg by atomic absorption. Soil reaction was read with a pH meter using a 1:1 soil water ratio and organic matter was determined by loss on ignition at 500° C. Soil analyses by the Michigan State University Soil Testing Laboratory showed the following soil characteristics:

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[1] Quebec Provincial Nursery.

pH	Organic Matter (%)	Available Nutrients (ppm)					
		NH_4-N	NO_3-N	P	K	Ca	Mg
5.8	4.2	5.0	4.0	81	73	840	18

The screened soil (6.2 mm mesh) was first moistened, then divided in three parts. A part was autoclaved at 121° C and a pressure of 1.06 kg/cm^2 for two and a half hours. A second part was fumigated with methyl bromide (bromomethane). The pots (9.45 kg of soil) were put in large polyethylene bags and the fumigant was injected in the bags at a rate of 0.9 kg MB/9.3 square meters of soil surface. After two days at room temperature (22° C) the bags were opened to permit aeration for three days. The third part of soil was kept as control. Fertilizer treatments were applied after the autoclaving, or before the fumigation (Table 1). In each pot we mixed with the soil potassium sulfate at a rate of 90 kg K/ha. Phosphorus (336 kg P/ha) as superphosphate was applied as a band 2.5 cm below the soil surface. On April 13, half rate (rate: 112 kg N/ha) of N sources were applied and a month later we applied the second half of the treatment.

Red pine and white spruce seeds stratified in moist medium for a month were sown and pots placed on a greenhouse

Table 1. Nitrogen and phosphorus treatments[1] used in experiment 1.

FERTILIZER N	FERTILIZER P	TREATMENT
O	O	Control
O	Rock	Rock Phosphate (8.8% P)
O	Super	Superphosphate (20% P)
O	Bone	Bone Meal (4.8% P)
NO_3	O	Sodium nitrate (16% N)
NO_3	Rock	Sodium nitrate + rock phosphate
NO_3	Super	Sodium nitrate + superphosphate
NO_3	Bone	Sodium nitrate + Bone meal
NH_4	O	Ammonium sulfate (20% N)
NH_4	Rock	Ammonium sulfate + rock phosphate
NH_4	Super	Ammonium sulfate + superphosphate
NH_4	Bone	Ammonium sulfate + Bone meal

[1]Nitrogen at 112 kg of N/hectare and phosphorus at 336 kg of P/hectare: equivalent to 100 lbs N/acre and 300 lbs P/acre.

bench on March 10, 1968. The pots were periodically watered with distilled water to adjust soil moisture to approximately 20 per cent by weight as determined by weighing the pots. Several pairs of fluorescent lights were arranged parallel in the center of the plant bed 64.0 cm from the plant foliage level. These lights were automatically controlled and synchronized with the day photoperiod to provide artificial light for 18 hours per day. The experiment was arranged in a randomized complete block design with 4 replications.

Sixteen weeks after sowing, measurements were made of seedling length from root collar to terminal bud, dry weight of both shoots and roots. We also made observations on the mycorrhizal development of the root system.

The plants were dried at 70^{o} C for 48 hours in a mechanical convection oven, ground in a Wiley mill. Total N was determined on shoots by the micro-Kjeldahl method. Phosphorus was measured using photoelectric spectrometer at the MSU Horticulture Laboratory. The data were subjected to analysis of variance and treatment means were compared using planned orthogonal contrast and Tukey's w-procedure.

Field (Experiment 2)

In this experiment we wanted to test the interaction of vapam (sodium methyl dithiocarbamate) and methyl bromide fumigants with adjustment of N and P fertility in red pine and white spruce seedbeds at the Berthierville nursery in Quebec in the Spring of 1968. The fertility of the sandy loam nursery soil is outlined below:

	Organic	Available nutrients (ppm)					
pH	Matter (%)	NH_4-N	NO_3-N	P	K	Ca	Mg
5.2	3.8	8.0	4.0	120	290	740	45

A split-plot design with a factorial arrangement of treatments was used. Fumigant treatments were the main units, whereas fertilizer treatments (Table 1) were the sub-units.

The P fertilization (505 kg of P/ha) was applied on May 11, but N (112 kg of N/ha) application was delayed until July 2 after seedling germination. Methyl bromide (0.9 kg per 9.3 square meters) and vapam (600 cubic cm per 9.3 square meters) were applied to the soil on May 13. Non-fumigated seedbeds were used as a control. Stratified seeds were sown on May 30-31.

Total precipitation at the field plot site for the 1968 growing season was inferior as compared to the eight precedent years. Spring and early summer precipitation was nearly normal but July, August and September were below normal and we supplied by overhead irrigation. Total precipitation for the 1969 growing season was a little above normal as compared to the 1959 to 1967 seasons. Above normal precipitations were recorded in April, May, June and July, but below normal in August. A comparison between the experimental seasons 1968 and 1969 and the period of record is shown below:

	Growing Season Precipitation (cm)						
	April	May	June	July	August	Sept.	Total
Period of Record (1959 - 1967)	6.3	5.7	7.5	9.2	10.3	7.7	46.7
1968 Growing Season	4.8	7.6	7.1	5.5	4.4	3.3	32.7
1969 Growing Season	9.4	11.7	8.8	12.1	5.8	7.4	55.2

At the end of August, the seedlings were counted and, on September 4, 25 seedlings were collected in every sub-plot. The physical growth measurements, plant chemical analyses, and tests for significance among treatment means

were the same as described previously. A complete soil sampling was also made and soil samples were subsequently analyzed.

At the end of the second growth season (October 69) 10 white spruce seedlings were collected in every sub-plot and their total length, their dry weights of shoots and roots were measured.

Growth Chamber (Experiment 3)

Since it was apparent that mycorrhizal fungi are killed by fumigation we wanted to inoculate our soil in order to give it the microorganisms necessary to a good mycorrhizal development of seedling roots.

To inoculate the fumigated soil, forest soil and pure cultures of mycorrhizal fungi were used. The soil inoculum was a soil from a red pine plantation located at Kellogg Experimental Forest near Battle Creek, Michigan. The isolates of mycorrhizal fungi were kindly furnished by Dr V. Slankis[1] and Dr E. Hacskaylo.[2] The isolates were first cultivated on "Hagem" agar in petri dishes

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[1]Dr Vladislavis Slankis, Department of Fishery and Forestry, Canadian Forestry Service, Maple, Ontario.

[2]Dr Edward Hacskaylo, Plant Physiologist, Forest Physiology Laboratory, Beltsville, Maryland 20705.

(Appendix Table 21). Chunks of mycelium were used to inoculate fumigated soil with mycorrhizal fungi (Table 2).

Table 2. Mycorrhizal fungi inoculum used in growth chamber (Experiment 3).

SEEDLING SPECIES	INOCULUM SOURCE
Red Pine	Nil
	Forest soil
	Suillus luteus
	Amanita rubescens
	Rhizopogon roseolus
White Spruce	Nil
	Forest soil
	Suillus granulatus
	Cenococcum graniforme
	Telephora terristris

Soil collected in August 68 at Berthierville nursery was dried and screened using a 6.2 mm mesh. The soil characteristics are outlined below:

	Organic	Available Nutrients (ppm)					
pH	Matter (%)	NH_4-N	NO_3-N	P	K	Ca	Mg
5.2	3.8	6.5	5.5	70	130	450	24

The soil was first moistened and then put in 4.4 liter plastic containers containing 2500 g soil and 1200 g quartz sand. These pots were fumigated with methyl bromide (0.9 kg/9.3 square meters) or vapam (840 liters/ha). Pots were kept in polyethylene bags during three days and then aerated for four days before sowing.

Red pine and white spruce seeds were sterilized with 30% hydrogen peroxide for one minute, washed five times with sterile water and put on water agar to germinate. At sowing time chunks of mycelium were buried in the soil at a depth of 3 to 4 cm and germinated seeds (radicles 0.2 - 0.5 cm long) were put in the soil. The soil inoculum (5 gm) was mixed in the 4 top cm of soil.

The environmental conditions maintained throughout this experiment were a 18 hour light period at 26.6° C, a 6 hour dark period at 16° C, and a relative humidity of approximately 65 per cent. The light intensity was approximately 2600 foot candles at the plant foliage level. The pots were periodically watered with sterile distilled water to adjust soil moisture to approximately 20 per cent by weight as determined by weighing the pots.

The experiment was arranged in a randomized complete block design with 4 replications. After 16 weeks, the number of mycorrhizal and unmycorrhizal short roots

was counted using a 10X magnification, and also the number and the length of long roots were recorded (4 seedlings per pot). Numerous root sections were fixed in formaldehyde-acetic acid-alcohol and sections were stained and examined under greater magnification to be sure of the presence or absence of mycorrhizae. Green and dry weight measurements were made of seedling shoots and roots. The total length of the seedlings was also recorded. Moreover the P concentration of seedling shoot tissue was determined for a few samples. The data were subjected to analysis of variances as described previously.

Greenhouse (Experiment 4)

In the previous experiments superphosphate or forest soil inoculum addition to fumigated nursery soil brought a better seedling growth than any other treatments. In this experiment these two treatments are put together to check their possible interaction on conifer seedling development.

A soil similar to experiment 3 was used. Screened soil (2000 g) was placed in 3.7 liter plastic greenhouse containers and fumigated with vapam at a rate of 840 liters per hectare. As in the previous experiment pots were placed in sealed polyethylene bags for 3 days and then aerated for 6 days before sowing.

The P fertilizer (Triple superphosphate, 20% P) was placed in a band 3-4 cm below the soil surface (Table 3). The soil inoculum from a pine plantation located at Berthierville, Quebec, was mixed in the 4 top cm of soil at time of planting the germinated seeds as described in experiment 3. On January 9, 1970, they were transplanted in the pots when radicles were 0.5 to 1.0 cm long. The soil moisture level in each pot was periodically adjusted with distilled water to approximately 20 per cent by weight as determined by weighing the pots.

Several pairs of fluorescent lights were arranged parallel in the center of the plant bed 64.0 cm from the plant foliage level. These lights were automatically controlled and synchronized with the daily photoperiod to provide artificial light for a 16 hour period per day. This factorial arrangement of treatments was set in a randomized complete block design with 4 replications. After 19 weeks we collected the seedlings and made the same measurements as in experiment 3.

Table 3. Phosphorus and forest soil inoculum treatments used in Experiment 4.

No	Treatment
1	Control
2	Soil inoculum (3.0 g/pot)
3	Soil inoculum (6.0 g/pot)
4	Soil inoculum (9.0 g/pot)
5	Superphosphate (224 kg P/ha)
6	Soil inoculum (3.0 g/pot) + Superphosphate (224 kg P/ha)
7	Soil inoculum (6.0 g/pot) + Superphosphate (224 kg P/ha)
8	Soil inoculum (9.0 g/pot) + Superphosphate (224 kg P/ha)
9	Superphosphate (448 kg P/ha)
10	Soil inoculum (3.0 g/pot) + Superphosphate (448 kg P/ha)
11	Soil inoculum (6.0 g/pot) + Superphosphate (448 kg P/ha)
12	Soil inoculum (9.0 g/pot) + Superphosphate (448 kg P/ha)
13	Superphosphate (672 kg P/ha)
14	Soil inoculum (3.0 g/pot) + Superphosphate (672 kg P/ha)
15	Soil inoculum (6.0 g/pot) + Superphosphate (672 kg P/ha)
16	Soil inoculum (9.0 g/pot) + Superphosphate (672 kg P/ha)
17	Superphosphate (896 kg P/ha)
18	Soil inoculum (3.0 g/pot) + Superphosphate (896 kg P/ha)
19	Soil inoculum (6.0 g/pot) + Superphosphate (896 kg P/ha)
20	Soil inoculum (9.0 g/pot) + Superphosphate (896 kg P/ha)

Field (Experiment 5)

Since in experiment 4 we thought not to have attained the optimum growth of red pine and white spruce seedlings, we set up a field experiment with higher rates of P at the Berthierville nursery in Quebec in the Spring of 1970. The fertility of the sandy loam nursery soil is outlined below.

pH	Organic matter %	Available Nutrients (ppm)					
		NH_4-N	NO_3-N	P	K	Ca	Mg
5.0	4.5	3.0	1.6	122	122	325	45

A complete randomized block design with four repetitions was used.

The P fertilization (Table 4) was applied on May 21. Phosphorus as triple superphosphate was incorporated in the first four inches of seedbed soil.

Methyl bromide (0.9 kg per 9.3 square meters) was applied to the soil on the same day. Stratified seeds were sown on June 4. Nitrogen fertilization, applied at a rate of 56 kg N/ha to all plots, was delayed to July 17.

Total precipitation at the field plot site for the 1970 growing season was normal as compared to the eleven precedent years. April and May precipitation were normal,

Table 4. Rates of phosphorus applied to nursery soil (Experiment 5).

Treatment no	P kg/ha
1	- - -
2	336
3	672
4	1008
5	1344
6	1680

but June, July and August was below normal and we supplied by overhead irrigation. Meanwhile in September the precipitation was more than twice the amount of the precedent years. A comparison between the 1970 season and the period of record is shown below.

	Growing Season Precipitation (cm)						
	April	May	June	July	August	Sept.	Total
Period of Record (1959 - 1969)	6.4	6.4	7.5	9.1	9.3	7.2	45.9
1970 Growing season	5.9	6.9	4.3	5.4	6.3	16.5	45.3

During the season observations were made on seedling growth. On October 7, 10 seedlings were collected in every plot and their length was measured. We also recorded their dry shoot and dry root weights. Moreover the seedling shoots were analysed for P concentration using a method modified from Jackson (1958). Our data were submitted to analysis of variance and regression.

CHAPTER IV

RESULTS AND DISCUSSION

The influence of eradicants on mycorrhiza forming fungi varies considerably depending on the nature and concentration of chemicals. A better growth of conifer seedlings is often related to soil fumigation (Wright, 1964), but it also happens that fumigants are shown to reduce drastically the growth of conifer seedlings and preclude the uptake of even available nutrient elements (Henderson and Stone, 1970). This research presents experimental evidence that fumigation significantly affects the mycotrophic mechanism of nursery soils and subsequently P uptake by conifer seedlings. The influence of N and P fertilization, inoculation with pure cultures of mycorrhizal fungi or forest soil were evaluated for their effect on the growth of conifer seedlings raised in fumigated soil. The experiments were conducted in the greenhouse, field and controlled environment chambers.

A- NITROGEN AND PHOSPHORUS FERTILIZATION OF FUMIGATED OR HEAT STERILIZED NURSERY SOIL - GREENHOUSE (1) AND FIELD (2) EXPERIMENTS

1- Morphological Characteristics

Greenhouse - About 5 weeks after germination P deficiency symptoms appeared on white spruce and red pine seedlings growing in fumigated pots not having received any supplemental P. This reddish purple discoloration lasted and intensified until the end of the growth period. The discoloration of the seedlings was accompanied by a severe decrease in growth (Tables 5, 6). In pots having received rock phosphate or bone meal this reddish purple discoloration appeared at the beginning, but disappeared with the lengthening of the period. Healthy and dark green seedlings grew in pots treated with superphosphate fertilizer.

The growth and quality of seedlings, grown in sterilized soil without supplemental P, were not improved by the addition of N (Figure 1). In pots without supplemental N the addition of P significantly increased the size and quality of seedlings independently of the P source. Meanwhile in unsterilized soil the addition of either N or P had not significant influence on morphological characteristics of red pine and white spruce seedlings (Tables 5, 6).

Table 5. Effect of soil sterilization and fertilizer treatments on morphological characteristics of 16 weeks old red pine seedlings (Experiment 1)

FERTILIZER		CONTROL			HEAT STERILIZATION			METHYL BROMIDE		
			DRY WEIGHT			DRY WEIGHT			DRY WEIGHT	
N	P	HEIGHT cm	SHOOT mg	ROOT mg	HEIGHT cm	SHOOT mg	ROOT mg	HEIGHT cm	SHOOT mg	ROOT mg
0	0	3.7	51.2	30.7	3.7	71.2	53.7	3.7	69.2	49.5
0	Rock	3.5	58.0	42.2	3.9	107.2	71.7	4.1	102.7	76.2
0	Super	3.8	60.0	39.2	4.0	88.2	74.0	4.0	126.2	85.0
0	Bone	4.1	88.0	39.5	4.2	122.0	67.0	4.1	108.7	79.7
NO_3	0	4.2	113.5	43.7	3.6	70.2	48.0	3.7	72.0	51.7
NO_3	Rock	4.9	104.7	52.7	4.3	147.7	93.0	4.5	172.5	100.0
NO_3	Super	4.4	120.7	60.5	4.4	147.5	89.0	4.5	157.5	112.7
NO_3	Bone	4.3	99.7	41.0	4.1	118.0	60.7	4.3	124.0	83.0
NH_4	0	4.4	126.0	51.5	3.5	68.0	40.0	3.6	71.0	47.2
NH_4	Rock	4.6	129.0	64.0	4.5	136.0	74.0	4.9	191.7	100.0
NH_4	Super	4.6	140.0	66.7	4.5	150.0	93.0	4.7	204.0	103.5
NH_4	Bone	4.6	126.2	57.0	4.4	173.0	85.0	4.4	156.2	92.0
Tukey's w (.05)		0.6	37.6	23.2	0.5	41.5	21.5	0.7	56.9	33.1
	(.01)	0.7	44.6	27.6	0.6	49.3	25.6	0.9	67.6	39.3

Table 6. Effect of soil sterilization and fertilizer treatments on morphological characteristics of 16 weeks old white spruce seedlings (Experiment 1)

FERTILIZER		CONTROL			HEAT STERILIZATION			METHYL BROMIDE		
			DRY WEIGHT			DRY WEIGHT			DRY WEIGHT	
		HEIGHT	SHOOT	ROOT	HEIGHT	SHOOT	ROOT	HEIGHT	SHOOT	ROOT
N	P	cm	mg	mg	cm	mg	mg	cm	mg	mg
0	0	3.0	28.7	11.2	1.9	16.0	9.2	1.9	17.5	10.7
0	Rock	2.8	25.0	13.2	3.7	74.0	43.0	3.7	79.7	41.5
0	Super	3.0	39.5	20.2	3.4	57.0	41.2	3.4	58.7	33.5
0	Bone	3.4	50.0	17.5	3.4	58.0	26.7	3.6	80.7	41.0
NO_3	0	3.7	64.5	16.0	1.9	18.0	8.7	2.0	18.0	10.0
NO_3	Rock	3.6	56.2	19.7	4.4	95.5	43.0	4.4	97.0	44.2
NO_3	Super	4.8	95.5	26.0	4.5	100.7	49.7	4.4	119.0	55.0
NO_3	Bone	3 4	51.5	16.7	3.5	66.7	23.0	4.0	89.0	38.0
NH_4	0	3.4	55.2	13.5	1.9	17.7	10.7	2.1	20.2	12.0
NH_4	Rock	4.2	84.0	26.2	4.5	111.0	53.0	4.9	131.7	52.7
NH_4	Super	4.3	90.2	27.7	4.5	100.0	53.0	4.8	143.5	54.5
NH_4	Bone	3.8	70.2	19.7	4.5	119.0	42.0	4.4	126.0	54.7
Tukey's w	(.05)	0.9	37.1	9.4	0.9	34.8	19.3	1.2	59.4	30.6
	(.01)	1.1	44.1	11.1	1.0	41.4	22.9	1.4	70.5	36.4

Figure 1. Effect of methyl bromide soil fumigation, N and P addition on 16 weeks old red pine (left) and white spruce (right) seedling development (Treatments: 91, 11 = $(NH_4)_2SO_4$; 158, 7 = Control; 14, 2 = $(NH_4)_2SO_4$ + superphosphate).

Although significant main effects due to soil sterilization occurred, the important features are the interactions between soil sterilization, N and P (Appendix Table 22). It is noteworthy to know that seedlings growing in sterilized soil with added P are better developed and have a greater shoot and root dry weight than those raised in untreated soil. To promote growth of red pine and white spruce seedlings superphosphate seems to be a better source of P than either rock phosphate or bone meal. Superphosphate alone or combined with N treatments from either NO_3 or NH_4 sources generally resulted in a better height growth and a greater shoot and root dry weight than with other treatments. Meanwhile NH_4 sulfate and superphosphate appear to be the best combination for raising red pine and white spruce seedlings. The shoots and roots of these seedlings were at least two times heavier than the control (Figure 2).

Field - By the end of July P deficiency symptoms appeared on white spruce seedlings growing in fumigated nursery sub-plots not having received any supplemental P. This reddish purple discoloration lasted and intensified until the end of the season. This phenomenon also appeared in sub-plots treated with rock phosphate or bone meal, but to a much lesser degree. Generally, the discoloration of

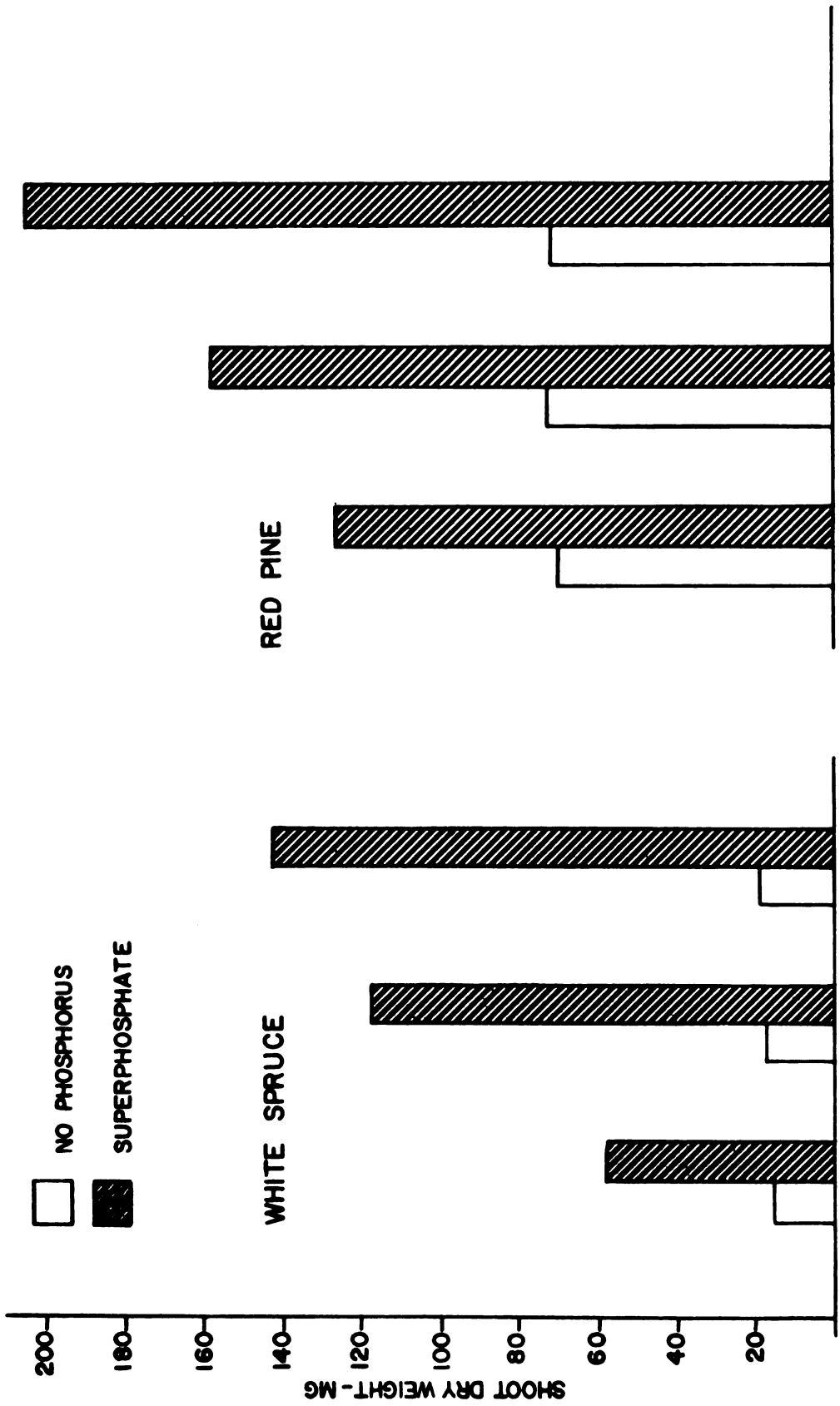

Figure 2. Influence of N sources and superphosphate addition on the shoot dry weight of 16 weeks old white spruce and red pine seedlings grown in methyl bromide fumigated soil (Experiment 1).

the seedlings was accompanied by decrease in growth. Healthy and dark green seedlings were growing in sub-plots treated with superphosphate fertilizer. The same observations apply to the red pine seedlings but the discoloration appeared later and was much less intense.

First sampling date (14 weeks) - Although, as in the greenhouse experiment, significant main effects occurred, the important features are the interaction between soil fumigation and P, and N and P (Appendix, Table 23).

Seedlings growing in untreated soil were generally not significantly affected by the addition of N or P. The addition of rock phosphate or superphosphate to fumigated soil was related to a significant increase in seedling growth and development, while bone meal did not influence them. Still larger seedlings were obtained when N and P were added together to the same soil.

As reported for the greenhouse experiment, superphosphate alone or combined with N treatments from either NO_3 or NH_4 sources resulted in a better height growth and a greater dry weight of shoots and roots than with any other treatments (Tables 7, 8). Again NH_4 sulfate and superphosphate appeared to be the best combination for raising red pine and white spruce seedlings (Figure 3). The shoots of

Table 7. Effect of soil fumigation and fertilizer treatments on morphological characteristics of 14 weeks old red pine seedlings (Experiment 2).

FERTILIZER		CONTROL			METHYL BROMIDE			VAPAM		
			DRY WEIGHT			DRY WEIGHT			DRY WEIGHT	
N	P	HEIGHT cm	SHOOT mg	ROOT mg	HEIGHT cm	SHOOT mg	ROOT mg	HEIGHT cm	SHOOT mg	ROOT mg
0	0	3.8	43.0	17.2	4.0	47.2	17.5	4.0	52.5	16.2
0	Rock	3.8	43.2	17.0	4.1	49.7	18.5	4.0	57.7	17.2
0	Super	3.8	44.7	16.5	4.3	68.7	20.7	4.4	80.0	21.2
0	Bone	3.9	36.0	10.6	4.1	54.0	15.0	4.1	56.0	14.2
NO_3	0	3.7	41.0	15.0	4.2	51.5	16.7	4.1	50.0	15.2
NO_3	Rock	3.8	37.7	13.0	4.0	50.7	16.2	4.1	50.7	15.2
NO_3	Super	3.7	42.0	13.5	4.4	70.7	21.0	4.3	66.0	19.5
NO_3	Bone	3.6	29.2	10.0	3.9	49.2	12.5	4.1	46.2	13.2
NH_4	0	3.9	52.0	18.0	4.2	55.5	19.2	4.0	56.0	18.0
NH_4	Rock	4.0	48.7	17.7	4.5	73.2	24.2	4.2	69.7	22.0
NH_4	Super	4.1	57.2	17.2	4.7	93.0	25.0	4.6	92.0	23.2
NH_4	Bone	3.7	37.5	10.5	4.1	49.0	13.7	3.8	53.0	15.7
Tukey's w	(.05)	0.5	16.2	5.4	0.5	12.3	4.9	0.5	14.6	4.3
	(.01)	0.6	19.1	6.4	0.6	14.5	5.8	0.6	17.1	5.1

Table 8. Effect of soil fumigation and fertilizer treatments on morphological characteristics of 14 weeks old white spruce seedlings (Experiment 2).

FERTILIZER		CONTROL			METHYL BROMIDE			VAPAM		
			DRY WEIGHT			DRY WEIGHT			DRY WEIGHT	
N	P	HEIGHT cm	SHOOT mg	ROOT mg	HEIGHT cm	SHOOT mg	ROOT mg	HEIGHT cm	SHOOT mg	ROOT mg
0	0	3.2	17.7	6.2	2.9	15.2	5.3	2.8	14.5	5.2
0	Rock	3.2	17.0	5.5	3.2	19.0	7.5	3.2	19.0	6.5
0	Super	3.6	25.0	7.5	4.8	41.2	11.5	4.5	35.2	9.0
0	Bone	3.5	25.5	6.7	3.4	23.7	7.0	3.2	20.0	6.0
NO_3	0	2.9	15.5	5.5	3.1	15.0	4.7	2.6	13.5	4.0
NO_3	Rock	2.9	15.0	4.7	3.0	17.0	6.2	3.0	16.5	5.7
NO_3	Super	3.2	20.2	5.5	4.5	41.7	10.0	4.5	37.5	9.0
NO_3	Bone	3.1	19.5	5.5	3.0	18.5	6.5	2.8	14.0	4.0
NH_4	0	2.8	16.5	5.5	2.9	15.2	5.5	2.8	14.5	5.0
NH_4	Rock	3.1	20.0	6.2	3.7	28.7	9.7	3.9	26.5	8.2
NH_4	Super	4.0	32.7	7.5	5.1	55.0	11.5	4.9	54.7	13.5
NH_4	Bone	3.4	29.2	7.2	3.3	24.5	8.0	3.1	16.2	5.2
Tukey's w	(.05)	0.5	6.1	1.8	0.7	9.6	2.1	0.7	9.9	3.5
	(.01)	0.6	7.2	2.2	0.9	11.3	2.5	0.8	11.6	4.2

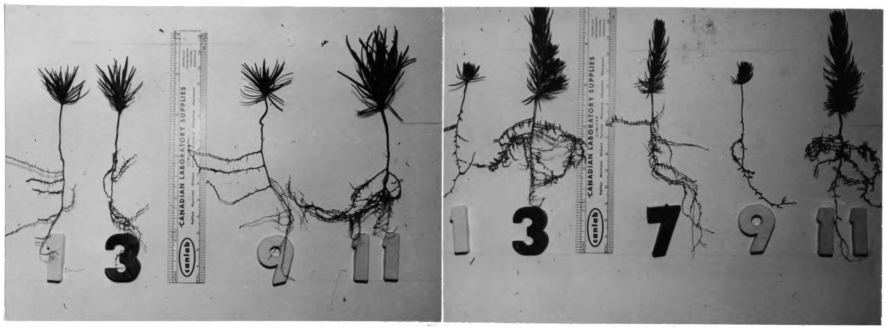

Figure 3. Effect of N and P addition on the shoot and root growth of red pine (left) and white spruce (right) grown in methyl bromide fumigated seedbed - Berthierville nursery (Treatments: left, 1 = control, 3 = superphosphate, 9 = $(NH_4)_2SO_4$, 11 = $(NH_4)SO_4$ + superphosphate; right, 1 = control, 3 = superphosphate, 7 = $NaNO_3$ + superphosphate, 11 = $(NH_4)_2SO_4$ + superphosphate.)

these seedlings were two to four times heavier and the roots three times heavier than the control (Figure 4).

Second sampling date (17 months) - White spruce seedlings grown in fumigated soil showed a much better growth and a healthier appearance than those grown in untreated soil. The main effects of experimental factors are highly significant (Appendix Table 24) and only the interaction of fumigation and phosphorus application is showing high significance (0.01 level).

At the end of the second growing season seedlings were dark green, sturdy (Figure 5) and showed a balanced root system. We can see the beneficial effect of N and mainly P fertilization on the seedling development. Looking at N sources the superiority of NH_4 over NO_3 when used in combination with P becomes much more evident than at the 1-0 stage (Table 9). Indeed the addition of N alone did not improve seedling growth. Rock phosphate did improve the growth, but not significantly; meanwhile superphosphate increased the shoot or root seedling dry weight to ten times when the soil had been fumigated with vapam. In unfumigated soil the growth increase of superphosphate addition was not so large, about three times the control (Figure 6).

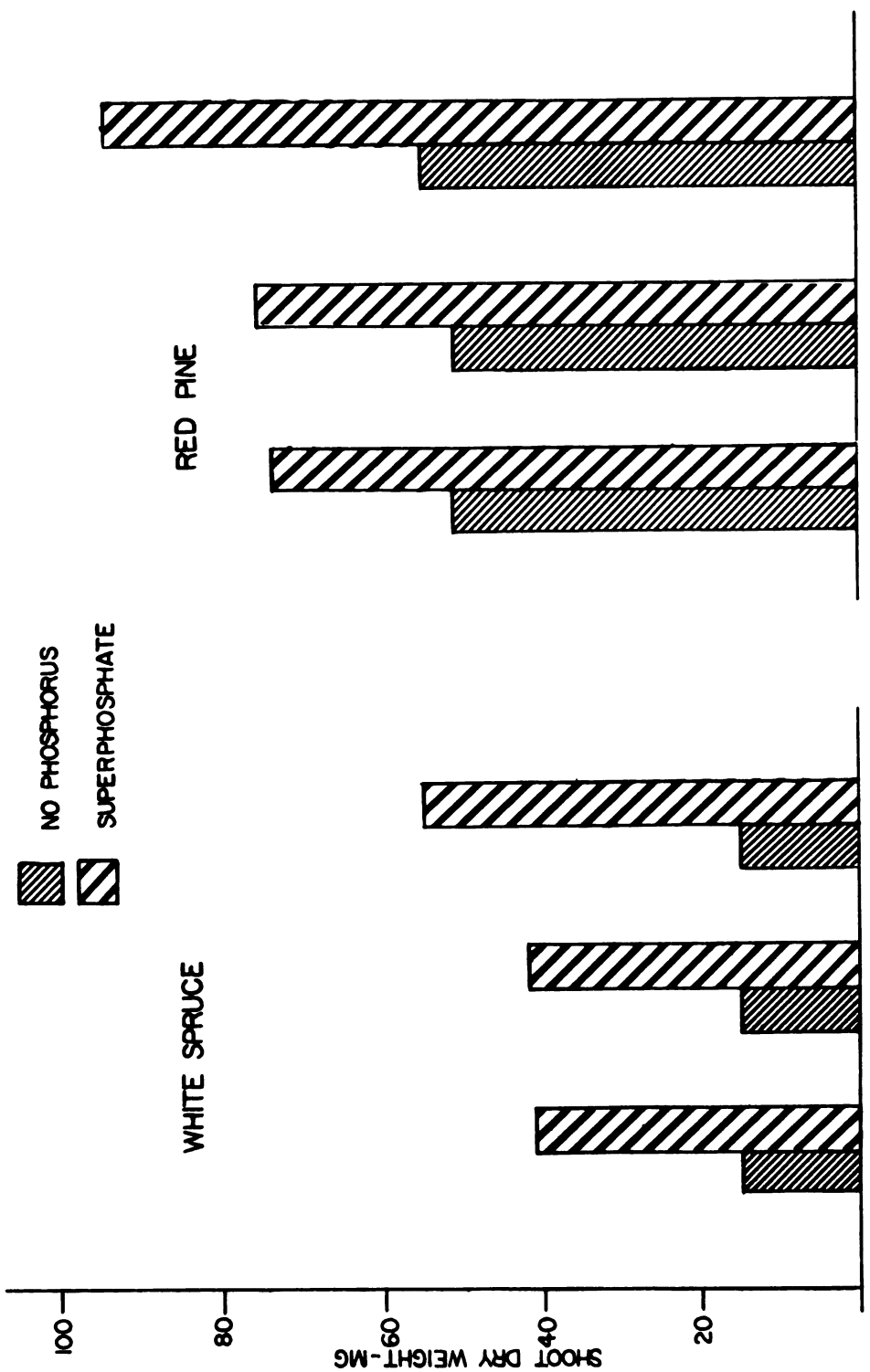

Figure 4. Influence of N sources and superphosphate addition on the shoot dry weight of 14 weeks old white spruce and red pine seedlings grown in methyl bromide fumigated seedbed (Experiment 2).

Figure 5. Effect of N and P addition on shoot growth of 2-0 white spruce seedlings grown in methyl bromide fumigated seedbed - Berthierville nursery (Treatments: A = $(NH_4)_2SO_4$ + superphosphate; B = control).

Table 9. Influence of experimental factors on morphological characteristics of white spruce seedlings after two growth seasons (Experiment 2).

FERTILIZER		CONTROL			VAPAM		
			DRY WEIGHT			DRY WEIGHT	
N	P	Height cm	Shoot mg	Root mg	Height cm	Shoot mg	Root mg
0	0	7.6	371	193	8.3	317	104
0	Rock	6.3	291	160	10.3	504	170
0	Super	7.2	402	245	15.4	1711	528
NO_3	0	8.2	538	250	7.0	236	84
NO_3	Rock	7.1	433	214	10.1	626	202
NO_3	Super	8.1	533	246	17.5	1810	505
NH_4	0	8.4	556	259	8.4	328	129
NH_4	Rock	9.5	628	289	11.7	938	380
NH_4	Super	11.9	1052	443	23.4	3093	1019
Tukey's w(.05)		4.4	621	228	5.5	923	299
	(.01)	5.3	751	275	6.7	1115	361

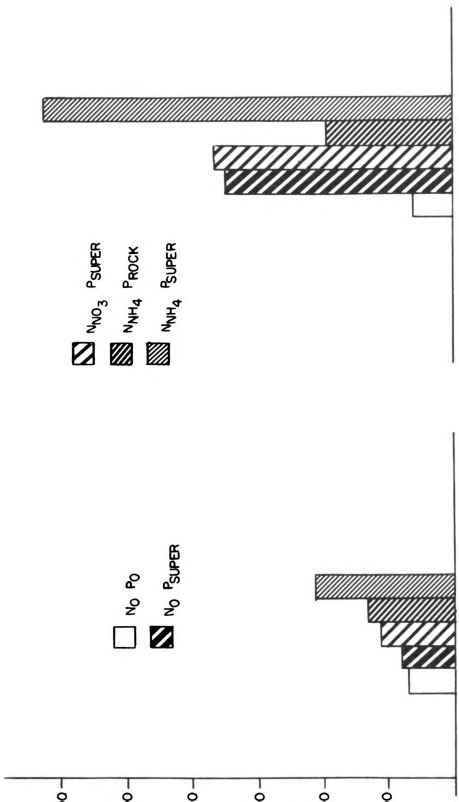

Figure 6. Influence of soil fumigation, N and P addition on the shoot dry weight of 2-0 white spruce seedlings (Experiment 2).

Discussion - Thorough soil sterilization with methyl bromide, vapam, or heat, duplicated in greenhouse and field experiments the adverse effects on seedling growth observed in routine nursery practice. In both experiments we reached statistical significance.

The severe decrease in growth and seedling development following soil sterilization could in part be attributed to the fact that this soil treatment had killed most soil microorganisms and fungi capable of forming symbiont association with seedling short roots. Mycorrhizal formation having been restricted, there was a lesser root surface for absorption, and then P absorption and growth could have been decreased (Hatch, 1937). Henderson and Stone (1970) observed that both growth and P content of non-mycorrhizal coniferous seedlings grown in fumigated soil without P fertilization were less than any other treatments; they attributed this phenomenon to the absence of mycorrhizae. It seems that, in the absence of mycorrhizal development of roots, the young seedlings can only absorb P when present in inorganic form at high concentration in the soil solution.

From the three P sources used, only the superphosphate addition was consistently related to a very good growth rate and a dark green seedling color. Meanwhile at sampling time, despite a fairly good root development, we observed few

if any mycorrhizae. It is possible that mycorrhizae were still not visible at that time. Larger seedlings could also result from a higher soil fertility level which can provide adequate nutrition even in the absence of mycorrhizae (Howe and Clifford, 1962; Wright, 1964; Henderson and Stone, 1970).

Nitrogen alone had very little influence on seedlings grown in sterilized soil. But addition of N and P were related to larger seedlings than P alone. Furthermore NH_4-N was consistently a better source of N than NO_3-N. At pH around 5.0 the NH_4 form of N is probably more available than the NO_3 form to conifer seedlings (McFee and Stone, 1968). The seedlings may absorb NH_4-N faster than NO_3-N, or because N is lost by leaching in the NO_3 form.

It becomes apparent that the best combination would be a fumigated soil, a NH_4 and a superphosphate addition. Seedlings produced in this manner compare favorably in size with standards set by Armson and Carman (1961).

Comparison with the unfumigated controls shows that following fumigation harmful pathogens are eliminated and seedlings growing in soil with adequate amounts of N and P make good first season growth and well formed buds for the next season. Since in conifers the growth conditions prevailing in one season determine to a large part the next

year's growth, the preforming of a good shoot and terminal bud is prerequisite to a healthy seedling.

2- Mineral nutrient concentration of shoot

Greenhouse - The significance of mineral nutrient concentration of seedling shoot tissue seemed to be due not only to the main effects of the experimental factors but also to their interaction (Appendix Table 25).

In untreated soil red pine and white spruce shoot tissue had a significant lower N percentage than those raised in sterilized seedbeds (Tables 10,11). The seedlings grown in sterilized soil not receiving any N also had a low level of this element in their shoot tissue. The addition of N as NH_4 or NO_3 sources significantly increased the shoot tissue concentration of this element. In the white spruce and red pine seedlings there is a significant interaction of P and N fertilizers. Indeed the addition of either superphosphate or rock phosphate is related to a sensible decrease of the N level in shoot tissue (Figure 7). This could be related to a better development of the seedlings accompanied by a dilution of N concentration in shoot tissue.

The addition of any P source is related to a significant increase of P concentration in shoot tissue, either for

Table 10. Effect of soil sterilization and fertilizer treatments on shoot nitrogen and phosphorus concentration (%) of 16 weeks old red pine seedlings (Experiment 1).

FERTILIZER		CONTROL		HEAT STERILIZATION		METHYL BROMIDE	
N	P	N %	P %	N %	P %	N %	P %
0	0	0.94	.201	1.53	.077	1.68	.073
0	Rock	0.87	.229	1.02	.154	1.06	.138
0	Super	1.05	.243	1.07	.154	1.16	.127
0	Bone	1.33	.204	1.59	.108	1.68	.089
NO_3	0	1.48	.165	2.32	.080	2.27	.092
NO_3	Rock	1.62	.214	1.76	.137	1.47	.147
NO_3	Super	1.47	.198	1.54	.160	1.63	.158
NO_3	Bone	1.93	.204	2.26	.113	2.28	.097
NH_4	0	1.32	.195	2.48	.080	2.21	.080
NH_4	Rock	0.98	.251	1.85	.164	1.66	.138
NH_4	Super	1.43	.222	1.55	.156	1.37	.147
NH_4	Bone	1.85	.233	1.39	.117	1.96	.139
Tukey's w (.05)		.53	.113	.82	.133	.64	.074
	(.01)	.63	.135	.97	.158	.75	.088

Table 11. Effect of soil sterilization and fertilizer treatments on shoot nitrogen and phosphorus concentration (%) of 16 weeks old white spruce seedlings (Experiment 1).

FERTILIZER		CONTROL		HEAT STERILIZATION		METHYL BROMIDE	
N	P	N %	P %	N %	P %	N %	P %
0	0	0.97	.205	2.07	.105	2.02	.086
0	Rock	1.00	.251	0.88	.160	1.00	.190
0	Super	0.87	.262	0.94	.175	1.09	.211
0	Bone	1.20	.193	1.72	.112	1.49	.155
NO_3	0	1.56	.175	2.18	.082	2.21	.102
NO_3	Rock	1.57	.187	1.52	.157	1.45	.230
NO_3	Super	1.55	.231	1.44	.201	1.48	.241
NO_3	Bone	1.63	.167	1.84	.127	1.88	.165
NH_4	0	1.50	.155	2.49	.103	2.95	.098
NH_4	Rock	1.49	.214	1.32	.158	1.52	.182
NH_4	Super	1.55	.225	1.56	.168	1.58	.183
NH_4	Bone	1.62	.190	1.79	.132	1.56	.184
Tukey's w(.05)		0.36	.059	.42	.133	.51	.054
	(.01)	0.43	.070	.50	.158	.61	.064

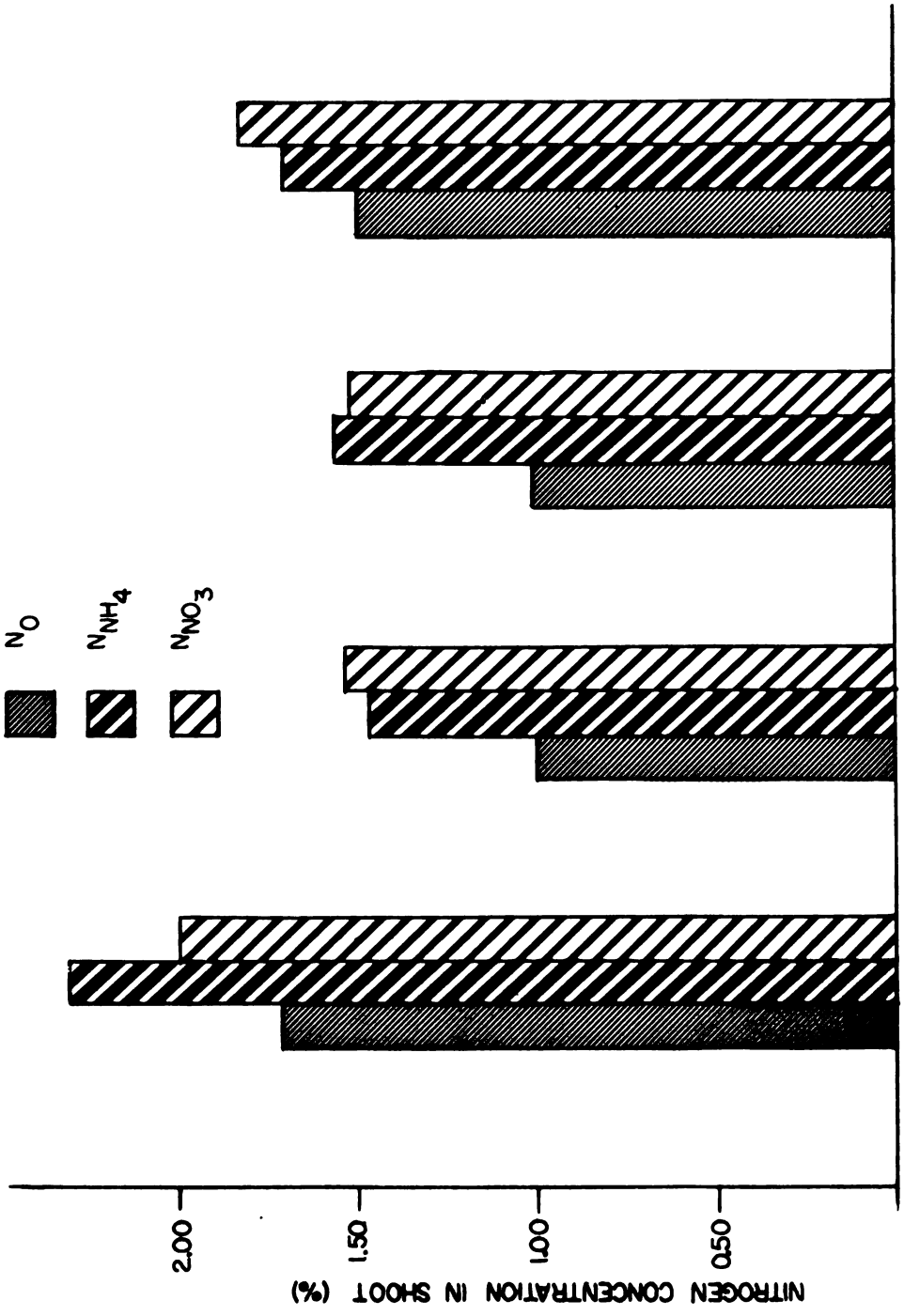

Figure 7. Influence of P and N fertilization on the N concentration (%) in shoot tissue of 16 weeks old white spruce seedlings (Experiment 1).

red pine or white spruce seedlings. The greatest increase was obtained with rock phosphate and superphosphate, as shown below.

P Sources	Phosphorus Concentration in Shoot (% Dry Weight)	
	Red Pine	White Spruce
None	.116*	.123*
Rock phosphate	.174	.192
Superphosphate	.174	.211
Bone meal	.145	.158

*Means of 36 determinations.

Following soil sterilization, the P concentration of seedling shoot tissue was lower than for seedlings grown in untreated soil. This reduction of P uptake was really severe when no P fertilizer was added to the soil (Figure 8). The addition of rock phosphate or superphosphate to sterilized soil brought a net increase of the shoot P concentration and a good rate of growth. This appears to be a typical effect of soil fumigation or sterilization on the uptake of P by young conifer seedlings. Red pine and white spruce seedlings grown in untreated soil showed a P level considered sufficient for an optimum growth, even when no P was added to the soil.

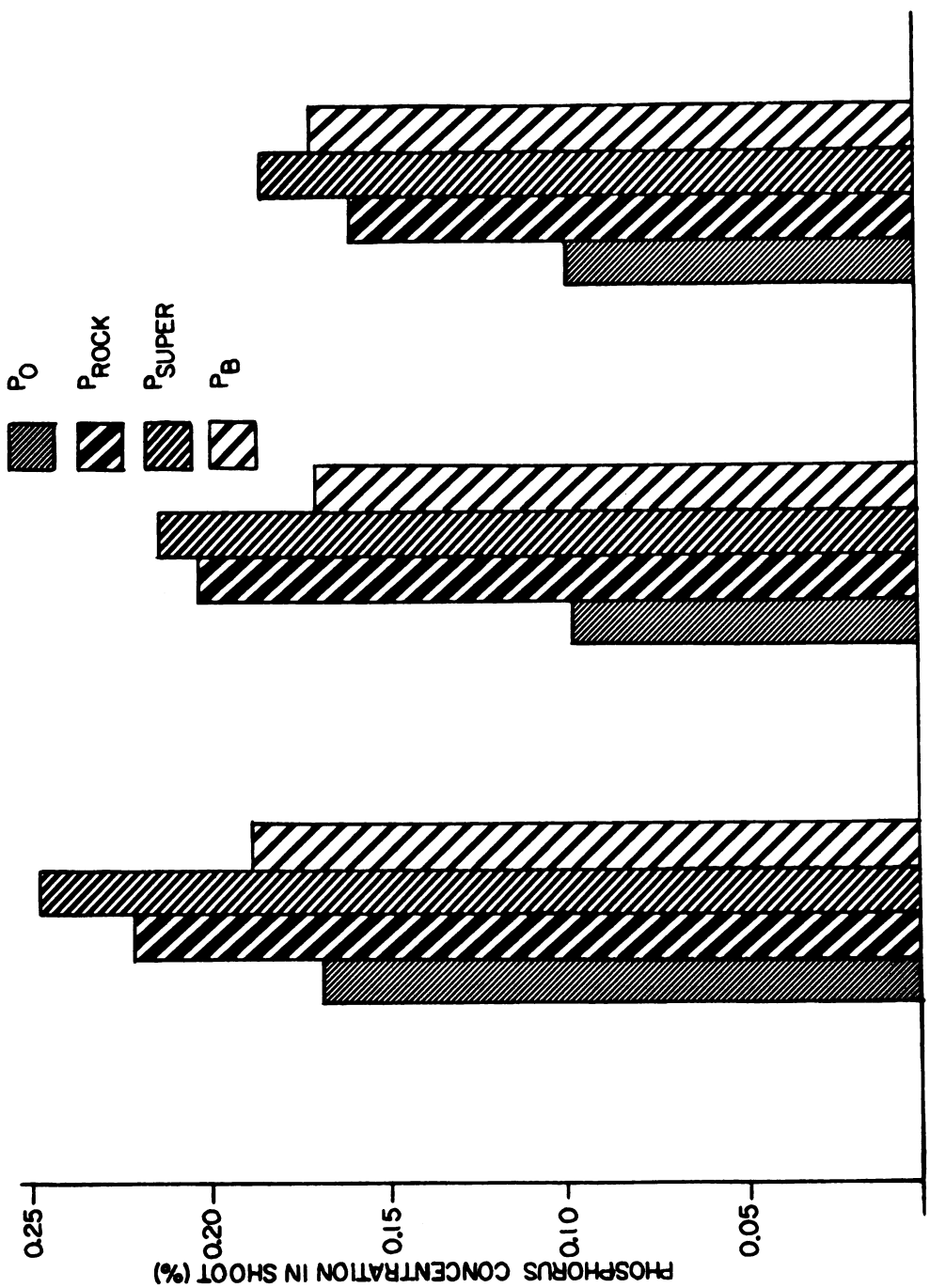

Figure 8. Influence of soil sterilization and P sources on P concentration (%) in shoot of 16 weeks old white spruce seedlings (Experiment 1).

Field - The significance of mineral nutrient concentration of seedling (14 weeks old) shoot tissue seemed to relate more to the main effects of the experimental factors than to their interaction (Appendix Table 26). Soil sterilization was related to greater accumulation of N in shoot tissue, whereas it significantly decreased the P concentration. The addition of N as NH_4 significantly increased the shoot tissue concentration of this element. Indeed we had a greater N concentration following a NH_4 than a NO_3-N addition (Figure 9).

The addition of any P source to fumigated soil is related to a significant increase of P shoot tissue concentration either for red pine or white spruce seedlings. The greatest increase was obtained with superphosphate. Rock phosphate or bone meal addition brought a much lower P concentration (Figure 10). This fact supports the visual observation of a P deficiency in seedlings growing in plots with no supplemental P or treated with rock phosphate or bone meal. The non-deficiency level was reached only when superphosphate was added to the fumigated soil (Tables 12, 13).

The addition of superphosphate to soil is related to a significant increase of foliar K concentration for both red pine and white spruce seedlings.

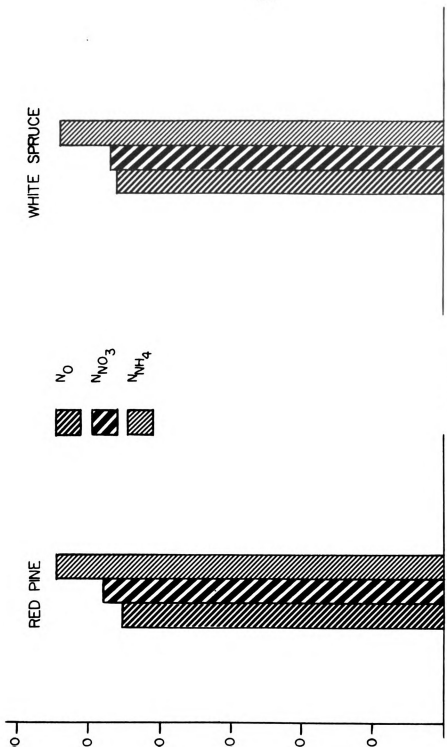

Figure 9. Influence of N sources on N concentration (%) in shoot of 14 weeks old red pine and white spruce seedlings (Experiment 2).

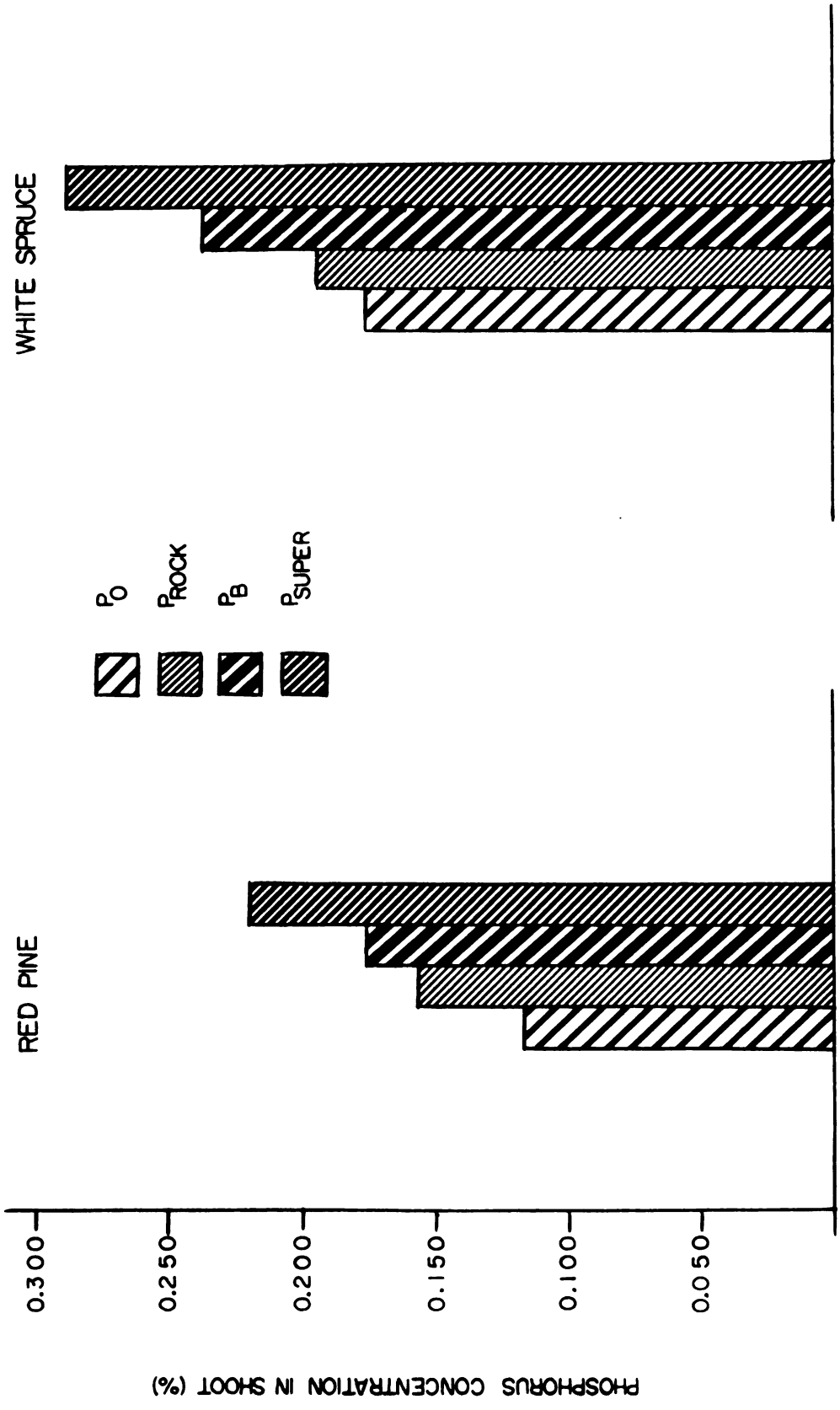

Figure 10. Influence of P sources on P concentration (%) in shoot of 14 weeks old red pine and white spruce seedlings (Experiment 2).

Table 12. Effect of soil fumigation and fertilizer treatments on shoot nitrogen, phosphorus and potassium concentration (%) of 14 weeks old red pine seedlings (Experiment 2).

FERTILIZER		CONTROL			METHYL BROMIDE			VAPAM		
N	P	N %	P %	K %	N %	P %	K %	N %	P %	K %
0	0	1.91	.217	.662	2.25	.135	.542	2.25	.134	.572
0	Rock	1.93	.218	.637	2.26	.148	.617	2.23	.133	.597
0	Super	2.02	.260	.667	2.39	.211	.860	2.34	.210	.737
0	Bone	2.11	.228	.650	2.64	.163	.642	2.51	.153	.690
NO_3	0	2.04	.173	.502	2.51	.134	.675	2.31	.128	.567
NO_3	Rock	2.27	.183	.487	2.44	.140	.600	2.31	.135	.600
NO_3	Super	2.17	.204	.490	2.55	.213	.742	2.49	.203	.780
NO_3	Bone	2.28	.267	.760	2.71	.151	.670	2.53	.148	.687
NH_4	0	2.64	.189	.662	2.59	.133	.567	2.49	.137	.530
NH_4	Rock	2.50	.189	.670	2.68	.162	.455	2.55	.155	.637
NH_4	Super	2.43	.221	.697	2.90	.255	.750	2.84	.273	.790
NH_4	Bone	2.55	.213	.577	2.85	.159	.582	2.60	.173	.500
Tukey's w(.05)		.50	.074	.20	.28	.032	.40	.32	.034	.24
	(.01)	.59	.088	.23	.33	.039	.48	.38	.041	.29

Table 13. Effect of soil fumigation and fertilizer treatments on shoot nitrogen, phosphorus, and potassium concentration (%) of 14 weeks old white spruce seedlings (Experiment 2).

FERTILIZER		CONTROL			METHYL BROMIDE			VAPAM		
N	P	N %	P %	K %	N %	P %	K %	N %	P %	K %
0	0	2.04	.273	1.05	2.07	.158	0.93	2.01	.145	0.92
0	Rock	2.07	.281	1.01	2.13	.176	0.92	2.25	.163	1.09
0	Super	1.98	.356	0.99	2.08	.253	1.09	2.33	.306	1.36
0	Bone	2.08	.318	1.13	2.74	.228	0.93	2.67	.200	0.93
NO_3	0	2.15	.233	0.82	2.30	.168	0.92	2.14	.158	0.83
NO_3	Rock	2.05	.231	0.52	2.28	.155	0.82	2.21	.170	0.90
NO_3	Super	2.08	.315	0.81	2.37	.268	1.04	2.42	.293	1.24
NO_3	Bone	2.18	.259	0.93	2.91	.231	0.74	2.77	.179	0.84
NH_4	o	2.78	.201	0.86	2.47	.152	0.77	2.48	.164	0.90
NH_4	Rock	2.47	.205	0.92	2.75	.198	0.85	2.79	.223	1.06
NH_4	Super	2.35	.266	0.94	2.71	.310	1.11	2.77	.324	1.19
NH_4	Bone	2.27	.281	1.00	3.18	.278	0.89	2.89	.218	0.87
Tukey's w(.05)		.28	.064	.42	.28	.089	.30	.32	.064	.19
(.01)		.33	.076	.50	.34	.105	.36	.38	.076	.22

Discussion - In both experiments a greater amount of N was accumulated by seedlings growing in sterilized soil than in untreated soil. This could be in part due to the fact that after fumigation N accumulates in the soil as NH_4 and this form of N is easily taken up by conifer seedlings. This NH_4 accumulation could be related to a near annihilation of the soil nitrifier population by sterilization. Both tree species appeared to prefer NH_4-N over NO_3-N either for growth or N uptake in their shoot tissue. The increase in shoot N content following soil sterilization seems to be also related to a better growth of seedlings following P fertilization in sterilized soil than in untreated soil.

The red pine and white spruce seedlings showing evident P deficiency symptoms gave a P concentration as low as .090 per cent when grown in the greenhouse, although those raised in the nursery beds showed a little higher P content for the same symptoms.

Swan (1960) reported evident P deficiency symptoms at 0.070 per cent in shoots of Pinus banksiana and at 0.100 per cent in Picea glauca and P. mariana raised in greenhouse. Fowells and Krauss (1959) reported symptoms in Pinus taeda and P. virginiana at 0.100 per cent P in the leaves. Ingestad (1962) found that deficiency symptoms are generally related to a foliar P concentration of 0.060 - 0.090 for

pines and 0.050 - 0.110 for spruces. On the other hand, Armson and Carman (1961) reported as deficient a P level lower than .200 per cent for red pine and .250 per cent for white spruce seedlings raised in nursery beds. Our data agree very well with findings of these workers.

The very low level of P in shoot tissue of seedlings growing in sterilized soil, without addition of P, was associated with a very poor seedling development and an absence of lateral and short roots. They showed a reddish purple discoloration of their foliage which is symptomatic of P deficiency. It is probable that soil sterilization severely reduced mycorrhizal fungi and other soil microorganisms necessary to a good availability and uptake of mineral nutrients (Iyer, Chesters and Wilde, 1968).

Mechanisms which make nutrients available to plant roots are: (1) Root interception, (2) mass-flow, and (3) diffusion. The concentration of soluble P in the soil solution is always low except close to sites of applied fertilizer. Moreover P is not normally considered to be a mobile nutrient and mass-flow is known to supply less than one per cent of the P needed by corn (Barber, 1964). Apparently, the rate of diffusion of P varies with soil moisture and is greater when more water is present. This makes

more P available in the root zone where root interception and contact feeding take place.

What has been learnt about pine root distribution and what is known about the way P moves in the soil give reason to believe that mycorrhizae do in fact help with P nutrition. The absorbing portion of a growing root remains functional for only 5-10 days, but mycorrhizae continue to absorb nutrients for a much longer period - possibly for as long as a year. Under these circumstances P from a solid particle will diffuse much further whatever the soil conditions are. The exploitation of soil reserves is thereby increased substantially. Moreover the hyphae, being much thinner than root hairs, can insinuate themselves into finer soil pores and this does increase the degree of soil exploitation.

It is known that mycorrhizal fungi can store large amounts of P. This facility may be important in trapping P when large amounts suddenly become available, such as after a P fertilizer has been applied, and making it available to the plant at a later stage. Experiments have also shown that mycorrhizae can absorb a number of organic compounds not normally taken up by higher plants.

The fumigation must have also had an effect on other rhizosphere microorganisms. The interactions of soil

fumigation, rhizosphere microorganisms on the availability of soil P for plant uptake could here be related to a lesser rate of seedling growth and a lower P content of shoot tissue. Indeed Gerretsen (1948), studying the influence of rhizosphere microorganisms on phosphate uptake in plants grew a variety of crop plants in sterilized and non-sterilized soil, to which various insoluble phosphates were added. He observed a greater phosphate absorption in the plants growing in non-sterilized soil.

Phosphorus as adenosine triphosphate and numerous phosphorylated products participates in nearly all synthetic reactions of the plant. It is essential for the development of meristematic tissues and it is associated with the general process of respiration. We can infer that any reduction in P absorption by coniferous seedlings due to unavailability of the P source and root surface factors will have a strong influence on seedling growth and the concentration of shoot P.

The level of K in shoot tissue is in the range set by Armson and Carman (1961) for an optimum growth of 1-0 conifer seedlings. The greater K uptake following superphosphate soil addition is probably related to the Ca content of this fertilizer. Superphosphate addition significantly increased the Ca available in soil. Jacobson et al (1961) reported an

increased K absorption by barley (Hordeum vulgare), corn (Zea mays), and sunflower (Helianthus annuus) roots, when Ca was present in the nutrient solution.

3- Soil Fumigation, Phosphorus Addition and Seedling Survival - As reported by other workers (Howe and Clifford, 1962; Wright, 1964) soil fumigation greatly increased first year survival (Table 14). This increase was particularly high when the test species was red pine. In unfumigated soil high losses from damping-off were usually observed.

The addition of P did not affect survival, except that addition of bone meal was significantly correlated with a reduced survival of seedlings. Bone meal seemed to have been related to a lower germination and a high damping-off mortality either for red pine and white spruce. Wahlenberg (1930) also reported that addition of bone meal or dried blood caused increased damping-off of conifer seedlings. The addition of superphosphate slightly decreased white spruce stocking. Benzian (1965), summing up experiments covering many soils and seasons in English nurseries reported that superphosphate had been found safe although occasionally survival can be slightly decreased by phosphate applications.

Table 14. Effect of experimental factors on first year survival of red pine and white spruce seedlings (Experiment 2).

	RED PINE	WHITE SPRUCE
Soil fumigants	No seedlings/ linear meter	No seedlings/ linear meter
None	8	52
Methyl bromide	24	79
Vapam	30	67
Tukey's w(.05)	4	16
(.01)	6	23
P. sources		
0	25	86
Rock	26	81
Super	23	77
Bone	8	26
Tukey's w(.05)	3	6
(.01)	4	8

4- Effects of Soil Fumigation on Soil Characteristics - Soil reaction has an important effect on growth of most plants since it alters availability of nutrients and the constitution of soil micro-flora and fauna. Soil fumigation significantly increased the pH level in soil whereas here the addition of either NO_3 or NH_4-H lowered this level (Appendix Table 27). The acidifying effect of NO_3 cannot be easily explained, except by an unknown interaction. As foreseen NH_4 sulfate addition significantly decreased soil pH (Table 15). The soil pH was not altered by superphosphate or rock phosphate sources. The addition of bone meal markedly reduced soil acidity. This effect is probably due to the high organic and Ca content of this P source.

As reported by others (Good and Carter, 1965; Driessche, 1969), our nursery soil fumigation either with methyl bromide or vapam delayed nitrification for the first growing season and was characterized by NH_4 accumulation (Table 15). It is generally recognized that the nitrifiers are among the most sensitive of soil bacterial flora to injury by fumigant chemicals (Martin and Pratt, 1958). Wolcott et al (1960) observed that the nitrifying activity of a muck soil was sharply reduced by fumigation with Telone at 385 liters per hectare. There was an 8-week lag before a significant recovery occurred. Winfree and Cox (1958) reported

Table 15. Influence of experimental factors on pH, NH_4 and NO_3, soil available phosphorus, and exchangeable potassium, calcium, and magnesium in nursery soil (Experiment 2).

Experimental Factor	pH	NH_4 kg/ha	NO_3 kg/ha	P kg/ha	K kg/ha	Ca kg/ha	Mg kg/ha
A) Fumigation							
1) None	5.0	47	130	260	298	1168	59
2) Methyl bromide	5.4	163	62	242	349	1021	81
3) Vapam	5.2	143	82	254	340	1067	66
Tukey's w(.05)	.08	39	30	46	67	217	19
(.01)	.11	54	44	72	97	317	28
B) Nitrogen sources							
1) None	5.4	63	32	245	323	1093	70
2) NO_3	5.2	57	170	248	331	1085	66
3) NH_4	5.0	234	73	264	335	1078	70
Tukey's w(.05)	.06	20	14	37	19	84	10
(.01)	.07	29	21	54	28	122	15
C) Phosphorus sources							
1) None	5.0	90	96	116	348	900	58
2) Rock	5.1	92	96	132	342	969	58
3) Super	5.1	94	76	564	321	1291	76
4) Bone	5.5	196	98	197	305	1180	83
Tukey's w(.05)	.07	23	16	42	22	97	12
(.01)	.09	34	24	62	33	144	17

A) and B) Means of 48 determinations; C) Means of 36 determinations.

that either chloropicrin (412 liters per ha.) or methyl bromide (.1 kg per square meter) caused an initial accumulation of NH_4-N at the expense of NO_3-N. This NH_4 accumulation in treated nursery soil could be a beneficial effect of soil fumigation since conifer seedlings seem to have a better growth when N is available in NH_4 form (McFee and Stone, 1968).

The addition of P had little effect on NH_4 or NO_3 level in soil except for bone meal which significantly increased the NH_4 soil content. This is evidence that proteins in bone meal were rapidly decomposed.

Superphosphate, made by treating raw rock phosphate with suitable amounts of sulfuric acid (Buckman and Brady, 1968) seems to be an effective and economical carrier of phosphatic acid for conifer seedling production. The amount of P available at the end of the season in the soil fertilized with 505 kg of P as superphosphate was as high as 564 kg of P. If we consider the amount in the soil before any P addition it seems as though the major part of this added P is in an available form at the end of the season. P also could have been made available from organic sources mineralized during the season. The efficiency of P fertilizers with regard to the P supply to plants depends on the extent, and for how long, the fertilizer is able to increase the availability

of P in the vicinity of the plant roots. In this experiment superphosphate increases the soil test for P much more than either rock phosphate or bone meal (Table 15). This superiority of superphosphate as a P source is also shown in the size of seedlings grown following the addition of this fertilizer to the soil.

Experimental factors were related to a few significant increases or decreases of exchangeable amounts of other nutrient elements. However to explain them would require additional experiments with these specific elements.

B- INOCULATION OF METHYL BROMIDE OR VAPAM FUMIGATED NURSERY SOIL - GROWTH CHAMBER (EXPERIMENT 3).

In this experiment both fumigants, methyl bromide and vapam, showed a very similar effect on morphological characteristics of red pine and white spruce seedlings (Tables 16, 17).

Meanwhile the inoculation of fumigated nursery soil with forest soil was associated with a much better growth and development of seedlings than control or other inoculation with pure cultures. There were highly significant (0.01 level) differences for all the studied seedling characteristics for both test species (Appendix Table 28). The green and dry weights of the seedling shoots and roots grown in

Table 16. Effect of methyl bromide soil fumigation and mycorrhizal fungi inoculation on morphological characteristics of 16 weeks old red pine and white spruce seedlings (Experiment 3).

Fungi Inoculation	Height	Green Weight Shoot	Green Weight Root	Dry Weight Shoot	Dry Weight Root	Mycorrhizal Short Roots
	cm	mg				%
RED PINE						
None	4.9	398	672	259	153	0.0
Forest Soil	6.3	3427	1530	991	349	30.2
Amanita rubescens	4.3	535	621	167	133	0.0
Rhizopogon roseolus	5.1	1314	950	422	240	7.5
Suillus luteus	4.7	438	564	147	119	0.2
Tukey's w(.05)	1.1	1650	847	491	198	28.4
(.01)	1.4	2137	1097	636	256	36.0
WHITE SPRUCE						
None	3.2	151	134	53	31	0.0
Forest Soil	7.7	2233	483	606	108	14.0
Cenococcum graniforme	3.4	213	141	68	32	0.0
Suillus granulatus	3.5	209	187	69	31	0.0
Telephora terrestris	3.5	188	163	63	26	0.0
Tukey's w(.05)	2.5	1889	405	491	98	3.9
(.01)	3.3	2246	525	636	127	5.1

Table 17. Effect of vapam soil fumigation and mycorrhizal fungi inoculation on morphological characteristics of 16 weeks old red pine and white spruce seedlings (Experiment 3).

	Height	Green Weight		Dry Weight		Mycorrhizal Short Roots
		Short	Root	Shoot	Root	
	cm	-mg-				%
RED PINE						
None	4.4	323	442	122	103	0.0
Forest Soil	6.5	3775	1739	1220	485	27.7
Amanita rubescens	4.3	343	424	131	95	0.0
Rhizopogon roseolus	4.5	334	386	120	90	0.0
Suillus luteus	4.4	346	431	128	93	0.0
Tukey's w(.05)	0.5	158	355	103	72	9.4
(.01)	0.6	204	460	134	93	12.2
WHITE SPRUCE						
None	2.9	80	58	31	16	0.0
Forest Soil	8.7	2204	507	609	124	12.2
Cenococcum graniforme	2.9	79	62	29	16	0.0
Suillus granulatus	3.1	100	80	37	20	0.0
Telephora terrestris	3.5	223	71	67	19	0.0
Tukey's w(.05)	2.4	1005	211	284	69	9.0
(.01)	3.1	1302	274	367	89	11.0

the forest soil inoculated pots were two to ten times greater than those grown in control pots (Figure 11). The former seedlings were dark green and were showing a healthy condition and a balanced rate of growth at sampling time, while the latter were showing a reddish purple discoloration of needles, and signs of P deficiency.

Unfortunately, the pure cultures of fungi inoculation failed except for a very few pots. In most pots in this serie the seedlings showed a reddish purple discoloration of their needles and poor growth. One of four pots inoculated with pure culture of Rhizopogon roseolus was particularly responsive to this inoculation. These red pine seedlings were well developed and comparable to those grown in forest soil inoculated pots and their roots showed numerous mycorrhizae. Failure to get a consistent response to pure culture inoculation may be explained in several ways. First it is known that mycorrhizae fungi are not competitive in the soil unless they are in symbiosis with tree roots. A few fungi (Ex. Trichoderma viride) quickly reinvade fumigated soil and outgrow other fungi for a certain period of time (Martin and Pratt, 1958). Soil inoculation and sowing of germinated seeds were done the same day. It probably took too much time for the young rootlets to make contact

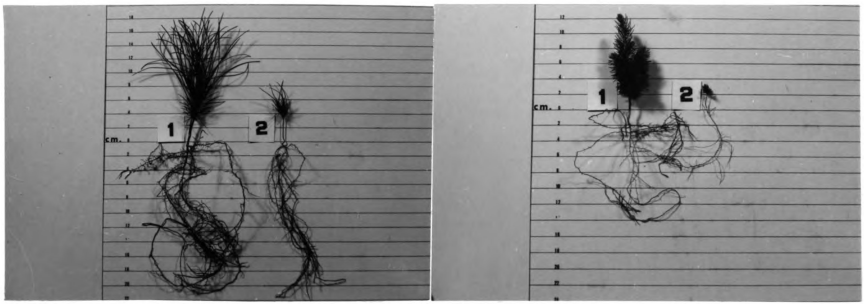

Figure 11. Effect of forest soil inoculum addition on the growth of red pine (left) and white spruce (right) grown in methyl bromide fumigated soil (Treatments: 1 = Soil inoculum; 2 = Control).

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with the chunks of mycorrhizal fungi. Seed radicles (0.25 to 0.5 cm) were not developed enough at sowing time and consequently there was a delay before these rootlets could secrete exudates or growth factors which stimulate mycorrhizal fungi growth to envelop the rootlets with a dense sheat of hyphae: the first sequence in mycorrhizal formation. Melin (1954) concluded that pine roots produce one or more growth promoting metabolites, which are essential to the growth of tree root mycorrhizae.

It is also possible that some toxic compounds from fumigants were still present in the soil at inoculation time despite a four day aeration period. These toxic compounds were probably detrimental to the life and the development of the mycorrhizal fungi.

Mycorrhizal formation - The seedlings grown in pots inoculated with forest soil showed abundant mycorrhization of short roots at sampling time (Tables 16, 17). Very good mycorrhizal development was present on the roots of red pine seedlings grown in a pot with *Rhizopogon roseolus* inoculation. In pine these fungi formed compact mantles of mycelium on the surface of the short roots. The infected roots were shorter than the non-mycorrhizal roots and they were dichotomously branched once or many times.

Microscopic studies of mycorrhizae fixed in formalin - acetic acid - alcohol confirmed the visual observation. Those obtained following a Rhizopogon roseolus inoculation showed the hyphae mantle on the surface of the roots and also that the fungi had dissolved the middle lamellae of the epidermal and outer cortical cells and then the hyphae had surrounded the walls of those cells to form a pattern called a "Hartig net" (Figure 12). In non-mycorrhizal roots this pattern was not observed, but a regular arrangement of cells.

At sampling time a large percentage of short roots of seedlings grown in fumigated soil inoculated with forest soil appeared to be ectotrophic mycorrhizae. But microscopic studies of these short roots did not show substantial evidence of true ectotrophic mycorrhizae. Hand-made cross-sections of these dichotomies showed a thin mycelium sheath and a disorganized cell structure. The cortex cells showed the presence of intra and intercellular hyphae, but most of the time in an irregular pattern (Figure 13). These hyphae could be due to a mycorrhizal fungus invading the cortex cells, but too young to have a definite pattern. These dichotomies could also be what are called: "ectendotrophic mycorrhizae". The intracellular hyphae could also be hyphae from a pathogenic fungus. It is also probable that the use of a

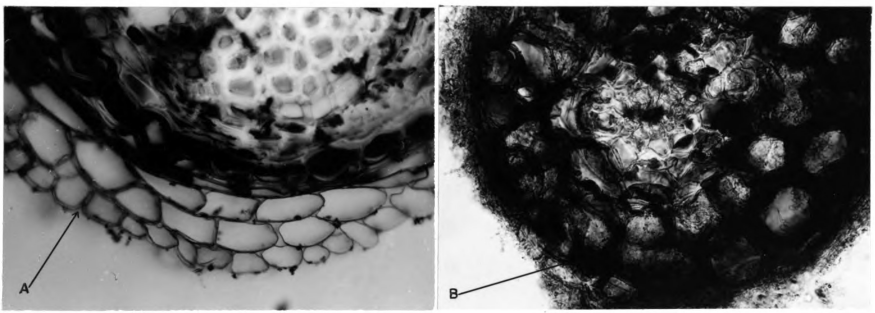

Figure 12. Cross-sections of non-mycorrhizal (left), and mycorrhizal (right) short root from red pine raised in Rhizopogon roseolus inoculated pot (900X). A: Thin intracellular space. B: Hyphae (Hartig net) around cortical cells of the short root.

Figure 13. Cross-sections of dichotomous short root of red pine showing (A) intra and (B) intercellular hyphae, and (C) a thin fungal sheath (3500X).

microtome would have given short root cross-sections of better quality and permitted an easier study of the phenomenon.

Meanwhile we believe that these dichotomous short roots are true mycorrhizae, due to the morphological characteristics of these seedlings and the visual observation of mycorrhizal characteristics on the short roots. There is evidence that most of the time one cannot conclude to presence of true ectotrophic mycorrhizae only by visual observation, but the need of further microscopic studies is obvious.

The red pine seedlings grown in pots inoculated with a pure culture of Amanita rubescens showed abnormal roots and malformed short roots which looked like mycorrhizae. These malformations could be due to incompatibility of this fungus with red pine or possibly it could also be related to the soil fumigation itself. Wilde and Persidsky (1956) reported malformations of mycorrhizae in Monterey pine seedlings following soil biocide treatment. A microscopic study of these roots showed that residual toxicants from the fumigant caused the formation of pseudo-mycorrhizae and malformations of the roots. The hyphae mantle and the Hartig net, characteristics of true ectotrophic mycorrhizae, were absent. Meanwhile the cells seem normal inside these malformations (Figure 14).

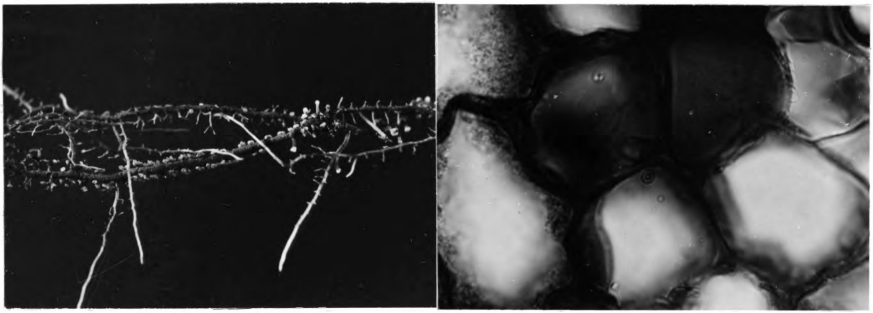

Figure 14. Abnormal root (left) of red pine seedling grown in pot inoculated with Amanita rubescens. Cross-sections (right) of these malformations (3500X).

Shoot phosphorus concentration in inoculated pots - Only the seedlings grown in pots kept as control and those where forest soil had been added at sowing time were analysed for P concentration. The data indicate that seedlings grown in fumigated soil without any supplement have a very low P concentration and P uptake (Figure 15). This corroborates the P deficiency symptoms and the decreased growth observed at sampling time. The addition of 5.0 grams of forest soil per pot (2.0 kg) was related to a very good growth rate and a P concentration of 0.135 per cent for red pine and 0.165 per cent for white spruce. These results are generally in good agreement with earlier data. Ingestad (1962) reported that the optimum P range should be between 0.15 - 0.40 per cent in the pine leaves and 0.10 - 0.30 in the spruce leaves. Fowells and Krauss (1959) found 0.14 - 0.18 per cent as optimum in Pinus taeda and P. virginiana leaves. Leyton (1958) reported 0.15 per cent as optimum in Corsican pine leaves and 0.13 per cent as an optimum in Sitka spruce. Moderate deficiency corresponds to 0.08 - 0.15 per cent P and the minimum content seems to be about 0.05 - 0.06 per cent in pines (Ingestad, 1962). In spruce, Ingestad also reports that minimum content seems to be about 0.04 - 0.05 per cent and moderate deficiency corresponds to 0.07 - 0.10

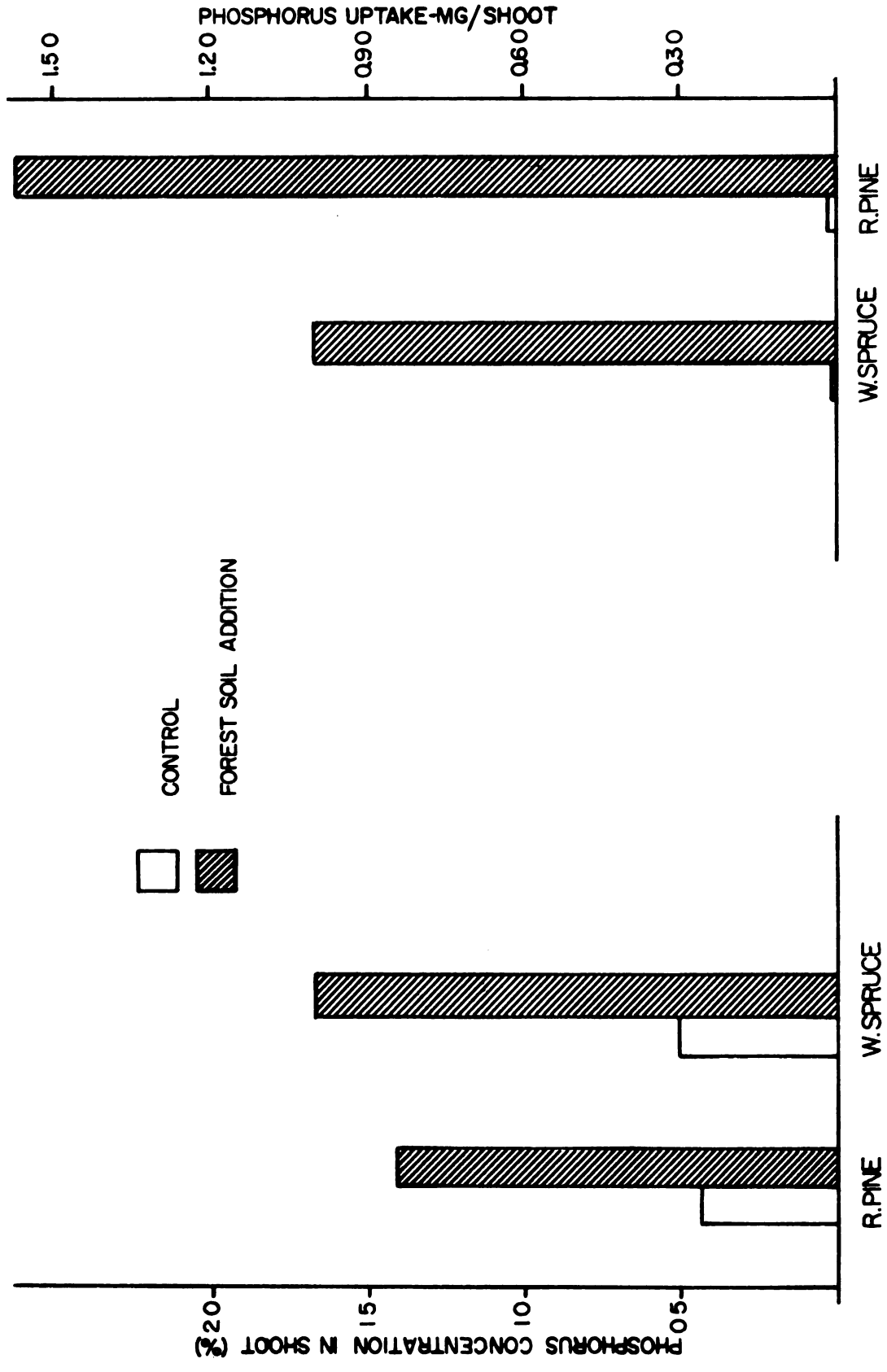

Figure 15. Influence of forest soil addition on the shoot P concentration and uptake of red pine and white spruce seedlings grown in vapam fumigated soil (Experiment 3).

per cent. In this study, at 0.04 per cent of P (control pots) the seedlings showed a severe deficiency and a poor growth (Figure 11).

C- SUPERPHOSPHATE AND FOREST SOIL INOCULUM ADDITION TO VAPAM FUMIGATED NURSERY SOIL - GREENHOUSE (EXPERIMENT 4).

This experiment was set to test the influence of different levels of forest soil inoculum and superphosphate, alone or in combination, on the growth and the development of red pine and white spruce seedlings grown in vapam fumigated soil. As in the growth chamber study the addition of forest soil inoculum increased the growth of both species of seedlings, but adding different levels of P resulted in a greater difference than soil inoculum in the development of the seedlings (Table 18).

Soil inoculum addition (3.0 g per 2.0 kg soil/pot) significantly increased seedling height. However tripling the amount (9.0 g) did not bring a significant increase over the 6.0 g addition (Appendix Table 29). When the dry shoot of both species is considered the soil inoculum was related to a significant increase (.05 level). For white spruce the low level (3.0 g) was also significantly different from the other two levels (6.0 and 9.0 g). Meanwhile the addition of soil inoculum improved the dry root weight of either red pine

Table 18. Effect of forest soil inoculum and different phosphorus levels on morphological characteristics of 19 weeks old red pine and white spruce seedlings grown in a vapam fumigated soil (Experiment 4).

Treatment			Red Pine			White Spruce		
				Dry Weight			Dry Weight	
No	Soil Inoculum g	P kg/ha	Height cm	Shoot mg	Root mg	Height cm	Shoot mg	Root mg
1	- -	- -	4.8	104	60	3.5	33	23
2	3.0	- -	6.0	287	95	7.0	149	33
3	6.0	- -	6.9	456	95	8.1	195	45
4	9.0	- -	6.3	337	92	8.0	194	46
5	- -	224	7.0	530	158	9.8	302	82
6	3.0	224	6.8	531	129	9.8	287	73
7	6.0	224	7.2	609	136	11.7	426	90
8	9.0	224	7.2	587	113	11.5	450	90
9	- -	448	7.0	645	159	12.9	528	127
10	3.0	448	7.1	680	152	13.2	497	120
11	6.0	448	7.3	729	154	13.2	508	108
12	9.0	448	7.5	734	155	11.7	486	102
13	- -	672	6.7	653	173	12.3	489	113
14	3.0	672	7.2	803	192	11.4	457	106
15	6.0	672	7.3	710	172	11.5	457	98
16	9.0	672	7.3	682	184	12.9	547	117
17	- -	896	7.2	747	204	13.7	550	120
18	3.0	896	6.9	719	160	13.4	525	105
19	6.0	896	7.4	743	153	14.1	555	121
20	9.0	896	7.9	793	162	14.2	564	119
Tukey's w(.05)			1.0	277	55	3.0	203	52
(.01)			1.2	318	63	3.5	233	60

or white spruce seedlings, but not significantly. Moreover the different levels of soil inoculum are related to only a slight difference between them.

There were significant differences (0.01 level) in morphological characteristics of the seedlings after P addition. The height, dry shoot or dry root weight were much greater for seedlings of both species grown in soil with added P. A significant difference (.01 level) was found between the low and the higher levels of P addition (Table 18, Appendix Table 29). A logarithmic equation ($y = a + b \log x$) characterizes well the relation between the growth of seedlings (shoot dry weight) and the P addition (Figure 16).

Dark green and well balanced seedlings were obtained at the highest level with no evidence of a harmful effect (Figure 17). Benzian (1965), in England, reported that at the high rate of application (144 kg P/ha) Sitka spruce seedlings grew exceptionally well. There was no indication of a harmful effect; on the contrary she thought that higher rates had to be applied to establish the full shape of the curve.

At sampling time the presence of very few if any mycorrhizae was observed on roots of seedlings grown in P fertilized soil. The addition of soil inoculum alone to the fumigated soil increased their numbers. This scarity of mycorrhizae can be related to the soil fumigation with vapam and also to the

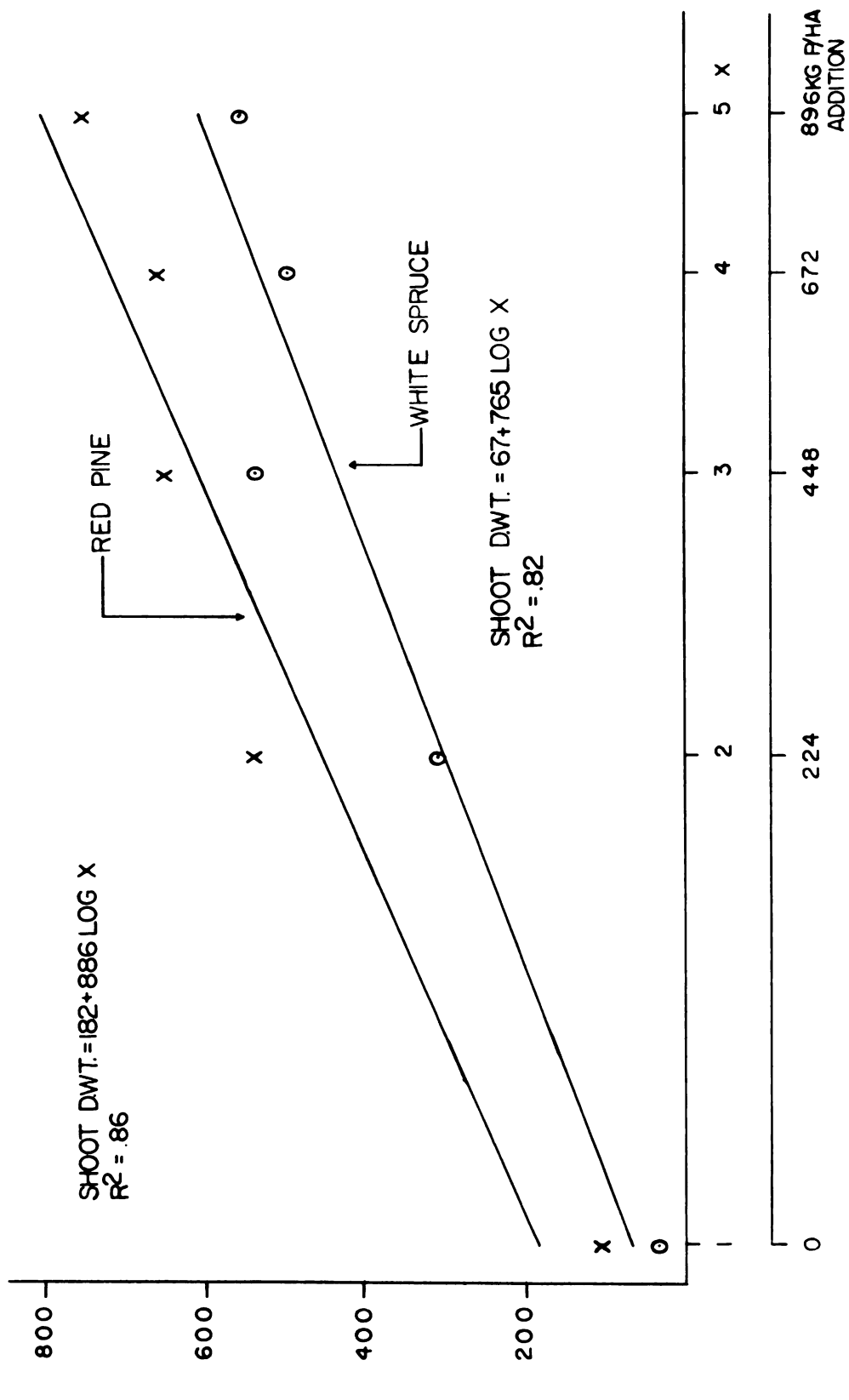

Figure 16. Relation between the shoot dry weight of red pine and white spruce seedlings and the amount of P added to a vapam fumigated soil (Experiment 4).

Figure 17. Effect of P addition on red pine (left) and white spruce (right) seedlings grown in a vapam fumigated greenhouse soil - (Treatments: left, A = control, B = 224 kg P/ha, C = 896 kg P/ha; Right. A = Control, B = 672 kg P/ha, C = 896 kg P/ha).

high P level in soil. Indeed mycorrhizae seem to need a low level of P to develop. Fowells and Krauss (1959) confirmed the findings of Hatch (1937) that mycorrhizae are generally more abundant with low levels of P and N. Hacskaylo and Snow (1959) further support these conclusions: prevalence of mycorrhizae on pines was greatest in soils with no nutrient added.

Phosphorus concentration in shoot tissue - The significance of different P level addition is shown in percentage of shoot dry weight and in uptake in mg/shoot (Table 19, Appendix Table 30). Phosphorus addition to soil is related to a highly significant difference (.01 level) in shoot concentration and uptake for both red pine and white spruce. Low addition of P brought results significantly different (.01 level) from the highest level of P fertilization. Ingestad (1962) reported that a P concentration of .152 per cent corresponds to a moderate deficiency for pine. Tamm (1956) also reported that the deficiency level in current spruce leaves is 0.07 - 0.08 per cent. The values obtained were identical to those mentioned by these authors. The relation between soil P addition and shoot P concentration of seedlings can be characterized by a logarithmic equation (Figure 18). The equation shows that these two variables are much more closely related for white spruce

Table 19. Effect of soil phosphorus addition on the shoot phosphorus concentration and uptake of red pine and white spruce grown in the greenhouse for 19 weeks; Experiment 4 (means of 4 replications).

Treatment No	Phosphorus	Shoot Phosphorus[1] Conc.[2]		Uptake		% P Increase over Control Conc.	Uptake
	kg/ha	%		mg/shoot			
				RED PINE			
1	0	.152		.16		- - -	- - -
2	224	.231	**	1.22	**	52	655
3	448	.234	**	1.48	**	54	820
4	672	.223	**	1.46	**	46	804
5	896	.245	**	1.82	**	61	1032
Tukey's	(.05)	.042		.33			
	(.01)	.055		.43			
				WHITE SPRUCE			
1	0	.069		.02		- - -	- - -
2	224	.210	**	.64	*	204	2738
3	448	.246	**	1.29	**	256	5621
4	672	.266	**	1.30	**	285	5679
5	896	.305	**	1.67	**	342	7307
Tukey's	(.05)	.065		.53			
	(.01)	.084		.69			

[1] Determined on a dry weight basis. See Appendix Table 30 for statistical significance.

[2] * Significantly greater than control at .05 level.
** Significantly greater than control at .01 level.

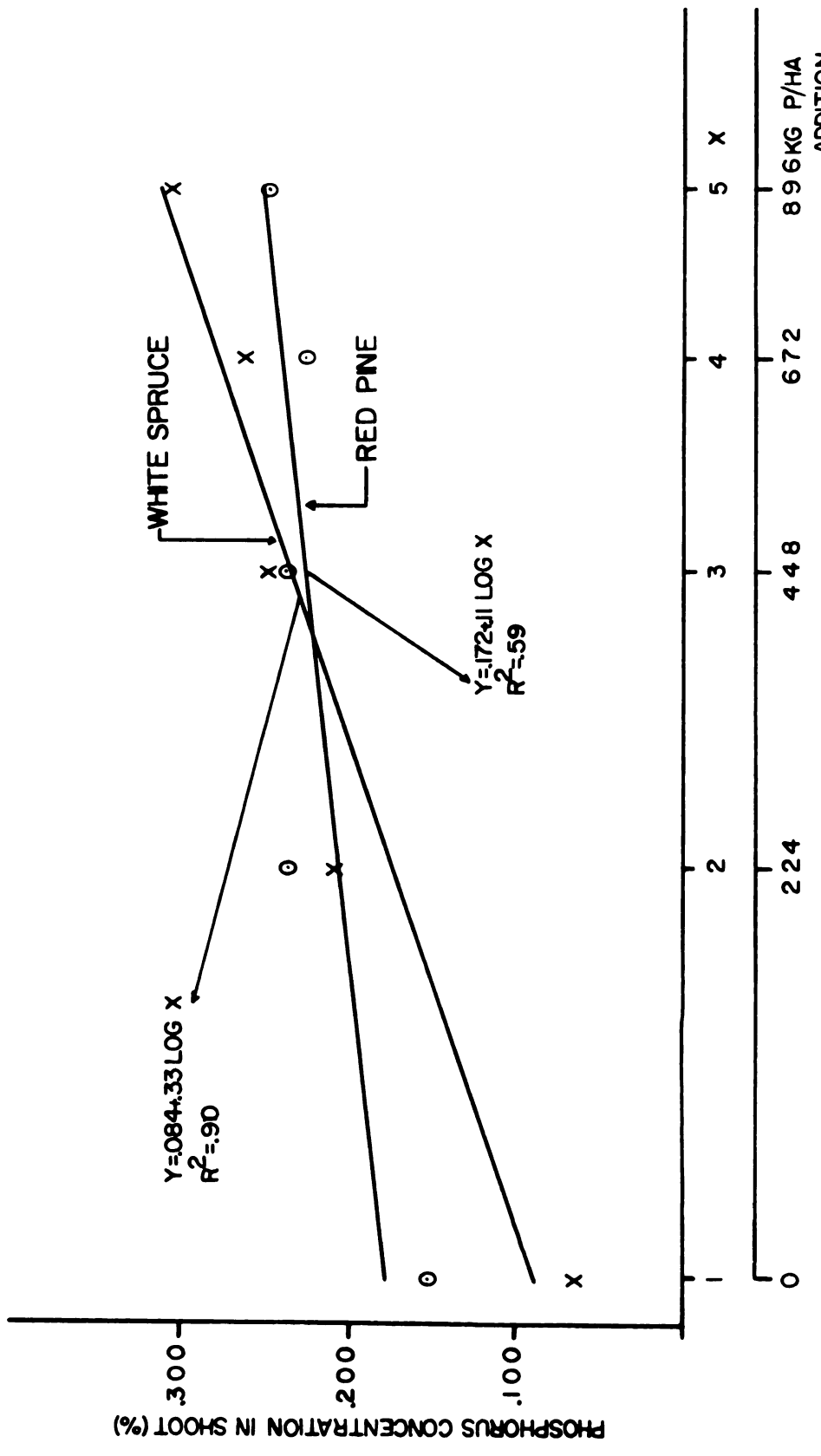

Figure 18. Relation between the shoot P concentration of red pine and white spruce seedlings and the amount of P added to a vapam fumigated soil (Experiment 4).

(R^2 = .90) than for red pine (R^2 = .59). It seems that P concentration in white spruce was more influenced than in red pine by the level of P in soil. In a fumigated soil containing just a small amount of available P spruce grew very poorly and showed evident signs of P deficiency.

The P uptake in seedling shoots in relation to the different levels of P addition gave significant differences (.01 level) except for a few treatments (Appendix Table 30). The control seedlings had poor growth, low shoot P concentration, and consequently a very low uptake of P into shoots. Uptake at all the levels of P addition were different (.01 level) from the control. The increase in P taken up was from 655 to 1032 per cent depending on the levels of P addition for red pine seedlings. For white spruce seedlings the percentage P increase was even much greater (2738 to 7307 per cent) after P addition to soil (Table 19). These facts give evidence of the highly beneficial effect of a high P addition to soil for the development of seedlings grown in a fumigated soil.

D- SUPERPHOSPHATE ADDITIONS TO METHYL BROMIDE FUMIGATED SOIL - BERTHIERVILLE NURSERY (EXPERIMENT 5).

This experiment was set to test the influence of different levels of P addition to nursery seedbeds, as

superphosphate, on the growth and development of red pine and white spruce seedlings grown in a methyl bromide fumigated soil. Due to very poor germination of white spruce results are shown only for red pine.

Morphological characteristics - The addition of different levels of P increased significantly (.01 level) the growth and the development of seedlings. The height, the dry shoot and the dry root weight were much greater for red pine seedlings grown in soil with added P (Table 20, Appendix Table 31). There is even a significant difference between low and higher levels of added P. The first two levels of P addition brought a sharp increase in the growth and the development of the seedlings. The higher P additions were also related to an increase, but not at a proportional level. It appears that an optimum level of growth has been attained after an addition of 672 kg P/ha and higher amounts have not added too much to seedling development. A logarithmic curve of the form $y = a + b \log x$ usually characterizes well this kind of response (Figure 19).

At sampling time seedlings grown in plots with no added P had red purple needles, sign of a P deficiency. The plots where the low level of P (336 kg/ha) was added grew a

Table 20. Effect of soil phosphorus addition on the morphological characteristics, the shoot phosphorus concentration and uptake of red pine seedlings grown in nursery seedbeds for 17 weeks; Experiment 5 (means of 4 replications).

Treatment			Dry Weight		Shoot Phosphorus[1]		% P Increase over Control	
No	Phosphorus kg/ha	Height[2] cm	Shoot mg	Root mg	Conc. %	Uptake mg/shoot	Conc.	Uptake
1	0	3.8	68	31	.091	.06	- -	- -
2	336	4.2	108	53 *	.243 **	.25 *	167	314
3	672	4.3	164 **	67 **	.351 **	.57 **	285	800
4	1008	4.8 **	181 **	71 **	.376 **	.68 **	300	1010
5	1344	4.8 **	172 **	58 **	.376 **	.65 **	300	940
6	1680	4.8 **	189 **	61 **	.388 **	.73 **	320	1180
Tukey's w(.05)[2]		.7	43	19	.064	.16		
	(.01)	.9	55	24	.081	.20		

[1] Determined on a dry weight basis. See Appendix Table 31 for statistical significance.

[2] * Significantly greater than control at 0.05 level.
** Significantly greater than control at 0.01 level.

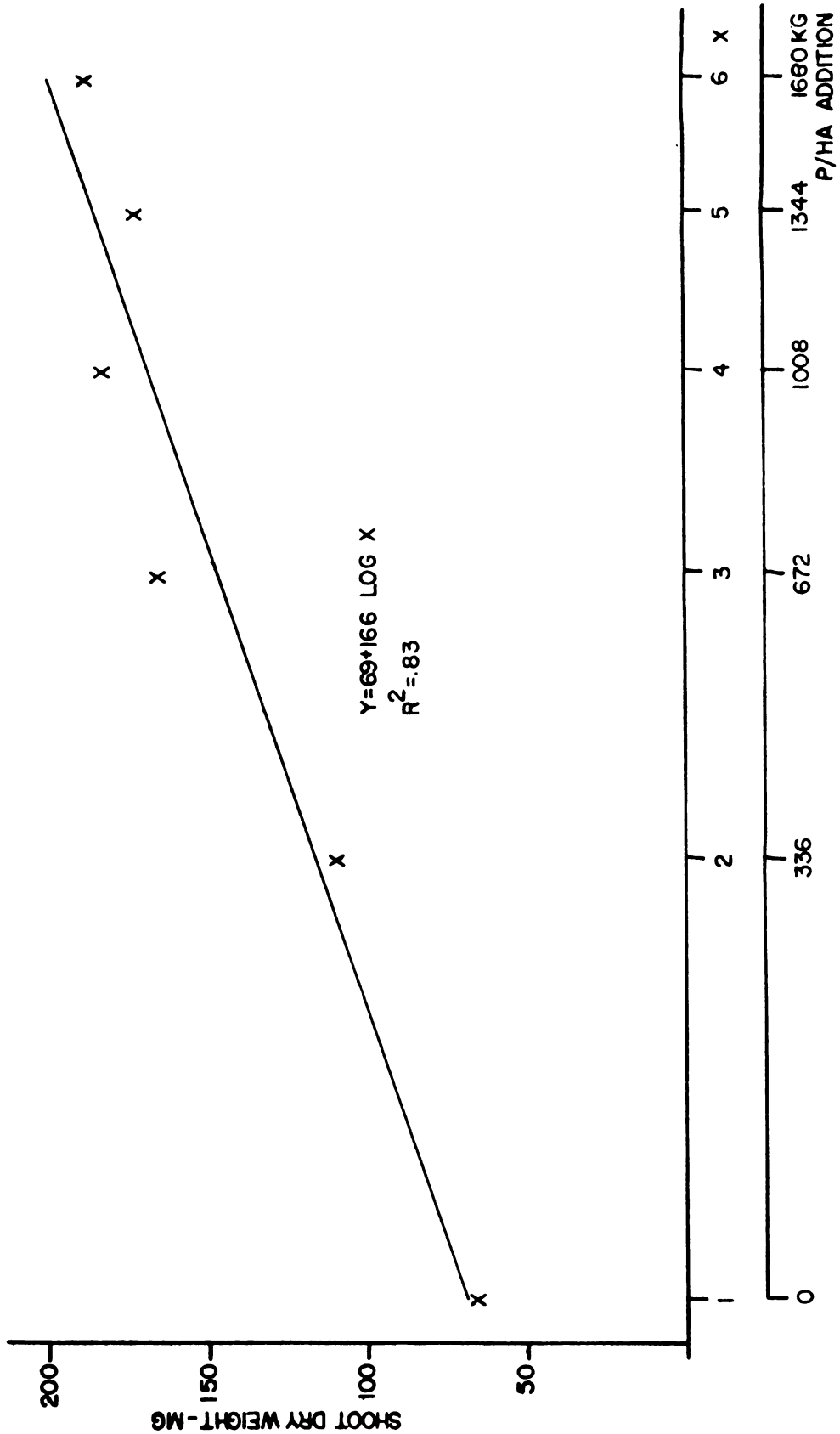

Figure 19. Relation between the shoot dry weight of 17 weeks old red pine seedlings and the amount of P added to a methyl bromide fumigated nursery soil (Experiment 5).

few red purple seedlings, but the majority of them was green. The plots with higher rates of P grew dark green and well developed red pine seedlings. In plots kept as control we got seedlings of small size (dry weight) based on the standards set by Armson and Carman (1961). On the contrary the seedlings, grown in plots having received 672 kg P/ha or more, were of very large size when compared to the same values.

No mycorrhizae were present on the short roots at sampling time. This is probably due to the fumigant effect on mycorrhizal fungi and also for a few treatments to the high level of P in the soil solution.

Shoot phosphorus concentration - Analysis of shoots confirmed a P deficiency condition in the seedlings grown in plots that were not fertilized with P (.091 per cent P concentration). Ingestad (1962) gave a level of P of 0.08 to 0.15 per cent as an evidence of deficiency in current leaves of pines. In this experiment the addition of the lowest level of P brought a major increase in the P concentration of pine leaves (.243 per cent). The higher P additions still increased the level of P in the pine needles (Table 20). These later values are in the range for an optimum growth (Armson and Carman, 1961; Ingestad, 1962).

As mentioned the addition of P brought a significant difference (.01 level) on the concentration of P in the pine leaves (Appendix Table 31). The lowest level of P addition (336 kg/ha) is related to an increase of 167 per cent in the P concentration in the seedling shoots. This increase goes to 320 per cent with the highest P addition to soil. Greater differences are shown for the P uptake in the seedling shoots (Table 20).

The relation between the soil P addition and the shoot P concentration and uptake is best characterized by a logarithmic curve ($y = a + b \log x$). Indeed we got R^2 values of .87 and .86 respectively (Figure 20). A sharp increase is related to the first P additions, and a much lower response to the last ones. This finding suggested an examination of the relation between the shoot P concentration (% D. WT.) and the shoot weight. Phosphorus concentrations of the red pine seedlings showed a consistent trend of increasing concentration with increase in seedling size; a trend characterized by a linear equation (Figure 21). Armson (1968) found a similar trend for white spruce, whereas for red pine the concentrations decreased somewhat. This relation seems to be reasonable in light of the importance of P for the plant. Phosphorus as adenosine

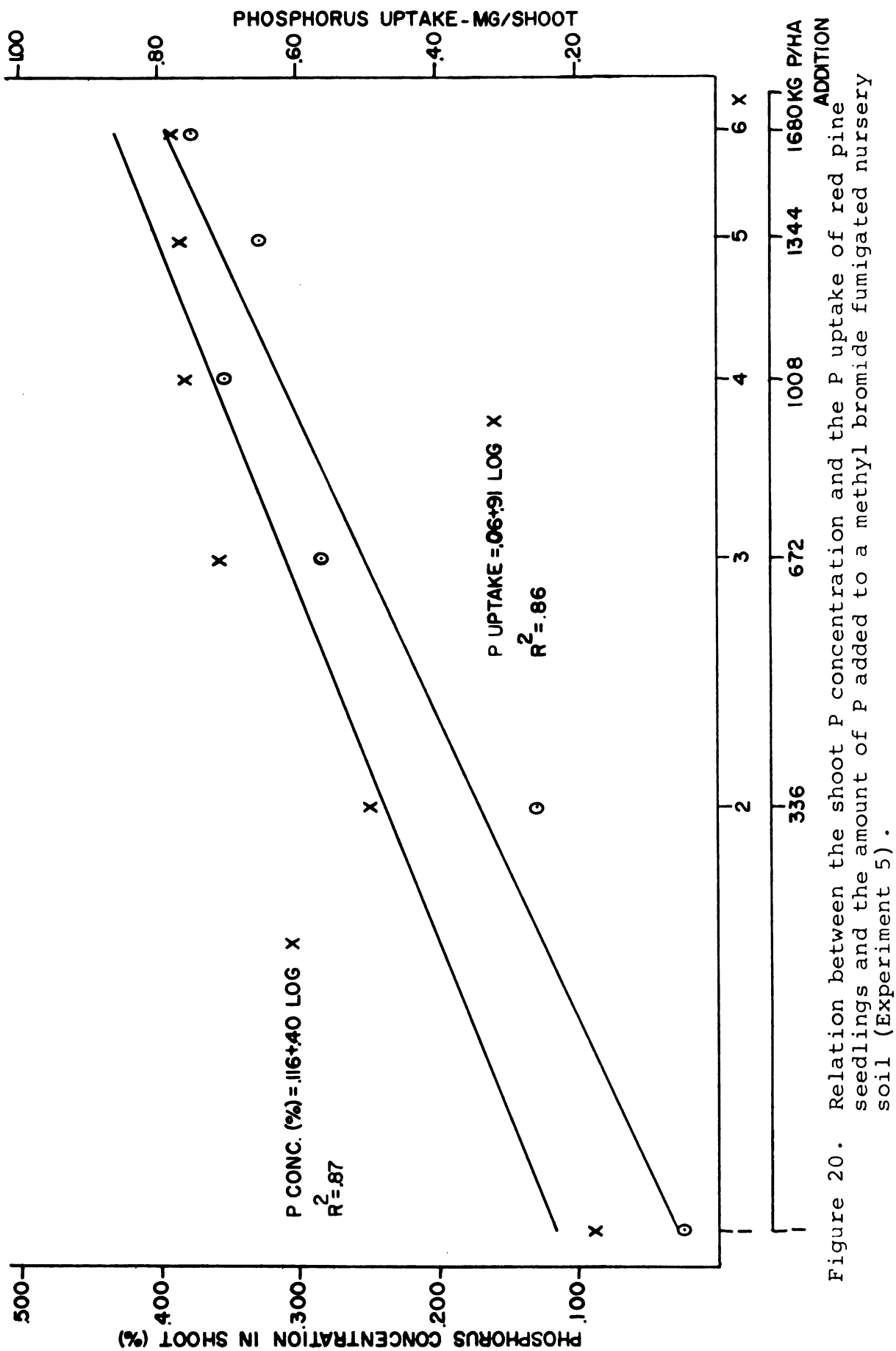

Figure 20. Relation between the shoot P concentration and the P uptake of red pine seedlings and the amount of P added to a methyl bromide fumigated nursery soil (Experiment 5).

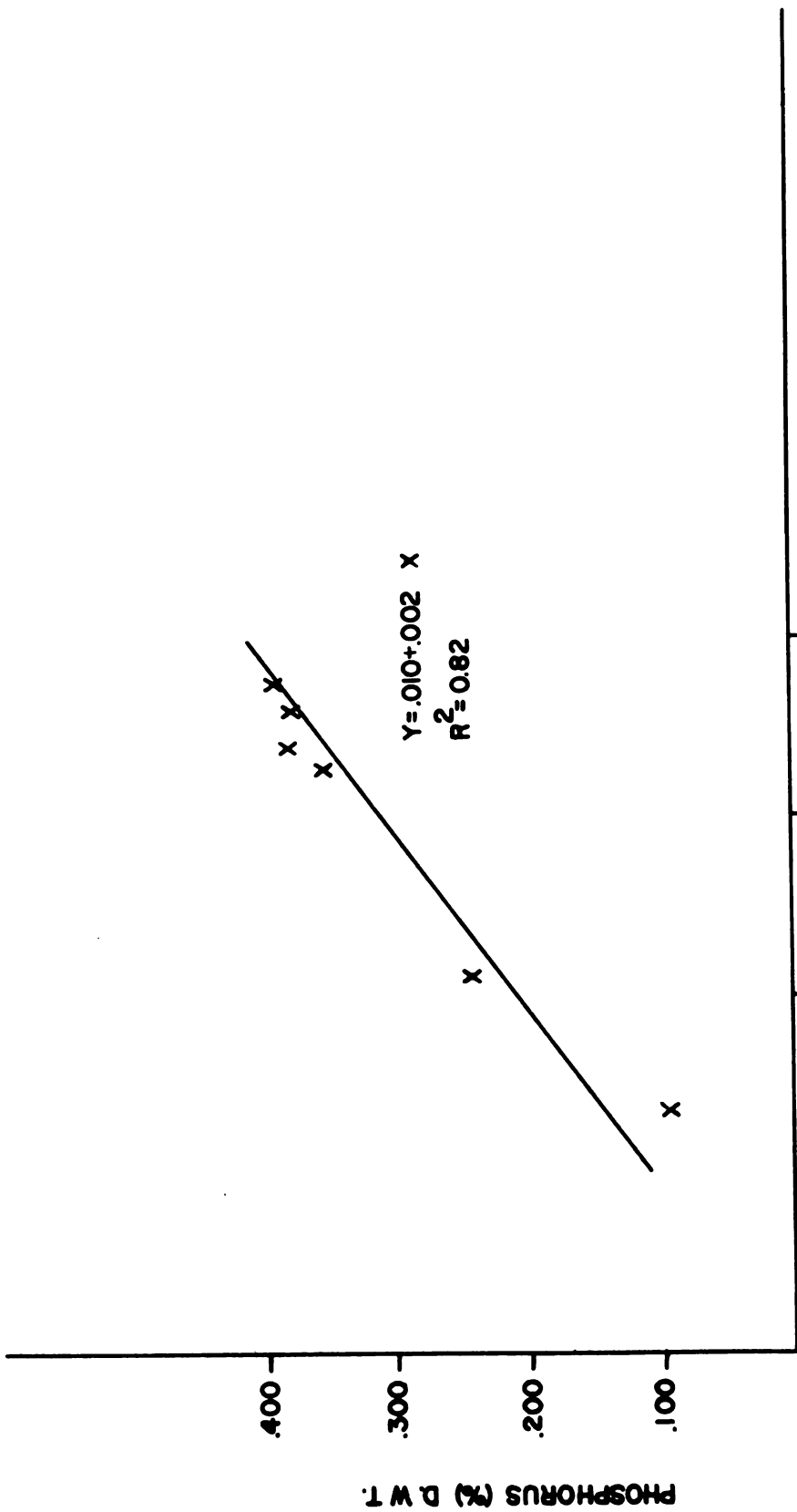

Figure 21. Relation between the P concentration in shoot and the shoot weight of 17 weeks old red pine seedlings (Experiment 5).

triphosphate and numerous phosphorylated products participate in nearly all synthetic reactions of the plant cell. It is essential for the development of the meristematic tissues and it is associated with the general process of respiration. It is apparent that adequate P nutrition will be related to a better development and growth of conifer seedlings.

CHAPTER V

CONCLUSIONS AND IMPLICATIONS FOR MANAGEMENT

The principal findings of this study are:

- Soil sterilization (methyl bromide, vapam or heat) severely depressed the development of red pine and white spruce seedlings grown in nursery soil without supplemental P.

- The addition of N alone to sterilized soil did not improve the growth of either red pine or white spruce. In combination with the P sources N generally appeared to enhance the development of the seedlings and their uptake of N. Ammonium-N was a superior source of N to NO_3-N.

- In fumigated soil red pine and white spruce seedlings raised with no supplemental P were P deficient according to shoot analysis.

- The combination of soil sterilization, NH_4-N and superphosphate fertilization produced larger seedlings and higher shoot P contents than any other treatment.

- Superphosphate was clearly the best source of P. The relatively cheap rock phosphate was associated with a better seedling development and P uptake than bone meal.

- Inoculation of fumigated soil with a small quantity of forest soil was associated with dark green seedlings showing a much better growth and higher P uptake than those from control or pure culture inoculation treatments.

Nursery Culture Implications

Soil fumigation is widely used in forest nurseries to control nematodes, damping-off, root rot fungi, and weeds. Occasionally fumigation is followed by strongly adverse responses. Entire beds of seedlings fail to grow much beyond the cotyledon stage, become purplish in color, and commonly die over winter or grow very poorly the next season.

Phosphorus is one of the most important elements in plant nutrition. All the experiments showed that the uptake of this element tends to be hindered by fumigation of forest nursery soil while it is normal in the untreated soil. This low P uptake is reflected in poor seedling growth, visual P deficiency symptoms, and low shoot P concentration.

In all the experiments it appeared that white spruce seedlings were more influenced than red pine by nursery soil

fumigation and low level of P; white spruce was also more responsive to high P addition.

It seems that the addition of a very small quantity of forest soil might be an efficient way of supplying mycorrhizal fungi inoculum to nursery soil. The use of soil from forest stands may introduce species of symbiotic fungi that would be well adapted to the environmental conditions encountered following seedling out-planting. This inoculation with forest soil would also strongly influence the microbial recolonization of nursery soil. This inoculation method can also present certain disadvantages. The cost of obtaining soil from forest stands and its application to nursery seedbeds can be high. Furthermore there also is the danger of reintroducing pathogens into seedbeds. Pathogens could become more virulent in the artificial nursery environment than in the natural forest soil.

A more effective way to inoculate fumigated nursery soil with pure cultures of mycorrhizal fungi is needed. This implies isolation of competitive mycorrhizal fungi well adapted to the species being grown. A pure culture inoculation technique adapted to practice would require the development of a growing culture medium that could be easily transferred into the nursery soil.

These studies showed that symptoms of P deficiency of seedlings can be overcome in part by an application of rock phosphate or bone meal, but only superphosphate fertilization significantly increased the seedling biomass and the shoot P concentration. At the same time NH_4-N seemed to be a better N source than NO_3-N.

In nursery experiments the seedling biomass and the shoot P concentration of conifer seedlings were closely related to the amount of P (superphosphate) added to fumigated nursery soil. The optimum level of P was 672 kg P/ha for conifer seedlings, although healthy seedlings and adequate shoot P concentration were obtained with lower rates. A good way to get this high rate of P into the soil is to drill superphosphate alongside the seeds, at sowing time. We found that it was difficult with mechanical seeders to band the P fertilizer close enough to the seeds. A suggestion not tried in these experiments would be to coat the seeds with finely ground superphosphate.

The experiments show that shoot P concentration is correlated with red pine seedling weight. This is another indication of the necessity of adequate P fertilization for optimum conifer seedling growth. Furthermore suitable P

fertilization and seedling P uptake may significantly influence: (1) seedling survival, (2) resistance to disease, and (3) resistance to insect attack. In addition an adequate amount of available P in fumigated nursery soil may influence a delayed nutritional gain in subsequent growing seasons in the nursery and even after outplanting in the field. This will shorten the period needed to raise seedlings in the nursery and permit the production of more uniform stock.

This research may provide useful guidelines to nurserymen who encounter stunted and P deficient seedlings following soil fumigation. It may also provide some guidelines for investigators of mycorrhizal fungi.

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APPENDIX

Appendix Table 21. Preparation of "Hagem" agar for culture of mycorrhizal fungi.

Agar	15.0 gm
Glucose	5.0 gm
Malt Extract	5.0 gm
$KH_2 PO_4$	0.5 gm
$MgSO_4 .7 H_2O$	0.5 gm
$NH_4 Cl$	0.5 gm
$FeCl_3$ (1% solution)	0.5 ml
H_2O	to 1000 ml

Appendix Table 22. Significance of experimental factors on morphological characteristics of red pine and white spruce seedlings grown in the greenhouse for 16 weeks (Experiment 1).[1]

Source	df	RED PINE			WHITE SPRUCE		
			DRY WEIGHT			DRY WEIGHT	
		Height cm	Shoot mg	Root mg	Height cm	Shoot mg	Root mg
Reps	3						
Soil Sterilization[2]	2	**	**	**	**	**	**
Control vs steril.	1	NS	**	**	**	**	**
Heat vs MB	1	**	**	**	**	**	*
N x P treatments	11						
N[2]	2	**	**	**	**	**	**
W/out N vs N	1	**	**	**	**	**	**
NH_4 vs NO_3	1	**	**	NS	*	**	**
Without supplemental N[2]							
No P vs P addition	1	**	**	**	**	*	**
Inorg. P vs Org. P	1	**	*	NS	NS	NS	NS
P_{rock} vs P_{super}	1	NS	NS	NS	NS	*	NS
NO_3-N[2]							
No P vs P addition	1	**	**	**	**	**	**
Inorg. P vs Org. P	1	NS	**	**	NS	NS	NS
P_{rock} vs P_{super}	1	NS	NS	NS	NS	NS	NS

Appendix Table 22 (Continued).

Source	df	RED PINE			WHITE SPRUCE		
			DRY WEIGHT			DRY WEIGHT	
		Height cm	Shoot mg	Root mg	Height cm	Shoot mg	Root mg
NH_4-N[2]							
No P vs P addition	1	**	**	**	**	**	**
Inorg. P vs Org. P	1	NS	NS	NS	**	NS	**
P_{rock} vs P_{super}	1	NS	NS	NS	*	NS	*
Soil steril. x N	4	**	**	**	*	NS	NS
Soil steril. x P	6	**	**	**	**	**	**
Soil steril. x N x P	12	NS	**	NS	NS	NS	NS
Error	105						
CV%		6.0	16.1	16.2	11.8	26.4	29.3

[1]See Tables 5, 6 for data.

[2]Orthogonal contrasts.
* Factor is significant at .05 level
** Factor is significant at .01 level
NS Factor is not significant.

Appendix Table 23. Significance of experimental factors on morphological characteristics of red pine and white spruce seedlings grown in nursery for 14 weeks (Experiment 2)[1].

Source	df	RED PINE			WHITE SPRUCE		
			DRY WEIGHT			DRY WEIGHT	
		Height cm	Shoot mg	Root mg	Height cm	Shoot mg	Root mg
Reps	3						
Soil sterilization[2]	2	*	**	**	**	**	*
Control vs steril.	1	**	**	**	**	**	*
MB vs vapam	1	NS	NS	NS	*	NS	NS
Error (a)	6						
N x P treatments	11						
N[2]	2	**	**	**	**	**	**
W/out N vs N	1	NS	*	NS	NS	*	NS
NH_4 vs NO_3	1	NS	**	**	**	**	**
Without supplemental N[2]							
No P vs P addition	1	NS	**	NS	**	**	**
Inorg. P vs Org. P	1	NS	**	**	**	*	**
P_{rock} vs P_{super}	1	*	**	*	**	**	**
NO_3-N[2]							
No P vs P addition	1	NS	NS	NS	**	**	**
Inorg. P vs Org. P	1	**	**	**	**	**	**
P_{rock} vs P_{super}	1	NS	**	**	**	**	**

Appendix Table 23 (Continued).

Source	df	RED PINE			WHITE SPRUCE		
			DRY WEIGHT			DRY WEIGHT	
		Height cm	Shoot mg	Root mg	Height cm	Shoot mg	Root mg
NH_4-N[2]							
No P vs P addition	1	NS	**	NS	**	**	**
Inorg. P vs Org. P	1	**	**	**	**	**	**
P_{rock} vs P_{super}	1	**	**	NS	**	**	**
Soil steril. x N	4	NS	NS	NS	NS	NS	NS
Soil steril. x P	6	NS	**	**	**	**	**
Soil steril. x N x P	12	NS	NS	NS	NS	NS	*
Error (b)	99						
CV(%)							
Error (a)		12.5	14.5	16.0	6.9	23.6	26.0
Error (b)		5.0	10.8	11.8	7.2	14.8	15.3

[1]See Tables 7, 8 for data.

[2]Orthogonal contrasts.
* Factor is significant at .05 level
** Factor is significant at .01 level
NS Factor is not significant.

Appendix Table 24. Significance of experimental factors on morphological characteristics of white spruce seedlings after two growth seasons (Experiment 2)[1].

Source	df	Height cm	Dry Weight Shoot mg	Dry Weight Root mg
Reps	3			
Soil sterilization	1	**	**	*
Error (a)	3			
N x P treatments	8			
N^2	2	**	**	**
W/out N vs N	1	**	**	**
NH_4 vs NO_3	1	**	**	**
Without supplemental N^2				
No P vs P addition	1	*	*	*
P_{rock} vs P_{super}	1	**	**	**
NO_3-N^2				
No P vs P addition	1	**	**	*
P_{rock} vs P_{super}	1	**	**	**

Appendix Table 24 (Continued).

Source	df	Height cm	Dry Weight Shoot mg	Dry Weight Root mg
NH_4-N[2]				
No P vs P addition	1	**	**	**
P_{rock} vs P_{super}	1	**	**	**
Soil steril. x N	2	NS	NS	NS
Soil steril. x P	2	**	**	**
Soil steril. x N x P	4	NS	NS	NS
Error (b)	48			
CV (%)				
Error (a)		21	44	40
Error (b)		20	33	36

[1] See Table 9 for data.

[2] Orthogonal contrasts.
* Factor is significant at .05 level
** Factor is significant at .01 level
NS Factor is not significant.

Appendix Table 25. Significance of experimental factors on shoot nitrogen and phosphorus concentration (%) of 16 weeks old red pine and white spruce seedlings (Experiment 1)[1].

Source	df		RED PINE N %	RED PINE P %	WHITE SPRUCE N %	WHITE SPRUCE P %
Reps	3					
Soil sterilization[2]	2		**	**	**	**
Control vs steril.		1	**	**	**	**
Heat vs MB		1	NS	*	NS	**
N x P treatments	11					
N[2]	2		**	NS	**	NS
W/out N vs N		1	**	NS	**	NS
NH_4 vs NO_3		1	**	NS	NS	NS
Without supplemental N[2]						
No P vs P addition		1	*	**	**	**
Inorg. P vs Org. P		1	**	**	**	**
P_{rock} vs P_{super}		1	NS	NS	NS	NS
NO_3-N[2]						
No P vs P addition		1	**	**	**	**
Inorg. P vs Org. P		1	**	NS	**	**
P_{rock} vs P_{super}		1	NS	NS	NS	*

Appendix Table 25 (Continued).

Source	df	RED PINE N %	RED PINE P %	WHITE SPRUCE N %	WHITE SPRUCE P %
NH_4-N					
No P vs P addition	1	**	**	*	*
Inorg. P vs Org. P	1	**	NS	*	NS
P_{rock} vs P_{super}	1	NS	NS	NS	NS
Soil steril. x N	4	NS	NS	*	*
Soil steril. x P	6	**	NS	**	*
Soil steril. x P x N	12	**	NS	*	NS
Error	105				
CV (%)		16.9	29.7	11.4	22.2

[1] See Tables 10, 11 for data.

[2] Orthogonal contrasts.
* Factor is significant at .05 level
** Factor is significant at .01 level
NS Factor is not significant.

Appendix Table 26. Significance of experimental factors on shoot mineral nutrient concentration (%) of 14 weeks old red pine and white spruce seedlings (Experiment 2)[1].

Source	df	RED PINE			WHITE SPRUCE		
		N	P	K	N	P	K
		%	%	%	%	%	%
Reps	3						
Soil Sterilization[2]	2	**	**	NS	**	**	NS
Control vs steril.	1	**	**	NS	**	**	NS
MB vs vapam	1	*	NS	NS	NS	NS	NS
Error (a)	6						
N x P treatments	11						
N^2	2	**	**	NS	**	*	**
W/out N vs N	1	**	NS	NS	**	NS	**
NH_4 vs NO_3	1	**	**	NS	**	*	**
Without supplemental N^2							
No P vs P addition	1	*	**	*	**	**	NS
Inorg. P vs Org. P	1	NS	**	**	*	**	**
P_{rock} vs P_{super}	1	NS	**	**	*	**	**
NO_3-N^2							
No P vs P addition	1	*	**	NS	**	**	NS
Inorg. P vs Org. P	1	*	NS	*	**	NS	NS
P_{rock} vs P_{super}	1	NS	**	*	*	**	**

Appendix Table 26 (Continued).

Source	df	RED PINE			WHITE SPRUCE		
		N %	P %	K %	N %	P %	K %
NH_4-N[2]							
No P vs P addition	1	NS	**	NS	**	**	**
Inorg. P vs Org. P	1	NS	**	**	**	NS	*
P_{rock} vs P_{super}	1	*	**	**	NS	**	**
Soil steril. x N	4	*	**	NS	NS	**	NS
Soil steril. x P	6	NS	**	*	**	**	**
Soil steril. x N x P	12	NS	*	NS	*	NS	NS
Error	99						
CV (%)							
Error (a)		7.8	11.5	39.4	5.4	25.8	26.3
Error (b)		6.3	11.7	18.9	5.0	13.0	13.4

[1] See Tables 12, 13 for data.

[2] Orthogonal contrasts.
* Factor is significant at .05 level
** Factor is significant at .01 level
NS Factor is not significant.

Appendix Table 27. Influence of experimental factors on pH, NH_4 and NO_3, soil available phosphorus, and exchangeable potassium, calcium and magnesium in nursery soil (Experiment 2)[1].

Source	df		pH	NH_4	NO_3	P	K	Ca	Mg
Reps	3								
Soil sterilization[2]	2		**	**	**	NS	NS	NS	NS
Control vs steril.		1	**	**	**	NS	NS	NS	NS
MB vs vapam		1	NS	NS	NS	NS	NS	NS	NS
Error (a)	6								
N[2]	2		**	**	**	NS	NS	NS	NS
W/out N vs N		1	**	**	**	NS	NS	NS	NS
NH_4 vs NO_3		1	**	**	**	NS	NS	NS	NS
Soil steril. x N	4		**	**	**	NS	NS	NS	NS
P[2]	3								
No P vs P addition		1	**	**	NS	**	**	**	**
Inorg. P vs Org. P		1	**	**	**	**	**	NS	**
P_{rock} vs P_{super}		1	NS	NS	**	**	**	**	**

Appendix Table 27 (Continued).

Source	df	pH	NH_4	NO_3	P	K	Ca	Mg
Soil steril. x P	6	**	**	NS	NS	**	NS	NS
N x P	6	NS	**	**	NS	**	NS	NS
Soil steril. x N x P	12	NS	NS	NS	NS	NS	NS	NS
Error (b)	99							
CV (%)								
Error (a)		2.3	60	59	28	30	35	40
Error (b)		2.3	30	28	21	8	13	26

[1] See Table 15 for data.

[2] Orthogonal contrasts.
* Factor is significant at .05 level
** Factor is significant at .01 level
NS Factor is not significant

Appendix Table 28. Significance of experimental factors on morphological characteristics of red pine and white spruce seedlings grown in the growth chamber for 16 weeks (Experiment 3)[1].

Source	df	Height	Shoot	Root	Shoot	Root	Mycorrhizal short roots
		cm		-mg-			%
				RED PINE			
Reps	3						
Fumigant (A)	1	NS	NS	NS	NS	NS	NS
Inoculum (I)	4	**	**	**	**	**	**
A x I	4	NS	NS	NS	NS	NS	NS
Error	27						
CV (%)		8	49	38	45	36	143
				WHITE SPRUCE			
Reps	3						
Fumigant (A)	1	NS	NS	NS	NS	NS	NS
Inoculum (I)	4	**	**	**	**	**	**
A x I	4	NS	NS	NS	NS	NS	NS
Error	27						
CV (%)		26	117	77	110	88	114

[1] See Tables 16, 17 for data.

*Factor is significant at .05 level
**Factor is significant at .01 level
NS Factor is not significant.

Appendix Table 29. Significance of experimental factors on morphological characteristics of 19 weeks old red pine and white spruce seedlings grown in a vapam fumigated soil (Experiment 4)[1].

Source	df	RED PINE			WHITE SPRUCE		
			DRY WEIGHT			DRY WEIGHT	
		Height cm	Shoot mg	Root mg	Height cm	Shoot mg	Root mg
Reps	3						
Inoculum (I)[2]	3	**	*	NS	**	*	NS
Control vs I	1	**	**	NS	**	*	NS
I (3.0 g vs 6.0 and 9.0 g)	1	**	NS	NS	**	**	NS
I (6.0 g vs 9.0 g)	1	NS	NS	NS	NS	NS	NS
Phosphorus (P)[2]	4	**	**	**	**	**	**
Control vs P	1	**	**	**	**	**	**
P (224 vs 448,672 and 896 kg/ha)	1	NS	**	**	**	**	**
P (448 vs 672 and 896 kg/ha)	1	NS	NS	**	NS	NS	NS
P (672 vs 896 kg/ha)	1	**	NS	NS	**	**	NS
I x P	12	*	NS	*	**	NS	NS
Error	57						
CV (%)		5.8	17.5	14.4	10.5	18.8	21.7

[1] See Table 18 for data.

[2] Orthogonal contrasts.
* Factor is significant at .05 level
** Factor is significant at .01 level
NS Factor is not significant.

Appendix Table 30. Significance of phosphorus addition on the shoot phosphorus concentration and uptake by 19 weeks old red pine and white spruce seedlings grown in a vapam fumigated soil (Experiments 4)[1].

Source	df	RED PINE		WHITE SPRUCE	
		Shoot Conc. %	P Uptake mg/shoot	Shoot Conc. %	P Uptake mg/shoot
Reps	3				
P levels[2]	4	**	**	**	**
1 vs 2, 3, 4, 5[3]	1	**	**	**	**
2 vs 3, 4, 5	1	NS	**	**	**
3 vs 4, 5	1	NS	NS	*	NS
4 vs 5	1	NS	**	NS	NS
Error	12				
CV (%)		8	12	13	24

[1]See Table 19 for data.

[2]Levels of P addition

1) No addition
2) 224 kg/ha
3) 448 kg/ha
4) 672 kg/ha
5) 896 kg/ha.

[3]Orthogonal contrasts.

* Factor is significant at .05 level
** Factor is significant at .01 level
NS Factor is not significant.

Appendix Table 31. Significance of phosphorus addition to a methyl bromide fumigated nursery soil on morphological characteristics, shoot phosphorus concentration and uptake of 17 weeks old red pine seedlings (Experiment 5)[1].

Source	df	Height cm	Dry Weight Shoot mg	Dry Weight Root mg	Shoot P Conc. %	Shoot P Uptake mg/shoot
Reps	3					
P levels[2]	5	**	**	**	**	**
1 vs 2, 3, 4, 5, 6[3]	1	**	**	**	**	**
2, 3 vs 4, 5, 6	1	**	**	NS	**	**
2 vs 3	1	NS	**	*	**	**
4 vs 5, 6	1	NS	NS	*	NS	NS
5 vs 6	1	NS	NS	NS	NS	NS
Error	15					
CV (%)		9	13	15	8	14

[1] See Table 20 for data.

[2] Levels of P addition

1) No addition
2) 336 kg/ha
3) 672 kg/ha
4) 1008 kg/ha
5) 1344 kg/ha
6) 1680 kg/ha.

[3] Orthogonal contrasts.

* Factor is significant at .05 level
** Factor is significant at .01 level
NS Factor is not significant.