EFFECTS OF GLYPHOSATE ON SEEDLINGS OF CONIFER AND BROADLEAF TREES SPECIES NATIVE TO BRITISH COLUMBIA, WITH PARTICULAR REGARD TO ROOT-FUNGUS INTERACTIONS

by

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ABSTRACT

The hypothesis that soil fungi can enhance the herbicidal activity of glyphosate on seedlings of red alder, paper birch, Douglas-fir, lodgepole pine, ponderosa pine and interior spruce was investigated. No significant media-associated differences were detected in the glyphosate sensitivities of conifer seedlings growing in sterilized and non-sterilized soil in containers. Similarly, no significant differences were seen when sensitivities of seedlings growing in media amended and not amended with selected spp. of *Pythium* and *Fusarium* were compared. Application of glyphosate to field-grown seedlings resulted in significant increases in root colonization by *Pythium* and *Fusarium* in three conifer and two broadleaf species. Application of glyphosate to seedlings in May caused more mortality and was more damaging than was application in September. Effects of sub lethal doses of glyphosate included reduced growth of all species, top dieback, forking, and multiple leaders in Dougals-fir and lodgepole pine, and shorter branches on spruce. Paper birch was the most glyphosate-sensitive species, followed by red alder. Douglas-fir and lodgepole pine showed similar and intermediate levels of glyphosate sensitivity, while spruce was the least sensitive of the observed species. Conifers treated in the fall showed reduced growth in the following growing season, but resumed normal rate of growth in the second growing season. In forest plantations, the cost of reduced growth for one year would likely be offset by release from competition by brush following operational application of glyphosate.

Dedication :

To Ibu, Papa, Leo, Annisa and Marissa

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CHAPTER I

Introduction

1.1. British Columbia Forests, Biogeoclimatic Zones and Physiography

British Columbia (BC) is more variable physically and biologically than any other region in Canada (Alaback *et al.*, 1994). It covers 948,600 km^2 and spans 11° of latitude and 25 ° of longitude. Approximately 85% of land in BC is covered by predominantly coniferous forest, comprising approximately 50% of Canada's softwood volume (Meidinger and Pojar, 1991). The distribution of coniferous and broadleaf tree species in BC forests is influenced by topography and regional climate (Fosberg, 1967). One economically important broadleaf species is red alder, *Alnus rubra*, a fast growing pioneer species that forms dense stands on clear cut areas and otherwise disturbed areas.

The high diversity of vegetation in BC is a result the highly variable topography, a severe climate over a large continental land mass, and the moderating climatic effect of the Pacific Ocean along the coast. Sizes and densities of plants decrease with increasing elevation and latitude (Meidinger and Pojar, 1991). BC's vegetation falls into 14 biogeoclimatic zones characterized by distinct associations between plants and their microclimates in relation to regional climate (Pojar, 1983; Meidinger and Pojar, 1991).

On a broad scale, BC is also broken into four physiographic regions based on distribution of soils, climate, vegetation and terrain (Meidinger and Pojar, 1991). The western most region lines along the Coast Mountains and coastal islands.

The second region is comprised of a series of interior plateaus and low mountain ranges. The third is a series of mountain ranges that rises toward the east and culminates in the grandeur of the Rocky Mountains. Lastly are the interior plains, a region that dominates BC's northeast corner, the Peace River District.

1. 2. Forest Vegetation Management

In BC's working forests vegetation management is often required to control weed species that compete with coniferous crop trees for light, water and sources of nutrients. Vegetation management has been described as any process used to control non-crop vegetation, in an effort to improve the growth of crop species (Newton *et al.*, 1991; Hart and Comeau,1992), or as the practice of controlling the growth of non crop species, so that sunlight, moisture and nutrients can be channeled to trees that produce useable forest products (Campbell and Long,1995). Research and operational experience have demonstrated significant increases in the growth of desirable tree species through management of non-crop vegetation (Clason, 1993; Glover *et al.*, 1993; Harrington *et al.*, 1995; Miller *et al.*, 1991).

Vegetation management can be achieved by controlling the growth of the undesirable competitor species, or by enhancing the competitive ability of the crop trees. These two methods can also be used alone or in concert (Titus *et al.*, 1989).

Landowners might benefit financially from vegetation management in the following ways (Campbell and Long ,1995):

1) high density of crop trees in the absence of competing vegetation;
2) short rotations because trees reach merchantable size at an early age;
3) lowered cost of timber sale preparation and logging because of better access and good visibility in a stand; and
4) reduced costs of regeneration, because of there is less residual vegetation.

Until the early 90's the majority of vegetation management in both Canadian forestry and agriculture involved the use of herbicides. Glyphosate (Roundup or Vision) is the most widely used herbicide in Canada (Lange *et al.*, 1973; Carlson and Prasad, 1981; Titus *et al.,* 1989). In forestry today other vegetation management techniques are commonly used, including manual removal, mechanical removal and fire, albeit on limited areas (Titus *et al.,* 1989; Burton, 1992; Clement and Keeping, 1996). Both chemical and alternative methods have particular advantages and disadvantages, and the method of choice will depend on the specific plant species, plant density, growing conditions, soil texture, weather and cost.

1.2.1. Manual Removal

Manual removal of vegetation involves the use of chain saws brushcutter saws, girdling tools, and sandviks to girdle or to remove undesirable weed species. Manual removal is labor-intensive, and therefore costly. It is most feasible

in relatively level sites that have more large stems than dense brush, and is recommended in environmentally sensitive and populated areas, because it is perceived to have little impact on environmental or human health (Rennie *et al.*, 1985). However, the use of tools can result in serious injury, even in the hands of properly trained personnel following a strict safety protocol. In addition, where there is rapid re-growth of brush species, manual removal is less effective than chemical removal or prescribed burning.

Manual removal, the oldest method of managing vegetation, has increased in BC from 3000 ha in 1981-1982 (36% of all vegetation management in forestry) to over 60,000 ha in 1989-1990, but has stayed constant at 57,000 ha since then (Boateng, 1996).

1.2.2. Mechanical Brushing

The main purpose of mechanical brushing is to disrupt existing vegetation so that competition of weeds with crop trees will be reduced (Campbell and Long, 1995). Herbicides may often be used in combination with mechanical methods, e.g if it is desirable to kill the roots, or achieve a more lasting effect.

Mechanical methods include bulldozing, shearing, crushing, chopping, disking, bedding, scalping and scarification. Mechanical methods are feasible and most efficient on gentle topography and on relatively dry soils lacking large rocks, boulders, stumps, or large decaying logs. Even on gentle slopes, however, the use of heavy machinery, may create an erosion hazard. Because of the rapid growth of weed species, neither manual nor mechanical methods have had much success in

vegetation management in coastal BC (Hart and Comeau, 1992), although some foresters believe that if used judiciously these methods will be satisfactory on coastal sites (Jones and Boateng, 1983). Mechanical scarification is employed mostly in the boreal forest, but its cost effectiveness over other methods must still be evaluated.

1.2.3. Prescribed Burning

Prescribed burning has been used in forest vegetation management for centuries. In BC, aboriginal people used fire to create and maintain wildlife habitat and to stimulate the growth of edible herbs and berries (Burton, 1992). Burning residual brush and slash after harvest is far less common now than before 1970, but is used in some cases to prepare sites for planting. The brown and burn technique of site preparation involves desiccation of advanced weedy vegetation after very light aerial application of herbicides, followed by burning to remove the aerial biomass, but is very rarely used, because competition from sprouting vegetation may necessitate later application of a herbicide for conifer release. In broken topography and on steep slopes it is difficult to burn uniformly and to control a fire. Combinations of prescribed fire, mechanical and chemical vegetation management are often needed for adequate site preparation and conifer release treatments (Scagel and Caldicott, 1991).

One advantage of prescribed fire is the increased survival and growth of conifers planted on burned sites (Low and Hamilton, 1991; Todd, 1991; Haeussler, 1990). It is a cost-effective way to create the required number of planting spots on

some sites (Scagel and Caldicott, 1991), improves planter mobility, allows better identification of suitable microsites (Todd, 1991; Haeussler,1990) and lowers costs of planting and subsequent treatments (Scagel and Caldicott, 1991). On wet and rich sites, a well timed burn of the correct intensity can result in 1 - 2 years of relief from competing vegetation (Scagel and Caldicott, 1991). Other advantages of prescribed fire are reducing the stocking level of advance regeneration in coastal ecosystems (Scagel and Caldicott, 1991), and improved forest health through elimination of residual trees infected with dwarf mistletoe. In some sites, prescribed fire has resulted in undesirable vegetation shifts (Todd, 1991).

Prescribed fire also has some disadvantages with respect to seedling survival and growth of seedlings. Low and Hamilton (1991) found that some burned sites have shown nutrient deficiencies in N, Cu and Fe, which may have long term ramifications on growth. The impact of nutrient losses can be critical in shallow soils, on steep slopes, and on sites where leaching and surface erosion are common (Hawkes *et al.*, 1990).

At the same time, disadvantages include decreased forest health through promotion of certain weevils, black army cutworm and fungal activity associated with feeder root disease (Hawkes *et al.,* 1990).

1.2.4. Herbicides

Herbicides offer a safe and cost-effective method of both site preparation and conifer release. Herbicides can present less overall risk to human health than other methods and can require less energy to implement (Walstad *et al.*, 1987).

Several types of ground and aerial application equipment can be used in applying herbicides to suppress unwanted woody vegetation, grasses and forbs. In BC, herbicides are most commonly used as a brushing tool after reforestation, but they have also proven to be an effective site preparation tool in some situations (Burton, 1992). In1981-93, 57% of brushing activity in B C was accomplished using herbicides (Boateng, 1996).

Release of conifers from competing hardwood vegetation is essential during the critical years of establishment in order to ensure survival and improve growth rates. For this purpose, aerial application of herbicides tends to be the most effective (Payne *et al.,* 1990), and is often the only choice, especially where roads are lacking or where large areas must be treated within a short time.

The main aim of conifer release is to divert the resources of the site, such as soil moisture, nutrients, and sunlight, from the competing hardwood vegetation to conifer seedlings. The complete removal of a number of plant species from a plant community is not considered desirable, however, because of the potential for invasion of a site by other species, which either may be strong competitors or may be difficult to control (Campbell and Long, 1995).

Herbicide applications are often more economical than other methods of vegetation management. For example in a 136 ha area of red pine - black spruce in Wisconsin five manual operations (chain saw, axe, etc.) at a total cost of $85,000 were needed to obtain the level of conifer release produced by one chemical treatment (2,4-D), which cost only $6,120 using aerial application or $30,000 by ground equipment (USDA Forest service 1977). Other advantages of

using chemical herbicides are that they have a more lasting effect on competing vegetation than other methods and create little site disturbance. With chemical site preparation, organic matter is left in place, which improves the nutrient status and water retention of the site. If herbicides are used to thin juvenile conifer stands, there is less combustible fuel left on the ground than after manual thinning, and treated stands are less prone to damage by wind, snow, sun, and insects (Finnis, 1967).

Many factors must be considered before deciding to use herbicides in controlling vegetation, such as the type and species of vegetation to be controlled, the duration of control desired, environmental factors such as soil type and climate, and costs and types of herbicide available for use.

1.3. Glyphosate

The herbicidal characteristics of glyphosate (N-phosphonomethylglycine) were discovered by John E. Fanz in 1970 (Franz, 1974). The commercial product Roundup (a.i. 37% glyphosate), released in 1974, is a postemergence, broad spectrum and non selective herbicide, effective for control of annual and perennial weeds (Franz, 1985). Glyphosate is symplastically translocated to the meristems of growing plants. It causes shikimate accumulation through inhibition of 5-nolpyruvyl shikimate-3-phospate (EPSP) synthase (Jaworski, 1972; Steinrucken and Amrhein, 1980 and Stalker *et al.*, 1985). In the early 1980's it became the first individual pesticide with world wide sales of over $1 billion US (Franz *et al.*, 1997).

1.3.1. Use of Glyphosate in Forest Vegetation Management

In Canada, glyphosate (as Vision) is registered for forestry site preparation and conifer release by ground and aerial application (Canadian Pulp and Paper Association, 1986). It is the most extensively used herbicide in forest management in BC and the US Pacific Northwest. It is the only herbicide currently used for aerial spraying in forestry, and its use is growing rapidly (Environment Canada, 1998) . Glyphosate has been a substitute brush killer for 2,4-D and 2,4,5-T since 1977 (Hallet and Dufour, 1983). Conifer seedlings can be susceptible to herbicide damage depending on time and rate of application (Newton and Knight, 1981; Sutton, 1978). Conifers are most susceptible to glyphosate applications during the period of active growth and shoot elongation (Radosevich *et al.*, 1980). Therefore, glyphosate is applied in late summer in forest sites, after conifers have "hardened off", i.e. they have stopped growing and formed the following year's buds (Lund-Hoie, 1985).

Injury to crop species caused by glyphosate drift has been reported in several studies. The Nova Scotia Department of Lands and Forests (1989) reported that glyphosate caused slight burning of shoot tips of black spruce, and foliage damage on balsam fir. Application in July caused extensive needle loss, sometimes defoliating shoots up to 3 years old. Varying degrees of damage occurred when glyphosate was applied in forest nurseries in eastern Canada (Hallet, 1985). Reynolds *et al.* (1989) observed injury to western hemlock, following aerial application of glyphosate at Carnation Creek, BC. Moorhead

(1998) reported damage to southern pines after glyphosate was applied to control herbaceous weeds prior to seeding cotton. Low-vigor, over-mature trees or newly-planted seedlings may exhibit more die-back or mortality than vigorously growing saplings (Moorhead, 1998). A significant decrease in growth of containerized black spruce was caused by application of glyphosate in late November, when seedlings were dormant (Lanteigne, 1987).

1.3.2. Mode of Action of Glyphosate

The mode of action is the overall manner in which the active ingredient of a herbicide affects a plant at the tissue or cellular level. Herbicides with the same mode of action will have the same translocation pattern and produce similar injury symptoms. Selectivity on crops and weeds, and behavior in the soil are less predictable, but are often similar for herbicides with the same mode of action (Gonsolus and Curran, 1998).

Cole (1985) and Coggins (1989) have reviewed the uncertain mode of action of glyphosate. Studies with *Rhizobium* sp. *Escherchia coli*, *Chylamydomonas einhardii*, carrot and soybean cell cultures and *Lemna gibba* (Jaworski, 1972; Roisch and Lingens, 1974; Hederlie *et al.*, 1977; Gresshoff, 1979) strongly imply that the aromatic amino acid biosynthetic pathway is a site of glyphosate action. However, glyphosate treatment on intact plants affects basic physiological process, such as chlorophyll synthesis (Hollander and Amrheim, 1980; Lee, 1981), protein synthesis (Cole *et al.*, 1983), auxin production (Lee,

1982) and ammonia accumulation (Cole *et al.,* 1980). Other researchers interpreted these as secondary effects.

Steinrucken and Amrhein (1980) identified the shikimic acid pathway as site of action of glyphosate in bacteria. Studies by Jaworski (1983) supported a similar mode of action in plants. This hypothesis was confirmed by other studies (Amrhein *et al.*, 1983; Nafziger *et al.,* 1984), which demonstrated that both plant cell cultures and bacterial cultures grown in the presence of moderate concentrations of glyphosate were able to adapt and grow at nearly normal rates by over expressing genes responsible for EPSP synthase. Comai *et al.* (1983) showed that glyphosate resistance in bacteria could arise by the acquisition of a glyphosate-resistant form of EPSP synthase by mutation.

The production of glyphosate-resistant transgenic plants provides definitive evidence for the inhibition of EPSP synthase as the mode of action of glyphosate (Comai *et al.*, 1985; Fillati *et al.*, 1987). Inhibition of this enzyme prevents formation of aromatic amino acids and precursor compounds needed for secondary metabolism and defensive reactions in plants.

1. 3.3. Effect of Glyphosate on Soil Microflora

One important effect of glyphosate in soil is a shift in microbial community structure that could alter litter degradation and plant-microflora (or pathogen) interactions (Franz *et al.*, 1997). Glyphosate is moderately persistent in soil, with an estimated average half-life of 47 days (Wauchope, *et al.*, 1992). Glyphosate tenaciously binds to most soil particles (Glass, 1987), and is readily metabolized

by soil microorganisms to produce the plant nutrient phosphoric acid, ammonia and carbon dioxide (Sprankle *et al.*, 1975; Ruepple *et al.,* 1977). Several studies indicate that glyphosate binds to soil through the phosphonic acid moiety in its phosphonate anion form (Sprankle *et al.,* 1975; Glass, 1986, 1987).

The use of pure culture technique to determine the influence of herbicides on soil microorganisms has been widely criticized for the following reasons (Wardle and Parkinson, 1990) :

1) isolated organisms may be atypical of their form in the soil;
2) microorganisms are normally stimulated to artificially high metabolic rates by growth in normal laboratory media;
3) isolated organisms are removed from their normal ecological associations; and
4) interpretation of results is difficult, and extrapolation to field situations is impossible.

It is not surprising, therefore, that results on the effect of glyphosate on microorganisms in pure culture in the laboratory may be different from those obtained in soil. Because glyphosate is adsorbed tightly by most soil particles (Glass, 1987) and is believed to be inactivated rapidly in soil, it may have little effect on soil microorganisms (Sprankle *et al.*, 1975).

1.4. Role of Soil Microorganisms in Herbicidal Action of Glyphosate

Rahe *et al.* (1990), and Levesque and Rahe (1989) have reviewed to role of soil microorganisms in the herbicidal action of glyphosate. It appears that certain soil fungi function as glyphosate synergists that increase herbicidal efficiency.

By growing bean seedlings in both heat-treated and untreated soil, Johal and Rahe (1984) demonstrated that higher doses of Roundup were required to kill plants growing in treated than untreated soil. In a related study (Levesque *et al.*, 1992) found that about 10 times more glyphosate was required to kill bean seedlings growing in autoclaved than in untreated soil. In contrast, no differences were found in the quantity of foliar-applied 2,4-D or paraquat needed to kill 50% of bean seedlings grown in autoclaved or untreated soil. Differential sensitivity of plants grown in untreated and heat-treated soil is not common for herbicides in general (Levesque and Rahe, 1992).

1.4.1. Glyphosate Effect on Microflora-Plant Interactions

Glyphosate can indirectly affect some components of the soil microflora (Levesque and Rahe, 1992). Glyphosate-treated herbaceous plants (Lynch and Penn, 1980; Brown and Sharma, 1984) were rapidly colonized by fungi. Rapid colonization of the roots of several weed species by *Fusarium* spp. occurred after glyphosate treatment in the field (Lesveque *et al.*, 1987). These studies are in contrast to those that have shown that glyphosate inhibits the growth of many microorganisms. The "inert" formulation may also inhibit fungal growth (Harris and Grossbard, 1979; Liu, 1995). At normally used concentrations of glyphosate , in both laboratory and field experiments, Roundup herbicide had no effect on stimulated straw decomposition (Grossbard, 1985), but at concentrations higher than those normally used in agriculture, glyphosate inhibited cellulolytic fungi in the soil.

1.4.2. Glyphosate - Plant Pathogen Interactions

When herbicides are introduced to an ecosystem, they may either stimulate or inhibit the growth of soil microflora (Grossbard, 1985). Few data exist on the potential of glyphosate for inhibiting spore production by pathogens on treated plant residues (Grossbard, 1985). Inhibitory effects of glyphosate on spore formation in pathogenic fungi were observed in *Ryncosporium secalis* on barley (Grossbard, 1985) and on *Pynerophora triticirepentis* grown in a greenhouse (Sharma *et al.*, 1989).

Conversely, several studies have shown that glyphosate stimulates growth of soil microorganisms including plant pathogens, by creating a nutrient source. Whigham and Stoller (1979) and Cerkauskas *et al.* (1982), reported that when glyphosate was applied as a crop desiccant, it provided fungal pathogens with a suitable substrate for colonization, consequently, allowing further invasion or re-infection. Another study reported that volunteer barley plants killed by paraquat or glyphosate, served as a source of inoculum for *Rynchosporium secalis* (Stedman, 1982).

Johal and Rahe (1990) showed that glyphosate inhibited the production of phaseolin, phaseollinisaflavan, phaseollidin and kievitone in bean, possibly reducing natural defense mechanisms and making the plant susceptible to fungal colonization well in advance of overt symptoms of phytotoxicity (Rahe *et al.*, 1990). Similarly, Brammal and Higgins (1988) showed that colonization of root tissue in

tomato seedlings genetically resistant to *Fusarium oxysporum* f.sp. *radicis-lycopersici* occurred following exposure to sublethal doses of glyphosate. They observed that the glyphosate-induced colonization was associated with a reduced efficiency of production of phenolic materials that normally are formed as a resistance response to this pathogen. The inhibition of glyceollin synthesis in soybean by glyphosate is reported to cause the plant to become susceptible even to incompatible fungi (Keen *et al.,* 1982). On the other hand, glyphosate is reported to protect soy bean plants against red crown-rot disease caused by *Calonetrica crotalariae* (Berner *et al.,* 1991)

Pythium spp. have been reported to cause root rot of cereal seedlings in Western Australia. Extensive crop losses occurred following the use of glyphosate immediately before seedlings were planted (Blowes, 1987). Smiley *et al.* (1992) showed that *Rhizoctonia solani* can be a major problem in no-till spring barley when glyphosate is applied 2 or 3 days before planting, but not when it is used 3 weeks before planting or 1 or 2 days after direct drilling.

1.5. Objectives

Glyphosate is the most extensively used herbicide in forest management in BC and the US Pacific Northwest. It is the only herbicide currently used in aerial spraying in forestry and its use in growing rapidly (Environment Canada Atlantic Region, 1998). In 1990, over 200,000 ha of forest in Canada was treated with herbicide, and 81% of that amount was treated with glyphosate (Campbell, 1990). In 1996, glyphosate was applied on 171,000 ha of Canadian forest land, about

eight times more than all other herbicides used in forestry that year (Canadian Council of Forest Ministers, 2000).

Lynch and Penn (1980), and Brown and Sharma (1984) showed that glyphosate-treated plants were rapidly colonized by fungi. Levesque *et al.* (1990) reported that roots of several weed species in the field were rapidly colonized by *Fusarium* spp. after their shoots were sprayed with glyphosate. Glyphosate-treated bean seedlings growing in untreated soil died due to glyphosate-induced colonization of their roots by *Pythium* and *Fusarium* spp., but glyphosate-treated seedlings growing in vermiculate or sterilized soil were not killed (Johal and Rahe, 1984). The plant-herbicide-fungus relationship was termed glyphosate synergistic interaction (GSI). Levesque *et al.* (1992) showed that seedlings of six different plant species were less sensitive to glyphosate when growing in heat-treated soil than in untreated soil. The interaction of glyphosate synergistic fungi and glyphosate in herbaceous seedlings under field condition appears to be rather general and unavoidable (Rahe *et al.*, 1990).

The possibility of GSI and the potential sub lethal effects of glyphosate on growth in woody species have not been investigated. These possibilities comprise the focus of this research.

My first objective was to answer the question " does GSI occur in conifer seedlings? " Two kinds of experiments addressed this objective. One compared the sensitivity of conifer seedlings to glyphosate when growing in sterilized and untreated soils, and in soil not amended and amended with *Pythium* and *Fusarium*

spp. The other investigated the effect of glyphosate on fungal colonization of the roots of selected conifer and broadleaf species in a field nursery.

A second objectives was to test the hypothesis that glyphosate treatment of selected conifer and broadleaf seedlings would create a favorable substrate for invasion by soil microorganisms, and thus could reduce colonization of the roots by *Armillaria ostoyae*. My remaining objectives were to compare the sensitivities of the selected conifer and broadleaf seedlings to glyphosate when treated in May and September, and to monitor sublethal effects of glyphosate on subsequent growth and development of these seedlings in a field nursery.

CHAPTER II

Does Glyphosate Synergistic Interaction (GSI) Occur in Conifer seedlings?

2.1. Introduction

Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase; this inhibition prevents the synthesis of chorismate-derived aromatic amino acids and secondary metabolites in plants (Steinrucken and Amrhein, 1980). Mortality of herbaceous plants treated with glyphosate involves certain soil fungi that function as glyphosate synergists (Johal and Rahe, 1984). This relation was first detected through comparison of the dosages of glyphosate required to kill herbs in heat-treated *versus* untreated soils. Seedlings growing in heat-treated soil survived doses of glyphosate that killed seedlings growing in untreated soil. *Pythium* and *Fusarium* spp. were shown to be the agents synergizing the herbicidal activity of glyphosate in the untreated soil (Johal and Rahe, 1984). In the field, glyphosate increased the sensitivity of *Ranunculus repens* and *Holcus lanatus,* but not *Plantago lanceolata* to colonization by *Fusarium* spp. (Levesque *et al.,* 1987). Levesque *et al.* (1992, 1993), confirmed the herbicidal action of *Pythium* spp. and *Fusarium* spp. as the main synergistic colonizers of glyphosate treated seedlings. Glyphosate appears to make the roots of treated plants permissive to fungal colonization before symptoms of phytotoxicity are apparent, by interfering with the roots' natural defense mechanisms (Johal and Rahe, 1988, 1990; Liu *et al.*, 1997). No data are available on the occurrence or mechanisms of glyphosate synergistic

synergistic interaction (GSI) in woody species. My objective was to investigate whether GSI occurs in conifer seedlings.

There were three components to this investigation. One involved seedlings produced from surface sterilized seed (experiment 1) and another (experiment 2) utilized 1-year old nursery grown seedlings. The second approach (experiment 3) compared glyphosate sensitivity of seedlings growing in soil amended and not amended with *Pythium* and *Fusarium* spp. The *Pythium* and *Fusarium* isolates used in experiment 3 were obtained from roots of glyphosate-treated conifer seedlings and selected for their ability to enhance the phytotoxicity of glyphosate on bean seedlings.

2.2. Materials and Methods

2.2.1. Soil, Plant Materials and Maintenance

The soil used for experiments 1 and 2 was a loamy sand containing about 2.75% organic matter collected in the spring 1996 from a field near Aldergrove, BC , 2 months prior to the start of the study. It was stored outside in covered,100 L plastic containers. Prior to use, the soil was passed through a 4.5 mm sieve. Two-kg quantities of soil in autoclavable plastic bags were heat-treated at 121°C for 1h, and then stored in the sealed bags for at least 1 week before use. For experiment 2, a 3:1 mixture of heat-treated and untreated soil was used.

For experiment 1, seedlings of Douglas-fir, *Pseudotsuga menziesii,* and lodgepole pine, *Pinus contorta* var. *latifolia* were grown from seeds obtained from the Seed Center, B C Ministry of Forests, Surrey, B C and kept at 4°C until

needed. Seeds were soaked in distilled water for 24 h at 4°C, then soaked in 1% NaOCl for 5 min, rinsed thoroughly with distilled water and planted individually in 1.5 cm depressions in 175-200 g of heat- treated or untreated soil in deep plastic containers (RLC-4 Cone-tainers, Stuewe, Corvallis OR). The seedlings were watered twice per week, and every 4 weeks for the first 12 weeks they were irrigated with a solution of N-P-K (20-20-20) soluble fertilizer(4 g per 1 L of water). The containers were kept in growth chambers at 25°C with a 16:8 h light cycle until the seedlings were 2 months old, and then were moved to a greenhouse with ambient light and temperature ranging from 4.5 to 25 ° C.

For experiment 2, 1 year old (1-0) seedlings of ponderosa pine, *Pinus ponderosa* and interior spruce, *Picea engelmannii* [1], seedlings were obtained from the Surrey Nursery, B C Ministry of Forests, Surrey, B C. These seedling's roots were washed prior to transplanting into either treated or untreated soil in 4 L pots. Potted seedlings were kept in the greenhouse and watered daily for 1 week and every second day thereafter. Fertilizer solution was applied as above at 2 weeks, 2 months and 4 months after transplantation. This experiment was carried out between the spring of 1996 and the winter of 1996.

Soil dilution plating was performed just prior to application of glyphosate on portions of the soils that were used in experiment 1 and 2 , to detect the presence of *Pythium* spp. and *Fusarium* spp. Ten g samples of soil were taken from each of five randomly selected pots. A 100-fold dilution series was prepared from each

[1] Spruce seedlings (other than Sitka spruce) obtained from the BC Ministry of Forests are classed as "interior spruce" unless they are clearly from seed zones in which only Engelmann or white spruce are found.

sample, and 0.2 mL aliquots were spread onto replicate plates containing PDA Rose Bengal. Beginning on day 2, colonies were marked on the bottoms of the plates as they appeared, using different colors of felt tip markers. Colonies were subcultured and examined under a compound microscope when the genus of fungus was uncertain based on visual examination. Numbers of *Pythium* and *Fusarium* colonies were calculated at day 5 .

Experiment 3 was carried out between the spring of 1996 and the spring of 1999. One year old white spruce and lodgepole pine seedlings were transplanted into soil in 4 L plastic pots in April 1996. These plants were maintained out doors and watered every 3 days throughout the summer.

2.2.2. Application of Treatments

Individual seedlings of similar size were treated in experiment 1 with 1%, 0.33%, or 0.11% concentrations of Vision® (356 g/L glyphosate) or with water (control) on 11 November 1996 (N=18 - 20 seedlings per treatment). Seedlings in their containers were placed individually on a rotating base, and sprayed from above with a hand operated atomizer while spinning. A piece of pre-weighed paper was placed beneath each plant to absorb any spray not intercepted by the plant. The weight of spray intercepted by the paper was substracted from the estimated weight of spray delivered from the atomizer to determine the quantity of glyphosate received by each seedling. Each seedling received 0.13 $\pm$ 0.01 g of spray. Thus individual seedlings treated with 1%, 0.33%, 0.11% and 0% Vision received 46.3, 15.4, 5.1 and 0.0 µg of glyphosate, respectively.

Seedlings in experiment 2 were treated with the same concentrations of Vision and by the same method as in experiment 1(N=20 seedlings per treatment). Plants were transplanted in March 1996 and treated in October 1996.

Glyphosate treatments were applied similarly to the seedlings used in experiment 3 except that seedlings were treated with 1%, 0.5% or 0% Vision, and thus received an estimated 46.3, 23.33 and 0.0 µg of glyphosate. Soil was amended with 25 mL of macerated mycelial suspension of *Pythium* spp. and *Fusarium* spp. in July 1998. Two, amendments , 1 X and 4 X concentrations of mycelia respectively contained 1 g and 4 g of mycelia of each isolate macerated in 100 ml of 0.08% sterile water agar. Seedlings were treated with glyphosate the following day (N=25 seedlings per treatment).

2. 2.3. Methods of Choosing *Pythium* and *Fusarium* Isolates for Fungal Amendment in Experiment 3

Isolates of *Pythium* and *Fusarium* spp. were obtained from roots of glyphosate-treated conifer seedlings. The potential of each isolate to enhance the herbicidal efficacy of glyphosate was assessed by comparing LD_{50} values using bean seedlings grown under controlled conditions in the presence and absence of isolates of *Pythium* and *Fusarium* spp. Estimation of LD_{50}'s (Descalzo,1996) involved planting surface-sterilized bean, *Phaseolus vulgaris*, seeds in plastic trays (55 x 28 x 7 cm) filled with 3.5 kg of autoclaved soil. Twenty seeds were planted in each of five equally spaced rows oriented across the width of each tray. The plants were placed in a growth chamber under a 16:8 h light and 25:19°C

temperature regime. Distilled water was added as needed to keep the soil moist. A dilute solution of 4 g of N-P-K (20-20-20) fertilizer was applied 1 week after planting.

Pythium isolates were grown individually in stationary culture in V8-cholesterol broth (Ayers and Lumsden, 1975) for 2 weeks. *Fusarium* isolates were grown in potato dextrose broth for 8 days. The cultures were incubated in the dark at 20-25°C. To prepare inoculum of an isolate, the culture was decanted into a Buchner funnel and the liquid removed by vacuum filtration through Whatman #1 filter paper. The mycelial mat was rinsed with sterile distilled water, and then aseptically cut into 1 cm^2 pieces using a scalpel. The pieces of mycelium were suspended in 0.08% sterile water agar (WA) at a concentration of 1 g per 100 mL, and macerated in a sterile blender for 20 sec. Inoculum was applied 1 day before glyphosate treatment by drenching 25 mL of the mycelial suspension between each row of seedlings once the seedlings had grown two fully expanded primary leaves (approximately 2 weeks after seeding).

Glyphosate treatment was applied 1 day later as two 1 µL droplets of aqueous dilution of Roundup herbicide (356 g a.i/L) applied to the stem at the cotyledonary node. Glyphosate doses (0,5,10,25 and 100 µg of glyphosate per plant) were assigned at random to each row of plants, with all plants in a row receiving the same dose.

Six *Fusarium* and seven *Pythium* isolates were evaluated twice each for their effect on LD_{50} based on number of plants dead per number of plants treated

(Appendix 1). The estimation of LD_{50} was done using linear regression of transformed mortality of plants to glyphosate [log(1-y/y)] *versus* glyphosate log (dose+1). When the observed mortality was 0 % or 100%, the number of dead plants was increased or decreased by 0.01 , respectively, when calculating (1-y/y). LD_{50} values were plotted by using the regression lines.

Isolates of *Pythium* spp. and *Fusarium* spp. varied in their ability to act as glyphosate synergists on bean (Table 1). Isolates 4 and 5 of *Pythium* spp. showed strong glyphosate-synergistic activity, with $LD's_{50}$ of 6.17 and 5.45 µg, respectively. Similarly, *Fusarium* spp. isolate 2 and 4 had $LD's_{50}$ of 4.73 and 9.12 ℓg, respectively. These four isolates were selected for use in experiment 3.

2.2.4. Collection of Mortality Data of Seedlings

Seedlings in experiments 1 and 2 were recorded as dead or living 12 weeks and 1 year after herbicide treatment, respectively (Appendix 2,3). Mortality data for seedlings in experiment 3 were recorded in August 1999. Herbicide injury in living seedlings was assessed as the percentage of needle injury (browned or dropped needles), and divided into three groups based on degree of injury (< 60%, 60-90% and 100%)

Comparisons of mortality were made between seedlings grown in heat-treated and untreated soil amended with two different concentrations of glyphosate synergistic *Pythium* spp. and *Fusarium* spp. (4X and 1X concentrations) and not amended with fungal extract .

Table 1. Estimates of glyphosate LD_{50} for bean seedlings growing in soil amended with different isolates of *Pythium* spp. and *Fusarium* spp. Estimates are based on linear regression of plant response (log 1-y/y) *versus* [log(dose+1)].

Treatment	LD_{50} (ug per plant)	R^2
Pyhtium isolate 1	37.00	0.913
Pythium isolate 2	9.12	0.809
Pythium isolate 3	7.80	0.927
Pythium isolate 4*	6.17	0.944
Pythium isolate 5*	5.45	0.811
Pythium isolate 6	9.46	0.970
Pythium isolate 7	33.60	0.994
Fusarium isolate 1	22.66	0.892
Fusarium isolate 2*	4.73	0.564
Fusarium isolate 3	15.28	0.567
Fusarium isolate 4*	9.12	0.995
Fusarium isolate 5	20.31	0.677
Fusarium isolate 6	11.22	0.973
Control	> 100	0.25

* selected for use in experiment 3

2.2.5. Analysis of Data

Data were analyzed using the General Linear Model procedure in SAS (SAS Institute Inc, 1985), testing for the effect of doses of glyphosate and soil type on seedling mortality, $p < 0.05$.

2.3. Results

2.3.1. Experiment 1

Lodgepole pine and Douglas-fir showed no significant differences in sensitivity to glyphosate between heat-treated and untreated soil (Table 2). Based on mortality, Douglas-fir and lodgepole pine tend to have similar sensitivity to glyphosate. GLM analysis showed that dose was a significant source of variation ($p<0.05$), and soil amendment with fungal mycelium was not a significant source of variation (Table 3).

2.3.2. Experiment 2

Interior spruce and ponderosa pine seedlings 18 months old in experiment 2 were less sensitive than younger Douglas-fir and lodgepole pine seedlings in experiment 1, and none of the treatments produced 50% mortality. As in experiment 1, there appeared to be no difference in the sensitivity to glyphosate of ponderosa pine and interior spruce seedlings grown in heat-treated or untreated soil to glyphosate (Table 4). Ponderosa pine seedlings tended to have higher sensitivity to glyphosate than spruce seedlings (Appendix 4). As in experiment 1, dose was as a significant source of variation ($p<0.05$), and soil was not (Table 5).

Soil plating of samples taken at the time of treatment of seedlings with glyphosate showed higher populations of *Pythium* and *Fusarium* spp in untreated soil than in heat-treated soil (Table 6).

2.3.3. Experiment 3

Low mortalities were observed 1 year after treatment of 1 year old ponderosa pine and interior spruce seedlings. Because only 1 of 50 seedlings was killed by 0.5% Vision, and foliage injury was low, only data with 1% Vision are shown in Table 7.

Amendment of growing media with glyphosate synergistic *Pythium* spp. and *Fusarium* spp. did not cause visible symptoms and did not change the sensitivity of potted seedlings to glyphosate. Symptoms of herbicide injury including tip burn, necrotic or brown and dry needles and leader mortality appeared as soon as 5 days after treatment. Spruce seedlings appeared to be less sensitive to glyphosate than ponderosa pine.

Table 2. Estimates of glyphosate LD50 values for Douglas-fir and lodgepole pine seedlings growing in untreated and treated soil, for glyphosate applied on 6 and 9 month old seedlings. Estimates are based on linear regression of plant response (log1-y/y) *versus* [log(dose+1)].

Seedlings species and age	untreated soil		treated soil	
	LD_{50} •	R^2	LD_{50}	R^2
6 month old				
Douglas-fir	26.9	0.781	27.2	0.798
Lodgepole pine	26.3	0.794	28.2	0.772
9 month old				
Douglas-fir	32.9	0.898	33.9	0.836
Lodgepole pine	27.5	0.779	26.9	0.781

6-month old seedlings treated in November 1996

9-month old seedlings treated in march 1997
*③g glyphosate / seedling

Table 3. Results of GLM analysis on seedling mortality of 6 and 9 month old Douglas-fir and lodgepole pine seedlings grown in pots in experiment 1

Seedlings species	Statistical results Dependent variable : Mortality				
Douglas-fir					
6 months	Source	DF	SS	F Value	Pr > F
	Model	4	7.3371	53.3000	0.0041
	Error	3	0.1032		
	Corrected Total	7	7.4403		
	R-Square		C.V		Response Mean
	0.9861		7.3076		2.5387
	Source	DF	SS	F Value	Pr > F
	DOSE	3	7.2708	70.4200	0.0028
	SOIL	1	0.0662	1.9200	0.2595

Lodgepole pine 6 months

Source	DF	SS	F Value	Pr > F
Model	4	11.2774	23.7700	0.0131
Error	3	0.3558		
Corrected Total	7	11.6332		

R-Square	C.V	Root MSE	Response Mean
0.9694	14.0055	0.3444	2.4588

Source	DF	SS	F Value	Pr > F
DOSE	3	11.0023	30.9800	0.0093
SOIL	1	0.2551	2.1500	0.2387

Douglas-fir 9 months

Source	DF	SS	F Value	Pr > F
Model	4	4.9325	36.7800	0.0070
Error	3	0.1006	0.0335	
Corrected Total	7	5.0331		

R-Square	C.V	Root MSE	Response Mean
0.9800	8.5183	0.1831	2.1496

Source	DF	SS	F Value	Pr > F
DOSE	3	4.9249	48.9600	0.0048
SOIL	1	0.0077	0.2300	0.6641

Lodgepole pine	Source	DF	SS	F Value	Pr > F
9 months	Model	4	6.9650	166.1000	0.0008
	Error	3	0.0314		
	Corrected Total	7	6.9964		

R-Square	C.V	Root MSE	Response Mean
0.9955	4.0158	0.1024	2.5496

Source	DF	SS	F Value	Pr > F
DOSE	3	6.9389	220.6400	0.0005
SOIL	1	0.0260	2.4800	0.21333

Table 4. Number of seedlings dead in experiment 2 in relation to different doses of glyphosate applied to 18 month old seedlings of spruce and ponderosa pine. Glyphosate applied in October 1996; data collected 11 months after treatment. N=15 seedlings/treatment

Species	Treatment	1%	0.33%	0.11%	0%
Ponderosa pine	Treated soil	6	6	2	0
	Untreated	7	5	3	0
Spruce	Treated soil	3	1	0	0
	Untreated	4	1	0	0

Table 5. Results of GLM analysis on seedling mortality of 18 month old spruce and ponderosa pine grown in pots

Species	Statistical results Dependent Variable : Mortality				
Spruce	Source	DF	SS	F value	Pr> F
	Model	4	4.8660	135.5500	0.0010
	Error	3	0.0269		
	Corrected Total	7	4.893		

R-Square	C.V	Root MSE	Response Mean
0.9944	13.2217	0.0947	0.7165

Source	DF	SS	F Value	Pr > F
DOSE	3	4.857	180.400	0.0007
SOIL	1	0.0089	1.0000	0.3910

Ponderosa pine

Source	DF	SS	Mean Square	F Value	Pr > F
Model	4	8.0301	2.0075	74.1300	0.0025
Error	3	0.0812	0.0271		
Corrected Total	7	8.1114			

R-Square	C.V	Root MSE	Response Mean
0.9899	10.1841	0.1646	1.6159

Source	DF	SS	Mean Square	F Value	Pr > F
DOSE	3	8.0188	2.6729	98.7000	0.0017
SOIL	1	0.0133	0.0133	0.4200	0.5643

Table 6. Comparative frequency of *Pythium* and *Fusarium* spp estimated by dilution plating of untreated and heat-treated soil used in experiment 1 and 2.

Experiment No.	Time of sampling: (Months after planting)	Soil treatment	CFU* *Pythium* spp.	CFU* *Fusarium* spp.
1				
	6	heat- treated	1.7×10^2	2.3×10^2
		untreated	32.0×10^2	22×10^2
	9	heat- treated	1.5×10^2	1.45×10
		untreated	36.8×10^2	20.6×10^2
2	6	heat-treated	3.4×10^2	28.4×10^2
		untreated	2.4×10^2	4.4×10^2

* Colony-forming units g^{-1} weight of soil estimated by dilution plating

Table 7. Response of ponderosa pine and interior spruce seedlings in experiment 3 to foliar application of glyphosate (1% Vision) after treatment with 1 or 4 gram of mycelial suspension per seedling. Fungal amendment and glyphosate treatment in July 1998, data collected in August 1999.

Percentage of needle injury	Number of seedlings per injury category and fungal amendment (N=25)					
	ponderosa pine			spruce		
	no fungus	1g	4g	no fungus	1g	4g
100% (death)	2	2	4	0	1	0
60% - 90%	5	10	7	1	1	0
< 60%	18	13	14	24	23	25

2.4. Discussion:

My results with Douglas-fir and lodgepole pine grown from surface sterilized seeds showed no difference in susceptibility to glyphosate between seedlings grown in heat-treated soil or untreated soil. Similarly, seedlings of ponderosa pine and interior spruce that were transplanted into heat-treated or untreated soils showed no difference in glyphosate sensitivity. These results are in contrast to earlier findings with herbaceous plants (Johal and Rahe, 1984; Levesque, 1990; Levesque *et al.*, 1992). It is possible that GSI does not occur in the conifers, or that fungi capable of GSI were not present in the soil used in my experiments. Because 6 or 9 months elapsed after heat treatment, glyphosate synergistic fungi could have inadvertently colonized treated soil prior to its treatment with glyphosate. However, tests performed before glyphosate treatment was applied showed very low levels of *Pythium* spp. and *Fusarium* spp. in heat-treated soil (Table 6).

Levesque (1990) hypothesized that higher sensitivity to glyphosate for bean, wheat and apple seedlings grown in raw soil than in heat-treated soil was due to quantitative and qualitative changes in microflora following heat treatment. Addition of raw soil water extract to heat-treated soil restored both *Pythium* spp. and herbicidal efficacy in soil where *Pythium* spp. were re-established. Before beginning my study, I repeated a portion of Levesque's study using bean seedlings, verifying the above result in the soil that I later used for experiments on conifers.

Descalzo (1996) showed that a variety of *Pythium* spp. were capable of glyphosate synergistic interaction (GSI) in seedlings of several dicot species,

including those of beans. In this study, I used bean seedlings to select two isolates each of *Pythium* spp. and *Fusarium* spp. with high GSI potential. Even with these potent isolates the results of experiment 3 revealed no significant effect of soil amendment with *Pythium* spp. and *Fusarium* spp. on the sensitivity of either lodgepole pine or spruce seedlings to glyphosate.

I have not been able to demonstrate that glyphosate synergistic interaction occurs on seedlings of Douglas-fir, interior spruce, ponderosa pine or lodgepole pine. Thus my results suggest that GSI does not occur in conifers, and indicate that GSI is not a general phenomenon in all plants.

CHAPTER III

Effect of Glyphosate on Fungal Colonization of The Roots of Selected Conifer and Broadleaf Species Growing in a Field Nursery

3.1. Introduction

In higher plants , glyphosate normally moves from aerial, chlorophyll-containing parts to areas of merismatic activity and roots through phloem and xylem tissues (Sprankle *et al.,* 1975; Franz, 1985; Sanberg *et al.*, 1980; Schultz and Burnside, 1980; Caseley and Coupland, 1985). Glyphosate has a rapid effect on many biochemical processes (Hoagland and Duke, 1982; Cole *et al.*, 1983). Biochemical studies have shown that the primary metabolic target is the enzyme 5-enolpyruvylshikimate-3-phosphate synthase of the shikimic acid pathway, from which is derived the aromatic amino acid phenylalanine (Steinrucken and Amrhein, 1984). Phenylalanine is necessary for the production of lignin and C_6 - C_3 phenolic compounds involved in plant disease resistance (Steinrucken and Amrhein, 1984).

The shikimic acid pathway occurs in many microorganisms that are glyphosate sensitive (Jaworski, 1972; Amrhein *et al.*, 1980). The reportedly minimal effects of glyphosate on soil microflora are presumably due to its rapid inactivation in soil (Sprankle *et al.,* 1975), but some reports show that glyphosate can have significant effect on soil microflora. Organic matter mineralization was inhibited in the field for 445 days after glyphosate applications (Bliev, 1983). Lynch and Penn (1980) as well as Brown and Sharma (1984) showed that glyphosate-treated plants were rapidly colonized by fungi. *Fusarium* spp. rapidly colonized several weed species after glyphosate treatment in the field (Levesque *et al.*,

1987). Glyphosate at sub-lethal doses predisposed the roots of treated bean seedlings to rapid colonization by soil fungi, that in turn contributed substantially to the herbicidal activity of glyphosate, a phenomenon termed glyphosate synergistic interaction (GSI) (Johal and Rahe,1990; Levesque and Rahe, 1992). Subsequently Descalzo *et al.*, 1996) showed that glyphosate had a similar effect in several other herbaceous species. This study was undertaken to test the hypothesis that treatment with glyphosate predisposes roots of selected conifer and broadleaf woody species grown under field conditions to fungal colonization.

3.2. Materials and methods

3.2.1. Experimental Treatment

Five species were used: Douglas-fir, *Pseudotsuga menziesii*, lodgepole pine, *Pinus contorta* var. *latifolia*, interior spruce, *Picea engelmannii*, paper birch, *Betula papyrifera* and red alder, *Alnus rubra*. One-year-old (1-0) seedlings provided by the BC Ministry of Forests (BCMOF) Green Timber Nursery, Surrey, BC, were planted in the field at the BCMOF Surrey Nursery, Surrey, BC in February 1996 with 1 m between seedlings and 2 m between rows for conifers, and 2 m between seedlings and 3 m between rows for broadleaf species. Supplemental irrigation was provided as required from mid April to mid September. During May 1996, single bags containing 10 g of slow release fertilizer were placed 15 cm deep approximately 5 cm from the stem of each conifer seedling. Vegetation between rows was mowed every spring; no other weed control method was used.

Glyphosate at four concentrations (0, 0.11%, 0.33%, and 1% of Vision®,

356 g glyphosate/L, Monsanto Canada) was sprayed on the foliage, at three application (September 1996, May 1997, September 1997; for September 1997 red alder seedlings were not treated), using a backpack sprayer to uniformly wet the foliage of each seedling. All treatment days were rain-free.

3.2.2. Estimating Fungal Colonization in Roots

At 1, 2 and 3 weeks after treatment, three or four plants within each treatment were randomly selected and uprooted, using a shovel. The roots were excised at the root collar, placed in labeled plastic bags, and transported in a cooler to the laboratory, where they were carefully washed to remove most of the soil. Then individual root clusters, each containing several intact tips, were excised 2.5 - 3.5 cm from tips of the central root axes, placed on a sieve (0.6 mm mesh), rinsed in distilled water for 10 min, surface sterilized and placed onto agar-based media, 4-5 per plate.

In fall 1996, root clusters were surface sterilized for 5 min in 2% NaOCl, washed with 50% ethanol and then sterile distilled water, and plated on PDA Rose Bengal and incubated in darkness at room temperature. The plates were examined daily and colonies were marked on the bottoms of the plates as they appeared, using a color-coded felt tip marker for each day. Each colony was subcultured and characterized on PDA, V8 agar and CMA using a single plate of each medium for each plant that was sampled.

In spring 1997, surface sterilization was reduced to 3 min in 1% NaOCl followed by a single rinse with sterile distilled water. During the sterilization process, 10 segments 1 cm long were cut from each root and plated onto PDA Rose Bengal.

Roots were examined on days 2 - 7 under a dissecting microscope, and emerging colony boundaries were circled on the bottoms of the dishes, as described above. Colonies were subcultured when the genus of fungus was uncertain based on visual examination. Fresh mounts from original plates and subcultures were examined at 250 X and 400 X magnification to observe spore morphology and mycelial characteristics. These characteristics were used to identify the fungi using published keys (Domsch *et al.*,1980). For roots collected in the fall 1997, the surface sterilization protocol was the same as for spring 1997, but 10 pieces of roots were plated onto each of PDA Rose Bengal medium, *Pythium spp.* -selective medium (Mircetich, 1971) and *Fusarium*-selective medium (Nash and Snyder, 1962). Plated roots were examined and colonies counted on PDA Rose Bengal after 4 days, and on the selective media after 6 days.

3.2.3. Analysis of Data

Data were analyzed by linear regression using the Systat statistical package testing each species separately for effect of dose on colony numbers at weeks 1, 2 and 3 after treatment. In all cases $\alpha < 0.05$.

3.3. Results

Fungal colonies began to appear about 1-2 days after plating, and after 2 days began to show distinctive color and texture. Colony boundaries began to overlap by day 6. Fungi that emerged from the roots of trees treated in fall 1996 and spring 1997 were categorized into seven groups: *Pythium* spp., *Fusarium*

spp., *Trichoderma* spp., *Cylindrocarpon* spp., and yellow, black and unknown white fungi. Among these seven groups, those with strong pigmentation (black fungi) occurred in greater numbers than fungi any of the other groups. Fungi grew well on PDA, moderately well on V8 agar, and poorly on CMA. Summaries of mean numbers of fungal colonies that emerged from roots that were sampled after application of glyphosate in the fall 1996 and the spring 1997 are presented in Appendix 5.

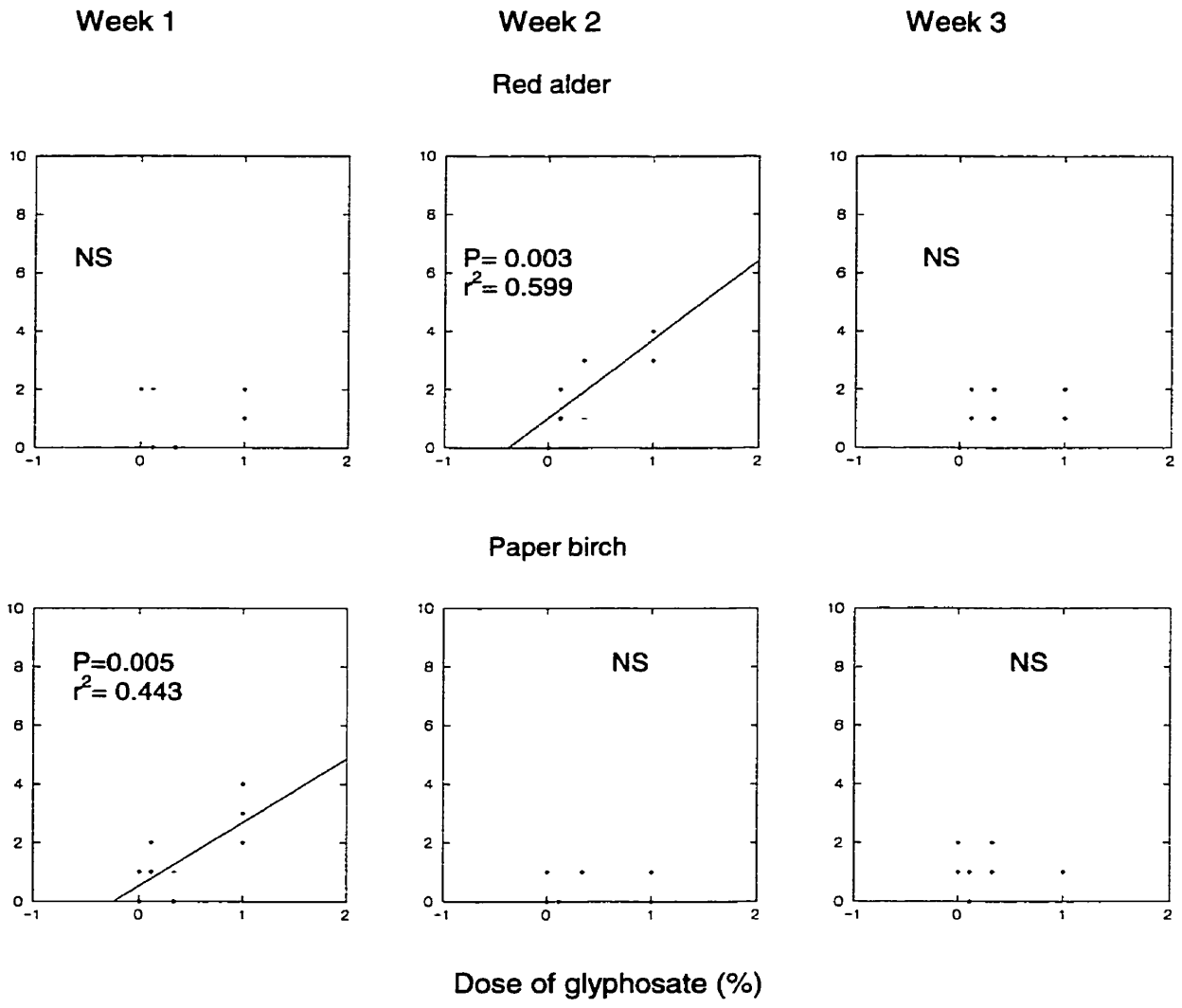
Glyphosate caused 15 significant–dose dependent increases in the numbers of *Pythium* spp. colonies that emerged from surface-sterilized root segments that were sampled from each of five woody species at each of the three time treatments (Figures 1-3), with the single exception of spruce treated in the fall of 1997 (Figure 3). The dose–dependent increases were observed at only one of the three sampling times, except for Douglas-fir that was treated in the fall 1997, when the effect was seen at both 1 and 2 weeks after foliar application of glyphosate.

In contrast, glyphosate caused significant dose–dependent increases in colonization of roots by *Fusarium* spp. in only seven cases: in red alder sampled 2 weeks after foliar application of glyphosate in May, 1997, in paper birch sampled 2 weeks after treatments in September of both 1996 and 1997, in Douglas-fir sampled at both 1 and 2 weeks after treatment in September, 1997, in lodgepole pine sampled 3 weeks after treatment in May, 1997, and in spruce sampled at 1 week after treatment in September, 1996 (Figures 4- 6).

Significant dose–dependent decreases in colonization by *Pythium spp.* or *Fusarium* spp. occurred in three instances all in paper birch. Colonization by both *Pythium* spp. and *Fusarium* spp. showed dose–dependent declines in roots sampled 2 weeks after foliar application of glyphosate in September, 1997, in both cases following significant dose–dependent increases in colonization at 1 week after treatment (Figures 3, 6). The remaining dose–dependent decline in colonization was by *Fusarium* spp. in roots sampled at 2 weeks after treatment in May, 1997 (Figure 4).

None of the other groups of fungi (*Trichoderma* spp., *Cylindrocarpon* spp., yellow, black and unknown white fungi showed a consistent pattern of dose – dependent change in colonization in response to foliar application of glyphosate.

Figure 1. Number of colonies of *Pythium* spp. emerging from roots of seedlings of selected woody species sampled at 1, 2 and 3 weeks after foliar application of glyphosate on 17 September, 1996. A regression line, r^2 and P value are shown in cases where $P < 0.05$; absence of a regression line and NS indicate that $P > 0.05$


Week 1
Week 2
Week 3
Red alder
NS
P= 0.003
r2= 0.599
NS
Paper birch
P=0.005
r2= 0.443
NS
NS
Dose of glyphosate (%)

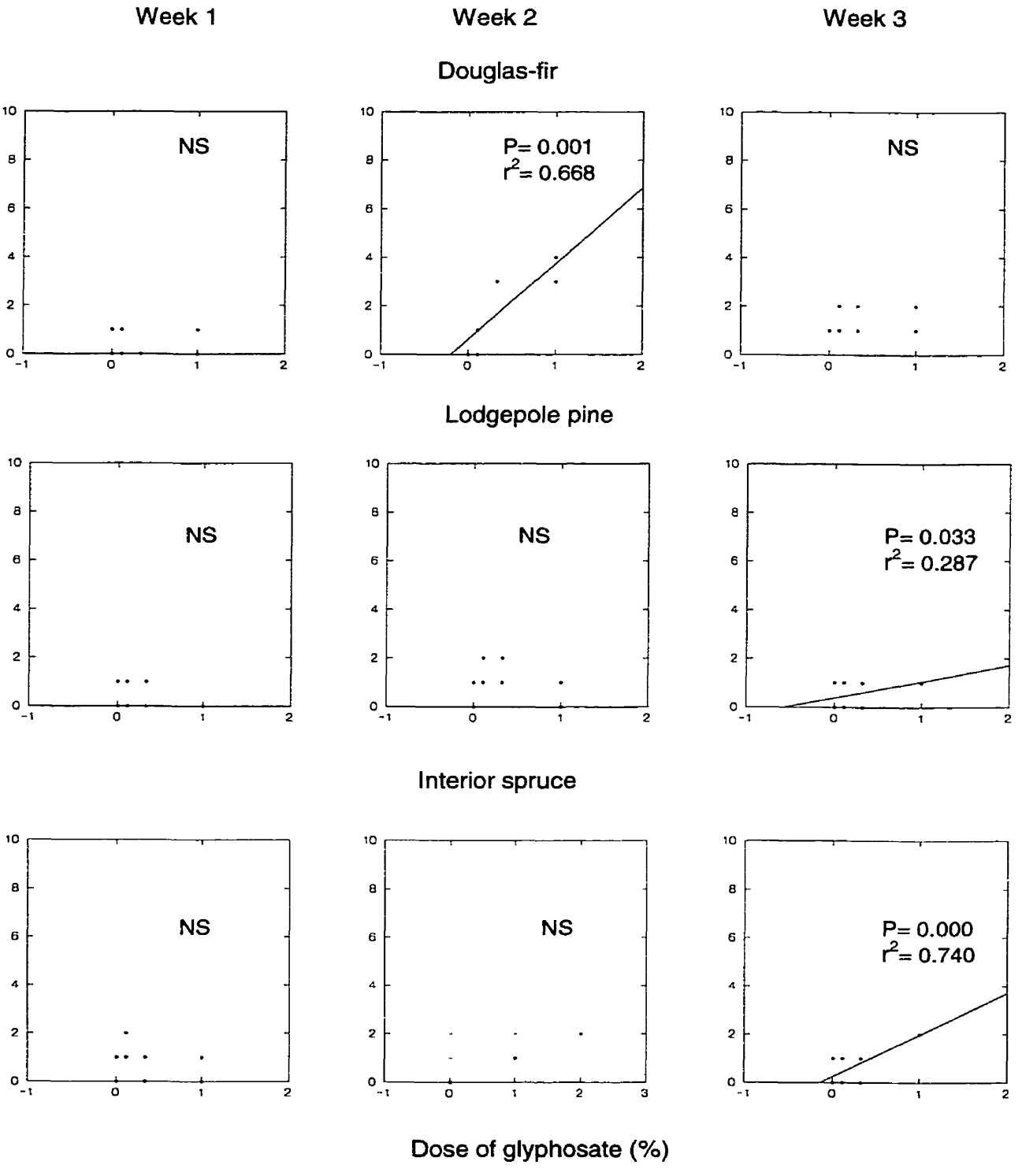
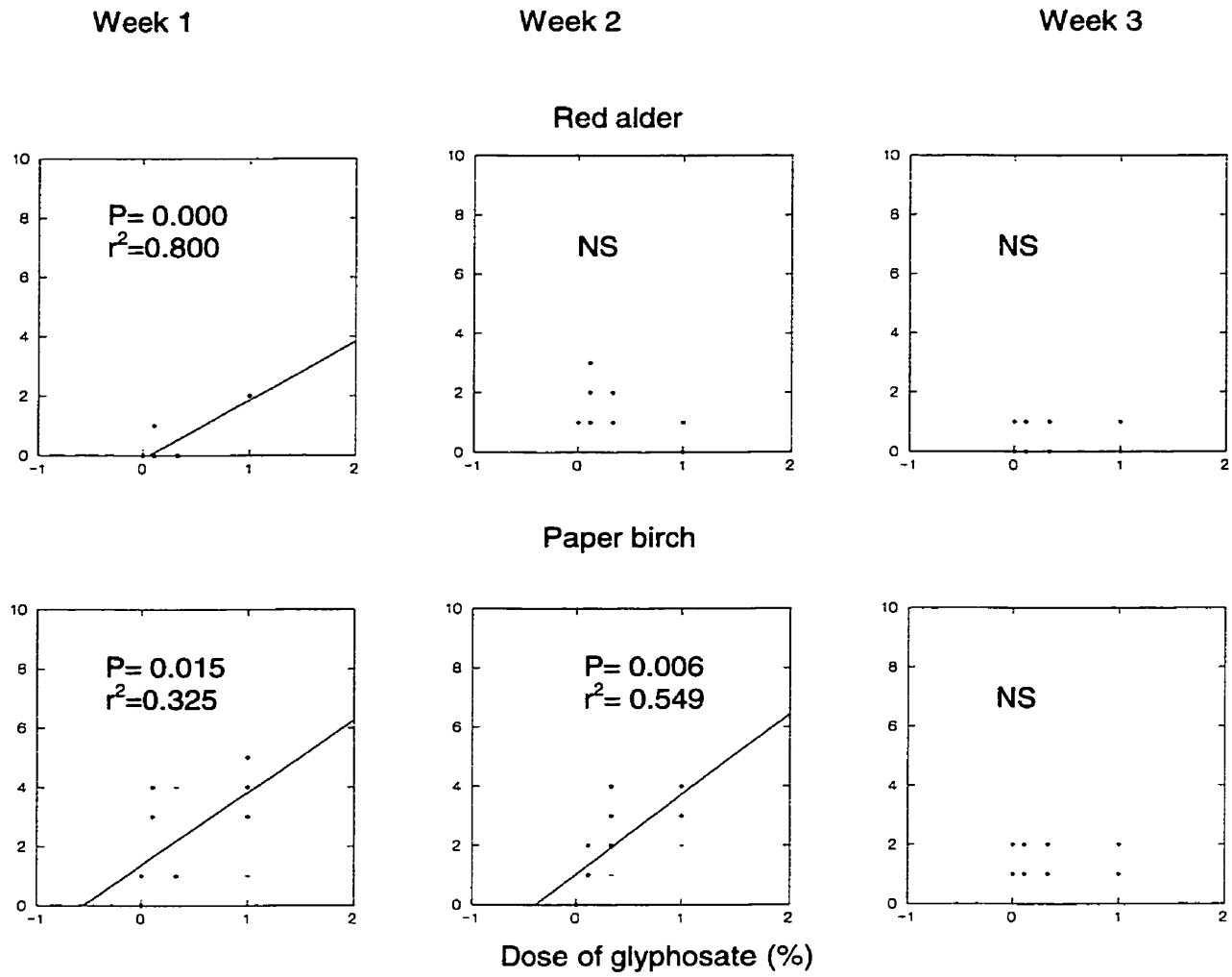

Week 1
Week 2
Week 3
Douglas-fir
NS
P= 0.001
r2= 0.668
NS
Lodgepole pine
NS
NS
P= 0.033
r2= 0.287
Interior spruce
NS
NS
P= 0.000
r2= 0.740
Dose of glyphosate (%)

Figure 2. Number of colonies of *Pythium* spp. emerging from roots of seedlings of selected woody species sampled at 1, 2 and 3 weeks after foliar application of glyphosate on 16 May 1997. A regression line r^2 and P value are shown in cases where $P < 0.05$; absence of a regression line and NS indicate that $P > 0.05$.


Week 1
Week 2
Week 3
Red alder
P= 0.000
r2=0.800
NS
NS
Paper birch
P= 0.015
r2=0.325
P= 0.006
r2= 0.549
NS
Dose of glyphosate (%)

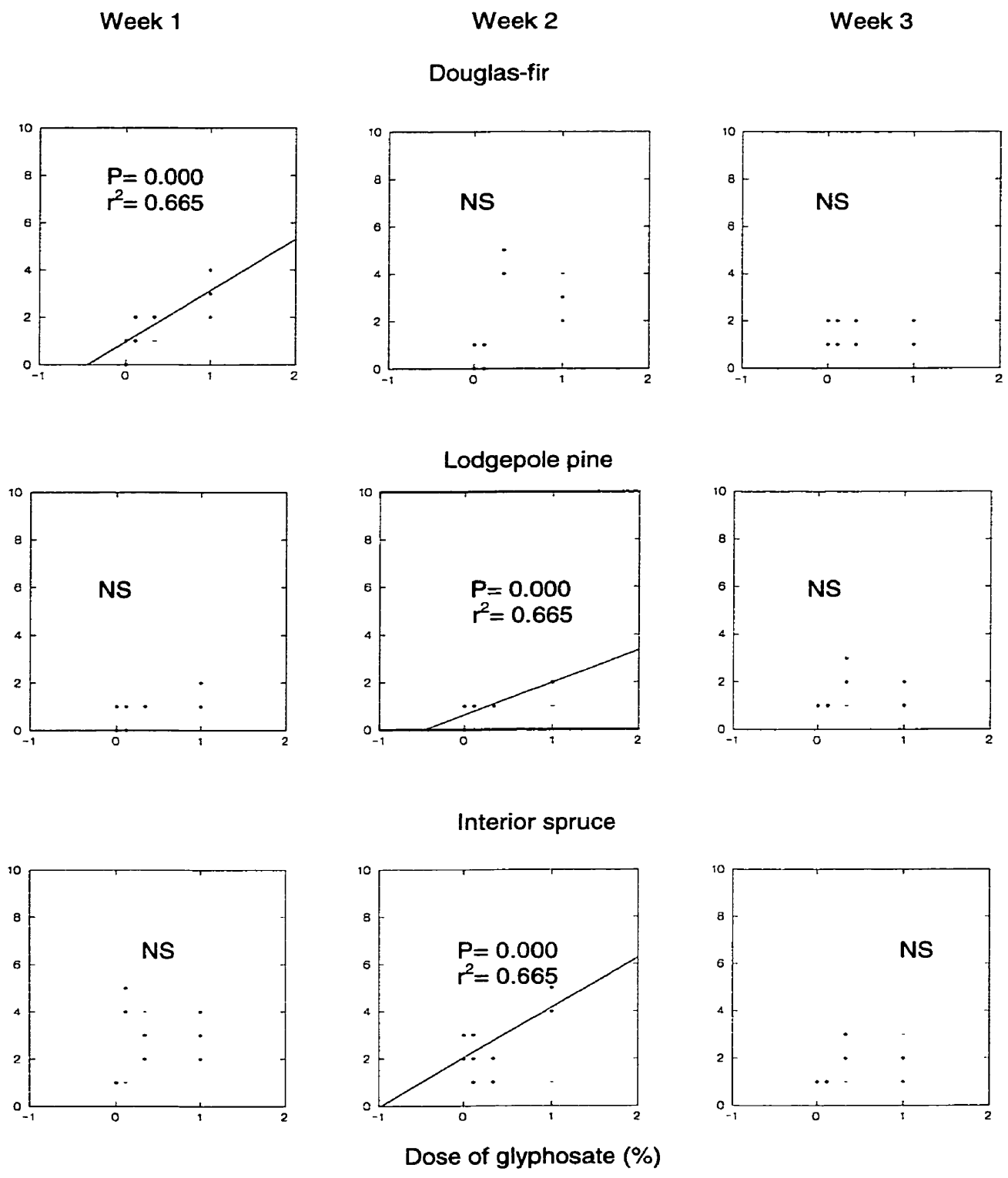
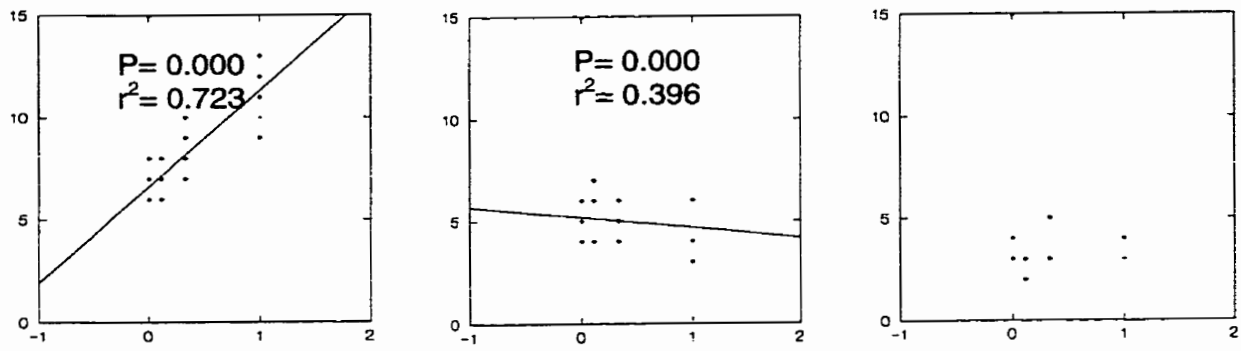

Week 1
Week 2
Week 3
Douglas-fir
P= 0.000
r2= 0.665
NS
NS
Lodgepole pine
NS
P= 0.000
r2= 0.665
NS
Interior spruce
NS
P= 0.000
r2= 0.665
NS
Dose of glyphosate (%)

Figure 3. Number of colonies of *Pythium* spp. emerging from roots of seedlings of selected woody species sampled at 1, 2 and 3 weeks after foliar application of glyphosate on 24 September 1997. A regression line r^2 and P value are shown in cases where $P < 0.05$; absence of a regression line and NS indicate that $P > 0.05$.

Week 1 Week 2 Week 3

Paper birch



Dose of glyphosate (%)

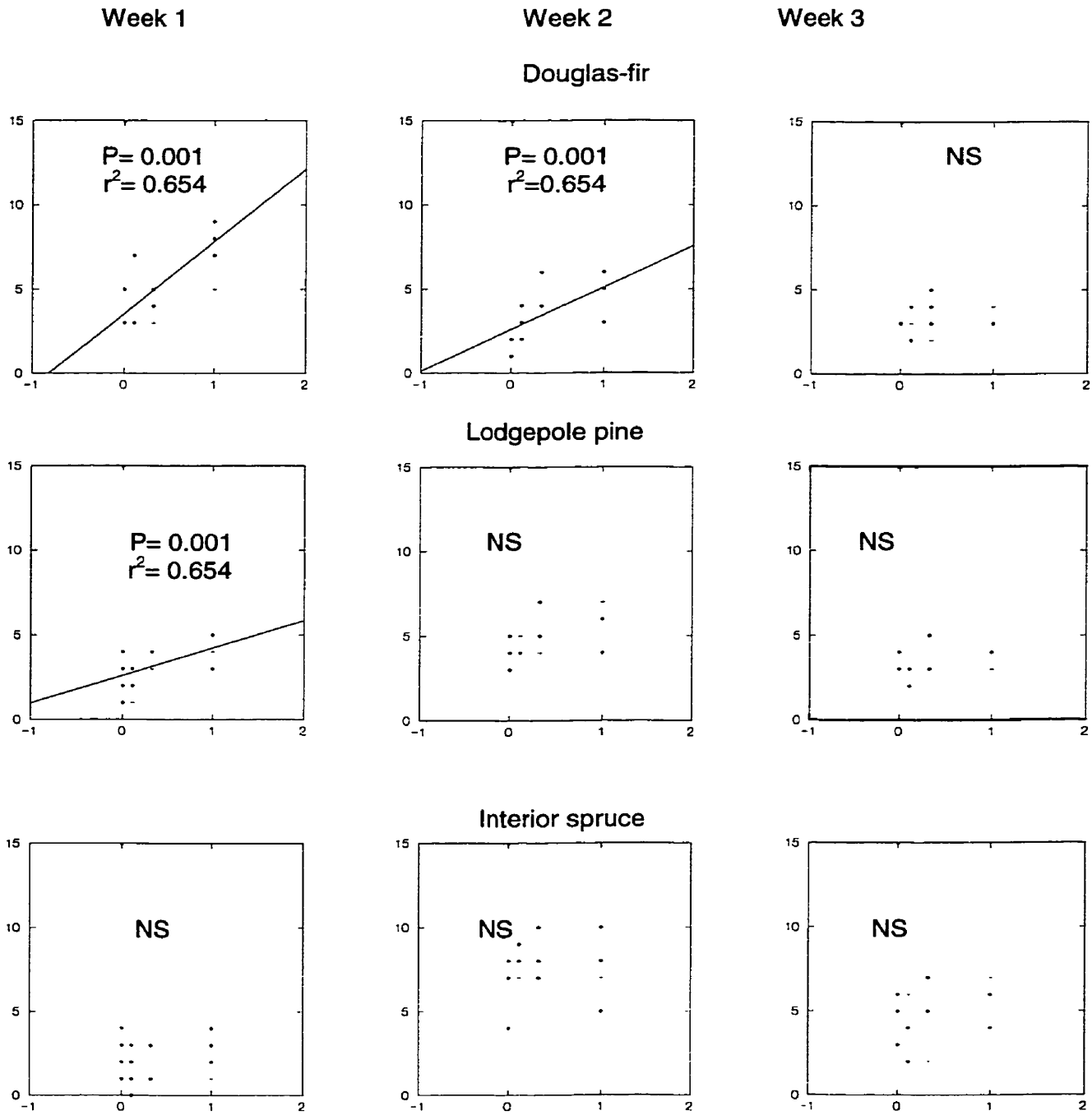
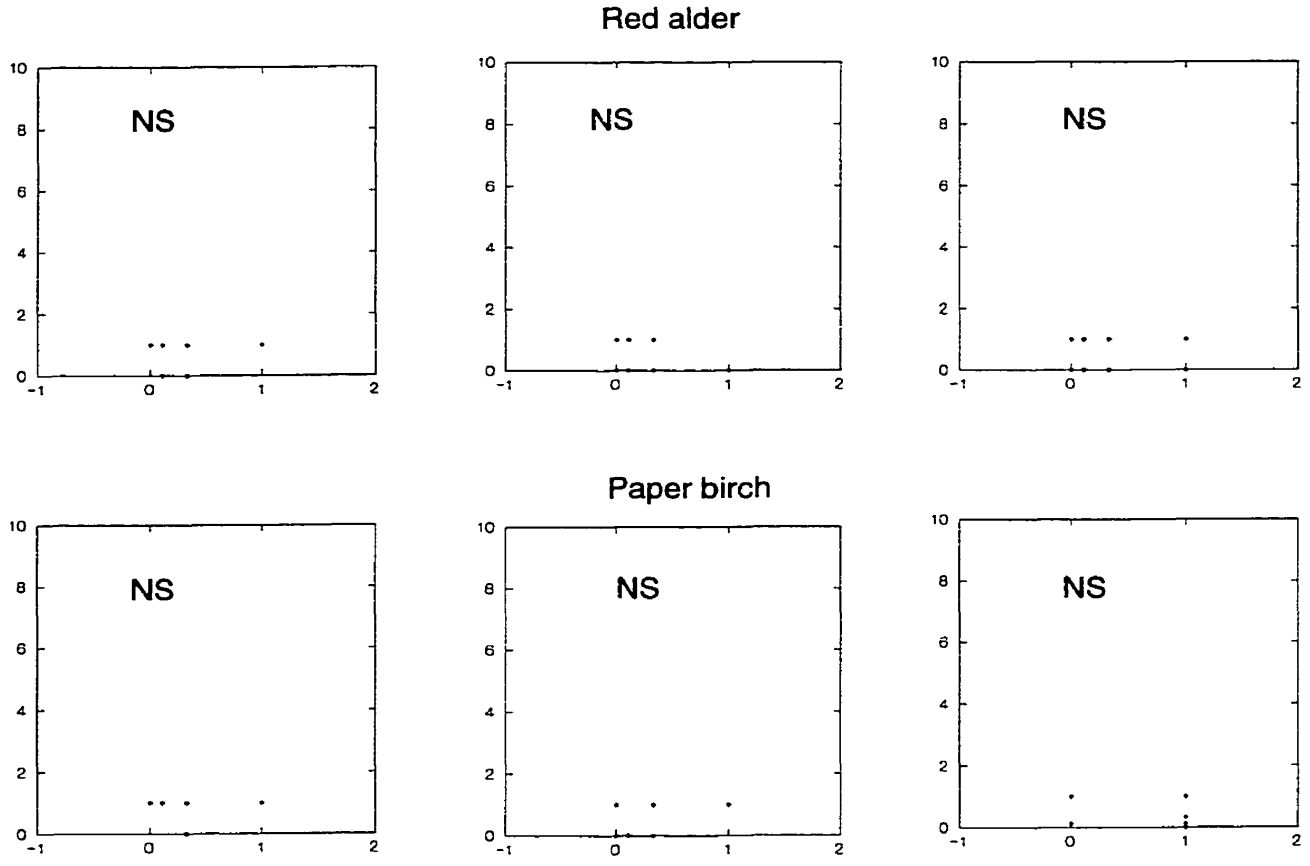

Week 1
Week 2
Week 3
Douglas-fir
P= 0.001
r2= 0.654
P= 0.001
r2=0.654
NS
Lodgepole pine
P= 0.001
r2= 0.654
NS
NS
Interior spruce
NS
NS
NS
Dose of glyphosate (%)

Figure 4. Number of colonies of *Fusarium* spp. emerging from roots of seedlings of selected woody species sampled at 1, 2 and 3 weeks after foliar application of glyphosate on 17 September, 1996. A regression line, r^2 and P value are shown in cases where $P < 0.05$; absence of a regression line and NS indicate that $P > 0.05$.


Week 1
Week 2
Week 3
Red alder
NS
NS
NS
Paper birch
NS
NS
NS
Dose of glyphosate (%)

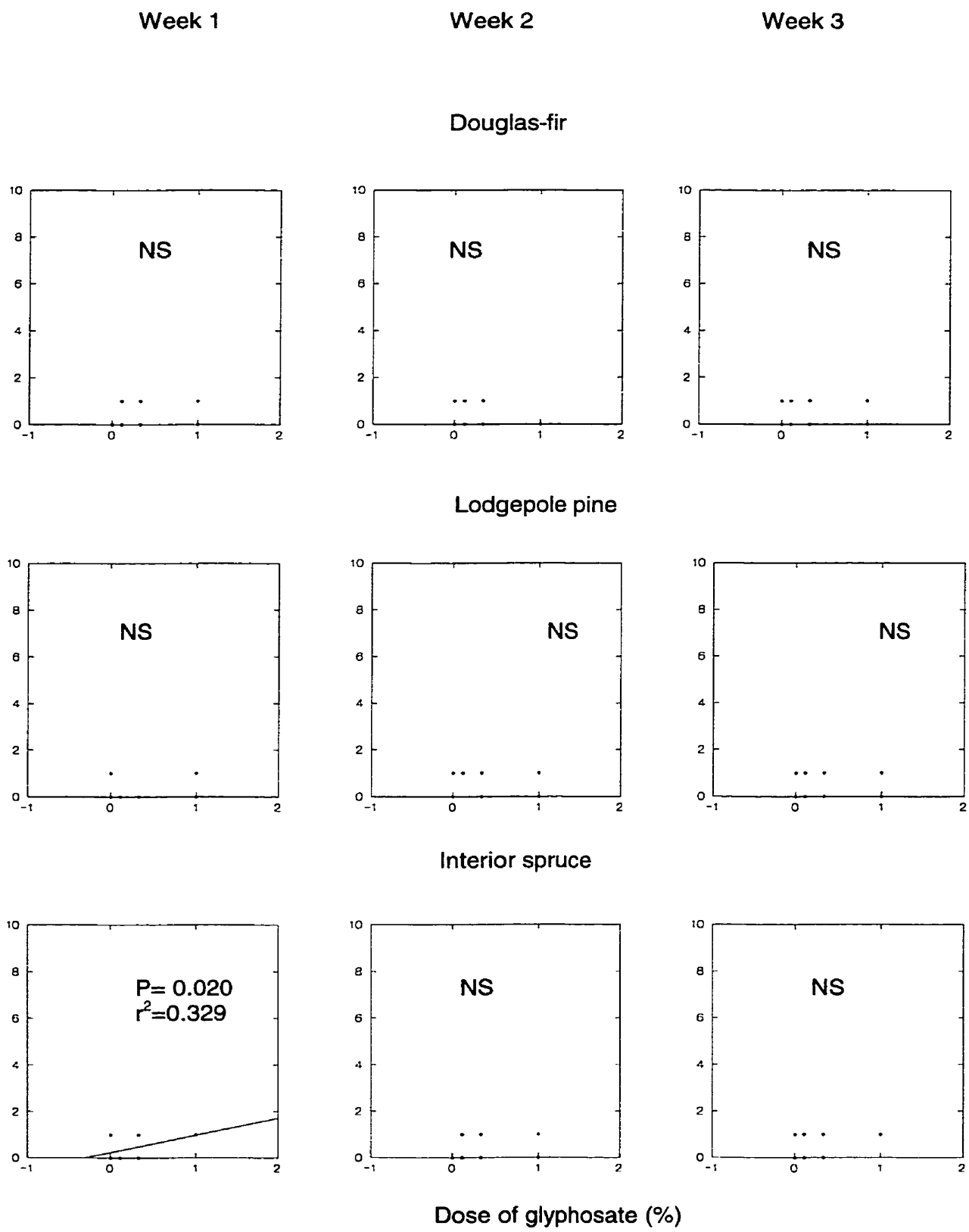
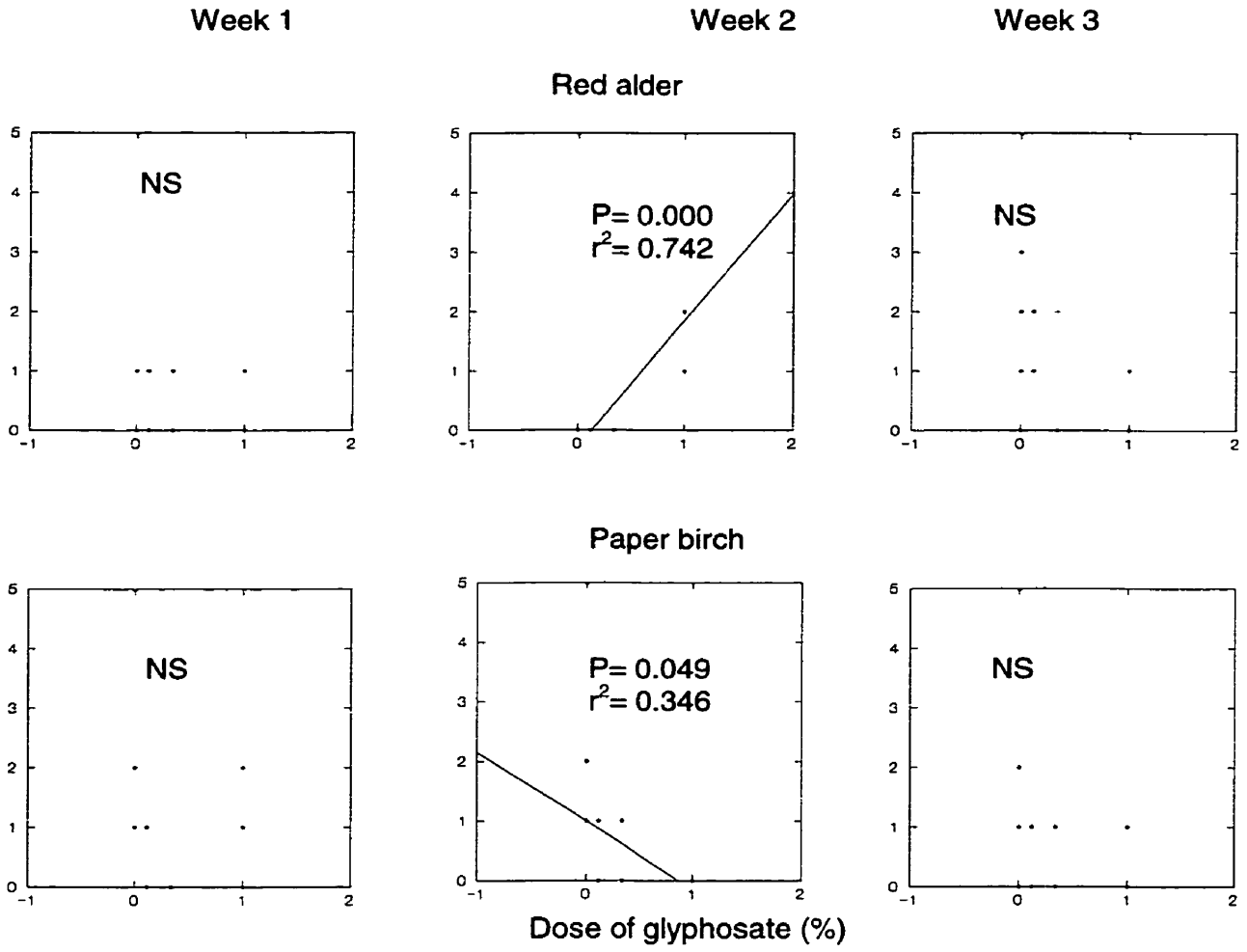

Week 1
Week 2
Week 3
Douglas-fir
NS
NS
NS
Lodgepole pine
NS
NS
NS
Interior spruce
P= 0.020
r²=0.329
NS
NS
Dose of glyphosate (%)

Figure 5. Number of colonies of *Fusarium* spp. emerging from roots of seedlings of selected woody species sampled at 1, 2 and 3 weeks after foliar application of glyphosate on 16 May, 1997. A regression line, r^2 and P value are shown in cases where $P < 0.05$; absence of a regression line and NS indicate that $P > 0.05$.


Week 1
Week 2
Week 3
Red alder
NS
P= 0.000
r2= 0.742
NS
Paper birch
NS
P= 0.049
r2= 0.346
NS
Dose of glyphosate (%)

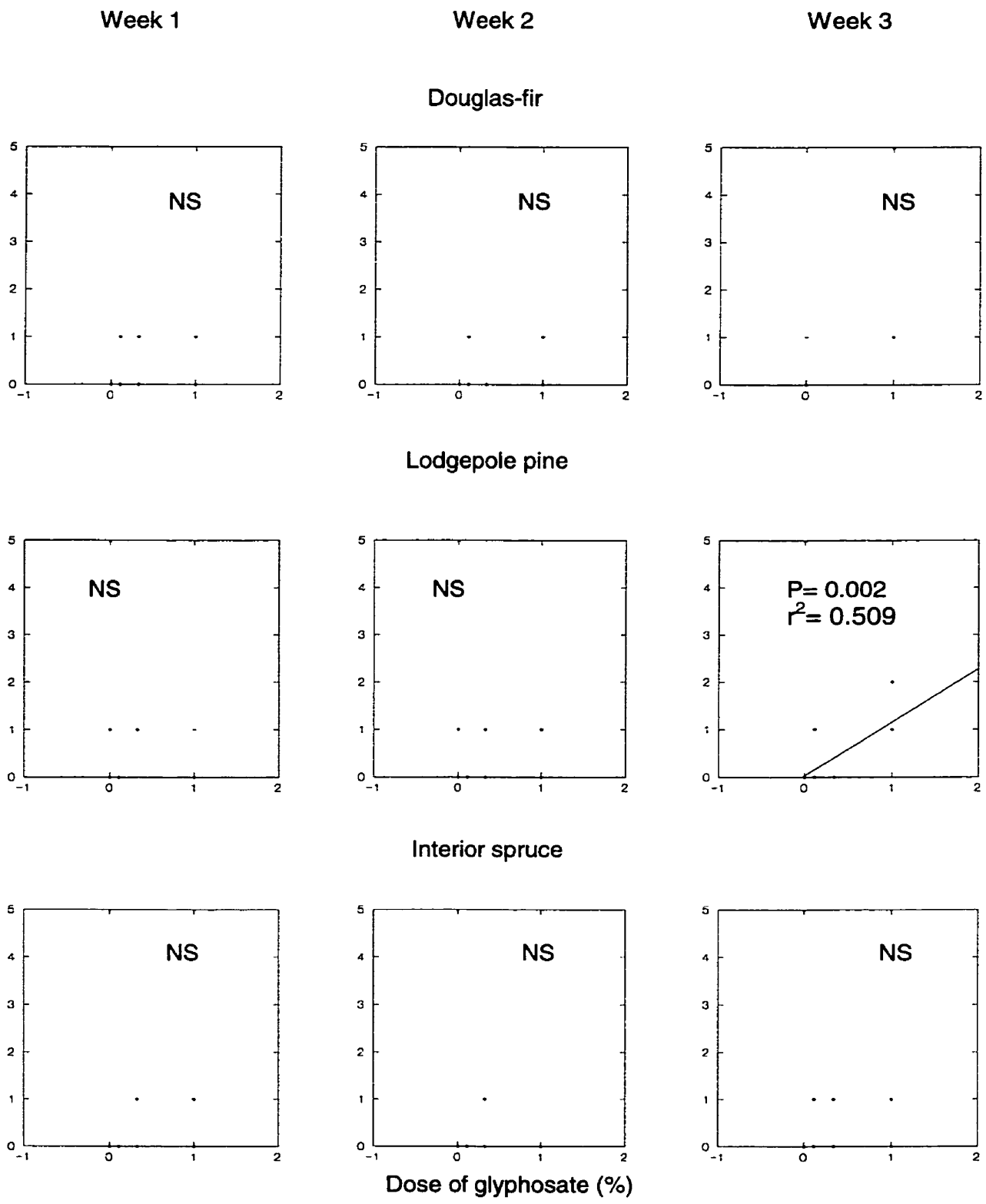
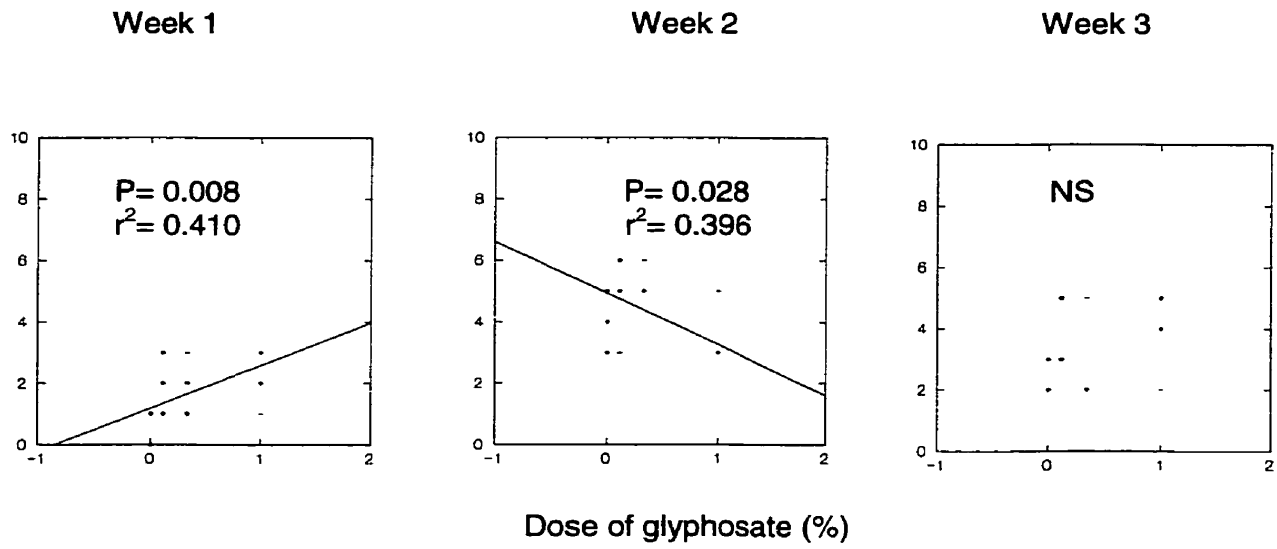
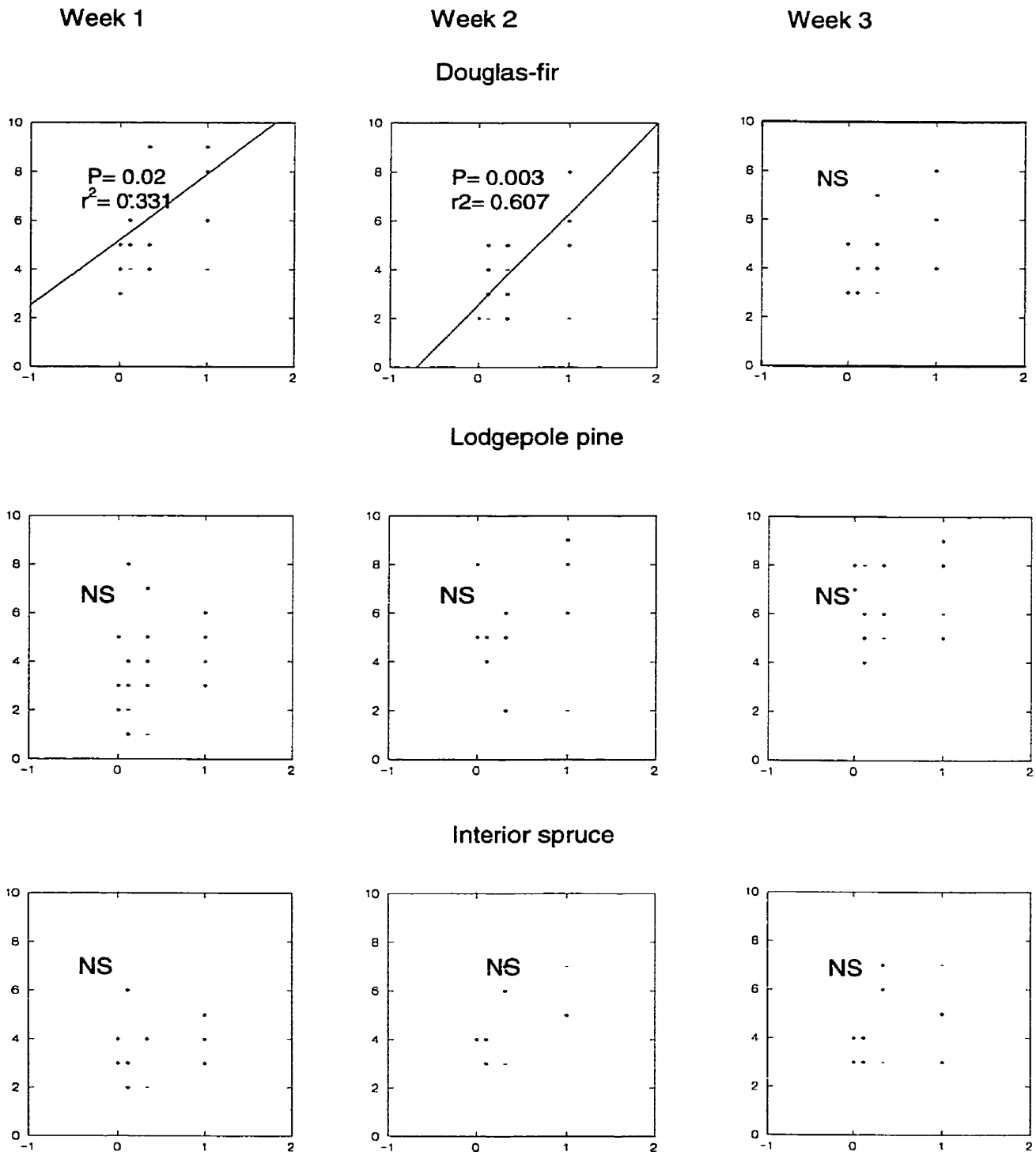

Week 1
Week 2
Week 3
Douglas-fir
NS
NS
NS
Lodgepole pine
NS
NS
P= 0.002
r2= 0.509
Interior spruce
NS
NS
NS
Dose of glyphosate (%)

Figure 6. Number of colonies of *Fusarium* spp. emerging from roots of seedlings of selected woody species samples at 1, 2 and 3 weeks after foliar application of glyphosate on 24 September, 1997. A regression line, r2 and P value are shown in cases where $P < 0.05$; absence of a regression line and NS indicate that $P > 0.05$.


Week 1
Week 2
Week 3
P= 0.008
r2= 0.410
P= 0.028
r2= 0.396
NS
Dose of glyphosate (%)


Week 1
Week 2
Week 3
Douglas-fir
P= 0.02
r2= 0.331
P= 0.003
r2= 0.607
NS
Lodgepole pine
NS
NS
NS
Interior spruce
NS
NS
NS
Dose of glyphosate (%)

3.4 Discussion

A general picture of the effect of foliar application of glyphosate on colonization of roots by fungi was sought in the experiments conducted in September 1996 and May 1997. The root plating procedure was modified for the experiment conducted in September 1997 to focus on *Pythium* spp. and *Fusarium* spp. because root colonization by these fungi appeared to be selectively influenced by the glyphosate treatments. Fungi in these two genera were the predominant colonizers in roots of glyphosate-treated herbaceous plants (Johal and Rahe, 1984; Levesque and Rahe, 1992). These fungi are commonly associated with root disease in seedlings (Agrios, 1988).

Results of this study showed that glyphosate enhanced colonization of roots by *Pythium* spp., and to a lesser extent, *Fusarium* spp. These experiments were conducted in different years, different seasons and with seedlings of different ages. The glyphosate-induced enhancement of colonization of roots by *Pythium* spp. is similar to the greatly increased colonization by *Pythium* spp. in roots of beans, wheat, corn, mustard and apple seedlings that were reported by Levesque (1990). Apple seedlings represent the only example of glyphosate-induced enhancement of root colonization by fungi leading to increased sensitivity to glyphosate in a woody species (Levesque, 1990). In this study, the enhancement of colonization in roots, in almost all instances, was observed in only 1 of the 3 weekly sampling intervals, and in week 3 in only three occasions. This suggests that after colonization well established colonized tissues in decaying roots may have been partially lost during the sampling or root washing procedure.

Johal and Rahe (1984) proposed that glyphosate, by blocking the shikimic acid pathway, prevented the synthesis of materials needed to produce or maintain natural defenses in bean roots and thereby allowed increased colonization of roots by *Pythium* spp and *Fusarium* spp. These results provide evidence that the phenomenon of glyphosate mediated colonization of roots by fungi may also occur in woody plants. While the results of these experiments demonstrate glyphosate-enhanced colonization by *Pythium* spp. and *Fusarium* spp. in five woody species, they do not provide evidence about whether such colonization contributed to the herbicidal efficacy of foliar-applied glyphosate, or simply allowed these fungi to exploit tissue that had been made less resistant than normal to invasion by fungi.

CHAPTER IV

Does Glyphosate Affect Colonization of Roots of Selected Conifer and Broadleaf Species by *Armillaria ostoyae* ?

4.1. Introduction

Root rot caused by *Armillaria* spp. affects many species of trees throughout the world (Kile *et al.*, 1991). Until recently, Armillaria root disease was thought to affect only severely stressed trees (Wargo and Harrington, 1991), and with the disease were associated certain site characteristics such as soil texture and moisture that are generally related to stress susceptibility (Wiensczyk *et al.*, 1997).

As more species of *Armillaria* were discovered, some species became recognized as primary pathogens, e.g. *Armillaria ostoyae* (Gregory *et al.*, 1991) and others as secondary pathogens, e.g. *Armillaria mellea* (Wargo and Harrington, 1991). In western North America, Armillaria root disease caused by *A. ostoyae* is abundant in conifers (Wargo and Shaw, 1985). In Canada, Armillaria root disease is found in all forest regions and causes significant losses in coniferous and deciduous tree stands of all ages (Hiratsuka, 1987; Myern, 1994). In BC an estimated 105,000 m^3 of wood are lost annually to Armillaria root disease (Taylor, 1986). There is little impact of Armillaria root disease in coastal trees > 25 years old, but the disease can cause serious damage in interior stands (Morrison, 1981; Wargo and Shaw, 1985). Ecosystem type and

microhabitat may affect the epidemiology of Armillaria root disease (Morrison, 1981; Byler *et al.*,1986; Shearer and Tippett, 1988).

Despite the extensive research that has been done on Armillaria root disease, it remains difficult to control. In my overall plan of study of the effect of glyphosate on fungal colonization of roots of selected forest tree species, it was considered valuable to include among the experimental models a specialized root pathogen as well as the opportunistic fungi such as *Pythium* and *Fusarium* spp. Thus, the aim of this study was to determine whether glyphosate might alter root colonization of selected conifer and broadleaf species by *A. ostoyae.*

4.2. Materials and Methods

4.2.1 Plant Materials and Source of Inoculum

One year-old (1-0) seedlings of Douglas-fir and paper birch were provided by the B.C Ministry of Forests Green Timbers Nursery, Surrey, B. C. Seedlings were transplanted into 4 L plastic pots in April 1996 using the loamy sand potting soil described in Chapter III. Plants were kept outside and watered every 3 days throughout the summer. A glass test tube was set into each pot, 2 cm from the stem of the transplant, to provide a hole for inserting an inoculum stick of *A. ostoyae* at a later time.

Three isolates of *A. ostoyae* (two of the isolates were from interior sites and one from a Vancouver island site) were provided by Mike Cruickshank of the Pacific Forestry Centre, Canadian Forest Service, Victoria, B C. Each isolate was recultured onto malt extract broth with 1.5% agar (MEBA) via a small

pieces of colonized source colony. Inoculated plates were kept in the dark at room temperature. Ethanol was added to the autoclaved medium at 100 ppm or 500 ppm. The isolate that grew best and consistently produced rhizomorphs on MEBA was chosen for use as inoculum.

4.2.2. Inoculum Stick Preparation

Inoculum was provided as colonized alder branch segments of about 2 cm diam. x 11 cm long (Redfern, 1975). Branch segments were autoclaved in metal trays, containing 300 ml of water and 40-50 segments per tray, for 1 h. Then 300 ml of malt extract dextrose peptone broth (MDPD) (3% w:v malt extract, 2 % dextrose, 0.5% peptone in distilled water) was added to each tray before autoclaving for an additional 20 min. Twenty one-day old Armillaria cultures on MEBA in 9 cm diameter petri dishes were comminuted in 100 ml of sterile water in a sterile blender and then added aseptically to the trays containing the branch segments and MDPB at a rate of four cultures/tray. Inoculated branch segments were kept at room temperature in the dark for 3 month.

4.2.3. Inoculation and Glyphosate Treatments

In early May 1998, the glass test tubes were carefully replaced with an inoculum stick, which was then covered with loose, fine soil. Glyphosate was applied to the seedlings as foliar spray in one of three doses (0%, 0.5% and 1%

Vision ®) as described in Chapter II. Twenty five seedlings per treatment were used.

Above ground symptoms and mortality in the treated seedlings were recorded monthly. Viability of the inoculum was verified 6 month after inoculation by removing pieces of the inoculum sticks from 5 pots of each seedling species, and reculturing them on MEBA amended with 2g/l streptomycin. Isolates were subcultured to obtain axenic cultures, and these were maintained for 3-4 weeks until characteristics of *Armillaria* on MEBA were apparent.

One year after glyphosate treatment, roots of seedlings were excised, examined for evidence of infection, oven-dried and weighed. Inoculum sticks were examined for surviving inoculum and presence, type and size of rhizomorphs.

4.2.4. Analysis of Data

The data were analyzed using ANOVA to compare the root biomass between different treatments. A Bonferroni test was used to compare multiple means of biomass. In all cases .= 0.05.

4.3. Results

4.3.1.Growth of *Armillaria ostoyae* on MEBA

New white mycelia of *A. ostoyae* appeared by 7 days after inoculation on MEBA. Rhizomorphs began to form at day 15 (Figure 7), followed by the disappearance of white mycelia and appearance of brown crustose material.

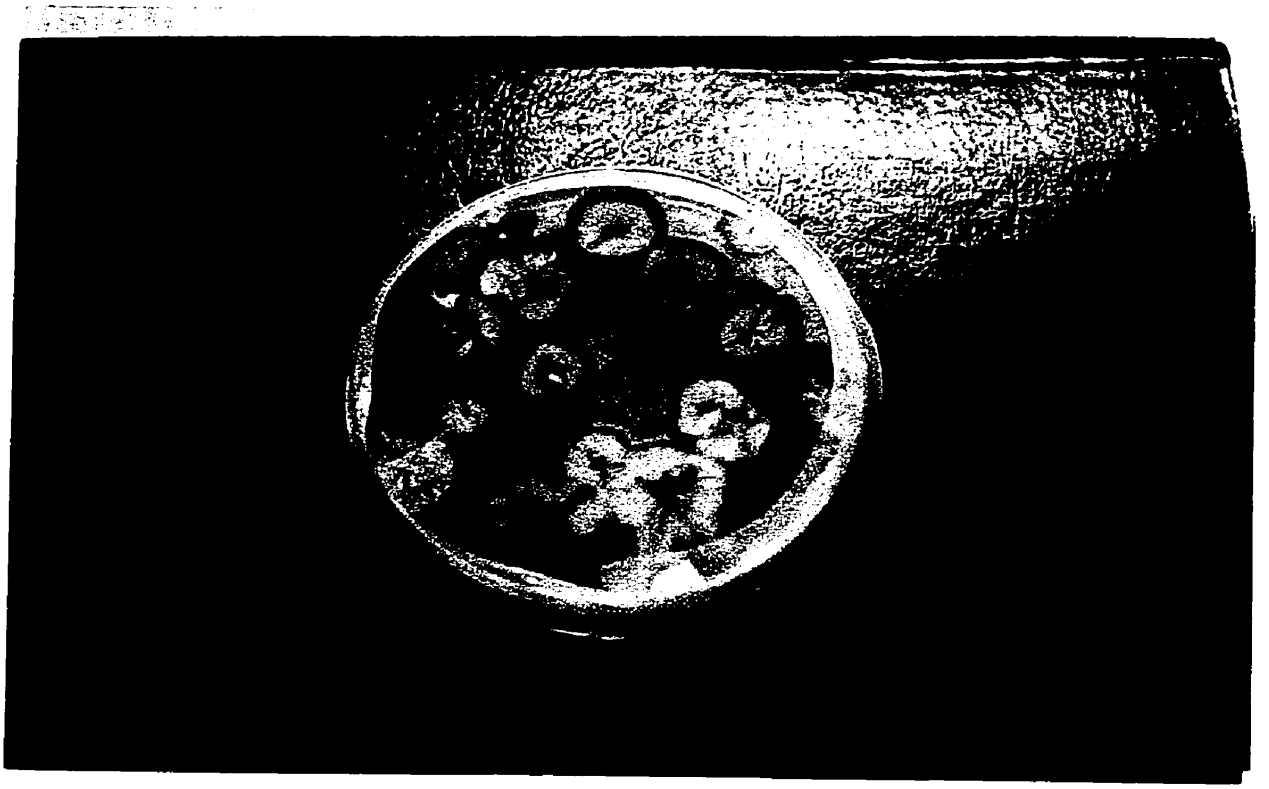


Figure 7. Rhizomorphs of *Armillaria ostoyae* on MEBA + 500 ppm ethanol at day 15

Rhizomorphs grew faster and were more abundant on media with 500 ppm then 100 ppm of ethanol or no ethanol. Thick white mycelia, a brown crustose appearance and long dichotomous rhizomorphs were present by 2 months of inoculation (Figure 8). After 3 month , inoculum sticks were covered by white mycelia, and some rhizomorphs were observed on the surface of the sticks (Figure 9).

4.3.2. Survival of *Armillaria ostoyae* on inoculum sticks in soil

Six month after placement in the soil, rhizomorphs were present on three of five inoculum sticks from pots containing paper paper birch, and two of five pots containing Douglas-fir. One year after glyphosate treatment, viable *A. ostoyae* confirmed by re-isolation on MEBA, was present on only 60% of inoculum sticks in pots with Douglas-fir and 53% of pots with paper paper birch (Table 8).

4.3.3. Growth of rhizomorphs, infection sites and weights of roots

Dichotomously branching rhizomorphs of *A. ostoyae* were found under the bark of inoculum sticks (Figure 10), but much less frequently than in MEBA. Most did not extend far enough from the inoculum sticks to come into contact with roots.

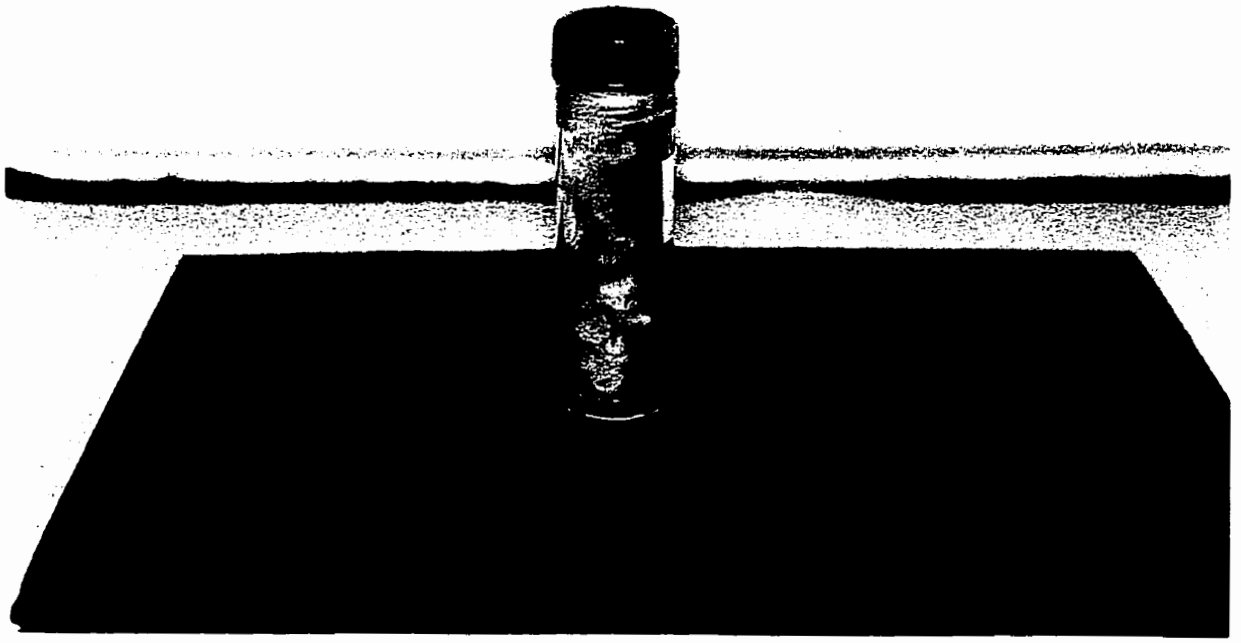


Figure 8. Growth of rhizomorph in MEBA containing 500 ppm ethanol, 3 months after inoculation

Figure 9. Inoculum sticks covered by white mycelia, and some rhizomorphs of *Armillaria ostoyae*

Table 8. Percent of inoculum sticks with viable *Armillaria ostoyae* one year after placement in soil

Species of seedlings	Percent of inoculum sticks (N=25) with *A. ostoyae*		
	0% Vision®	0.5% Vision®	1% Vision®
Douglas-fir	68	52	60
Paper birch	64	56	40

of contact sites between glyphosate-treated and untreated seedlings (Table 9). No mycelia were observed on these infection sites. Significant dose-related reductions of root biomass were associated with glyphosate treatment of paper birch and Douglas-fir seedlings (Figures 11, 12). In Douglas-fir, there was no further reduction in root biomass in the six seedlings showing visible sign of infection (Figure 11).

Figure 10. Brown dichotomous rhizomorph of *Armillaria ostoyae* on inoculum stick from soil

Table 9. Number of *Armillaria ostoyae* rhizomorphs-root contacts, and number of contacts with associated visible evidence of infection

Species of seedlings	Number of root contact Treatment with glyphosate (as % Vision®)			Number of root contacts with visible sign of infection Treatment with glyphosate (as % Vision®)		
	0%	0.5%	1%	0%	0.5%	1%
Douglas-fir	8	9	7	4	3	3
Paper birch	6	8	5	0	0	0

Figure 11. Root biomass of Douglas-fir seedlings 1 year after treatment with glyphosate . Treatments and number of replicates as follows:

TRT1, inoculated, 0% glyphosate, N= 4
TRT2, inoculated, 0.5% glyphosate, N= 3
TRT3, inoculated, 1% glyphosate, N= 3

Bars with same letter are not significantly different, $p < 0.05$

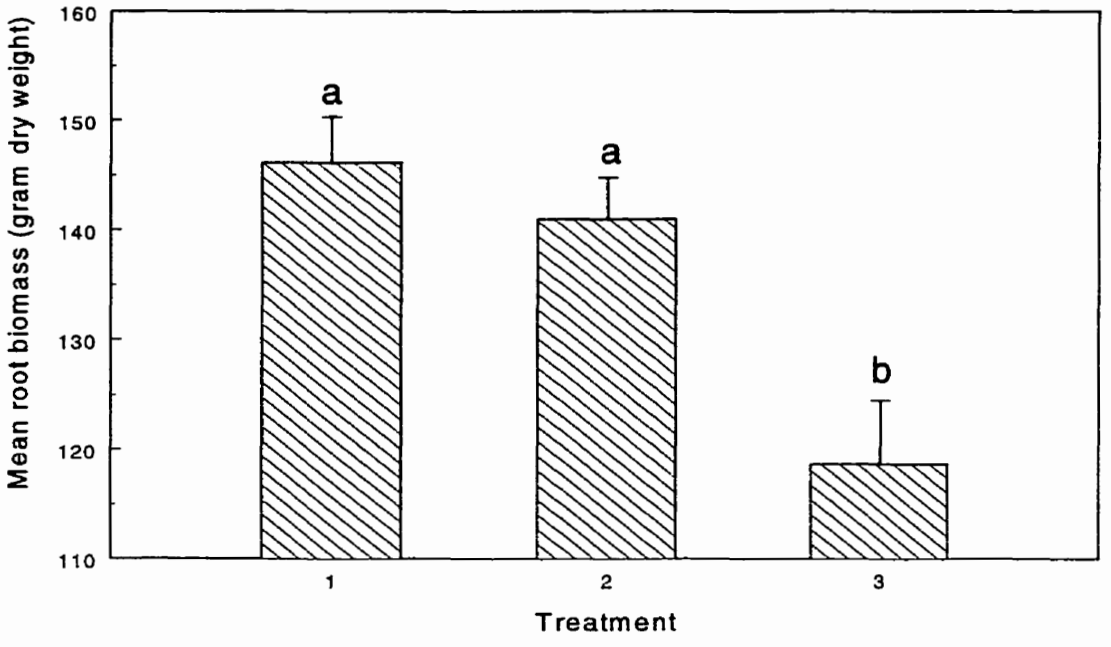
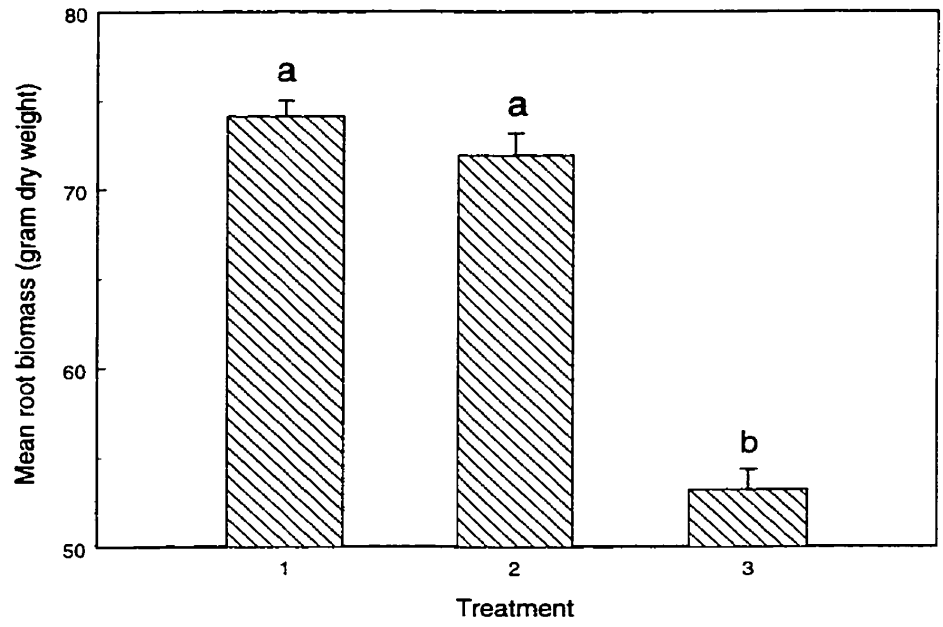


Figure 12. Root biomass of paper birch seedlings 1 year after treatment with glyphosate . Treatments (N=10) as follows:

TRT1, inoculated, 0% glyphosate
TRT2, inoculated, 0.5% glyphosate
TRT3, inoculated, 1% glyphosate

Bars with same letter are not significantly different, $p < 0.05$



4.4. Discussion

The stimulation of rhizomorph growth caused by addition of 500 ppm ethanol to MEBA is similar to that reported by Weinhold (1963) and Allerman and Sotkjaer (1973) for ethanol and other low molecular weight alcohols. Despite the ability of the chosen isolate to produce rhizomorphs on MEBA, a low frequency of infection sites was found on roots of Douglas-fir inoculated with alder sticks colonized by the isolate. Most rhizomorphs did not grow far enough from the inoculum to contact the roots.

There are several possible explanations for why very few infection sites were observed on Douglas-fir and none on paper birch. First, invasion of soil by rhizomorphs appeared to be infrequent, and there was a low viability of

A. ostoyae on the inoculum sticks by the end of study. Soil conditions could have been antagonistic to, or did not support growth of rhizomorphs in the pots. The pots were placed outside, so it is possible that during the study pots experienced excessive water content due to rainfall, which might have suppressed rhizomorph growth and infection of roots (Ono, 1970). Alternatively, heat stress during the summer could also have inhibited rhizomorph growth by drying the film of water over the rhizomorph tips that is required for rhizomorph growth (Smith and Griffin, 1971). Below critical soil moisture level the tips become melanized and

growth ceases. Other possibilities are that the small inoculum sticks had too low an inoculum potential to infect 2-year old seedlings (Cruickshank *et al.*, 1997) or that the nutrient availability in the soil was too high (Singh, 1983).

Although this study demonstrated a dose-dependent effect of non-lethal treatments with glyphosate on root biomass in Douglas-fir and paper birch, the results do not support any conclusion regarding the effect of glyphosate on root colonization by *A. ostoyae*.

CHAPTER V

Effects of Glyphosate on Mortality and Subsequent Growth of Surviving Seedlings of Selected Conifer and Broadleaf Species Growing in a Field Nursery

5.1. Introduction

Glyphosate is a competitive inhibitor of 5-enolpyruvyl shikimate-3-phosphate (EPSP) synthase (Amrhein *et al.,* 1982). In plants, the biosynthesis of all aromatic compounds involved in primary metabolism proceeds by way of the shikimate pathway (Amrhein *et al.*, 1982). The aromatic end products of this pathway are essential for protein synthesis and are precursors of numerous secondary products, such as lignin and tannins. Various studies have shown that glyphosate affects diverse physiological processes: protein synthesis (Cole *et al.,* 1983), chlorophyll synthesis (Hollander and Amrhein, 1980; Lee, 1981), auxin production (Lee, 1982) and accumulation of ammonia (Cole *et al.,* 1980). These physiological effects have been interpreted as secondary effects of glyphosate.

When glyphosate is applied to vegetation, it kills weeds and other plants it contacts. The susceptibility of plants to glyphosate will depend on the species and age of plant. Flowering species seem less susceptible to glyphosate at flowering than either before or after this stage (Lund-Hoie, 1975). The tolerance of conifer species is generally higher during the period when top shoot growth has ceased and new shoots are mature, rather than during active shoot elongation (Lund-Hoie, 1975).

Glyphosate is readily translocated throughout plants. Thus not only aerial parts of plants are affected, but also stem and root tissues (Chase and Appleby, 1979). Few histological studies on the effect of glyphosate have been published (Cole, 1985; Vaughn and Duke, 1986; Canal *et al.*, 1990). Glyphosate has been found to inhibit growth of seedlings (Ali and Fletcher, 1978). Several studies have been documented the effect of glyphosate drift onto crop species. The degree of herbicide injury to a crop is dependent on species, stage of growth and the concentration of herbicide that contacts the plants (Jordan, 1977; Kelley *et al.*, 1984).

Although many studies on the effect of glyphosate on herbaceous species have been published, relatively little is yet known about the impact of glyphosate on woody species. The present study was conducted to investigate the consequences of exposure of seedlings of selected woody species to glyphosate, as might occur from the use of glyphosate on broadleaf species for conifer release, and from accidental drift or unavoidable contact associated with the use of glyphosate in silvicultural operations.

5.2. Materials and Methods

Five species were used in some or all of the experiments. Douglas-fir lodgepole pine, interior spruce, paper birch and red alder were used for assessment of mortality in response to different doses of glyphosate. Only the three conifer species were assessed for histological effects, sub lethal dose effects on growth post treatment, and root and shoot biomass following exposure

to sublethal doses of glyphosate. Glyphosate treatments consisted of sprays of aqueous dilutions of the commercial herbicide product Vision ®, which contains glyphosate as the isopropylamine salt at 356 g/L. Treatments were applied with a backpack sprayer to provide thorough coverage of the foliage with a uniform deposit of fine droplets but not to the point of runoff. Treatments were applied to individual trees on September 17, 1996, May 16, 1997 and September 24, 1997. Individual trees were sprayed only once. Treatments consisted 1%, 0.33%, 0.11% and 0% (control) Vision. Trees receiving each treatment were selected randomly, which resulted in a completely randomized design for the experiments. There were 20 replicate trees of each species for each treatment and time of exposure, except 15 replicates for red alder. Red alder seedlings were treated only in September 1996 and May 1997, because the seedlings already too big to be sprayed by September 1997.

Mortality data were collected in the second and third month following the treatments, and confirmed in April of the following year. Qualitative assessments of growth form (leader forking, curvature) in surviving seedlings of Douglas-fir and lodgepole pine were made in April 1998 and 1999. Different assessment were done on spruce (length and number of branches). From the beginning of the study until the second year following glyphosate treatment many of the spruce seedlings had more than one leader, making it more difficult to monitor the growth of leader and to assess the growth form than in Douglas-fir and lodgepole pine. Tree heights and diameters at 10 cm above the root collar were measured monthly using a long stick ruler and calipers for all surviving conifer

seedlings. At the termination of the experiment, fall 1998-spring 1999, three or four randomly selected trees from each treatment were dug up for assessment of root and shoot biomass. These were taken to the lab, separated into roots, needles and stems, dried at 80° C to constant weight (20-24 h), and weighed to the nearest 0.1 g.

Three to four replicate stem segments 10 cm long were excised at 7, 13 and 20 days post treatment from 5-10 cm below the tips of leaders of lodgepole pine, spruce and Douglas-fir seedlings that were treated on September 21, 1997 The excised segments were divided into 3 to 4 small pieces, fixed in 2M glutaraldehyde phosphate buffer at pH 7 (Sabatini *et al.*, 1963; Ledbetter and Gunning, 1964). After 4-7 days, the segments were transferred to 30% ethanol for 1 week, then to 50% ethanol for 1 week, and lastly to 70% ethanol.

A sledge microtome was used to produce sections approximate 15 µthick. The sections were stained in various ways for the detection of plant biochemicals as follows:

1) lignin: sections placed in 1ml of 1% (w/v) phloroglucinol in 70% ethanol for 3-6 min, and then transferred to 11 N NHCL for 2-5 min; positive reaction: light-violet color (Gatenby and Beams, 1950);
2) lipid: sections flooded with Sudan IV made from several drops of saturated Sudan IV in 70% ethanol for 15 min, and then rinsed quickly with 50% ethanol; positive reaction: light orange pink color (Johansen, 1940);
3) starch: sections placed in IKI solution (0.2 g I_2 + 2 g KI in 100 ml H_2O) for 3-6 min; positive reaction: dark blue-black color (Johansen, 1940); and

4) tannins: sections placed in 1% ferric sulfate for up to 2 h: positive reaction : blue-green color (Johansen, 1940).

Stained sections were mounted on glass microscope slides in glycerin jelly and examined under a stereo microscope Meiji, EMZ series and a Zeiss light microscope at 10 x 10 magnification.

5.2.1. Analysis of Data

Data for mortality and effect of dose on growth form (number of leaders, length and number of branches) analyzed using GLM. The Bonferroni test was used to compare multiple means for biomass and growth. In all cases $\alpha < 0.05$.

5.3. Results

5.3.1 Effect of Glyphosate Treatment on Seedling Mortality

Higher mortality was found among broadleaf seedlings than conifer seedlings (Figure 13). The highest dose of glyphosate used (1% Vision) killed 100% of paper birch seedlings. Among the conifer species, Douglas-fir and lodgepole pine exhibited similar levels of sensitivity to glyphosate, while spruce was the least sensitive. Mortality of all species was highest after a spring application (May 1997) of glyphosate. There was a highly significant effect of both treatment and glyphosate dose on mortality of paper birch seedlings

Figure 13. Seedling mortality as of April 1999 for application of glyphosate (from Vision® applied as foliar spray) made 17 September, 1996

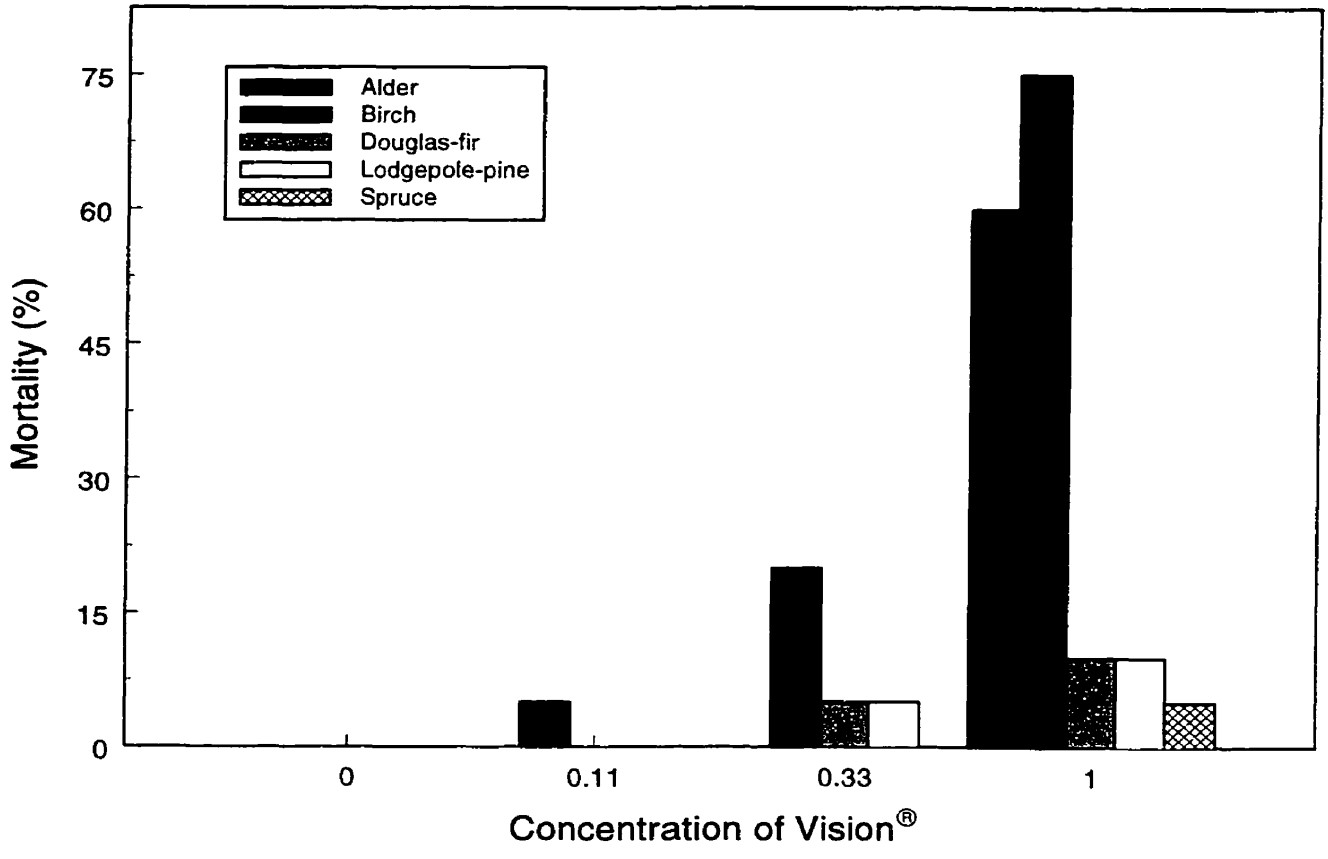


Figure 14. Seedling mortality as of April 1999 for application of glyphosate (from Vision® applied as foliar spray) made 16 May, 1997

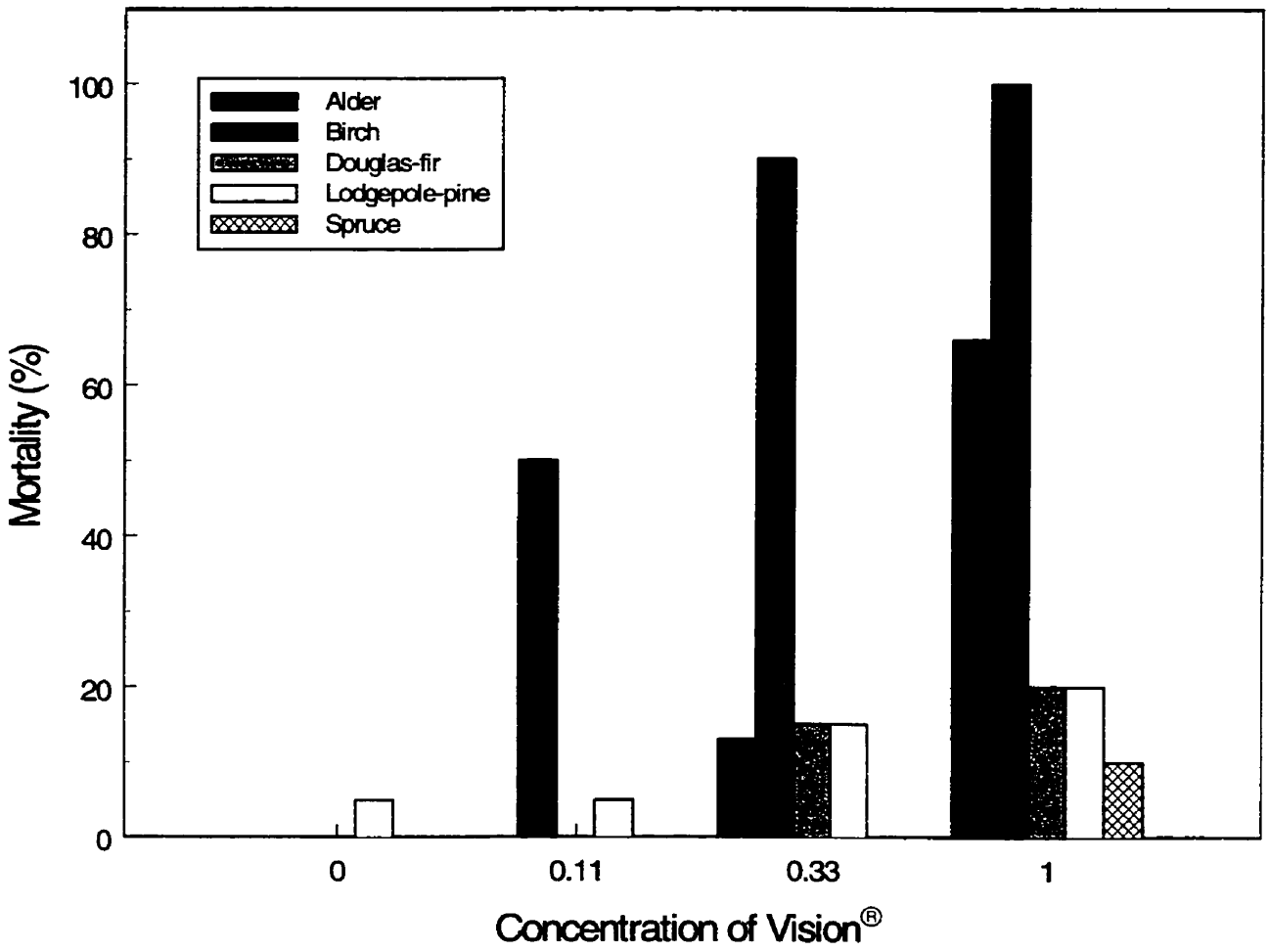
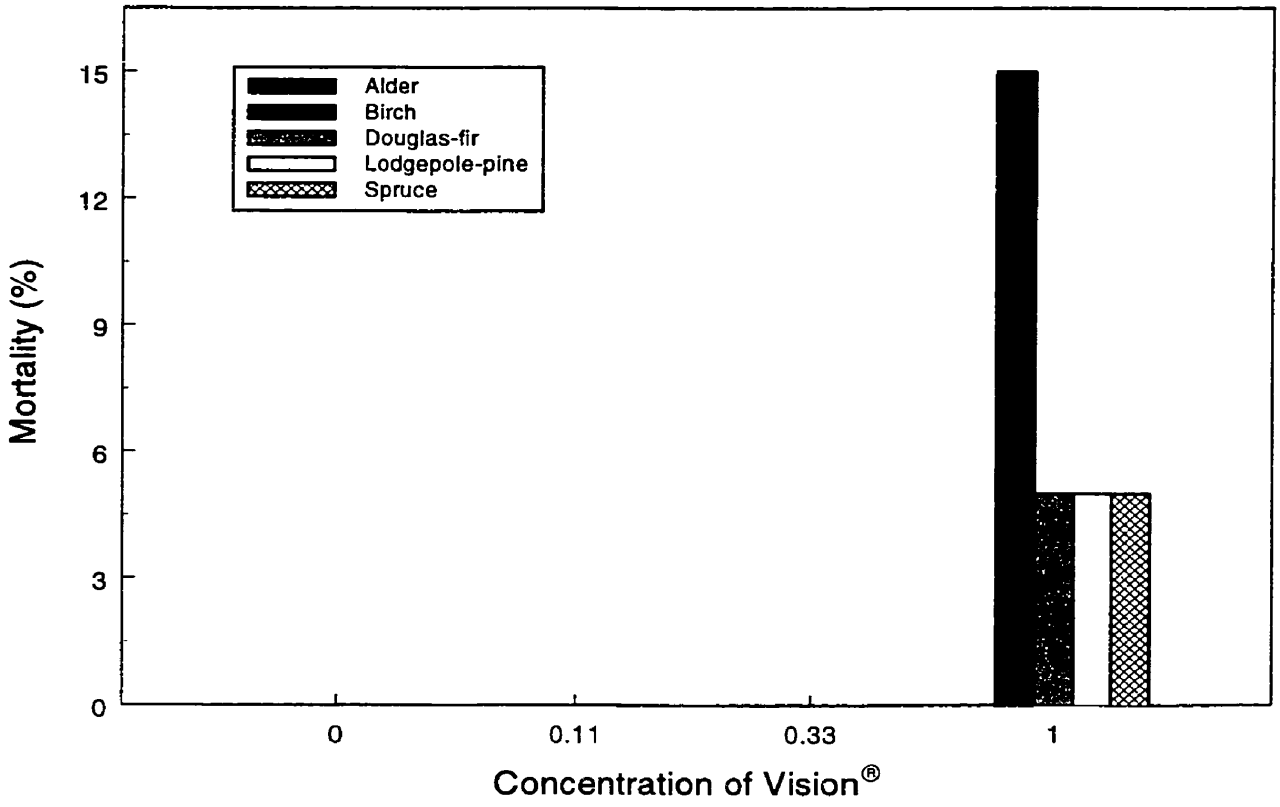


Figure 15. Seedling mortality as of April 1999 for application of glyphosate (from Vision® applied as foliar spray) made 24 September 1997



($p < 0.05$). Seedling mortality of red alder, Douglas-fir and lodgepole pine treated with 1% and 0.33% of glyphosate was significantly higher than mortality of untreated seedlings or those treated with 0.11% glyphosate.

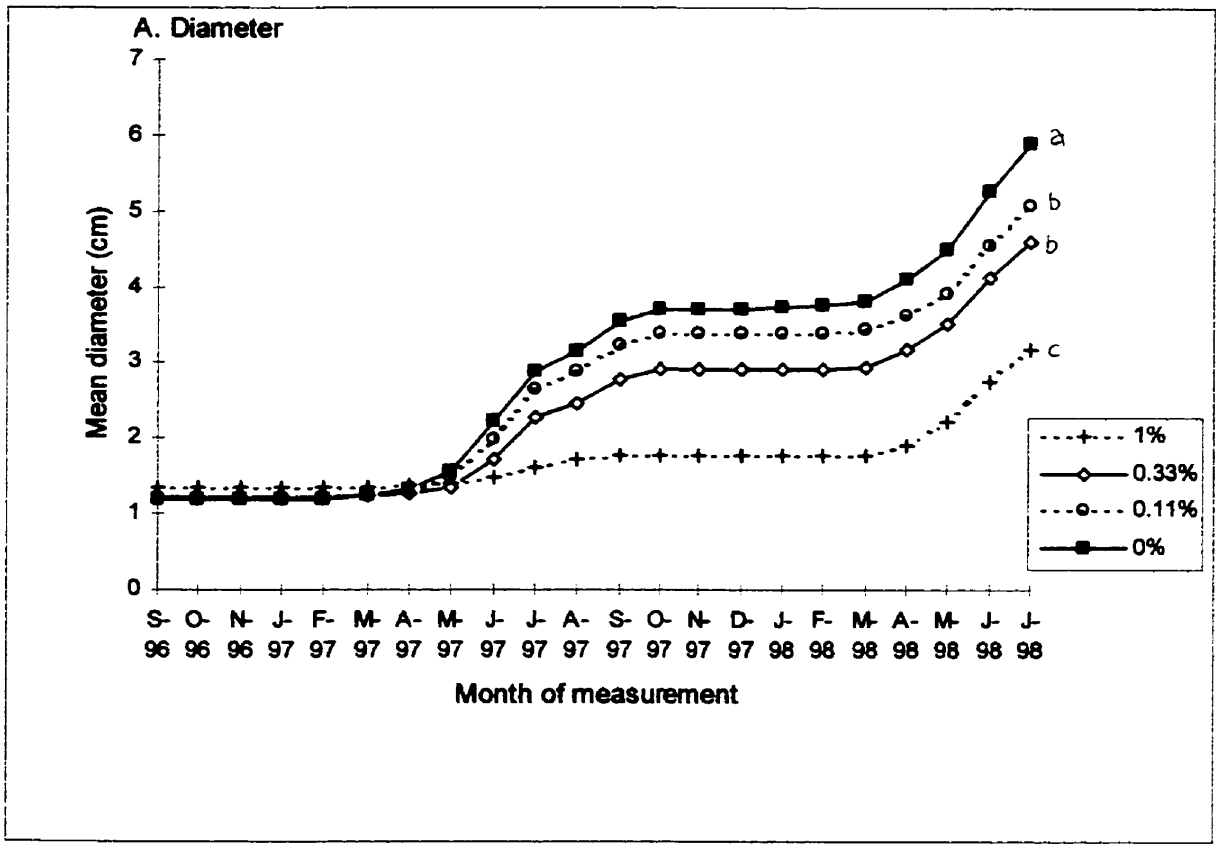
Paper birch seedlings showed less sensitivity to glyphosate in fall 1997 than in fall 1996, possibly because they were one year older and more resistant.

5.3.2. Effect of Glyphosate Application on Diameter and Height Growth

Application of glyphosate to both broadleaf and conifer seedlings caused suppression of both diameter and height growth in a general dose-dependent manner (Figures 16-30). The effect was more pronounced and significant in the two hardwood species than in the three conifers. In fact, severe mortality of paper birch (Figure 13) made it impossible to track the growth of trees treated with 1.00% Vision in September 1996 and those treated with either 1.00 or 0.33% Vision in May 1997.

The growth of trees treated in September 1996 was monitored for two successive growing seasons (Figures 16, 18, 21, 23, 27). Almost invariably, sublethal exposure to glyphosate in the fall slowed the growth rate in the following spring in a dose-dependent manner. One year later, most treated trees grew at almost the same rate as untreated control trees. However, they did not compensate for lost growth in the previous year, accounting for statistically significant differences in both height and diameter by the end of the experiment.

Figure 16. Mean diameter (A) and height (B) of red alder seedlings treated with glyphosate on 17 September 1996, 7 months after planting in the field. Measurements within either A or B followed by the same letter are not significantly different, Bonferroni test, $P < 0.05$


A. Diameter
Mean diameter (cm)
0
1
2
3
4
5
6
7
a
b
b
c
1%
0.33%
0.11%
0%
S-96 O-96 N-96 J-97 F-97 M-97 A-97 M-97 J-97 J-97 A-97 S-97 O-97 N-97 D-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98
Month of measurement

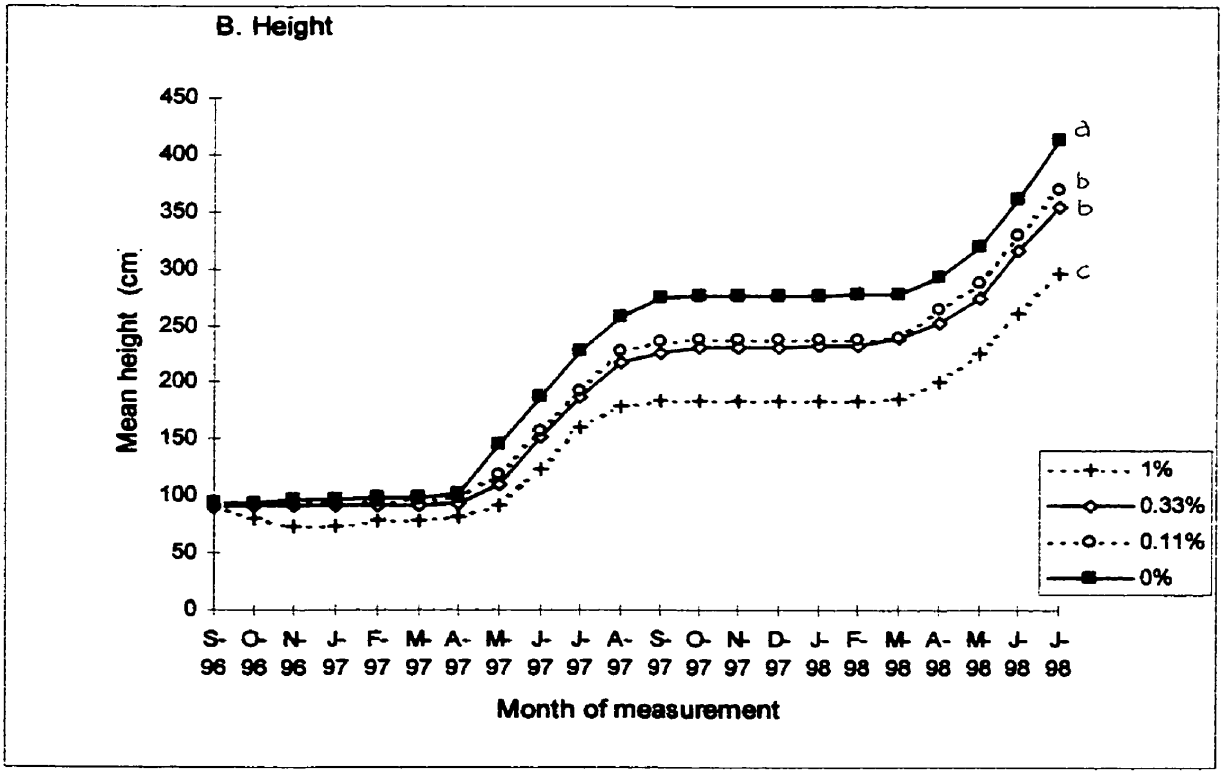
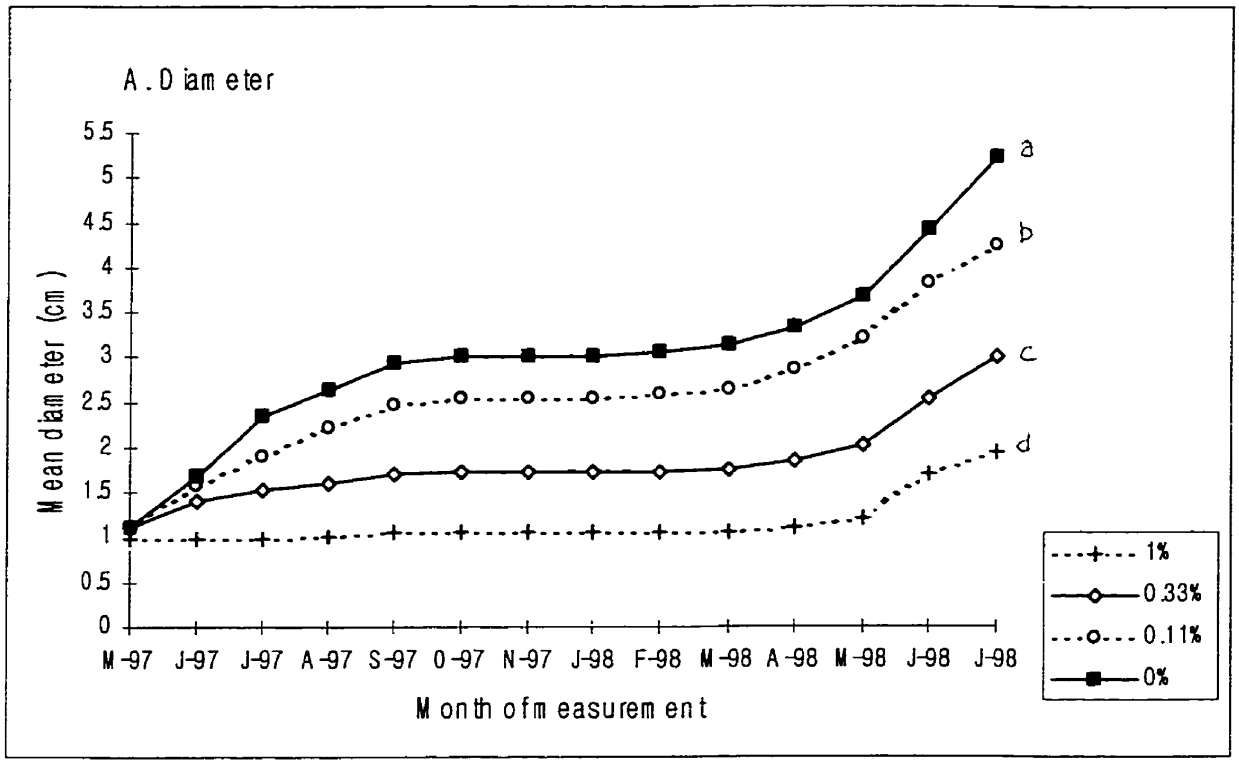

B. Height
Mean height (cm)
0
50
100
150
200
250
300
350
400
450
a
b
b
c
1%
0.33%
0.11%
0%
S-96 O-96 N-96 J-97 F-97 M-97 A-97 M-97 J-97 J-97 A-97 S-97 O-97 N-97 D-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98
Month of measurement

Figure 17. Mean diameter (A) and height (B) of red alder seedlings treated with glyphosate on 16 May 1997, 15 months after planting in the field. Measurements within either A or B followed by the same letter are not significantly different, Bonferroni test, $P<0.05$


A. Diameter
Mean diameter (cm)
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
M-97 J-97 J-97 A-97 S-97 O-97 N-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98
Month of measurement
a
b
c
d
1%
0.33%
0.11%
0%

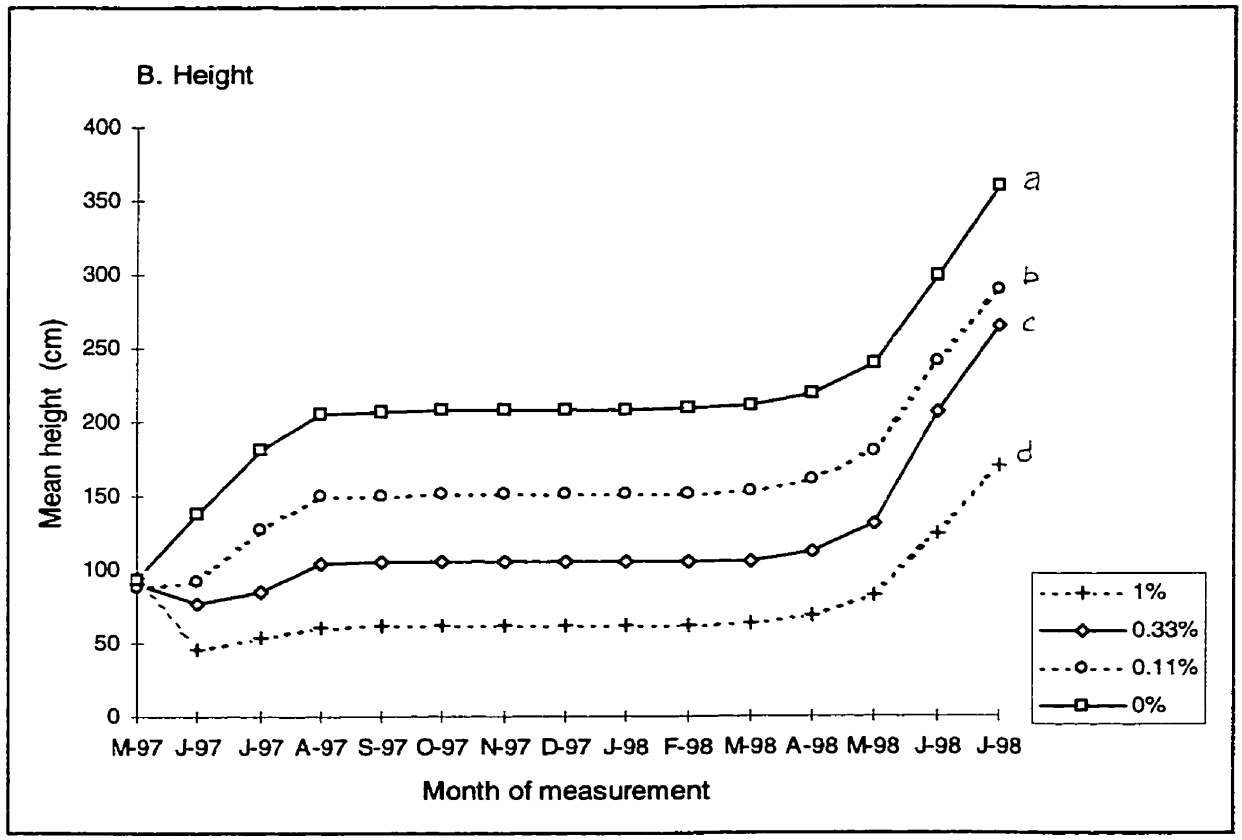
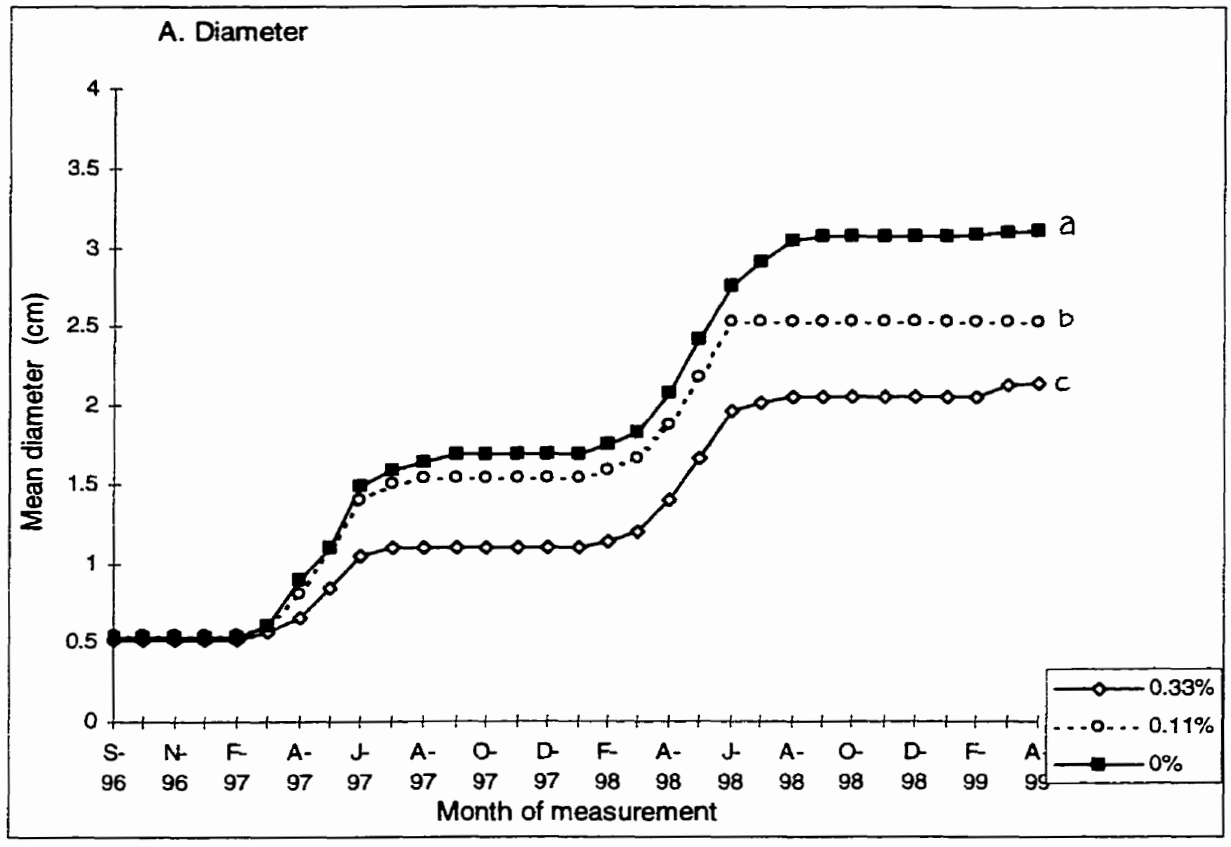

B. Height
Mean height (cm)
400
350
300
250
200
150
100
50
0
M-97 J-97 J-97 A-97 S-97 O-97 N-97 D-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98
Month of measurement
a
b
c
d
1%
0.33%
0.11%
0%

Figure 18. Mean diameter (A) and height (B) of paper birch seedlings treated with glyphosate on 17 September 1996, 7 months after planting in the field. Measurements within either A or B followed by the same letter are not significantly different, Bonferroni test, $P < 0.05$


A. Diameter
Mean diameter (cm)
4
3.5
3
2.5
2
1.5
1
0.5
0
a
b
c
0.33%
0.11%
0%
S-96
N-96
F-97
A-97
J-97
A-97
O-97
D-97
F-98
A-98
J-98
A-98
O-98
D-98
F-99
A-99
Month of measurement

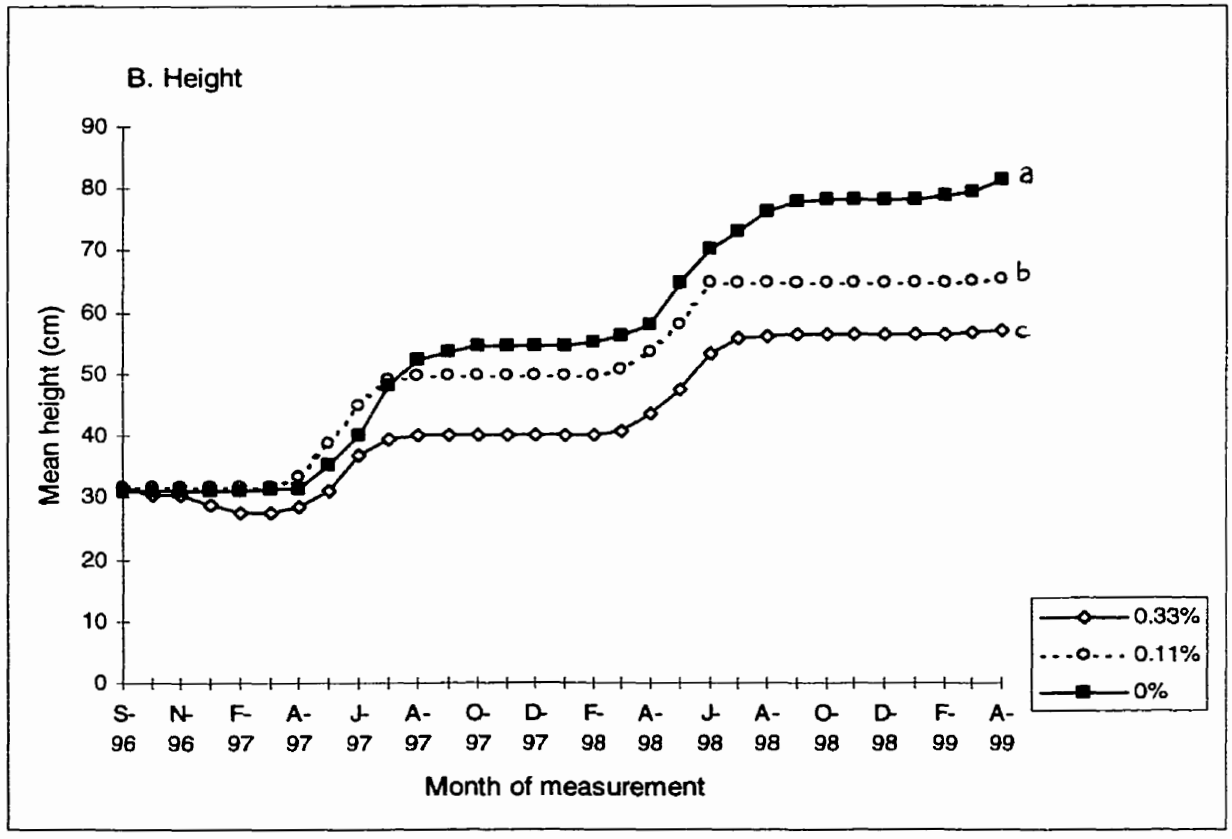
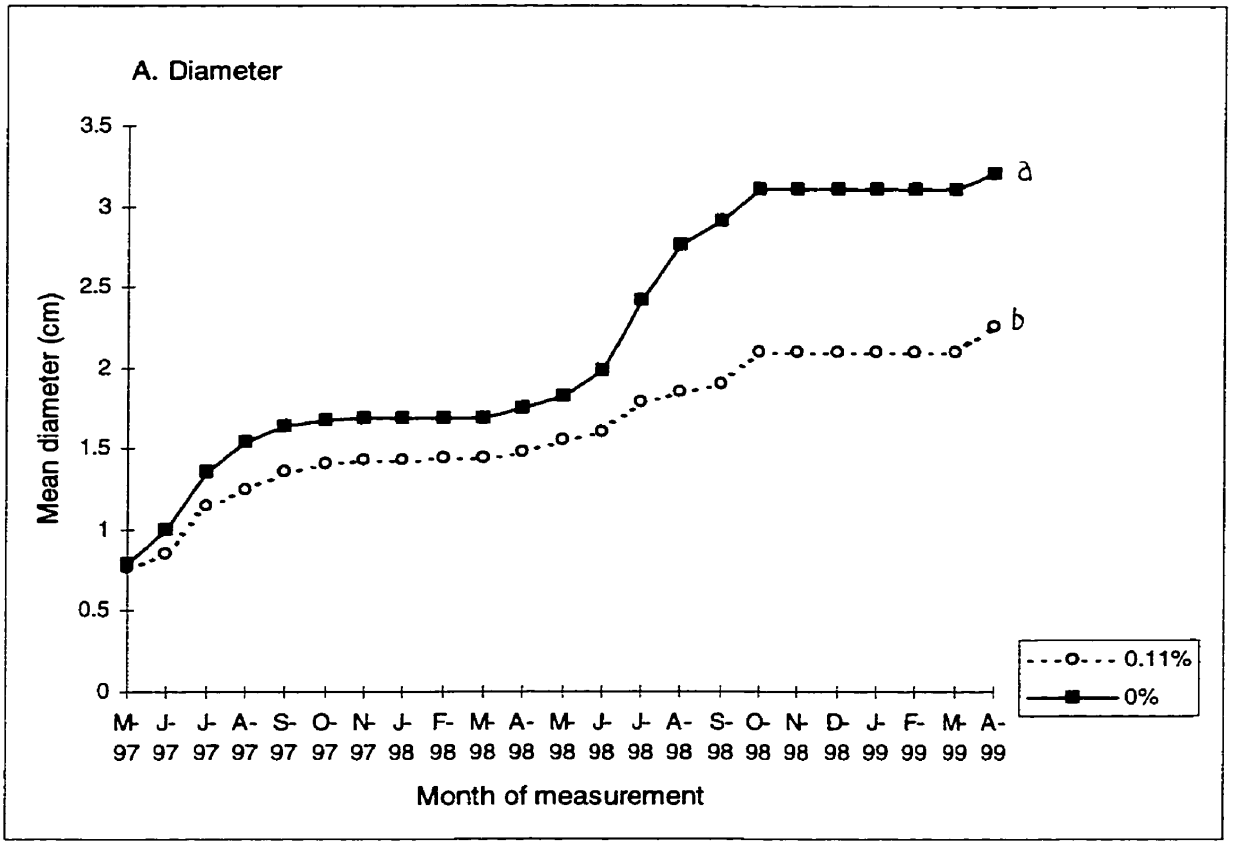

B. Height
Mean height (cm)
90
80
70
60
50
40
30
20
10
0
a
b
c
0.33%
0.11%
0%
S-96
N-96
F-97
A-97
J-97
A-97
O-97
D-97
F-98
A-98
J-98
A-98
O-98
D-98
F-99
A-99
Month of measurement

Figure 19. Mean diameter (A) and height (B) of paper birch seedlings treated with glyphosate on 16 May 1997, 15 months after planting in the field. Measurements within either A or B followed by the same letter are not significantly different, Bonferroni test, $P<0.05$


A. Diameter
Mean diameter (cm)
3.5
3
2.5
2
1.5
1
0.5
0
a
b
0.11%
0%
M-97 J-97 J-97 A-97 S-97 O-97 N-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98 A-98 S-98 O-98 N-98 D-98 J-99 F-99 M-99 A-99
Month of measurement

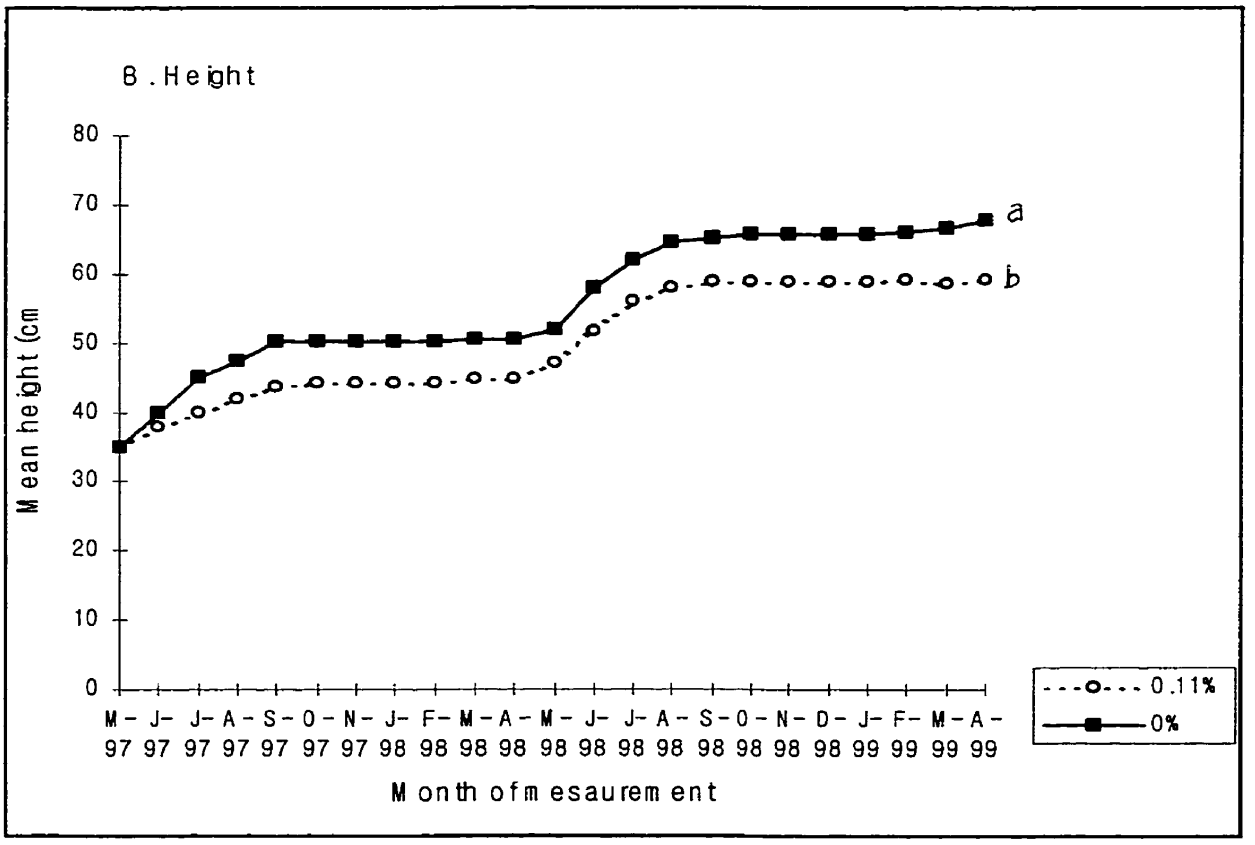
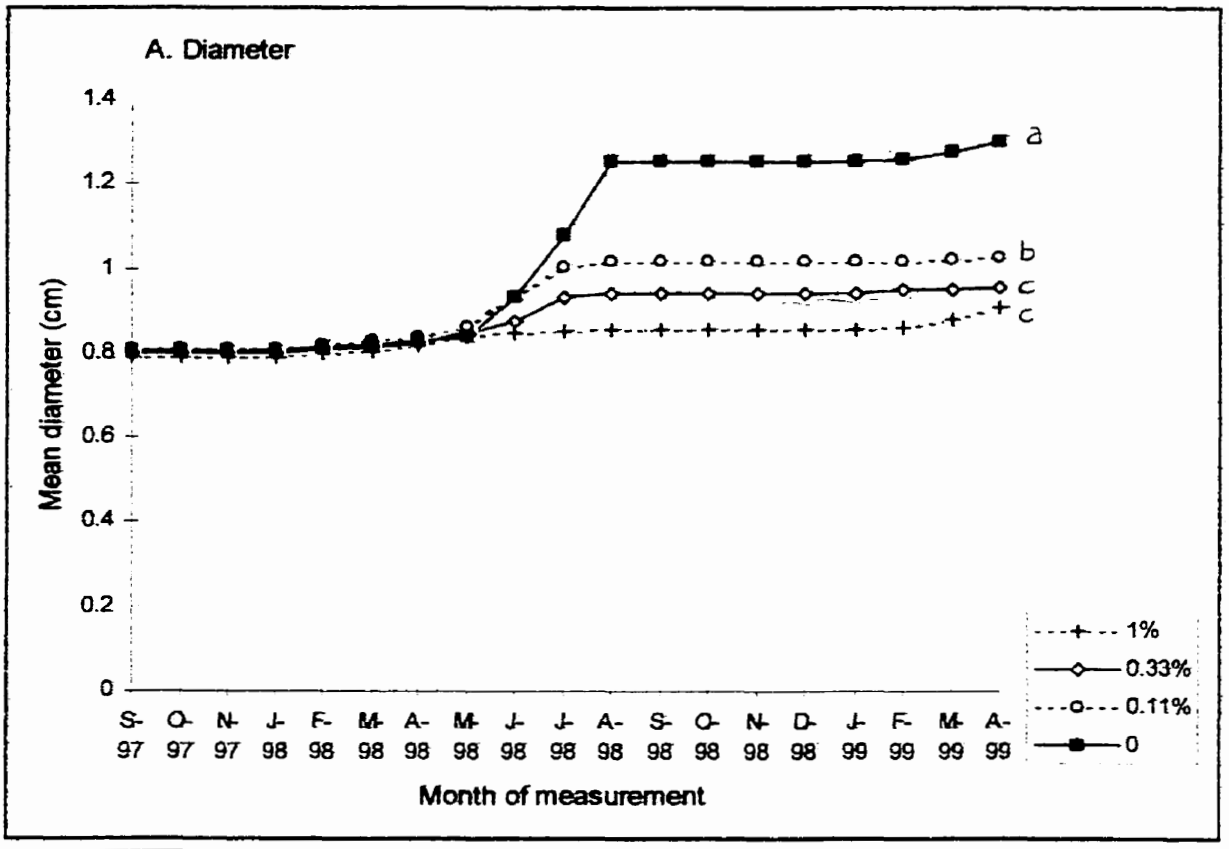

B . Height
Mean height (cm
80
70
60
50
40
30
20
10
0
a
b
0.11%
0%
M-97 J-97 J-97 A-97 S-97 O-97 N-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98 A-98 S-98 O-98 N-98 D-98 J-99 F-99 M-99 A-99
Month of measurement

Figure 20. Mean diameter (A) and height (B) of paper birch seedlings treated with glyphosate on 24 September 1997, 19 months after planting in the field. Measurements within either A or B followed by the same letter are not significantly different, Bonferroni test, $P<0.05$


A. Diameter
Mean diameter (cm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
b
c
c
S-97 O-97 N-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98 A-98 S-98 O-98 N-98 D-98 J-99 F-99 M-99 A-99
Month of measurement
1%
0.33%
0.11%
0

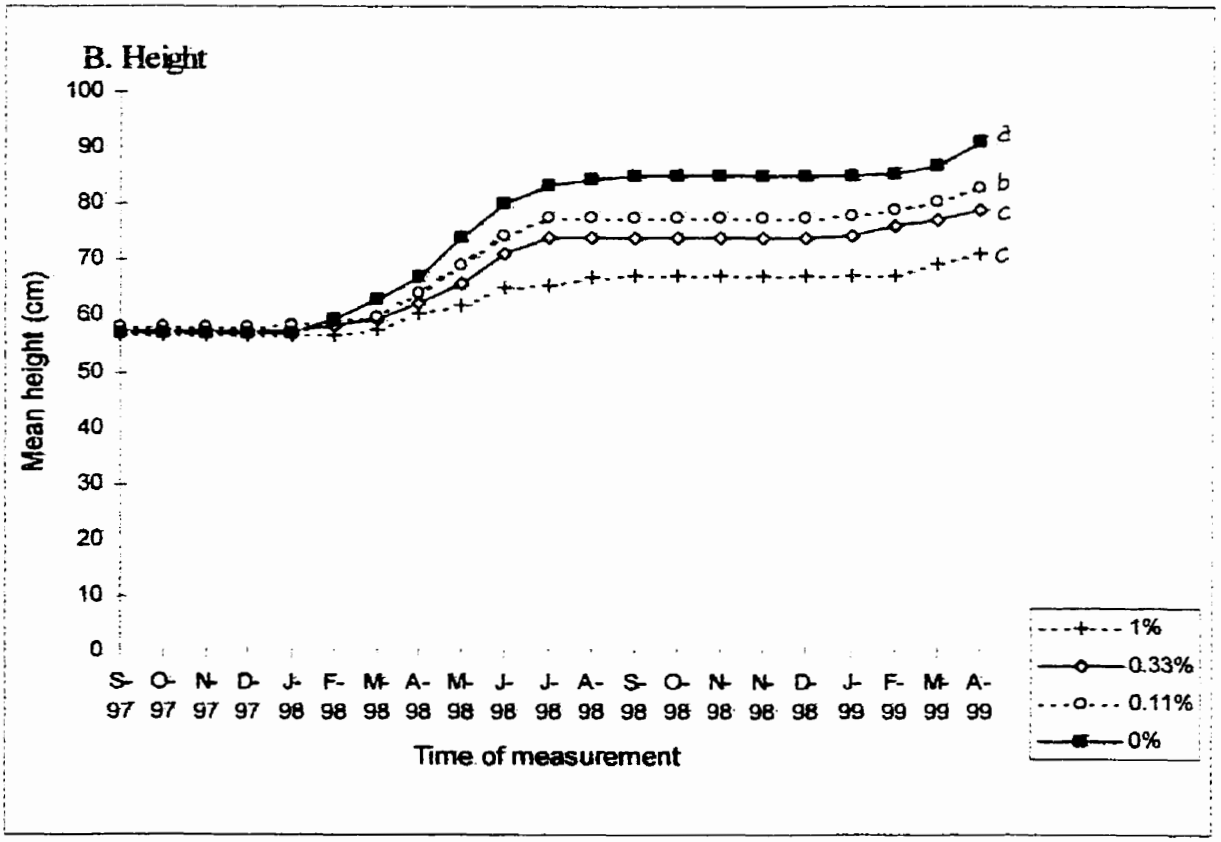
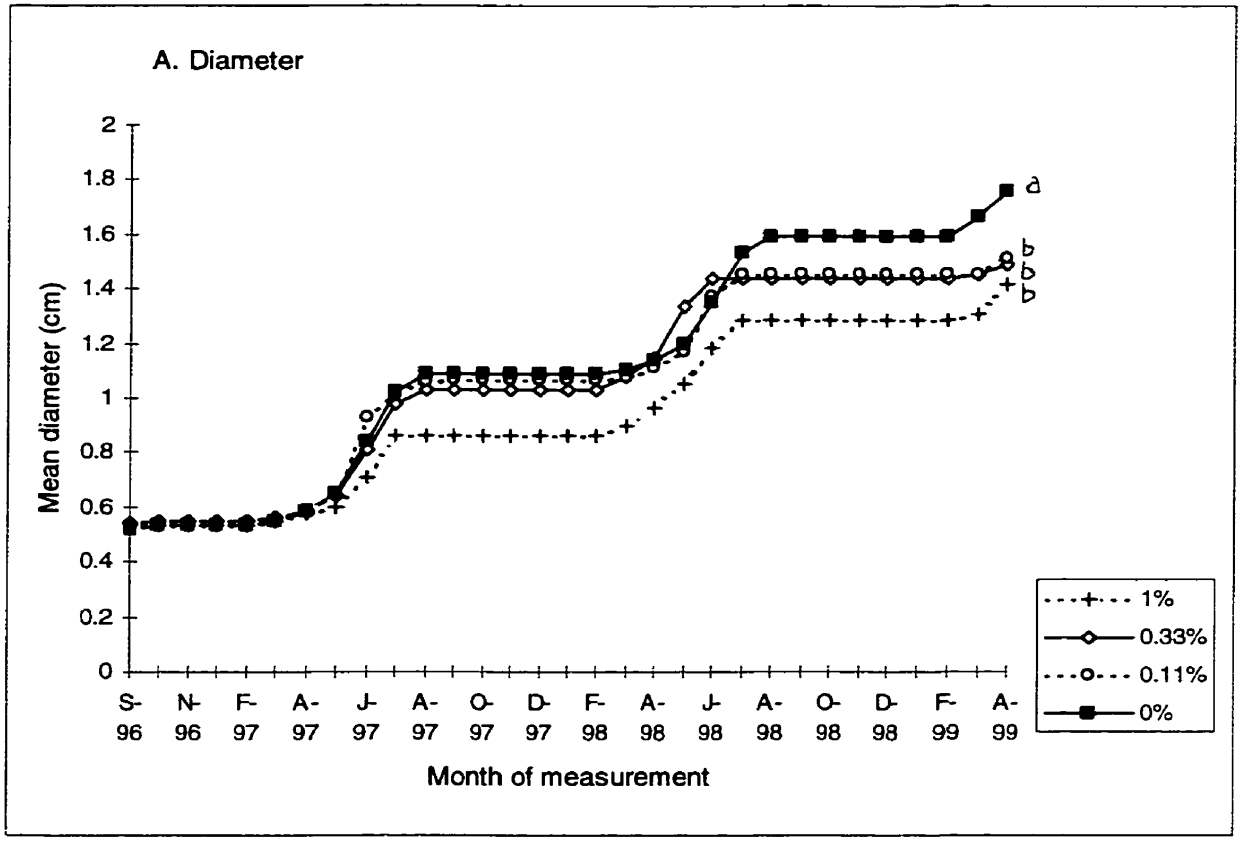

B. Height
Mean height (cm)
100
90
80
70
60
50
40
30
20
10
0
a
b
c
c
S-97 O-97 N-97 D-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98 A-98 S-98 O-98 N-98 N-98 D-98 J-99 F-99 M-99 A-99
Time of measurement
1%
0.33%
0.11%
0%

Figure 21. Mean diameter (A) and height (B) growth of Douglas-fir seedlings treated with glyphosate on 17 September 1996, 7 months after planting in the field. Measurements within either A or B followed by the same letter are not significantly different, Bonferroni test, $P < 0.05$


A. Diameter
Mean diameter (cm)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
b
b
b
S-96
N-96
F-97
A-97
J-97
A-97
O-97
D-97
F-98
A-98
J-98
A-98
O-98
D-98
F-99
A-99
Month of measurement
1%
0.33%
0.11%
0%

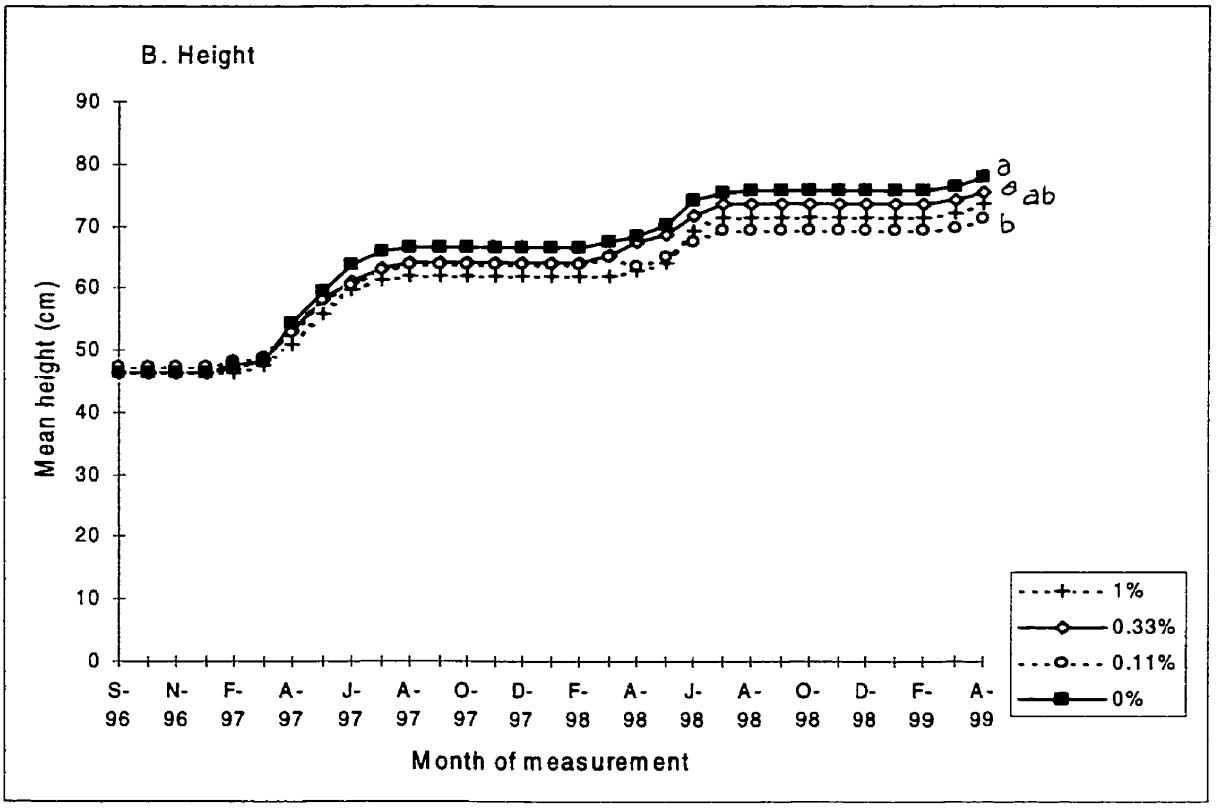
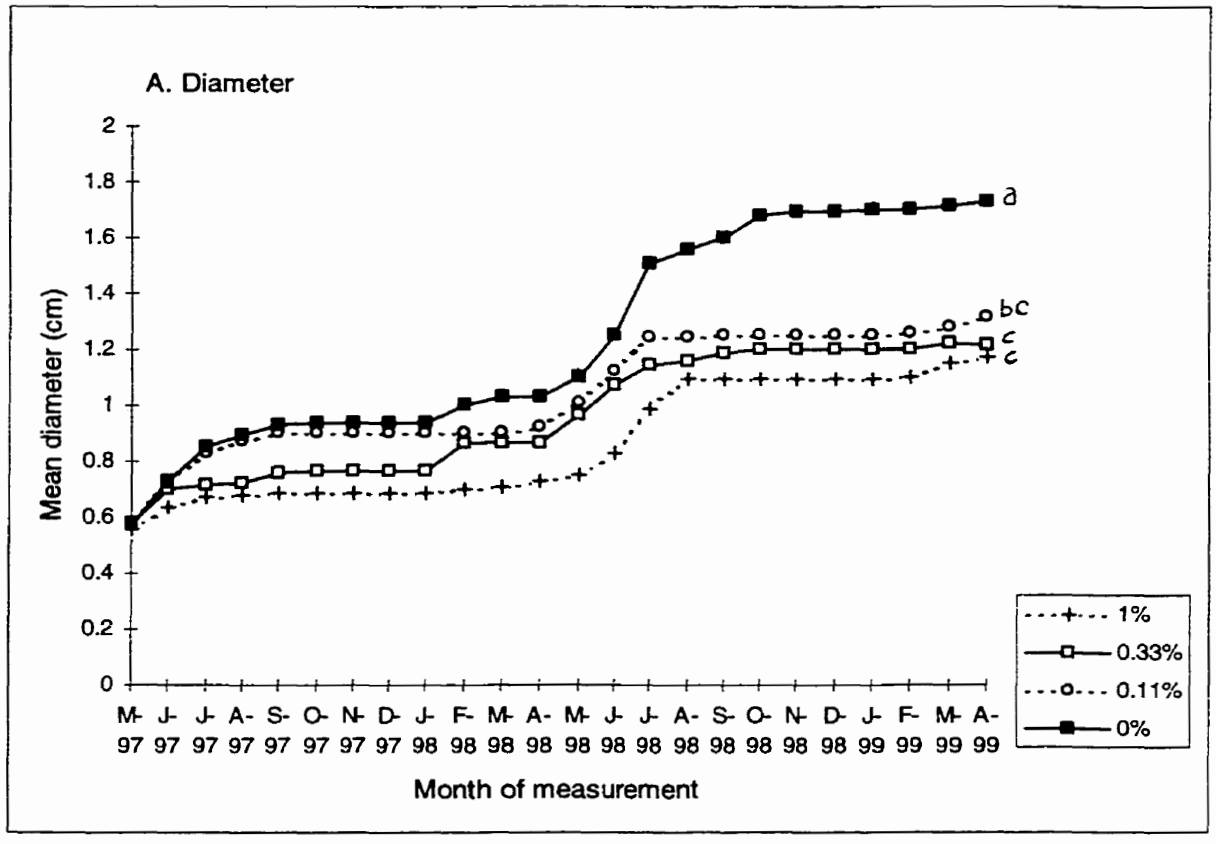

B. Height
Mean height (cm)
90
80
70
60
50
40
30
20
10
0
a
a
ab
b
S-96
N-96
F-97
A-97
J-97
A-97
O-97
D-97
F-98
A-98
J-98
A-98
O-98
D-98
F-99
A-99
Month of measurement
1%
0.33%
0.11%
0%

Figure 22. Mean diameter (A) and height (B) of Douglas-fir seedlings treated with glyphosate on 16 May 1997, 15 months after planting in the field. Measurements within either A or B followed by the same letter are not significantly different, Bonferroni test, $P<0.05$


A. Diameter
Mean diameter (cm)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
bc
c
c
M- 97
J- 97
J- 97
A- 97
S- 97
O- 97
N- 97
D- 97
J- 98
F- 98
M- 98
A- 98
M- 98
J- 98
J- 98
A- 98
S- 98
O- 98
N- 98
D- 98
J- 99
F- 99
M- 99
A- 99
Month of measurement
1%
0.33%
0.11%
0%

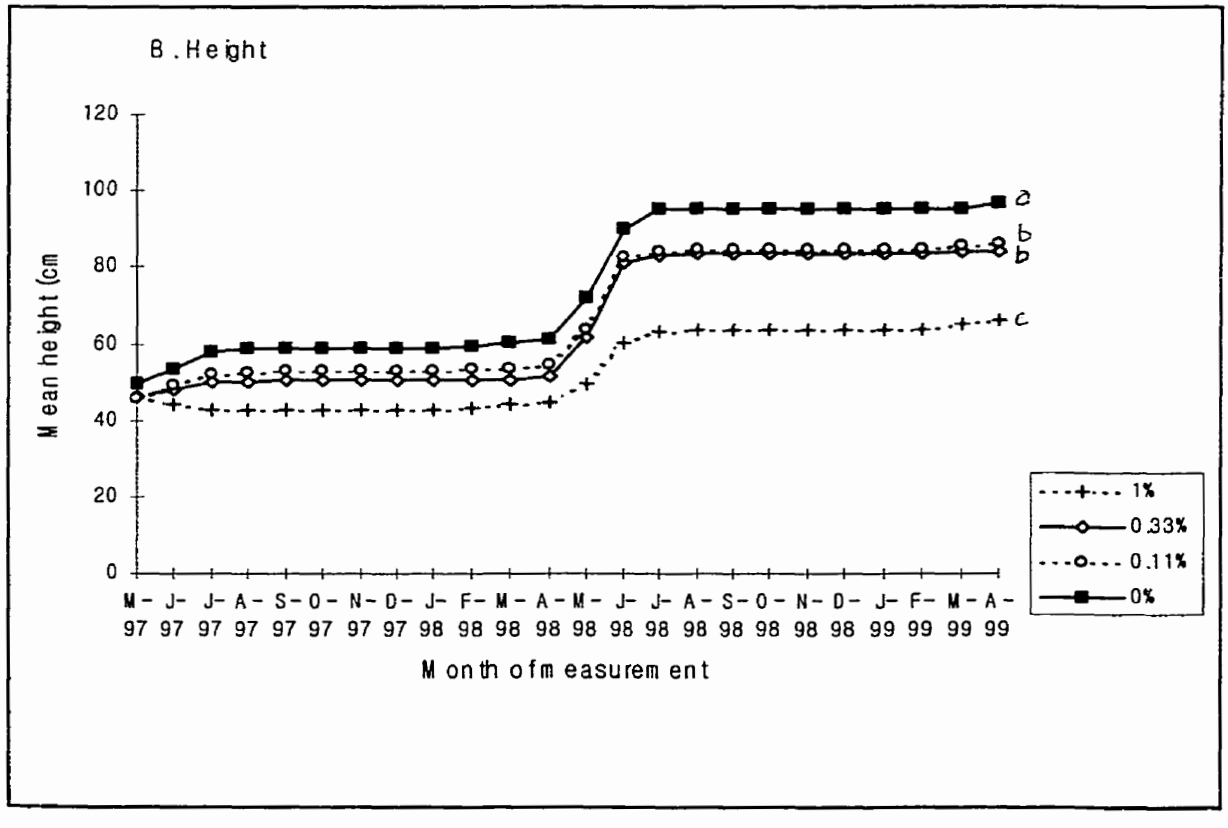
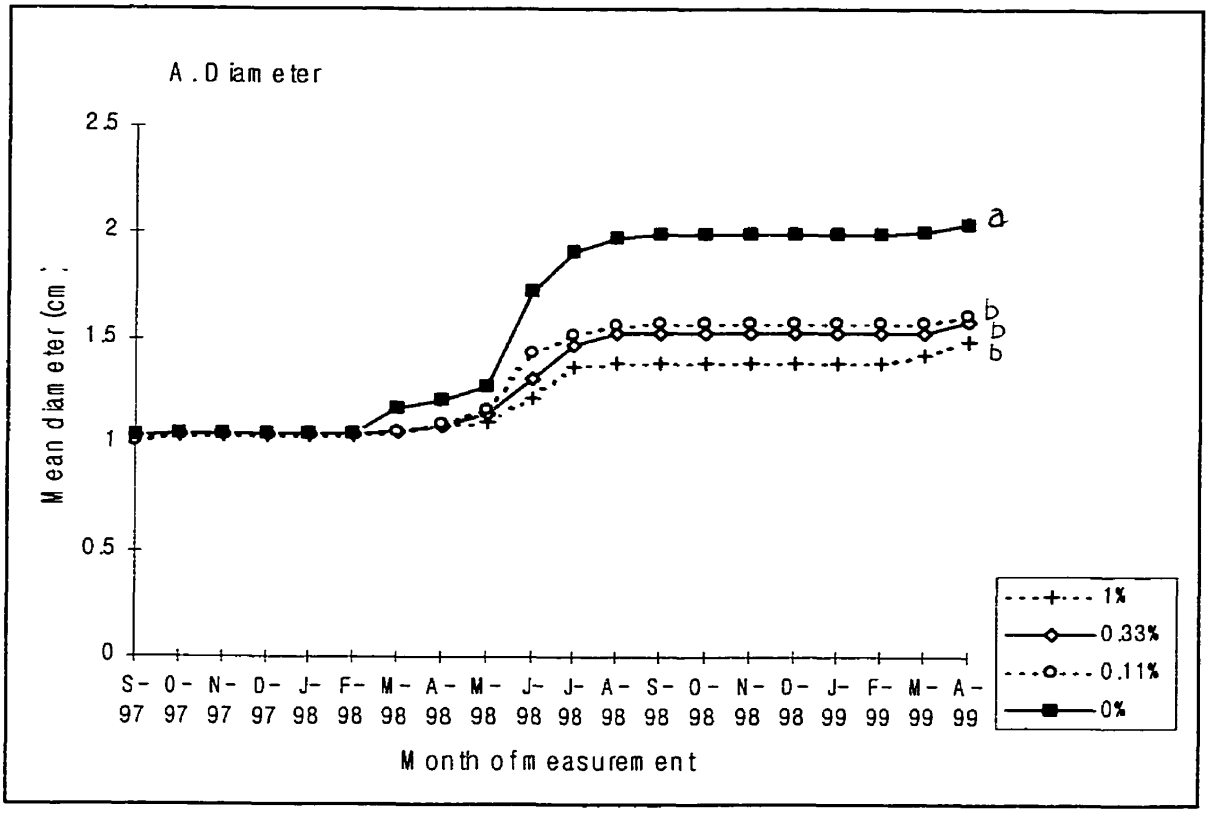

B . Height
Mean height (cm
120
100
80
60
40
20
0
a
b
b
c
M- 97
J- 97
J- 97
A- 97
S- 97
O- 97
N- 97
D- 97
J- 98
F- 98
M- 98
A- 98
M- 98
J- 98
J- 98
A- 98
S- 98
O- 98
N- 98
D- 98
J- 99
F- 99
M- 99
A- 99
Month ofm easurem ent
1%
0.33%
0.11%
0%

Figure 23. Mean diameter (A) and height (B) of Douglas-fir seedlings treated with glyphosate on 24 September 1997, 19 months after planting in the field. Measurements within either A or B followed by the same letter are not significantly different, Bonferroni test, $P<0.05$


A. Diameter
Mean diameter (cm)
2.5
2
1.5
1
0.5
0
a
b
b
b
S-97 O-97 N-97 D-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98 A-98 S-98 O-98 N-98 D-98 J-99 F-99 M-99 A-99
Month of measurement
1%
0.33%
0.11%
0%

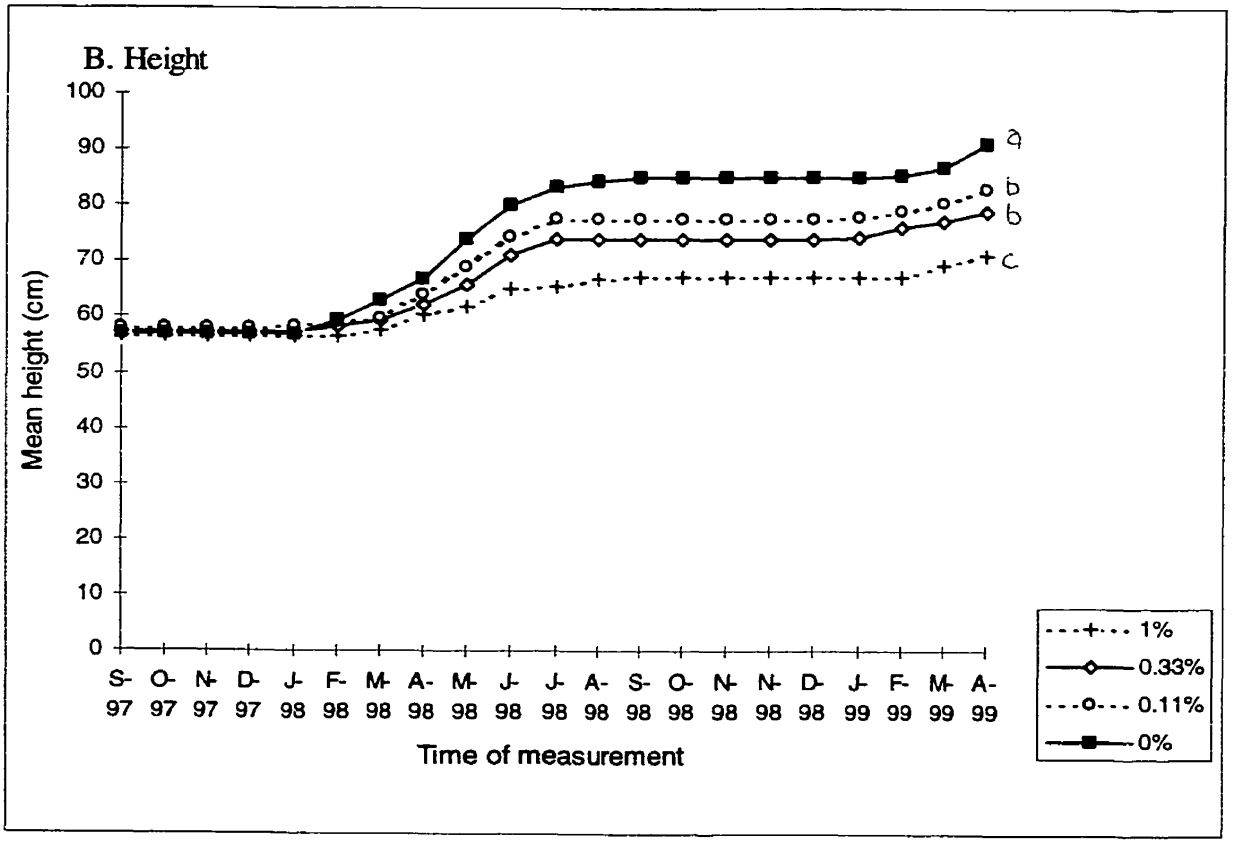
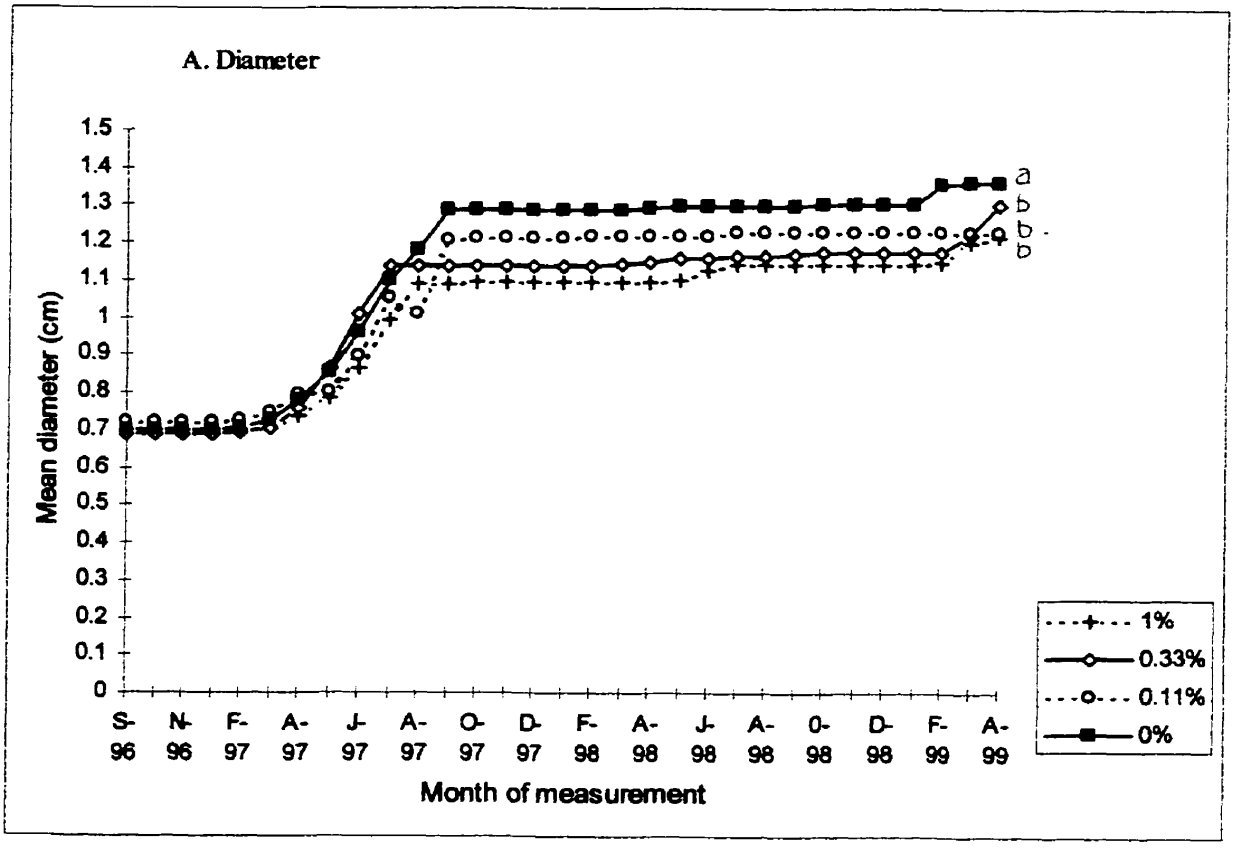

B. Height
Mean height (cm)
100
90
80
70
60
50
40
30
20
10
0
a
b
b
c
S-97 O-97 N-97 D-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98 A-98 S-98 O-98 N-98 N-98 D-98 J-99 F-99 M-99 A-99
Time of measurement
1%
0.33%
0.11%
0%

Figure 24. Mean diameter (A) and height (B) of lodgepole pine seedlings treated with glyphosate on 17 September 1996, 7 months after planting in the field. Measurements within either A or B followed by the same letter are not significantly different, Bonferroni test, $P < 0.05$


A. Diameter
Mean diameter (cm)
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
S-96
N-96
F-97
A-97
J-97
A-97
O-97
D-97
F-98
A-98
J-98
A-98
0-98
D-98
F-99
A-99
Month of measurement
a
b
b
b
1%
0.33%
0.11%
0%

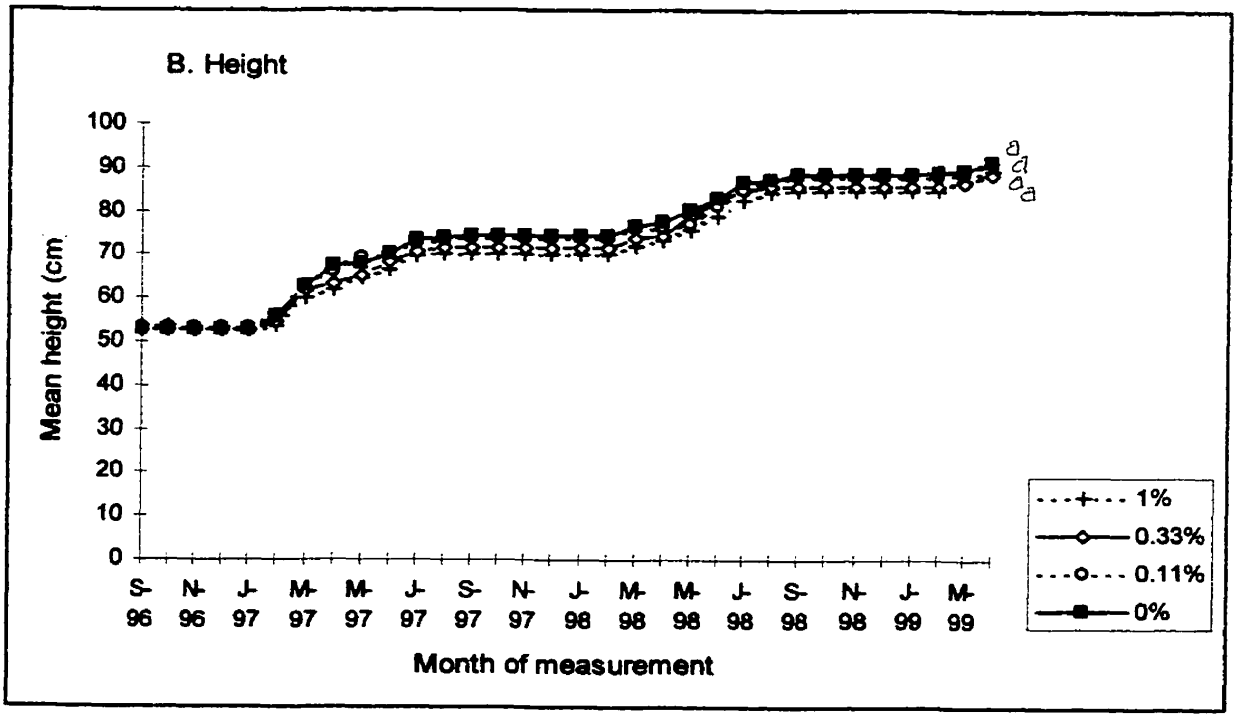
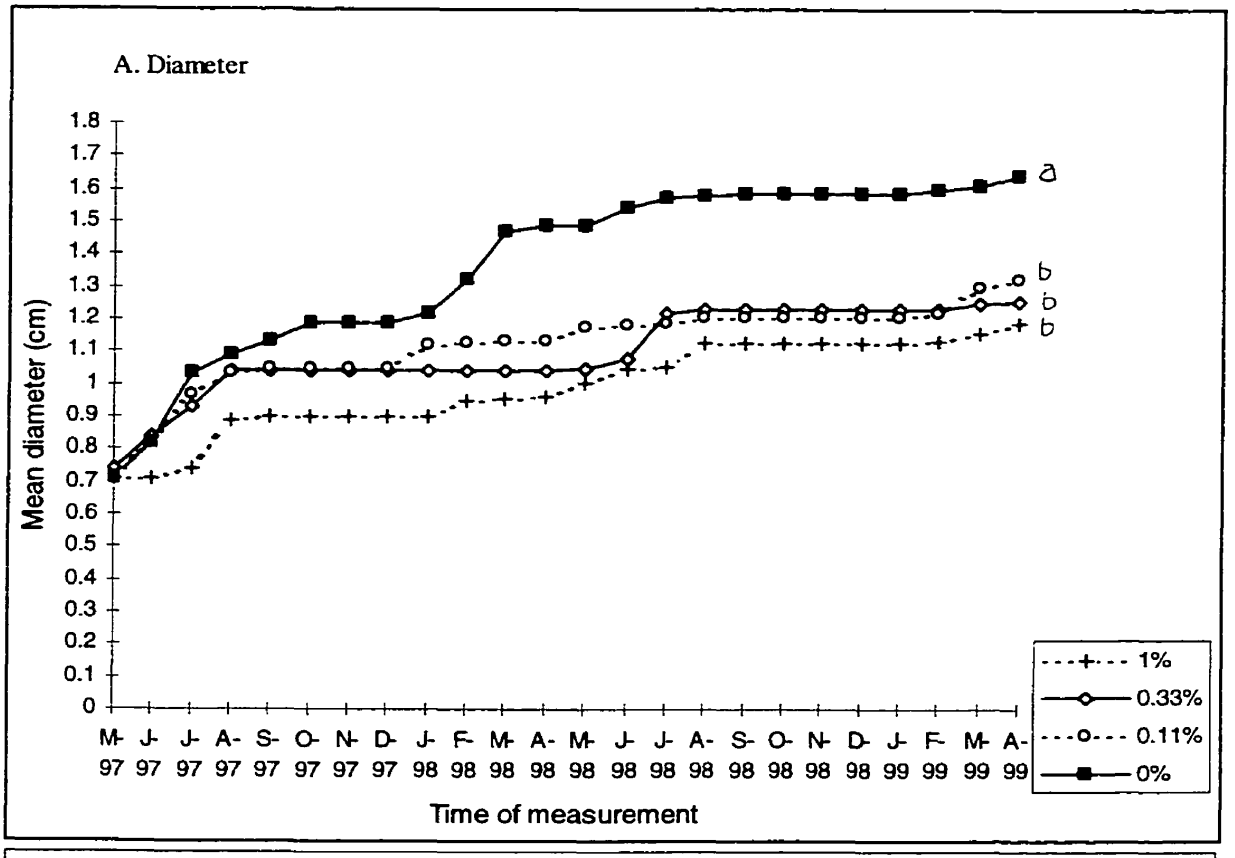

B. Height
Mean height (cm)
100
90
80
70
60
50
40
30
20
10
0
S-96
N-96
J-97
M-97
M-97
J-97
S-97
N-97
J-98
M-98
M-98
J-98
S-98
N-98
J-99
M-99
Month of measurement
a
a
a
1%
0.33%
0.11%
0%

Figure 25. Mean diameter (A) and height (B) of lodgepole pine seedlings treated with glyphosate on 16 May 1997, 15 months after planting in the field. Measurements within either A or B followed by the same letter are not significantly different, Bonferroni test, $P<0.05$


A. Diameter
Mean diameter (cm)
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
M-97 J-97 J-97 A-97 S-97 O-97 N-97 D-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98 A-98 S-98 O-98 N-98 D-98 J-99 F-99 M-99 A-99
Time of measurement
a
b
b
b
1%
0.33%
0.11%
0%

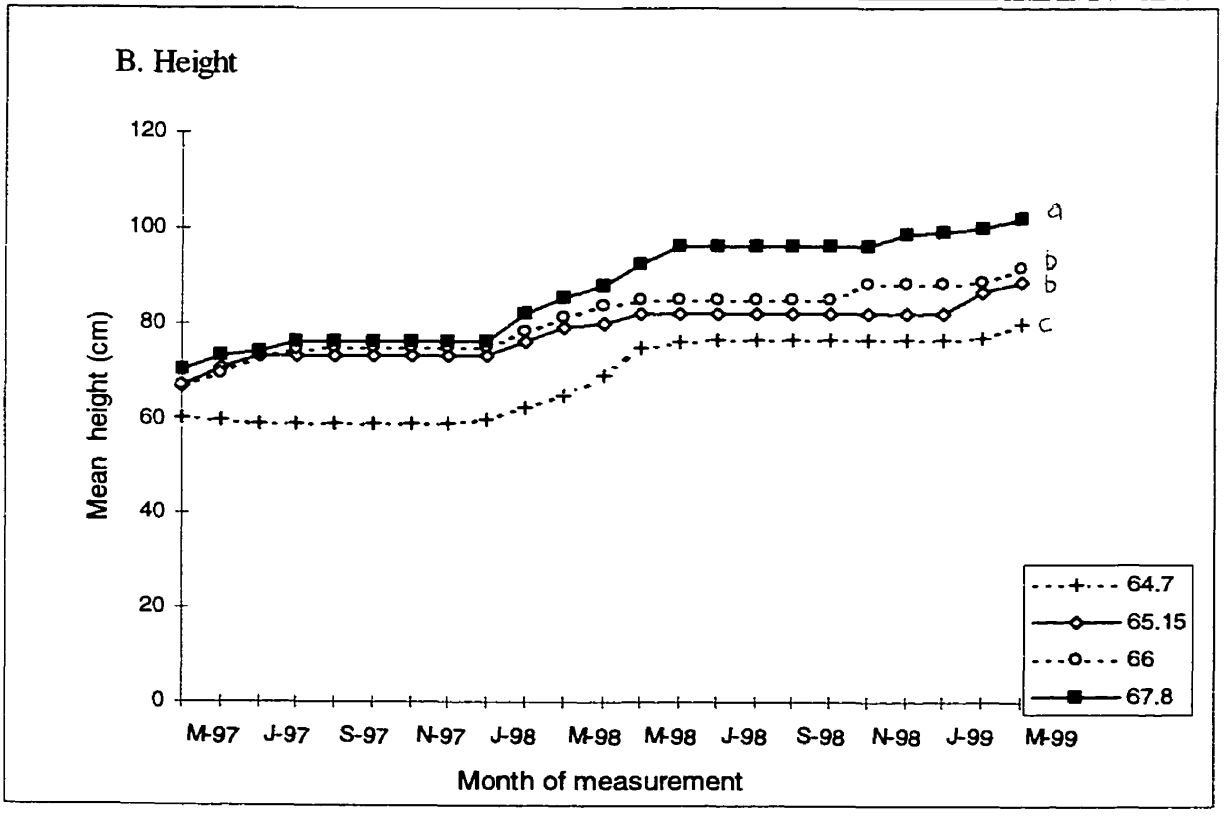
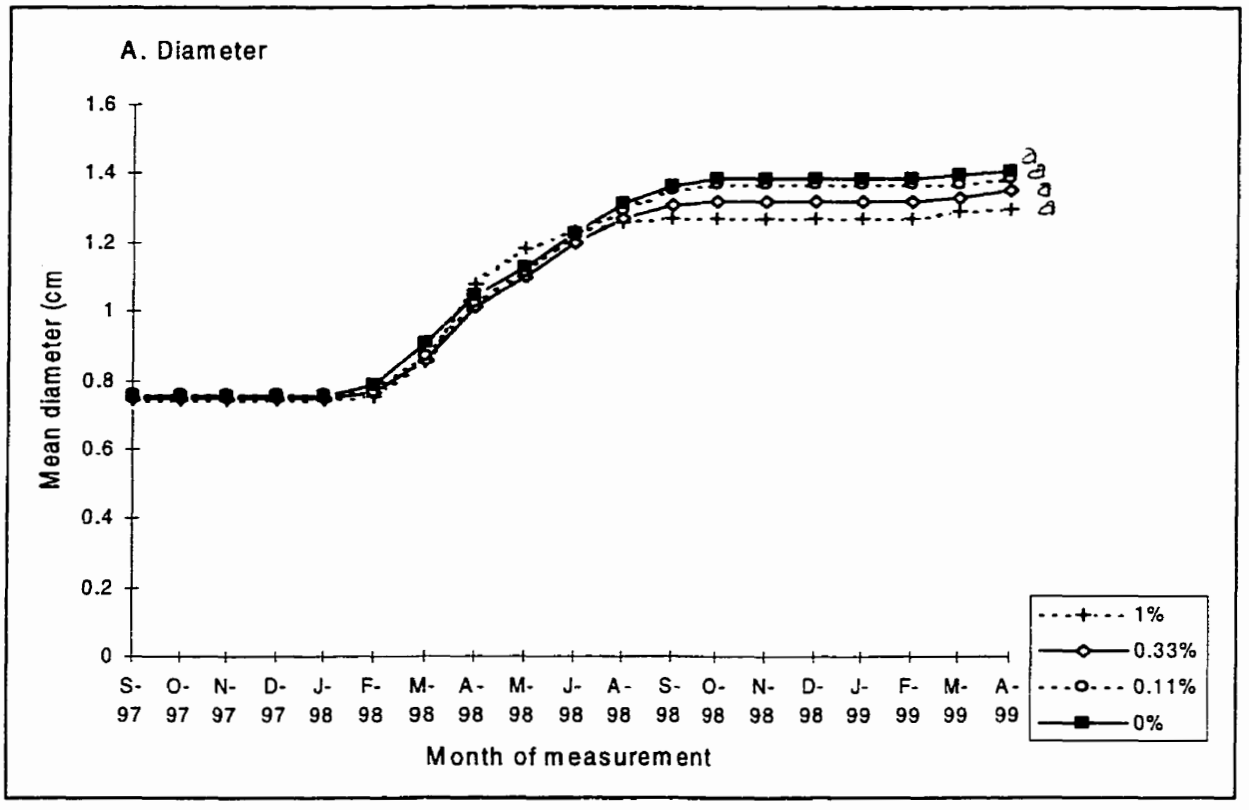

B. Height
Mean height (cm)
120
100
80
60
40
20
0
M-97 J-97 S-97 N-97 J-98 M-98 M-98 J-98 S-98 N-98 J-99 M-99
Month of measurement
a
b
b
c
64.7
65.15
66
67.8

Figure 26. Mean diameter (A) and height (B) of lodgepole pine seedlings treated with glyphosate on 24 September 1997, 19 months after planting in the field. Measurements within either A or B followed by the same letter are not significantly different, Bonferroni test, $P<0.05$


A. Diameter
Mean diameter (cm
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
S- 97
O- 97
N- 97
D- 97
J- 98
F- 98
M- 98
A- 98
M- 98
J- 98
A- 98
S- 98
O- 98
N- 98
D- 98
J- 99
F- 99
M- 99
A- 99
Month of measurement
1%
0.33%
0.11%
0%
a
a
a
a

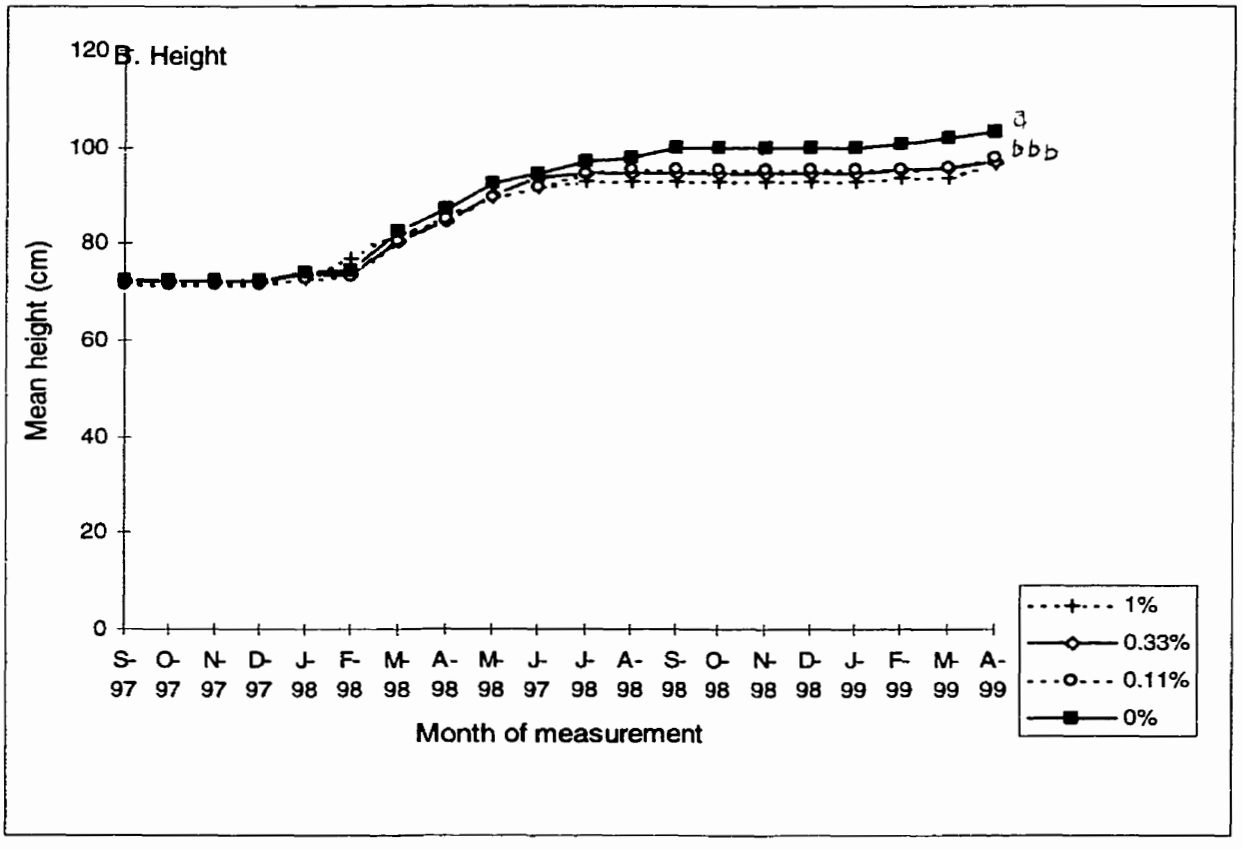
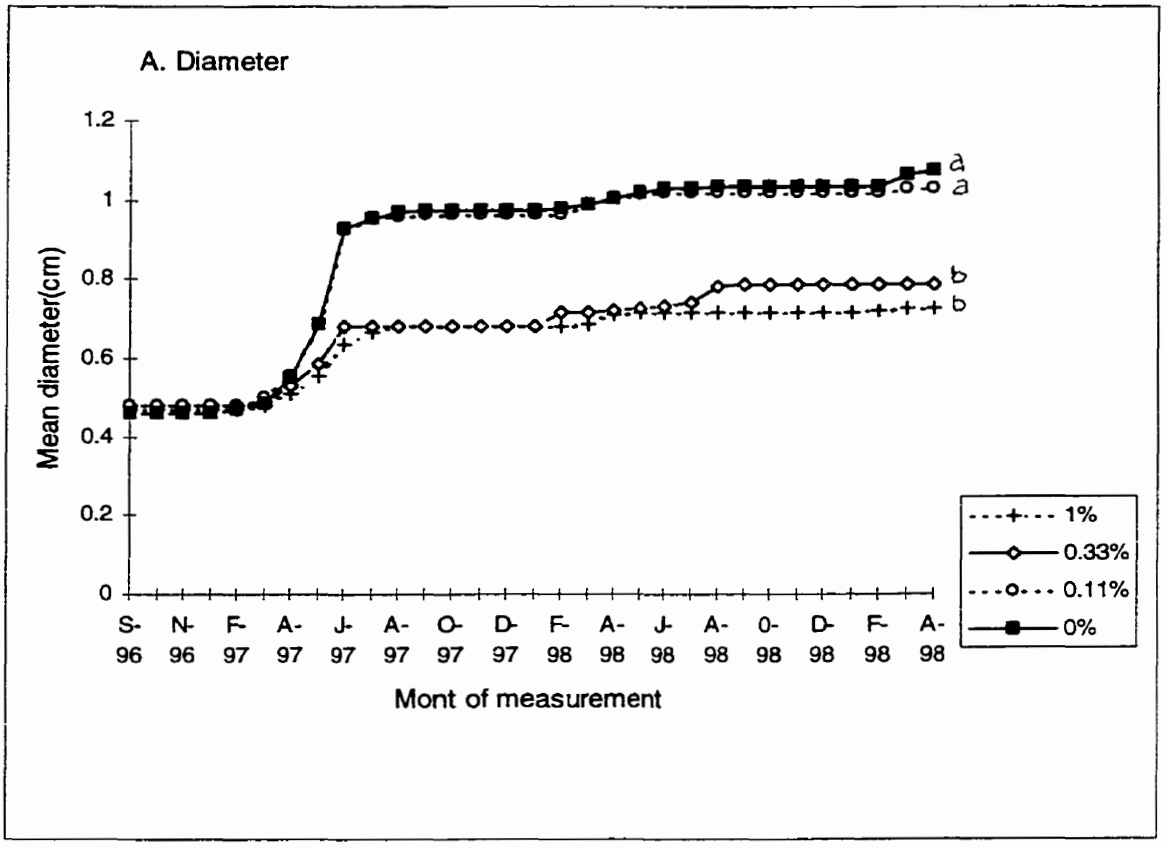

B. Height
Mean height (cm)
120
100
80
60
40
20
0
S- 97
O- 97
N- 97
D- 97
J- 98
F- 98
M- 98
A- 98
M- 98
J- 97
J- 98
A- 98
S- 98
O- 98
N- 98
D- 98
J- 99
F- 99
M- 99
A- 99
Month of measurement
1%
0.33%
0.11%
0%
a
b
b
b

Figure 27. Mean diameter (A) and height (B) of spruce seedlings treated with glyphosate on 17 September 1996, 7 months after planting in the field. Measurements within either A or B followed by the same letter are not significantly different, Bonferroni test, $P < 0.05$


A. Diameter
Mean diameter(cm)
1.2
1
0.8
0.6
0.4
0.2
0
a
a
b
b
S-96
N-96
F-97
A-97
J-97
A-97
O-97
D-97
F-98
A-98
J-98
A-98
0-98
D-98
F-98
A-98
Mont of measurement
1%
0.33%
0.11%
0%

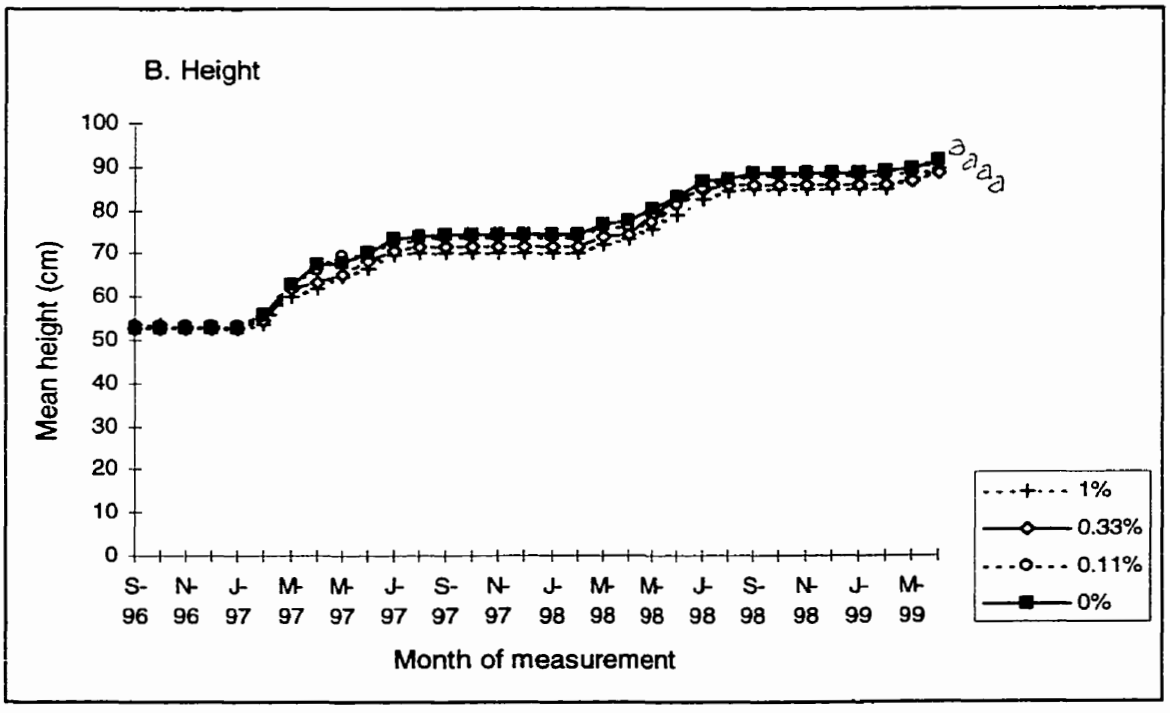
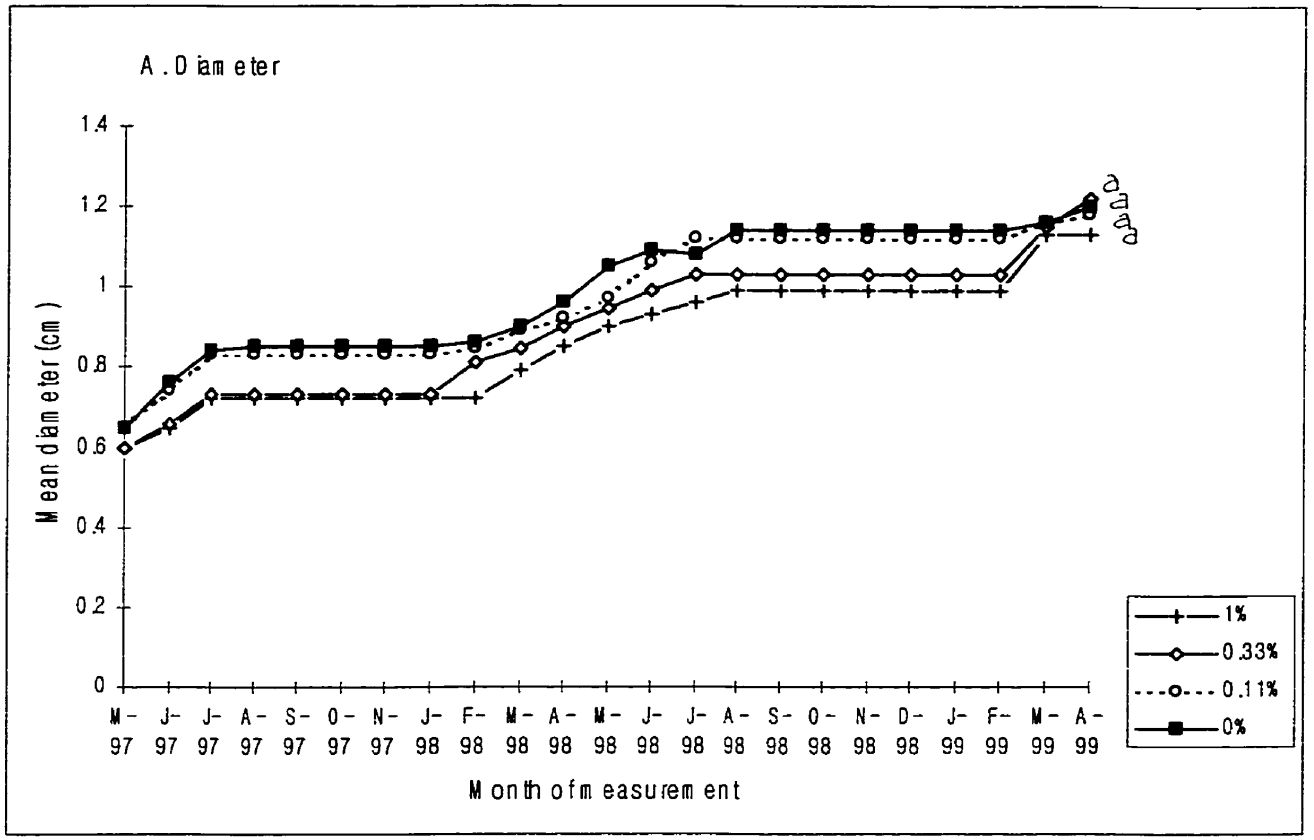

B. Height
Mean height (cm)
100
90
80
70
60
50
40
30
20
10
0
a a a a
S-96
N-96
J-97
M-97
M-97
J-97
S-97
N-97
J-98
M-98
M-98
J-98
S-98
N-98
J-99
M-99
Month of measurement
1%
0.33%
0.11%
0%

Figure 28. Mean diameter (A) and height (B) of spruce seedlings treated with glyphosate on 16 May 1997, 15 months after planting in the field. Measurements within either A or B followed by the same letter are not significantly different, Bonferroni test, $P < 0.05$


A. Diameter
Mean diameter (cm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
M-97 J-97 J-97 A-97 S-97 O-97 N-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98 A-98 S-98 O-98 N-98 D-98 J-99 F-99 M-99 A-99
Month of measurement
1%
0.33%
0.11%
0%
a
a
a
a

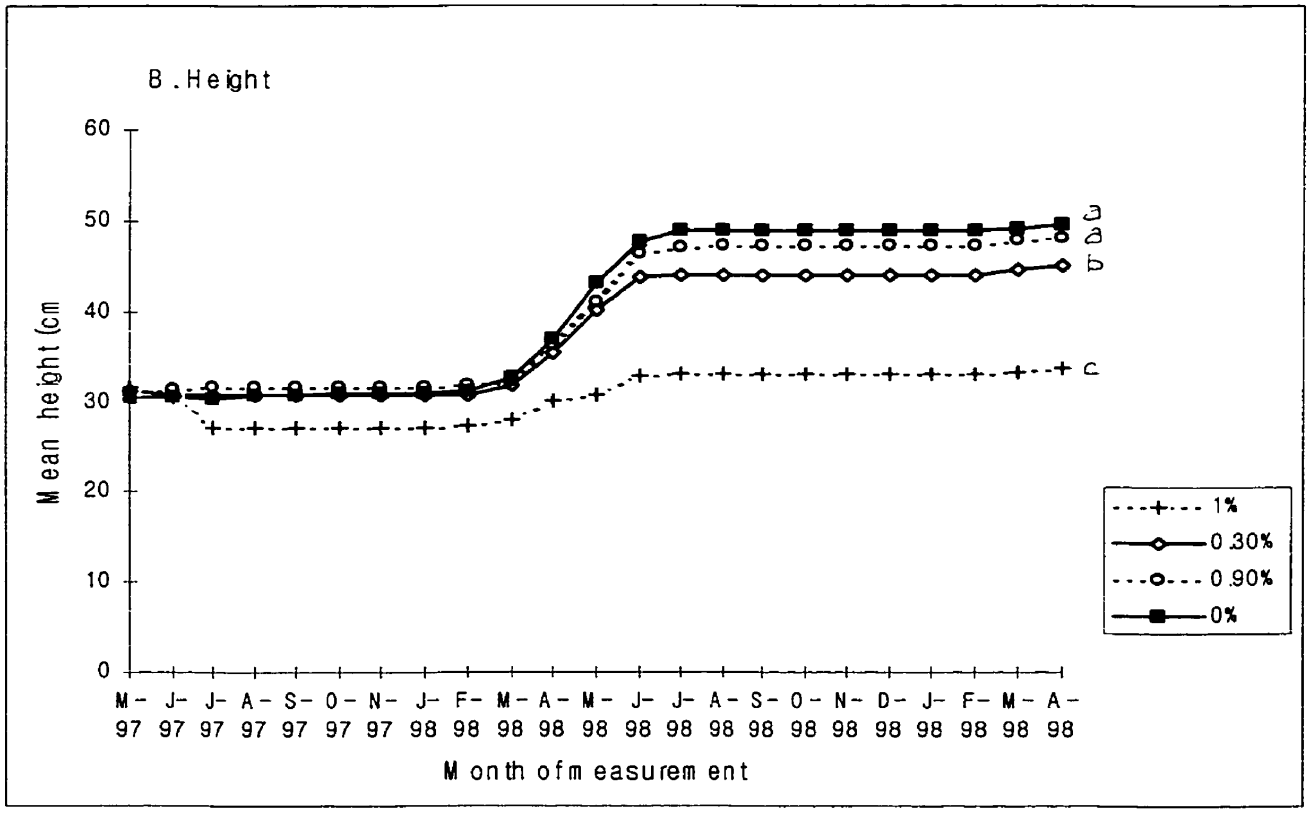
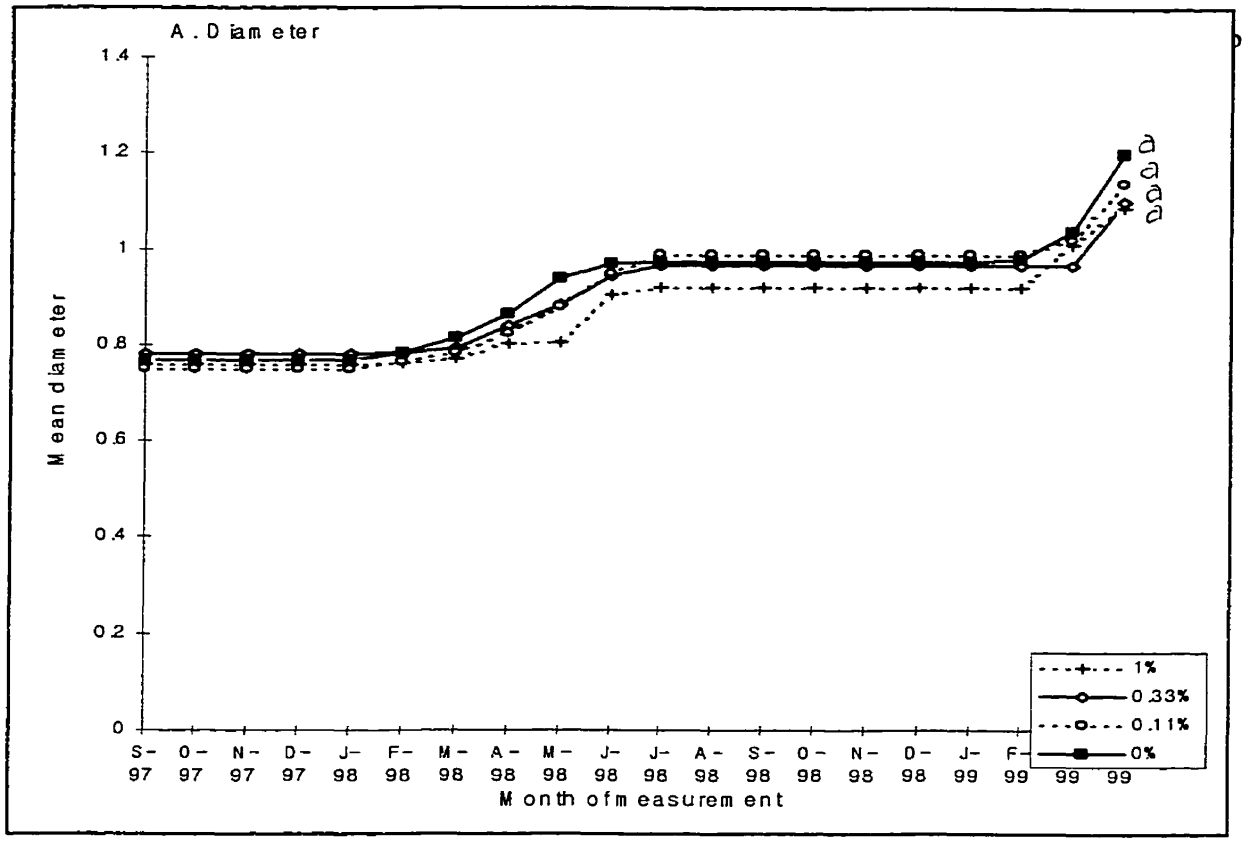
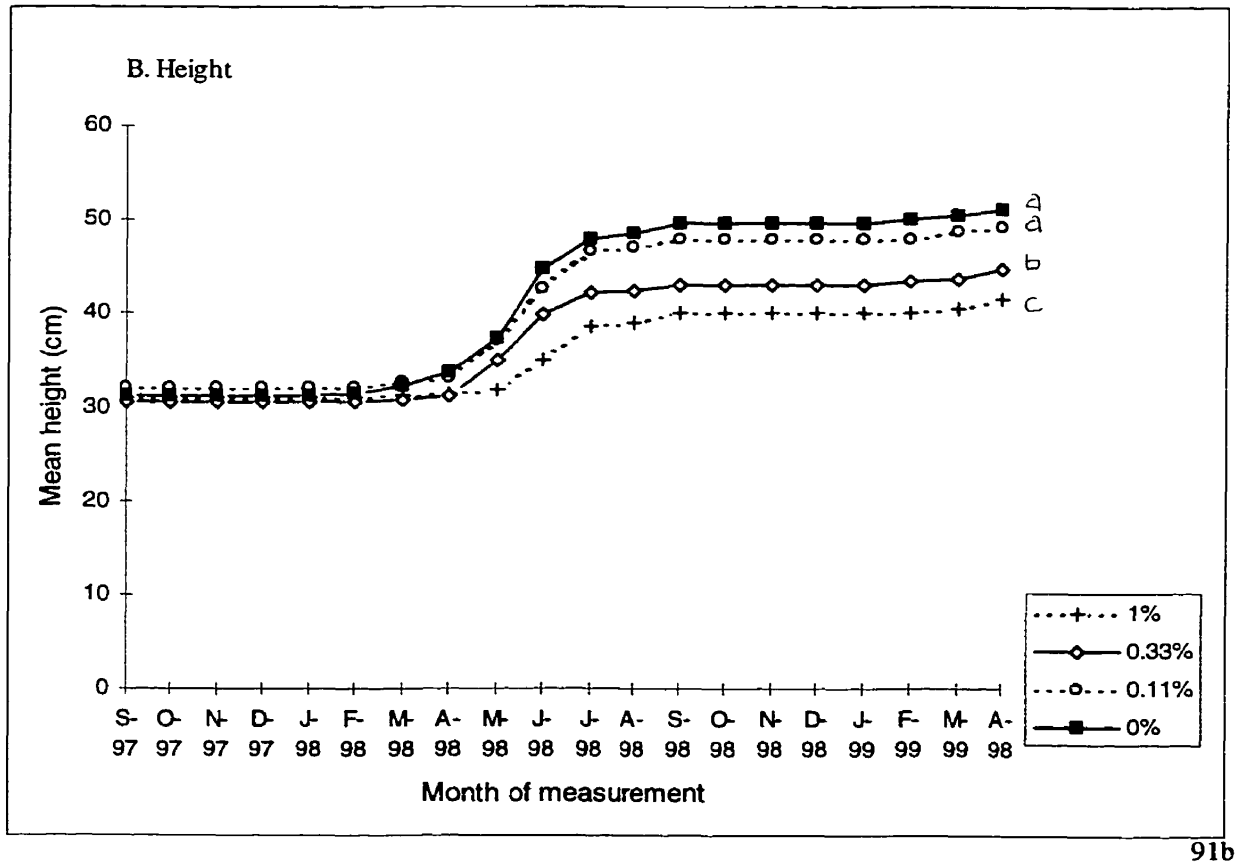

B. Height
Mean height (cm
60
50
40
30
20
10
0
M-97 J-97 J-97 A-97 S-97 O-97 N-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98 A-98 S-98 O-98 N-98 D-98 J-98 F-98 M-98 A-98
Month of measurement
1%
0.30%
0.90%
0%
a
a
b
c

Figure 29. Mean diameter (A) and height (B) of spruce seedlings treated with glyphosate on 24 September 1997, 19 months after planting in the field. Measurements within either A or B followed by the same letter are not significantly different, Bonferroni test, $P<0.05$


A. Diameter
Mean diameter
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
a
a
a
1%
0.33%
0.11%
0%
S-97 O-97 N-97 D-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98 A-98 S-98 O-98 N-98 D-98 J-99 F-99
Month of measurement


B. Height
Mean height (cm)
60
50
40
30
20
10
0
a
a
b
c
1%
0.33%
0.11%
0%
S-97 O-97 N-97 D-97 J-98 F-98 M-98 A-98 M-98 J-98 J-98 A-98 S-98 O-98 N-98 D-98 J-99 F-99 M-99 A-98
Month of measurement

In the same cases there was apparently negative height growth after exposure to glyphosate, particulalrly after treatment in May 1997 (Figures 15B, 20, 23B, 26B), this was caused by wilting and dieback of the terminal shoot, even though the tree remained alive.

5.3.3. Effect of Sublethal Glyphosate on Subsequent Growth and Development of Seedlings

Glyphosate application significantly affected the subsequent growth of leaders of Douglas-fir and lodgepole pine seedlings. This effect began with injury or death of the leader, reflecting the negative height growth observed in some instances. The effect in spruce was restricted to reduction in number of branches and branch size (Table 10). Multiple leaders and bent new leaders indicated the sensitivity of seedlings species to certain doses.

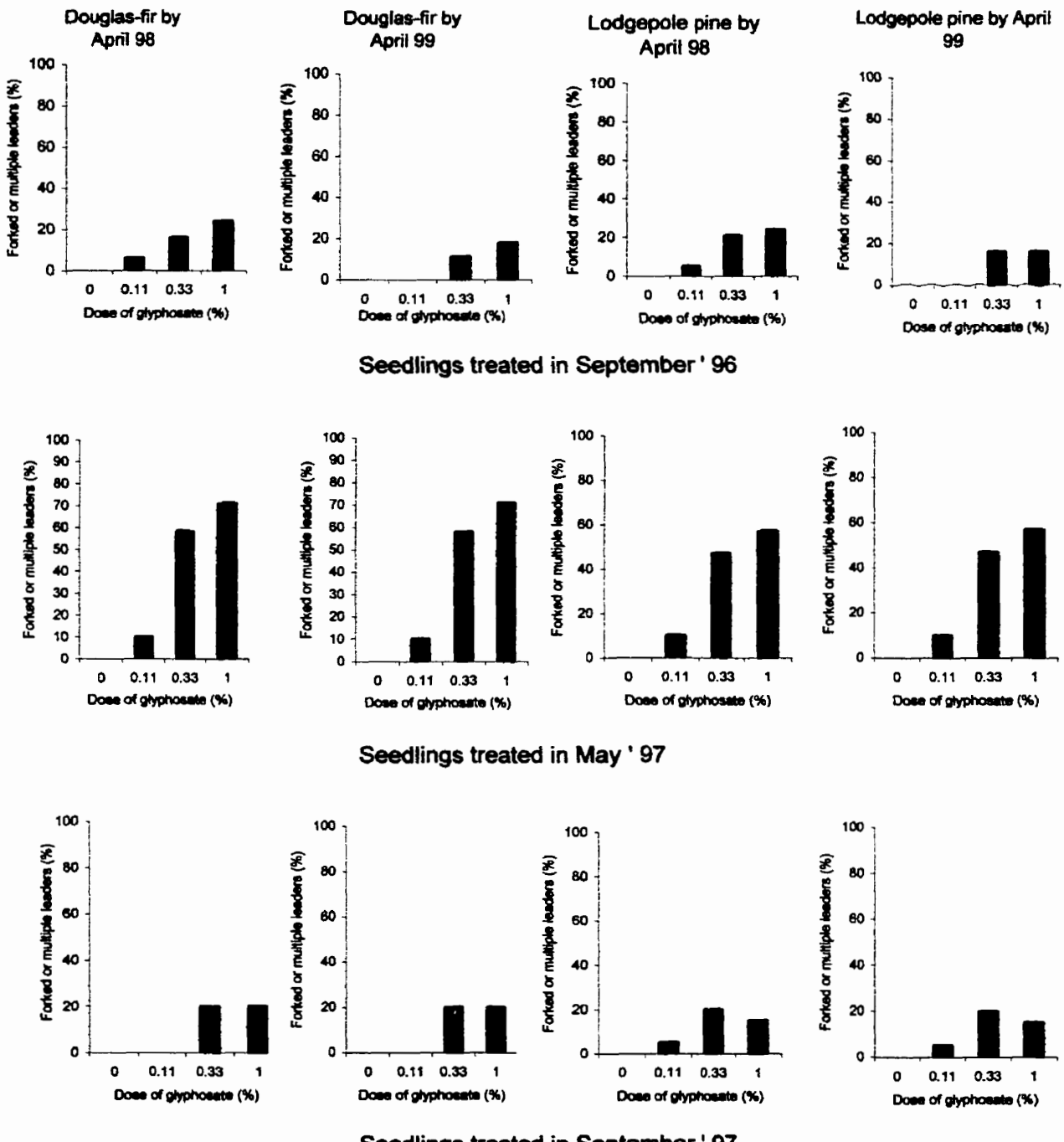
Spring application caused the most leader damage. The leaders of those seedlings that survived grew back in the following season, but many were forked (Figures 28, 29, 30,31,32). There was no evidence that glyphosate application had any effect on subsequent growth form of spruce seedlings. Damage to spruce was restricted to the needles of the current year's leader, whereas defoliation of the shoot and abnormalities in leader growth persisted to the end of the study in Douglas-fir and lodgepole pine treated with 1% of Vison.

Table 10. Total number length of branches of interior spruce following application of Vision

Treatment Date	Measurement date	Treatment (% Vision)	N	Total branches	Branch length (cm) ($\chi \pm SE$)[a]
Sept/96	Apr/98	1.00	10	21.4 ± 0.6 a	14.6 ± 0.5 a
		0.33	10	21.2 ± 0.5a	15.07 ± 0.4 a
		0.11	10	20.9 ± 0.6 a	14.1 ± 0.3 a
		0.0	10	26.7 ± 0.5 b	15.4 ± 0.5 a
May/97	Apr/99	1.00	10	20.8 ± 0.4 a	13.8 ± 0.6 a
		0.33	10	21.5 ± 0.6 a	14.1 ± 0.5 a
		0.11	10	26.4 ± 1.5 b	16.0 ± 0.6 b
		0.00	10	28.2 ± 1.0 b	18.0 ± 0.6 b
Sept/97	Apr/99	1.00	10	20.4 ± 0.4 a	14.1 ± 0.9 a
		0.33	10	22.6 ± 0.6 b	14.8 ± 0.8 a
		0.11	10	24.2 ± 0.6 b	15.2 ± 1.0 a
		0.00	10	27.8 ± 0.7 c	18.5 ± 0.6 b

[a] means within a column, species and treatment date followed by the same letter are not significantly different, Bonferroni test , $p < 0.05$.

Figure 30. Percentage of seedlings of Douglas-fir and lodgepole pine that developed forked or multiple leaders following foliar application of glyphosate in different seasons



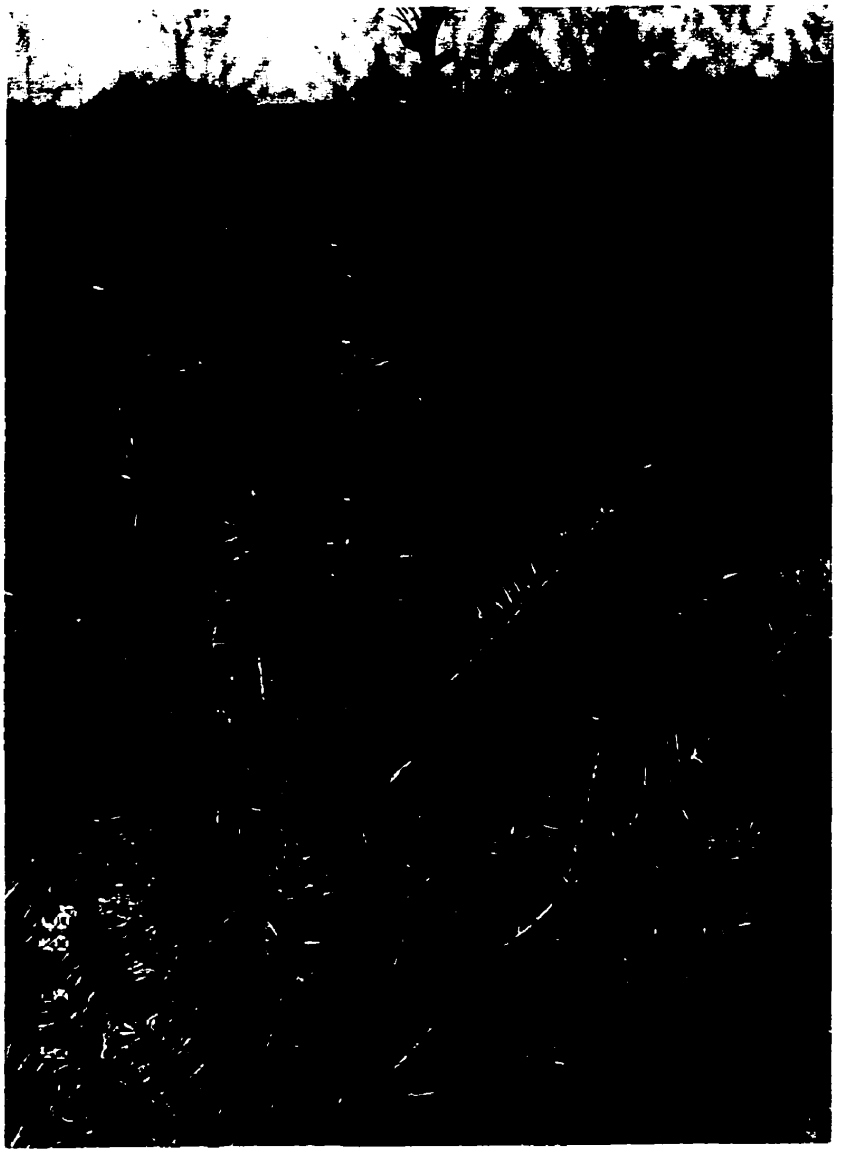


Figure 31. Forked leader on Douglas-fir seedling following foliar application of glyphosate in September 1996; photograoh 30 months after treatment

Figure 32. Multiple leader on lodgepole pine seedling photographed 2 years after foliar treatment with glyphosate (0.33% Vision) applied in May 1997

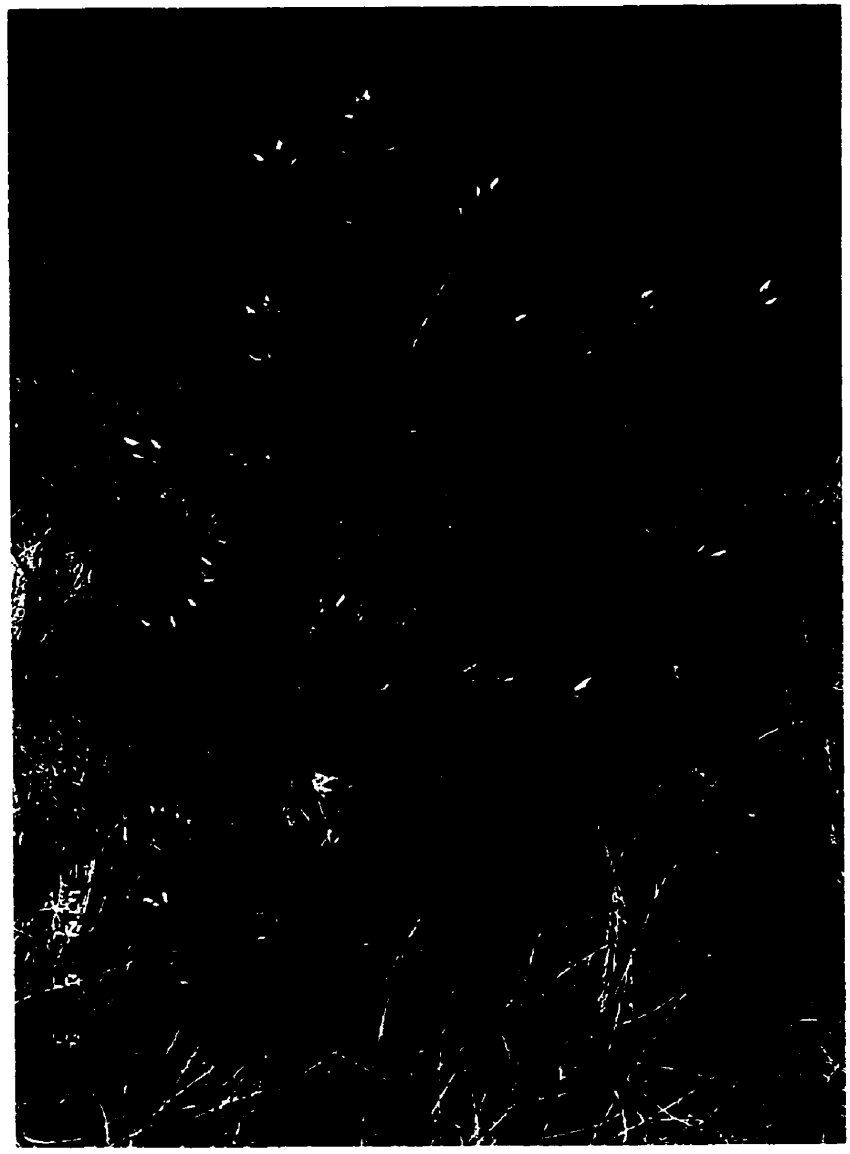


Figure 33. Abnormal growth of Douglas-fir seedling photographed 32 months after foliar treatment with glyphosate (1% Vision)in May 1997

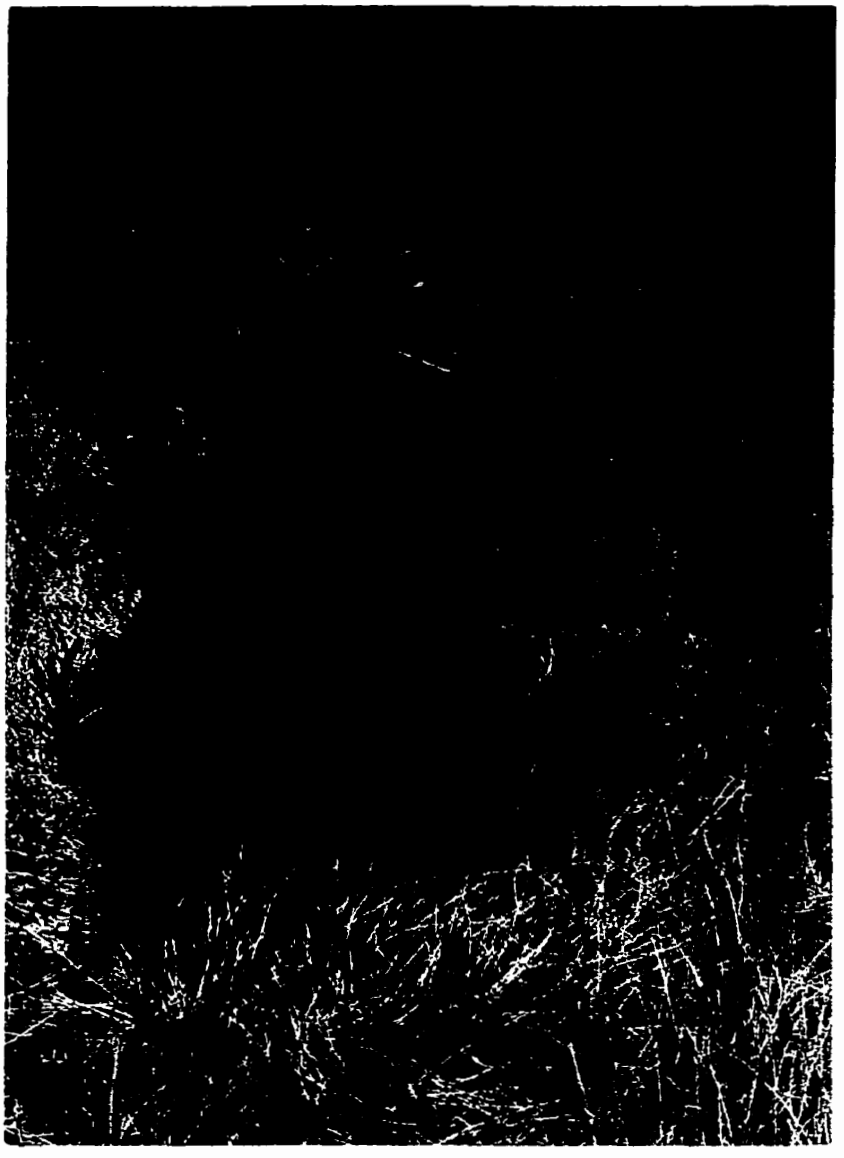


Figure 34. Abnormal growth of lodgepole pine seedling photographed 32 months after treatment with glyphosate (1% Vision) in May 1997

5.3.4. Effect of Sublethal Glyphosate on Root-shoot Biomass of Conifer Seedlings

In all cases, there was a significant, dose-dependent effect of reduced root and shoot biomass in Vision-treated conifer seedlings, compared to untreated controls (Table 11). In general, the root: shoot ratios remained relatively similar, regardless of dose, indicating equal, if reduced, allocation of resources to all parts of the plants, possibly because because of a uniform systemic effect of translocated glyphosate. However, particularly in lodgepole pine, but also in spruce seedlings, two years after treatment in September 1996 the root: shoot ratio declined significantly with dose of Vision, suggesting a reduced allocation of resources to roots over time in seedlings that had received the highest doses of Vision.

There was a similar trend among seedlings treated in fall 1997 to that observed in the spring. Root biomass of seedlings treated with 1% glyphosate was significantly lower than in seedlings treated with lower doses. There were no significant differences between seedlings treated with 0.33% and 0.11%, although both had significantly lower biomass than untreated seedlings.

5.3.5. Effect of Sublethal Glyphosate on Content of Starch, Tannin, Lignin and Lipid in Stems

Overall analysis of starch, tannin, lignin and lipid content revealed no significant differences between untreated and treated seedlings at any measurement time (1,2, 3 weeks, 18 and 24 months) after treatment . There was no observable effect of glyphosate treatment on cell structure.

Table 11. Root and shoot biomass of field-grown seedlings of Douglas-fir, lodgepole pine and interior spruce treated with glyphosate (as Vision®) in September 1996, May 1997 and September 1997, and evaluated in the fall and winter 1998/1999.

Species and Treatment date	Dose of Vision (%)	N	Biomass (g) ($\chi \pm$ SE) [a] Roots	Shoots	Root: shoot ratio ($\chi \pm$ SE) [a]
Douglas-fir					
Sept/96	1.00	4	25.5 ± 1.4 a	76.4 ± 1.6 a	33.4 ± 0.6 a
	0.33	4	31.3 ±1.2 b	89.3 ± 1.5 b	35.1 ± 0.8 a
	0.11	4	31.7 ±1.3 b	87.7 ± 1.5 b	36.1 ± 0.4 a
	0.00	4	35.8 ± 1.3 b	101.2 ±1.6 c	35.4 ± 0.9 a
May/97	1.00	4	16.8 ± 0.9 a	44.2 ±0.72 a	38.0 ± 0.8 a
	0.33	4	42.5 ± 0.6 b	128.0 ± 0.85 b	33.1 ± 0.6 a
	0.11	4	45.8 ± 1.6 bc	132.2 ± 1.2 b	34.6 ± 1.2 a
	0.00	4	49.9 ± 1.0 c	144.3± 1.5 c	34.6 ± 0.9 a
Sept/97	1.00	4	27.6 ± 0.9 a	70.6 ± 0.5 a	39.1 ± 0.4 a
	0.33	4	35.1 ± 0.7 a	78.4 ± 0.7 b	44.8 ± 0.8 a
	0.11	4	41.1 ± 0.6 ab	119.2 ± 1.0 c	34.5 ± 0.4 a
	0.00	4	48.8 ± 1.3 b	140.8 ± 1.0d	34.6 ± 1.1 a

Lodgepole pine					
Sept/96	1.00	4	28.1 ± 1.4 a	147.7 ± 1.1 a	19.0 ± 1.6 a
	0.33	4	47.7 ±1.3 b	147.8 ± 0.7 a	32.3 ± 0.9 b
	0.11	4	58.2 ± 1.6 b	173.7 ± 1.3 b	33.5 ± 1.3 b
	0.00	3	100.0 ± 0.9 c	164.2 ± 0.4 b	60.9 ± 0.8 c
May/97	1.00	4	31.4 ± 0.5 a	105.7 ± 0.9 a	29.7± 1.3 a
	0.33	4	53.2 ± 0.6 b	176.6 ± 1.6 b	30.1 ± 0.6 a
	0.11	4	64.8 ± 1.2 c	187.5 ± 0.6 c	34.6 ±1.1 a
	0.00	4	63.4 ± 1.8 c	188.5 ± 0.5 c	33.9 ±1.0 a
Sept/97	1.00	4	40.8 ± 1.4 a	23.5 ± 1.5 a	33.0 ± 1.2a
	0.33	4	39.0 ± 1.7 a	128.3 ± 1.6 a	30.4 ± 0.4a
	0.11	4	53.3 ± 1.4 b	194.5 ± 0.4 b	27.4 ± 0.6 a
	0.00	4	56.8 ± 0.4 b	213.4 ± 0.3b	26.1 ± 0.8 a
Interior spruce					
Sept/96	1.00	4	30.2 ± 1.2 a	75.8 ± 0.4 a	39.8 ± 1.2 a
	0.33	4	33.3 ± 1.0 a	78.1 ± 0.8 a	42.6 ± 1.8 ab
	0.11	4	41.5 ± 0.6 b	88.2 ± 1.1b	48.2 ± 2.1 b
	0.00	4	44.0 ± 1.3 b	90.6 ± 0.7 b	48.6 ±1.1b

May/97	1.00	4	22.7 ± 1.2 a	52.6 ± 1.4 a	43.2 ± 0.4 a
	0.33	4	32.3 ± 1.4 b	80.9 ± 0.9 b	39.9 ± 0.6 a
	0.11	4	34.3 ± 1.6 b	75.0 ± 1.2 b	45.7 ± 0.7 a
	0.00	4	38.8 ± 1.1c	108.9 ±1.7 c	35.6 ± 1.0 a
Sept/97	1.00	4	34.2 ± 0.8 a	82.1 ± 0.9 a	41.6 ± 0.5 a
	0.33	4	38.2 ± 1.1 b	85.7 ± 1.1 b	44.6 ± 0.4 a
	0.11	4	39.2 ± 1.2 b	94.2 ± 0.5 b	41.6 ± 0.4 a
	0.00	4	43.2 ± 0.9 c	103.2 ± 1.3 c	41.9 ± 0.7a

[a] Means within a column, species and treatment date followed by the same letter are not significantly different, Bonferroni test , $p < 0.05$.

5.4 Discussion

Results on seedling mortality following treatment with Vision (Figure 13) suggest that broadleaf seedlings are more susceptible to glyphosate than conifer seedlings. This hypothesis is supported by the greater impact of glyphosate in diameter and the growth of the two broadleaf species compared with that in conifers (Figures 14, 15, 16, 17, 18). Tolerance of Vision in conifers can be attributed to the anatomical structure of conifer needles, which limit the quantity of glyphosate that can enter the plant. Conifer needles have a smaller surface area to intercept spray droplets, and are covered by a thick cuticle and epicular . The cuticular membrane is composed of cutin, a polimer of hydroxyfatty acids (Holloway, 1982), and a complex mixture of very long chain lipids or cuticular wax that is embedded within the cell matrix (Baker, 1974). The desposition of epicuticular wax begins very early in conifer needle development (Gunthard-Goerg, 1987). All of these structural element would contribute to restricted needle penetration by Vision , which is probably an important basis for the tolerance to glyphosate shown by most conifers (Lund-Hoie, 1980). Low growth rate and low transpiration potential in conifers may also contribute to tolerance to glyphosate (Radosevich *et al.,* 1980). Late-season increases in tolerance by conifers to glyphosate are probably due to advanced dormancy (Lund-Hoie, 1985, McCormark, 1981).

High mortality of seedlings (Figure 13), reduced growth (Figures 14-27) and pronounced morphological defects (Figures 15, 17, 20, 23, 25, Table 10) of seedlings following application of Vision in the spring (May 1997) are probably

related to the growth activity of the plants. Most plants in temperate regions are actively growing from March to July, and are most sensitive to glyphosate when shoots are actively elongating.

The higher susceptibility of paper birch to glyphosate than red alder may be because red alder seedlings grew faster than paper birch seedlings at the study site and therefore were bigger when treated with glyphosate. In woody species, glyphosate is translocated in both phloem and xylem. The larger the distance from foliage to roots, the more glyphosate will concentrated in the youngest aerial shoots, with diminished toxic effect on the roots (Lund-Hoie, 1985). Because paper birch seedlings were short when treated, much of the applied glyphosate would have been concentrated in the roots, increasing the toxic effect and killing the entire plant. The low root: shoot ratio in lodgepole pine and interior spruce treated in September 1996 (Table 11) when they were short also supports this hypothesis. Finally, the highest dose of Vision killed 75% of paper birch seedlings when applied in September, but only 45% of seedlings when applied in September 1997 when the seedlings were bigger.

Of the three conifers tested in this study, interior spruce was the most tolerant to glyphosate. Douglas-fir and lodgepole pine showed similar sensitivity. Prasad (1989) suggested that species-specific differences in susceptibility occur. He found that spruce species harden off faster at the end of summer than Douglas-fir and thus are reasonably tolerant to glyphosate when Vision is applied in late summer or fall. The growth impact of sub lethal doses of Vision applied in the fall occurred primarily in the following spring, with resumed apparently normal

growth in the spring thereafter. Thus, the year of reduced growth following recommended operational applications in the fall (Nova Scotia Department of Land and Forests, 1989) may be a small cost to pay for the increased growth of crop trees following release from competition with brush species. However, the morphological affects of sub lethal doses of Vision, particularly to the leaders may persist as hidden defects many years later when the trees are harvested. Therefore, applications of Vision should probably be delayed until the potential crop trees are overtopped by deciduous brushy competitors, reducing their exposure to aerially applied glyphosate.

CHAPTER VI

General Discussion and Conclusion

6.1 Glyphosate Synergistic Interaction (GSI)

What causes death in plants treated with the herbicide glyphosate? Do plants die as a direct result of inhibition by glyphosate of the shikimic acid pathway? If so, why does glyphosate appear to act selectively in the roots of herbaceous plants? Participation by certain soil fungi, notably *Pythium* spp. and *Fusarium* spp., in the herbicidal activity of glyphosate was first reported by Johal and Rahe (1984). They showed that bean seedlings growing in heat treated soil were markedly more tolerant to glyphosate than were similar seedlings growing in untreated soil. Full sensitivity to the herbicide was restored by drenching heat treated soil or inoculating seedlings growing in heat treated soil with macerated mycelium from pure cultures of *Pythium* spp. or *Fusarium* spp. that had been isolated from the roots of glyphosate treated seedlings. Treatment of bean seedlings with metalaxyl blocked the synergistic effect of *Pythium* drenches or inoculations on the glyphosate sensitivity of bean seedling growing in heat-treated soil.

Subsequent research showed that diverse *Pythium* spp. could enhance the glyphosate sensitivity of seedlings growing in soils in which indigenous populations of *Pythium* and *Fusarium* had been eliminated by heat treatment, and that the phenomenon of GSI between certain soil fungi and the roots of plants treated with glyphosate occurs in several plant species (Johal and Rahe,

1990; Levesque and Rahe, 1992). Putative evidence for GSI is provided when a difference in glyphosate sensitivity of seedlings growing in heat-treated soil and untreated soil is observed. Seedlings of the dicot species bean, *Phaseolus vulgaris*, pepper, *Capsicum* sp. and sunflower, *Helianthus* sp. were 20X to 30X more tolerant (mortality, LD_{50}) to glyphosate when growing in heat treated soil compared with their sensitivities in untreated soil. In contrast, the glyphosate tolerance of seedlings of the monocot species corn, *Zea mays* and wheat, *Triticum aestivum* growing in heat treated soil and untreated soils was only 2X to 3X higher in heat treated than in untreated soil.

There are few reports concerning the possible occurrence of GSI in woody species. Levesque *et al.* (1992) reported that 6-week old seedlings of apple, *Malus pumila,* grown in heat treated soil were approximately 7X less sensitive to glyphosate than were similar seedlings growing in untreated soil, and that full sensitivity to glyphosate was restored by drenching the heat- treated soil with selected isolates of *Pythium* and *Fusarium* . Wanner (1994), and Fraser (1989) tested the hypothesis that application of glyphosate to stumps would enhance colonization of the roots of mature Douglas-fir by soil fungi.
In these studies there was no control of translocation of glyphosate into the roots, and the levels of glyphosate detected varied greatly. Wanner (1994) reported that the frequency of stain fungi as the primary fungal colonizers of Douglas-fir stumps was not different between treated and control stumps.

Although the above studies suggest that GSI does not occur in mature conifers, it can be argued that only large structural roots were examined, and that

possible effects of glyphosate on colonization in small feeder roots of conifers remain unknown.

The broad objective of the present study was to seek evidence for the possible occurrence of GSI in feeder roots of selected hardwood and conifer species. The following hypotheses were tested using 1- and 2- year old seedlings and examining effects in portion of root systems that include the actively growing feeder root tips of the seedlings.

1) Foliar application of glyphosate, such as might occur during commercial use of glyphosate herbicides such as Vision for forest vegetation management, will cause lower mortality on seedlings growing in heat treated than in untreated soil.
2) Foliar application of glyphosate to seedlings growing in untreated soil will cause enhanced colonization of roots by fungi.
3) Phytotoxicity of glyphosate to selected conifer species will be enhanced by amendment of soil with selected *Pythium* and *Fusarium* spp.
4) Colonization of conifer roots by the pathogen, *Armillaria ostoyae*, will be enhanced following foliar application of glyphosate.

Extensive research produced little evidence in support of any of these hypotheses, except for the second hypothesis, where enhanced colonization by *Pythium* and *Fusarium* spp. was consistently observed following foliar application of glyphosate. The results showed also arge differences in the sensitivities of the selected species with regard to mortality, and with regard to

effects on growth and form of seedlings up to 2 years following treatment with glyphosate.

6.2 Detection of GSI

In studies of GSI, seedlings growing in heat treated soil survived doses of glyphosate that killed seedlings growing in untreated or autoclaved soil infested with *Pythium* spp. (Lesveque, *et al.,* 1992; Lesveque , 1990). *Pythium* spp. and *Fusarium* spp. were found to be the agents synergizing the herbicidal activity of glyphosate in the untreated soil (Johal and Rahe, 1984; Levesque, *et al.*, 1987; Descalzo, 1996). The higher sensitivity to glyphosate for bean, wheat and apple seedlings grown in untreated soil than in heat-treated soil was due to quantitative and qualitative changes in microflora following heat treatment (Levesque, 1990). Addition of water extract of untreated soil to heat-treated soil restored both *Pythium* spp. and herbicidal efficacy in soil where *Pythium* spp. were re-established.

Before beginning my study, I repeated a portion of Levesque's (1990) study using bean seedlings, verifying the above result in the soil that I later used for experiments on conifers seedlings.

My results with Douglas-fir and lodgepole pine grown from surface sterilized seed showed no differences in the glyphosate sensitivity of seedlings growing in heat-treated and untreated soils. Similarly, 1 year old seedlings of ponderosa pine and spruce that were transplanted into heat-treated and untreated soils after thorough washing of their roots, were equally sensitive to glyphosate in either of the soil treatments. Thus, I was unable to detect significant differences in the

glyphosate sensitivity of conifer seedlings growing in heat-treated and untreated soils from surface sterilized seeds, or from 1-year-old seedlings after washing of the roots. These results are in contrast to earlier finding with herbaceous plants (Johal and Rahe, 1984); Rahe *et al.*, 1990, Levesque *et al.*, 1992).

Although my experiments provide no evidence for the occurrence of GSI in conifer seedlings, the conclusion that GSI does not occur in conifer seedlings is not definitive because I was unable to prevent the reintroduction *Pythium* into the heat-treated soils during the 6 and 9 months that were required to complete these two sets of experiments. The populations of *Pythium* and *Fusarium* that were present in the heat-treated soils at the times of treatment of the seedlings with glyphosate were lower than the corresponding populations in the untreated soils. The strongest conclusion that can be drawn is that if *Pythium* spp. or *Fusarium* spp. are able to enhance the herbicidal efficacy of glyphosate on conifer seedlings, their contribution is largely academic since these fungi are omnipresent under the natural conditions and difficult to exclude from the long term experiments that are required to demonstrate GSI in a woody species under laboratory conditions.

6.3 Effect of Glyphosate on Subsequent Growth of Conifer Seedlings Treated with Sublethal Doses of Glyphosate

Of the three conifers used in this study, spruce was the most tolerant species to glyphosate. Douglas-fir and lodgepole pine showed similar sensitivity to glyphosate. Prasad (1989) suggested that the degree of susceptibility appears

to be species specific, probably because of different rates of hardening off in late summer, when Vision is applied in forest plantations.

My results support other studies (Lund-Hoie, 1975; Nova Scotia Department of Lands and Forests, 1988; 1989) that have reported conifer species to be more tolerant to glyphosate than broadleaf species. This is probably due to the anatomical structure of conifer needles, which limits the quantity of glyphosate spray that enters the plant. Conifer needles have a small surface area to intercept spray droplets, and they are covered by thick cuticle and epicuticular wax. Lund-Hoie (1980) reported that structure and composition of cuticule contributed to restricted uptake glyphosate by needles.

The higher mortality of seedlings that I observed following application of Vision in May than in September is probably related to differences in metabolic activity of plants at these times. Most plants in temperate regions are actively growing from March to July, and are most sensitive to glyphosate when shoots are elongating (Lund-Hoie, 1985).

There are two scenarios for the effect of exposure to sub lethal doses of glyphosate on subsequent growth in conifer seedlings. One is a long-term persistent reduction in growth rate subsequent to exposure to glyphosate. Such an effect could affect the quality of wood and could have a severe negative impact on forest productivity. The other scenario is short-term reduction in the growth rate, followed by return to normal rate of growth. In this scenario, the effect of glyphosate would disappear and growth rate would return to normal

following one or two seasons of suppressed growth after glyphosate is applied. My data support the second scenario. They suggest that a temporary loss in growth rate may be offset by reduced competition by brushy species killed by glyphosate. Therefore, the continued use of glyphosate for conifer release is justified, as long as it can be applied in such manner that defects in form of exposed conifers can be minimized.

6.4 Conclusions

Based on data presented here, the following conclusions can be made.

1) Glyphosate enhanced colonization of roots by *Pythium* spp., and *Fusarium* spp.
2) No evidence was found for GSI in selected woody species.
3) Broadleaf species were more sensitive to glyphosate than conifers.
4) Conifers were more sensitive to glyphosate in spring than fall
5) Douglas-fir and lodgepole pine were more sensitive to glyphosate than spruce.
6) Glyphosate in surviving conifer seedlings caused multiple and forked leaders, and reduced rate of growth in first year following treatment.

The second conclusion suggests that the mode of efficacy of glyphosate in woody species may differ from that in dicot herbaceous species, and may be primarily a direct effect of biochemical toxicity. The last four conclusions support the results and hypotheses of other researchers, and are soundly based on

replicated experiments involving up to two years of comparative observations on two broadleaf and three conifer species exposed to a range of lethal and sub lethal dose of glyphosate. They support the continued but careful use of glyphosate herbicide for conifer release in commercial plantations.

References :

Agrios, G.N. 1988. Plant Pathology. Third edition. Academic Press, San Diego.

Alaback, P., Antos, J., Goward, T., Lertzman, K., MacKinnon, A., Pojar, J., Pojar, R., Reed, A., Turner, N. and Vitt, D. 1994. *In* Pojar, J and MacKinnon, eds. Plants of Coastal British Columbia including Washington, Oregon & Alaska. The B.C. Ministry of Forests and Lone Pine Publishing, Vancouver.

Ali, A. and Fletcher, R.A. 1978. Phytotoxic action of glyphosate and amitrole on corn seedlings. Canadian Journal of Botany 56:2196-2202.

Allermann, K.. and Sortkjaer, O. 1973. Rhizomorph formation in fungi, II. The effect of 12 different alcohols on growth and rhizomorph formation in *Armillaria mellea* and *Clitocybe geotropa*. Physiologia Plantarum 28: 51-55.

Amrhein, N., Schab, J. and Steinrucken, H.L. 1980. The mode of action of the herbicide glyphosate. Naturwissenschaften 67: 356-357.

Amrhein, N., Johanning., D., Schab, J. and Schultz. 1983. Biochemical basis for glyphosate-tolerance in a bacterium and a plant tissue culture. Federation of European Biochemist Societies. Federation of European Biochemistry Societies Letters 157: 191-196.

Amrhein, N., Hollander-Czytko, H., Leifeld, J., Schulz, A., Steinrucken, H.C. and Topp, H. 1982. Inhibition the shikimate pathway by glyphosate. *In* Boudet, A.M and Rajeva, R. , eds. Journees internationales d' etude du Groups Polyphenols. Bulletin de Liaison, vol. II. Tolouse, France. Pp. 21-30.

Ayers, W.A. and Lumsden, R.D. 1975. Factors affecting production and germination of oospores of three *Pythium* species. Phytopathology 65: 819-821.

Baker, E. A. 1974. The influence of environment on leaf wax development in *Brassica oleracea* var. *gemmifera*. New Phytologist 73: 955-966.

Berner, D.K., Berggren, G.T. and Snow, J.P. 1991. Effect of glyphosate on *Colonetrica crotalariae* and red crown rot of soybean. Plant Disease 75: 809-813.

Bliev,, Y.K. 1983. Influence of glyphosate on the organic matter mineralization and enzymatic activity of the soddy-podzolic soil. Pochvovedeneie 4:84-79.

Blowes, W.M. 1987. Effect of rye grass residues, knockdown herbicides, and fungicides on the emergence of barley in sandy soils. Australian Journal of Experimental Agriculture 27:785-790.

Boateng, J. 1996. Past and future trends in forest management. *In* Comeau , P.G, Harper, G.J Blache, M.E, Boateng, J.O and Gilkeson, L.A, eds. Integrated Forest Vegetation Management: Options and Applications. Proceeding of the Fifth B.C Forest Vegetation Management Workshop. FRDA Report. No.251. Pp. 1-4.

Brammal, R.A. and Higgins, V.J. 1988. The effect of glyphosate on resistance of tomato to Fusarium crown root rot disease and on the formation of host structural defensive barriers. Canadian Journal of Botany 66:1547-1555.

British Columbia Ministry of Forests. 1998. Ecosystem of British Columbia. Ministry of Forests Research Branch, Victoria, British Columbia.

Brown, A.E. and Sharma, H.S.S. 1984. Production of polysaccharide-degrading enzymes by saprophytic fungi from glyphosate-treated flax and their involvement in retting. Annals of Applied Biology 105:65-74

Burton, G.D. 1992. Alternatives to prescribed burning. Unpublished Report. Prepared for John R. Cuthbert, Chief Forester, BC Ministry Forests., Victoria B. C.

Byler, J.W., Marsden, M.A and Hagle, S.K. 1986. Opportunities to evaluate root disease incidence and damage using forest inventory and permanent growth plots. *In*: Proceedings of the 34th Annual Western International Forest Disease Work Conference, 8-12 September 1986. Juneau, Alaska. USDA Forest Service, Portland, Oregon. Pp. 52-56

Campbell, R.A.1990. Herbicide use for forest management in Canada: where we are and where we are going. Forestry Chronicle. 66:355-360.

Campbell, P. and Long, A. 1995. Vegetation management in Florida's private non-industrial forests. Cooperative Extensive Service. University of Florida.

Canadian Council of Forest Ministers. 2000. National Forestry Database Program-Compendium of Canadian Forestry Statistics [online] . http://www.nfdp.ccfm.org/cp95/data_e/tab96e_l.htm

Canadian Pulp and Paper Association (CPPA) 1986. Herbicides registered for forest and woodlands management, Forest Protection Committee.

Canal, M.J., Albuerne, M., Sanchez Tames, R. and Fernandez, B. 1990. Glyphosate injury on *Cyperus esculentus* leaves and basal bulbs: histological study. Weed Research 30: 117-122.

Carlson, L.W and Prasad, R. 1981. Herbicides and Forestry. Environment Canada., Canadian Forestry Service., Forest Pest Management Institute, Sault Ste. Marie, Ontario.

Carter, M.C., Holt, H.A., and Richard, W. 1978. Alternative methods of vegetation management for timber production. *In* Symposium on the Use of Herbicides in Forestry, Feb. 21-22, 1978. U.S. Department of Agriculture Office Secretariat., Washington, D. C.

Caseley, J.C. and Coupland, D. 1985. Environmental and plant factors affecting glyphosate uptake, movement and activity. *In* Grossbard, E and Atkinson, D, eds. The Herbicide Glyphosate. Butterworths, London. Pp. 92-124.

Cerkauskas, R.F., Dhingra, O.D. and Sinclair, J.B.1982. Effect of herbicides on competitive saprophytic colonization by *Marcophomina phaseolina* of soybean stems. Transactions of the British Mycological Society 79: 201-205.

Chase, R.L. and Appleby, A. P. 1979. Effect of humidity and moisture stress on glyphosate control of *Cyperus rotundus* L. Weed Research 19: 241-246.

Clason, T.R. 1993. Hardwood competition reduces loblolly pine plantation productivity. Canadian Journal of Forest Research 23:2133-2140.

Clement, C. and Keeping, B. 1996. Evaluation of forest vegetation community dynamics on the Bush River brushing trial site. Canadian Forest Service and British Columbia Ministry of Forests., Victoria, B.C. FRDA Rep. 241.

Coggins, J.R. 1989. The shikimate pathway as target for herbicides. *In* Dodge, A. D, ed. Herbicides and Plant Metabolism. Society foe Experimental Biology. Seminar Series 38. Pp 97-112.

Cole, D. J. 1985. Mode of action of glyphosate- a literature analysis. *In* Grossbard, E and Atkinson, D, eds. The Herbicide Glyphosate. Butterworths, London. Pp 48-74.

Cole, D.J., Dodge, A.D. and Caseley, J.C. 1980. Some biochemical effects of glyphosate in plant meristems. Journal of Experimental Botany 55: 1665-1674.

Cole, D.J., Dodge, A.D. and Caseley, J.C. 1983. Influence of glyphosate on selected plant processes. Weed Research 23: 173-183.

Comai, L., Sen, L.C. and Stalker, D.M. 1983. An altered aroA gene product confers resistance to the herbicide glyphosate. Science 221: 370-744.

Comai, L., Faciotti. D., Hiatt, W.R., Thompson. G., Rose., R and Stalker., D. 1985. Expression in plants of a mutant aroA gene from *Salmonella typhimurium* confers tolerance to glyphosate. Nature 317:741-744.

Cruickshank, M.G., Morrison, D.J. and Punja, Z.K. 1997. Incidence of *Armillaria* species in precommercial thinning stumps and spread of *Armillaria ostoyae* to adjacent Douglas-fir trees. Canadian Journal of Forest Research 27: 481-490.

Descalzo, R. 1996. The Role of *Pythium* as Glyphosate Synergistic Fungi. Ph.D Thesis. Simon Fraser University, Burnaby, BC. Canada.

Descalzo, R.D., Punja, Z.K., Lesveque, C.A. and Rahe, J.E. 1996. Assessment of host specificity among different species of glyphosate synergistic *Pythium.* Mycological Research 100: 1445-1453

Domsch, K.H., Gams, W. and Anderson, T. 1980. Compendium of Soil Fungi . Volume 1 and 2. Academic Press. London, San Francisco.

Environment Canada. 1998. Land Polution-Atlantic Region Green Lane. [online]. http://www.ns.ec.gc.ca/pollution/land.html

Environmental Canada. 1998. Pesticide and the Environment in the Atlantic Region [online] available : http://www.ns.ec.gc.ca/epb/fiddle/index.html

Fillatti, J.J., Kiser, J., Rose, R. and Comail, L. 1987. Efficient transfer of a glyphosate tolerance gene into tomato using a binary *Agrobacterium tumefaciens* vector. Biotechnology 5:726-730.

Finnis, J.M. 1967. Precommercial thinning with chemicals. *In* Symposium Proceeding Herbicide Vegetation Management. Oregon State University, Corvallis, Oregon. Pp. 161-166

Fosberg, F.R. 1967. A classification of vegetation for general purposes. *In* Peterken, G.F. ed. Guide to the check sheet for IBP areas. IBP Handbook No.4. Blackwell Scientific Publication, Oxford and Edinburgh. Pp 73-133

Franz, J.E. 1974. N-Phosphonomethylglycine phytotoxicant composition. U. S. Patent No. 3,399, 758.

Franz, J.E. 1985. Discovery, development and chemistry of glyphosate. *in* . Grossbard, E. and Atkinson, D, eds. The Herbicide Glyphosate. Butterworths. London. Pp. 3-17.

Franz , J.E., Mao, M. Kard., Sikorski, J.A. 1997. Glyphosate: a unique global herbicide. American Chemistry Society . Monograph 189.

Fraser, R.G. 1989. Management of laminated root rot caused by *Phellinus weirii* (Murr.) Gilbertson in Douglas-fir. M.P.M. Professional paper. Simon Fraser University. Burnaby, B. C.

Gatenby, J. B. and Beams, H. W. 1950. The Microtomist's Vade-mecum. J. and A. Chruchill. London.

Glass, R.L. 1986. 192nd National Meeting of the American Chemical Society. Abstract No. AGRO 94, September 7-12. Anaheim, California.

Glass, R.L.1987. Adsorption of glyphosate by soils and clay minerals. Journal of Agriculture and Food Chemistry 35: 497-500.

Glover, G.R., Zutter, B.R. and Gjerstad. 1993. Loblolly pine and mixed hardwood stand dynamics for 27 years following chemical, mechanical and manual site preparation. Canadian Journal of Forest Research 23:2126-2132.

Gregory, S.C., Rishbeth, J. and Shaw, C.G, III. 1991. Pathogenicy and virulence. *In* Shaw, C.G. III and Kile, G. A. eds. Armillaria Root Disease Agriculture Handbook No. 691. USDA Forest Service, Washington, D.C. Pp. 76-87.

Gresshoff, P.M. 1979. Growth inhibition by glyphosate and reversal of its action by phenylalanine and tyrosine. Australian Journal of Plant Physiology 6:177-185.

Grossbard, E.1985. Effects of glyphosate on microflora with reference to the decomposition of treated vegetation and interaction with some plant pathogens. *in* Grossbard, E. and Atkinson, D, eds.. The Herbicide Glyphosate Butterworths. London. Pp. 159-185.

Gonsolus, J.L. and Curran, W.S. 1998. Herbicide Mode of Action and Injury symptoms. North Central Regional Publication No. 377.

Gunthard-Goerg, M.S. 1978. Epicuticular wax formation on needles of *Picea abies* and *Pinus cembra. In* Stumpf, P. K ed. The Metabolism, Structure and Function of Plant Lipids. Plenum, New York. Pp. 557-559.

Haeussler, S. 1990. Prescribed Fire for Forest Vegetation Management. FRDA Memo No.198. FRDA and BC Ministry of Forests, Victoria, B.C.

Hallet, R. 1985. Weed control in forest nursery. Proceedings of the Canadian Forest Nursery Workshop. Fredericton, New Brunswick.

Hallet, R.. and Dufour, A.J. 1983. Intensive site preparation for research plantations in forest areas. Environment Canada, Canadian Forest Service, Maritime Forest Research Centre, Fredericton, New Brunswick. Technical Note No. 57.

Harrington, T.B., Wagner, R.B., Radosevich, S. R. and Walstad, J.D. 1995. Interspecific competition and herbicide injury influence 10-year response of coastal Douglas-fir and associated vegetation to release treatments. Forest Ecology and Management 76:55-67

Harris, D and Grossbard, E. 1979. Effect of the herbicides gramoxone and Roundup on *Septoria nodorum.* Transactions of the British Mycological Society 73:27-34.

Hart, D. and Comeau, P.G. 1992. Manual brushing for forest vegetation management in British Columbia: A review of current knowledge and information needs. B. C. Ministry Forest Research Branch, Victoria B.C. Land Management Report 77.

Hawkes, B., Feller, M.C. and Meehan, D. 1990. Site preparation: Fire. *In* Regenerating British Columbia's Forests. Lavender, D. [*et. al.*,] eds. U.B.C Press, Vancouver. Pp. 131-149.

Hederlie, L.C., Widholm, J.M. and Slife, F.W. 1977. Effect of glyphosate on tobacco cells. Plant Physiology 60: 40-43.

Hiratsuka, Y. 1987. Forest Tree Diseases of the Prairie Provinces. Canadian Forestry Service, Northern Forest Centre, Edmonton, Alberta. Information Report. No. NOR-X-286.

Hoagland, R.E. and Duke,S.O. 1982. Biochemical effects of glyphosate (N-(phosphonomethyl) glycine). *In* Moreland, D.E, St John, J.B and Hess, F.D. eds. Biochemical Responses Induced by Herbicides. America Chemical Society, Symposium Series, No. 181. Pp. 175-205.

Hollander, H. and Amrhein, N. 1980. The site of inhibition of the shikimate pathway by glyphosate, I. Inhibition by glyphosate of phenylpropanoid synthesis in buckwheat (*Fagopyrum esculentum* Moench.). Plant Physiology 66:823-829.

Holloway, P.J. 1982. The chemical constitution of plant cutin. *In* Cutler, D.F Alvin. K.L and Price, C.E. eds., The Plant Cuticle. Academic Press. New York. Pp. 45-86.

Jaworski. E.G. 1972. Mode of action of N-phosphonomethylglycine: inhibition of aromatic amino acid biosynthesis. Journal of Agriculture and Food Chemistry 20:1195-98.

Jaworski, E.G. 1983. Herbicide target sites, mode of action, and detoxification: chloroacetanilides and glyphosate. *In* Thomber, J.P., Staehelin, L.A and Hallick, R.B. eds. Biosynthesis of the Photosynthesis Apparatus: Molecular Biology, Development, and Regulation: Proceedings. UCLA Symposium, Keystone, Colorado, April 21-26, 1983. Pp. 335-349.

Johal, G.S and Rahe, J.E. 1984. Effect of soilborne plant-pathogenic fungi on the herbicidal action of glyphgosate on bean seedlings. Phytopathology 4:950-955.

Johal, G.S. and Rahe, J.E. 1988. Glyphosate, hypersensitivity and phytoalexin accumulation in the incompatible bean anthracnose host-parasite interaction. Physiological and Molecular Plant Pathology 32:267-281.

Johal, G.S. and Rahe, J.E. 1990. Role of phytoalexins in suppression of resistance of *Phaseolus vulgaris* to *Colletotrichum lindemunthianum* by glyphosate. Canadian Journal of Plant Pathology 12: 225-235.

Johansen, D.A. 1940. Plant Microtechnique. McGraw-Hill, New York.

Jones, R.C and Boateng, J.O. 1983. Weeds and Forestry. Expert Committee on Weeds, West Sector Meeting. November 29 - December 1, 1983, Vancouver, B.C.

Jordan, T.N. 1977. Hooded sprayer for glyphosate application in cotton. Proceeding South Weed Science Society 30:417.

Keen, N.T. Holliday, M.J. and Yoshikawa, M. 1982 Effect of glyphosate on glyceollin production and the expression of resistance to *Phytophthora megasperma f.sp. glycinae* in soybean. Phytopathology 72:1467-1470.

Kelley, P.E., Thullen, R.J., Carter, C.H. and Miller, J.H. 1984. Control of Johnsongrass (*Shorghum halepense*) in cotton (*Gossypium hirsutum*) with glyphosate. Weed Science 32: 306-309.

Kile, G.A., McDonald, G.I. and Byler, J.W. 1991. Ecology and disease in natural forests. *In* Armillaria Root Disease. Agriculture Handbook No. 691. USDA Forest Service, Washington, D.C. Pp. 102-121.

Lange, A., Kempen, H., McHenary, W and Leonard, O. 1973. Roundup-a new perennial weed killer. California Agriculture 26: 6-7.

Lateigne, L.J., Burns, T.W and Hallet, R.D. 1987. Effect of various herbicides on the growth of black spruce container seedlings. *In* Vegetation Management Activities of Forestry Canada: A Compendium of Summaries 1988/89. Forestry Canada's Vegetation Management Working Group. Forestry Canada Ottawa.

Ledbetter, M.C. and Gunning, B. E. S. 1964. Glutaralehyde-osmic fixation of plant cells. Symposium On Botanical Application of Electron Microscopy. Journal of the Royal Microscopic Society. 83, 331

Lee, T.T. 1981. Effects of glyphosate on synthesis and degradation of chlorophyll in soybean and tobacco cells. Weed Research 21: 161-164.

Lee, T.T. 1982. Mode of action of glyphosate in relation to metabolism of indole-3-acetic acid. Physiologia Plantarum 54:289-294.

Lesveque, C.A. 1990. The nature and significance of fungal colonizers in the herbicidal effect of glyphosate. Ph.D Thesis. Simon Fraser University, Burnaby, BC

Levesque, C.A. and Rahe, J.E. 1989. Fungal colonization of glyphosate treated plants. Canadian Journal Plant Pathology 10:193-194.

Levesque, C.A. and Rahe, J.E. 1992. Herbicide interactions with fungal root pathogens, with special reference to glyphosate. Annual Review of Phytopathology 30:579-602

Levesque, C.A., Rahe, J.E. and Eaves, D.M. 1987. Effect of glyphosate on *Fusarium* spp.: its influence on root colonization of weeds, propagule density in the soil, and crop emergence. Canadian Journal of Microbiology 33:354-360.

Levesque, C.A., Rahe, J. . and Eaves, D.M. 1992. The effect of soil heat treatment and microflora on the efficacy of glyphosate in seedlings. Weed Research 32: 363-373.

Levesque, C.A. and Rahe, J.E. and Eaves, D.M. 1993. Fungal colonization of glyphosate-treated seedlings using a new root plating technique. Mycological Research 97:299-306.

Liu, L. 1995. Studies of mechanisms of predisposition by glyphosate on bean roots (*Phaseolus vulgaris L.*) to colonization by *Pythium spp.* Ph.D thesis. Simon Fraser University. Burnaby, B. C. Canada.

Liu, L., Punja, Z.K. and Rahe, J .E. 1997. Altered root exudation and suppression of induced lignification as mechanisms of predisposition by glyphosate of bean roots (*Phaseolus vulgaris* L.) to colonization by *Pythium* spp. Physiological and Molecular Pathology 51: 111-127

Low, S and Hamilton, E. 1991. Alternatives to prescribed burning: a review of the reforestation information bank (RIB). B. C. Min. For. Victoria, B. C.

Lund-Hoie, K. 1985. Efficacy of glyphosate in forest plantation. *In* Grossbard, E and Atkinson, D. eds. The Herbicide Glyphosate. Butterworths. London. Pp.328-338.

Lund-Hoie, K. 1980. The impact of helicopter application of glyphosate on the management of Norwegian forest plantations. Proceedings of Weed Control in Forestry Conference. Pp. 73-81.

Lund-Hoie, K. 1975. N-phospohonomethylglycine (glyphosate), an alternative to commercial pre and postemergence herbicides for the control of unwanted plant species in forest plantation in Norway. Scientific Reports of the Agricultural University of Norway 54.

Lynch, J.M. and Penn, D.J. 1980. Damage to cereals caused by decaying weed residues. Journal of the Science of Food and Agriculture 31: 321-324.

McCormack, M.L., Jr. 1981. Chemical weed control in northeast forest. *In* Herbicides in Forestry Proceedings .Byrnes, W.H and Holt , H.A . eds. J.S. Wright Forestry Conference, Purdue Univesity, West Layfayette.

McDonald, G.I., Martin, N.E., and Harvey, A.E. 1987. Occurrence of *Armillaria* spp. in forests of the northern Rocky Mountains. Res. Pap. INT-381. U.S. Department of Agriculture Intermountain Research Station. Ogden, UT.

Meidinger, D. and Pojar, J. 1991. Ecosystems of British Columbia. B. C. Ministry of Forests, Victoria, B. C. Canada.

Miller, J.H., Zutter, B.R., Zedaker, S.M., Edwards, M.B., Haywood, J.D. and Newbold, R.A. 1991. A regional study on the influence of woody and herbaceous competition on early loblolly pine growth. Southern Journal of Applied Forestry 15:168-179.

Mircetich, S.M. 1971. The role of *Pythium* in feeder roots of diseased trees and in orchard soils in peach tree decline. Phytopathology 61:357-360.

Morrison, D.J. 1981. Armillaria root disease. A guide to disease diagnosis, development and management in British Columbia. Canadian Forestry Service. Pacific Forest Centre. Victoria, B.C. No. BC-X-203.

Moorhead, D.J. 1998. Pine damage from cotton herbicides and defoliants. Bugwood Publication No. 98-001. The University of Georgia, USA.

Myern, D.T. 1994. Tree Diseases of Eastern Canada. Natural Resources Canada. Canadian Forestry Service, Sustainable Development Dir., Ottawa, Ontario.

Nafziger, E.D., Widholm, J.M., Steinrucken, H.C. and Killmer, J.L. 1984. Selection and characterization of a carrot cell line tolerant to glyphosate. Plant Physiology 76:571-574.

Nash, S.M. and Snyder, W.C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in the field. Phytopathology 52:567-572.

Newton, M. and Knight, F. 1981. Handbook of Weed and Insect Control Chemicals for Forest Resource Managers. Timber Press. Beaverton, Oregon.

Newton, M., Cole, E.C., McCormack, M.L. and White, D.E. 1991. Young spruce-fir forest released by herbicides, II: Conifer response to residual hardwoods and overstocking. Northern Journal of Applied Forestry 9:130-135.

Nova Scotia Department of Lands and Forests. 1988. Efficacy of glyphosate in controlling competing vegetation in Nova Scotia. Forest Research Report, No. 3

Nova Scotia Department of Lands and Forests. 1989. Conifer Susceptibility to Glyphosate in Nova Scotia. Forest Research Report, No. 10

Ono, K. 1970. Effect of soil conditions on the occurrence of Armillaria root rot of Japanese larch. Review of Plant Pathology 50:2001.

Payne, N.J., Feng, J.C. and Reynolds, P.E. 1990. Off-target deposits and buffer zones required around water for aerial glyphosate application. Pesticide Science 30:183-198.

Pojar, J. 1983. Ecosystem classification by the British Columiba Ministry of Forest. *In* Watt, S.B. ed. Forestry Handbook for British Columbia. Faculty of Forestry, University of British Columbia, Vancouver, B.C. Pp. 225-247.

Prasad, R. 1989. Crop tolerance of three west coast conifer species to glyphosate. Pp 189-196 in *Proceeding of the Carnation Creek Herbicide Workshop*. Reynolds, P.E. ed. Forest Pest Management Institute Forestry Canada. Ontario.

Radosevich, S.R., Roncoroni, E.J., Conard, S.G. and McHenry, W.B. 1980. Seasonal tolerance of six coniferous species to eight foliage-active herbicides. *Forest Science* 26:3-9.

Rahe, J.E., Lesveque, C.A. and Johal, G.S. 1990. Synergistic role of soil fungi in the herbicidal efficacy of glyphosate. Pp260-275 in *ACS Symposium Series 439, Microbes and Microbial Products as Herbicides*. Hoagland, R.E, ed. Washington DC: American Chemical Society.

Redfern, D.B. 1975. The influence of food base on rhizomorph growth and pathogenecity of *Armillaria melea* isolates. *In* Bruehl, G. W. ed. Biology and Control soil-borne Plant Pathogens. St. Paul, MN: The American Phytopathological Society: 69-73.

Rennie, D.A., Klassen, W.A., Fuller, G.A., Kenneth, E.H. 1985. Report of the task force on use of herbicides in forest management. Saskatoon Department of Parks and Renewable and Resources, Saskatoon, Saskatchewan

Reynolds, P.E., Pitt, D.G., Whitehead, R. and King, K. 1989. Three-year herbicide efficacy, crop tolerance and crop growth response results for a 1984 glyphosate conifer release trial at Carnation Creek, British Columbia. FRDA Report March 1989.

Roisch, V.F. and Lingens, F.1974. Effect of the herbicide N-phosphonomethylglycine on the biosynthesis of aromatic amino acids. Angewandte Chemie 13:400.

Rueppel , M.L., Bright, B.B, Schaefer, J. and Marvel, J.T. 1977. Metabolism and degradation of glyphosate in soil and water. Journal of Agriculture Food Chemistry 25: 517-528.

Sabatini, D. D., Bensch, K. and Barnett, R. J. 1963. The preservation of cellular ultrastucture and enzymatic activity by aldehyde fixation. The Journal of Cell Biology 17: 19-58

Sanberg, C.L., Meggitt, W.F. and Penner, D. 1980. Absorption, translocation and metabolism of ^{14}C-glyphosate in several weed species. Weed Research 20:195-200

SAS Institute Inc. 1985. SAS® User's Guide: Statistics, Version 5 Edition. Cary, NC.

Scagel, R. and Caldicott, N. 1991. Silvicultural aspects of site preparation- a coastal perspective of slashburning and its alternatives. Unpublished. Report . Ministry Forests. Silviculture Branch, Victoria, B. C.

Schultz, M.E. and Burnside, O.C. 1980. Absorption, translocation and metabolism of 2,4-D and glyphosate in hemp dogbane (*Apocynum cannabinum*). Weed Science 28:13-20.

Sharma, V., Adee, E.A. and Pfender, W.F. 1989. Effect of glyphosate herbicide on pseudothecia formation by *Pyrenophora triticirepentis* in ingested wheat straw. Plant Disease 73:647-650.

Shearer, B.L. and Tippett, J.T. 1988. Distribution and impact of *Armillaria luteobubalina* in the *Eucalyptus marginata* forest of south-western Australia. Australian Journal of Botany 36: 433-445.

Singh, P. 1983. Armillaria root rot: Influence of soil and nutrients and pH on the susceptibility of conifer species to the disease. European Journal of Forest Pathology 13: 92-101.

Smiley, R.W., Ogg, A.G.Jr. and Cook, R.J. 1992. Influence of glyphosate on Rhizoctonia root rot, growth, and yield of barley. Plant Disease 76:937-942.

Smith, A.M. and Griffin, D.M. 1971. Oxygen and the ecology of *Armillaria elegans* Heim. Australian Journal of Biological Sciences 24:231-162.

Sprankle, P. Meggitt, W.F. and Penner, D. 1975. Absoprtion, action, and translocation of glyphosate. Weed Science 23: 235-240

Stalker, D.M., Hiatt, W.R., Comai, L. 1985. A single amino acid substitution in the enzyme 5-enolpyruvylshikimate-3- phosphate synthase confers resistance to the herbicide glyphosate. Journal of Biological Chemistry 260: 4724-4728.

Stedman, O.J. 1982. The effect of three herbicides on the number of spores of *Rhynchosporium secalis* on barley stubble and volunteer plants. Annals of Applied Biology 100:271-279.

Steinrucken, H.C. and Amrhein, P. 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. Biochemical and Biophysical Research Communication 94:1207-1212.

Steinrucken, H.C. and Amrhein, N. 1984. 5-enolpyruvilshikimate-3-phosphate synthase of *Klebsiella pneumoniae* 2. Inhibition by glyphosate [N(phosphonomethyl) glycine]. European Journal of Biochemistry 143: 351-357.

Sutton, R.F. 1978. Glyphosate herbicide: an assessment of forestry potential. Forestry Chronicle 54: 24-28.

Taylor, S.P. 1986. Forest insect and disease impacts in timber supply areas. Pest Management Report No.6. British Columbia. Ministry of Forests, Forest Protection Branch, Victoria, B. C.

Titus, B., Lanteigne, L., Delisle, C., Wood, J., Sutto, R., Althen, F., Reynolds, P., Pitt, D., Brace, L., Feng, L., Sidhu, S., Ball, N., Oswald, E., Hall,P. J. eds. 1989. Vegetation Management Activities of Forestry Canada: A Compendium of Summaries 1988/89. Forestry Canada's Vegetation Management Working Group. Forestry Canada Ottawa

Todd, A.M.D. 1991. A documentation of mechanical site preparation alternatives to the use of prescribed fire. Unpublished Report B. C. Ministry of Forests, Victoria, B. C.

USDA Forest Service, 1977. The use of herbicide in the eastern region. Eastern Region, Milwaukee, Wisconsin. Draft Environmental Statement.

Vaughn, K.C. and Duke, S.O. 1986. Ultrastuctural effects of glyphosate on *Glycine max* seedlings. Pesticide Biochemistry and Physiology 26:56-65.

Walstad, J.D., Newton, M and Gjerstad, D.H. 1987. Overview of vegetation management alternatives. *In* Walstad, J. D. and . Kuch, P.J. eds. John Forest Vegetation Management for Conifer Production. Wiley & Sons. New York.

Wanner, K. 1994. Microbial and insect colonization of Douglas-fir stumps and roots with special reference to *Phellinus weirii* (Murr.) Gilbertson and glyphosate application. M.P.M. Thesis. Simon Fraser University. Burnaby, B.C.

Wardle, D.A and Parkinson, D. 1990. Determination of bacterial and fungal fumigation Kc factors across a soil moisture gradient. Soil Biology and Biochemistry 22:811-816.

Wargo, P.M. and Harrington, T.C. 1991. Host stress and susceptibility. *In* C. G. Shaw, III and G.A. Kile, eds. Armillaria Root Disease. Agriculture Handbook No. 691. USDA Forest Service. Washington, D.C. Pp. 88-101.

Wargo, P.M. and Shaw, III, C.G. 1985. Armillaria root rot: The puzzle is being solved. Plant Disease 69: 826-832.

Wauchope, R.D., Buttler, T.M., Hornsby A.G., Augustijn Beckers, P.W.M. and Burt, J.P. 1992. SCS/ARS/CES Pesticide properties database for environmental decision making. Reviews of Environmental Contamination and Toxicology 123: 1-157.

Weinhold, A.R. 1963. Rhizomorph production by *Armillaria mellea* induced by ethanol and related compounds. Science 142:1065-1066.

Wiensczyk, A.M., Dumas, M.T. and Irwin, R.N. 1997. Effect on northwestern Ontario forest ecosystem classification treatment units on the infection levels of *Armillaria* in black spruce plantations. Canadian Journal of Forest Research 26: 1248-1255.

Whigham, D.K. and Stoller, E.W. 1979. Soybean desiccation by paraquat, glyphosate and ametryn to accelerate harvest. Agronomy Journal 71: 630-633.

Appendix 1. Mortality of glyphosate treated bean seedlings growing in heat-treated soil amendment with different isolates of *Pythium* and *Fusarium* .

Isolates	Doses of glyphosate (ug) 0	5	10	25	100
Pythium 1	0/19 **	3/20	4/17	11/20	13/20
*Pythium*2	0/23	9/25	16/25	14/24	20/20
*Pythium*3	0/20	8/18	11/22	17/23	21/24
*Pythium*4*	0/18	10/21	16/20	22/25	20/21
*Pythium*5*	0/20	15/19	16/22	22/25	22/22
*Pythium*6	1/21	4/18	13/25	18/20	25/25
*Pythium*7	0/21	4/16	5/16	9/19	13/20
*Fusarium*1	0/24	2/22	11/24	12/24	18/21
*Fusarium*2*	0/22	8/17	11/18	17/18	14/16
*Fusarium*3	0/17	5/16	10/18	13/20	11/18
*Fusarium*4*	0/20	3/16	12/20	17/18	21/21
*Fusarium*5	0/18	2/20	5/19	15/18	13/18
*Fusarium*6	0/14	0/15	10/20	8/14	18/24
Control	0/17	0/19	0/20	0/20	2/21

* used in experiment 3.

** number of plants killed/number of plants treated

Appendix 2: Number of seedlings dead in relation to the total number of seedlings treated. Seedlings treated after 6 months. Treatment applied in November 1996, data were collected 2 months after treatment

	0%	:	0.11%	:	0.33%	:	1%
Douglas fir							
Treated soil	1/20	:	7/20	:	8/20	:	11/20
Untreated	1/20	:	7/20	:	9/20	:	15/20
Lodgepole pine							
Treated soil	0/19	:	8/20	:	8/20	:	12/20
Untreated	1/20	:	7/20	:	10/20	:	14/20

Appendix 3: Number of seedlings dead in relation to the total number of seedlings treated. Seedlings were treated at 9 months of age. Treatment applied in March 1997, data were collected 2 months after treatment

	0%	:	0.11%	:	0.33%	: 1%
Douglas fir						
Treated soil	1/20	:	4/20	:	7/20	: 8/20
Untreated	1/20	:	3/20	:	8/20	: 10/20
Lodgepole pine						
Treated soil	1/19	:	7/20	:	10/20	:10/18
Untreated	1/20	:	7/20	:	11/20	:12/20

Appendix 4. Estimates of glyphosate LD_{50} value for yellow pine and spruce seedlings growing in untreated and treated soil, glyphosate applied on 18 month old seedlings. Estimates are based on linear regression of plant response (log1-y/y) versus [log(dose+1)].

	untreated soil		treated soil	
	LD_{50}	R^2	LD_{50}	R^2
Yellow pine	125.00	0.946	>138.9	0.952
Spruce	>138.9	0.501	>138.9	0.416

Appendix 5. Mean number of fungal colonies per plate isolated from roots of selected woody species after foliar application of vision

Woody species	Treatment time	Fungal identification	Dose (%) of Vision	Mean number of colonies per plate by time after treatment 1 week	2 week	3 week
Alnus rubra	Fall 1996	*Pythium spp.*	0.00	2.00	0.00	0.00
			0.11	0.00	1.67	1.50
			0.33	0.00	3.00	1.75
			1.00	1.50	3.33	1.50
		Fusarium spp.	0.00	1.00	0.67	0.50
			0.11	0.50	0.33	0.25
			0.33	0.50	0.33	0.25
			1.00	1.00	0.00	0.50
		Cylindrocarpon spp.	0.00	0.75	1.00	0.50
			0.11	0.75	1.33	0.50
			0.33	0.75	1.00	0.75
			1.00	1.00	1.00	0.75
		Trichoderma spp.	0.00	0.50	0.67	0.50
			0.11	0.75	1.33	0.50
			0.33	0.75	0.67	0.50
			1.00	1.25	2.00	1.50
		Black fungus	0.00	5.50	10.67	7.00
			0.11	5.00	6.33	5.50
			0.33	3.75	3.33	4.75
			1.00	4.50	3.00	2.00
		Yellow fungus	0.00	0.75	1.00	0.50
			0.11	0.50	1.00	0.50
			0.33	0.75	1.67	0.50
			1.00	0.50	0.67	0.50
		White fungus	0.00	1.00	1.67	0.75
			0.11	1.00	1.33	1.00
			0.33	1.50	2.67	1.75
			1.00	1.00	2.33	1.00
	Spring 1997	*Pythium spp.*	0.00	0.00	1.00	0.75
			0.11	0.33	2.00	0.50

			0.33	0.00	1.67	0.75
			1.00	2.00	0.67	0.50
		Fusarium spp.	0.00	0.25	0.00	1.50
			0.11	0.25	0.00	1.50
			0.33	0.25	0.00	0.00
			1.00	0.25	2.00	0.50
		Cylindrocarpon spp.	0.00	0.33	0.33	0.00
			0.11	0.67	1.00	0.75
			0.33	0.33	0.00	0.00
			1.00	1.00	1.00	0.25
		Trichoderma spp.	0.00	0.67	1.00	0.50
			0.11	0.33	0.33	0.00
			0.33	0.67	1.00	0.75
			1.00	0.33	0.33	0.00
		Black fungus	0.00	12.00	13.50	12.33
			0.11	16.67	18.25	19.00
			0.33	18.67	20.75	22.00
			1.00	18.00	19.75	21.00
		Yellow fungus	0.00	2.00	2.33	1.75
			0.11	1.00	1.33	0.75
			0.33	5.33	8.66	4.50
			1.00	1.67	2.66	1.50
		White fungus	0.00	2.67	3.33	2.75
			0.11	7.00	8.33	5.25
			0.33	3.33	8.66	3.75
			1.00	2.33	3.33	2.75
Betula papyrifera	Fall 1996	*Pythium spp.*	0.00	0.50	0.50	1.50
			0.11	1.75	0.00	0.75
			0.33	0.00	1.00	1.50
			1.00	3.00	0.25	1.00
		Fusarium spp.	0.00	1.00	0.50	1.00
			0.11	1.00	0.00	0.50
			0.33	0.00	0.50	1.00
			1.00	1.00	1.00	0.50

	Cylindrocarpon spp.	0.00	0.25	0.25	0.25
		0.11	0.75	0.75	0.75
		0.33	0.50	0.50	0.25
		1.00	0.75	1.25	1.25
	Trichoderma spp.	0.00	0.50	0.50	0.25
		0.11	0.75	0.75	0.50
		0.33	1.75	1.50	1.50
		1.00	1.25	2.00	1.75
	Black fungus	0.00	2.50	4.75	5.00
		0.11	2.50	3.75	1.75
		0.33	1.75	1.50	2.00
		1.00	2.50	3.25	2.50
	Yellow fungus	0.00	0.50	0.25	0.25
		0.11	0.50	0.50	0.25
		0.33	0.25	0.50	0.25
		1.00	0.50	0.50	0.25
	White fungus	0.00	1.00	1.00	0.75
		0.11	1.50	1.00	0.75
		0.33	1.75	1.25	1.50
		1.00	2.00	1.25	1.00
Spring 1997	*Pythium spp.*	0.00	0.50	0.00	1.00
		0.11	3.75	1.67	1.50
		0.33	1.00	3.00	1.75
		1.00	4.00	3.33	1.50
	Fusarium spp.	0.00	1.25	1.66	0.75
		0.11	0.25	0.33	0.25
		0.33	0.00	0.33	0.25
		1.00	1.25	0.00	0.50
	Cylindrocarpon spp.	0.00	0.75	1.33	1.00
		0.11	0.25	1.00	0.50
		0.33	0.50	2.00	1.00
		1.00	1.50	4.33	2.00
	Trichoderma spp.	0.00	0.75	1.33	1.00
		0.11	0.25	0.66	0.50
		0.33	0.50	1.33	0.50

			1.00	1.25	3.33	1.50
		Black fungus	0.00	14.00	15.33	15.00
			0.11	13.33	18.33	16.00
			0.33	18.67	22.00	20.00
			1.00	14.00	19.33	16.67
		Yellow fungus	0.00	0.00	0.00	0.25
			0.11	0.75	1.66	1.00
			0.33	0.25	0.66	0.50
			1.00	0.00	1.00	0.25
		White fungus	0.00	1.00	1.00	1.00
			0.11	1.25	3.00	2.00
			0.33	2.25	4.66	3.00
			1.00	1.75	5.66	3.50
	Fall 1997 (selective media)	*Pythium spp.*	0.00	6.50	5.25	3.33
			0.11	6.75	5.25	2.33
			0.33	8.50	4.75	4.33
			1.00	11.25	4.75	4.00
		Fusarium spp.	0.00	0.75	4.00	2.33
			0.11	1.75	5.33	3.66
			0.33	1.75	5.00	2.00
			1.00	2.50	3.00	4.33
Pseudotsuga menziesii	Fall 1996	*Pythium spp.*	0.00	0.50	0.00	1.00
			0.11	0.50	1.00	1.50
			0.33	0.00	3.00	1.75
			1.00	0.50	3.33	1.50
		Fusarium spp.	0.00	0.50	0.67	0.75
			0.11	0.50	0.33	0.25
			0.33	0.00	0.33	0.25
			1.00	0.50	0.00	0.50
		Cylindrocarpon spp.	0.00	0.75	1.00	0.50
			0.11	0.75	1.00	0.75
			0.33	1.00	0.66	0.75
			1.00	1.00	1.00	0.75
		Trichoderma spp.	0.00	0.50	0.66	0.50
			0.11	0.75	1.33	0.50
			0.33	0.50	0.66	0.75

		1.00	0.50	1.33	1.00
	Black fungus	0.00	7.75	10.00	5.00
		0.11	4.00	6.66	6.25
		0.33	4.50	5.00	3.50
		1.00	3.00	5.33	2.50
	Yellow fungus	0.00	0.75	1.00	0.50
		0.11	0.50	1.00	0.50
		0.33	0.75	1.66	0.50
		1.00	0.50	0.66	0.50
	White fungus	0.00	1.25	1.00	1.25
		0.11	1.00	1.33	0.75
		0.33	1.25	2.33	2.00
		1.00	1.00	1.66	1.50
Spring 1997	*Pythium spp.*	0.00	0.50	0.33	1.25
		0.11	1.50	0.67	1.50
		0.33	2.00	4.33	1.75
		1.00	3.00	2.33	1.50
	Fusarium spp.	0.00	0.00	0.00	1.00
		0.11	0.50	0.25	0.25
		0.33	0.50	0.00	0.75
		1.00	0.25	0.25	0.75
	Cylindrocarpon spp.	0.00	0.50	1.00	0.50
		0.11	0.75	1.33	0.50
		0.33	1.00	2.66	1.75
		1.00	0.25	0.66	0.50
	Trichoderma spp.	0.00	1.75	2.66	2.50
		0.11	3.25	4.33	5.00
		0.33	2.00	2.00	2.25
		1.00	3.00	5.00	3.50
	Black fungus	0.00	20.25	23.33	21.50
		0.11	22.25	29.30	26.50
		0.33	23.50	29.00	28.00
		1.00	20.00	28.67	28.00
	Yellow fungus	0.00	1.00	1.33	0.75
		0.11	0.00	0.00	0.25
		0.33	0.50	0.67	0.25
		1.00	2.00	2.66	1.75

		White fungus	0.00	1.50	3.00	2.25
			0.11	1.00	1.66	1.75
			0.33	3.75	6.33	3.00
			1.00	1.00	2.00	2.50
						1.50
	Fall 1997 (selective media)	*Pythium spp.*	0.00	3.66	1.66	3.00
			0.11	4.33	3.00	3.33
			0.33	4.33	4.66	4.00
			1.00	8.00	5.66	3.00
		Fusarium spp.	0.00	4.25	2.00	4.33
			0.11	6.50	4.00	3.66
			0.33	6.25	3.33	5.33
			1.00	7.75	6.33	6.00
Pinus contorta						
	Fall 1996	*Pythium spp.*	0.00	0.25	0.50	0.25
			0.11	0.25	1.50	0.50
			0.33	0.00	1.50	0.75
			1.00	0.00	0.50	1.00
		Fusarium spp.	0.00	0.50	0.50	0.50
			0.11	0.00	1.00	0.50
			0.33	0.00	0.50	0.75
			1.00	0.50	0.50	0.50
		Cylindrocarpon spp.	0.00	0.75	0.75	0.75
			0.11	1.25	1.00	1.00
			0.33	1.75	0.75	1.00
			1.00	0.75	1.00	0.50
		Trichoderma spp.	0.00	1.50	2.00	1.50
			0.11	1.25	1.75	0.75
			0.33	0.75	0.75	0.75
			1.00	2.25	2.00	1.75
		Black fungus	0.00	3.00	5.00	4.00
			0.11	3.50	1.50	4.00
			0.33	2.50	2.75	2.25
			1.00	2.50	2.25	2.25
		Yellow fungus	0.00	0.75	5.00	3.00
			0.11	0.50	1.50	1.00
			0.33	0.25	2.75	2.00
			1.00	0.00	2.25	1.00

	White fungus	0.00	0.75	0.75	3.00
		0.11	1.25	1.25	5.00
		0.33	1.75	1.50	5.00
		1.00	1.25	1.50	3.00
Spring 1997	*Pythium spp.*	0.00	0.50	0.50	1.00
		0.11	0.75	1.00	1.00
		0.33	1.00	1.00	2.50
		1.00	1.50	2.00	1.50
	Fusarium spp.	0.00	0.50	0.50	0.00
		0.11	0.00	0.00	0.50
		0.33	1.00	1.00	0.00
		1.00	0.25	0.00	1.25
	Cylindrocarpon spp.	0.00	0.75	1.25	1.00
		0.11	0.50	0.75	0.75
		0.33	1.25	1.50	1.25
		1.00	0.25	1.00	0.75
	Trichoderma spp.	0.00	0.75	0.75	0.75
		0.11	0.25	1.00	1.00
		0.33	0.00	0.50	0.50
		1.00	1.25	2.00	1.25
	Black fungus	0.00	14.25	14.50	15.50
		0.11	10.25	12.00	11.50
		0.33	18.50	20.50	18.25
		1.00	17.25	18.75	17.75
	Yellow fungus	0.00	0.50	0.75	0.50
		0.11	0.75	2.25	1.50
		0.33	0.50	0.50	1.00
		1.00	0.25	0.25	0.25
	White fungus	0.00	4.00	5.50	4.75
		0.11	2.50	3.75	2.25
		0.33	2.50	5.25	3.75
		1.00	4.50	6.00	4.75
Fall 1997 (Selective media)	*Pythium spp.*	0.00	2.50	4.00	3.33
		0.11	2.25	4.00	2.33
		0.33	4.00	5.66	4.33

			1.00	4.00	4.66	4.00
		Fusarium spp.	0.00	3.25	6.00	7.66
			0.11	4.00	4.33	5.00
			0.33	4.75	4.33	6.66
			1.00	4.50	7.66	7.33
Picea sp						
	Fall 1996	*Pythium spp.*	0.00	0.50	0.50	0.25
			0.11	1.50	2.00	0.50
			0.33	0.75	0.75	0.75
			1.00	0.50	1.50	2.00
		Fusarium spp.	0.00	0.50	0.00	0.50
			0.11	0.00	0.50	0.50
			0.33	0.50	0.50	0.50
			1.00	1.00	0.50	1.00
		Cylindrocarpon spp.	0.00	0.50	0.50	0.50
			0.11	0.75	1.00	0.50
			0.33	0.75	0.75	0.75
			1.00	0.50	0.75	0.50
		Trichoderma spp.	0.00	2.75	2.25	2.75
			0.11	2.00	1.75	1.75
			0.33	2.75	2.00	1.25
			1.00	2.50	2.50	1.25
		Black fungus	0.00	3.00	2.50	1.75
			0.11	4.00	3.00	3.75
			0.33	4.50	5.00	3.00
			1.00	2.50	4.50	4.25
		Yellow fungus	0.00	0.00	0.00	0.25
			0.11	0.25	0.25	0.25
			0.33	0.25	0.25	0.25
			1.00	0.25	0.50	0.25
		White fungus	0.00	1.25	1.00	1.75
			0.11	0.75	1.00	0.75
			0.33	1.50	1.25	1.50
			1.00	1.00	1.00	1.25
	Spring 1997	*Pythium spp.*	0.00	1.00	2.75	1.00
			0.11	4.50	2.25	1.00

		0.33	2.50	1.75	2.33
		1.00	3.00	4.50	1.75
	Fusarium spp.	0.00	0.00	0.00	0.00
		0.11	0.00	0.00	0.25
		0.33	0.25	0.25	0.25
		1.00	0.25	0.00	0.25
	Cylindrocarpon spp.	0.00	0.00	0.00	0.00
		0.11	0.25	0.00	0.00
		0.33	0.25	0.50	0.25
		1.00	0.50	0.75	0.75
	Trichoderma spp.	0.00	3.25	2.50	5.00
		0.11	2.50	2.75	1.50
		0.33	3.75	4.00	2.50
		1.00	3.75	3.75	3.75
	Black fungus	0.00	6.67	7.00	6.75
		0.11	5.00	6.25	6.00
		0.33	11.00	16.50	15.75
		1.00	7.67	9.75	8.75
	Yellow fungus	0.00	0.00	0.00	0.00
		0.11	0.00	0.00	0.00
		0.33	0.00	0.00	0.00
		1.00	0.00	0.00	0.00
	White fungus	0.00	0.75	1.25	1.50
		0.11	2.00	2.50	1.75
		0.33	2.50	3.75	2.50
		1.00	0.75	1.75	1.50
Fall 1997 (selective media)	*Pythium spp.*	0.00	2.50	6.66	4.66
		0.11	1.50	8.66	2.66
		0.33	1.50	8.33	5.66
		1.00	3.00	7.66	4.66
	Fusarium spp.	0.00	3.50	4.00	3.66
		0.11	3.75	3.66	3.33
		0.33	4.00	6.66	6.66
		1.00	3.75	5.00	4.33