SEEDBORNE *FUSARIUM* AND ROOT COLONIZATION OF CONTAINER-GROWN DOUGLAS-FIR SEEDLINGS

by

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ABSTRACT

The role of seedborne *Fusarium* in root colonization of container-grown Douglas-fir seedlings was studied in two coastal Douglas-fir seedlots; one contaminated with *Fusarium* and the other with minimal *Fusarium.* Seedlots were treated using either a standing water imbibition, or a running water imbibition with a post-stratification hydrogen peroxide sanitation treatment. The sanitation treatment significantly reduced the number of *Fusarium*-contaminated seeds. Seedlings were grown in an operational conifer nursery and seedling infection and root colonization by *Fusarium* was assessed throughout the growing season. The number of seedlings with *Fusarium* root infections increased throughout the season, and remained highest for the standing water imbibition treatment of the contaminated seedlot. Seedborne *Fusarium* was an important source of inoculum in one of the two years of the study. Other sources of inoculum may have been pallets or debris, but planting mix and irrigation water used in the study did not appear to contain *Fusarium.* Seed sanitation was associated with a significant increase in average height, root collar diameter, and shoot and root dry weight for the seedlings from the contaminated seedlot but not for the uncontaminated seedlot. Contamination of seed by *Fusarium* during cone and seed processing was also investigated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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CHAPTER 1 GENERAL INTRODUCTION

In recent years, *Fusarium* spp. have been associated with increasing amounts of disease in British Columbia's forest nurseries (Sutherland, personal communication). For example, most diseased conifer seedlings sent to the Seedling Pest Clinic at the Pacific Forestry Centre, Victoria, harboured *Fusarium* (Dennis, 1990). In addition, 66.9% of 251 coastal Douglas-fir seedlots assayed at the clinic contained seedborne *Fusarium* at incidence levels of 0.2 to 75.4%; most of them having contamination levels of less than 15% (John Dennis, unpublished data). However, Axelrood *et al.* (unpublished data) showed that even moderate contamination levels may increase during seed stratification. Fungi in the genus *Fusarium* can be particularly pathogenic on Douglas-fir (*Pseudotsuga menziessii* (Mirb.) Franco). In British Columbia (B.C.) about $200,000 worth of seedlings of a crop worth $22 million were killed in 1992 via damping-off and top blight, or were culled due to insufficient growth resulting from *Fusarium*-related root rot (Gwen Shrimpton, personal communication). *Fusarium* causes economic losses directly through seedling death or culling and possibly indirectly by reducing seedling growth in nurseries or following outplanting.

This research project was limited to the study of *Fusarium* on Douglas-fir seedlings grown in styroblock containers in an operational conifer nursery.

1.1 The Genus *Fusarium*

Fungi in the genus *Fusarium* (Link) lack a sexual state and so are classified as Fungi Imperfecti (Booth, 1971). Some species produce a sexual or perfect stage, and these species have been placed in separate genera (e.g., *Gibberella* spp.). *Fusarium* is characterized by the presence of crescent-shaped macroconidia. Depending on the species

and environmental conditions, microconidia, macroconidia, and chlamydospores may be produced. Spores may be produced on monophialides or polyphialides (spore-producing cells). Some isolates produce pionnotal colonies with conidia in sporodochia. Macroconidia are straight to curved and 0-10 septate. Microconidia are 0-1 septate and are pyriform, fusoid or oval shaped. The pigment of colonies depends on the culture medium and can range from cream to orange, to salmon, to purple-blue to carmine red. There is disagreement as to the speciation of fusaria because the traditional classification systems are based on slight morphological differences (Toussoun and Nelson, 1976; Brayford, 1989). These classification systems cause difficulties in the communication of information because workers may not have the skills to correctly identify the species. However, this morphologically based classification system is still commonly used.

Not all fusaria are pathogenic on Douglas-fir seedlings, e.g., Axelrood *et al.* (unpublished data) found that seedborne *F. oxysporum* and *F. proliferatum* were the most pathogenic.

Fusarium is often a soilborne facultative parasite well adapted for survival in either dormant or saprophytic states (Bruehl, 1987). Dormant survival is most common, particularly during cold winters, and involves the storage of nutrients in thick-walled chlamydospores which have a greater inoculum potential and longevity than micro- or macroconidia (Bruehl, 1987). Saprophytic survival requires a more active role by the fungus which must colonize organic debris and also remain active long enough to utilize the substrate. This mode of survival is characterized by the ability to be the primary colonizer of a substrate, e.g., by living on dead plant tissue following parasitic colonization that occurred during the growing season (Bruehl, 1987). Dead conifer root pieces may provide *Fusarium* with a potentially important source of organic debris for its saprophytic survival (Bloomberg, 1976).

1.2 The Host

Pseudotsuga menziessii (Mirb.) Franco (Douglas-fir) is a coniferous tree native to B.C. It is an economically valuable species and is commonly planted on southern coastal reforestation sites. Because of its shade intolerance, and its status as a pioneer species in forest succession, it is critical that Douglas-fir seedlings be vigorous and able to deal with brush competition.

In B.C., most Douglas-fir seedlings produced in forest nurseries are grown in styroblock containers rather than directly in soil (bareroot culture). One or more seeds are sown into cylindrical cavities containing a peat-vermiculite growing medium (Yalpani, 1979).

Prior to sowing, seeds are soaked in standing or running tap water for 24 hours (imbibition). They are then surface dried and stored at 2-4^{o}C for 3 weeks (stratification). This moist cold treatment helps to break the deep state of dormancy for most conifer tree seeds increasing germination speed and uniformity (Ching and Ching, 1962).

Depending on the type and size of seedlings required, seeds may be sown from January-April. Fertilization and irrigation regimes are tailored to the seedlot, weather, and outplanting specifications. They continue to grow until bud set and are usually harvested in December.

1.3 The Disease

Research on *Fusarium* diseases of conifer seedlings has been conducted on both nursery and outplanted seedlings. However, compared to the amount of research done on *Fusarium* diseases on conifers in nurseries, little has been done on seedlings planted on reforestation sites. Smith (1967) attempted to detect *F. oxysporum* Schlecht. in the roots of nursery-grown pine seedlings transplanted onto a reforestation site. Although

Fusarium was recovered initially, after 5 years *F. oxysporum* could not be detected. Roots of naturally regenerated seedlings from the site contained no *F. oxysporum.* Axelrood and Chapman (1992) compared the numbers of seedlings with *Fusarium* root infections for planted and naturally regenerated seedlings on 4-year old regeneration sites in coastal B.C. The numbers of seedlings with *Fusarium* root infection levels were low, and there was no significant difference between the numbers infected for the planted and naturally regenerated seedlings. These two studies indicate *Fusarium* probably causes little damage on regeneration sites. Several authors (e.g., Thornton, R.H., 1960; Park, D., 1963) have not been able to detect *F. oxysporum* in forest soils. It is likely that other fusaria are also rare in coniferous forest soils. Schisler and Linderman (1984) found only one of 14 forest soils assayed contained *Fusarium.* They postulated that the absence of fusaria was due to the activity of microorganisms particular to coniferous forest soils. This absence was believed to be due to a qualitative difference in the antagonistic species of bacteria, fungi, or actinomycetes present, rather than a quantitative one.

Since *Fusarium* spp. are infrequently isolated from coniferous forest soils, reforestation sites are not likely to increase the numbers of seedlings with *Fusarium* infections. However, losses due to *Fusarium* may still occur when nursery grown seedlings are transplanted in these areas. This is possible if much of the root system is affected, particularly for seedlings stressed by cold storage, transportation, or outplanting. Losses will usually be most severe in the first growing season on the regeneration site (James, 1985b). Besides mortality, growth losses may also accrue if *Fusarium*-caused root rot has resulted in root damage.

As the changeover of many forest nurseries from bareroot to container culture has been fairly recent, much of the research on *Fusarium* diseases in Douglas-fir has been done on bareroot seedlings. However, the disease symptoms have been studied for both types of cultural methods and have been found to be similar.

1.3.1 Symptomatology

It is important to clarify the terminology which will be used throughout this thesis. Infected, as used here, means that a tissue has been invaded by a microorganism. This is determined by isolation of the microorganism following surface-sterilization of the tissue. A latent infection is one where the tissue has been invaded but no disease symptoms develop. Disease is used to describe the situation where tissue has been invaded and disease symptoms are present.

Fusarium is similar to many other plant pathogens in that it requires both a susceptible host and favourable environmental conditions to cause disease symptoms (Agrios, 1988). However, *Fusarium* disease on conifers differs from other pathosystems in that *Fusarium* may infect seedling tissue without causing disease symptoms (latent infection). In most pathogen-plant interactions, if environmental conditions are suitable for penetration and infection by the pathogen, disease usually occurs. In this pathosystem, however, many conifer seedlings are infected by pathogenic *Fusarium* spp., but remain symptomless due to the absence of environmental conditions necessary for disease progression. Bloomberg (1966) frequently found *Fusarium* associated with shoots and roots of healthy Douglas-fir seedlings. He also found that *Fusarium* isolates from diseased and healthy seedlings were morphologically similar in culture. This was further studied by testing the pathogenicity of *Fusarium* isolates found in healthy Douglas-fir seedlings. These tests showed that *Fusarium* from healthy seedlings could be pathogenic (Bloomberg and Lock, 1972). James and Gilligan (1988) corroborated Bloomberg's findings by detecting healthy-looking seedlings that harboured *Fusarium* for fairly long periods of time. When such seedlings are stressed, these symptomless or latent infections may progress into typical *Fusarium* disease symptoms (James *et al.*, 1986).

The amount of seedling tissue infected by *Fusarium* is not predictive of disease development. James *et al.* (1986) found no correlation between the proportion of root

tissue infected with *Fusarium* and the severity of above-ground symptoms. James *et al.* (1989) worked with both healthy and diseased Douglas-fir seedlings and found that the relative disease potential of an isolate was not related to the source (diseased or latent) of the isolate. Sometimes, isolates from diseased seedlings were less pathogenic than isolates from healthy seedlings. Although healthy-looking seedlings harbour *Fusarium*, James *et al.* (1989) suggested that under certain environmental conditions and with a susceptible host, most fusaria will cause disease in conifer seedlings.

Damage by *Cylindrocarpon* (another common soilborne fungus with pathogenic members) to container-grown seedlings has not been documented, but it is commonly isolated with *Fusarium* in seedlings exhibiting root rot symptoms (Sutherland *et al.*, 1989). *Cylindrocarpon*, like *Fusarium*, has been isolated from the roots of healthy-looking seedlings (H. Kope, J. Dennis, P. Axelrood, unpublished). Axelrood and Peters (1993a) indicated that *Cylindrocarpon* may compete with *Fusarium* for infection sites on seedling roots. When *Cylindrocarpon* infection and root colonization levels decreased, *Fusarium* infection and colonization levels increased.

The environmental conditions required to produce *Fusarium* diseases in Douglas-fir seedlings may occur several times during the growing season. Specifically these diseases are known as: pre-emergence damping-off, post-emergence damping-off, *Fusarium* top blight, hypocotyl rot and a late season root rot (Sutherland *et al.*. 1989; Sutherland, 1990; Hamm, 1990). A relatively rare stem rot of older seedlings may also occur (Morgan, 1983).

Pre-emergence and post-emergence damping-off by *Fusarium* can result in substantial losses in conifer nurseries (Bloomberg, 1981). Pre-emergence damping-off occurs before the germinant emerges from the soil. This is apparent as a poor emergent crop. Attack by the fungus prior to germination results in the rotted seed which eventually dries out and disintegrates (Agrios, 1988). Upon germination, the seedling produces succulent tissues covered by a thin epidermis which is easily attacked by the pathogen.

Affected tissue becomes necrotic and the germinant is killed before it emerges (Boyce, 1961). Post-emergence damping-off is characterized by lesions on the stem near the groundline so that the seedling falls over. This may occur for 4-6 weeks following emergence or until stems have formed a periderm and are less susceptible to *Fusarium* (Sutherland *et al.*, 1989).

Both pre- and post-emergence damping-off in container and bareroot nurseries are favoured by cool, wet, compacted planting mix or soil. Also, high humidity and sowing more than one seed per container cavity, or dense sowing in bareroot seed beds, may contribute to the disease. Post-emergence damping-off in containers is rare and may occur in clumps, suggesting that it is probably due to the splashing of water and inoculum (Sutherland *et al.*, 1989). Both diseases can be caused by *F. oxysporum* (Bloomberg, 1971). James (1985b) found that *F. avenaceum* (Fr.) Sacc. and *F. acuminatum* Ell. et Kellerm. could cause pre- and post-emergence damping-off of Douglas-fir.

Fusarium-caused root rot occurs much later in the growing season after damping-off has ceased. The symptoms usually appear in mid-July and may continue into late autumn. High temperatures, drought, and seedling crowding result in stress, and induce root rot which leads to above-ground symptoms characterized by chlorosis of terminal needles, which then become flaccid and purple, turn brown, and dry out. The terminal of the seedling may be crozier-shaped (Sutherland, 1990). Root rot is distinguishable from post-emergent damping-off because the killed seedling remains upright. Roots of affected seedlings exhibit stunted root systems with few laterals. Extant roots are often blackish and distended and actively growing root tips are rare. The cambium is usually dark in colour (Sutherland, 1990). Tap roots and secondary roots may be rotted in the cortical region. In addition, secondary roots may be sparse and have poorly developed mycorrhizae (Merrill, 1981). *Fusarium* root rot usually kills seedlings but additional losses are incurred because diseased seedlings with poor root development are culled at harvest (Sutherland *et al.*, 1989). Species of *Fusarium* that cause root rot of Douglas-fir

are: *F. oxysporum* (Bloomberg, 1971; James, 1985b), *F. solani* (Merrill, 1981) *F. avenaceum* and *F. acuminatum* (James, 1985b).

Fusarium hypocotyl rot caused by *F. oxysporum* is the most common post-emergent seedling disease in bareroot nurseries in the western United States (Hamm, 1990). Hypocotyl rot of Douglas-fir also occurs on container-grown seedlings in British Columbia (Sutherland *et al.*, 1989). Symptoms usually appear in late June or July, after the first period of hot weather, and may extend into the fall. The above-ground symptoms of *Fusarium* hypocotyl rot are similar to those of *Fusarium* root rot. The distinguishing feature is a lesion on the hypocotyl which girdles the stem, killing the seedling. Initially, roots of killed seedlings are unaffected but eventually they decay in a fashion similar to that seen in *Fusarium* root rot.

Another similar disease is *Fusarium* top blight which occurs on both bareroot and container-grown Douglas-fir seedlings. Symptoms appear from mid-summer through fall and involve a browning at the base of the terminal leader, bud, or associated needles. The disease progresses down the seedling eventually killing all or part of the stem or needles. This disease also affects germinants and appears at the point where the seedcoat contacts the cotyledons. The disease spreads down the stem, eventually killing the germinant (Sutherland *et al.*, 1989). The causal organism is *F. oxysporum*.

Morgan (1983) reported an uncommon *Fusarium* stem disease which differs from *Fusarium* hypocotyl rot in that lesions appear on the stem above the cotyledons. He found the disease on 9-16 month-old bareroot Douglas-fir seedlings. The disease produced symptoms such as shoot chlorosis in the winter or early spring and lead to seedling death. The causal organisms were *F. avenaceum* and *F. sambucinum*.

1.3.2 Epidemiology

Epidemiology is the study of factors affecting the outbreak and spread of infectious (plant) diseases (Agrios, 1988). Although the symptomatology of *Fusarium*-caused diseases is similar on both bareroot and container grown Douglas-fir, disease epidemiology may differ for the two cultural systems.

Sources of Inoculum

In bareroot nurseries, inoculum is mainly soilborne. *Fusarium* is present in nearly all soil types due to its ability to survive as a saprophyte. In particular, bareroot seed beds which have harboured *Fusarium*-infected seedlings in the past are good sources of pathogenic *Fusarium* inoculum in the form of hyphae, conidia, or chlamydospores. Such propagules are able to survive by overwintering on and in conifer seedling root pieces (Bloomberg, 1976). Container-grown seedlings were once thought to be excluded from this inoculum source by the use of peat/vermiculite growing media whose acidity was thought to inhibit *Fusarium* growth and germination (Bloomberg, 1981). Although rare, contaminated container planting mix may be a source of *Fusarium* inoculum. It has been shown that *Fusarium* can survive a wide pH range including that for most seedling growing media (James, 1985a). *Fusarium* has also been isolated from the planting mix in some B.C. nurseries (P. Axelrood, John Dennis, personal communication). The importance of this source of inoculum is not known.

A second source of *Fusarium* inoculum for container nurseries may be used growing containers. Styroblocks are sanitized between growing seasons by submersion in hot water containing bleach. Axelrood and Peters (1993a) showed that 50% of the cavities in operationally sanitized styroblock containers contained *Fusarium*-infested root fragments. In addition, 60% of the growing cavities were contaminated with *Fusarium* on

the styrofoam surface inside the cavity. The importance of this source of inoculum in the epidemiology of *Fusarium* root diseases has not been studied.

Fusarium inoculum may be seedborne and this could be an important source for container-grown seedlings. Graham and Linderman (1983) showed that pre- and post-emergence damping-off can be caused by seedborne fusaria. In bareroot culture, this seedborne source is secondary in importance to the soilborne one, except perhaps in the case of seed beds densely sown with an infested seedlot. In container nurseries, the importance of seedborne *Fusarium* in the epidemiology of *Fusarium* root diseases is not known.

Other sources of *Fusarium* include airborne inoculum produced on vegetation around the nurseries. *Fusarium* has also been found on pallets used to hold growing containers and on organic debris beneath these pallets (Neumann and Axelrood, 1992). Contaminated equipment used to harvest Douglas-fir seed and during nursery culture practices may also increase inoculum levels (James, 1985a). Mittal and Wang (1987) suggested certain stages during extraction of pine and spruce seed lead to an increase in *Fusarium* contamination.

Dissemination of Inoculum

Fusarium inoculum can be disseminated in a variety of ways, the importance of which may depend on whether the seedlings are grown in bareroot nurseries or in containers. For example, irrigation water could disseminate *Fusarium* from contaminated container pallets to organic debris below and the *Fusarium* could later be wind-blown onto seedlings (Neumann and Axelrood, 1992). This could only be important in container culture where wooden pallets are used to support containers.

On both bareroot and container-grown seedlings, *Fusarium* spores which are produced in sporodochia at the stem base or on the shoots of diseased seedlings may be

spread to adjacent seedlings by wind, irrigation water or during fertilization (James, 1985a). Spores can also be spread in this manner if sporulation occurs on cast or uncast seed coats (Graham and Linderman, 1983). Both bareroot and container-grown seedlings may be contaminated by previous crops. In bareroot nursery soils, colonized root pieces from previous crops may harbour *Fusarium* inoculum (Bloomberg, 1976). Growing containers which have not been adequately sanitized may contain *Fusarium* in the growing cavities, or on contaminated organic debris within these cavities. In addition, multiple sowings per cavity in containers may increase the probability of introducing seedborne *Fusarium* to cavities and heavy sowing in bareroot seedbeds may spread inoculum to other seeds. Inoculum transfer may occur when contaminated seeds come in contact with non-contaminated seeds or seedlings.

1.3.3 Control Methods

Because of the nature of *Fusarium* diseases on Douglas-fir seedlings and the value of the crop, one tactic to minimize losses may be to prevent the disease.

Cultural and Chemical Controls

To maximize the efficacy of cultural or chemical control tactics, they must often be integrated; particularly since *Fusarium* may cause disease at any time during the growing season.

Practices such as seed stratification which increases germination speed will reduce pre- and post-emergence damping-off losses. In addition, adjusting fertilizer regimes to promote woody growth resistant to disease would be beneficial (Sutherland *et al.*, 1989). Avoiding excessive nitrogen levels and increasing the potassium content in fertilizers seems to minimize seedling mortality (Johnson *et al.*, 1989). Reducing seedling density,

and improving air circulation which decreases humidity reduces losses due to post-emergence damping-off (Sutherland *et al.*, 1989).

Since fusaria may enter the plant at an early stage, practices which decrease *Fusarium*-caused damping-off should reduce losses to *Fusarium* root disease later in the growing season. Root disease can also be minimized by sanitizing used containers and by culling infected seedlings throughout the growing season (Sutherland *et al.*, 1989). Irrigation regimes may play a significant role in decreasing *Fusarium* root rot and *Fusarium* hypocotyl disease losses, both of which can be reduced by preventing soils from getting too warm and at the same time reducing seedling moisture stress. Infrequent deep irrigation is recommended for *Fusarium* hypocotyl rot control in bareroot nurseries (Hamm, 1990).

One tactic to reduce inoculum sources would be to reduce the amount of *Fusarium* on the seed. In the United States, where work has been carried out on seed treatment methods, it is recommended that seeds be imbibed in running tap water prior to stratification (Campbell and Landis, 1990). This procedure is not phytotoxic and removes many seedborne pathogens. When more extreme measures are required chemical seed treatments such as bleach, ethanol, or hydrogen peroxide may be used before or after seed stratification (Campbell and Landis, 1990). A 10 minute soak in a 40% household bleach solution effectively reduces seedborne fungi without reducing germination (Wenny and Dumroese, 1987). Another study found a 10 second soak in 90% ethanol prior to stratification reduced seed infestation levels (Dumroese *et al.*, 1988) but it also detrimentally affected germination. Axelrood (unpublished) observed an inhibitory effect of ethanol on seed germination. Ethanol should only be used after stratification when seeds are less likely to absorb the material (Dumroese *et al.*, 1988). Trappe (1961) observed that a 30 minute soak in 35% hydrogen peroxide reduced fungal contamination and stimulated seed germination of many conifer species. A post-stratification soak for 5

hours in 3% hydrogen peroxide was best for reducing seedborne *Fusarium* levels without affecting germination (Dumroese *et al.*, 1988; Campbell and Landis, 1990).

In B.C. work on seed treatments of Douglas-fir is ongoing. Axelrood *et al.* (unpublished data) showed that running water imbibition decreased seedborne *Fusarium* levels over standing water imbibition for most seedlots. Consequently, the B.C. Ministry of Forests is now developing a method to incorporate this at an operational level (David Trotter, personal communication).

Fungicides are often applied to seeds and seed beds to control disease. Fungicidal drenches were applied to bareroot seed beds in both Canada and the United States (Bloomberg and Lock, 1974). Control of soilborne pathogens once they have invaded the plant is difficult and therefore the timing of soil treatments is critical. Bloomberg and Lock (1974) found that soil drenches of the fungicide Captan® needed to be timed such that protection of the seedlings from *Fusarium* was maintained for at least 10 days after emergence. In Canada there are no fungicide drenches registered for use on *Fusarium* in forest nurseries. The use of containers to grow conifers greatly restricts the choice of chemicals to those with minimal phytotoxicity. Because "the biological and chemical 'buffering capacity' of (container) media is low" (Sutherland *et al.*, 1989) the use of fungicide-treated seed is not recommended in container culture.

Biological Control

The use of biological controls for *Fusarium*-related diseases on conifers is not yet practical. However, there is much ongoing research to find economically viable biological controls for these diseases. For example, Sinclair *et al.*(1974 and 1982); Stack and Sinclair,(1974); Sylvia and Sinclair,(1983), suggest that ectomycorrhizal fungi may protect bareroot Douglas-fir from *Fusarium oxysporum*. *Laccaria laccata* colonizes the primary root very rapidly, allowing it to successfully compete with *Fusarium* for space in the root.

Also, *L. laccata* induces resistance in the cortex of primary roots and may have an antibiotic effect on the pathogen. *Paxillus involutus* also appears to suppress *Fusarium* root disease of *Pinus resinosus* via the synthesis of anti-fungal compounds (Duchesne *et al.*, 1989) These mycorrhizal fungi could be used operationally by inoculating Douglas-fir seed at sowing or shortly thereafter.

Some bacteria may reduce damping-off and root infections due to *Fusarium*. Myxobacteria of the genus *Cytophaga* produce extra-cellular chitinases but not cellulases. This suggests that they may destroy fungal cell walls without destroying the seedling cell walls (Hocking *et al.*, 1972). However, these same chitinases may kill mycorrhizal fungi of Douglas-fir seedlings. Another group of bacteria, the Pseudomonads, has recently received much attention as potential biocontrol agents. *Pseudomonas stutzeri* YPL-1 reportedly reduces *Fusarium* root rots (Lim *et al.*, 1991). The mechanism is thought to be an extracellular chitinase and laminarase. Kloepper *et al.* (1980) have suggested that the production of siderophores in rhizobacteria such as *Pseudomonas* spp. may be a biocontrol mechanism. Siderophores sequester available iron, making it unavailable to the pathogen which reduces pathogen growth. Hebbar *et al.* (1992) found that antibiotic rather than siderophore production was the mechanism behind the *in vitro* antagonism of *Pseudomonas* spp. to *F. moniliforme*. Axelrood *et al.* (1993b) have identified several *Pseudomonas* spp. which have significantly reduced *F. oxysporum* disease in laboratory assays on Douglas-fir. Preliminary results indicate some of these biocontrol candidates promoted seedling survival and new root growth in the field.

Non-pathogenic *Fusarium* may also be a biocontrol candidate for *Fusarium* diseases. In cucumber, non-pathogenic *F. oxysporum* may suppress *Fusarium* wilt by competing with the pathogen for nutrients and infections sites, and by inducing enhanced resistance in the host (Mandeel and Baker, 1991).

Because of the general movement away from chemical pesticides, the demand for efficient biocontrol agents could increase. However, these agents will need to be used in

conjunction with appropriate cultural practices to keep *Fusarium* disease losses at a minimum.

1.4 Summary

It is apparent that losses due to *Fusarium* diseases in British Columbia's forest nurseries are still occurring. *Fusarium* can cause disease at various times during the growing season, making control difficult. Most of the epidemiological research in *Fusarium* diseases has been carried out on bareroot seedlings, however there is a lack of information about the epidemiology of these diseases in containers. It has been suggested that the seed may be an important source of *Fusarium* inoculum for container-grown seedlings. However, this has not been shown conclusively. As a result it is not known whether it would be economically beneficial to adopt measures to control levels of seedborne *Fusarium*.

The objective of this research project was to determine the role of seedborne *Fusarium* in the root colonization of container-grown Douglas-fir seedlings. A second objective was to address the question of whether the cone and seed processing affects final seedborne *Fusarium* levels.

CHAPTER 2 THE ROLE OF SEEDBORNE *FUSARIUM* IN THE ROOT INFECTION OF CONTAINER-GROWN DOUGLAS-FIR AND ASSESSMENT OF SEEDLING GROWTH

2.1 Introduction

One of the earliest reports of seedborne *Fusarium* was by Bloomberg (1965) who isolated fusaria from asceptically grown seedlings, indicating that the source of the inoculum was probably the seed. Graham and Linderman (1983) isolated seedborne *Fusarium oxysporum* from Douglas-fir seed in Oregon, and found that isolates from seed caused pre- and post-emergence damping-off of greenhouse-grown Douglas-fir seedlings.

James *et al.* in Idaho (1989) reported that *Fusarium* isolated from Douglas-fir seed and seedling roots caused damping-off and disease of older seedlings. One of the most virulent *Fusarium* isolates was from apparently healthy seed.

In Canada, Axelrood *et al.* (unpublished data) made isolations from seeds of 12 coastal B.C. seedlots and indicated that *F. oxysporum* and *F. proliferatum*, were the most virulent on Douglas-fir seedlings. Subsequently, John Dennis (unpublished data) found that about 70% of 251 seedlots contained *Fusarium* at levels of 0.2-75.4%. The pathogenicity of these fusaria was not determined.

Although many seedborne fusaria are pathogenic in laboratory tests, it is difficult to demonstrate pathogenicity in the field. Bloomberg (1965) isolated morphologically identical fusaria from both healthy and diseased seedlings. James and Gilligan (1988a), found that over 90% of healthy pine seedlings had *Fusarium* root infections. A study of healthy bareroot Douglas-fir showed that although the roots of these seedlings were often extensively colonized by *Fusarium* there were no root lesions or effects on seedling growth (James and Gilligan, 1988b). In addition, *Fusarium* strains from healthy seedling roots were pathogenic on Douglas-fir seedlings (James *et al.*, 1989). Apparently most *Fusarium* isolates can extensively colonize the cortex of seedling roots without producing

disease. These latent infections may remain inactive or cause disease when seedlings are stressed (James *et al.*, 1989).

These studies show that seed may be a source of *Fusarium* inoculum and that such fusaria can be pathogenic. It is also clear that the presence of *Fusarium* in Douglas-fir seedling roots does not necessarily result in disease expression. However, these studies have not determined the role of seedborne *Fusarium* in seedling root infection in an operational container nursery.

One objective of the present research was to investigate the role of seedborne *Fusarium* in root colonization of container-grown Douglas-fir. Because disease symptoms are not always expressed, even in the presence of substantial root infection, the study of root colonization was thought to be one way to determine the potential importance of seedborne *Fusarium* as one factor in *Fusarium* root disease epidemiology.

To study this problem it was necessary to use two seedlots; one contaminated with *Fusarium*, and the other having minimal or no *Fusarium*. A control for the contaminated seedlot would be the same seedlot sanitized to minimize *Fusarium* levels. It was also preferable that the contaminated seedlot contain predominantly one species of *Fusarium* which did not occur in the uncontaminated seedlot. Presumably, this species could be used as an indicator in the roots of the seedlings grown from the contaminated seedlot. This indicator should determine whether *Fusarium* root infections of seedlings corresponded to the level and frequency of seedborne *Fusarium* species present prior to sowing.

The field experiment was used to test the following:

(1) The number of seedlings with *Fusarium* root infections from the unsanitized treatment of the contaminated seedlot should be greater than the number from the sanitized treatment of this seedlot.

(2) The number of seedlings with *Fusarium* root infections from the unsanitized treatment of the contaminated seedlot should be greater than the number from both treatments of the non-contaminated seedlot.

(3) The indicator seedborne species should be found more frequently in seedlings grown from the unsanitized treatment of the contaminated seedlot than in seedlings from the sanitized treatment of the contaminated seedlot or from both treatments of the non-contaminated seedlot.

The second objective of the field experiment was to assess other potential sources of *Fusarium* inoculum in the nursery. If the indicator species was found to be present in the nursery, it would not be possible to determine the origin of particular root infections. However, this should not affect the rationale behind (3) since the differences in the frequencies of indicator species infections between treatments should show what levels may be expected to be of nursery origin and which are likely to be of seed origin.

The third objective was to assess germination and seedling growth of the operational and sanitized seedlot treatments. If seedborne *Fusarium* is an important inoculum source, and seed sanitation necessary, it is required that the seed treatment not affect seed germination or seedling growth at economically damaging levels.

2.2 MATERIALS AND METHODS

Douglas-Fir Seedlots

Two coastal Douglas-fir seedlots were used; seedlot 476 which had high levels of seedborne *Fusarium* (Axelrood, personal communication) and seedlot 9983 which had very little *Fusarium* associated with the seed. In addition, seedlot 476 contained mainly *Fusarium proliferatum*, whereas seedlot 9983 contained *Fusarium avenaceum* only. Actual percentages are shown in Appendix 7.0, Table 7.0.1. Pathogenicity studies (Axelrood, personal communication) showed that the *F. proliferatum* present in seedlot 476 could cause damping-off of Douglas-fir seedlings.

Seed Stratification

Operational treatment:

Seeds were imbibed in de-chlorinated tap water (to approximate the well water used at the B.C. Ministry of Forests Tree Seed Centre) for 24 hours, surface-dried and incubated for 21 days at 4°C.

Sanitation treatment:

One of the prerequisites for this field experiment was to find a seed sanitation treatment which would reduce seedborne *Fusarium* levels as much as possible without adversely affecting seed germination. Seeds were imbibed for 24 hours in running tap water and incubated (stratified) as described above. Stratified seeds were soaked in 3% hydrogen peroxide for 4-8 hours or in 30% hydogen peroxide for 30-45 minutes for the preliminary assessment. An 8 hour soak in 3% hydrogen peroxide was selected for the nursery trials. All seeds were rinsed for 48 hours in running tap water following the hydrogen peroxide treatment. The control was rinsed in running tap water only.

Seedlot treatments used in the 1991 and 1992 field experiments included operational and sanitation treatments for seedlots 476 and 9983 and a sanitized non-experimental *Fusarium*-free seedlot for planting in the buffer regions between the experimental treatments.

Pre-sowing Assessment of Seed

Seeds from the operational and surface-sanitized treatments of seedlot 476 (260 seeds/treatment) and seedlot 9983 (500 seeds/treatment) were placed asceptically onto Komada's medium (1975) immediately prior to the 1991 nursery trial. Five hundred seeds from the border seedlot were also assayed to ensure no *Fusarium* was present. Seed assay

plates were incubated under fluorescent lights at 22-24°C for 14 days. The numbers of *Fusarium*-contaminated seeds were expressed as a percentage of seeds assayed. All *Fusarium* isolates were identified to the species level using the classification system proposed by Nelson, Toussoun, and Marasas (1983). Seedborne Fusarium was assessed in a similar manner in 1992 except that 500 seeds of each treatment were placed on Nash and Snyder's medium (1962). The medium was changed because *Trichoderma*, a common contaminant, grew more slowly on this medium while *Fusarium* grew as well as on Komada's medium.

Nursery Experiments

These were done during 1991 and 1992 at the B.C. Ministry of Forests Surrey Nursery.

1991 Nursery Experiment

Seeds were hand-sown June 17-18 in new Beaver 313B styroblocks (198 cavities per styroblock) in a randomized complete block design and were grown outdoors. There were 12 replicates of each of the four treatments and two treatments were sown in each styroblock. Seventy-two seeds were sown for each treatment replicate, giving a total of 864 seeds per treatment. There were six blocks with two replicates of each treatment randomized within each block (Figure 2.2.1). The weather at the time of sowing was cool and wet and remained so for several days. All replicates were bordered by a sanitized *Fusarium*-free non-experimental treatment seedlot as a buffer to prevent cross-contamination. The seedlings were fertilized with: 12-17-29 (410 g/1000 l), $MgSO_4$ (200 g/1000 l), and $CaNO_3$ (322 g/1000 l) during the majority of the growing season and were grown under standard nursery practices.

1992 Nursery Experiment

The experimental design was similar to that of 1991 except 42 seeds were sown per treatment replicate for a total of 504 potential germinants for each treatment. There were four blocks with three replicates of each treatment randomized within each block (Figure 2.2.2). Seeds were sown April 7-8 which is the usual period for sowing under operational conditions. The weather at the time of sowing was warm and dry and continued that way for a few days.

A	C	D	B	A	C	B	D
B	D	C	A	A	D	B	C
C	D	C	A	B	D	B	A
B	A	A	D	C	B	C	D
A	D	A	C	B	D	B	C
D	C	A	B	C	A	D	B

Figure 2.2.1 Diagram of experimental layout for the 1991 nursery experiment. (A=Operational 476, B=Sanitized 476, C=Operational 9983, D=Sanitized 9983)

C	B	D	A	B	D	C	C	B	D	A	A
B	A	D	C	C	B	D	A	B	D	A	C
D	B	A	D	C	A	C	B	A	C	D	B
A	B	A	B	C	D	C	A	C	D	B	D

Figure 2.2.2 Diagram of experimental layout for the 1992 nursery experiment. (A=Operational 476, B=Sanitized 476, C=Operational 9983, D=Sanitized 9983)

Assessment of Root Colonization

Seedling roots were assessed three times during the 1991 and 1992 field seasons; (1) when 90% of the germinants had dropped their seed coats (4-6 weeks after sowing), (2) when 80% of the seedlings were at least 5 cm tall (8 weeks after sowing), and (3) shortly before the time of seedling harvest (December).

At each sampling date, five seedlings were randomly selected from each of the 12 replicates of the four treatments (n=60). Seedling roots were carefully washed to remove all growing medium prior to assessment of *Fusarium* root colonization.

Schneider (1984) showed that exposure of celery roots to 0.21% sodium hypochlorite (bleach) for up to 2 minutes significantly reduced the number of *Fusarium* colonies recovered, but longer time periods showed no further reduction. The seedling root sterilization times for the current project were determined using this information plus evaluations of the age of the seedlings, the amount of root material, and the number of new roots.

1991 Nursery Experiment

Seedling roots from sample time 1 (July 16) were surface-sterilized for 30 seconds in a 10% commercial bleach solution (final concentration of 0.5% sodium hypochlorite), rinsed at least three times in sterile distilled water, and then blotted dry on sterile paper towels. The entire root sytem of two seedlings from each replicate sample were placed asceptically on Komada's medium while the other three root systems were incubated on Komada's medium amended with 1 ppm benomyl. The two media were used to see if benomyl reduced *Trichoderma* contamination without inhibiting *Fusarium*. The roots were incubated under fluorescent lights at 22-24^{o}C for 14 days and the seedlings with *Fusarium* and *F. proliferatum* root infections were counted. (Seedlings with *F. proliferatum* root infections were included in *F. proliferatum* totals even if root infection was not exclusively *F. proliferatum*). To obtain an index of root infection, the length of root colonized was measured and expressed as a proportion of root length assayed.

Seedlings from sample period 2 (August 20) were treated as above except the roots were soaked for 60 seconds in the bleach solution prior to rinsing with sterile distilled water. Depending on the amount of roots, either all root material or ~40cm was randomly selected from the middle 3 cm of the root mass for assessment.

At sample time 3 (December 1), 40 cm of washed roots were randomly sampled from the middle 3 cm of the root plug, surface-sterilized in 10% bleach solution for 3 minutes, and incubated and assessed as before.

1992 Nursery Experiment

Root infection assays were similar to 1991 except for the following differences. All roots were incubated on Nash and Snyder's medium (1962). The first set of samples was collected on May 25th. Because seedling roots from sample 2 in 1992 (July 7) appeared to be more numerous and woody than in 1991, the root surface-sterilization soak was increased to 3 minutes. Seedling roots from sample time 3 (December 5) were

surface-sterilized for 5 minutes and the assessment method was changed in that the roots were cut into 1 cm pieces prior to being placed on the medium. Forty of these pieces were placed on each medium plate and root infection by *Fusarium* was expressed as a proportion of the total number of root pieces. The reason for changing the assessment method in 1992 was because it was observed that some *Fusarium* species were growing much faster than others, and were over-represented in the measurement of the length of root colonized. Expressing root infection as a proportion of root pieces colonized was thought to give a more accurate measurement of the degree of root infection for those roots assayed. All root plates were incubated and assessed as done previously.

Data summaries for both years included the percentage of seedlings infected with all *Fusarium* species and the percentage of seedlings infected by *F. proliferatum*. These proportions were analyzed using a chi-square test to determine whether the observed proportions were independent of treatment method (Zar, 1984). Bonferroni's inequalities (Snedecor and Cochran, 1980) were used to set 'p levels' at $p=0.01$ so that multiple tests on data sets could be carried out at a 95% confidence level ($p=0.05$). Data shown as the average proportion of root colonized were for *Fusarium*-infected seedlings only (ie., seedling roots without *Fusarium* infections were not included in calculations for the average proportion of root colonized). Treatment differences between the proportion of root colonized in infected seedlings were tested using the Mann-Whitney test (1947). Operational treatments of the seedlots were compared with their corresponding sanitized control treatments for most of the statistical tests. Analyses were further restricted by carrying out tests within a sample date because of the different root surface-sterilization times.

Germination Assessment

The germination assessment was performed when approximately 90% of the emergent seedlings had dropped their seedcoats. In 1991, this occurred about 4 weeks after sowing (July 16). In 1992 the assessment was performed approximately 6 weeks after sowing (May 25). At this time the germinants for each treatment replicate were counted.

Since the germination data were best represented by a binomial distribution, a chi-square test was used to determine whether, within seedlots, the number of germinants observed for each treatment was independent of the treatment used.

Seedling Growth Assessment

Seedling growth was assessed on January 10th, 1992 for the 1991 experiment and November 8th, 1992 for the 1992 experiment. The field experiments each had a 7 month period between sowing and the growth assessment. Five seedlings were randomly selected from each replicate of the four treatments as described previously. Sixty seedlings from each treatment were carefully washed in tap water, and their heights and root collar diameters were measured. Root and shoot dry weights were obtained after drying seedlings for 48 hours at 60°C in 1991 and for 24 hours at 110°C in 1992.

An analysis of variance procedure with replicates nested within treatments was used to determine treatment differences. Analysis of covariance was used where justified.

Assessment of Other Sources of Inoculum at Surrey Nursery

Growing Medium

Seven samples of medium were collected prior to styroblock loading from the growing medium mixing machine at Surrey Nursery in 1991. Five gram samples were taken from each sample, placed into 500 ml of 10 mM sterile phosphate buffer (pH 7.0) and incubated on a shaker at 28°C for 24 hours. Fifty ml from each of the peat/phosphate buffer solutions was collected, centrifuged for 25 minutes at 4,000 rpm and the concentrated pellet suspended in 2.5 ml sterile phosphate buffer. A dilution series, ranging from 10^0 to 10^{-6} , was prepared in phosphate buffer and 100 µl spread onto Komada's medium supplemented with 200 µg/ml streptomycin sulfate and aeromycin sulfate to reduce bacterial contaminants from the peat samples. Plates were incubated under fluorescent lights at 22-24°C for 7 days and then examined for *Fusarium* species.

In 1992, five samples of planting mix were taken as in 1991. Ten grams from each sample were placed into 150 ml of sterile distilled water and shaken gently at 24°C for 1 hour. A dilution series, ranging from 10^{-1} to 10^{-4}, was prepared in phosphate buffer and 100 µl spread onto Nash and Snyder medium supplemented with 200 µg/ml streptomycin sulfate and aeromycin sulfate. Plates were incubated and assessed as before.

Irrigation Water

Water samples (50 ml) were taken from the irrigation system three times during the field season and assessed for *Fusarium* using two methods. The first method involved centrifuging the 50 ml water sample at 4,000 rpm for 30 minutes, removing the top 45 ml by pipet and sampling from the pellet suspended in the remaining 5 ml sample. A dilution series, ranging from 10^0 to 10^{-2} was prepared in phosphate buffer and 100 µl spread onto two plates each of Komada medium with 1 µg/ml benomyl and polychloronitrobenzene medium (PCNB, similar to Nash and Snyder medium). The other method consisted of

filtering a 50 ml irrigation water sample through a 0.2 μm sterile cellulose Millipore filter. Filters were then placed asceptically onto Komada medium with benomyl. The remaining two samples for 1991, and all three samples from 1992, were subsequently processed using the centrifugation method. Sample plates were incubated and examined as described for the growing medium assessment.

Pallets

Samples of wood from styroblock pallets were collected three times during the field season. In 1991 one of the styroblock pallets was new and untreated, while the other was old and scrubbed with a 10% bleach solution prior to use. In 1992 both pallets were scrubbed with a 10% bleach solution. A flame-sterilized knife was used to sample 70-140 cm of wood from each set of pallets. The pallet wood samples were then placed asceptically onto Nash and Snyder medium and incubated as previously described. The length of wood colonized by *Fusarium* was measured and expressed as a percentage of the total amount assayed.

Debris

In both years debris samples were taken from the experimental area. Ten areas (10 cm x 10 cm) were selected from below and around the styroblock pallets in 1991 and five areas were selected in the same manner in 1992. The debris mainly contained peat, grit, and dead plant material. The mass of each sample was measured and added to 50 ml of sterile distilled water. The debris/water samples were shaken and serial dilutions, ranging from 10^0 to 10^{-2} were prepared. In 1991, 100 μl from each sample was spread onto Komada and Nash and Snyder medium. In 1992 only the Nash and Snyder medium was used. The plates were incubated and examined as previously described. *Fusarium* colonies were counted and the number of colony forming units per gram of debris calculated.

2.3 RESULTS

Assessment of Sanitation Treatments

The sanitation treatment assay indicated that a running water imbibition, followed by an 8-hour soak in 3% hydrogen peroxide and a 48-hour running water rinse, provided the best control for *Fusarium* without a marked decrease in germination (Table 2.3.1).

Identification of *Fusarium* isolates from section Liseola

Difficulty was encountered identifying *F. proliferatum* because it may be confused with *F. moniliforme*. Both species belong to *Fusarium* section Liseola and are differentiated by the presence of monophialides in *F. moniliforme*, and both monophialides and polyphialides in *F. proliferatum*. Dr. K. Seifert (Agriculture Canada, Ottawa) examined a representative seed and root isolate from the operational 476 treatment for both years and identified the isolates as *F. proliferatum*. The isolate which predominates on seedlot 476 produces very few polyphialides. The taxonomy of section Liseola is considered controversial (Dr. K. Seifert, pers. com.).

Pre-sowing *Fusarium* Assessment of Seed

No *Fusarium* was found associated with the seed used for the border treatments in either year. In 1991 (Table 2.3.2), 68.8% of the seeds assayed in the operational treatment of seedlot 476 were contaminated with *Fusarium*, especially *F. proliferatum* (43.8% of all seeds tested). *Fusarium oxysporum* occurred on 5.8% of the seeds tested and other species of *Fusarium* were found on 20.0% of the seeds (ie., *F. solani, F. avenaceum, F. acuminatum*). Sanitation of seedlot 476 significantly reduced *Fusarium* levels to 1.2% (Appendix 1.1, Table A1.1.1). Similar results were observed in 1992 (Table 2.3.3). The proportion of seeds associated with *Fusarium* in the operational treatment of seedlot 476 was not significantly different between 1991 and 1992 (Table A1.1.3). Seed sanitation of seedlot 476 significantly reduced *Fusarium* levels compared

to the operational treatment (Table A1.1.2). Slightly more seeds were associated with *F. proliferatum* in the operational treatment of seedlot 476 in 1992 (Table A1.1.4). However, there were significantly fewer seeds with other *Fusarium* species in 1992 compared to 1991 (Table A1.1.4).

Pre-sowing *Fusarium* levels on the seedlot 9983 treatments were identical for both years and were much lower than those on the seedlot 476 treatments. In operationally treated seedlot 9983, *Fusarium* was associated with 0.4% of the seeds, which was not much different from the sanitized treatment (although there was too little data to test statistically, Appendix 1.1, Table A1.1.6). No *F. proliferatum* or *F. oxysporum* occurred in either treatment of this seedlot. *Fusarium avenaceum* was the only *Fusarium* species found in seedlot 9983. No *Fusarium* was detected in the sanitized treatment of this seedlot.

Table 2.3.1 Effects of seed sanitation methods on germination and level of *Fusarium* contamination of seedlot 476 (Laboratory--1991).

Sanitation Treatment	Cumulative Percent Germination*	Percentage of Seeds Contaminated by *Fusarium***
4 hours--3% H_2O_2	73.0 %	1.0 %
5 hours--3% H_2O_2	69.0	0.0
6 hours--3% H_2O_2	72.0	1.0
7 hours--3% H_2O_2	81.0	1.0
8 hours--3% H_2O_2	81.0	0.5
30 min.--30% H_2O_2	67.0	0.5
45 min.--30% H_2O_2	74.0	0.5
Control	77.0	60.0

*n=100 **n=200

Table 2.3.2 Pre-Sowing Levels of *Fusarium* Associated with Operational and Sanitized Treatments of Seedlots 476 and 9983-(1991).

Treatment	% of Seeds Contaminated by *Fusarium* (Total)	% of Seeds Contaminated by *F. proliferatum*	% of Seeds Contaminated by *F. oxysporum*	% of Seeds Contaminated by other*** *Fusarium* Species
Operational 476*	68.8 %	43.8 %	5.8 %	20.0 %
Sanitized 476*	1.2	0.0	0.0	1.2
Operational 9983**	0.4	0.0	0.0	0.4
Sanitized 9983**	0.0	0.0	0.0	0.0

* n=260
** n=500
*** *Fusarium* species other than *F. proliferatum* or *F. oxysporum*

Table 2.3.3 Pre-Sowing Levels of *Fusarium* Associated with Operational and Sanitized Treatments of Seedlots 476 and 9983-(1992).

Treatment	% of Seeds Contaminated by *Fusarium* (Total)	% of Seeds Contaminated by *F. proliferatum*	% of Seeds Contaminated by *F. oxysporum*	% of Seeds Contaminated by other* *Fusarium* Species
Operational 476	64.2 %	58.4 %	3.6 %	2.2 %
Sanitized 476	5.2	1.0	0.0	4.2
Operational 9983	0.4	0.0	0.0	0.4
Sanitized 9983	0.0	0.0	0.0	0.0

* *Fusarium* species other than *F. proliferatum* or *F. oxysporum*
n=500

Percentage of Seedlings Infected with *Fusarium*-1991 Nursery Experiment

It is important to note that visible disease symptoms did not occur during this growing season.

Seedling roots were considered to be infected if *Fusarium* was isolated from the surface-sterilized root pieces. Results of this assay indicated that the culture medium did not influence root colonization by *Fusarium,* and thus the seedling infection and root colonization results from both media were combined in the statistical analyses. Since there was no significant difference between blocks (Appendix 2.1, Table A2.1.1) data were combined for the chi-square tests.

The percentage of seedlings with *Fusarium* root infections increased during the field experiment for all treatments (Figure 2.3.1). For the operational treatment of seedlot 476, the percentage of seedlings with *Fusarium* root infections increased from 6.7% at sample time 1 (July 16), to 56.7% at sample time 3 (December 1). Smaller increases were observed for the other three treatments.

Sanitation significantly reduced the number of seedlings with *Fusarium* root infections for most sample times in seedlot 476 but not for seedlot 9983. There were not enough data for statistical analysis of either seedlot at sample time 1. Sanitation of seedlot 476 significantly reduced the number of seedlings with *Fusarium* root infections by sample time 2 (August 20, Appendix 2.2, Table A2.2.2). Treatments were not significantly different for seedlot 9983 (Table A2.2.5). By sample time 3, sanitation of seedlot 476 had signficantly reduced the number of seedlings with *Fusarium* root infections to 26.7% (Table A2.2.3). There was no significant treatment difference for seedlot 9983 (Table A2.2.6).

Figures 2.3.2 to 2.3.4 show the percentage of seedlings of each treatment with *F. proliferatum* root infections, relative to the percentage of seedlings with total *Fusarium*

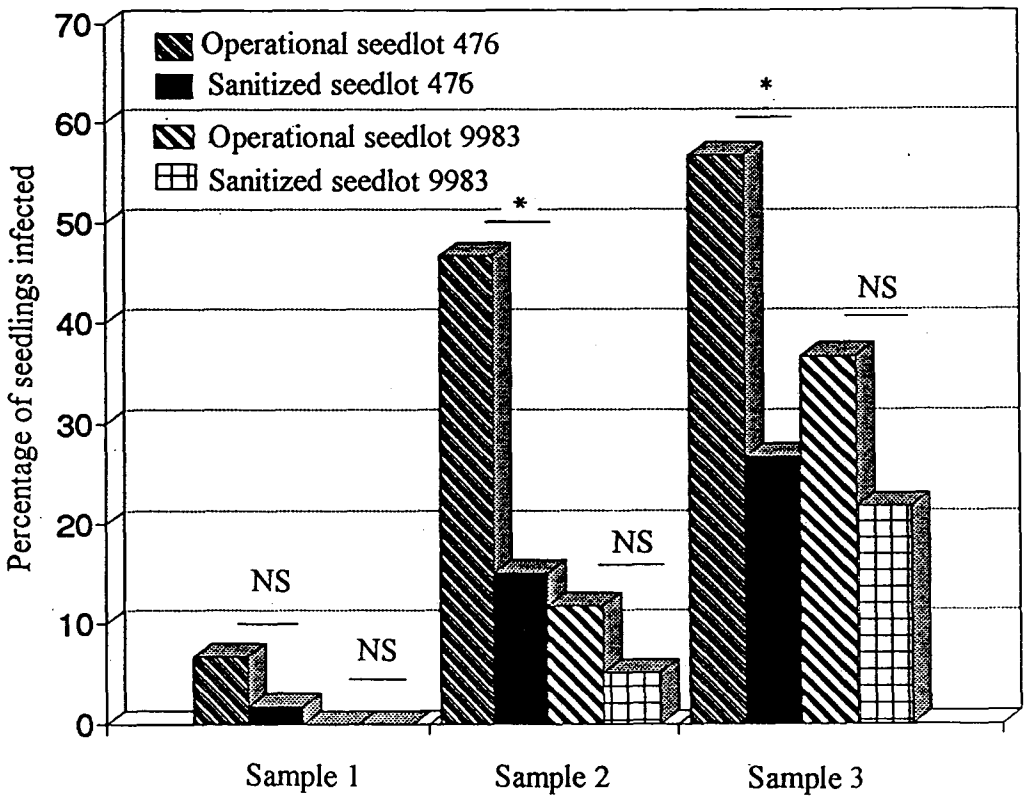

Figure 2.3.1 1991--Percentage of seedlings with *Fusarium* root infections for sample dates 1,2 and 3 (July 16, August 20, and December 1).

NS indicates that within a seedlot, treatments are not significantly different.
* indicates that within a seedlot, treatments are significantly different (p=0.01).
n=60

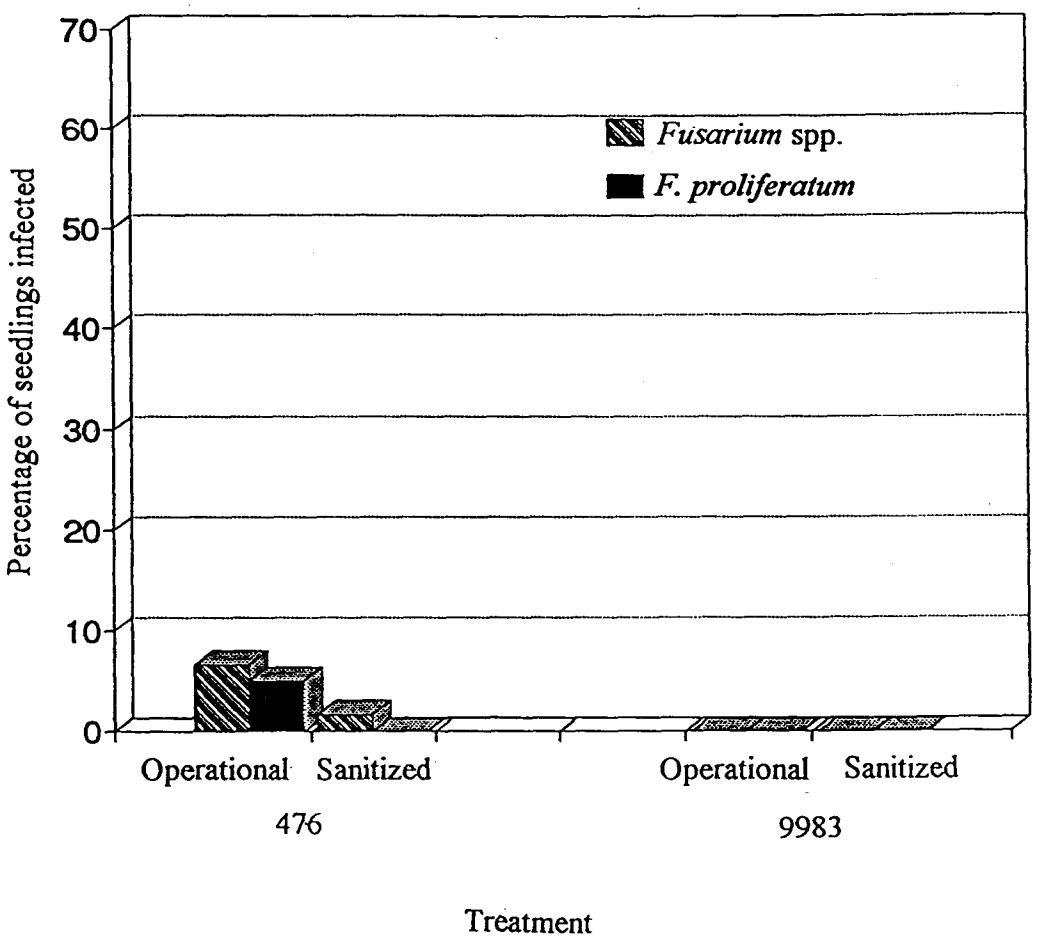

Figure 2.3.2 1991--Percentage of seedlings with *Fusarium* spp. and *F. proliferatum* root infections (sample time 1).

n=60

root infections (including *F. proliferatum*) for the three sample times in 1991. At sample time 1 (Figure 2.3.2), only seedlings from the operational treatment of seedlot 476 had any *F. proliferatum* root infections.

For the second sample time (Figure 2.3.3), most of the *F. proliferatum* root infections were observed in the operational treatment of seedlot 476. Of the 46.7% of seedlings with *Fusarium* root infections, the majority (36.7%) were represented by *F. proliferatum.* This was significantly higher than the sanitized 476 treatment, where only 11.7% of the seedlings contained *F. proliferatum* root infections (Appendix 2.3, Table A2.3.2). The roots of one seedling from the operational treatment of seedlot 9983 contained *F. proliferatum.*

The trend in seedlot 476 continued to be observed at sample time 3 (Figure 2.3.4). The majority of the infected seedlings in the operational 476 treatment had *F. proliferatum* root infections. Sanitation of this seedlot significantly reduced this percentage to 8.3% (Table A2.3.3). The percentages of seedlings with *F. proliferatum* root infections for seedlot 9983 were much lower and there was no significant difference between operational and sanitized treatments (Table A2.3.6).

A comparison of the number of seedlings with *Fusarium* root infections between the sanitized 476 and operational and sanitized 9983 treatments showed that there was no significant difference (Appendix 2.7, Table A2.7.1). A comparison of the proportion of infected seedlings with *F. proliferatum* root infections between these three groups revealed that the *F. proliferatum* to total *Fusarium* ratios were not significantly different (Appendix 2.7, Table A2.7.3).

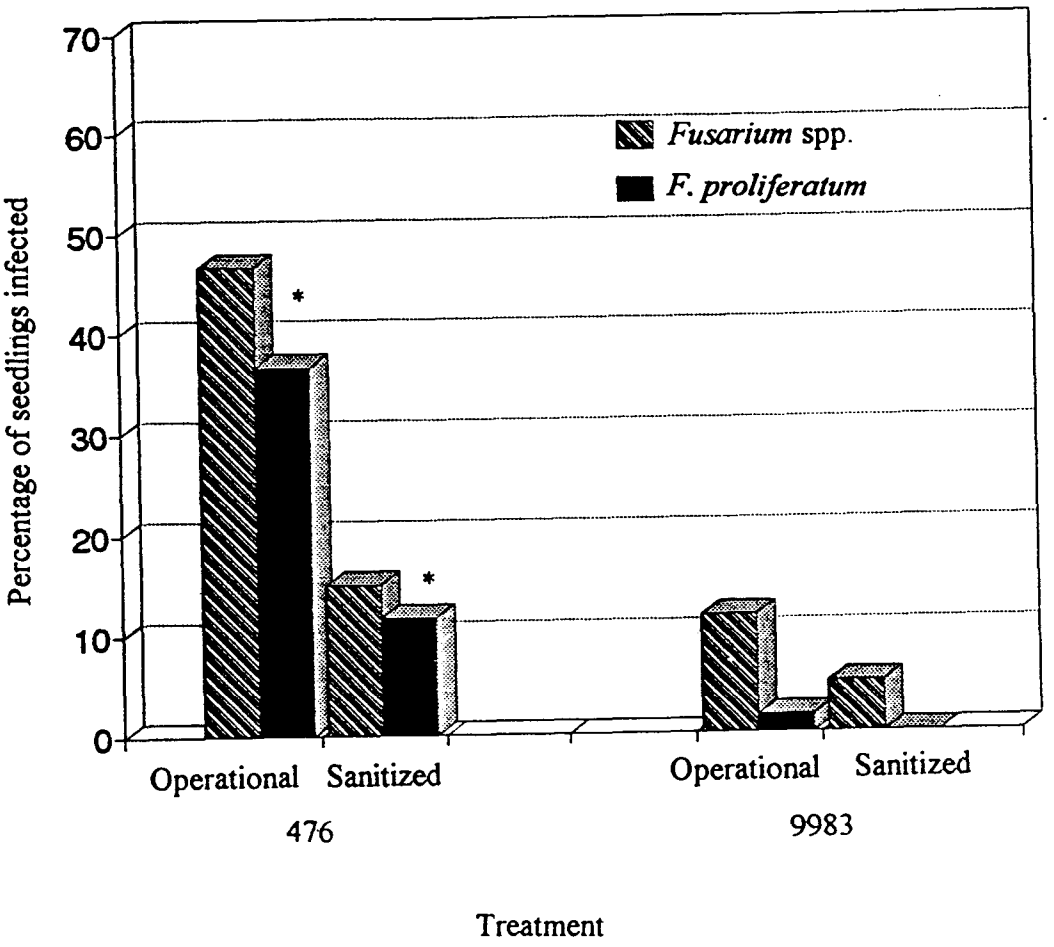

Figure 2.3.3 1991--Percentage of seedlings with *Fusarium* spp. and *F. proliferatum* root infections (sample time 2).

* indicates that within seedlots, the operational and sanitized treatments are different with respect to the numbers of seedlings with *F. proliferatum* root infections (p=0.01). n=60

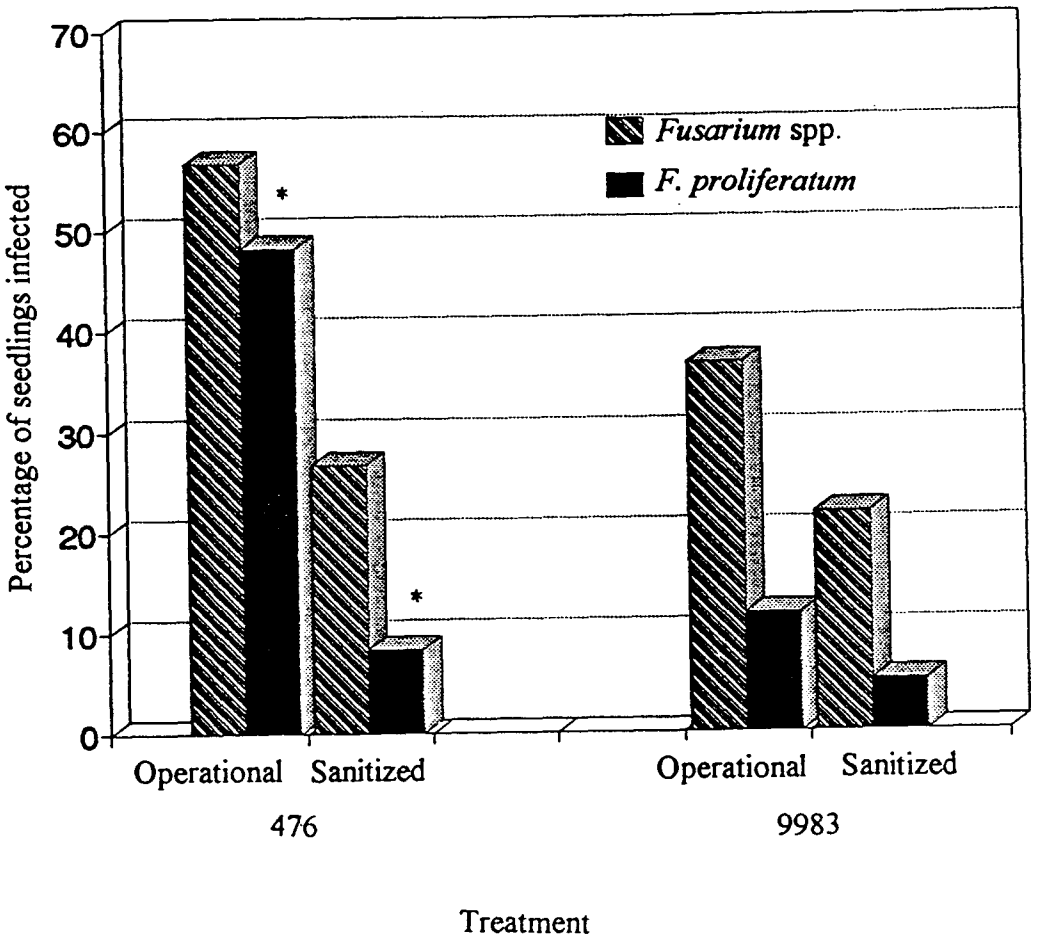

Figure 2.3.4 1991--Percentage of seedlings with *Fusarium* spp. and *F. proliferatum* root infections (sample time 3).

* indicates that within seedlots, the operational and sanitized treatments are different with respect to the numbers of seedlings with *F. proliferatum* root infections (p=0.01) n=60

Fusarium Root Colonization-1991 Nursery Experiment

Fusarium root colonization for the infected seedlings of each sample is summarized in Table 2.3.4. At sample time 1, the average percentage of roots colonized in the operational and sanitized treatments of seedlot 476 appeared to be similar but there was not enough data to test this statistically. There were no seedlings with *Fusarium* root infections for the seedlot 9983 treatments. For sample time 2, the percent of infected roots decreased for both treatments of seedlot 476, but the treatment differences were not significant (Appendix 2.8.1). There was no significant difference in the percentage of root colonization for seedlot 9983 treatments (Appendix 2.8.2). In the final assay (sample time 3) the percent root infection increased for all four treatments but, within seedlots, treatment differences were not significant (Appendix 2.8.1-2).

Table 2.3.4 1991--Average percentage of roots colonized by *Fusarium* for infected seedlings

Treatment	Sample time	Infected Seedlings	
		Number	Average percentage of roots colonized by *Fusarium*
Operational 476	1	4	19.9 %
Sanitized 476	1	1	18.8
Operational 9983	1	0	-
Sanitized 9983	1	0	-
Operational 476	2	28	9.2
Sanitized 476	2	9	4.4
Operational 9983	2	7	9.6
Sanitized 9983	2	3	5.4
Operational 476	3	34	21.8
Sanitized 476	3	16	13.2
Operational 9983	3	22	9.9
Sanitized 9983	3	13	11.5

Percentage of Seedlings Infected with *Fusarium*-1992 Nursery Experiment

There was no significant difference between blocks in the 1992 field trial (Appendix 2.1, Table A2.1.2) and therefore data were combined for chi-square tests. As in 1991, visible disease symptoms did not occur.

Like 1991, the percentage of seedlings with *Fusarium* root infections increased throughout the field season (Figure 2.3.5). All 476 and 9983 treatments were similar in the percentage of seedlings infected with *Fusarium* for the first sample period (May 25) with levels ranging from 1.7% to 5.0%. There were not enough data to test statistically for either seedlot (Appendix 2.4).

The percentage of seedlings with *Fusarium* infections had increased slightly for two of the treatments at sample time 2 (July 7). *Fusarium* root infections in the sanitized treatment of seedlot 476 increased to 10%. There was no significant difference between treatments for this seedlot (Table A2.4.2). For the operational treatment of seedlot 9983 *Fusarium* root infections decreased slightly to 1.7%, while in the sanitized version of this seedlot it increased to 15%. This treatment difference was significant (Table A2.4.5).

Increases in the percentage of seedlings with *Fusarium* root infections were observed for most of the treatments at sample time 3 (December 5). *Fusarium* root infections in the operational treatment of seedlot 476 increased nine-fold to 46.7% and increased to 28.3% in the sanitized treatment of this seedlot. These treatments were not found to be significantly different (Table A2.4.3). Root infections in the operational treatment of seedlot 9983 increased to a level of 33.3% which was not statistically different from the 16.7% observed in the sanitized treatment (Table A2.4.6).

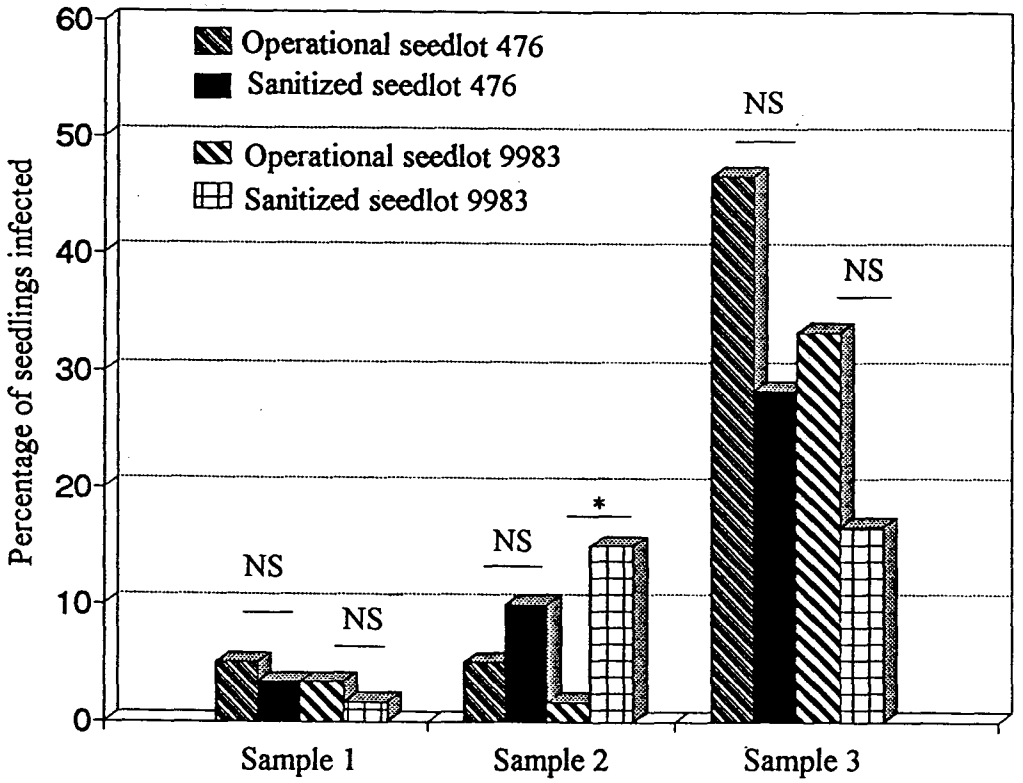

Figure 2.3.5 1992--Percentage of seedlings with *Fusarium* root infections for sample times 1,2, and 3 (May 25, July 7, and December 5)

NS indicates that within a seedlot, treatments are not significantly different.
* indicates that within a seedlot, treatments are significantly different (p=0.01).
n=60

In general there was very little *F. proliferatum* isolated from the roots of seedlings with *Fusarium* root infections. There was no *F. proliferatum* found in any of the four treatments for sample time 1 or 2. Figure 2.3.6 illustrates the percentage of seedlings with *F. proliferatum* root infections in relation to total *Fusarium* root infections (including *F. proliferatum*) for sample date 3. *Fusarium proliferatum* was isolated from the roots of seedlings sampled in the operational treatment of seedlot 476 (8.3%) and the sanitized treatment of seedlot 9883 (1.7%). There was no significant difference in the *F. proliferatum* root infections between treatments of seedlot 476 or seedlot 9983 (Table A2.5.3 and Table A2.5.6).

The numbers of seedlings with *Fusarium* root infections between the sanitized 476, and both 9983 treatments were compared and were not significantly different as was found in the 1991 field experiment (Appendix 2.7, Table A2.7.2). A comparison of *F. proliferatum*:total *Fusarium* for the infected seedlings in these three groups indicated that there were not enough data for statistical analysis (Table A2.7.4).

Combining data from sample time 3 for 1991 and 1992 showed there was no significant difference between the number of seedlings with *Fusarium* root infections in the operational 476 and 9983 treatments (Appendix 2.6, Table A2.6.1). Combining the 1991 and 1992 data for the operational and sanitized treatments of seedlot 476 at sample time 3 showed that seed sanitation significantly reduced *Fusarium* root infections (Table A2.6.2).

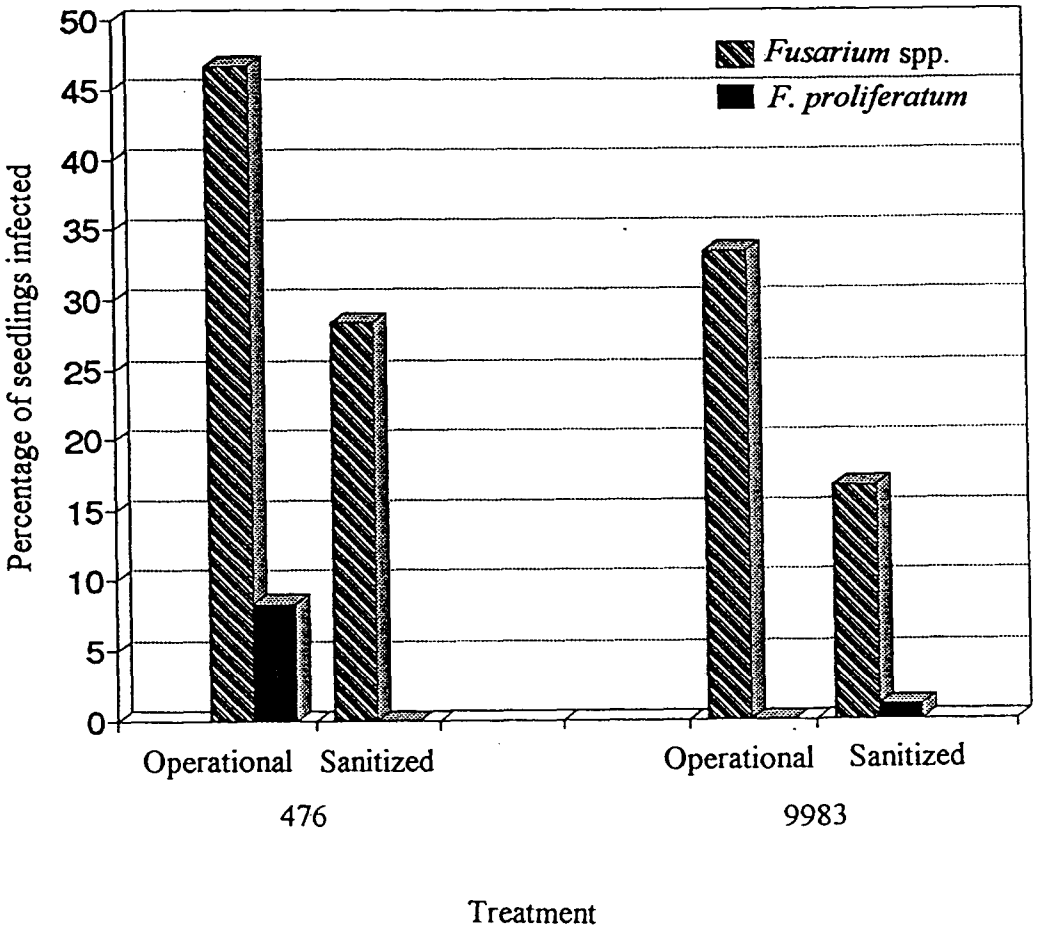

Figure 2.3.6 1992--Percentage of seedlings with *Fusarium* spp. and *F. proliferatum* root infections (sample time 3)

n=60

Fusarium Root Colonization-1992 Nursery Experiment

The average percentage of roots colonized by *Fusarium* in seedlings with root infections is summarized in Table 2.3.5. At sample time 1, the percentage of *Fusarium* root colonization differed between treatments, but the sample size was too small to determine whether this was significant. The level of root colonization appeared to be comparable for treatments within seedlots at sample time 2. The sample sizes were too small to test statistically for seedlot 9983 treatments, while the differences between the seedlot 476 treatments were not significant (Appendix 2.8.3-4). Average root colonization levels were similar for most treatments at sample time 3. There were no significant differences in the percentage of roots colonized for either treatment of seedlots 476 and 9983 (Appendix 2.8.3-4).

Table 2.3.5 1992--Average percentage of roots colonized by *Fusarium* for infected seedlings

Treatment	Sample time	Infected Seedlings	
		Number	Average percentage of roots colonized by *Fusarium*
Operational 476	1	3	16.2 %
Sanitized 476	1	2	37.4
Operational 9983	1	2	30.8
Sanitized 9983	1	1	3.6
Operational 476	2	3	21.4
Sanitized 476	2	6	22.7
Operational 9983	2	1	6.4
Sanitized 9983	2	9	7.8
Operational 476	3	28	17.0
Sanitized 476	3	17	11.4
Operational 9983	3	20	9.3
Sanitized 9983	3	10	17.4

Seed Germination-1991 and 1992 Nursery Experiments

Seed germination was significantly greater for the operational treatments of seedlots 476 and 9983 compared to corresponding seed sanitation treatments in 1991 (Figure 2.3.7, Appendix 3.1, Tables A3.1.1-2). The average germination for the operational seedlot 476 was 77.5% and sanitation resulted in a reduction in germination of almost 20%. The germination results were similar for seedlot 9983.

The effect of sanitation on seedlot 476 was more beneficial in 1992, where the average germination was 82.1%, which was significantly greater than in the operational treatment (73.8%)(Appendix 3.2, Table A3.2.1). There were no significant treatment differences for seedlot 9983 (Table A3.2.2).

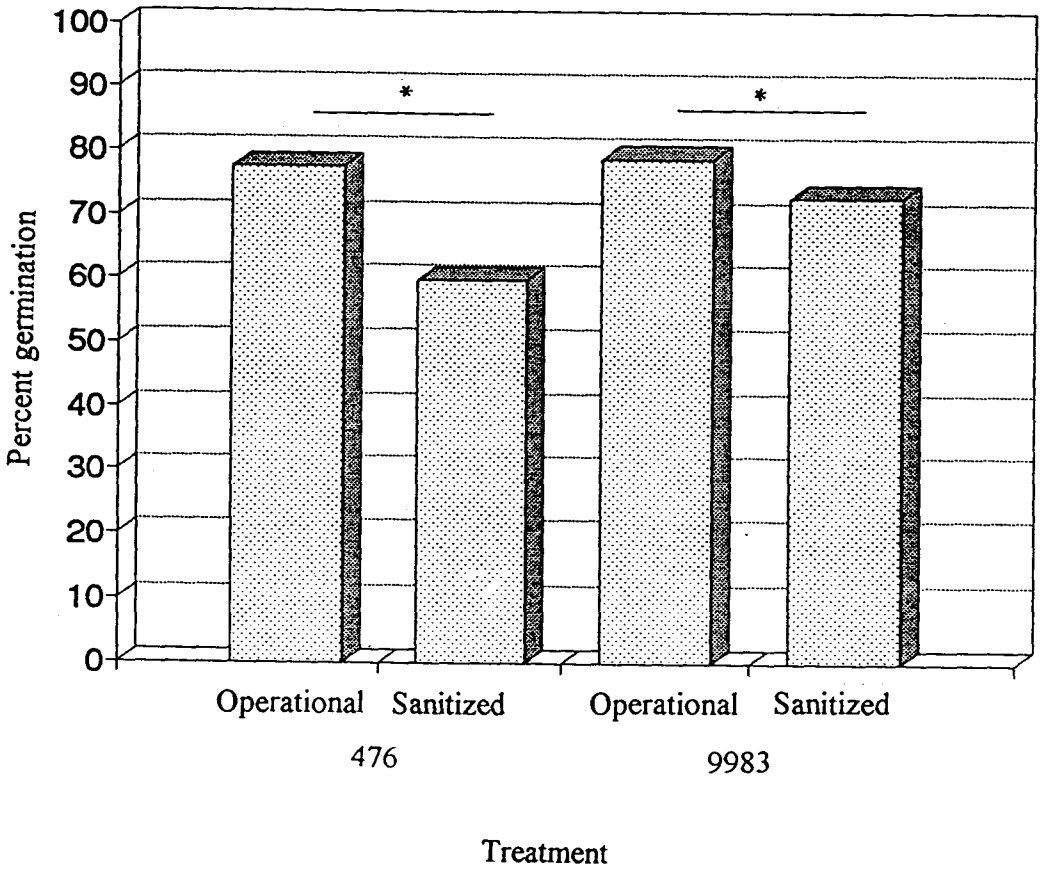

Figure 2.3.7 1991--Average percent seed germination.

* indicates that within seedlots, treatment differences are significantly different at the p=0.01 level
n=864

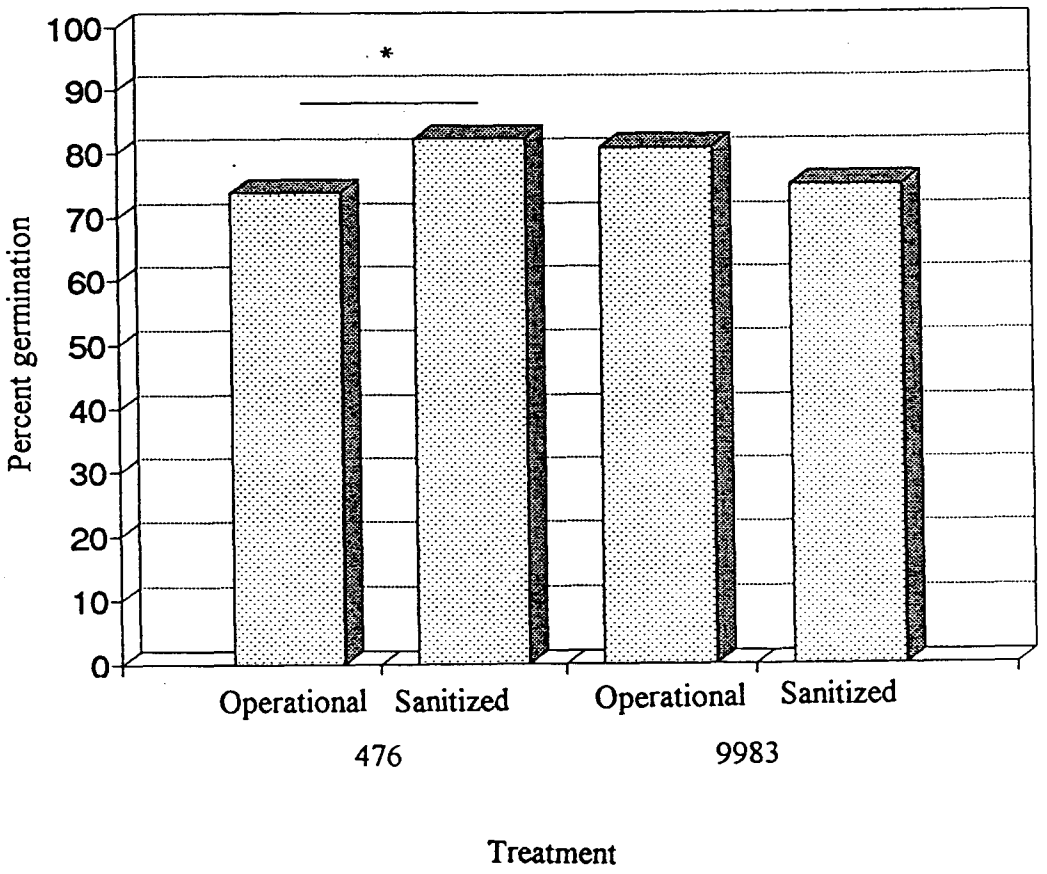

Figure 2.3.8 1992--Average percent seed germination.

* indicates that within seedlots, treatment differences are significantly different at the p=0.01 level
n=504

Seedling Growth-1991 and 1992 Nursery Experiments

Sanitation significantly increased seedling size for seedlot 476 while having little effect on seedlot 9983 for the 1991 and 1992 nursery experiments. The average height, root collar diameter, shoot and root dry weights are shown for each treatment in Tables 2.3.6-7. All statistical significance tests were performed at the p=0.05 level (Appendix 4.0).

The height of seedlings grown from the sanitized treatment of seedlot 476 was significantly greater than the corresponding operational treatment (Tables A4.1.1 and A4.2.1). Seedling height was increased by approximately 14% in 1991 and 9% in 1992. There was no significant difference in the average seedling height of the operational and sanitized treatments of seedlot 9983 for either year (Tables A4.1.2 and A4.2.2).

Sanitation resulted in significantly increased root collar diameters (16% in 1991 and 6% in 1992) for seedlot 476 (Tables A4.1.3 and A4.2.3). For seedlot 9983, sanitation did not significantly increase root collar diameter for either year of the study (Tables A4.1.4 and A4.2.4).

The average shoot dry weight of the seedlings in the sanitized treatment of seedlot 476 was also signficantly higher than the average for the operational treatment for both years of the study (Tables A4.1.5 and A4.2.5). There was no significant difference in seedling shoot dry weight associated with seed sanitation of seedlot 9983 in 1991 or 1992 (Tables A4.1.6 and A4.2.6).

Seed sanitation significantly increased root dry weights of seedlings grown from seedlot 476 (Tables A4.1.7 and A4.2.7). There was no significant treatment difference in average seedling root dry weight for seedlot 9983 in either year (Tables A4.1.8 and A4.2.8).

Table 2.3.6 Assessment of seedling growth (1991).

Treatment (n=60)	Height (cm)	Root collar diameter (mm)	Shoot dry weight (g)	Root dry weight (g)
Operational 476	6.2 *	1.7 *	0.21 *	0.23 *
Sanitized 476	7.2 *	2.0 *	0.28 *	0.32 *
Operational 9983	7.2	2.0	0.28	0.29
Sanitized 9983	7.4	2.1	0.30	0.31

* Within a seedlot, treatments are significantly different (p=0.05)

Table 2.3.7 Assessment of seedling growth (1992).

Treatment (n=60)	Height (cm)	Root collar diameter (mm)	Shoot dry weight (g)	Root dry weight (g)
Operational 476	24.0 *	2.6 *	1.15 *	0.41 *
Sanitized 476	26.0 *	2.8 *	1.51 *	0.52 *
Operational 9983	28.1	2.9	1.67	0.53
Sanitized 9983	27.4	2.8	1.56	0.49

* Within a seedlot, treatments are significantly different (p=0.05)

The number of germinants for each treatment may have led to some uncontrollable but measureable density-dependent effects on seedling size. An analysis of covariance with germination number as the covariate, and height, root collar diameter, and shoot and root dry weight as the dependent variables was used. One important prerequisite for this analysis is that the relationship between the two treatments being compared (in this case an operational and sanitized treatment of the same seedlot) be parallel for the variable being measured. As shown in Appendix 5, this was the case for the operational and sanitized treatments of seedlot 476 in 1991 only. Taking seed germination into account, the covariance analysis showed that average seedling height, root collar diameter, shoot dry weight, and root dry weight were still significantly greater for the sanitized 476 treatment (Appendix 6.0).

Other sources of *Fusarium* inoculum at Surrey Nursery

Container Mix and Irrigation Water

There was no *Fusarium* found associated with the container mix or irrigation water used in the experiments at Surrey Nursery in 1991 or 1992.

Pallets

The percentage of pallet wood colonized by *Fusarium* remained relatively constant throughout the field season in 1991 ranging between 24.8% and 38.1% except for the new pallet which had a much lower infestation level of 3.4% on the first sample date (Table 2.3.7). However, by sample time 2 the percent colonization of the new pallet had increased to 38.1% and remained fairly high for the rest of the field season. In 1992, the percentage of pallet wood colonized by *Fusarium* increased from 7.6% at sample time 1 to 63.3% at sample time 3 (Table 2.3.8). In 1992, *F. avenaceum* and *F. acuminatum*

were most frequently found on the pallets, with lesser amounts of *F. proliferatum*, *F. oxysporum*, and *F. sambucinum*.

Table 2.3.8 Assessment of Seedling Pallets for *Fusarium* (1991).

Date	Origin	Total Length of Wood Assayed	Total Length Colonized by *Fusarium*	Percentage of Length Colonized by *Fusarium*
August 6	New pallet (untreated)	99.9 cm	3.4 cm	3.4 %
	Old pallet (bleached)	67.5	17.9	26.5
September 3	New pallet (untreated)	87.3	33.3	38.1
	Old pallet (bleached)	68.7	24.4	35.5
December 13	New pallet (untreated)	103.4	27.4	26.5
	Old pallet (bleached)	73.9	18.3	24.8

n=5

Table 2.3.9 Assessment of Seedling Pallets for *Fusarium* (1992).

Date	Total Length of Wood Assayed	Total Length Colonized by *Fusarium*	Percentage of Wood Colonized by *Fusarium*	*Fusarium* Species Found (In order of frequency of occurrence)
June 18	138.8 cm	10.5 cm	7.6 %	*F. avenaceum* *F. acuminatum*
August 31	83.6	24.6	29.4	*F. proliferatum* *F. avenaceum* *F. sambucinum* *F. oxysporum*
October 24	116.0	73.4	63.3	*F. oxysporum* *F. avenaceum* *F. acuminatum* *F. proliferatum*

n=5

Debris

Fusarium was isolated from debris lying below the experimental pallets. For the first two sample periods of 1991, it was detected in nine of the ten samples from under the old pallet (Table 2.3.9). No *Fusarium* was detected under the new pallet for the first sampling time. This may be related to the low amounts of debris found below this pallet or to the low levels of *Fusarium* found on the pallet at this sampling time. *Fusarium* was detected on debris below the new pallet by the second sampling time. No *Fusarium* was found in any of the debris samples taken during the third sample date.

Two of the five debris samples were associated with *Fusarium* for the first sample period in 1992 (Table 2.3.10). The species present were *F. oxysporum*, *F. avenaceum*, and *F. sambucinum*. No *Fusarium* was detected under the pallets on the August 31st

sample date. This may have been related to the extremely small amounts of debris found at this time. However, on the third sample date, all five debris samples were found to contain *F. avenaceum*, *F. oxysporum*, and *F. proliferatum*.

Table 2.3.10 Assessment of the number of Colony Forming Units (CFU) of *Fusarium* for Debris Under Pallets (1991).

Date	Site	Sample	Mass of Debris	Mean CFU/g of Debris (Komada)	Mean CFU/g of Debris (Nash & Snyder)
August 6	Debris under old pallet	1	2.66 g	94	0
		2	3.29	0	0
		3	3.51	712	0
		4	4.15	2167	13360
		5	4.81	26	364
	Debris under new pallet	1	0.10	0	0
		2	0.29	0	0
		3	0.13	0	0
		4	0.20	0	0
		5	0.13	0	0
September 3	Debris under old pallet	1	2.36	2965	6672
		2	1.42	3514	88
		3	2.76	4527	4303
		4	0.59	6990	9774
		5	1.72	217	941
	Debris under new pallet	1	0.79	0	2347
		2	1.07	816	7463
		3	1.43	87	0
		4	0.49	0	0
		5	0.55	0	0
December 13	Debris under old pallet	1	1.71	0	0
		2	2.99	0	0
		3	5.53	0	0
		4	3.16	0	0
		5	2.53	0	0
	Debris under new pallet	1	0.68	0	0
		2	1.59	0	0
		3	1.85	0	0
		4	0.85	0	0
		5	1.91	0	0

Table 2.3.11 Assessment of the number of Colony Forming Units (CFU) of *Fusarium* for Debris Under Pallets (1992).

Date	Sample	Mass of Debris	Mean CFU/g of Debris	*Fusarium* Species Present
June 18	1	0.88 g	0	n/a
	2	0.47	0	n/a
	3	1.20	0	n/a
	4	0.69	1383	*F. oxysporum*, *F. avenaceum*, *F. sambucinum*
	5	1.01	390	*F. oxysporum*, *F. avenaceum*
August 31	1	0.23	0	n/a
	2	0.76	0	n/a
	3	1.52	0	n/a
	4	0.13	0	n/a
	5	0.28	0	n/a
October 24	1	1.52	163	*F. avenaceum* *F. oxysporum* *F. proliferatum*
	2	1.45	66	as above
	3	1.15	29	"
	4	1.78	48	"
	5	2.38	139	"

2.4 DISCUSSION

The *Fusarium* assessment of pre-sown seed indicated that the hydrogen peroxide sanitation treatment significantly decreased seedborne *Fusarium* for seedlot 476 in 1991 and 1992 field experiments. This agreed with results from other studies using similar treatments (Dumroese *et al.*, 1988; Campbell and Landis, 1990).

Seedborne *Fusarium* levels were much lower for both treatments of seedlot 9983 than for the operational treatment of seedlot 476 in 1991 and 1992 nursery experiments. Furthermore, the majority of the seed contamination in the operational treatment of seedlot 476 was from *F. proliferatum*, but this species was absent on both treatments of seedlot 9983.

Seedborne *Fusarium* appears to play a significant role in the root colonization of Douglas-fir. Over the 2 years, sanitation of seedlot 476 resulted in an average of 46% fewer seedlings with *Fusarium* root infections compared to the operational treatment of this seedlot (sample time 3). This difference was statistically significant in 1991. The same trend continued in 1992, however results were not significant. One of the assumptions of this experiment was that the seed sanitation treatment would only have an effect on seedborne inoculum, and not on any other inoculum source. Therefore, the difference in the amount of seedling infection observed between the operational and sanitized treatments of seedlot 476 probably represents infection due to seedborne inoculum.

A comparison of the operational treatment of seedlots 476 and 9983 over the 2 years indicated that seedlot 9983 resulted in a 29% reduction in the number of infected seedlings. However, the difference in the number of infected seedlings for the operational treatments of these two seedlots was not statistically significant. The most obvious difference between them was the almost complete absence of *Fusarium* from seedlot 9983 and therefore the difference in the number of seedlings with *Fusarium* root infections is

likely to be due to the seedborne inoculum present in operational seedlot 476. However, genetic differences relating to *Fusarium* resistance between the two seedlots may also be a factor.

The frequency of occurrence of *F. proliferatum* (the indicator species) provides evidence for the importance of seedborne *Fusarium*. The sanitized 476 and both 9983 treatments resulted in seedlings with comparable numbers of *Fusarium*-infected seedlings and *F. proliferatum* to total *Fusarium* ratios at sample period 3. In 1991, approximately 30% of infected seedlings in these treatments were infected with *F. proliferatum*. In contrast, about 85% of the total infections in the operational 476 treatment were *F. proliferatum*; 55% probably originated from the seed. The trend was similar but much less pronounced in the 1992 field season. The sanitized 476 and both 9983 treatments may represent *F. proliferatum* sources predominantly of nursery origin, while the additional amount of *F. proliferatum* observed in the operational 476 treatment was probably of seed origin.

The detection of *F. proliferatum* in the sanitized 476 and both treatments of seedlot 9983, as well as in pallet and debris samples meant that the source of particular root infections could not be determined. However, there were more *F. proliferatum* root infections found on seedlings grown from operational seedlot 476 and the only difference between treatments was seedborne *Fusarium* levels and species.

Fusarium root infections increased throughout the field season for all treatments and this could have an impact on the number of diseased seedlings. *Fusarium* root disease symptoms tend to appear late in the growing season during hot, dry weather (Sutherland, 1990) or during bud set (James *et al.*, 1986). By the time these environmental conditions occur, a large number of infected seedlings may express disease symptoms. The reason why the seedborne contribution of *Fusarium* inoculum does not appear early in the field season is unclear, but it is possible that the culturing technique inhibited its detection.

James *et al.* (1993) have reported that *F. proliferatum* is isolated from container-grown conifer seedling roots, usually at the end of the production cycle.

It appears that the contribution of seedborne *Fusarium* may vary in importance between years. Differences in the numbers of seedlings with *Fusarium* and *F. proliferatum* root infections between 1991 and 1992 may be related to sowing dates, environmental conditions, or to interactions with other microorganisms. It is possible that the earlier sowing date and weather conditions in 1992 resulted in fewer seedlings with *Fusarium* root infections. Pre- and post-emergence damping-off is favoured by cool, wet soil (Sutherland *et al.*, 1989), and while no damping-off was observed, the cool weather at the time of sowing in 1991 may have lead to an increase in root infections. The low levels or complete absence of *F. proliferatum* in the roots of seedlings sampled from all treatments in 1992 indicate that either this species was not present in large amounts at Surrey Nursery, or more likely, environmental conditions were not favourable for *F. proliferatum*. It is also possible that *Cylindrocarpon* levels were higher in 1992. Axelrood and Peters (1993a) indicated that *Fusarium* and *Cylindrocarpon* may compete for infection sites and this interaction could account for the differences between years. The change to Nash and Snyder's medium in 1992 reduced *Trichoderma* contamination but according to earlier testing should not have affected *Fusarium* detection.

In addition, the reason why the sanitized treatment of seedlot 9983 produced so many more infected seedlings during sample time 2 of 1992 (Figure 2.3.5) is not known. Since the experiment was a randomized block design, and seedlings were sampled randomly, "pockets" of *Fusarium* should not have been a factor.

The evidence for the importance of seedborne *Fusarium* in root colonization obtained in this project is corroborated by a smaller, but similar study performed in 1990 (Axelrood, Neumann, *et al.*, unpublished data). Again, the majority of infected seedlings came from the operational 476 treatment which also resulted in the most *F. proliferatum* infections (Appendix 7.0). Results of the thesis project can also be compared with a study

by Graham and Linderman (1983). They showed the importance of seedborne inoculum by recovering *F. oxysporum* from damped-off seedlings grown from seed which had been inoculated with *F. oxysporum*. James *et al.* (1993) reported that *F. proliferatum* was the fusaria most commonly isolated from the roots of healthy and diseased container-grown seedlings. They also found that many of these isolates caused damping-off and root disease. Although disease symptoms were not obvious in the current study, the virulence of seedborne *Fusarium* species could translate into unacceptable economic losses under conducive environmental conditions.

The proportion of roots assayed that were colonized by *Fusarium* remained below 25% for the majority of treatments and sample times. Seed sanitation did not significantly affect the proportion of roots colonized in infected seedlings for either seedlot. Sampling from the middle portion of the root system may not indicate colonization patterns for the entire root system. Also, the threshold percentage of root infection to result in disease symptoms is not known, but it has been shown by James *et al.* (1986) that the proportion of seedling roots colonized by *Fusarium* does not seem to be related to the severity of above-ground disease symptoms. Since these symptoms were not apparent in the results reported here, these factors could not be correlated. This problem is complicated by differential growth rates of *Fusarium* on selective media, resulting in root assays that may not accurately determine the proportion of tissue that is infected. More work is needed in this area to develop an accurate root colonization assay technique.

The effect of the 8-hour hydrogen peroxide sanitation treatment on germination of seedlots 476 and 9983 in the field is variable. This suggests this sanitation treatment may not be ideal in operational practice, despite the fact that it reduced *Fusarium* contamination to very low levels (Tables 2.3.2 and 2.3.3). Because laboratory tests do not mimic field conditions, and the sowing dates differed between the two field trials, more research is required to determine the effects of this type of seed sanitation. Trappe (1961) found that strong hydrogen peroxide (30%) greatly stimulated germination of Douglas-fir

seed while others report that 3% hydrogen peroxide is effective (Dumroese *et al.*, 1988; Campbell and Landis, 1990). For management purposes, a different seed treatment could be developed to control contamination problems below threshold levels without inhibiting germination.

Seed sanitation was associated with an increase in the overall size of seedlings grown from the 476 seedlot, but had little effect on seedlot 9983. Part of this increase in growth in the 1991 experiment could have been due to the presence of fewer germinants and reduced seedling competition. However, covariance analysis indicated treatment differences were still significant even when the number of germinants was taken into consideration. The sanitation treatment may have speeded up germination and this may have contributed to the increased growth seen in the sanitized 476 treatment. This increased growth could also have occurred because of little *Fusarium* or other deleterious microorganisms associated with the seed. Since sanitation of seedlot 476 was associated with fewer seedlings harbouring *Fusarium* root infections, more seedlings may have attained their maximum potential growth. One symptom of *Fusarium* root infection may be reduced growth (Smith, 1975). Sanitation of seedlot 9983 had little effect on the size of seedlings, probably in part because there was little *Fusarium* associated with the seed. However, it can not be shown conclusively that the reduction in seedborne *Fusarium* led to increased seedling size, since there are numerous other effects which could have produced the same results. Seed sanitation requires further study to determine its utility to assist contaminated seedlots to reach size specifications, while keeping germination losses within economically viable limits.

The absence of *Fusarium* from the planting mix and irrigation water suggests that these are not important sources of *Fusarium* inoculum. However, sample size and frequency could be increased to confirm these results.

Pallets and debris may be important sources of *Fusarium* inoculum, particularly if levels are permitted to build up from one season to the next. It is possible that water

dripping off of contaminated pallets may carry *Fusarium* inoculum to debris lying below them. *Fusarium* was found to be associated with debris and normal air circulation may transfer airborne inoculum onto the seedling crop (Neumann and Axelrood, 1992). The reason for the absence of *Fusarium* in the debris samples of the third sample date in 1991 is unclear; however, weather conditions may have affected the survival of inoculum. Because *Fusarium* inoculum from seedling pallets and debris may play an important role in the epidemiology of *Fusarium* root diseases, more research is needed in this area including the development of effective sanitation for seedling pallets. Finally, keeping seedling growing areas as clean as possible may reduce inoculum levels.

To conclusively determine whether seedborne *Fusarium* is an important inoculum source, a method to discriminate between *Fusarium* of seed origin and that of nursery origin would have to be developed. Attempts were made to develop a polymerase chain reaction based assay using restriction fragment length polymorphisms, and primers from the internal transcribed spacer region of ribosomal DNA (Vrain et al., 1993). However, the work had not progressed far enough to be utilized in this study. Future work may address this problem, and may also lead to the development of tools such as diagnostic kits for seed contamination assays.

Further work on seedborne *Fusarium* is required to determine its role in the epidemiology of root diseases in container-grown Douglas-fir. It appears that pathogenic seedborne *Fusarium* may colonize seedling roots, but whether this ultimately may lead to disease expression under operational conditions is not known. Furthermore, some seedborne fusaria may not be pathogenic. Studies on other species such as *Cylindrocarpon*, may provide more information about the expression of *Fusarium* root disease symptoms since there seems to be some interaction between the two fungi (Axelrood and Peters, 1993a). It is also not known whether seedborne *Fusarium* causes enough disease in container-grown seedlings to warrant the added costs of its control.

CHAPTER 3 *FUSARIUM* CONTAMINATION OF DOUGLAS-FIR SEED DURING CONE AND SEED PROCESSING

3.1 Introduction

Fusarium has been associated with both seed orchard and wild Douglas-fir seed (J. Dennis, personal communication). Neumann and Axelrood (1992) found seedborne *Fusarium* levels in contaminated seedlots ranged from 0.3 to 14%. Axelrood (personal communication) also found *Fusarium* levels as high as 95.4% prior to stratification. Evidence suggested that stratification may further increase seedborne levels, particularly for seedlots with moderate *Fusarium* contamination.

One way to control *Fusarium* root disease on Douglas-fir may be to reduce the sources of inoculum, such as minimizing seedborne *Fusarium*. However, it is not known exactly when Douglas-fir seed becomes contaminated. Graham and Linderman (1983) suggested that *Fusarium* may be associated with cones, cone and seed processing equipment and methods, or storage containers. Mittal and Wang (1987) found that the incidence of fungi on pine and spruce seeds was low at the time of cone harvest, but increased during air drying of cones and most of the cone and seed processing operations. However, in the final seed processing stage, fewer seeds were contaminated than at any other stage of cone or seed processing. They also found that seeds from cones which had lain on the ground for a period of time were frequently contaminated by fungi. The B.C. Ministry of Forests Tree Seed Centre, Surrey receives a number of cone collections originating from squirrel caches each year (Heather Rooke, personal communication). It is therefore possible that the same pattern of seed contamination observed by Mittal and Wang (1987) may occur at the B.C. Ministry of Forests Tree Seed Centre.

For Douglas-fir there is approximately 2 years from the beginning of gametophyte formation to the cleaned seed. The reproductive cycle of Douglas-fir is 17 months (Allen

and Owens, 1972), during which time *Fusarium* may be present to contaminate seeds. Another potential period for seed contamination is during cone harvest. Seed may be more susceptible to disease if cones are harvested before the seeds are mature (Bloomberg, 1969). Although efforts are made to collect only ripe cones, in moderate to heavy crop years there is a logistical problem of collecting a large volume of cones in a relatively short period of time (Heather Rooke, personal communication). In addition, the containers that harvested cones are put into may contaminate Douglas-fir seed. Cones are usually placed in burlap sacks which are then stored in outdoor drying sheds. Cone sacks are often re-used and may permit *Fusarium* inoculum to build up from season to season. Airborne inoculum could also contaminate seeds and cones. Since Douglas-fir tends to produce occasional, large crops of cones, they may be stored in burlap sacks for several months prior to seed processing. Seeds could become contaminated if the sacks are contaminated and environmental conditions during the storage period are favourable to the fungus (Mittal and Wang, 1987).

Fusarium contamination may also occur during cone and seed processing. Not only may seeds be brought into contact with contaminated equipment but clean seed may be in close physical contact with contaminated seed. The first step in the Douglas-fir cone processing operation at B.C. Ministry of Forests Tree Seed Centre is the pre-conditioning phase during which cones are stored for at least 4 weeks in an outdoor drying shed. They are then loaded onto trays at ambient kiln conditions for the drying process. Over the next 4 hours, the relative humidity is taken from 60% to 40% and the temperature is increased to 40°C. These conditions are held for 12-14 hours and when the majority of the cone scales are flexed, the temperature is reduced to 20°C and the relative humidity to 25-30%. This warm air drying opens the cones thereby facilitating maximum seed removal. Immediately after the kiln drying, cones are tumbled in rotating screened drums to loosen and extract the seed. Because small pieces of debris are often extracted along with the seed, the seed must then be cleaned via a two-stage screening process.

Next, the wings must be removed from the Douglas-fir seed since they interfere with the automatic seeders used by most conifer seedling nurseries. The wing of the Douglas-fir seed is part of the seedcoat and therefore must be mechanically broken off by rotating the dry seeds inside a drum for 30-45 minutes. This step is potentially damaging to the seed because of cracking or abrasion which leaves it more vulnerable to fungal contamination. However, adequate precautions (ie., not rotating seeds too fast or too long) usually eliminate seed damage at this step. A further cleaning to remove the remainder of the debris is then performed. This cleaning employs another screening step, which is followed by seed immersion in a liquid separation tank, through which there is a continuous flow of water that is recycled back into the tank containing the seed. Water is changed only between seedlot batches unless it appears 'dirty' or the seedlot batch is exceptionally large. During this liquid separation process, debris such as rocks, pitch and fully dehusked seeds sink to the bottom of the tank while the rest of the seeds float to the top. It is possible during this step that *Fusarium* on the seedcoat of contaminated seeds may be transferred to other non-contaminated seeds through the water.

Immediately following this flotation cleaning, wet seeds are removed and dried on trays that move through an hour-long, three-stage drying process going from 17°C, to 35°C with a humidity of 20-30%. The final stage of seed extraction process is grading. The seed grader divides seeds into four classes based on their size which are then further sorted into separate fractions based on specific gravity via a pneumatic process. The pneumatic process ensures that dead, large seeds are discarded, while viable but small seeds are not. The size classes are recombined prior to moisture, germination, and purity testing, following which, the seedlot is registered and stored at -18°C. Seedlots are usually re-tested every two years (Description of cone and seed processing--Heather Rooke, personal communication).

All processing equipment and areas are cleaned between seedlot batches using vacuuming, sweeping and air hosing methods. The liquid separation tank is rinsed with

water and wiped. However, no other sanitation measures are taken which may result in opportunities for the contamination of seed by *Fusarium*.

The objective of this study was to determine which method(s) in the cone harvesting or cone and seed processing operations could be potentially significant in final *Fusarium* contamination levels of Douglas-fir seed.

3.2 Materials and Methods

The source of seed was an orchard comprised of clonal and seedling coastal Douglas-fir donated by Canadian Pacific Forest Products, Saanichton, B.C.

All heavy to medium cone-producing trees were selected for harvest. Trees were visually divided into four quadrants: north upper, north lower, south upper, and south lower. All cones were harvested from each quadrant from each chosen tree on September 5-6, 1991. Cones from tree quadrants were bulked and half of the cones from each quadrant were placed in new burlap sacks while the rest were placed in old burlap sacks. The sacks were filled about two-thirds full as per operational standards. There were three replications (sacks) of each of these eight treatments. The burlap sacks were placed in the middle of a drying shed filled with sacks of cones from other orchards. The sacks were turned weekly for 8 weeks until they were taken to the B.C. Ministry of Forests Tree Seed Centre for further conditioning and processing.

The first sample was taken at the time of harvest. Ten cones from each of the four quadrants were randomly selected and cut in half with a modified-knife cone cutter (Winjum and Johnson, 1960). Seeds were asceptically removed and placed on Komada's medium. Four hundred seeds were assayed from each of the four treatments (quadrants). Both full and partially empty seeds were included in the sample. Seeds were incubated on the medium under continuous fluorescent lighting for 2 weeks. Seeds associated with *Fusarium* colonies were counted and identification was verified microscopically.

The second sample was taken after the 8-week drying period. Because it was apparent that the north-lower old burlap bag treatment was the most likely to contain *Fusarium*, all further sampling was restricted to this treatment. Ten cones were selected randomly from each of the three replicates of the north-lower old bag treatment. Cones were asceptically dissected and 400 seeds were assayed as described above.

The third set of samples was taken during cone processing and seed extraction. Cones from the three replicates were operationally processed for seed extraction at the Seed Centre. All three replicates were processed at the same time but were in separate compartments of the seed extraction equipment. Seed samples of 15 grams were taken after each step of the seed extraction process (Figure 3.2.1) for each replicate. Four hundred seeds from each replicate of the final seed product were assessed as described previously.

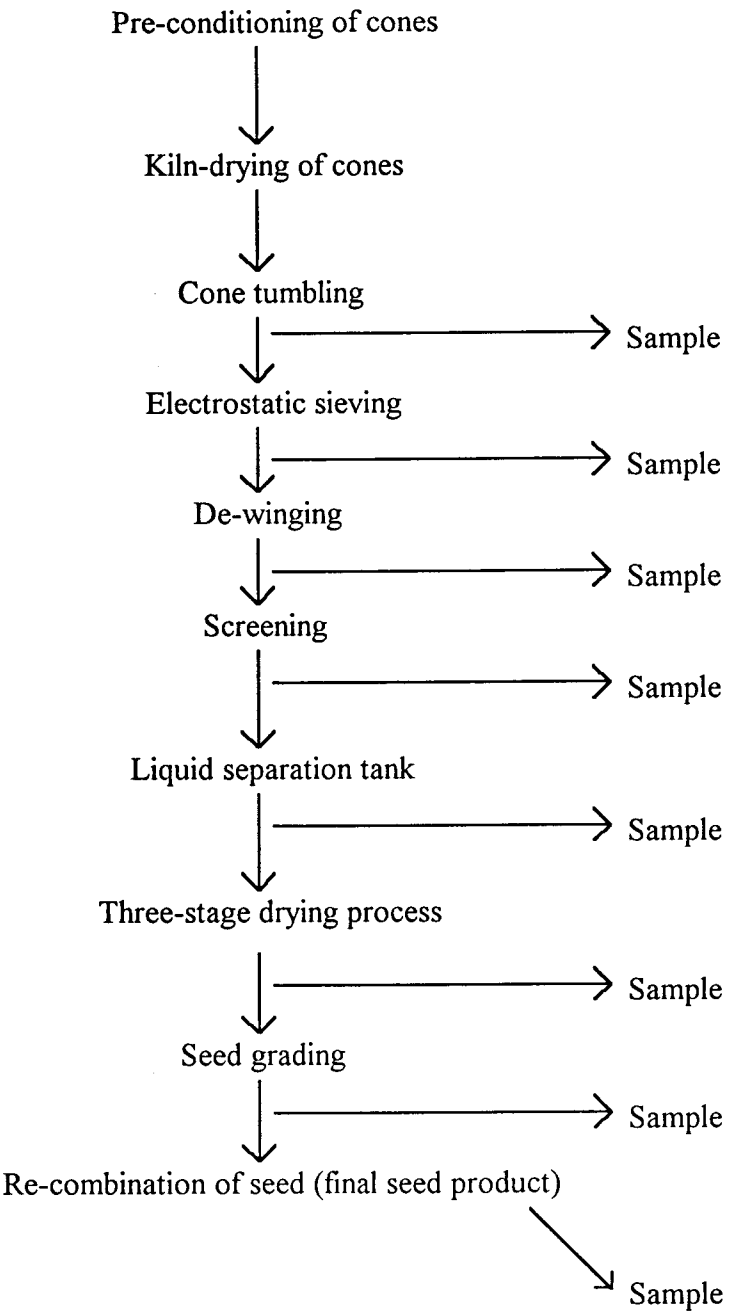

Figure 3.2.1 Flow-chart of sample points during cone and seed processing.

3.3 Results

The percentage of seeds contaminated with *Fusarium* immediately after cone harvest ranged from 0.0-0.75% depending on which quadrant of the tree the seeds originated from (Table 3.3.1). Seeds taken from cones on the north lower quadrant of the tree appeared to have the most *Fusarium*. *Fusarium* contamination of seed appeared to be minimal immediately after harvest.

Only the seeds from the replicates of the north-lower old bag treatment were assessed for the second sample time (after the 8-week drying period). The results of the first sample suggested that this treatment was the most likely to yield *Fusarium*. For replicate one, seedborne *Fusarium* levels had increased to 5.5%. Replicates two and three remained at a contamination level of 0.75% (Table 3.3.2).

Although seed samples were taken at every stage of the seed extraction process, seed assessments began with the final clean seed product (sample 3) which indicated there was no *Fusarium* present (Table 3.3.2) and it was therefore not worthwhile to assay the rest of the samples.

3.4 Discussion

Little *Fusarium* was found on the seeds which were assayed directly after cone harvesting. This is corroborated by Mittal and Wang (1987) who found that *Fusarium sporotrichoides* was absent on pine and spruce seeds at the time of harvest. After the 8-week drying period, one of the treatment replicates showed an increase in seedborne *Fusarium* while the other replicates remained the same. This increase could be due to the use of an old and possibly contaminated burlap sack, normal air circulation during the drying period, contact with other contaminated cones, or a combination of these factors.

Table 3.3.1 Percentage of seeds contaminated with *Fusarium* at the time of harvest. (Sample time 1)

Treatment (n=400)	Percentage of Seeds Contaminated with *Fusarium*
North-lower	0.75 %
North-upper	0.25
South-lower	0.25
South-upper	0.0

Table 3.3.2 Percentage of seeds in the north-lower old burlap bag treatment contaminated with *Fusarium* at sample time 2 (post eight-week drying) and at sample time 3 (final seed product)

NORTH-LOWER OLD BURLAP BAG TREATMENT		
Replicate (n=400)	Sample Time	% of Seeds Contaminated with *Fusarium*
1	2	5.5 %
2	2	0.75
3	2	0.75
1	3	0.0
2	3	0.0
3	3	0.0

Mittal and Wang (1987) also found that *Fusarium* levels on spruce and pine seeds increased during the air-drying process. The absence of *Fusarium* from the final seed product may indicate that the seed processing did not enhance *Fusarium* contamination of seed. Since the final seed product is 100% filled seed, it is likely that *Fusarium* contamination seen in previous samples was on seed which was empty or partially empty. Fungal species in general appear to occur less frequently in the final seed product compared to other stages in the seed extraction, probably as a result of the removal of empty and under-developed seeds (Mittal and Wang, 1987).

It is possible that the low levels of *Fusarium* seen here are a result of environmental conditions in the seed orchard unfavourable to seed contamination. Seed and cone processing equipment may not have been a source of *Fusarium* inoculum at the time of this study. Although *Fusarium* appeared to be eradicated in the final seed product, additional research is needed using cones collected from different locations and processed at several times during the cone and seed processing season. The presence of *Fusarium* in the final product of other seedlots processed at this seed centre indicates the need for more research into the impact of cone and seed processing on final seedborne *Fusarium* levels.

CHAPTER 4 GENERAL CONCLUSIONS

It appears that seedborne *Fusarium* may be an important source of inoculum in root colonization of container-grown Douglas-fir seedlings. The results of this study also suggest that the importance of this source may vary between years. Although root colonization by *Fusarium* is a prerequisite for *Fusarium* root disease, symptoms are not always expressed. Further research to determine the relationship between seedborne *Fusarium* and root disease will assist in the development of management tactics to prevent seedling losses.

The 8-hour hydrogen peroxide seed sanitation treatment may have contributed to some germination losses, although it was associated with increased seedling growth in seedlot 476. Part of this increase may have been due to reduced seedborne *Fusarium* levels. Further research into seed sanitation treatments is required to minimize germination losses and to ensure that seed sanitation contributes in an operationally significant way to seedling growth.

The study of the impact of cone and seed processing techniques on seedborne *Fusarium* levels resulted in undetectable levels of *Fusarium* in the final seed product. However, the existence of *Fusarium* in the final seed product of other seedlots processed at the B.C. Ministry of Forests Tree Seed Centre indicates that further research into cone and seed processing methods is required. Cone and seed processing methods may then be changed to reduce the magnitude of seed contamination by *Fusarium*.

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APPENDICES

Appendix 1.0 Pre-sowing chi-square tests.

Appendix 1.1 Chi-square tests for pre-sowing *Fusarium* assessment of seeds (1991 and 1992)

Statistical hypotheses used for all Chi-square tests in this section:

H_0= The proportion of seeds infested with *Fusarium* is independent of treatment (or year).
H_1= The proportion of seeds infested with *Fusarium* is associated with treatment (or year).

Table A1.1.1 Chi-square tests of independence in the proportions of seeds infested with *Fusarium* (total) in seedlot 476 (1991) .

Treatment	# of Seeds infested with *Fusarium* (Total)	# of Seeds not infested with *Fusarium*	Total
Operational 476	114	146	260
Sanitized 476	3	257	260
Total	117	403	260

$\chi_i^2 = 135.881$

$\chi^2_{.01,1} = 6.635$

Therefore, H_O may be rejected and it may concluded that the proportion of infected seeds is associated with treatment.

Table A1.1.2 Chi-square tests of independence in the proportions of seeds infested with *Fusarium* (total) in seedlot 476 (1992) .

Treatment	# of Seeds infested with *Fusarium* (Total)	# of Seeds not infested with *Fusarium*	Total
Operational 476	321	179	500
Sanitized 476	26	474	500
Total	347	653	1000

$\chi_i^2 = 383.768$

$\chi^2_{.01,1} = 6.635$

Therefore, H_O may be rejected and it may concluded that the proportion of infected seeds is associated with treatment.

Table A1.1.3 Chi-square tests of independence in the proportions of seeds infested with *Fusarium* (total) in the operational treatment of seedlot 476 in the two years (1991 and 1992).

Treatment	# of Seeds infested with *Fusarium* (Total)	# of Seeds not infested with *Fusarium*	Total
Operational 476-1991	179	81	260
Operational 476-1992	321	179	500
Total	500	260	760

$\chi_i^2 = 1.640$

$\chi^2_{.01,1} = 6.635$

Therefore, H_O may not be rejected and it may concluded that the proportion of infected seeds in the operational treatment of seedlot 476 is independent of year.

Table A1.1.4 Chi-square tests of independence in the proportions of seeds infested with *F. proliferatum* in the operational treatment of seedlot 476 in the two years (1991 and 1992).

Treatment	# of Seeds infested with *F. proliferatum*	# of Seeds not infested with *F. proliferatum*	Total
Operational 476-1991	114	146	260
Operational 476-1992	292	208	500
Total	406	354	760

$\chi_i^2 = 14.560$

$\chi^2_{.01,1} = 6.635$

Therefore, H_O may be rejected and it may concluded that the proportion of seeds infected with *F. proliferatum* in the operational treatment of seedlot 476 is associated with year.

Table A1.1.5 Chi-square tests of independence in the proportions of seeds infested with other *Fusarium* species in the operational treatment of seedlot 476 in the two years (1991 and 1992).

Treatment	# of Seeds infested with other *Fusarium* species	# of Seeds not infested with other *Fusarium* species	Total
Operational 476-1991	52	208	260
Operational 476-1992	11	489	500
Total	63	697	760

$\chi_i^2 = 71.287$

$\chi^2_{.01,1} = 6.635$

Therefore, H_O may be rejected and it may concluded that the proportion of seeds infected with other *Fusarium* species in the operational treatment of seedlot 476 is associated with year.

Table A1.1.6 Chi-square tests of independence in the proportions of seeds infested with *Fusarium* in the 9983 seedlot (1991).

Treatment	# of Seeds infested with *Fusarium*	# of Seeds not infested with *Fusarium*	Total
Operational 9983	2	498	500
Sanitized 9983	0	500	500
Total	2	998	1000

There were not enough data to test.

Appendix 2.0 Chi-square and Mann-Whitney tests for seedling root infections (1991 and 1992)

Appendix 2.1 Chi-square tests of independence in the proportions of seedlings with *Fusarium* root infections in each block.

Statistical hypotheses used for all Chi-square tests in this section:

$\mathbf{H_0}$= The proportion of seedlings with *Fusarium* root infections is independent of block.

$\mathbf{H_1}$= The proportion of seedlings with *Fusarium* root infections is associated with block.

1991

Table A2.1.1 Chi-square contingency table for proportion of infected seedlings in each block for 1991.

Block	# of seedlings with *Fusarium* root infections	# of seedlings without *Fusarium* root infections	Total
1	22	98	120
2	21	99	120
3	17	103	120
4	29	91	120
5	25	95	120
6	23	97	120
Total	137	583	720

$\chi_i^2 = 4.3844$

$\chi^2_{.01,5} = 15.086$

Therefore H_0 cannot be rejected and it may assumed that the proportion of infected seedlings is not associated with block.

1992

Table A2.1.2 Chi-square contingency table for proportion of infected seedlings in each block for 1992.

Block	# of seedlings infected with *Fusarium*	# of seedlings not infected with *Fusarium*	Total
1	31	149	180
2	26	154	180
3	18	162	180
4	27	153	180
Total	102	618	720

$\chi_i^2 = 4.0663$

$\chi^2_{.01,3} = 11.345$

Therefore H_0 cannot be rejected and it may assumed that the proportion of infected seedlings is not associated with block.

Appendix 2.2 Chi-square tests of independence in the proportions of seedlings with *Fusarium* root infections for 1991.

Statistical hypotheses used for all Chi-square tests in this section:

$\mathbf{H_0}$= The proportion of seedlings with *Fusarium* root infections is independent of treatment.

$\mathbf{H_1}$= The proportion of seedlings with *Fusarium* root infections is associated with treatment.

1991

Table A2.2.1 Chi-square contingency table for proportion of infected seedlings in seedlot 476--sample time 1.

Treatment	# of Seedlings with *Fusarium* Root Infections	# of Seedlings without *Fusarium* Root Infections	Total
Operational 476	4	56	60
Sanitized 476	1	59	60
Total	5	115	120

There were not enough data to test.

Table A2.2.2 Chi-square contingency table for proportion of infected seedlings in seedlot 476--sample time 2.

Treatment	# of Seedlings with *Fusarium* Root Infections	# of Seedlings without *Fusarium* Root Infections	Total
Operational 476	28	32	60
Sanitized 476	9	51	60
Total	37	83	120

$\chi_i^2 = 14.106$

$\chi^2_{.01,1} = 6.635$

Therefore, H_O may be rejected and it may concluded that the proportion of infected seedlings is associated with treatment.

Table A2.2.3 Chi-square contingency table for proportion of infected seedlings in seedlot 476--sample time 3.

Treatment	# of Seedlings with *Fusarium* Root Infections	# of Seedlings without *Fusarium* Root Infections	Total
Operational 476	34	26	60
Sanitized 476	16	44	60
Total	50	70	120

$\chi_i^2 = 11.108$

$\chi^2_{.01,1} = 6.635$

Therefore, H_O may be rejected and it may concluded that the proportion of infected seedlings is associated with treatment.

Table A2.2.4 Chi-square contingency table for proportion of infected seedlings in seedlot 9983--sample time 1.

Treatment	# of Seedlings with *Fusarium* Root Infections	# of Seedlings without *Fusarium* Root Infections	Total
Operational 9983	0	60	60
Sanitized 9983	0	60	60
Total	0	120	120

The Chi-square test is not necessary as it can be seen that the proportion of infected seedlings cannot be associated with treatment.

Table A2.2.5 Chi-square contingency table for proportion of infected seedlings in seedlot 9983--sample time 2.

Treatment	# of Seedlings with *Fusarium* Root Infections	# of Seedlings without *Fusarium* Root Infections	Total
Operational 9983	7	53	60
Sanitized 9983	3	57	60
Total	10	110	120

$\chi_i^2 = 1.746$

$\chi^2_{.01,1} = 6.635$

Therefore H_O cannot be rejected and it may assumed that the proportion of infected seedlings is not associated with treatment.

Table A2.2.6 Chi-square contingency table for proportion of infected seedlings in seedlot 9983--sample time 3.

Treatment	# of Seedlings with *Fusarium* Root Infections	# of Seedlings without *Fusarium* Root Infections	Total
Operational 9983	22	38	60
Sanitized 9983	13	47	60
Total	35	85	120

$\chi_i^2 = 3.266$

$\chi^2_{.01,1} = 6.635$

Therefore H_O cannot be rejected and it may assumed that the proportion of infected seedlings is not associated with treatment.

Appendix 2.3 Chi-square tests of independence of the proportions of seedlings with *F. proliferatum* root infections for seedlings with *Fusarium* spp. root infections (1991).

Statistical hypotheses used for all Chi-square tests in this section:

$\mathbf{H_0}$= The proportion of seedlings with *F. proliferatum* root infections is independent of treatment.
$\mathbf{H_1}$= The proportion of seedlings with *F. proliferatum* root infections is associated with treatment.

1991

Table A2.3.1 Chi-square contingency table for proportion of *F. proliferatum* infected seedlings in seedlot 476--sample time 1.

Treatment	# of Seedlings with *F. proliferatum* Root Infections	# of Seedlings without *F. proliferatum* Root Infections	Total
Operational 476	3	1	4
Sanitized 476	0	1	1
Total	3	2	5

There were not enough data to test.

Table A2.3.2 Chi-square contingency table for proportion of *F. proliferatum* infected seedlings in seedlot 476--sample time 2.

Treatment	# of Seedlings with *F. proliferatum* Root Infections	# of Seedlings without *F. proliferatum* Root Infections	Total
Operational 476	22	6	28
Sanitized 476	7	2	9
Total	29	8	37

$\chi_i^2 = 9.758$

$\chi^2_{.01,1} = 6.635$

Therefore H_O may be rejected and it may assumed that the proportion of *F. proliferatum* infected seedlings is associated with treatment.

Table A2.3.3 Chi-square contingency table for proportion of *F. proliferatum* infected seedlings in seedlot 476--sample time 3.

Treatment	# of Seedlings with *F. proliferatum* Root Infections	# of Seedlings without *F. proliferatum* Root Infections	Total
Operational 476	29	5	34
Sanitized 476	5	11	16
Total	34	16	50

$\chi_i^2 = 19.192$

$\chi^2_{.01,1} = 6.635$

Therefore, H_O may be rejected and it may concluded that the proportion of *F. proliferatum* infected seedlings is associated with treatment.

Table A2.3.4 Chi-square contingency table for proportion of *F. proliferatum* infected seedlings in seedlot 9983--sample time 1.

Treatment	# of Seedlings with *F. proliferatum* Root Infections	# of Seedlings without *F. proliferatum* Root Infections	Total
Operational 9983	0	0	0
Sanitized 9983	0	0	0
Total	0	0	0

There were not enough data to test.

Table A2.3.5 Chi-square contingency table for proportion of *F. proliferatum* infected seedlings in seedlot 9983--sample time 2.

Treatment	# of Seedlings with *F. proliferatum* Root Infections	# of Seedlings without *F. proliferatum* Root Infections	Total
Operational 9983	1	6	6
Sanitized 9983	0	3	3
Total	1	9	10

There were not enough data to test.

Table A2.3.6 Chi-square contingency table for proportion of *F. proliferatum* infected seedlings in seedlot 9983--sample time 3.

Treatment	# of Seedlings with *F. proliferatum* Root Infections	# of Seedlings without *F. proliferatum* Root Infections	Total
Operational 9983	7	15	22
Sanitized 9983	3	10	13
Total	10	25	35

$\chi_i^2 = 2.600$

$\chi^2_{.01,1} = 6.635$

Therefore H_0 cannot be rejected and it may assumed that the proportion of *F. proliferatum* infected seedlings is not associated with treatment.

Appendix 2.4 Chi-square tests of independence in the proportions of seedlings with *Fusarium* root infections for 1992.

Statistical hypotheses used for all Chi-square tests in this section:

$\mathbf{H_0}$= The proportion of seedlings with *Fusarium* root infections is independent of treatment.
$\mathbf{H_1}$= The proportion of seedlings with *Fusarium* root infections is associated with treatment.

1992

Table A2.4.1 Chi-square contingency table for proportion of infected seedlings in seedlot 476--sample time 1.

Treatment	# of Seedlings with *Fusarium* Root Infections	# of Seedlings without *Fusarium* Root Infections	Total
Operational 476	3	57	60
Sanitized 476	2	58	60
Total	5	115	120

There were not enough data to test.

Table A2.4.2 Chi-square contingency table for proportion of infected seedlings in seedlot 476--sample time 2.

Treatment	# of Seedlings with *Fusarium* Root Infections	# of Seedlings without *Fusarium* Root Infections	Total
Operational 476	3	57	60
Sanitized 476	6	54	60
Total	9	111	120

$\chi_i^2 = 1.082$

$\chi^2_{.01,1} = 6.635$

Therefore, H_O may not be rejected and it may concluded that the proportion of infected seedlings is not associated with treatment.

Table A2.4.3 Chi-square contingency table for proportion of infected seedlings in seedlot 476--sample time 3.

Treatment	# of Seedlings with *Fusarium* Root Infections	# of Seedlings without *Fusarium* Root Infections	Total
Operational 476	28	32	60
Sanitized 476	17	43	60
Total	45	75	120

$\chi_i^2 = 4.302$

$\chi^2_{.01,1} = 6.635$

Therefore, H_O may not be rejected and it may be concluded that the proportion of infected seedlings is not associated with treatment.

Table A2.4.4 Chi-square contingency table for proportion of infected seedlings in seedlot 9983--sample time 1.

Treatment	# of Seedlings with *Fusarium* Root Infections	# of Seedlings without *Fusarium* Root Infections	Total
Operational 9983	2	58	60
Sanitized 9983	1	59	60
Total	3	117	120

There were not enough data to test.

Table A2.4.5 Chi-square contingency table for proportion of infected seedlings in seedlot 9983--sample time 2.

Treatment	# of Seedlings with *Fusarium* Root Infections	# of Seedlings without *Fusarium* Root Infections	Total
Operational 9983	1	59	60
Sanitized 9983	9	51	60
Total	10	110	120

$\chi_i^2 = 6.982$

$\chi^2_{.01,1} = 6.635$

Therefore H_0 may be rejected and it may assumed that the proportion of infected seedlings is associated with treatment.

Table A2.4.6 Chi-square contingency table for proportion of infected seedlings in seedlot 9983--sample time 3.

Treatment	# of Seedlings with *Fusarium* Root Infections	# of Seedlings without *Fusarium* Root Infections	Total
Operational 9983	20	40	60
Sanitized 9983	10	50	60
Total	30	90	120

$\chi_i^2 = 4.446$

$\chi^2_{.01,1} = 6.635$

Therefore H_0 cannot be rejected and it may assumed that the proportion of infected seedlings is not associated with treatment.

Appendix 2.5 Chi-square tests of independence of the proportions of seedlings with *F. proliferatum* root infections for seedlings with *Fusarium* spp. root infections (1992).

Statistical hypotheses used for all Chi-square tests in this section:

$\mathbf{H_0}$= The proportion of seedlings with *F. proliferatum* root infections is independent of treatment.
$\mathbf{H_1}$= The proportion of seedlings with *F. proliferatum* root infections is associated with treatment.

1992

Table A2.5.1 Chi-square contingency table for proportion of *F. proliferatum* infected seedlings in seedlot 476--sample time 1.

Treatment	# of Seedlings with *F. proliferatum* Root Infections	# of Seedlings without *F. proliferatum* Root Infections	Total
Operational 476	0	3	3
Sanitized 476	0	2	2
Total	0	5	5

There were not enough data to test.

Table A2.5.2 Chi-square contingency table for proportion of *F. proliferatum* infected seedlings in seedlot 476--sample time 2.

Treatment	# of Seedlings with *F. proliferatum* Root Infections	# of Seedlings without *F. proliferatum* Root Infections	Total
Operational 476	0	3	3
Sanitized 476	0	6	6
Total	0	9	9

There were not enough data to test.

Table A2.5.3 Chi-square contingency table for proportion of *F. proliferatum* infected seedlings in seedlot 476--sample time 3.

Treatment	# of Seedlings with *F. proliferatum* Root Infections	# of Seedlings without *F. proliferatum* Root Infections	Total
Operational 476	5	23	28
Sanitized 476	0	17	17
Total	5	40	45

$\chi_i^2 = 5.900$

$\chi^2_{.01,1} = 6.635$

Therefore, H_O cannot be rejected and it may be concluded that the proportion of *F. proliferatum* infected seedlings is not associated with treatment.

Table A2.5.4 Chi-square contingency table for proportion of *F. proliferatum* infected seedlings in seedlot 9983--sample time 1.

Treatment	# of Seedlings with *F. proliferatum* Root Infections	# of Seedlings without *F. proliferatum* Root Infections	Total
Operational 9983	0	2	2
Sanitized 9983	0	1	1
Total	0	3	3

There were not enough data to test.

Table A2.5.5 Chi-square contingency table for proportion of *F. proliferatum* infected seedlings in seedlot 9983--sample time 2.

Treatment	# of Seedlings with *F. proliferatum* Root Infections	# of Seedlings without *F. proliferatum* Root Infections	Total
Operational 9983	0	1	1
Sanitized 9983	0	9	9
Total	0	10	10

There were not enough data to test.

Table A2.5.6 Chi-square contingency table for proportion of *F. proliferatum* infected seedlings in seedlot 9983--sample time 3.

Treatment	# of Seedlings with *F. proliferatum* Root Infections	# of Seedlings without *F. proliferatum* Root Infections	Total
Operational 9983	0	20	20
Sanitized 9983	1	9	10
Total	1	29	30

There were not enough data to test.

Appendix 2.6 Chi-square tests for sample time 3-1991 and 1992 combined

Statistical hypotheses used for all Chi-square tests in this section:

$\mathbf{H_0}$= The proportion of seedlings with *Fusarium* root infections is independent of treatment.
$\mathbf{H_1}$= The proportion of seedlings with *Fusarium* root infections is associated with treatment.

Table A2.6.1 Chi-square contingency table for proportion of *Fusarium* infected seedlings in the operational treatments of seedlots 476 and 9983--sample time 3 (1991 and 1992 combined).

Treatment	# of Seedlings with *Fusarium* Root Infections	# of Seedlings without *Fusarium* Root Infections	Total
Operational 476	62	58	120
Operational 9983	44	76	120
Total	106	134	240

$\chi_i^2 = 5.4746$

$\chi^2_{.01,1} = 6.635$

Therefore, H_0 cannot be rejected and it may be concluded that the proportion of *Fusarium* infected seedlings is not associated with treatment.

Table A2.6.2 Chi-square contingency table for proportion of *Fusarium* infected seedlings in the operational treatment of seedlot 476--sample time 3 (1991 and 1992 combined).

Treatment	# of Seedlings with *Fusarium* Root Infections	# of Seedlings without *Fusarium* Root Infections	Total
Operational 476	62	58	120
Sanitized 476	22	98	120
Total	84	156	240

$\chi_i^2 = 29.3042$

$\chi^2_{.01,1} = 6.635$

Therefore, H_O can be rejected and it may be concluded that the proportion of *Fusarium* infected seedlings is associated with treatment.

Appendix 2.7 Chi-square tests at sample period 3 for the sanitized 476, operational 9983, and sanitized 9983 treatments (1991 and 1992).

Statistical hypotheses used for all Chi-square tests in this section:

$\mathbf{H_0}$= The proportion of seedlings with *Fusarium* (or *F. proliferatum*) root infections is independent of treatment.
$\mathbf{H_1}$= The proportion of seedlings with *Fusarium* (or *F. proliferatum*) root infections is associated with treatment.

Table A2.7.1 Chi-square contingency table for the proportion of *Fusarium* infected seedlings in the sanitized treatment of seedlot 476 and both seedlot 9983 treatments--sample time 3 (1991).

Treatment	# of Seedlings with *Fusarium* root infections	# of Seedlings without *Fusarium* root infections	Total
Sanitized 476	16	44	60
Operational 9983	22	38	60
Sanitized 9983	13	47	60
Total	51	129	180

$\chi_i^2 = 3.7032$

$\chi^2_{.01,2} = 9.210$

Therefore, H_O cannot be rejected and it may be concluded that the proportion of *Fusarium* infected seedlings is not associated with treatment.

Table A2.7.2 Chi-square contingency table for the proportion of *Fusarium* infected seedlings in the sanitized treatment of seedlot 476 and both seedlot 9983 treatments--sample time 3 (1992).

Treatment	# of Seedlings with *Fusarium* root infections	# of Seedlings without *Fusarium* root infections	Total
Sanitized 476	17	43	60
Operational 9983	20	40	60
Sanitized 9983	10	50	60
Total	47	133	180

$\chi_i^2 = 4.5432$

$\chi^2_{.01,2} = 9.210$

Therefore, H_O cannot be rejected and it may be concluded that the proportion of *Fusarium* infected seedlings is not associated with treatment.

Table A2.7.3 Chi-square contingency table for the proportion of seedlings with *F. proliferatum* root infections for seedlings with *Fusarium* spp. root infections in the sanitized treatment of seedlot 476 and both seedlot 9983 treatments--sample time 3 (1991).

Treatment	# of Seedlings with *F. proliferatum* root infections	# of Seedlings without *F. proliferatum* root infections	Total
Sanitized 476	5	11	16
Operational 9983	7	15	22
Sanitized 9983	3	10	13
Total	15	36	51

$\chi_i^2 = 0.336$

$\chi^2_{.01,2} = 9.210$

Therefore, H_O cannot be rejected and it may be concluded that the proportion of *F. proliferatum* infected seedlings is not associated with treatment.

Table A2.7.4 Chi-square contingency table for the proportion of seedlings with *F. proliferatum* root infections for seedlings with *Fusarium* spp. root infections in the sanitized treatment of seedlot 476 and both seedlot 9983 treatments--sample time 3 (1992).

Treatment	# of Seedlings with *F. proliferatum* root infections	# of Seedlings without *F. proliferatum* root infections	Total
Sanitized 476	0	17	17
Operational 9983	0	20	20
Sanitized 9983	1	9	10
Total	1	46	47

There were not enough data to test.

Appendix 2.8 Mann-Whitney tests of percent root colonization.

Statistical hypotheses used for all Mann-Whitney tests in this section:

$\mathbf{H_0}$= Infected seedlings from both treatments have the same percent root colonization.
$\mathbf{H_1}$= Infected seedlings from both treatments do not have the same percent root colonization.

Appendix 2.8.1 Mann-Whitney tests for operational and sanitized treatments of seedlot 476 at sample times 2 and 3 (1991).

Sample time 2:

U test statistic = 174.500
$U_{0.05,(2),9,28}$ = 182.000

Therefore, H_o is not rejected and it is concluded that there is no difference in the percent root colonization for infected seedlings of the operational and sanitized treatments of seedlot 476.

Sample time 3:

U test statistic = 365.000
$U_{0.05,(2),16,34}$ = 367.000

Therefore, H_o is not rejected and it is concluded that there is no difference in the percent root colonization for infected seedlings of the operational and sanitized treatments of seedlot 476.

Appendix 2.8.2 Mann-Whitney tests for operational and sanitized treatments of seedlot 9983 at sample times 2 and 3 (1991).

Sample time 2:

U test statistic = 15.000
$U_{0.05,(2),3,7}$ = 20.000

Therefore, H_o is not rejected and it is concluded that there is no difference in the percent root colonization for infected seedlings of the operational and sanitized treatments of seedlot 9983.

Sample time 3:

U test statistic = 120.000
$U_{0.05,(2),13,22}$ = 201.000

Therefore, H_o is not rejected and it is concluded that there is no difference in the percent root colonization for infected seedlings of the operational and sanitized treatments of seedlot 9983.

Appendix 2.8.3 Mann-Whitney tests for operational and sanitized treatments of seedlot 476 at sample times 2 and 3 (1992).

Sample time 2:

U test statistic = 10.000
$U_{0.05,(2),3,6}$ = 17.000

Therefore, H_o is not rejected and it is concluded that there is no difference in the percent root colonization for infected seedlings of the operational and sanitized treatments of seedlot 476.

Sample time 3:

U test statistic = 243.000
$U_{0.05,(2),17,28}$ = 322.000

Therefore, H_o is not rejected and it is concluded that there is no difference in the percent root colonization for infected seedlings of the operational and sanitized treatments of seedlot 476.

Appendix 2.7.4 Mann-Whitney tests for operational and sanitized treatments of seedlot 9983 at sample time 3 (1992).

Sample time 3:

U test statistic = 94.000
$U_{0.05,(2),10,20}$ = 145.000

Therefore, H_o is not rejected and it is concluded that there is no difference in the percent root colonization for infected seedlings of the operational and sanitized treatments of seedlot 9983.

Appendix 3.0 Chi-square tests of seed germination in the field

Appendix 3.1 Chi-square tests of independence in the proportions of seeds which germinate and those that do not for 1991.

Statistical hypotheses used for all Chi-square tests in this section:

H_0= The proportion of seeds which germinated is independent of treatment.

H_1= The proportion of seeds which germinated is associated with treatment.

Table A3.1.1 Chi-square contingency table for the proportion of seeds which germinate in seedlot 476

Treatment	# of Seeds which germinated	# of Seeds which did not germinate	Total
Operational 476	670	194	864
Sanitized 476	505	359	864
Total	1175	553	1728

$\chi_i^2 = 72.402$

$\chi^2_{.01,1} = 6.635$

Therefore H_0 may be rejected and it may be assumed that the proportion of seeds which germinated is associated with treatment.

Table A3.1.2 Chi-square contingency table for the proportion of seeds which germinate in seedlot 9983

Treatment	# of Seeds which germinated	# of Seeds which did not germinate	Total
Operational 9983	680	184	864
Sanitized 9983	629	235	864
Total	1309	419	1728

$\chi_i^2 = 8.194$

$\chi^2_{.01,1} = 6.635$

Therefore H_O may be rejected and it may be assumed that the proportion of seeds which germinated is associated with treatment.

Appendix 3.2 Chi-square tests of independence in the proportions of seeds which germinate and those that do not for 1992.

Statistical hypotheses used for all Chi-square tests in this section:

H_0= The proportion of seeds which germinated is independent of treatment.

H_1= The proportion of seeds which germinated is associated with treatment.

Table A3.2.1 Chi-square contingency table for the proportion of seeds which germinate in seedlot 476

Treatment	# of Seeds which germinated	# of Seeds which did not germinate	Total
Operational 476	372	132	504
Sanitized 476	414	90	504
Total	786	222	1008

$\chi_i^2 = 10.190$

$\chi^2_{.01,1} = 6.635$

Therefore H_0 may be rejected and it may be assumed that the proportion of seeds which germinated is associated with treatment.

Table A3.2.2 Chi-square contingency table for the proportion of seeds which germinate in seedlot 9983

Treatment	# of Seeds which germinated	# of Seeds which did not germinate	Total
Operational 9983	407	97	504
Sanitized 9983	378	126	504
Total	785	223	1008

$\chi_i^2 = 4.844$

$\chi^2_{.01,1} = 6.635$

Therefore H_O may not be rejected and it may be assumed that the proportion of seeds which germinated is not associated with treatment.

Appendix 4.0 Analysis of variance for seedling growth assessments.

Appendix 4.1 Analysis of variance tables with replicates nested within treatments for the seedling growth assessments of 1991.

Statistical hypotheses used for all analysis of variance tests in this section:

$\mathbf{H_0}$= The difference in treatment means is not significantly different (p=0.05).

$\mathbf{H_1}$= The difference in treatment means is significantlydifferent (p=0.05).

Table 4.1.1 Analysis of variance table for the variable height (476).

SOURCE	DF	SS	MS	F	p
Residual	96	88.10	0.92		
Constant	1	5377.42	5377.42	5859.89	.000
Block	5	5.25	1.05	1.14	.342
Treatment	1	29.30	29.30	31.93	.000
Rep. within Treatment	2	1.26	0.63	0.69	.505

Table 4.1.2 Analysis of variance table for the variable height (9983).

SOURCE	DF	SS	MS	F	p
Residual	96	100.52	1.05		
Constant	1	6412.33	6412.33	6123.75	.000
Block	5	18.89	3.78	3.61	.005
Treatment	1	0.97	0.97	0.93	.338
Rep. within Treatment	2	11.83	5.92	5.65	.005

Table 4.1.3 Analysis of variance table for the variable root collar diameter (476).

SOURCE	DF	SS	MS	F	p
Residual	96	6.38	0.07		
Constant	1	421.25	421.25	6338.13	.000
Block	5	0.27	0.05	0.82	.540
Treatment	1	2.13	2.13	32.05	.000
Rep. within Treatment	2	0.06	0.03	0.46	.633

Table 4.1.4 Analysis of variance table for the variable root collar diameter (9983).

SOURCE	DF	SS	MS	F	p
Residual	96	6.29	0.07		
Constant	1	526.22	526.22	8026.27	.000
Block	5	0.35	0.07	1.06	.389
Treatment	1	0.00	0.00	0.00	.946
Rep. within Treatment	2	0.77	0.38	5.87	.004

Table 4.1.5 Analysis of variance table for the variable shoot dry weight (476)

SOURCE	DF	SS	MS	F	p
Residual	96	0.36	0.00		
Constant	1	7.17	7.17	1894.50	.000
Block	5	0.01	0.00	0.46	.803
Treatment	1	0.14	0.14	35.83	.000
Rep. within Treatment	2	0.00	0.00	0.56	.575

Table 4.1.6 Analysis of variance table for the variable shoot dry weight (9983).

SOURCE	DF	SS	MS	F	p
Residual	96	0.43	0.00		
Constant	1	10.00	10.00	2213.19	.000
Block	5	0.05	0.01	2.21	.060
Treatment	1	0.01	0.01	2.66	.106
Rep. within Treatment	2	0.07	0.04	7.78	.100

Table 4.1.7 Analysis of variance table for the variable root dry weight (476).

SOURCE	DF	SS	MS	F	p
Residual	96	0.56	0.01		
Constant	1	9.24	9.24	1577.46	.000
Block	5	0.04	0.01	1.28	.278
Treatment	1	0.23	0.23	39.44	.000
Rep. within Treatment	2	0.01	0.01	0.86	.426

Table 4.1.8 Analysis of variance table for the variable root dry weight (9983).

SOURCE	DF	SS	MS	F	p
Residual	96	0.40	0.00		
Constant	1	10.90	10.90	2603.34	.000
Block	5	0.05	0.01	2.59	.030
Treatment	1	0.01	0.01	2.71	.103
Rep. within Treatment	2	0.02	0.01	2.35	.101

Appendix 4.2 Analysis of variance tables with replicates nested within treatments for the seedling growth assessments of 1992.

Statistical hypotheses used for all analysis of variance tests in this section:

$\mathbf{H_0}$= The difference in treatment means is not significantly different (p=0.05).
$\mathbf{H_1}$= The difference in treatment means is significantlydifferent (p=0.05).

Table 4.2.1 Analysis of variance table for the variable height (476).

SOURCE	DF	SS	MS	F	p
Residual	96	1416.73	14.61		
Constant	1	75245.20	75245.20	5151.87	.000
Block	3	117.36	39.12	2.68	.051
Treatment	1	100.88	100.88	6.91	.010
Rep. within Treatment	4	45.92	11.48	0.79	.537

Table 4.2.2 Analysis of variance table for the variable height (9983).

SOURCE	DF	SS	MS	F	p
Residual	96	1317.91	13.73		
Constant	1	92313.17	92313.17	6724.34	.000
Block	3	355.33	118.44	8.63	.000
Treatment	1	17.56	17.56	1.28	.261
Rep. within Treatment	4	39.33	9.83	0.72	.583

Table 4.2.3 Analysis of variance table for the variable root collar diameter (476).

SOURCE	DF	SS	MS	F	p
Residual	96	28.18	0.26		
Constant	1	883.57	883.57	3404.05	.000
Block	3	0.40	0.13	0.51	.673
Treatment	1	1.01	1.01	3.91	.051
Rep. within Treatment	4	0.54	0.13	0.52	.721

Table 4.2.4 Analysis of variance table for the variable root collar diameter (9983).

SOURCE	DF	SS	MS	F	p
Residual	96	15.05	0.16		
Constant	1	980.64	980.64	6253.73	.000
Block	3	1.49	0.50	3.17	.028
Treatment	1	0.23	0.23	1.46	.230
Rep. within Treatment	4	0.89	0.22	1.42	.235

Table 4.2.5 Analysis of variance table for the variable shoot dry weight (476).

SOURCE	DF	SS	MS	F	p
Residual	96	22.94	0.24		
Constant	1	212.35	212.35	898.08	.000
Block	3	0.25	0.08	0.35	.789
Treatment	1	4.19	4.19	17.74	.000
Rep. within Treatment	4	0.27	0.07	0.28	.890

Table 4.2.6 Analysis of variance table for the variable shoot dry weight (9983).

SOURCE	DF	SS	MS	F	p
Residual	96	18.36	0.19		
Constant	1	311.89	311.89	1630.78	.000
Block	3	1.90	0.63	3.32	.023
Treatment	1	0.38	0.38	1.99	.161
Rep. within Treatment	4	0.72	0.18	0.95	.441

Table 4.2.7 Analysis of variance table for the variable root dry weight (476).

SOURCE	DF	SS	MS	F	p
Residual	96	6.71	0.07		
Constant	1	25.69	25.69	371.33	.000
Block	3	0.52	0.17	2.52	.062
Treatment	1	0.44	0.44	6.32	.014
Rep. within Treatment	4	0.17	0.04	0.63	.641

Table 4.2.8 Analysis of variance table for the variable root dry weight (9983).

SOURCE	DF	SS	MS	F	p
Residual	96	2.78	0.03		
Constant	1	31.44	31.44	1086.23	.000
Block	3	0.51	0.17	5.82	.001
Treatment	1	0.05	0.05	1.86	.176
Rep. within	4	0.21	0.05	1.79	.137

Appendix 5.0 Scatterplots of biometric variables vs. germination

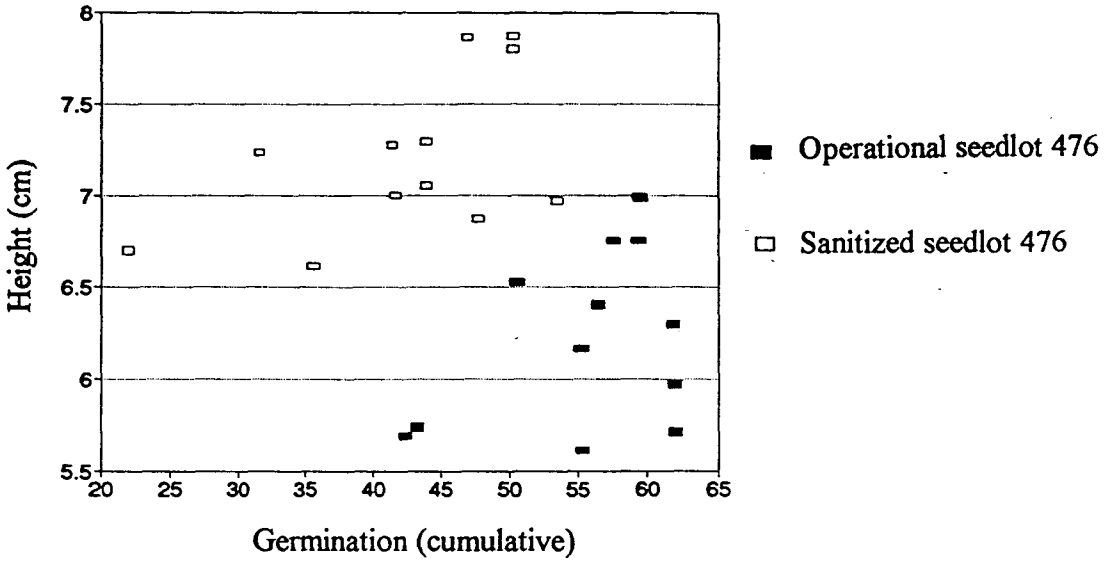

Figure 5.0.1 Height vs. germination for the operational and sanitized treatments of seedlot 476--1991.

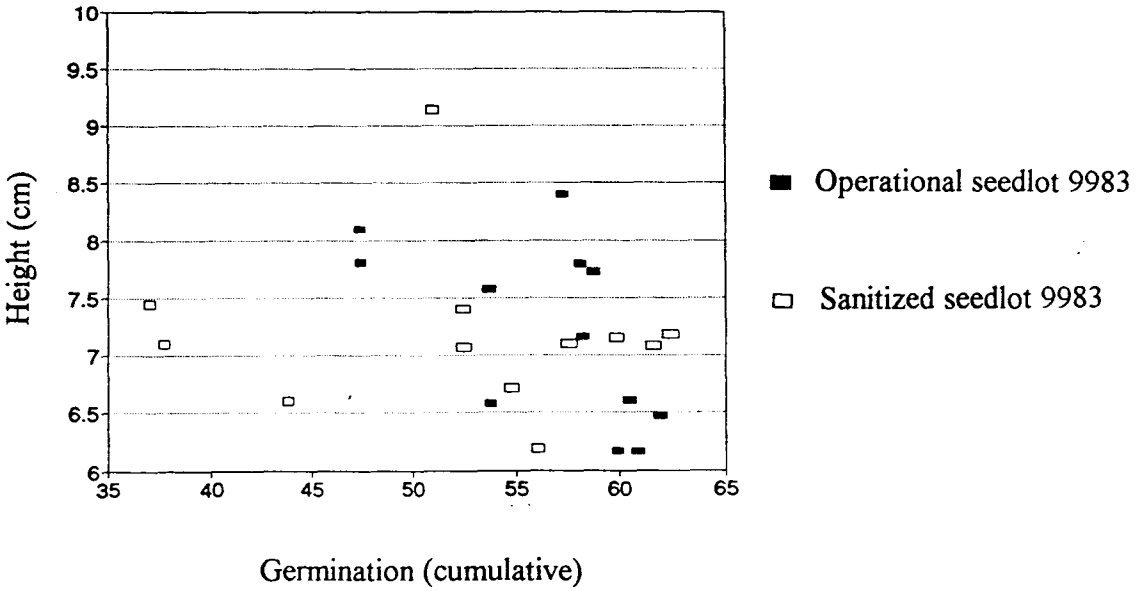

Figure 5.0.2 Height vs. germination for the operational and sanitized treatments of seedlot 9983--1991.

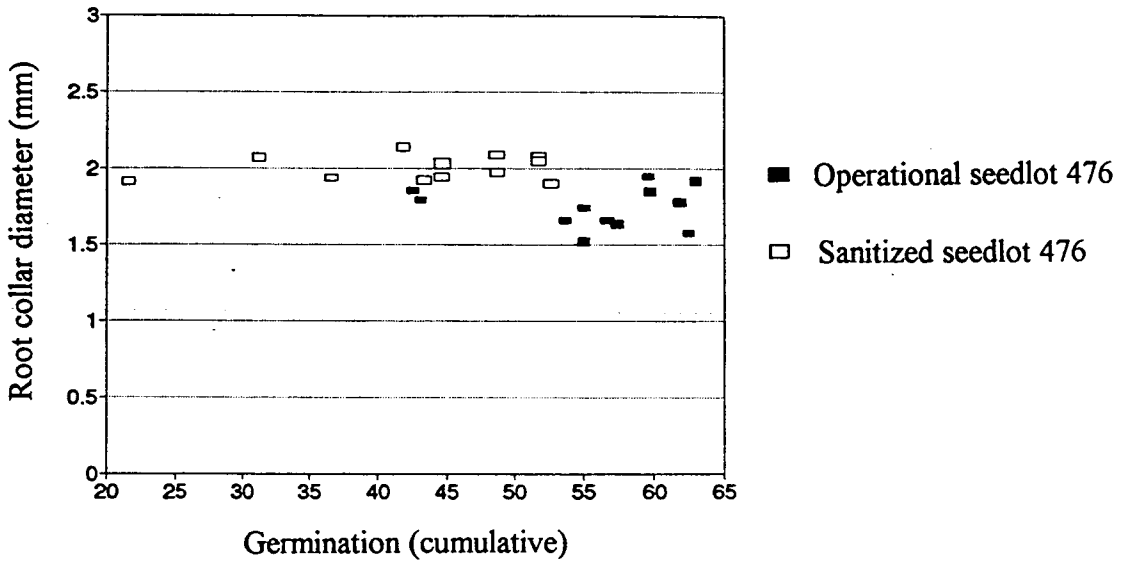

Figure 5.0.3 Root collar diameter vs. germination for the operational and sanitized treatments of seedlot 476--1991.

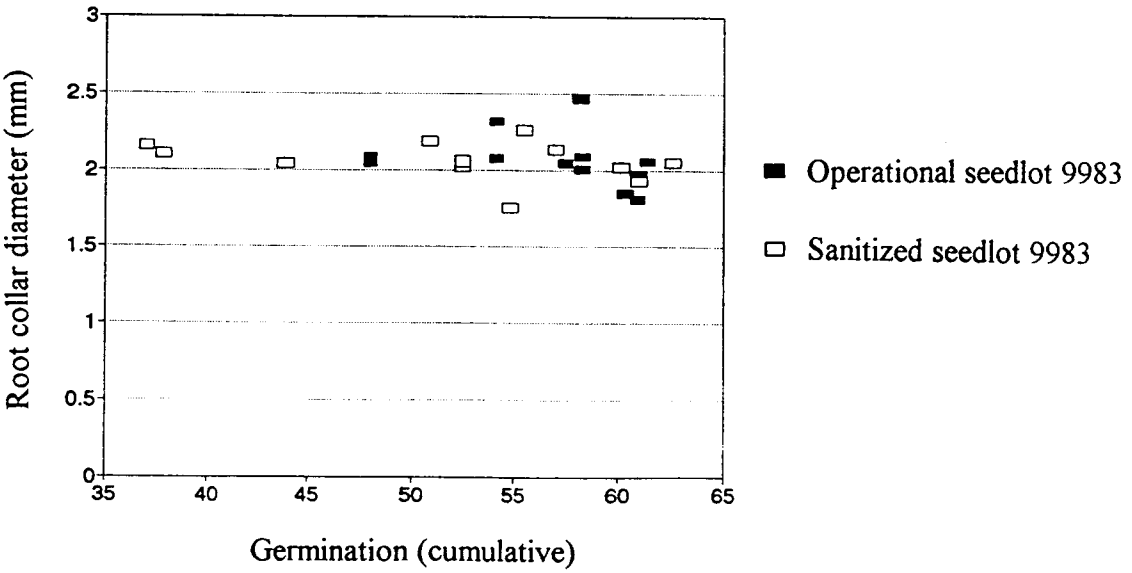

Figure 5.0.4 Root collar diameter vs. germination for the operational and sanitized treatments of seedlot 9983--1991.

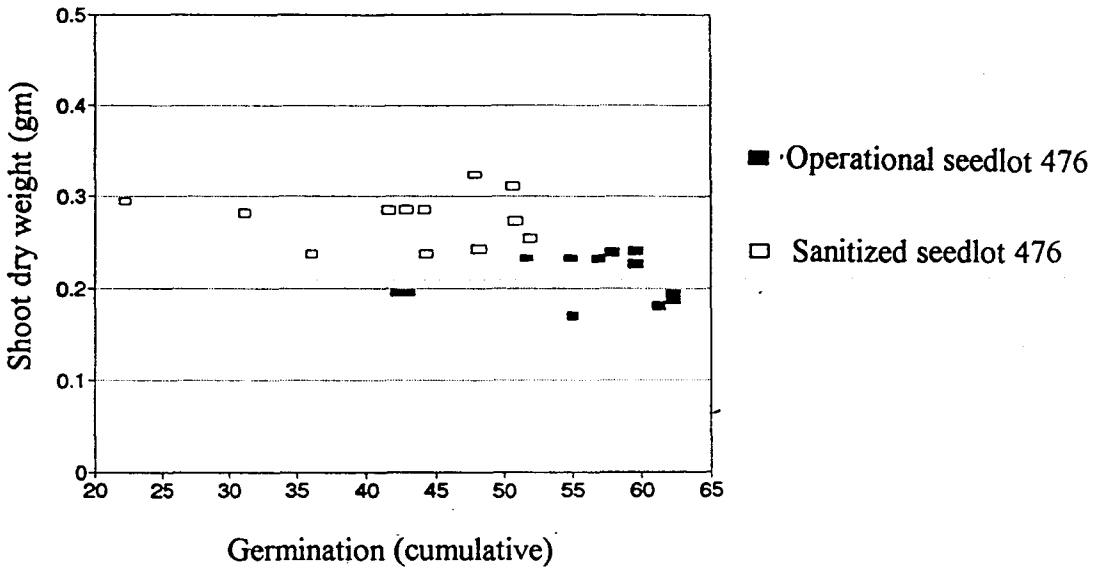

Figure 5.0.5 Shoot dry weight vs. germination for the operational and sanitized treatments of seedlot 476--1991.

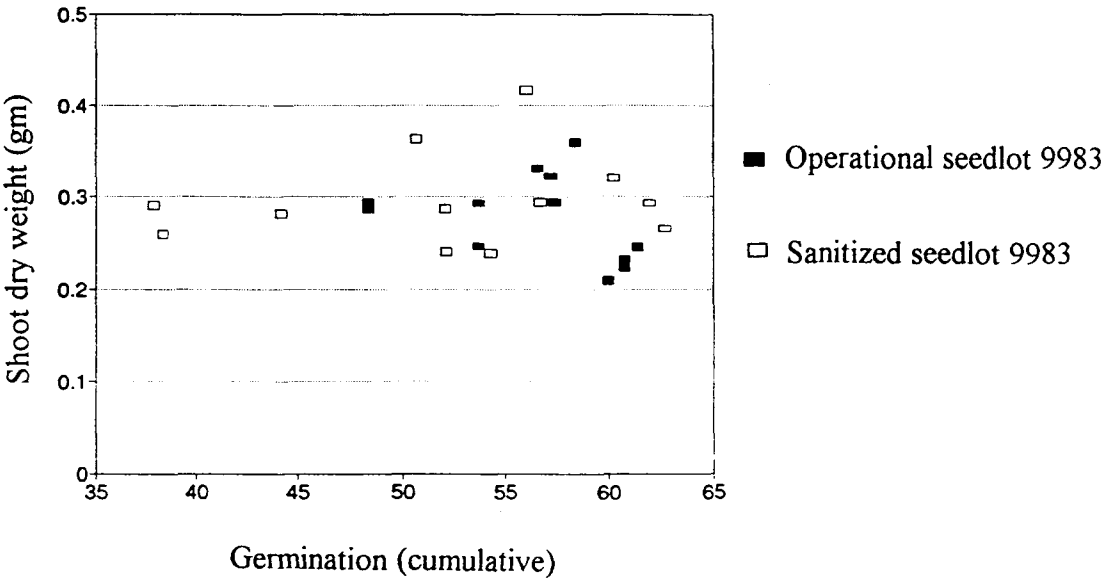

Figure 5.0.6 Shoot dry weight vs. germination for the operational and sanitized treatments of seedlot 9983--1991.

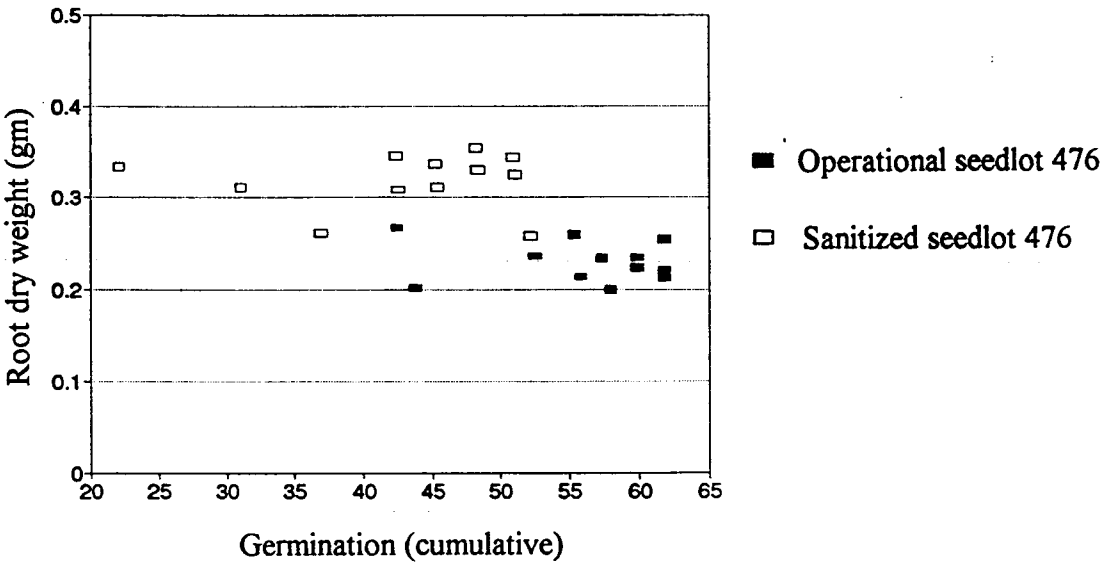

Figure 5.0.7 Root dry weight vs. germination for the operational and sanitized treatments of seedlot 476--1991.

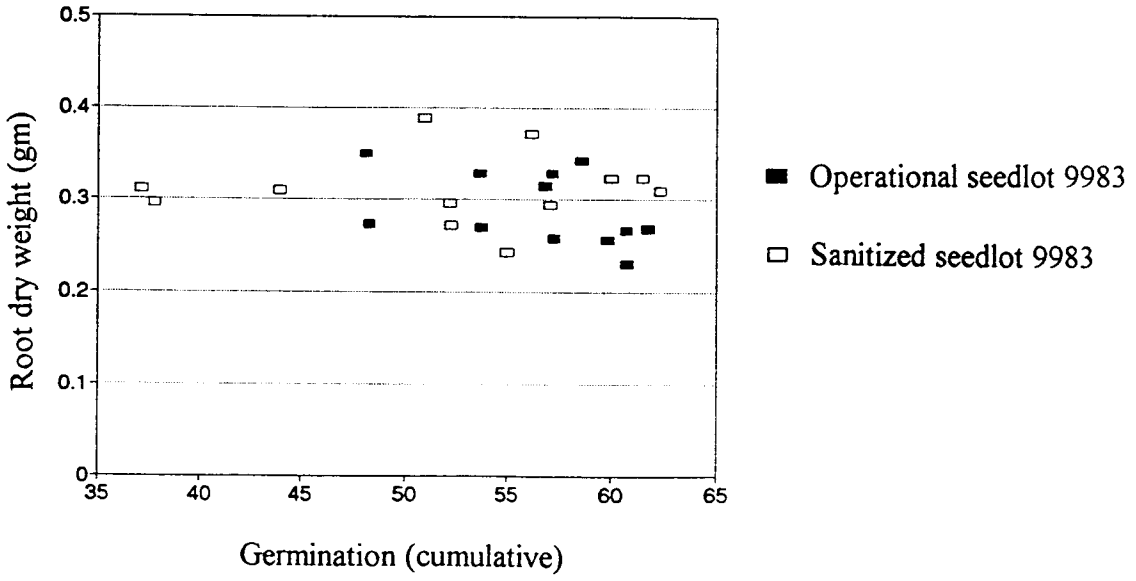

Figure 5.0.8 Root dry weight vs. germination for the operational and sanitized treatments of seedlot 9983-1991.

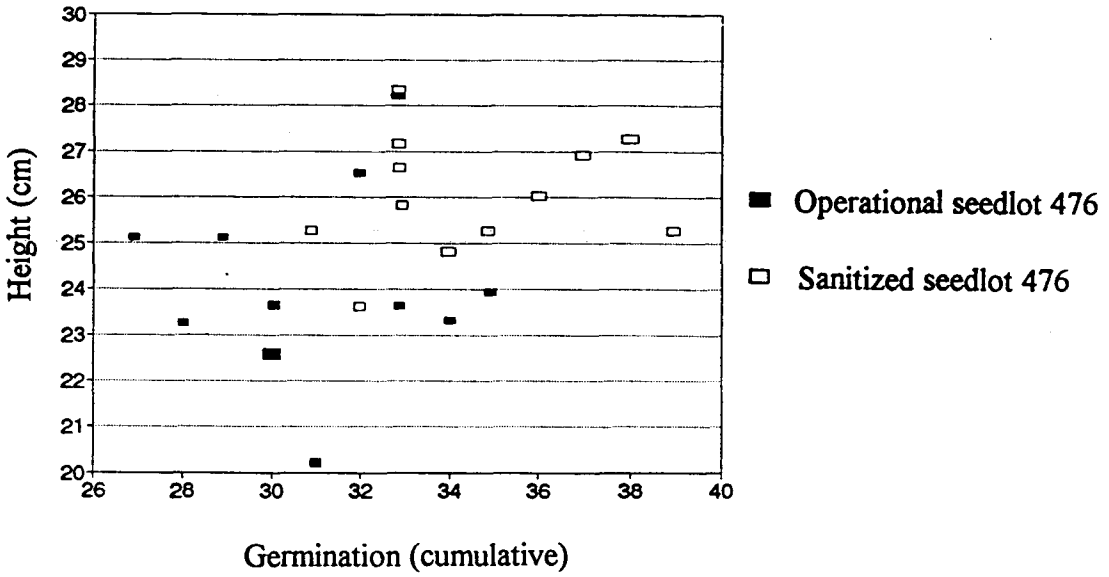

Figure 5.0.9 Height vs. germination for the operational and sanitized treatments of seedlot 476--1992.

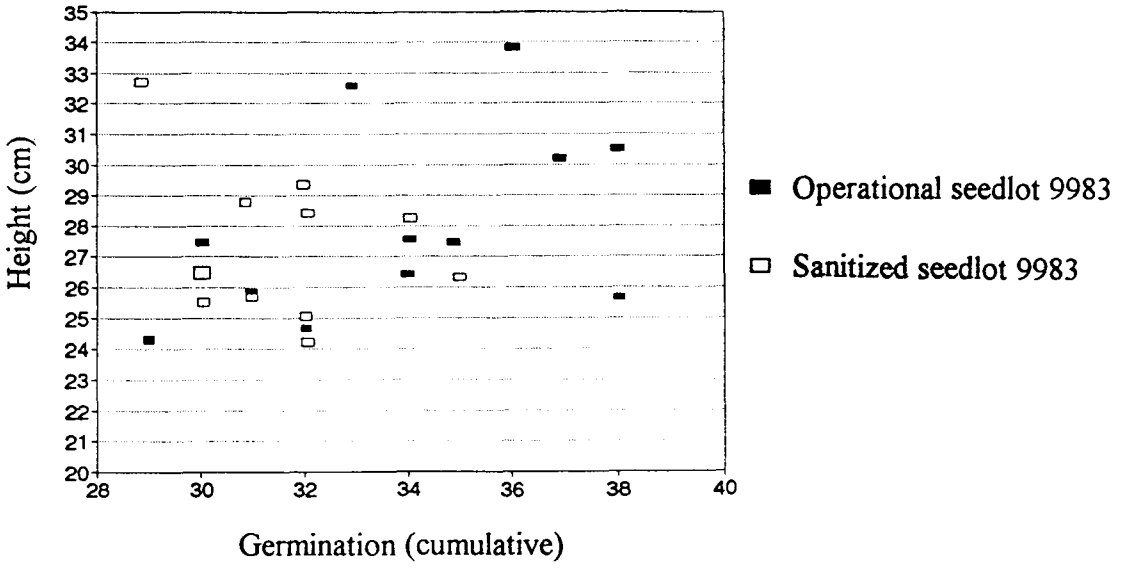

Figure 5.0.10 Height vs. germination for the operational and sanitized treatments of seedlot 9983--1992.

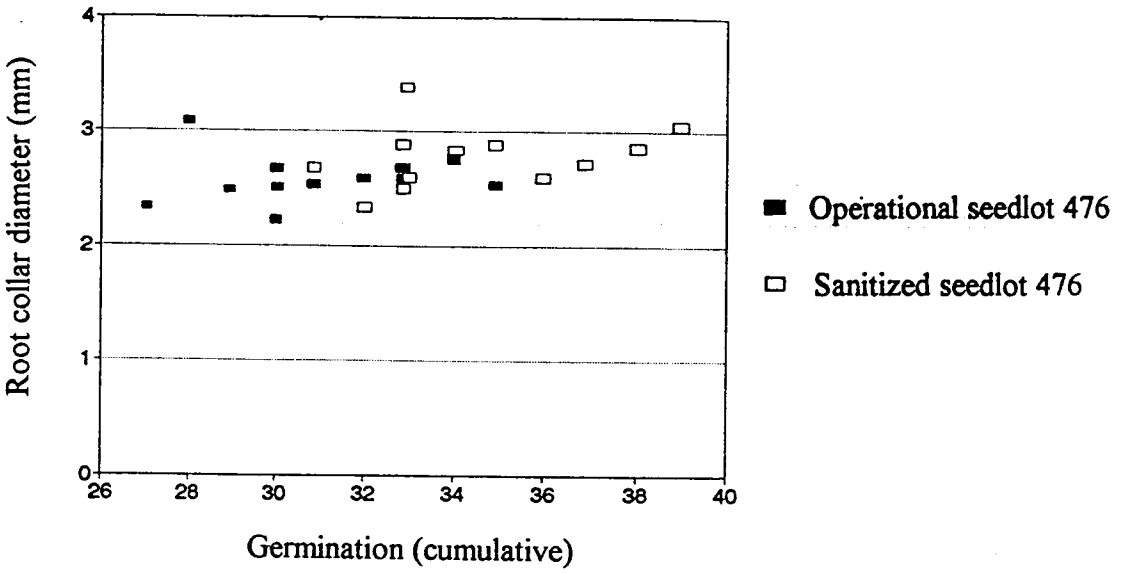

Figure 5.0.11 Root collar diameter vs. germination for the operational and sanitized treatments of seedlot 476--1992.

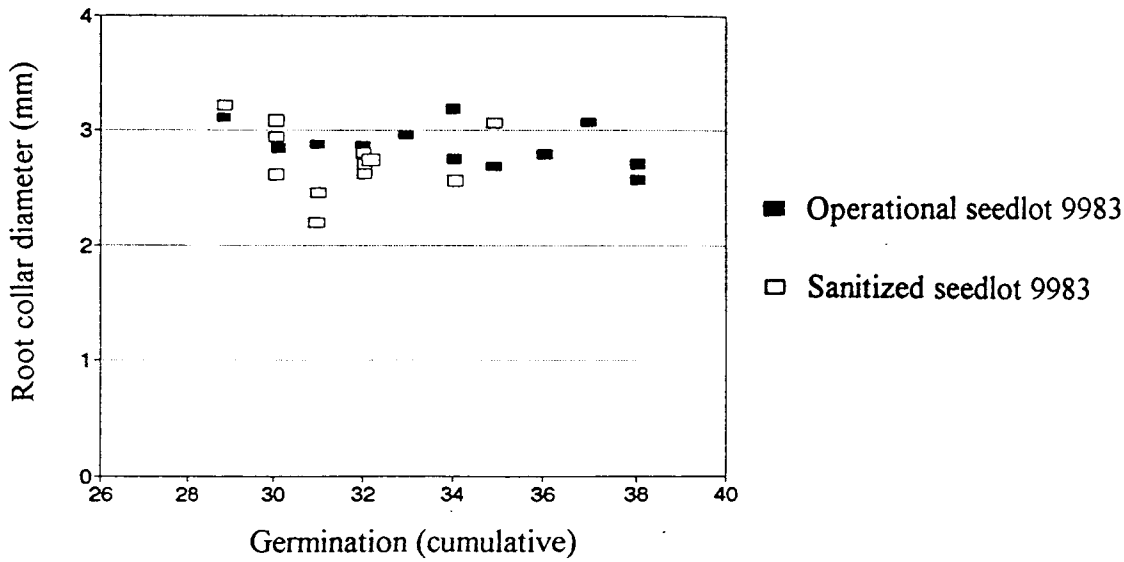

Figure 5.0.12 Root collar diameter vs. germination for the operational and sanitized treatments of seedlot 9983--1992.

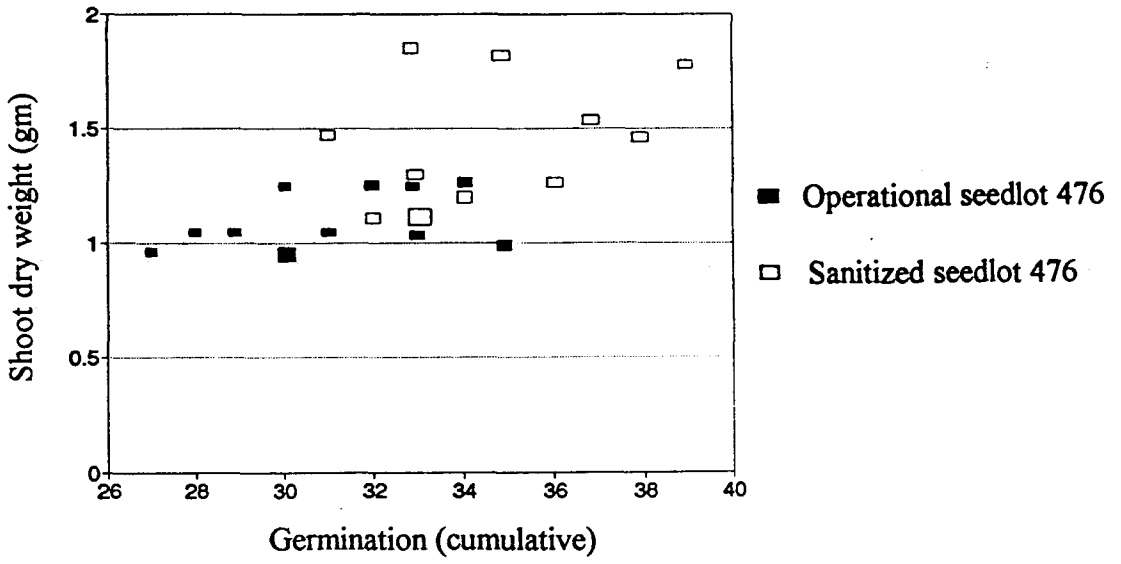

Figure 5.0.13 Shoot dry weight vs. germination for the operational and sanitized treatments of seedlot 476--1992.

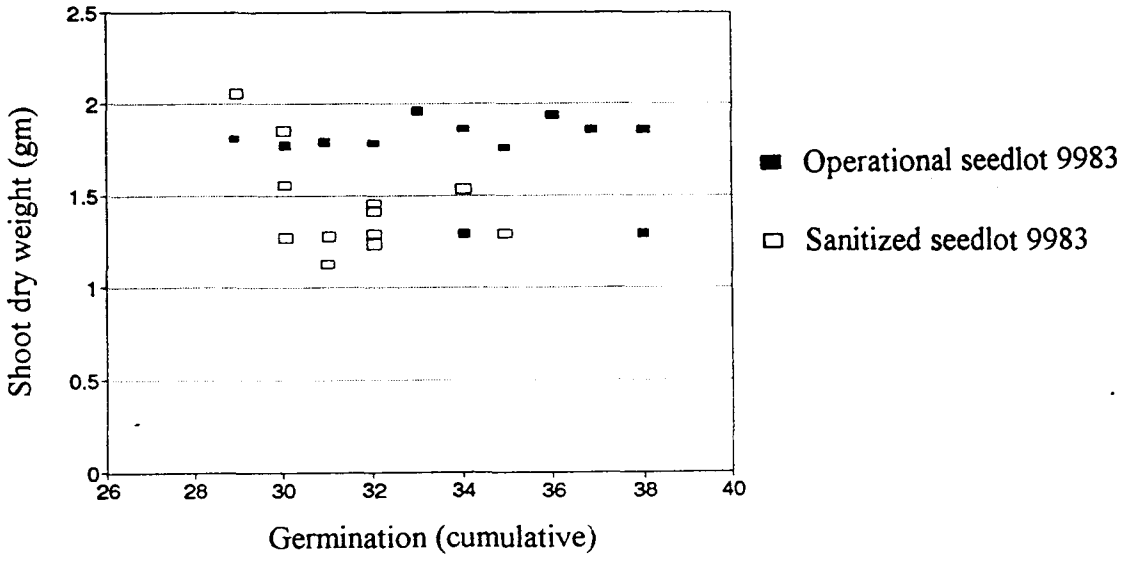

Figure 5.0.14 Shoot dry weight vs. germination for the operational and sanitized treatments of seedlot 9983--1992.

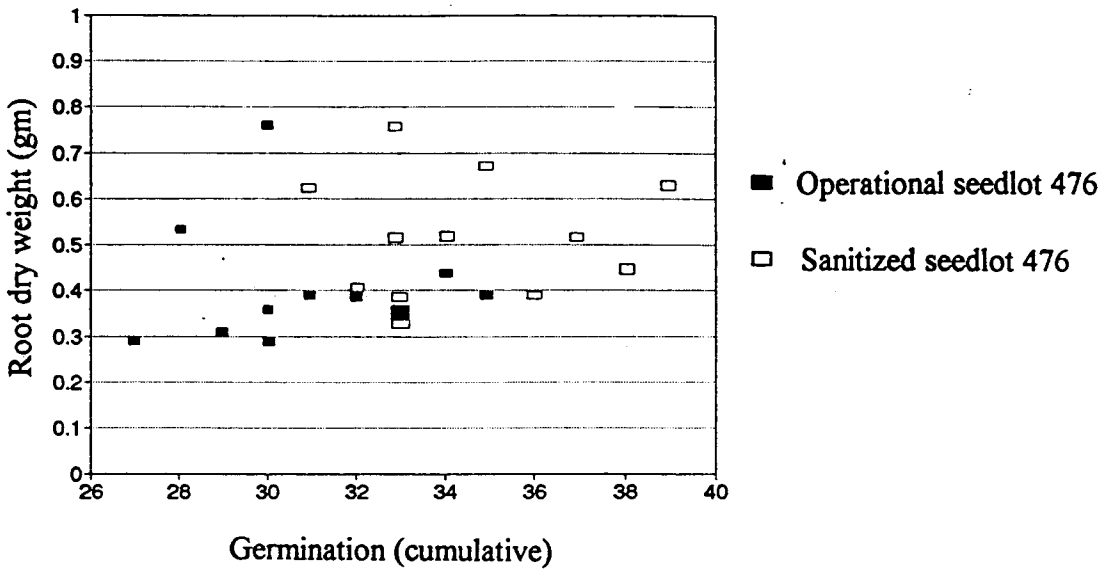

Figure 5.0.15 Root dry weight vs. germination for the operational and sanitized treatments of seedlot 476--1992.

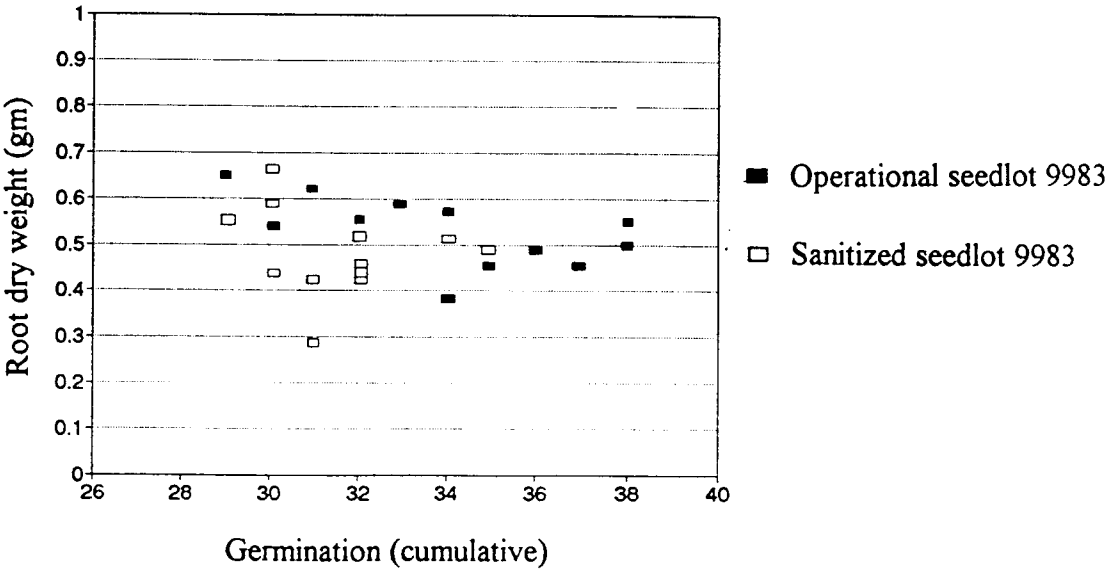

Figure 5.0.16 Root dry weight vs. germination for the operational and sanitized treatments of seedlot 9983-1992.

Appendix 6.0 Analysis of covariance for germination and biometric measurements.

Appendix 6.1 Analysis of covariance tables with replicates nested within treatments for seedlings grown from seedlot 476 in 1991.

Statistical hypotheses used for all analysis of variance tests in this section:

$\mathbf{H_0}$= The difference in treatment means is not significantly different (p=0.05).

$\mathbf{H_1}$= The difference in treatment means is significantlydifferent (p=0.05).

Table A6.1.1 Analysis of covariance table for height and germination.

SOURCE	DF	SS	MS	F	p
Residual	14	2.76	0.20		
Regression	1	0.30	0.30	1.50	.241
Constant	1	9.68	9.68	49.04	.000
Block	5	0.60	0.12	0.61	.694
Treatment	1	3.98	3.98	20.13	.001
Rep. within Treatment	2	0.03	0.02	0.08	.926

Table A6.1.2 Analysis of covariance table for root collar diameter and germination.

SOURCE	DF	SS	MS	F	p
Residual	14	0.23	0.02		
Regression	1	0.00	0.00	0.13	.728
Constant	1	0.95	0.95	57.78	.000
Block	5	0.05	0.01	0.55	.737
Treatment	1	0.21	0.21	12.94	.003
Rep. within Treatment	2	0.02	0.01	0.46	.639

Table A.6.1.3 Analysis of covariance table for shoot dry weight and germination.

SOURCE	DF	SS	MS	F	p
Residual	14	0.01	0.00		
Regression	1	0.00	0.00	0.98	.338
Constant	1	0.03	0.03	36.87	.000
Block	5	0.00	0.00	0.71	.629
Treatment	1	0.01	0.01	11.12	.005
Rep. within Treatment	2	0.00	0.00	0.85	.447

Table A6.1.4 Analysis of covariance table for root dry weight and germination.

SOURCE	DF	SS	MS	F	p
Residual	14	0.01	0.00		
Regression	1	0.00	0.00	0.00	.998
Constant	1	0.02	0.02	40.56	.000
Block	5	0.01	0.00	2.37	.093
Treatment	1	0.02	0.02	33.50	.000
Rep. within Treatment	2	0.00	0.00	1.46	.266

Appendix 7.0 Related work from 1990.

Table A7.0.1 Pre-sowing seed assessment of operational treatments of seedlots 476 and 9983.

Treatment (n=500)	% of Seeds Infested with *Fusarium* (Total)	% of Seeds Infested with *F. oxysporum*	% of Seeds Infested with *F. proliferatum*	% of Seeds Infested with other *Fusarium* Species
Operational 476	86.2 %	1.2 %	85.0 %	n/a
Operational 9983	0.0-0.05 %	0.0 %	0.0 %	0.0-0.5 %

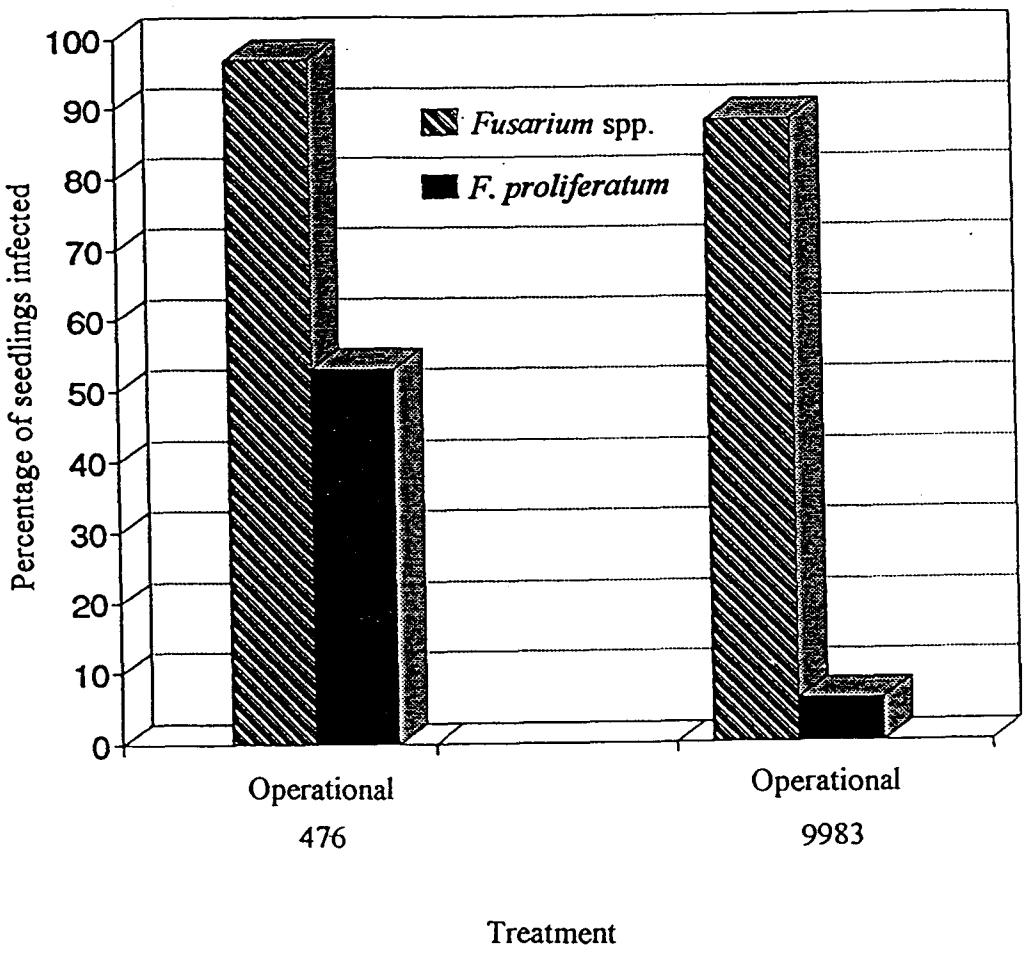

Figure A7.0.1 1990--Percentage of seedlings with *Fusarium* spp. and *F. proliferatum* root infections in the operational treatments of seedlots 9983 and 476.

n=32