AN ABSTRACT OF THE THESIS OF

Elizabeth Cameron Whiting for the degree of Master of Science in Forest Science presented on February 20, 1987.

Title: The Effects of Nursery Incurred Tap-root Wounds on Growth of Douglas-fir Seedlings

Abstract approved: Signature redacted for privacy.

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Tap-root wounds frequently occur on seedlings during lifting in forest tree nurseries. Data are needed to clarify guidelines for culling wounded seedlings. Two-year-old bareroot Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seedlings were wounded by hand on the tap-root to lengths of either 3/8, 1, or 3 inches. Wounded seedlings were used in three greenhouse experiments to determine the effects of moisture stress, wound length, potentially pathogenic fungi, and soil microflora on height growth, number of white root tips, root dry weight, and wound closure.

Results indicated that, regardless of moisture stress level, wound size had no significant effect on the number of white root tips and no effect on height growth. However, seedlings with 1- and 3-inch wounds tended, on the average, to have fewer new roots than controls or seedlings with 3/8-inch wounds. Moisture stress affected wound closure: seedlings with 3-inch wounds were sensitive to high soil moisture and formed much callus but left some xylem still exposed; smaller wounds closed almost completely under all soil moisture stress levels tested. Seedling response to inoculated potentially pathogenic fungi was unclear due to a bacterial problem with the fungal substrate. Soil microflora had no effect on seedlings with 1-inch wounds.

The Effects of Nursery Incurred Tap-root Wounds on Growth of Douglas-fir Seedlings

by

Elizabeth Cameron Whiting

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of
Master of Science

Completed February 20, 1987

Commencement June 1987

Acknowledgement

To Dr. Peyton Owston, my sincere gratitude for his continual guidance and support.

My thanks to Dr. Joe Zaerr for his helpful comments and advice.

I would like to thank Dr. Everett Hansen for serving as Minor Professor and for the use of the Ecology Laboratory.

Special thanks to Phil Hamm for his laboratory assistance.

I wish to acknowledge Dr. William Denison for his help in the identification of the fungi.

Thanks to Tami Webb for her helpfulness.

To Tom -- for support and encouragement.

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INTRODUCTION

Statement of Problem

During the time when nurseries are lifting seedlings for outplanting, many seedlings suffer damage to the tap-root from the lifting process. Mechanical lifters consist of a tractor-drawn undercutting blade with agitators which disturb the seedlings and loosen the soil from around the roots so that they can be manually removed from the ground (Duryea and Landis 1984). The blade may not always make a clean cut, and lateral roots may be ripped off--leaving an open lesion on the tap-root--or the tap-root may be gashed directly. Many of the larger seedlings with otherwise good growth potential seem to be most damaged during lifting. Seedlings with gashes may or may not have good survival potential after outplanting.

Many of the areas to be reforested are less than optimal for seedling growth due to poor soil or hot, dry summers (Hobbs 1983). Healthy and vigorous seedlings are necessary for these sites. The ability to grow roots quickly and re-establish contact with the soil is important for early survival--especially on dry planting sites. Therefore, damage to the root system could possibly affect seedling survival.

Nurseries have established sorting and grading guidelines for what is considered an acceptable seedling for outplanting. Some nurseries consider any damage to the root system to be unacceptable, while others consider single tap-root wounds less than 1/2-inch (1.3 cm) in length and less than 1/3 of the circumference to be acceptable. These guidelines are based on the intuition and experience of nursery managers and silviculturists.

Purpose of Study

The objective of this study is to provide data relating the size of tap-root wounds to subsequent top and root growth of bareroot 2-0 Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seedlings under conditions of moisture stress and inoculation with a potentially pathogenic fungus. Results from these experiments will help nursery managers and silviculturists to evaluate current guidelines for planting seedlings with root wounds.

The null hypotheses are:

1. The length of tap-root wound has no effect on wound closure or seedling growth.
2. Moisture stress level has no effect on wound closure or on root growth.
3. Fresh forest soil, pasteurized forest soil, or fungi isolated from within the wound area have no effect on height or root growth.

LITERATURE REVIEW

Root Wounds

During the lifting process, when seedlings are removed from the nursery beds, tap-roots often become wounded (Thompson 1985). Although many feeder roots are removed during pruning or damaged during lifting, it is the damage to the tap-root that is of concern. Feeder roots are not permanent, and are continually being sloughed off and regenerated (Manion 1981).

If the tap-root has been wounded, the outer protective cortex, an effective barrier separating the inner portion of the root from soil and micro-organisms, has been removed. This leaves the area open and vulnerable to invasion by micro-organisms until it can close (Garrett 1970; Hudler 1984; Biggs 1985). Tap-root wounds may provide an infection court for fungi already inhabiting the rhizosphere of the seedling from the nursery. Or, soil fungi in the planting site can be a potential problem to seedlings with open wounds. The root rot pathogens of forest soils usually attack older, larger trees (Manion 1981) and the problem of seedling root pathogens has not been extensively examined.

Wounding of the tap-root also removes lateral branches with many fine roots. This loss of absorptive area might have an effect on later root growth at or below the wound. Little information is available relating tap-root wounds to subsequent root growth.

Wound Closing

Tree response to wounding, whether from biotic or abiotic causes, involves biochemical and morphological changes. Wilcox (1955), in early work involving the wound closing response of pruned noble fir roots, described five "zones" leading to wound closing: a zone of dried-up cells on the pruned surface as a result of the knife used to cut the root; a zone which was infiltrated with "wound substances" and which showed signs of disorganization and necrosis; a region of callus tissue; a region of wound cork on the outer portion of the callus; and an area of transition to normal tissue. Tippett and Shigo (1981) in their observations of decayed roots found that in response to wounding, conifer roots increased the production of parenchyma cells and that these cells often accumulated polyphenols. Polyphenols are involved in a tree's biochemical response to injury and pathogen invasion. Chemically altered cells form discolored and dark areas in wood. This transformed area functions as a barrier zone and contributes to the defense against invading micro-organisms and probably corresponds to Wilcox's (1955) zone of "wound substances" (Dickinson and Lucas 1982).

As trees age, the protective tissue--bark--around stems, branches, and roots changes. The cortex and epidermis in young trees are replaced by phellogen and phellem cells. The phellogen, which produces the mature bark, is formed by a modification of the cortical cells during the first season of growth (Hudler 1984). If wounds to the bark are shallow, the phellogen produces phellem which then becomes suberized and protects the live tissues beneath. When phellogen is damaged by deeper wounds, a process called phellogen restoration occurs. This involves

transformation of phelloderm and phloem parenchyma cells into phellogen by cellular dedifferentiation. The phellogen then produces the cells referred to as necrophyllactic periderm, wound periderm, or the term used by Wilcox (1955), "wound cork" (Hudler 1984).

Wound closing is dependent on tree vigor and on the size of wound. Neely (1970) defined wound closure as the annual decrease in wound width. This decrease is determined by the annual increment of diameter growth at the site of the wound. Closing occurs during the season of stem growth--May, June, and July for temperate zone deciduous and evergreen trees.

Root Growth

Environments where seedlings are planted are usually less than optimal. Planting sites during the spring typically have cold soils which inhibit root growth and moisture absorption. As the air warms seasonally, transpiration increases and seedlings may experience moisture stress. A root system with many fine roots provides more absorbing area and enables the seedling to resist low soil moisture levels. Therefore, the resumption of root growth after outplanting is important and has been correlated with high seedling survival rates (Stone 1955; Ritchie and Dunlap 1980; Feret and Kreh 1985).

Recent studies on white spruce roots by Johnson-Flanagan and Owens (1985) indicate that growth of individual roots may be cyclic and independent. Roots elongate for 2-3 weeks, stop, develop a metacutization layer (become brown), then resume growth again. The presence of a metacutization layer does not necessarily indicate that a

root is dormant. Krueger and Trappe (1967) observed active root tips on Douglas-fir seedlings throughout the year. Webb's work with sugar maple also indicated that roots which have stopped elongating are capable of renewed growth (Webb 1976). Yet, within the total root system, there are general trends in seasonal root growth. For both white spruce and Douglas-fir, peaks in root activity occur before bud burst in the spring and after shoot growth ceases in the fall (Krueger and Trappe 1967; Johnson-Flanagan and Owens 1985).

New root growth relies on currently produced photosynthate (Webb 1976; Marshall and Waring 1985). Photosynthate produced in the older needles is translocated through the phloem to the roots where it is used in new growth and also deposited as starch reserves for later use in maintenance (Marshall and Waring 1985). As long as the roots are in a favorable environment, growth proceeds using currently produced photoassimilates, but if the environment becomes unfavorable for a long period of time, stored reserves are used (Webb 1976; Marshall and Waring 1985).

Effects of Moisture Stress on Growth

Water is involved in many plant processes and therefore deficiencies can have wide-ranging effects, depending on the physiological condition of the plant, the season when stress occurs, and the duration of stress (Hsiao 1973). Moisture stress directly reduces growth by reducing cell turgor. Cell expansion, or growth, is dependent on the maintenance of cell turgor and is the process most sensitive to moisture stress. Cell division is somewhat less sensitive to moisture

stress than cell enlargement because cells need to reach a minimal size before the next division can occur (Hsiao 1973). Johnson-Flanagan and Owens observed that before brown roots can elongate, there must first be an accumulation of undifferentiated cells through increased mitotic activity (cell division), and increased metabolic activity (Johnson-Flanagan and Owens 1985). Reductions in turgor pressure also affect guard cells which regulate stoma opening and closing. Osmotic regulation of solutes in the guard cells seems to be involved. As guard cells lose turgor, the stomata begin to close and transpiration is reduced (Hsiao 1973). With the stomata closed, CO_2 assimilation decreases and photosynthesis also becomes affected by the increasing drought.

Tension within the xylem vessels can increase with a decrease in moisture availability. If some of the water columns in the xylem break, water flow through the xylem can be interrupted (Hsiao 1973).

Although shoot growth of conifers is largely predetermined by the conditions of the previous season when the buds were originally formed, current conditions can affect how much of that potential is realized. A drought early in the season when shoots are expanding can reduce cell size and therefore reduce growth (Lotan and Zahner 1963; Glerum and Pierpoint 1968).

Root growth, as it involves cell expansion, is also affected by reductions in moisture level (Kaufman 1968; Hsiao 1973). In pine seedlings, root dry weight and length of roots decreased as soil water decreased (Kaufman 1968).

The loss of a lateral root by wounding also reduces the amount of

absorptive area relative to transpiring area; the shoot-to-root ratio is increased. Lopushinsky and Beebe (1976) found that survival of fir and pine seedlings with large root systems was higher than seedlings with smaller root systems. A low shoot-to-root ratio is especially important for seedlings planted on dry sites where having a large number of absorbing roots can increase water uptake and meet transpirational demands (Sutton 1980).

MATERIALS AND METHODS

Scope of Study

Three experiments were planned to test the effects of tap-root wounds over a period of three to four months. The first experiment involved three different levels of moisture stress and three wound lengths to determine how these affect seedling growth and wound closure. The second experiment was designed to test the effects of pasteurized and fresh forest soil on seedling growth and wound closure. The third experiment involved planting wounded seedlings into soil that had been inoculated with a potentially pathogenic fungus to test the effects of this fungus on seedling growth and wound closure.

To control environmental variability and provide a means of comparing wound and growth responses, the two-year-old seedlings were potted and grown in a greenhouse. Wounds occurring from nursery lifting commonly showed wide variation in length, shape, and depth. In order to control this variation, seedlings that were otherwise suitable for planting were wounded with a knife.

Stock Description

Seedlings for the studies were grown at the D.L. Phipps State Forest Nursery located in Elkton, Oregon, and were lifted during early February, 1986. Two-year-old bareroot Douglas-fir seedlings, seed zone

491, elevation 1,500 ft. (457 m), from the Mt. Scott area in the southern Cascade Range were used.

Handling and Wounding

The seedlings to be wounded were selected from those that had successfully passed through the operational grading and sorting process at the Phipps State Nursery, Elkton, Oregon. On February 14, 1986, seedlings were checked for the absence of root wounds and for uniform height between 28-38 cm and stem diameter between 3-5 mm. Also, trees having a straight tap-root were selected for ease of wounding.

Wounds were made by hand in order to control their length and depth. Wound sizes were chosen to include lengths both less than and greater than the 1/2-inch cut-off used in some nurseries' grading standards. Wound lengths were also chosen to correspond to those which occur during the lifting process. The three lengths were--3/8 inch (1.0 cm), 1 inch (2.5 cm), and 3 inches (8.0 cm). Since nursery grading practice uses the English system, wound sizes will be given in these units. Wounding was done within a few days after lifting at the nursery in an outside covered area to keep the seedlings cool and to reduce the amount of handling and root exposure.

Six hundred seedlings were divided randomly into groups according to the wound length to be received, and wounds of the same size were made at the same time for all studies. To aid in the wounding process, a board was marked to correspond to the different wound lengths.

Wounds were made with a 3-1/4-inch (8.3 cm) long carbon-blade Opinel® knife. A seedling was placed on the board and the cotyledon

scar lined up with the taped edge. The seedling was held with one hand and marks perpendicular to the tap-root were made, marking the beginning and end of the wound. The cut could be made in one smooth motion and the even pressure created a wound with even depth and width along the length. The piece of cut root could be lifted out, leaving a clean wound. Measurements were taken at three equidistant spots on three seedlings per wound size for each wound length to determine the average wound width. The 3/8-inch wound width averaged 3.0 mm; the 1-inch wound width averaged 3.0 mm; the 3-inch wound width averaged 3.5 mm. The areas exposed by the wounds were 30.0 mm^2, 80.0 mm^2, and 254.0 mm^2 respectively.

After wounding, seedlings were gathered into bundles of 20-25; the roots were placed in plastic bags, tied, labeled, and placed in waxed cardboard shipping boxes. Wounding took about nine seconds per tree. Since controls were to be treated like non-controls, seedlings without wounds (controls) were placed on the board in bundles of ten and left for nine seconds. The controls were then bundled the same as the treated seedlings and placed in the waxed boxes.

Transport to the Corvallis Forestry Sciences Laboratory was in an unheated station wagon with the windows open for added coolness and ventilation. At the laboratory, the boxes were stored in coolers maintained at approximately 33-35^{o}F (0.5-1.67^{o}C) until time of planting.

Experiment 1--Wound Closure Under Moisture Stress

Seventy-five seedlings from each wound category and seventy-five non-wounded controls were planted on March 3, 1986. Seedlings were planted one to a pot in fiber pots, 15 inches (38 cm) long and 6 inches (15 cm) wide. Well-drained forest soil, which came from the Burnt Woods area west of Corvallis, Oregon, was obtained from the Oregon State University (OSU) Forest Research Laboratory. Soil from the Burnt Woods area was originally used in the OSU Seedling Vigor tests and was considered free of pests. Therefore, it was not pasteurized for this study.

The experimental design was a 4X3 factorial arranged as a split-plot. Moisture stress was the main-plot factor at three different levels. Tap-root wounds were the sub-plot factors at four levels. Four greenhouse benches were used with each one comprising a replication. Each replication (bench) consisted of three groups of seedlings. Each group had four trees from each of the four wound categories, or sixteen trees randomly arranged within the group. The three moisture stress treatments were randomly assigned to each of the groups of seedlings on a bench, but all three stress treatments occurred on every bench. Stress treatments were arranged in groups in order to facilitate watering.

Seedlings were potted, placed in a greenhouse, and grown for four weeks before beginning stress treatments. Temperatures in the greenhouse were maintained at $70^{o}F$ ($21^{o}C$) day and $55^{o}F$ ($13^{o}C$) night. No supplemental lighting was used. Moisture stress was measured by the pressure chamber technique. Seedlings were watered and allowed

to dry until the pre-dawn xylem pressure potential for the low stress treatment reached -5 bars (-0.5 MPa); the moderate stress treatment was between -8 and -13 bars (-0.8 and -1.3 MPa); and the high stress treatment was between -15 and -20 bars (-1.5 and -2.0 MPa). Treatment levels were chosen on the basis of a relationship between moisture stress readings and plant response. For 2-0 Douglas-fir seedlings, water stress is not limiting to plant growth above -8 bars (-0.8 MPa); between -9 and -12 bars (-0.9 and -1.2 MPa) phloem transport becomes limited and height and diameter growth are affected, and between -13 and -20 bars (-1.3 and -2.0 MPa) photosynthesis begins to slow (Cleary and Zaerr 1984). Below -50 bars (-5.0 MPa) mortality of seedlings and young stock occurs (Cleary and Zaerr 1984).

Plant moisture stress (PMS) readings were taken with a PMS Model 600 Pressure Chamber (PMS Instrument Co., Corvallis, Oregon). Extra seedlings were placed on another bench and arranged the same as the experimental seedlings. These seedlings were used for the readings.

A lateral branch was cut from the sample seedling and the bark stripped back about 1 inch (2.5 cm) from the cut end. The branch was then inserted into a rubber stopper and inserted into the chamber with the cut end exposed. Nitrogen gas was slowly released into the chamber, increasing the pressure until xylem water was seen on the exposed cut surface of the branch. The pressure reading taken from the gauge at that moment was an estimate of the water potential of the seedling (Cleary and Zaerr 1980).

Seedlings were watered thoroughly during the experiment, then allowed to dry until the pre-dawn pressure chamber readings were within

the treatment levels. The seedlings were then re-watered and allowed to dry again. The drying cycles began on April 1, 1986.

Sample trees for the moisture stress readings were randomly chosen from the extra bench, and one pot was placed in the center of a bench for each of the stress treatments and replicates. A pot placed in the center of a group of pots was thought to represent the average environment experienced during the drying cycle. After two readings, the sample seedling was replaced with another from the extra bench. Trees near the edges of the bench dried faster; trees near the greenhouse walls dried slower. A row of empty pots was placed next to the wall in order to provide more air circulation and uniformity during drying. Trees in the center of a bench experienced higher relative humidity, transpired less, and therefore depleted soil moisture more slowly. Soil moisture evaporation was also reduced. Taking this into consideration, readings, and therefore stress levels of sample trees, were kept within the middle of the treatment range rather than at the lower or upper ends. Seedlings on the edges, which might have drier soil, would still theoretically fall within treatment ranges, but at the lower end.

Cycles for the treatments ran approximately seven days for the low stress treatment, twelve days for the moderate treatment, and sixteen days for the high stress treatment, depending on environmental conditions. Seedlings were watched for signs of stress--wilting, needle browning, needle loss. These observations, along with days since last watering, soil dryness, and weather conditions gave an indication of when to begin readings. Readings were taken on the extra trees until

they reached the water potential required. Four readings per stress level were taken, one reading from each replicate.

During the first week of July, 1986, seedlings were unpotted, the soil loosened from around the root mass, and roots were washed free of soil. At this time damage to the tap-root and lack of fine roots on several seedlings was observed. Weevil larvae were found in the soil during unpotting and were confirmed as being the probable cause of the damage. Seedlings were initially potted according to wound size--3/8, 1, 3, Controls--and damage seemed to follow this pattern. Five trees in the 3/8-inch group, 3 trees in the 1-inch group, and 1 tree in the 3-inch group had weevil damage. This damage was spread over three replications and involved all of the stress treatments. Damaged seedlings were removed from the data set. One other seedling that did not break bud or have a dominant lateral from which to measure height growth was also removed from the data set. Six seedlings out of sixteen from the 3/8-inch category were removed; 3 seedlings from the 1-inch category, and one from the 3-inch category were removed. The means of the remaining seedlings were used for the analysis of variance.

Height was measured to the nearest 0.5 cm from the cotyledon scar to the terminal bud-scale scar. New growth was recorded in centimeters from the bud scale scar to the tip of the current season's terminal bud. Stem diameter was measured to the nearest 0.5 mm just above the cotyledon scar. White root tips $\geq$ 1 cm long were counted.

Wound widths were measured at three equidistant spots along the length of the wound. One point of a Staedtler Mars Masterbow® draughtsman's compass was placed on the spot where callus tissue met

exposed xylem, and the other point was placed on the opposite side of the wound where xylem was exposed from the callus tissue. The compass points were then placed on a ruler and wound width was recorded to the nearest 0.5 mm.

Data were analyzed for differences among means by analysis of variance. The Statistical Analysis System (SAS) statistical package (SAS Institute 1979) was used for the analysis. Where significance occurred, multiple pairwise comparisons were calculated, using Tukey's Honestly Significant Difference test at the 95% level.

Experiment 2--Effect of Microflora in Forest Soil Compared to Absence of Microflora in Pasteurized Soil on Wounded Seedlings

The forest soil for this test came from a site in the Cascades around Sweet Home, Oregon, elevation 3,700 ft (1128 m). The site had been clear cut and burned a season earlier. The soil was sieved through a 2 cm X 2 cm mesh screen to remove large debris and stones, and three-quarters of it was pasteurized at 180°F (82°C) for 30 minutes.

Eighteen uniform seedlings per treatment were potted, one to a pot, in 450-ml plastic tree containers. The pots had been soaked overnight in a commercial 1% sodium hypochlorite solution diluted 1/2 gallon (1.89 l) sodium hypocholorite to 30 gallons (114 l) of water. The pots were then rinsed and air-dried.

Treatments for the 2x2 factorial experiment consisted of seedlings with 1-inch-long tap-root wounds and non-wounded controls, potted into

either fresh forest soil or pasteurized forest soil. Seedlings were arranged in a completely randomized design and watered thoroughly, approximately every two days, to keep them well-moistened. Greenhouse temperatures were maintained at 70°F (21°C) day and 55°F (13°C) night; natural lighting was not supplemented.

At the end of twelve weeks, the seedlings were unpotted and roots were washed free of soil. Initial height was recorded to the nearest 0.5 cm from the cotyledon scar to the previous terminal bud scale scar. New growth from the previous bud-scale scar to the tip of the terminal bud was measured to the nearest 0.5 cm, and stem diameter was measured just above the cotyledon scar to the nearest 0.5 mm. Wound closing was noted and coded on a scale of 1 to 4; 1--a closed wound with a flat wound callus; 2--a closed wound but with a ridged callus area; 3--a slight gap with some exposed xylem; and 4--a gap exposing xylem along most of the wound. Roots were cut at the cotyledon scar, placed in small paper bags and oven dried at 65°C for 63 hours. Root dry weight was recorded to the nearest 0.01 gram.

Root dry weight and new top growth were analyzed for differences among means by analysis of variance using the Statistical Analysis System (SAS) package.

Experiment 3--Growth of Seedlings with Wounds in Soil Inoculated with Fungi

Inoculum Preparation

Fungi used for Experiment 3 were isolated from dead seedlings with

tap-root wounds. The trees had been planted on a site in southwest Oregon. Seedlings were dug and brought to the laboratory for isolation. The tap-root was cleaned thoroughly. Cross-sectional discs, 1 mm thick, were cut from the wounded area and surface sterilized in 1% sodium hypochlorite for one minute. The discs were rinsed twice in sterile water and blotted on sterile filter paper. They were then plated on potato dextrose agar (PDA). After seven days, two plates with unidentified colonies were sub-cultured onto fresh PDA. Two weeks later plates were read; the fungal genera (isolated from within the wound area) identified included Fusarium, Phoma, Trichoderma, and four colonies of unknown fungi. Fusarium and Phoma are important pathogens of seedlings in forest tree nurseries (Forest Insect & Disease Conditions in the U.S. USDA Publ. 1984); Trichoderma is a common saprobic soil fungus (Webster 1980).

The unknown fungi (identified later and discussed in the Results section) were chosen to serve as the inoculum. While Fusarium and Phoma are important pathogens, much information is already known about them. Moreover, the dead seedlings did not exhibit symptoms of root rot or top blight, suggesting that while the fungi causing these symptoms were recovered from within the seedlings, they were not the probable cause of mortality. The presence of the unknown fungi in the wounded area of the dead seedlings was of interest. To determine what effect they would have on seedlings with tap-root wounds, they were to be used as the inoculum. The unknown isolates appeared to represent two types based on colony appearance in culture. A representative of each type was subcultured for further work.

A supply of rolled oats was available which could be used as a substrate for growing the fungi for soil infestation. A preliminary test was done to see if the fungi would grow on the oats. Three-and-a-half one-quart-size Mason jars full of oats were placed in a dishpan, covered with water, and soaked overnight. They were then drained through a collander lined with three layers of cheesecloth and squeezed to remove excess water. Four clean Mason jars were filled with 500 ml of oats and autoclaved at 15 psi for 25 minutes.

Inoculation of the cooled oats was done under a laminar flow hood using aseptic technique. Small pieces of the two isolates of the cultured fungi were transferred into the four jars--two jars of each isolate. Every two days the jars were shaken to distribute the fungi evenly throughout the substrate.

In ten days the fungi were growing on the oats; fungal mycelium, visible as a white cottony mass, was present in all jars. Since the fungi appeared to be growing well, this procedure was repeated on a larger scale for inoculating the soil with the oats. Eighteen jars, each filled with 500 ml of oats, five of each fungus and eight jars with no fungus, were prepared. The fungi did not do as well as in the previous test. The fungi in some jars were growing very slowly; in some jars there was no visible mycelium. The jars with no visible mycelium were inoculated again.

Pasteurized forest soil, described earlier, was mixed with oats containing a mixture of the two fungi or with only sterile oats. A ratio of one part oats to four parts soil was used. The mixing was done

in a clean plastic bag to distribute the oats uniformly throughout the soil.

Prior to potting, cultures were taken from each jar used to inoculate the soil and plated on PDA as a record of the inoculum used.

Treatments and Methods

Thirty-six trees with 1-inch-long wounds were potted, one to a pot (soil and pots described in Experiment 2). Half of the trees were potted in the sterile oat mixture and half of the trees potted in the inoculated oat mixture. Thirty-six trees without wounds (controls) were potted similarly, half into sterile oats, half into inoculated oats.

The seedlings were arranged in a randomized design and watered thoroughly. Thereafter they were watered approximately every two days. Greenhouse temperatures were $70^{o}F$ ($21^{o}C$) day and $55^{o}F$ ($13^{o}C$) night.

At the end of twelve weeks, seedlings were unpotted and roots washed free of soil. Initial height, new growth, stem diameter, and root dry weight were measured as in Experiment 2. Wound callus was also recorded as a coded value from one to four as in Experiment 2.

New growth and root dry weight data were analyzed for differences among means by analysis of variance. The Statistical Analysis System (SAS) statistical package (SAS Institute 1979) was used for the analysis.

Isolations from the wound area were carried out on a sample of nine trees per treatment. For the non-wounded controls, a one-inch segment beginning three inches below the cotyledon scar was used for isolation. The area for isolation was washed with tap water, and a soft toothbrush

was used to clean soil out of the callused area. The wound area or a one-inch segment of the non-wounded controls was cut from the tap-root and sliced longitudinally exposing the xylem. Any stem discoloration was noted. One-millimeter-wide cross-sectional discs were cut from one of the stem segments, the surfaces sterilized one minute in 1% sodium hypochlorite, rinsed twice in sterile water, and blotted on sterile filter paper. Seven discs from each tree were placed onto PDA with streptomycin (an anti-bacterial agent), 0.4cc/250 ml, and placed under fluorescent lights.

Four days after potting the trees for Experiment 3, bacteria were found growing in the petri dishes plated for a record of the inoculum used for the oat-soil mixture. As it was not known whether the bacteria were present in the oat-soil mixture or occurred only later in the petri dishes, the experiment was repeated to avoid a possible contamination problem.

Additional oats were prepared, as before, but they were autoclaved for an extra ten minutes or for 35 minutes total at 15 psi. Fungi for the inoculum came from record plates because the colonies were already at room temperature and growing quickly. Only areas free of bacteria were used to inoculate the oats; suspicious areas were also avoided.

Pasteurized soil was available from the previous experiments and seventy-two additional trees were wounded. Two weeks after Experiment 3 was begun, a second trial was potted. Identical procedures to those in the first trial were used, and the test was also a randomized design, consisting of four treatments with eighteen trees per treatment.

Oats used to inoculate the soil were also plated on PDA agar as a

record. It was noted in mixing the soil and oats the second time that the oats were not as sticky nor did they have the sweet-vinegarish smell noted on those used for the first trial of Experiment 3.

Histological Examination From All Three Experiments

Samples of healed and unhealed 3/8-inch, 1-inch, and 3-inch wounds were examined histologically. One millimeter-thick discs from the wound area were mounted on a Tissu-Freez® freezing microtome, (Bailey Instruments, Saddle Brook, NJ 07662). Transverse slices were made, 5 and 10 microns thick, then transferred to glass slides and examined microscopically.

RESULTS

Experiment 1--Wound Healing Under Moisture Stress

Early readings showed that wound size did not affect plant moisture stress. This observation was verified by readings taken at the end of the experiment before the trees were harvested. Sixteen trees, four trees from each wound size from each replication, were evaluated for moisture stress within each of the three stress treatments. Results show that at the end of the drying cycles, trees with wounds were within the required stress range as were the controls. In addition, the three stress levels were significantly different from one another at the 1% level (table 1). The number of drying cycles performed were 10 for low stress, seven for moderate stress, and six for high stress treatment.

Among the four wound categories, counts of white root tips $\geq$ 1 cm for seedlings grown under low, moderate, and high moisture stress regimes were not significantly different ($p < .05$) (table 2). Examining the means, the number of white root tips per seedling did not appear to be affected by moisture stress. However, there seemed to be a trend for the 1-inch and 3-inch wounds in the low and high stress treatments to have lower white root counts than the controls and 3/8-inch wounded trees. The moderate stress treatment appeared to be more favorable for root growth for seedlings with 1-inch and 3-inch wounds, but the controls and 3/8-inch wounded seedlings developed more root tips under the higher stress level (drier treatment).

Table 1. Final mean pre-dawn plant water potential readings taken from a sub-sample of four seedlings per wound size and stress level. Taken at the end of the last dry-down cycle before harvest.

	Wound Size				
Stress	Control	3/8	1	3	
	Water Potential Reading (bars)				Mean
Low	-5.0	-5.4	-5.3	-5.1	-5.2a
Moderate	-10.0	-11.4	-9.6	-9.7	-10.2b
High	-18.2	-22.2	-16.1	-18.2	-18.7c
Mean	-11.1d	-13.0d	-10.3d	-11.0d	

S.E. Stress $\pm$ 0.7

S.E. Wound $\pm$ 0.8

Note: Treatment means not followed by the same letter are significantly different at the 1% level.

Table 2. Effect of moisture stress level on number of white roots.

Wound Size	Count of white root tips ≥ 1 cm			
	Low	Moderate	High	Mean*
Control	81	84	92	86
3/8-inch	87	79	87	84
1-inch	65	74	66	68
3-inch	67	83	64	71
Mean*	75	80	77	

S.E. Wound ± 6

S.E. Stress ± 7

*Means not significantly different at the 5% level.

There were no significant differences in seedling height growth due to either level of moisture stress or length of wound (table 3). However, there was a trend for height to decrease with increasing moisture stress. After two drying cycles, seedlings in the high stress treatment did show symptoms of stress and were beginning to lose needles. Many of the remaining green needles were shorter than those of the low stress treatment and some were turning brown.

Table 3. Effect of wound size and moisture stress on height growth.

	Wound Size				
Stress	Control	3/8	1	3	Mean*
	New Growth (cm)				
Low	13.2	10.9	11.2	11.3	11.6
Moderate	10.8	10.2	10.9	10.3	10.5
High	11.0	8.8	10.2	10.2	10.0
Mean*	11.7	9.9	10.8	10.5	

S.E. Stress ± 0.4

S.E. Wound ± 0.4

*Means not significantly different at the 5% level.

Amount of wound closure was analyzed separately for each wound size. There were no significant differences in the amount of xylem left exposed under the different stress levels for the 3/8-inch and 1-inch wounds (figure 1). The average width (measured at three points) of exposed xylem along the length of the wound was 0.1 mm (avg. area 1 mm^2) for the 3/8-inch wound compared to initial width of 3.0 mm (avg. area 30 mm^2), and 0.2 mm (avg. area 5 mm^2) for the 1-inch wound compared to 3.0 mm (avg. area 80 mm^2). Effectively, the 3/8-inch wounds closed completely (figure 2). The 1-inch wounds did not close as well overall as the 3/8-inch wounds, but did show closure in many seedlings (figure 3).

With the 3-inch wound, stress level was highly significant in the amount of wound closure. Contrary to expectations, the low stress treatment resulted in a decrease in the amount of tissue formed and therefore in closing, while the high stress treatment showed greater closing. The average amount of xylem exposed along the 3-inch wound at all three stress levels was 0.5 mm (avg. area 34 mm^2) compared to 3.5 mm (254 mm^2) exposed initially. The overall closure in the 3-inch wound treatment was poor compared to the 3/8-inch and 1-inch wounds (figure 4). Where closure did occur, it was uneven, and rarely did the entire wound close completely.

Replication was significant for both the 1-inch and 3-inch wounds. Seedlings in replications one and two had less wound closure than in replications three and four. Replication had no effect on seedlings with the 3/8-inch wounds.

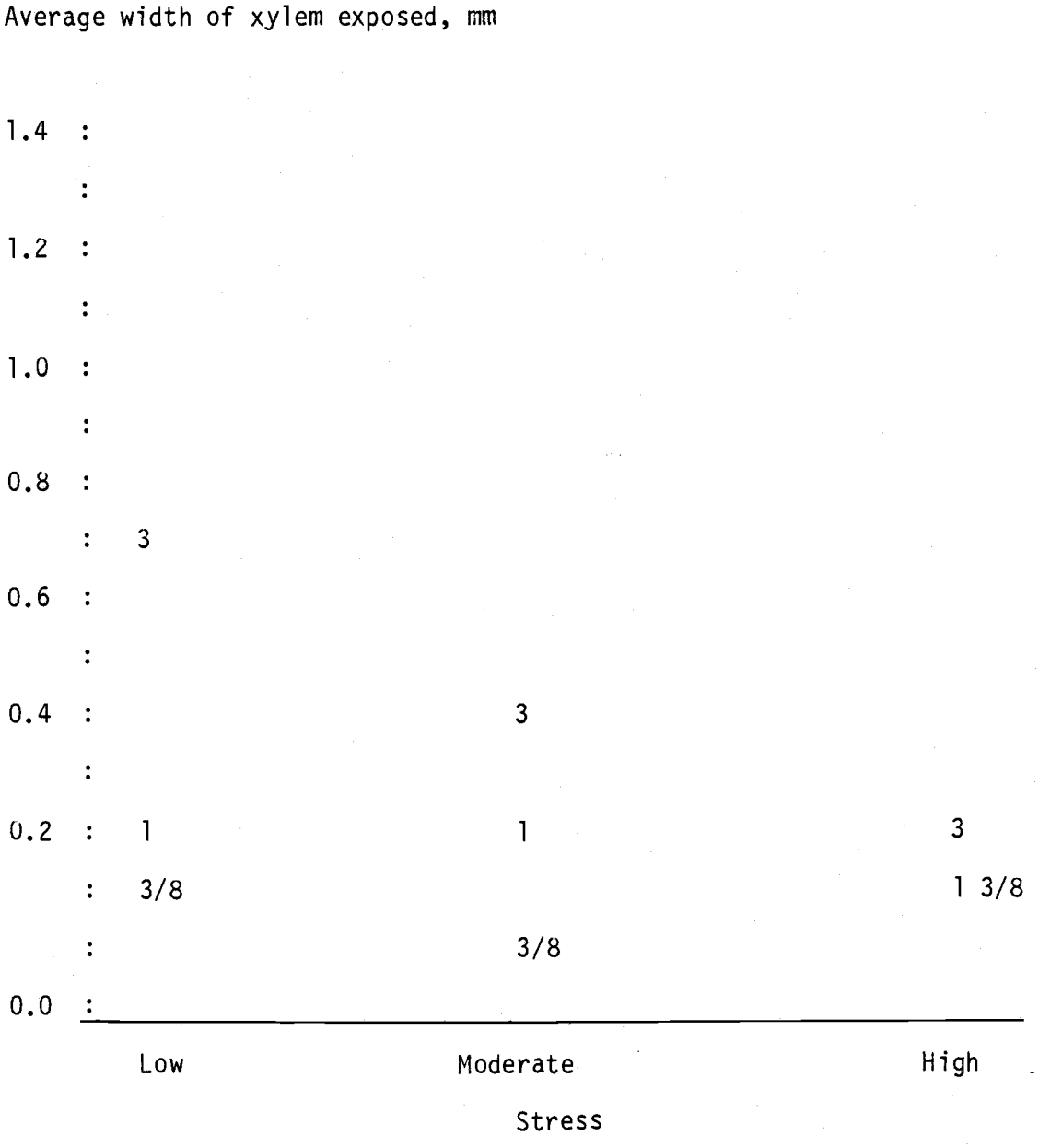


Figure 1. Plot of average amount of exposed xylem taken at 3 equidistant points along the wound. Symbol is the length, in inches, of the three wounds.

Figure 2. Wound closure of 3/8-inch wounds at moderate moisture stress treatment after 3 months.

Figure 3. Wound closure of the 1-inch wounds at high moisture stress level after 3 months.

Figure 4. Wound closure of the 3-inch-long wounds at low moisture stress after 3 months.

Experiment 2--Effect of Microflora in Forest Soil Compared to Absence of Microflora in Pasteurized Soil on Wounded Seedlings

New growth of wounded and non-wounded seedlings was not significantly different when the seedlings were planted in either fresh forest soil or pasteurized forest soil. However, there was a tendency for seedlings in pasteurized soil to have more growth (table 4).

Root dry weight was also not significantly different for seedlings planted in either of the two soils. The presence of a 1-inch wound, even in non-pasteurized soil, had no effect on seedling root weight (table 5).

Table 4. Average height growth per seedling by soil type and wound treatment.

	Forest soil	Pasteurized soil	
	Amount of New Growth (cm)		Mean*
Control	7.6 ±.5	8.0 ±.3	7.8 ±.3
Wound 1"	7.3 ±.4	8.5 ±.6	7.8 ±.4
Mean*	7.5 ±.3	8.1 ±.3	

*Means not significantly different at the 5% level.

Table 5. Average root dry weight per seedling by soil type and wound treatment.

	Forest Soil	Pasteurized Soil	
	Amount of Root Dry Weight (g)		Mean*
Control	3.19 ±.23	2.97 ±.17	3.08 ±.20
Wound 1"	3.00 ±.18	3.31 ±.15	3.15 ±.10
Mean*	3.09 ±.20	3.14 ±.10	

*Means not significantly different at the 5% level.

Experiment 3--Growth of Seedlings with Wounds in Soil Inoculated with Fungi

Seedling response to inoculated soil differed between the two trials of this experiment. Height growth was not affected by the presence of the fungi used as the inoculum in the soil in the first trial. There were no significant differences in height growth between seedlings planted in soil with sterile oats or seedlings planted in fungal-inoculated oats (table 6).

Table 6. Average height growth of seedlings planted in soil with sterile oats or oats inoculated with fungi (Trial 1).

	Non-inoculated oats	Inoculated oats	
	Amount of New Growth (cm)		Mean*
Control	4.9 ±.5	5.4 ±.4	5.1 ±.3
Wound 1"	6.1 ±.4	4.9 ±.3	5.5 ±.3
Mean*	5.6 ±.3	5.1 ±.3	

*Means not significantly different at the 5% level.

Differences in height growth for seedlings in the second trial were highly significant ($P<.01$) (table 7). Seedlings with a 1-inch wound and growing in the inoculated soil grew approximately 1 cm less than non-wounded seedlings growing in the inoculated soil, and were approximately 1.5 cm shorter than those with wounds growing in non-inoculated soil. Initial height was used as a covariate.

Table 7. Amount of new growth in centimeters of wounded and non-wounded seedlings grown in soil inoculated with fungi (Trial 2).

	Non-inoculated soil	Inoculated soil	
	Amount of New Growth (cm)		Mean
Control	5.8 ±.4 a	5.3 ±.3 ab	5.6 ±.3
Wound 1"	6.2 ±.3 a	4.3 ±.3 b	5.4 ±.3
Mean	6.0 ±.3	4.9 ±.2	

Note: Treatment means not followed by the same letter are significantly different at the 1% level.

Root dry weight was not significantly affected by the inoculation in the first trial. Root mass of seedlings planted in the inoculated soil showed no significant differences from those planted in the non-inoculated soil (table 8). Stem diameter was used as a covariate to remove the effect of large tap roots on root weight.

Table 8. Average root dry weight of seedlings planted in soil with sterile oats or oats inoculated with fungi (Trial 1).

	Non-inoculated oats	Inoculated Oats	
	Root Dry Weight (g)		Mean*
Control	2.67 ±.10	2.52 ±.11	2.60 ±.10
Wound 1"	2.54 ±.10	2.71 ±.10	2.62 ±.10
Mean*	2.61 ±.10	2.62 ±.10	

*Means not significantly different at the 5% level.

In the second trial, root dry weight was affected by the presence of the fungi. There were significant differences ($P<.05$) in root mass between non-wounded seedlings and those with a 1-inch wound. Non-wounded seedlings weighed approximately 0.75 grams more than wounded seedlings. Stem diameter was used as a covariate to remove the effect of large tap roots on root weight. Again, differences in root mass were due to treatment effects over and above the variation associated with stem diameter (table 9).

Table 9. Average root dry weight per seedling of wounded and non-wounded seedlings grown in soil inoculated with fungi (Trial 2).

	Non-inoculated soil	Inoculated soil	
	Root Dry Weight (g)		Mean
Control	3.46 ±.20 ac	3.65 ±.14 a	3.56 ±.10
Wound 1"	2.88 ±.15 bc	2.81 ±.16 b	2.84 ±.10
Mean	3.16 ±.10	3.24 ±.10	

Note: Treatment means not followed by the same letter are significantly different at the 5% level.

Wound Closure in the Soil and Inoculation Experiments

Results indicated that there were no significant differences in closure due to the different treatments. On the average, in each of the experiments, 1-inch wounds closed completely. Averages of the coded values were 1.9 for Experiment 2, and 2.0 for both trials of Experiment 3. Category 2 signified wounds which had callused and had no xylem exposed (table 10).

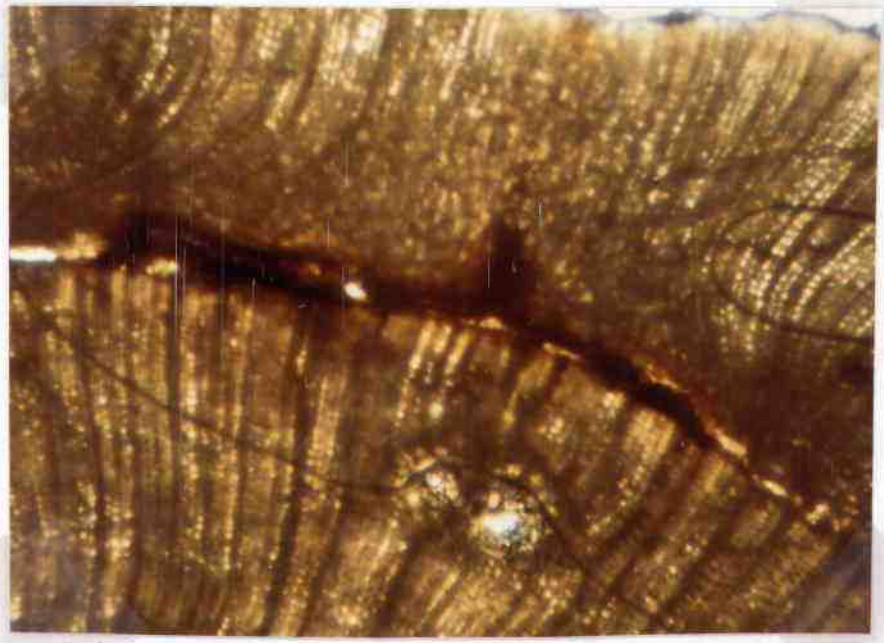
Histological Examination

The results of the histological examination showed that a distinct wound response tissue was formed (figure 5). Where the original wound was made, the cells were irregular in size, and filled with materials giving them the dark coloration usually associated with a wound response (Tippet and Shigo 1981). In completely closed 3/8-inch wounds, the tracheids and ray parenchyma had returned to normal and the wound was completely enclosed in new tissue. In the larger wounds, 1-inch and 3-inch, more irregular callus tissue and disorganized tracheids were formed between the original wound and where the periderm joined in the center, termed "callus inrolling" by Shigo (1986). A wound buried inside new tissue is considered completely closed, whereas a wound covered by the inrolling of callus and periderm can still leave hairline cracks (Shigo 1986).

Table 10. Average wound closure of 1-inch wounds as a coded value 1-4 with 1 representing a completely closed wound.

Treatment		Mean
Forest Soil	Pasteurized Soil	
1.9	1.9	1.9 ±0.1
Non-inoculated Soil (T1)	Inoculated Soil (T1)	
2.0	1.9	2.0 ±0.1
Non-inoculated Soil (T2)	Inoculated Soil (T2)	
2.0	2.0	2.0 ±0.2

a.



b.

Figure 5. Photomicrographs of unstained transverse sections through healed wounds showing wound-associated tissue. Dark area indicates original wound. Normal tissue, tracheids and rays, are formed towards the outside of the root, 125X.
a. 3/8-inch wound; b. 1-inch wound.

Results of Final Isolations

Out of the 36 trees sampled, the fungi used as the inoculum were recovered from 31 of the seedlings originally planted in soil with inoculated oats. Of the 36 seedlings, 17 out of 18 seedlings with wounds yielded the fungi, while 14 out of 18 seedlings without wounds yielded the fungi. Of the seedlings with wounds in the first trial, the fungi were recovered from 40 out of 57 chips (70%) removed from the wound. In the second trial, fungi were recovered from 40 out of 66 chips (61%). In seedlings without wounds, chips for isolation were taken from a 1-inch stem segment located 3-inches below the cotyledon scar. In non-wounded seedlings in the first trial, fungi were recovered from 1-inch stem segment chips in 56 out of 57 chips (98%). In the second trial, fungi were recovered from only 5 trees out of 9, and from 29 out of 62 chips (47%). No stem discoloration was observed from within the wounded area or from within the 1-inch segment in the controls. The fungi were not recovered from any of the 28 wounded or non-wounded seedlings planted in non-inoculated soil (table 11).

Isolations were also made from seedlings from Experiment 2. Wounded and non-wounded seedlings were planted in fresh forest soil and pasteurized forest soil which was not inoculated with fungi. Results of the isolations show that fungi resembling those used for the inoculum for Experiment 3 (identified and discussed in the next section) were recovered from some of the seedlings. In non-wounded trees in forest soil, the fungi were recovered from 3 trees out of 10, and from 4 chips out of 58 (7%). No fungi were recovered from 4 wounded seedlings planted in forest soil. In pasteurized forest soil, fungi were

recovered from 2 out of 9 non-wounded seedlings--in 3 out of 56 chips (5%). Fungi were recovered from 5 out of 9 wounded seedlings--from 8 out of 56 chips, or from 14% of the chips (table 11).

Table 11. Recovery of Chaetomium sp. from Experiments 2 and 3 after twelve weeks.

Treatment	Control			1-inch wound		
	Trees	Chips	%	Trees	Chips	%
Exp. 2						
Forest soil	3/10	4/58	7	0/4	0	0
Past. forest soil	2/9	3/56	5	5/9	8/56	14
Exp. 3-Trial 1						
Non-inoculated	0/4	0	0	0/8	0	0
Inoculated	9/9	56/57	98	8/9	40/57	70
Exp. 3-Trial 2						
Non-inoculated	0/8	0	0	0/8	0	0
Inoculated	5/9	29/62	47	9/9	40/66	61

Identity and Ecology of Fungi

The identities of the unknown fungi isolated originally from the seedlings have been established as two different species of the genus Chaetomium Kunze ex Fr. 1821, class Ascomycetes. This genus is characterized by the presence of long hairs growing from the fruiting body and by the dark color of the ascospores. Chaetomium species are common soil saprophytes important in the decomposition of cotton and cellulose materials (Domsch, Gams, and Anderson 1980). They have been found to cause soft-rot in wood. Through enzymatic action the fungus feeds on the cellulose component of cell walls. The hyphae ramify within the secondary cell walls, forming elongated cavities which contribute to the disintegration and instability of the walls (Duncan 1960; Boyce 1961). Some Chaetomium sp. are also seed-borne in many kinds of seeds (Neergaard 1977).

Species identification was done from visual characteristics of the cultures and from microscopic observation. One species was most probably C. cochliodes. Distinguishing features used to make the identification were the following:

perithecia.....311 x 266 μm

ascus.....clavate

ascospores.....olive-brown, lemon-shaped

9 x 7.2 - 8 μm

terminal hairs.....unbranched

formed coils towards the tips

1 to 5 coils

colony in culture....homothallic

very pronounced odor.

According to the keys by Domsch, Gams, Anderson (1980), and of Skolko and Groves (1953), this species is similar to C. angustrum but does not have any straight terminal hairs. Further, the species is similar to C. bostrychoides, which has some branched terminal hairs and more coils per hair than C. cochliodes, smaller perithecia and ascospores, and no pronounced odor. It is also similar to C. globosum, but again, there is no pronounced odor to the culture and the hairs are wavy and not usually coiled.

C. cochloides has been isolated from soil, plant materials and seeds (Domsch, Gams, Anderson 1980; Skolko and Groves 1953). It is one of the species capable of causing soft-rot in wood by attacking the secondary cell walls (Duncan 1960; Boyce 1961; Manion 1981). It has also been shown to be an antagonist to the seed-borne pathogens, Drechslera victoriae and Fusarium nivale (Neergaard 1977).

The second species was identified as probably C. fusiforme. Distinguishing features observed in this fungus were the following:

perithecia.....less than 311 x 266 μm

ascus.....clavate

ascospores.....dark colored

fusiform (some with flattened side)

12.6 x 5.4 μm

terminal hairs.....curved with recurved or circinate tips

dark colored

septate

colony.....homothallic

red pigment in media (PDA)

no pronounced odor.

C. fusiforme is very similar to *C. aureum* which has wider terminal hairs that do not form coils; moreover, the ascospores of *C. aureum* are irregularly ellipsoid and not as long as those of *C. fusiforme* (Skolko and Groves 1953).

DISCUSSION

Wounds and Moisture Stress

Results of the experiment involving growing seedlings with root wounds under different moisture stress levels indicated that moisture stress and tap-root wounds had no effect on height growth. These results need to be interpreted with caution since earlier research indicates that moisture stress can affect height growth. Lotan and Zahner (1963) and Glerum and Pierpoint (1968) conclude that during the period of optimal growth, shoot expansion is very sensitive to drought. Hsiao (1973) in his work concerning plant responses to water stress also agreed that cell expansion is very sensitive to water stress. Severe wounds to the tap-root, which reduce conductive tissue, would further contribute to problems in meeting transpirational demands in a seedling already stressed by low soil moisture. The amount of available soil water is reflected in the ability of cells to elongate, which results in an increase in growth. Even though bud primordia are formed during the previous season and are conditioned by the environment at that time, the current environment, during which the buds expand, can affect the expression of that growth potential.

Lack of positive results could be related to at least three factors. One, during the early part of the experiment, April and May, when shoots would be expanding, it was cloudy, transpirational demand was low, and drying was slow. Second, as soon as the seedlings reached the required stress level they were all re-watered immediately. Increasing the duration that the seedlings were stressed might have

affected them differently. Third, seedlings also had a chance to recover during the re-watering period following the dry-down. By the time the soil was drying faster due to increasing solar radiation and increased seedling transpirational demand, shoot growth was almost completed.

Growth involves not only cell expansion but also cell initiation and cell division, processes which may not be as sensitive to water stress as elongation (Hsiao 1973). Results of the counts of white root tips seem to agree with this observation. There were no statistically significant differences in the number of white root tips between the three different moisture stress treatments, indicating that water stress had little effect on this parameter. Results of root count data, however, do show a trend for root counts of the 1- and 3-inch wounded seedlings to be less than the controls or the 3/8-inch wounded seedlings in the low and high stress treatments. The reduction in the low stress treatment could be due to fine root death in response to high soil moisture (and accompanying low O_2 level), while the reduction in the high stress treatment could actually be due to lack of adequate soil moisture. Also, the roots in the drier treatment did appear to be shorter than those in the low stress treatment. Moisture stress might affect the length of roots rather than the number (Kaufmann 1968). In this case, length of white root tips might have been a better indicator of seedling response to moisture stress than counts alone. During the re-watering period, seedlings were probably able to initiate new roots and elongate those already present before the available soil moisture became depleted.

Wounds -- Soil and Fungus

Results of planting seedlings in either fresh forest soil or in pasteurized soil show that where there are no known disease problems, the microorganisms in fresh forest soil do not affect the growth of seedlings with 1-inch root wounds. In an unstressed environment (greenhouse, abundant moisture), seedlings with wounds grew as well as controls in fresh soil or in pasteurized soil.

Most forest soils contain a larger number and wider variety of microflora than those found in agricultural soils (Pritchett 1979). The diversity of plant species and organic substrates in a forest environment supports organisms with differing nutritional requirements and ecological niches. Moreover, these organisms must compete with one another for their share of the available substrate. And, for an organism to successfully infect plant roots, there must not only be an available food supply, but environmental factors must favor the invading organism, and there must be sufficient infective units in contact with the potential host (Garrett 1970). Although there was no evidence that some of the soil microflora did not enter the wound area, at low populations the seedlings' natural resistance could be enough to overcome opportunistic invasion (Garrett 1970).

The results from the second trial of Experiment 3, unlike the first, indicate that the presence of the Chaetomium did have an effect on seedling growth. Even though Chaetomium is commonly a soil saprophyte and degrades dead organic materials, these results seem to show that some species of Chaetomium are able to invade a living host. Wounded seedlings growing in inoculated soil had reduced top growth

when compared to wounded seedlings in non-inoculated soil, (table 7). In the inoculated soil the presence of a wound was important because the Chaetomium was recovered from within the wound. Root dry weight results also show that wounded seedlings weighed less than non-wounded seedlings in inoculated soil (table 9). Again, the presence of a wound was important only in the inoculated soil. The wound, then, allowed the Chaetomium greater access to plant tissue. These results are consistent with the theory of wounds acting as avenues for pathogen infection (Garrett 1970; Lucas and Dickinson 1982).

It seems that wounded seedlings, in the presence of a sufficiently high population of a saprophyte, can become infected and show reductions in growth. But differences, while statistically significant, are still very small: 1 to 1.5 cm in height and .75 grams in root weight. In the first trial there were no differences found in height and root weight. Since the two trials showed different results, another experiment would be required to verify the results of the second trial.

Even so, relating results from artifically inoculated soils to natural conditions can present problems. High populations of the inoculum could influence the ability of an organism to infect a host or affect the host's natural resistance to infection (Garrett 1970).

During the inoculation experiments, there were problems with the oats used for the fungal substrate. As mentioned in the Materials and Methods section, a possible contamination problem prompted the addition of another experiment duplicating the one in which bacteria were found in the record plates. The rolled oats used as the fungal substrate were

autoclaved for ten minutes longer in the second trial to make sure that the bacteria did not come from them. Fungus gnats were attracted to the oats mixed in with the soil in the two trials of the inoculation experiment and before they could be controlled caused some root damage from larval feeding. At first it was thought that the stressed appearance of the seedlings was due to the treatments, but upon inspection of the root mass, larvae were found. In large numbers, the larvae feed on fine root hairs (PNW Insect Control Handbook). Seedlings were given two soil drenches, two weeks apart, in a solution of 4.5 grams 50 W Diazinon per gallon (3.8 l) of water. After the second drench, there were no signs of larvae or flying adults.

Results of final isolations

Isolations from both wounded and non-wounded seedlings in the inoculation experiment show that Chaetomium spp. were recovered from within seedlings that had been inoculated with the fungal oats. Species identifiation had not been completed at the time of the final isolations, so the percent recovery refers only to the fact that a Chaetomium species was recovered but which species, or the number of different species, was not recorded. That the fungus was recovered from non-wounded trees also indicates that a wound is not necessary for it to gain entrance. It was thought that possibly the fungus was originally a surface contaminant on the seedlings from the nursery, but this idea was later rejected. The fungus was not recovered from seedlings in the non-inoculated soil and a small sample of seedlings from the moisture stress experiment were tested for the presence of Chaetomium, and none was found.

The fact that Chaetomium spp. are common soil inhabitants (Domsch, Gams, Anderson 1980) is evidenced by their recovery from seedlings growing in fresh forest soil (table 11). Surprisingly, though, an unidentified Chaetomium species was recovered in pasteurized forest soil. There are two species of Chaetomium which are not very sensitive to heat: C. globosum and C. thermophile (Domsch, Gams, Anderson 1980). Since the fungi were found in both forest and pasteurized soils, it would seem that they would also be recovered from the pasteurized soil with the non-inoculated oats, but Chaetomium was not recovered. As the only difference between the two treatments was the non-inoculated oats, lack of recovery in that treatment could be due to the presence of the oats. Or, the distribution of the Chaetomium in the soil could have been localized. Soil from this particular area might have been used in Experiment 2, but not in Experiment 3.

What does seem apparent is that some Chaetomium species are present in the soil and can penetrate planted seedlings, regardless of whether they are wounded or not. What effect the fungi have on the seedlings is not clear because the results of the two trials of the inoculation experiment differ. Further investigation would be required to determine the effect.

Wound Closure

Closure of the 3/8-inch and 1-inch wounds was generally very good. Moisture stress or the presence of the Chaetomium did not affect wound closing for the smaller sizes of wounds. Only in the 3-inch wound did water stress significantly affect closing. The larger wounds closed

better at the higher stress level rather than, as would be expected, at the lower stress level. There was some tissue necrosis around the larger wounds on trees in the low stress treatment. Puritch and Mullick (1975) found with Abies grandis that water stress above -1.5 MPa (-15 bars) had no effect on the non-suberized tissue formed following wounding. And, with increasing water stress, the rate of healing was slowed, but returned to the previous level on re-watering (also Hudler 1984). This agrees with the results for the smaller wounds, but is contrary to the results for the seedlings with 3-inch wounds.

The necrosis around the larger wounds could be due to high soil moisture in the low stress treatment. Esau (1965) found that adequate aeration is important in the suberization of wounds and that excessive moisture inhibited suberization and the formation of cork. The larger wounds, because of more surface area exposed and amount of tissue to be produced during closing, were more sensitive than the smaller wounds to high levels of moisture. The high moisture content of the soil in the low stress treatment probably exacerbated problems for wound closing for the larger, 3-inch-long wounds. This might also explain why replications one and two showed significance in the statistical analysis. These replications seemed not to dry as quickly as the other replications, and so, the extra moisture inhibited wound healing. This reaction confirms the experimental results and is consistent with Esau's explanation of wound healing.

Changes in Techniques; Further Research

The low moisture stress treatment in Experiment 1 seemed to keep the soil overly moist; because of the high moisture (and probable reduced oxygen level), wound closure seemed to be adversely affected. An analysis of soil moisture characteristics for the potting soil could be done prior to potting to provide better information for the low stress treatment. Or, a faster draining potting soil could be used. A faster draining soil would enable seedlings to become dry faster and reach stress faster.

Due to the great amount of variation in root counts, increasing the number of seedlings per treatment would give a better indication of root growth.

An outplanting should be done to reflect natural conditions and provide more rapidly fluctuating environmental stresses. Investigating the effects of rapidly induced stress versus slow stress induction would be helpful.

Initial caliper should be taken along with final caliper to show diameter growth over time, or growth rate. As wound closing depends on diameter growth at the wound site, the rate of tissue growth, as millimeters per unit of time, would give an indication of photosynthate partitioning and therefore tree vigor.

Root counts, often used in root growth potential experiments (Ritchie 1980), did not reflect significant differences due to wound length or moisture stress. Since root elongation is adversely affected by high moisture stress, it would seem that measuring root length, as it reflects cell enlargement, would be a better indicator of seedling response to moisture stress than root counts provide. Since the data

have important implications for grading and there is a trend for seedlings with larger wounds to have less root growth, further studies should be made using length of white roots along with counts.

Further research could focus on outplanting wounded seedlings on different types of sites. Seedlings with different wound sizes could be planted on moist and dry sites to determine the effect of available moisture on growth and wound closure. On dry sites, wounded seedlings might be at a disadvantage in terms of water uptake and wound closing.

The fungal-inoculation part of Experiment 3 could be combined with Experiment 1, forming a 3-factor factorial experiment. Combining them would test the effects of moisture stress level and fungal interaction on wound healing and seedling growth response. Further research might involve the interaction between bacteria and fungi insofar as they are competitors for nutrients. Also, the relationship between live sapwood and microorganisms existing in the sapwood might be of interest--especially non-pathogenic decay fungi.

CONCLUSION

Conclusions regarding the null hypotheses, based on experimental results:

Wound Length

Length of tap-root wound affected wound closure. Smaller, 3/8-inch-long wounds closed completely. One-inch-long wounds closed on many seedlings, but not as often as did the smaller wounds. The larger, 3-inch-long wounds formed much callus tissue which inrolled over the wound and left gaps where tissue did not meet. These gaps left areas of xylem still exposed at the end of 3 months. Length of wound had no significant effect on the number of white root tips $\geq$ 1 cm, but there was a trend for seedlings with larger wounds to have less root growth. Length of wound had no effect on seedling height growth or root dry weight.

Moisture Stress

Moisture stress level had no significant effect on number of white root tips $\geq$ 1 cm or on height growth. But, due to problems during the stressing, the no-effect conclusion on height growth must be suspect. Moisture stress did have an effect on wound closure. High levels of soil moisture appeared to inhibit wound healing. The 3-inch-long wounds were most sensitive and had areas of necrosis and bark dieback along the wound margins. The 1-inch-long wounds were somewhat sensitive to

moisture levels, while the 3/8-inch-long wounds closed under all soil moisture levels.

Soil Microbes and Fungal-inoculum

The effect of the fungi, Chaetomium sp., on seedling growth is not clear at this time. In both trials of Experiment 3, the fungi were recovered from wounded seedlings and also from controls in the inoculated soil, yet there was no stem discoloration indicating plant response or infection. In trial 1 of Experiment 3 there was no effect on seedling height growth or root dry weight. But, in the second trial, wounded seedlings in the inoculated soil did show significant differences in height growth. Experimental results are contradictory and therefore more information seems necessary in order to reach a conclusion.

Resident soil microorganisms normally found in forest soils do not affect seedlings with 1-inch-long tap-root wounds. Seedlings showed no symptoms of infection--stem or root discoloration, reduced height growth or reduced root growth.

RECOMMENDATIONS FOR NURSERY MANAGEMENT

Seedlings with tap-root wounds up to 1/2-inches long should not be culled, if the seedling is otherwise acceptable for planting. Based on experimental results, seedlings with 3/8-inch-long tap-root wounds grew as well as non-wounded controls, wounds were completely closed at the end of three months, and wound closing was not inhibited by high soil moisture. In addition, seedlings with even larger wounds (1 inch) were not adversely affected by resident soil microorganisms when planted in non-pasteurized forest soil.

However, seedlings with wounds exceeding 1/2-inches long should be culled. Root growth seemed to be affected and wound closing was sensitive to high soil moisture levels.

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APPENDIX

Table A1. ANALYSIS OF VARIANCE TABLE FOR WATER POTENTIAL FINAL READINGS--Moisture Stress Experiment

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR F
Model	20	1636.1512500	81.8075625	10.35	0.0001
Rep	3	10.9491667	3.6497222	0.46	0.7113
Stress	2	1485.0387500	742.5193750	87.23	0.0001
Rep*Stress	6	51.0745833	8.5124306	1.08	0.4007
Wound	3	49.1875000	16.3958333	2.07	0.1272
Stress*Wound	6	39.9012500	6.6502083	0.84	0.5493
Wound*Rep+ Wound*Stress*Rep	27	213.4212500	7.9044907		
Total	47	1849.5725000			

Table A2. ANALYSIS OF VARIANCE TABLE FOR NEW GROWTH--
Moisture Stress Experiment

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR F
Model	20	64.686270	3.23431	1.43	0.1891
Rep	3	5.227539	1.74251	0.77	0.5194
Stress	2	20.618345	10.30917	4.72	0.0587
Rep*Stress	6	13.108507	2.18475	0.97	0.4648
Wound	3	18.627423	6.20914	2.75	0.0620
Stress*Wound	6	7.104456	1.18408	0.53	0.7842
Wound*Rep+ Wound*Stress*Rep	27	60.891819	2.25525		
Total	47	125.578089			

Table A3. ANALYSIS OF VARIANCE TABLE FOR ROOTS

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR F
Model	20	9697.31279	484.86564	1.01	0.4847
Rep	3	125.63021	41.87674	0.09	0.9666
Stress	2	227.04716	113.52358	0.13	0.8797
Rep*Stress	6	5200.03733	866.67289	1.80	0.1367
Wound	3	2834.43692	944.81231	1.96	0.1433
Stress*Wound	6	1310.16117	218.36019	0.45	0.8360
Wound*Rep+ Wound*Stress*Rep	27	12995.33941	481.30887		
Total	47	22692.65220			

Table A4. ANALYSIS OF VARIANCE FOR 3/8-inch WOUND CLOSURE

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR F
Model	5	0.09023277	0.01350244	1.34	0.3628
Rep	3	0.05216821	0.01738940	1.29	0.3611
Stress	2	0.03806456	0.01903228	1.41	0.3149
Rep*Stress	6	0.08101466	0.01350244		
Total	11	0.17124743			

Table A5. ANALYSIS OF VARIANCE FOR 1-inch WOUND CLOSURE

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR F
Model	5	0.38614005	0.07722801	3.03	0.1049
Rep	3	0.37607703	0.12535901	4.92	0.0468
Stress	2	0.01006301	0.00503151	0.20	0.8261
Rep*Stress	6	0.15300283	0.02550047		
Total	11	0.53914288			

Table A6. ANALYSIS OF VARIANCE FOR 3-inch WOUND CLOSURE

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR F
Model	5	1.23320152	0.24664030	11.29	0.0052
Rep	3	0.68806263	0.22935421	10.50	0.0084
Stress	2	0.54513889	0.27256944	12.48	0.0073
Rep*Stress	6	0.13104424	0.02184071		
Total	11	1.36424576			

Table A7. ANALYSIS OF VARIANCE FOR NEW GROWTH--Forest Soil and Pasteurized Forest Soil

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR F
Treatment	3	13.28600654	4.42866885	1.17	0.3294
Error	66	250.66199346	3.79790899		
Total	69	263.94800000			

Table A8. ANALYSIS OF VARIANCE FOR ROOT DRY WEIGHT--Forest Soil and Pasteurized Forest Soil

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR F
Treatment	3	1.40579951	0.46859984	0.78	0.5101
Error	66	39.72259477	0.60185750		
Total	69	41.12839429			

Table A9. ANALYSIS OF VARIANCE FOR NEW GROWTH--
Inoculation Experiment 3-Trial 1

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR F
Treatment	3	17.15849673	5.71949891	2.05	0.1163
Error	64	178.95915033	2.79623672		
Total	67	196.11764706			

Table A10. ANALYSIS OF VARIANCE FOR ROOT WEIGHT--
Inoculation Experiment 3-Trial 1

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR F
Treatment	3	0.47449722	0.15816574	0.89	0.4507
Error	64	11.35975572	0.17749618		
Total	67	11.83425294			

Table A11. ANALYSIS OF VARIANCE FOR NEW GROWTH--
Inoculation Experiment 3-Trial 2

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR F
Treatment	3	33.2580397	11.0860132	5.28	0.0025
Initial Height	1	4.9399911	4.9399911	2.35	0.1299
Error	66	138.5894207	2.0998397		
Total	70	177.3098592			

Table A12. ANALYSIS OF VARIANCE FOR ROOT WEIGHT--
Inoculation Experiment 3-Trial 2

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	F REQ 5%	F REQ 1%
Treatment	3	0.912	0.304	3.853	2.75	4.10
Error	67	5.288	0.079			
Total	70	6.200				