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THE RELATIONSHIP BETWEEN *PHOMOPSIS OCCULTA* TRAV.
AND THE POST HARVEST DISORDER OF
COLORADO BLUE SPRUCE (*PICEA PUNGENS GLAUCA* ENGELM.)

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By

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ABSTRACT

THE RELATIONSHIP BETWEEN *PHOMOPSIS OCCULTA* TRAV.
AND THE POST HARVEST DISORDER OF
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Using artificial inoculation techniques, *Phomopsis occulta* Trav. was shown to be a causal agent of a post-harvest disorder that plagues nursery grown Colorado blue spruce in Michigan. Field-grown trees declined significantly following fall harvest. Initially, post-harvest symptoms were characterized by needle drop from lower branch areas where inconspicuous stem cankers had developed. In spring, newly developed shoots appeared wilted, then browned and died. In 1988, a drought year, August-harvested plants were less symptomatic than later harvest dates. In 1989, a more normal year, August-harvested plants were more symptomatic than later harvested plants. Various fertility regimes had little affect on the symptom severity of harvested plants during the first year of the study. Plants of the highest foliar nitrogen content during the second year of the study were less symptomatic than plants that were denied fertilizer for two years. Benomyl showed potential as a chemical control.

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INTRODUCTION

Colorado blue spruce (*Picea pungens glauca* Engelm.) is a popular evergreen tree valued in the Midwest for its bluish-green foliage and perfect symmetry. Several hundred thousand are harvested annually in Michigan and are sold as landscape specimens or as Christmas trees. Colorado blue spruce sales in Michigan account for three percent of the total nursery stock sales in the state. Landscape plants are field grown and harvested in the spring or in the fall. Trees are commonly available in sizes that range from 12-15 inches to 3-4 feet and larger. To ensure early spring shipping from Michigan, smaller sizes are commonly over-wintered in quonset style structures while larger sizes are heeled-in.

Since 1986, Michigan nursery growers have been reporting poor post-harvest quality of Colorado blue spruce. The rapid decline of trees after harvest has rendered up to 30% of the plants unsalable. Plants that appear healthy in the field begin to shed needles from the lower third of the plant within a few weeks after harvest. Plants that remain in the field are symptomless except for an occasional flagging branch.

Cutting plant roots during the harvest procedure can reduce the vigor of plants. Plants that are weakened by environmental stresses such as nutrient deficiency, drought, flooding, extreme temperatures, or transplanting; suddenly become susceptible to otherwise nonaggressive

pathogens (38).

Samples of Colorado blue spruce trees exhibiting post-harvest damage were submitted to the Michigan State University Plant Diagnostic Clinic. Stem cankers were revealed by scraping away the bark. Samples of this cankerous tissue consistently yielded cultures of *Phomopsis occulta*.

In 1943, *P. occulta* was considered only a saprophyte by Hahn (14, 15), although White (45) reported in 1929 two cases of spruce blight caused by *P. occulta*. White reported that various species of spruce were susceptible to a blight resembling juniper blight caused by *Phomopsis juniperovora*. In one case spruce were transplanted late and excessively irrigated. In the other case seedlings were grown in overcrowded beds.

Sanderson and Worf have provided recent evidence of the susceptibility of spruce to *P. occulta*, proving the pathogenicity of this fungus (36). Sanderson and Worf artificially inoculated four spruce species and found that Colorado blue spruce was the most susceptible of the 4 spruce species tested.

The purpose of this thesis was to determine if there is a causal relationship between *Phomopsis occulta* and the post-harvest disorder among Colorado blue spruce grown in Michigan, and to study the effects of certain cultural practices upon the severity of the disorder.

LITERATURE REVIEW

Phomopsis Sacc. was originally included as a subdivision of a much larger genus--*Phoma*, by Saccardo in 1884 (14). Saccardo later (1905) described *Phomopsis* as its own genus (14). The separation of the two taxa was based on the recognition of two distinct spore types produced by *Phomopsis* (14). *Phoma* produced only one type (14). The filiform spores produced by *Phomopsis* had previously been disregarded by Saccardo, who in 1905 was still uncertain of the origin and function of these structures (14). In 1911, Diedicke confirmed that the filamentous spores were true spores and designated them as beta spores (14). The beta spores are long, slender and hooked at the apex; guttules (oil droplets) are absent. Alpha spores are cylindrical, with a rounded taper at each end. Alpha spores usually contain two oil droplets (guttules), one at each end. Hahn regarded the beta spores as non-functional bodies because they resisted germination when conditions conducive to germination were provided (14).

Over 400 taxa of *Phomopsis* have been described (41). Many are pathogens of agricultural and horticultural crops. Wehmeyer (43, 44) collected considerable cultural evidence that linked *Phomopsis* with *Diaporthe*, which is now accepted as the perfect stage of *Phomopsis*. Wehmeyer recognized many species of *Diaporthe* to be weak parasites at best, while Phomopsis stages caused serious diseases on citrus fruits,

legumes, and potatoes (44). *Phomopsis* causes blights of azalea, blueberry, and juniper; stem cankers of gardenia, cottonwood, European black alder, and Russian olive; cane, leaf and berry spot of grape; and stem end rot of citrus fruits. *Phomopsis* is also commonly reported living as an endophyte or saprophyte on many conifers (4, 13, 14, 15, 35, 40).

Phomopsis Fruit Rots

Phomopsis vaccinii Shear causes a fruit rot of blueberry (*Vaccinium corymbosum* L.) (20). Milholland (20) observed that infections were obtained with artificial inoculations from the time the fruit was in the small green stage until it was ready for harvest; but rot symptoms were not seen until the fruit had matured.

In reviewing New York extension newsletters, Pscheidt and Pearson (32) found that in years with high levels of rainfall (>100 mm) during bloom, *P. viticola* (Sacc.) Sacc. was able to incite a fruit rot disease of grape (*Vitis* sp.). Pscheidt and Pearson (32) inoculated grape clusters at all stages of development and found that berries were most susceptible just after bloom. Inoculation of clusters at later stages did not show significantly more fruit rot than in the controls. However, as in Phomopsis fruit rot of blueberry, this disease does not manifest itself until the fruit has matured (32).

Phomopsis citri Fawcett is found in stem end rots of oranges grown in Florida (2). A similar *Phomopsis* is found in lemons grown in California (9). The California *Phomopsis* is much less virulent than the Florida *Phomopsis*. In California, *Phomopsis* was only found on 1) a few twigs

after careful searching in the orchard and 2) on old mature fruits in storage (9). *P. citri* infected Florida oranges only after the abscission layer formed, thereby creating a natural entrance for the fungus (2).

Blights, Cankers, and Diebacks Caused by Phomopsis

Although *Phomopsis* has been implicated in several fruit rots, most diseases caused by *Phomopsis* can be characterized as blights, cankers and diebacks. Literature concerning some of these diseases will be reviewed, followed by a review of Phomopses specifically on conifers.

Phomopsis vaccinii causes a serious canker and dieback disease that affects 20% of the highbush blueberries in Michigan (25). In North Carolina, Phomopsis twig blight reduces blueberry yield by 2-3 pints per bush (19). Conidia are released from pycnidia in lesions of infected blueberry bushes during the spring from flower budbreak through petal fall (19, 25). The blueberry in North Carolina is primarily infected where conidia fall on the flower buds. After infection the fungus enters the vascular system and can proceed 50-150 mm down the stem (19). Wilcox (46) found that when young plants were sprayed with spores, *P. vaccinii* entered at the tip of succulent new growth, rapidly grew downward, and finally girdled the second-year wood. The rapid downward progression averaged 5.5 cm. in 2 months. While succulent tissue was killed within five weeks after inoculation, woody tissue inoculated with spores or mycelium developed only small lesions. Wilcox (46) found that *P. vaccinii* was virulent on succulent shoots regardless of wounding. Parker (25) reported

that wounding was necessary to obtain an infection with mycelia or conidia in two year-old stems inoculated from spring to fall.

Phomopsis viticola causes a severe cane and leaf spot disease of grapevine during wet springs in California (7). Lake Erie region grape growers in New York saw greater disease development during a 1986 spring of heavy rains than in 1987 when pre-bloom conditions were dry (31). Until 1978, it was thought that *P. viticola* caused the "dead-arm" disease in grapes. Then it was proven that *Eutypa armeniacae* Hansf. and Carter caused the "dead-arm" disease and not *P. viticola* (21).

Gardenia cankers are caused by *Phomopsis gardeniae* Hansen and Barrett (30). *Phomopsis gardeniae* enters the gardenia through wounds inflicted during cultivation or insect feeding (30). For propagation purposes, commercial growers remove leaves on cuttings before sticking the cutting into the rooting medium (30). Cankers formed on rooted cuttings at wound sites created by leaf removal (30).

Phomopsis macrospora T. Kobayashi and Chiba causes a stem canker on poplar in Minnesota (11) and on cottonwood (*Populus deltoides*) in Mississippi (10). French and Bergdahl (11) reported a natural occurence of Phomopsis canker on 84% of nursery grown 'Robusta' poplar. Most of the diseased trees had basal cankers not aerial cankers. Although cankered trees were rarely killed, diseased trees could not be sold. Occasionally, infected plants were sold when lethal cankers were not evident. Trees that developed cankers during bare-root storage were a complete loss (11).

P. macrospora was one of three pathogenic fungi responsible for lethal cankers on young cottonwoods in Mississippi (10). Wound inoculations performed in September, 1963, on rooted cuttings resulted in girdling of 75% of the plants held in a greenhouse and 100% of those held in a lathhouse. Plants that were wound inoculated and held in a growth chamber did not die. This pathogen may not be serious unless the plants are stressed, but Filer (10) found no relationship between the level of bark moisture and the incidence of disease when bark turgidity was measured on 2 samples every 2 months. Filer (10) suggests that light, temperature and humidity strongly influence rate of disease development.

In Michigan, *Phomopsis elaegni* Arnold and Carter is responsible for most cankers found on Russian-olive (18). Maffei and Morton (18) usually found cankers at the base of epicormic branch clusters or at branch tips. Infections were always obtained when spores or mycelium were used to artificially wound inoculate Russian-olive seedlings. However, they found pathogenicity variable among different isolates used as inoculum.

In 1983, Oak and Dorset (22) first reported *Phomopsis alnea* (Sacc.) Hoehn. causing a canker on European black alder (*Alnus glutinosa*) in a field planted for seed production. The field began to decline within several months after planting. Basal cankers were found on all sides of the main stem. Preliminary inoculations with *Phomopsis* conidia on unwounded succulent stems did not result in infection. When water-stressed plants were wound inoculated, small localized cankers developed. The primary symptom was an inhibition of callus formation at wounds. Epidemic years

were attributed to poor planting sites, temperatures of 20-30 C and frequent rainfall in the fall prior to the epidemic.

Bedwell reported *Phomopsis* among other fungi occurring on Asiatic chestnuts planted at 112 sites in 22 states (1). Bedwell tested the pathogenicity of *Phomopsis* by wound inoculating twigs of two-year-old plants. The typical dieback and canker resulted only when plants were wounded and inoculated. Cankers were often found at the intersection of the twig and limb. Plants that were dormant or just breaking bud were most susceptible to wound inoculations.

Phomopsis Occurring on Conifers

For purposes of differentiation, Hahn extensively studied, in nature and in culture, eight species of *Phomopsis* occurring on conifers (13, 14, 15). Within species of *Phomopsis*, Hahn found pycnidial stromatic structure variable in culture, but found growth characteristics stable in culture (14). Size and shape of spores were consistent in nature and in culture. Characterization of spores can be relied on for species identification (14). However, there was some variability in the relative numbers of alpha and beta spores produced (14).

Hahn (13, 14) accounted for the presence of *Phomopsis* on many coniferous hosts: *Abies, Chamaecyparis, Cupressus, Juniperus, Larix, Picea, Pinus, Pseudotsuga, Sequoia, Taxodium,* and *Taxus.* The most well studied of the Phomopses occurring on conifers is *Phomopsis juniperovora* Hahn., a widespread virulent parasite which devastates Eastern red cedar,

Juniperus virginiana L.

Hahn (13) found that *P. juniperovora* could also parasitize other coniferous hosts as vigorously as it infected *J. virginiana*. Artificial wound inoculations were performed with *P. juniperovora* and resulted in canker formation and tip blight of the various host plants. Infections showed signs of expansion within five to tens days of inoculation of young stems; and quickly girdled small stems less than one-half inch in diameter. Growth was primarily in a longitudinal direction within the inner bark, killing the cambium, and staining the wood. Entire plants were killed when the fungus entered the main stem through a lateral. Older trees are rarely killed since only the smaller stems are girdled (28). Peterson and Hodges (28) explained that *P. juniperovora* infects new foliage and then spreads to the stem tissue. As the needles age, they become less susceptible to infection (26, 28). Pycnidia can form within 3 to 4 weeks and appear partially embedded in needles and stems (26, 28). Viable spores can be produced for up to two years after infected tissue has died (28).

High humidity and high temperatures enhance infection and disease development on junipers (26, 28). Growth of *P. juniperovora* in culture is optimum at 24-26 C (26). Schoeneweiss (37) reported severe disease development occurring in the cool, wet springs of 1966-1968 in Illinois. Epidemics have occurred with the use of overhead irrigation in fields with a few infected plants (13, 28).

When Hahn began his study of coniferous Phomopses (13), he observed that *P. juniperovora* and *Phomopsis occulta* Trav. were

morphologically very similar. Both were found on hosts belonging to Cupressaceae, although only the former was considered parasitic (13, 15). The alpha spores of the two species are very similar, but a significant difference was found in the spore length. The alpha spores of *P. occulta* are shorter (14). The straighter beta spore of *P. juniperovora* is distinguished from the hooked beta spore of *P. occulta* (14). The most obvious difference was illustrated when the two fungi were grown in culture. Bright orange crystals are formed in cultures of *P. juniperovora*, while cultures of *P. occulta* on the same medium are dull colored (15).

Hahn found *P. occulta* widely distributed in Europe and North America; and living on fourteen genera of conifers (14). In rare instances, Hahn associated the perfect stage of *P. occulta* with *Diaporthe conorum*. Hahn further demonstrated that cultures of monoascospore isolates of *D. conorum* gave rise to *P. occulta* (14). In turn, monopycnidiospore cultures gave rise to the ascogenous form (14). *D. conorum* is now accepted as the perfect stage of *P. occulta*. It is commonly found in Europe, but its presence in North America has not been substantiated (15).

After considerable study, Hahn (15) concluded that *P. occulta* is a saprophyte, or secondary organism, infecting host plants after injuries caused by frost, transplanting, drought, or after infection by other parasitic fungi. *P. occulta* has been found living saprophytically on all plant parts: cones, leaves, stems, and trunks (14). Sieber (40) isolated *P. occulta* from twigs of healthy and diseased Norway spruce (*Picea abies* (L.) Karst.) and white fir (*Abies alba* Mill.) at three sites in Switzerland. *P. occulta* was

found more frequently on diseased Norway spruce than on healthy Norway spruce at one site which was warmer and dryer. Sieber did not consider this coincidence as evidence for the pathogenicity of *P. occulta*. However, there was a report in 1927 of *P. occulta* causing a blight on Colorado blue spruce (*Picea pungens* Engelm.) that was similar to the disease of juniper caused by *P. juniperovora* (45). Even after this report, the organism was not considered a pathogen. In 1986, Sanderson and Worf (36) reported the pathogenicity of *P. occulta*, which caused a shoot blight of Colorado blue spruce in Wisconsin. Symptoms were observed on nursery plants and included downward curling of expanding shoots, browning of the needles and tips, and subsequent death of the tip or entire shoot. Sanderson and Worf (36) found small cankers and sap exudate on diseased branches. They also found pycnidia on dead shoots and dead needles.

Sanderson and Worf (36) sprayed a *P. occulta* spore suspension to inoculate healthy, one-year-old Colorado blue spruce seedlings. Symptoms appeared within 13 days after inoculation. Thirty percent of the plants became symptomatic. Greatest symptom development was achieved at a higher temperature (25 C) and higher humidities (75-90% RH). These researchers suggested that plants may be susceptible to infection for only a short time in the spring, when conditions are cool and relatively dry. Infection may occur without further symptom development, until the weather becomes warmer and more humid. This correlates well with the more commonly observed symptoms of disease during the warmer months of June and July (36).

Artificial inoculation of several coniferous hosts has proven the potential pathogenicity of *P. occulta* (36). Sanderson and Worf (36) obtained symptom development when *Picea abies*, *P. glauca*, and *P. obovata* as well as *P. pungens* were inoculated with spores of *P. occulta*. *P. pungens* showed much greater symptom severity than any of the other susceptible spruce investigated. *Abies balsamea* and *Abies concolor* were not susceptible.

Predisposing Factors and Latent Infections

In reviewing the literature on *Phomopsis* diseases, many studies have shown that the wounding or weakening of the host plant encourages disease development. Hahn (13) reported that transplanting, cultivating and pruning wounds predisposed plants to attack by *P. juniperovora*. In 1961, Raniere (34) attributed the sudden occurrence of extensive cane blighting and death of blueberry plants by Phomopsis canker and Botryosphaeria canker to low temperature injury. Damage predisposed "plants to invasion by various weak pathogens normally unable to cause any measurable injury to vigorous plants (34)." Brown (3) found that *Phomopsis* was a weak pathogen on oak and hickory trees and required wounds to enter the host plant. Wounding or injury as a consequence of drought, low temperatures, mechanical injuries, animal damage, or poor planting site was a prerequisite for the development of a parasitic relationship between *Phomopsis* and Asiatic chestnuts (1).

In such instances, the fungus may have penetrated the host without

the development of symptoms until the presence of predisposing elements. This condition is termed a latent infection (42). Fungi enter susceptible and resistant hosts with equal frequency (40, 39). After entry, disease development depends on the environmentally influenced, genetic response of the host to the presence of the organism (40). Predisposition refers to the environmental factors that affect the susceptibility of the host (40, 38).

Verhoeff (42) described latent infections of fungi in fruits that appeared during senescence. *Phomopsis* has produced latent infections in fruit of blueberry (20), grape (32), cranberry (6), and citrus fruits (2, 9). Symptoms of fruit rot did not appear until the fruit was wounded or began to senesce. *P. batatae,* Harter and Field, *P. phaseoli* (Desm.) Grove and *P. sojae* Lehm. were shown to infect seedlings of sweet potato (*Ipomoea batatas* (L.) Lam.) and 16 legumes when artificially inoculated (17). However symptoms did not appear until the onset of senescence. Cerkauskas and Sinclair (5) used paraquat to detect latent infections in soybeans. Formation of pycnidia was observed two weeks before pycnidia were seen on untreated controls. Pscheidt and Pearson were also able to utilize paraquat to aid in detection of *P. viticola* infections in grape vines and fruits (31, 32). Shoots 2.5 cm. long were inoculated but did not develop symptoms. An application of paraquat resulted in 78% of the shoots showing disease symptoms after 1 week (31).

Schoeneweiss (38) observed that canker and dieback diseases were more prevalent on plants subjected to environmental stress before symptom development. The organisms attacking environmentally stressed plants

were often nonaggressive pathogens or facultative parasites (38). The most common environmental stresses predisposing woody plants to disease are drought, freezing, and in nursery or landscape plants-- transplanting (38).

Botryosphaeria dothidea is considered a weak pathogen that invades peach trees through wounds or lenticels followed by a latent infection that can last from two weeks to many months (33). Pusey (33) drought stressed one-year-old potted peach trees. Plants that were inoculated and watered daily showed no sign of disease until 19 weeks after inoculation. Plants that were denied daily irrigation for six days out of an eight day cycle began to exude gum 5 weeks after inoculation. Plants showing different levels of water stress at the time of inoculation, showed no difference in disease development. When water stress was imposed 2-6 months after inoculation disease severity was significantly increased.

Colorado blue spruce is predisposed to Cytospora canker by drought (16, 39). Kamiri and Laemmlen saw the development of greater numbers of cankers, produced in a shorter time, in drought stressed trees than in the well watered controls (16). Schoeneweiss (39) reported that bark cankers appeared on wound-inoculated stems of 5-year-old spruce when plant water potentials fell to between -20 and -30 bars. In the same study, spruce were not predisposed to Cytospora canker by freezing temperatures between -20 and -30 C.

Control of *Phomopsis*

When English covered freshly pruned branch stubs of Kadota fig trees with plastic caps, Phomopsis canker was controlled by 60% (8). When 1% phenyl mercuric acetate or a Bordeau paste was painted onto pruning wounds, in addition to plastic caps, 75% control was attained (8). When pruning was performed later in the dormant season (April) cankering was reduced (8).

Cucuzza and Sall found sodium arsenite applied as a dormant treatment controlled Phomopsis cane and leaf spot on grapevines. Satisfactory control was also achieved with a dormant treatment of dinoseb and treatment with captan at 100% budbreak (7). Pscheidt and Pearson found that two applications of mancozeb during bloom, or two treatments with captan during shoot growth, significantly reduced fruit rot and rachis lesions on grapes (32). In another study, Pscheidt and Pearson found that hand pruning grape vines reduced the amount of disease compared to those vines that are hedged. Hedging creates more nodes and more dead branches, increasing the level of inoculum (31).

Phomopsis blight of juniper could easily be controlled by planting resistant plants once they are identified. Schoeneweiss found notable variation in resistance to *P. juniperovora* among species and among some cultivars within species of host plants (37). The progeny of 86 *J. virginiana* were evaluated for resistance to *P. juniperovora*. Progeny from twenty of the selected trees were found to have some degree of resistance (27).

Peterson and Hodges suggest avoiding 1) planting juniper seed next

Peterson and Hodges suggest avoiding 1) planting juniper seed next to beds containing juniper stock, 2) planting on poorly drained areas, 3) overhead irrigation that will not allow plants to dry before nightfall, 4) use of shade frames that can prolong wet periods 5) using junipers or other hosts as windbreaks (28). Chemical control of *P. juniperovora* has been achieved in several trials using benomyl to control (12, 23, 24). Otta and coworkers obtained in vitro control of *P. juniperovora* with benomyl which restricted growth and pycnidia formation. Chemical control in the field resulted in a significant decrease in disease severity on individual infected trees, the percent infected trees bearing pycnidia, the amount of diseased tissue bearing pycnidia, the percent pycnidia with spores, and the spread of infection from inoculum sources. Although benomyl did decrease the production of pycnidia and production of spores within the pycnidia, it did not inhibit germination of spores. Foliar applied benomyl was not redistributed systemically but roots translocated low levels of benomyl through out the plant. Successful recommendations were to "spray weekly with benomyl during the entire growing season and to rogue all plants with any dead foliage every 7-10 days (24)." Benomyl must be applied often enough to assure protection of new growth (29).

Summary

From a review of the literature, it is clear that some species of *Phomopsis* merit pathogen status since they cause many different symptoms on a wide range of host plants. *Phomopsis* species range in

Phomopsis seem to infect new succulent growth while woody tissue is less susceptible. Often disease is related to stress or wounding and latent infections are sustained for variable periods. *P. juniperovora* is considered a virulent pathogen causing serious losses, but control measures have been recommended. Little is known about the pathogenicity of *P. occulta*, which until recently was considered only a saprophyte. Little is known about the control of *P. occulta*.

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CHAPTER I

PATHOGENICITY, GROWTH, AND CONTROL OF *PHOMOPSIS OCCULTA* ASSOCIATED WITH THE POST-HARVEST DISORDER OF COLORADO BLUE SPRUCE (*PICEA PUNGENS GLAUCA* ENGELM.)

ABSTRACT

Phomopsis occulta Trav. was shown to be a causal agent of a post-harvest disorder that affects nursery grown Colorado blue spruce (*Picea pungens glauca* Engelm.). Symptoms of the post-harvest decline appear several weeks after fall harvest. Symptoms are characterized by needle loss primarily from the lower third of the plant and inconspicuous stem cankers occurring in all areas of the plant. This has been a serious concern for Michigan nursery growers who rouge thousands of symptomatic spruce each season. All samples collected from symptomatic harvested plants yielded cultures of *Phomopsis occulta*. Koch's postulates were satisfied by means of artificial inoculation of healthy Colorado blue spruce seedlings. Both mycelia and conidia of *P. occulta* were shown to cause symptoms similar to those symptoms on field-harvested spruce. Wounding and simulated harvest were significant factors in the development of lesions on plants inoculated with a mycelial slurry. Wounding was not required for the penetration of stem tissue by conidia. Cankers formed most rapidly on younger tissue regardless of the inoculum type.

Optimal growth of *P. occulta* in potato dextrose agar (PDA) cultures occurred at 25°C. When mycelial plugs were grown on fungicide amended PDA, benomyl stopped growth at all concentrations tested. Iprodione, chlorothalonil, and cupric hydroxide reduced growth of *P. occulta* only at

higher concentrations. Cupric hydroxide stimulated growth at lower concentrations. When fungicides were sprayed on naturally inoculated field plants, adequate control was not achieved with any of the fungicides. While these fungicides may be effective when used to protect healthy plants, they were not effective in controlling the development of cankers in infected harvested plants.

INTRODUCTION

In 1986, Michigan nursery growers noticed a post-harvest decline of Colorado blue spruce (*Picea pungens glauca*, Engelm.) trees of sizes ranging from 30-40 cm to 2-3 m in height. Plants that remained in the field appeared symptomless except for an occasional flagging branch. Affected plants displayed symptoms several weeks after harvest. Symptoms were characterized by needle loss concentrated on the lower part of the plant. Extreme cases resulted in plant death.

Since 1986, Michigan nursery growers have reported severe annual losses of harvested Colorado blue spruce. In one report, unmarketable plants resulted in a loss of 30% of the harvested crop. Additional losses occur because many affected trees require cosmetic pruning before sale, trees must be sold at a lower cost because of poor form, dead plants must be rogued out, warranteed plants must be replaced and the growers reputation is questioned.

Symptoms of this disorder appeared similar to those caused by a common canker-causing pathogen of mature Colorado blue spruce, *Cytospora kunzei* Sacc. var. *picea* Waterman, which is considered the most destructive disease of Colorado blue spruce in Michigan (4). Samples of spruce affected by the post-harvest disorder were submitted to the Michigan State University Plant Diagnostic Clinic. Samples yielded cultures of *Phomopsis* only.

The only previous study suggesting that *Phomopsis* is a pathogen of spruce was done in 1986 by Sanderson and Worf (7). By means of artificial spore inoculation, Sanderson and Worf found that *Phomopsis occulta* caused disease symptoms on four spruce species: *Picea abies*, *P. obovata*, *P. glauca* cv. 'Densata', and *P. pungens glauca*. Colorado blue spruce was the most susceptible of the four spruce in their research.

This study was initiated to determine whether *Phomopsis* is a pathogen of young spruce, determine the role of *Phomopsis* in the post-harvest disorder of Colorado blue spruce in Michigan, find the optimal temperature for growth of the suspect organism, and measure the effects of chemical controls on the growth of *Phomopsis*.

MATERIALS AND METHODS

Koch's Postulates were used to verify a causal relationship between *P. occulta* and the post-harvest disorder of Colorado blue spruce. Requirements include isolation of suspect organism from symptomatic plants, inoculation of healthy plants resulting in the reproduction of symptoms found on initially symptomatic plants, and resiolation of suspect organism from artificially inoculated plants.

Isolations. Samples were collected in 1988 and 1989 from nursery-grown Colorado blue spruce, harvested from a large Michigan nursery in the fall of each respective year. Fifty samples exhibiting die-back symptoms were collected each year from ten plants that displayed symptoms of post-harvest decline. Samples were prepared by rinsing under running tap water for several minutes, surface sterilizing for ten to fifteen minutes in 20% bleach (5.25% NaOCl), and then rinsing twice with sterile water. The outer bark was removed and 4 small sections of each shoot were taken from the margin of the canker and were plated directly on potato dextrose agar (PDA) in 90-mm-diameter Petri plates. PDA cultures were incubated at room temperature for seven days before evaluation.

Inoculum. Several isolates of *Phomopsis occulta* were obtained from the margins of expanding cankers of symptomatic Colorado blue spruce. Cultures were grown on PDA and incubated at room temperature for fourteen days. Two fourteen day old cultures of the same isolate were

randomly chosen and then macerated for two minutes in a commercial blender with 30 milliliters of sterile water to produce a mycelial inoculum. Sterile PDA inoculum was prepared in the same manner, macerating two Petri plates of sterile PDA.

Several cultures of each of five isolates were incubated under continuous fluorescent lighting to encourage pycnidia formation. Sixteen-day-old cultures were flooded with sterile water to obtain a conidial suspension. The water was allowed to sit for thirty minutes; the surface of the culture was scraped using a sterile teasing needle; and the suspension was filtered through four layers of cheesecloth. The concentration of alpha conidia was determined using a hemacytometer.

Pathogenicity. Six inoculation experiments were conducted in 1990 and were performed as completely randomized designs. Mycelial inoculum was used in the first four experiments and a conidial suspension was used as inoculum in the last two trials. Fresh inoculum was prepared for each experiment as previously described. Half of the plants in each treatment were selected for reisolation when the experiments were terminated. Samples were prepared for isolation and were cultured on PDA as described previously. Reisolation from symptomatic plants and comparison to the original culture was the last step in verifying Koch's Postulates.

One-year-old Colorado blue spruce plugs were potted in a peat-based, soilless commercial mix (Baccto, Michigan Peat Company) three months prior to inoculation. Seedlings were placed in a greenhouse with sixteen

hour supplemental fluorescent lighting and computer controlled day/night temperatures set at 24° C / 20° C. Seedlings were well established at the time of inoculation.

In the first trial, (started Jan. 8, 1990) 5 trees were wounded 10 cm above the soil line by pushing a sterile pin, 1 mm in diameter, through the stem. Needles were clipped off of the main stem within 1 cm surrounding the wound. A sterile hypodermic needle was used to inject inoculum into the wound until a small amount of inoculum was seen exuding from each side of the wound. Five control trees were wounded in the same manner, but received an injection of sterile PDA. All wounds were wrapped with a small strip of Parafilm. To simulate a harvest procedure, the root mass of half of the inoculated trees and half of the control trees was reduced by cutting away the lower six cm from a 10 cm long root plug. Treatments were arranged as a completely randomized design in a 2 X 2 factorial experiment. Trees were observed periodically and at the end of six months, lesion length was measured by scraping away the outer bark and measuring the length of the necrotic region.

A second trial was conducted (started Feb. 5, 1990) using the aforementioned treatments but two wounds were inflicted on each plant instead of one to investigate the susceptibility of tissues of different ages. The first wound was located 10 cm above the soil line and the second wound was located on the terminal leader, in the center of the most recent flush of growth. Both wounds on one plant were treated alike. Ten plants were inoculated with *Phomopsis*, and 10 were inoculated with sterile PDA. The

lesion length at each wound location was measured five months later.

In order to develop a successful inoculation technique, the third inoculation experiment (started Feb. 5, 1990) was conducted to determine which factors would be most effective in producing disease symptoms. Wounding, sealing the wound with Parafilm, and inoculating, at two locations on the plant were evaluated. These factors composed 16 treatments in a 4-way factorial experiment with ten replications in a completely randomized design. Each plant was wounded in a lower and an upper location as described previously. Lesion length was measured after a four month incubation time.

The final mycelial inoculation experiment (started Feb. 15, 1990) investigated three factors: inoculation, wounding, and simulated harvest. Plants were wounded only within the most recent flush of growth of the terminal leader. Five plants served as replicates in each of eight treatments in a 2 X 2 X 2 factorial experiment. Lesion length was measured three months after inoculation.

Two techniques of wounding were used to determine if wounds were required for conidial penetration (started Feb. 15, 1990). Ten plants were wounded by cutting needles and 10 plants were wounded by pruning branches. Ten control plants remained unwounded. Five branches on each of ten plants were wounded by pruning three cm from the tips of branches. Another set of ten trees was injured by cutting needles in half on five branches per tree. A conidial suspension of 1.74×10^4 conidia per milliliter, was sprayed on half of the trees with an atomizer until small

droplets formed and fell from branches. Control plants were sprayed with sterile water to the point of runoff. Each plant was covered with a clear polyethylene bag for 72 hours. Plants were periodically observed for the development of symptoms. The number of cankered branches was recorded four months after inoculation. Data were analyzed as a completely randomized 2-way AOV with 10 plants per treatment.

One month later (March 13, 1990) another inoculation trial was initiated, using a spore concentration of approximately 1.47 X 10^6 conidia per milliliter. Spores were brushed directly on three branches per tree. Control trees were brushed with sterile water. In a third treatment, the spore suspension was injected through syringe needle wounds into three different stems per tree, four cm from the branch tips. Control trees were injected with sterile water. There were twenty plants in each treatment, and three branches treated per plant. All plants were covered with clear polyethylene bags for 72 hours. Plants were evaluated when the experiment was terminated three months later.

Effect of temperature on growth. The radial growth of one isolate of *Phomopsis occulta* was measured at the following temperatures: 0, 5, 10, 15, 20, 25, and 30 °C. Mycelial plugs, 10 mm in diameter, taken from the margin of 10-day-old cultures were placed in the center of 90-mm-diameter Petri plates containing PDA. Plates had been incubated over-night at the assigned temperature prior to inoculation. Twenty replicate plates were incubated in the dark at each temperature. Radial growth was measured every 24 hours.

In-vitro fungicide screening. The poison agar technique was used to evaluate four fungicides: benomyl (Benlate: 50% methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, Dupont Agrichemicals), iprodione (Chipco 26019: 23.3% 3-(3,5-dichlorophenyl)-N- (1-methylethyl)-2,4-dioxo-1-imidazolidine-carboxamide, Rhone-Poulenc Inc.), chlorothalonil (Daconil 2787: 40.4% tetracholorisophthalonitrile, Fermenta, Plant Protection), and cupric hydroxide (Kocide 606: Kennecott Copper Corporation). Fungicides were added to warm PDA for final concentrations of 1, 10, 100, and 1000 ppm active ingredient. Fungicide amended PDA, in 90-mm-diameter Petri plates were inoculated with 10-mm mycelial plugs cut from the margin of 10-day-old cultures. Twenty PDA plates were prepared for each concentration of each fungicide, and 20 unamended plates were used as controls. Radial growth was measured every 24 hours.

Field evaluation of fungicides. The same fungicides used in the *in vitro* study were sprayed on a field of 38-45 cm Colorado blue spruce trees located in a large commercial nursery. A nursery worker separately mixed, 454 g of Benlate, 454 g of Chipco, 1 liter Daconil, and 2 liters Kocide with 379 liters of water each to cover 46.45 m^2. Sprays were applied to one field divided into beds. Each bed contained three rows of trees spaced 50 cm apart with trees in a row spaced 45 cm on center. Treated beds were separated by two unsprayed beds in order to act as a buffer in case of fungicide drift. One bed of control plants did not receive a fungicide application. Three fungicide sprays were applied by a trained nursery applicator in 1989 on June 24, July 24, and August 30.

Ten plants from each treatment were randomly selected by the researcher, hand dug and placed in pots by nursery workers in November, 1989. Plants were immediately transported to Michigan State University, Horticulture Teaching and Research Center in East Lansing, Michigan. Plants were placed in unheated quonset style houses covered with 4-mil white polyethylene and stored for the winter. Plants were periodically monitored until final evaluation July 1, 1990.

RESULTS AND DISCUSSION

Isolations. All samples collected in 1989 and 1990 from symptomatic Colorado blue spruce yielded cultures of *P. occulta* and rarely any other fungus. Pycnidia were formed in PDA cultures within 10 days, when isolates were incubated at room temperature under continuous fluorescent lighting. Within fourteen days, pycnidia were exuding tendrils of alpha and beta conidia typical of *Phomopsis* species. The identification of *Phomopsis occulta* was confirmed by Dr. David L. Roberts and Dr. Alvin Rogers of the Department of Botany and Plant Pathology, Michigan State University. Identification was based on conidia size and morphology of conidia.

Until recently, *P. occulta* was only considered a common saprophyte found growing on many conifers (2, 3, 11). Nearly all stress-related diseases are caused by organisms that usually grow as saprophytes on the host plant (8). Suspicions of the pathogenicity of *P. occulta* arose in 1929, when White published a New Jersey report of stressed *P. pungens* with blighted tips from which *P. occulta* was isolated (12). In 1986, firm evidence of the potential of *P. occulta* to cause disease was reported by Sanderson and Worf who artificially inoculated four species of spruce and two species of fir with conidia of *P. occulta* (7). The inoculum was isolated from Colorado blue spruce that were found growing in nurseries and landscapes in Wisconsin. Suspect plants were showing necrosis and death of new

growth. This was the first report of *P. occulta* causing disease on Colorado blue spruce in Wisconsin. There are no previous reports of *P. occulta* causing disease on spruce grown in Michigan, hence this is the first report of Phomopsis shoot blight on Colorado blue spruce in Michigan.

Pathogenicity. Inoculated plants in all pathogenicity trials were significantly more symptomatic than control plants (Tables 1.1-1.7). Plants that were inoculated with mycelia 10 cm above the soil line on the main stem in the first pathogenicity experiment were slow to show any symptoms. After six months, the mean lesion length of inoculated plants was significantly longer than the lesion length in control plants (Table 1.1). Wounding caused by piercing the main stem with a 1 mm pin caused some splitting of the stem. Wounded stems inoculated with sterile agar slowly closed while seedlings inoculated with *P. occulta* showed an inhibited callus formation in wounded zones when compared with control plants. The wound of inoculated plants was characterized by an open lesion with inconspicuous necrotic tissue extending beyond the open lesion. The extended canker was measured by carefully scraping away the outer bark with a razor blade and then measuring the length of the necrotic zone.

In experiment one, removing approximately 60% of the root mass of plants had a significant effect on the mean lesion length when plants were also inoculated (Table 1.1). Lesion length in plants that were inoculated with sterile agar was unaffected by root reduction. After six months the greatest lesion length achieved was 14.4 mm, but none of the plants showed

Table 1.1. *Pathogenicity Experiment One.* The effect of inoculation and reduction of 10 cm root plugs to 4 cm on the development of lesions in one-year-old wound-inoculated Colorado blue spruce seedlings, six months after inoculation.

Inoculum		Mean Lesion Length (mm) Roots Intact	Roots Trimmed
Sterile PDA		3.7	6.0
P. occulta		8.2	14.4
Source of variation			
Inoculation (I)	**		
Root treatment (Rt)	**		
I X Rt	*		

*,** Significant at P=0.05, 0.01 respectively, according to F test.

Table 1.2. *Pathogenicity Experiment Two.* The effect of inoculation and reduction of 10 cm root plugs to four cm on the lesion length (mm) at two wound locations on one-year-old Colorado blue spruce seedlings after five months incubation.

	Mean Lesion Length (mm)			
	Wound Loc. One[x]		Wound Loc. Two	
Inoculum	Roots Intact	Roots Trimmed	Roots Intact	Roots Trimmed
Sterile PDA	4.3	4.2	2.8	4.4
Phomopsis occulta	8.0	32.2	53.3	64.3

Source of variation	
Inoculum (I)	**
Root treatment (Rt)	NS
I X Rt	NS
Location (L)	**
I X L	**
Rt X L	NS
I X Rt X L	NS

[x] Each tree was wounded 10 cm above the soil line, identified as wound location one, and in the center of the most recent flush of terminal leader growth, identified as wound location two.

NS,** Nonsignificant or significant at P = 0.01, respectively, according to the F test.

Table 1.3. *Pathogenicity Experiment Two.* Symptom development on one-year-old Colorado blue spruce seedlings one month after wound-inoculating and reducing 10 cm root plugs to 4 cm or leaving roots intact.

Inoculum	No. Trees With Blighted Terminal Leaders[x]
Sterile PDA	
Roots intact	0
Roots trimmed	0
Phomopsis occulta	
Roots intact	5*
Roots trimmed	9*

[x] A total of ten plants per treatment were each inoculated on the main stem 10 cm above the soil line and in the center of the terminal leader.

*Values marked with an asterisk are significantly different from each other and from the control treatments at P = 0.05 according to the Chi-square analysis.

Table 1.4. *Pathogenicity Experiment Three.* Efficacy of wounding, inoculating, and sealing wound with Parafilm, in the production of lesions at two locations on one-year-old Colorado blue spruce, after four months incubation.

Inoculum	Mean Lesion Length(mm)			
	Wound Inoculated		Surface Inoculated	
	Parafilm	Exposed	Parafilm	Exposed
Sterile agar				
Wound location one[x]	3.3	3.4	0	0
Wound location two	3.8	4.4	0	0
Phomopsis occulta				
Wound location one	9.0	13.7	0	0
Wound location two	42.9	66.2	0	0

Source of variation	
Inoculum (I)	**
Wounding (W)	**
I X W	**
Parafilm (Pf)	NS
I X Pf	NS
W X Pf	*
I X W X Pf	*
Location (L)	**
I X L	**
W X L	**
I X W X L	**
Pf X L	NS
I X Pf X L	NS
W X Pf X L	NS
I X W X Pf X L	**

[x] Each of ten plants per treatment was wounded in two locations. Each wound per plant was treated in the same manner. Wound location one is defined as the wound inflicted on the main stem 10 cm above the soil line. Wound location two is the wound located in the center of the terminal leader.

NS, *, ** Nonsignificant or significant at P = 0.05, 0.01, respectively, according to F test.

Table 1.5. *Pathogenicity Experiment Four.* Mean lesion length measured three months after Colorado blue spruce seedlings were inoculated within the center of the terminal leader when roots were left intact or when 10 cm root plugs were trimmed to four cm.

Inoculum	Mean Lesion Length (mm) Wound Inoculated	Surface Inoculated
Sterile PDA		
Roots intact	2.4	0.0
Roots trimmed	2.8	0.0
Phomopsis occulta		
Roots intact	21.8	7.6
Roots trimmed	32.6	0.0

Source of variation	
Inoculum (I)	**
Wounding (W)	**
I X W	**
Root treatment (Rt)	NS
I X Rt	NS
W X Rt	NS
I X W X Rt	NS

NS,** Nonsignificant or significant at P = 0.01, respectively, according to F test.

Table 1.6. *Pathogenicity Experiment Five.* Mean number of shoots blighted per tree on injured one-year-old Colorado blue spruce seedlings sprayed with 1.74 X 10^4 conidia per milliliter of *Phomopsis occulta*, four months after inoculation.

	Mean Number of Blighted Shoots		
Inoculum	Stem Injury	Needle Injury	No Injury
Sterile water	0.0	1.8	0.0
Phomopsis occulta	4.7	7.1	8.8
Source of variation			
Inoculum (I)	**		
Wounding (W)	NS		
I X W	NS		

NS,** Nonsignificant and significant at P = 0.01 respectively, according to F test.

Table 1.7. *Pathogenicity Experiment Six.* Mean number of blighted shoots on one-year-old Colorado blue spruce seedlings, with tips that were wound-inoculated or surface-inoculated with a concentrated conidial suspension (1.47 X 10^6 conidia per milliliter), three months after inoculation.

Inoculum	Mean Number of Blighted Shoots	
	Wound Inoculated	Surface Inoculated
Sterile water	0.4	0.0
Phomopsis occulta	2.9	4.2

Source of variation	
Inoculum (I)	**
Wounding (W)	NS
I X W	*

NS,*, ** Nonsignificant or significant at P = 0.05, or 0.01 respectively, according to F test.

symptoms of needle loss or tip death, in fact all the plants produced new growth.

When plants were wounded in two locations, lesions that developed on younger tissue were significantly longer than lesions that developed on older stems (Table 1.2). Location of wound was not a significant factor in the development of lesions when plants were inoculated with sterile agar. This indicates that younger tissue may be more susceptible to colonization by *P. occulta* than older stems.

The effect of root reduction on lesion length in this experiment was only significant when comparing the lesions that developed at the older region of wound-inoculated plants (Table 1.2). Lesions in plants with reduced root systems were much longer than lesions in plants without root disturbance. Although the effect of root reduction on lesion length in inoculated terminal leaders was not significant, 90% of the plants that were inoculated and had roots trimmed developed girdling cankers that killed terminal leaders within one month after inoculating (Table 1.3). Only 50% of plants that were wound-inoculated without root treatment developed lethal girdling cankers. This difference was significant according to the Chi-square analysis at the 5% confidence level. None of the control plants developed cankers. Growth of cankers was primarily longitudinal, but when roots were trimmed, small, young stems were quickly girdled.

Pathogens that cause stem cankers are mostly nonaggressive pathogens that only attack weakened or wounded hosts (8). Host vigor is reduced by stresses such as drought, flooding, freezing, defoliation, and

transplanting. Cutting plant roots reduces stored food reserves, and reduces the absorptive surface area for taking up water and nutrients (9). Transplanting will induce stress in most plants until an adequate root system can be reestablished (8). The experimental simulation of harvest stress in Colorado blue spruce in the previously discussed experiments has shown that stressed plants were more symptomatic than nonstressed plants. Drought stressed Colorado blue spruce have also been shown to be more susceptible to Cytospora canker, caused by *Cytospora kunzei* Sacc. var. *picea* Waterman, when artificially wound-inoculated (4, 10).

When all permutations of inoculating, wounding, and sealing with Parafilm, were evaluated as techniques for creating disease symptoms at two wound sites, all factors were found to be dependent on one another (Table 1.4). Parafilm was found to have a significant effect only when plants were also wound-inoculated. When wounds were wrapped with Parafilm, the mean lesion length was shorter than lesion length at wound zones without Parafilm. The moist wound environment maintained by a Parafilm wrap could have either inhibited growth of the pathogen, encouraged growth of competitor organisms, or enhanced compartmentalization efforts by the plant.

The greatest mean lesion length, 66.2 mm, was achieved by leaving the wound exposed (not wrapped with Parafilm) and by wound-inoculating the younger region of the main stem. As demonstrated in an earlier experiment, (experiment two), the newer tissue was more susceptible to colonization than older tissue. Half of the plants that were wound

inoculated developed girdling cankers on the terminals. In the absence of wounding, no lesions developed and the mycelial inoculum was unable to penetrate even the newest most succulent tissue. The surface inoculum was still viable after four months of inoculating as indicated by reisolation from the surface.

The role of wounding was further demonstrated when only one wound per plant was inflicted on the terminal leader (Table 1.5). Plants that were wounded and inoculated developed the longest lesions. When plants were unwounded no lesion developed except when a mean lesion length of 7.6 mm was obtained when plants were surface inoculated while roots remained intact. This large value can be explained by accidental wounds that were inflicted on several plants when needles were clipped from the terminal leader at the inoculation point. This was done to facilitate the wrapping of the wound with Parafilm. The new growth was very tender and easily injured by scissors that slightly pulled needles from the stem.

No terminal leader was girdled in any of the treatments in this fourth pathogenicity experiment. Of the four experiments discussed previously, the only treatments that caused girdled terminal leaders were those that involved two wounds on the main stem. When two wounds were inflicted and the roots remained intact, 15 out of 30 plants developed girdling cankers (experiment two and three). When roots were trimmed, 9 out of 10 plants developed girdling cankers (experiment two). Although comparisons between experiments are of limited value because

experiments were performed at different times with different isolates, plants that were wounded in two locations developed girdling cankers on the terminal leaders while those plants wounded only in an upper or lower location did not, regardless of root pruning. The lower wound on the main stem may have been stressing the terminal leader tissue and predisposing it to colonization. Root pruning would further aggravate this condition.

P. occulta was successfully reisolated from all samples of discolored lesion tissue that had been originally wound-inoculated with the same fungus. Cankers extended primarily longitudinally, although some staining of the xylem tissue was evident within the first 1 mm of stem tissue. *P. occulta* was isolated from discolored xylem tissue.

In the previously discussed experiments, *P. occulta* mycelia was shown to be infectious, causing small cankers that developed slowly. The conidial suspension was shown to be even more infectious (Table 1.6, 1.7). When plants were sprayed with a 1.74 X 10^4 conidia per milliliter suspension, new shoots had very small purplish cankers on the tips within 10 days of inoculation. After three weeks, cankers were barely visible as purple-bronze streaks extending backwards from the tips. Ten uninjured plants sprayed with the conidial inoculum developed an average of 8.8 shoots blighted per tree after four months. An average tree contained only 15 tips per tree (n=140). Wounding did not produce significantly more shoots blighted per tree as might be expected if wounding aided penetration.

Plants were successfully inoculated when concentrated amounts of conidia were brushed directly on the surface of the most susceptible tissue

or injected into syringe wounds (Table 1.7). The main effect of wounding was not a significant factor in symptom development. However, the interaction between inoculating and wounding was significant. When unwounded plants were inoculated with conidia, 4.2 blighted shoots developed per tree while those that were injected with conidia developed only 2.9 blighted shoots per tree. Since only three branches were treated per tree some branches on trees that were surface inoculated were either contaminated by unnoticed dripping when applying spores with a paintbrush or developed natural infections. Injection of conidia with a syringe was a cleaner technique that left a smaller volume of the conidial suspension on each plant. Two plants that received injections of sterile water were also contaminated by *P. occulta*. *P. occulta* was recovered from the few dead shoots on these control plants and from all shoots that were symptomatic.

Wounding is necessary for the infection of shoots by mycelia but not for the infection by spores. In nurseries, open wounds are commonly created by pruning practices. Open wounds were experimentally shown to be more susceptible to canker development than those wounds covered by Parafilm. If Colorado blue spruce are pruned when highly susceptible new growth is present, the plants are at risk of infection by *P. occulta* mycelia growing saprophytically on the stem. Also, it may be possible for pruned shoots to become inoculated by contaminated pruning tools. Furthermore, pruning plants would increase the quantity of new growth, thereby greatly increasing the number of potential infection sites for conidia. If pruning is

to be done, waiting until growth has matured and hardened would provide fewer chances for infection.

Effect of temperature on growth. Optimal growth of *P. occulta* occurred in cultures incubated at 25° C (Figure 1.1). Moderate growth was observed at 15° C, 20° C and 30° C. No growth occurred in cultures incubated at 0° C and 5° C after 30 days. These low temperatures were not lethal. When cultures were brought to room temperature, growth resumed.

If growth of *P. occulta* within host plants is also optimal at 25° C, symptom development would be expected to be greatest during warmer months. Sanderson and Worf observed greater symptom development on artificially inoculated Colorado blue spruce when plants were incubated at around 25° C and at a relatively high humidity (7). They suggest that plants may be susceptible to infection for a short time in the spring but that further symptom development does not occur until the weather becomes warmer and more humid.

In-vitro fungicide screening. Benlate was the only fungicide that showed complete control of the fungus at all concentrations (Figure 1.2). The mycelial plug was killed by Benlate concentrations of 10, 100, and 1000 ppm active ingredient. Chipco and Daconil provided good control at 10 and 100 ppm a.i., and complete control at 1000 ppm a.i. Kocide controlled growth at 1000 ppm a.i. but gave no control at 1, 10, and 100 ppm a.i. Kocide slightly encouraged growth of *P. occulta* at lower concentrations when compared to control cultures.

Figure 1.1. Effect of temperature on the radial mycelial growth of one isolate of *Phomopsis occulta*. Measurements were made ten days after PDA plates were inoculated with 10-mm-diameter disks of mycelia. Each treatment was replicated 20 times. The LSD (P=0.05) for comparing means is 1.85 mm.

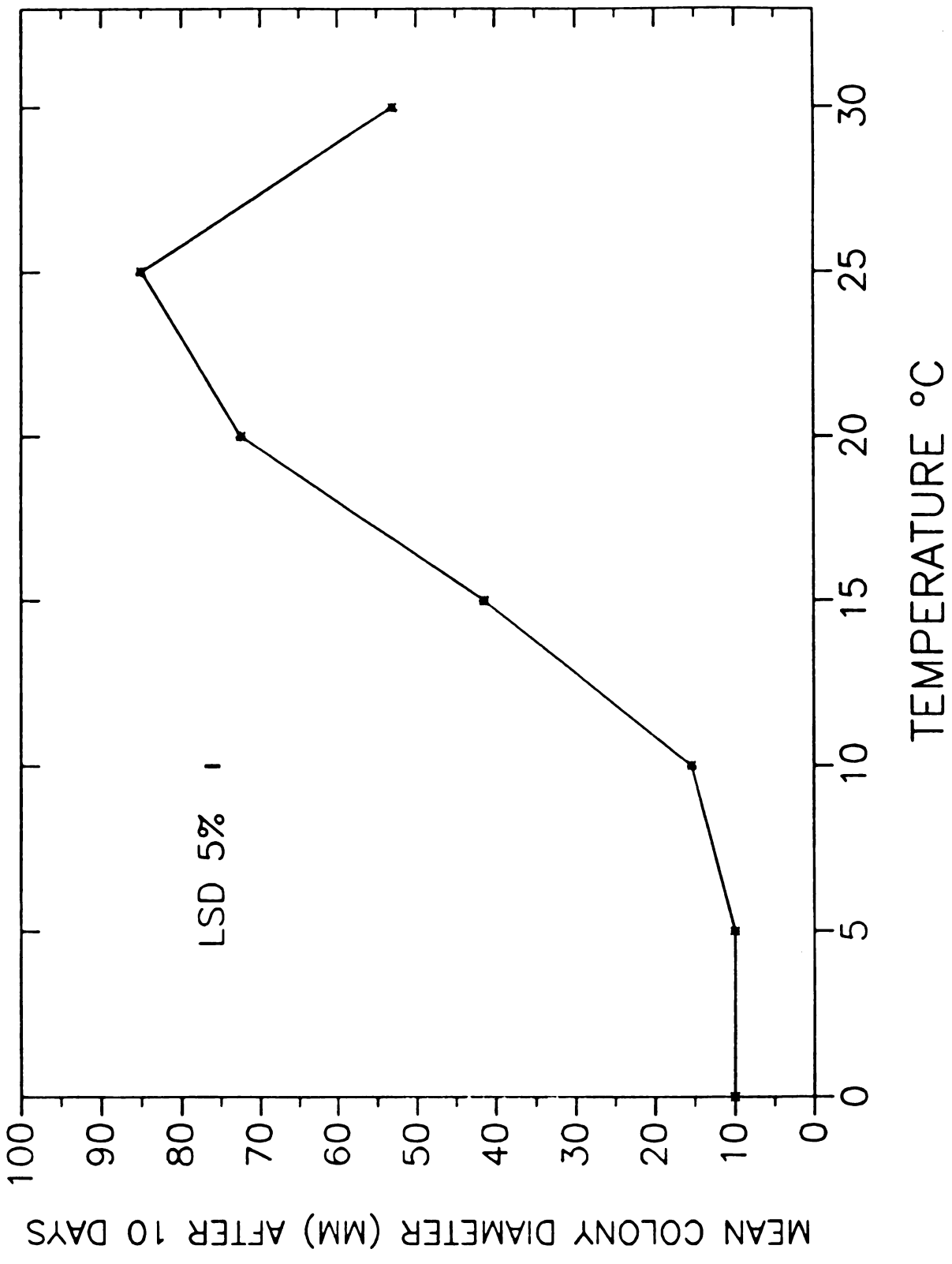
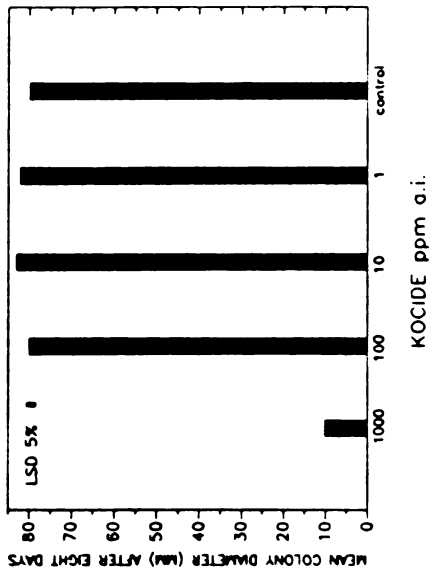
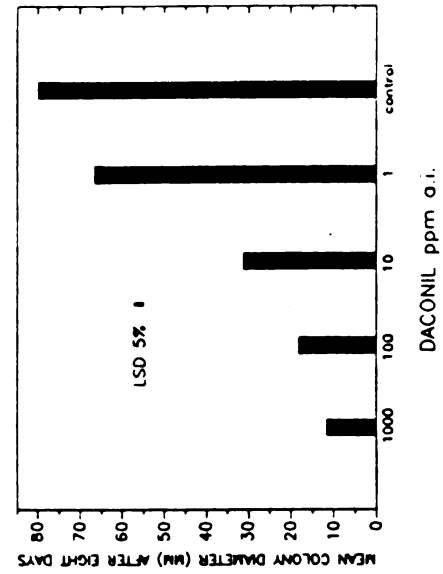
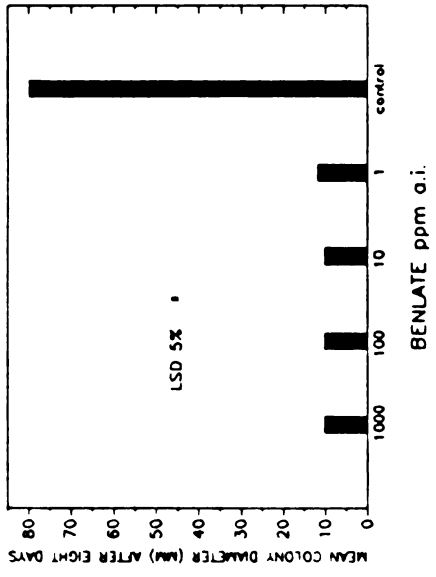
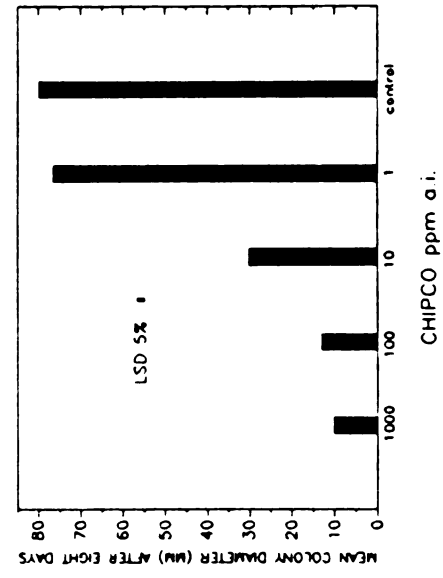

MEAN COLONY DIAMETER (MM) AFTER 10 DAYS
100
90
80
70
60
50
40
30
20
10
0
LSD 5%
0
5
10
15
20
25
30
TEMPERATURE °C

Figure 1.2. Effect of four fungicides on the radial mycelial growth of *Phomopsis occulta* growing in fungicide amended PDA cultures incubated at room temperature for eight days. *Significantly different from the control at P = 0.05 according to Tukey's HSD. Each treatment was replicated 20 times. The LSD (P=0.05) for comparing means is Benlate= 1.3, Chipco= 1.6, Daconil= 2.0, Kocide=2.1.


MEAN COLONY DIAMETER (MM) AFTER EIGHT DAYS
0
10
20
30
40
50
60
70
80
LSD 5%
1000
100
10
1
control
KOCIDE ppm a.i.


MEAN COLONY DIAMETER (MM) AFTER EIGHT DAYS
0
10
20
30
40
50
60
70
80
LSD 5%
1000
100
10
1
control
DACONIL ppm a.i.


MEAN COLONY DIAMETER (MM) AFTER EIGHT DAYS
0
10
20
30
40
50
60
70
80
LSD 5%
1000
100
10
1
control
BENLATE ppm a.i.


MEAN COLONY DIAMETER (MM) AFTER EIGHT DAYS
0
10
20
30
40
50
60
70
80
LSD 5%
1000
100
10
1
control
CHIPCO ppm a.i.

Field test of fungicides. When the same fungicides were tested on a nursery plot of 38-45 cm Colorado blue spruce known to have infections of *P. occulta*, no fungicides provided any control of the expansion of stem cankers when compared to unsprayed control plants (Table 1.8). Symptom severity was greatest on plants that were treated with Kocide. On a scale of one to five, with five equating a dead plant, Kocide treated plants had a mean rating of 4.7 which was significantly worse than the rating of plants that received no fungicide treatment. Although only ten plants were evaluated, Kocide may actually have a stimulating effect on the growth of *P. occulta* as suggested by the results of the in-vitro screening.

Even though this field test of fungicides was unsuccessful, the potential for using chemical controls needs further evaluation. Test plants in the nursery plot were known to be naturally infected by *P. occulta.* Chemical controls, even systemic fungicides, are not likely to be effective on plants that are already infected (8). Timing of fungicide application would also be important since plants are most susceptible during the short, spring period of shoot growth. Fungicides applied to field plants in this experiment were applied after shoot growth had ceased. The best means of controlling a stress-related pathogen is to maintain plant vigor by implementing a proper pruning, watering and fertilization program (9).

Table 1.8. Mean symptom severity on 15-18 inch Colorado blue spruce, field grown in a commercial nursery and known to be naturally inoculated with *Phomopsis occulta*, when treated with a fungicide in the summer of 1989 and evaluated eight months after hand-digging.

Treatment[x]	Symptom Severity[y]
Benlate	3.5
Chipco 26019	4.3
Daconil 2787	3.5
Kocide 606	4.7*
None	3.4

[x]All fungicides were applied on June 24, July 24, and August 30, 1989, at the rate of 1 lb Benlate/100 gallons water, 1 lb Chipco/100 gallons water, 2 pints Daconil/100 gallons of water, and 4 pints Kocide/100 gallons water to cover 46.45 m^2 (500 ft^2).

[y]1=no symptoms of disease, 2=less than one-third of the plant surface exhibiting symptoms of needle loss or discoloration, 3=more than one-third, but less than two -thirds of the plant surface showing signs of disease, 4=more than two-thirds of the plants showing symptoms of needle loss, needle discoloration, extensive cankering, and death of shoots, 5=entire plant killed.

*Significantly different from the unsprayed control at P = 0.05 according to Tukey's HSD.

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CHAPTER II

INFLUENCE OF HARVEST DATE AND FERTILIZER RATE ON THE SEVERITY OF THE SHOOT BLIGHT DISEASE OF HARVESTED COLORADO BLUE SPRUCE (*PICEA PUNGENS GLAUCA* ENGELM.)

ABSTRACT

Michigan grown Colorado blue spruce (*Picea pungens glauca*, Engelm.) are susceptible to infection by *Phomopsis occulta* Trav. Latent infections present at the time of harvest are manifested several weeks after harvest. Needles were initially lost from the lower third of the plant, but as cankers rapidly expanded with warmer temperatures, total plant death occurred in some cases. Fertilizer rate and harvest date studies showed that harvest date significantly influenced the post-harvest symptom development in 1988 and 1989, while fertilizer rates increased the quality of specimens only in the second year of the study. In 1988, a drought year, plants that were harvested in August were less symptomatic than plants harvested in September, October, or November. In 1989, a more normal year, August-harvested plants were the most symptomatic. Those harvested in October were the least symptomatic. Plants that were fertilized had higher foliar nitrogen content than those plants that were not fertilized. Fertility rate made no difference in the length of terminal leader growth. However, spring growth of undisturbed plants was 2-3 times that of plants harvested the previous fall. In 1989, plants with the highest foliar nitrogen content, were less symptomatic than trees that had received no fertilizer in two years.

INTRODUCTION

In some Michigan nurseries, large losses of field grown Colorado blue spruce (*Picea pungens glauca* Engelm.) occur several weeks after trees are harvested. This post-harvest decline has been associated with infections of *Phomopsis occulta* Trav. (Chapter one). *Phomopsis occulta* was long regarded as only a saprophyte (6, 7) until 1986 when Sanderson and Worf gathered evidence of its pathogenicity to spruce in Wisconsin nurseries and landscapes (13). In Wisconsin, the Phomopsis disease was characterized by the wilting appearance of new shoots, browning of tips, and the presence of cankers and pycnidia on dead shoots.

Many saprophytic fungi are nonaggressive pathogens that cause diebacks, cankers, and declines but only attack weakened plants (14, 15). The development of disease symptoms is dependent on the relative aggressiveness of the pathogen and the vigor of the host. Vigor of nursery grown plants is reduced by transplanting procedures that cause drastic reductions in tree root systems. Common nursery transplanting techniques can reduce a tree's root system by up to 98 percent which leads to water stress within the plant (25). Drought stress is the major factor in the predisposition of Colorado blue spruce to Cytospora canker (*Cytospora kunzei* Sacc. var. *picea* Waterman), the most destructive canker causing fungus of Colorado blue spruce in Michigan (8, 16).

Rapid regeneration of roots is critical to the reestablishment of

transplanted trees. Some difficult to transplant species may not rapidly recover from transplanting stress. In a study where 3-year-old Corsican pine and Atlas Cedar seedlings were transplanted in October and watered regularly, plants remained water stressed for a minimum period of 218 days and 190 days respectively (9). The length of time needed for the regeneration of the original root system is much longer for larger trees than for seedlings (24).

The time of year that trees are transplanted will influence the transpirational water loss, desiccation resistance and the root regeneration potential. Root regeneration potential of some deciduous species is reduced during spring growth when reserve carbohydrates are low (25). A similar trend has been found in coniferous species (1, 2). Growth and survival of outplanted loblolly pine seedlings, was positively correlated with the root growth potential (RGP) at the time of lifting (11, 12). However when seedlings are planted in low temperature soil, the correlation between survival and RGP may be low (4). When loblolly pine seedlings were planted in colder soils, the correlation between RGP and survival was low and the correlation between root/shoot ratio and survival was high. Dewald and Feret concluded that root regeneration of loblolly pine seedlings was more closely related to shoot phenology and to a lesser extent seasonal changes in the environment (2). Five year-old Colorado blue spruce lifted at various times of the year and held under greenhouse conditions for one month had more new roots when seedlings were lifted in October or March (1).

Plant nutrition is an important factor in maintaining plant vigor. Nitrogen fertilization can positively influence the root growth and subsequent shoot growth of transplanted coniferous seedlings (11, 12, 20, 21, 22). Outplanting success is indirectly influenced by nitrogen fertilization rate through its affect on shoot growth (11, 20). In Canada, N fertilization increased survival of coastal Douglas-fir and Sitka spruce slightly but decreased survival of presumably stressed interior Douglas-fir (21).

Optimal nitrogen fertilization can also increase the host plant vigor and tolerance of disease. Many agricultural crops become more susceptible to disease under high N-fertilization regimes but because of increased plant vigor, yields are increased (5). Either excess or insufficient fertilization often leads to reduced vigor and disease of forest plants (3). High levels of nitrogen often increase the susceptibility of seedling conifers to fungal disease (23). However, low vigor conifer seedlings grown on nutrient deficient soils developed more infections of *Armillaria mellea* and had lower survival than those grown on nutrient sufficient soils (17).

In this study, four fall harvest dates and a range of fertilizer rates were evaluated in a factorial experiment to determine the effect on the development of post-harvest disease symptoms on field grown Colorado blue spruce infected with *Phomopsis occulta*.

MATERIALS AND METHODS

One uniform field of Colorado blue spruce in a large commercial nursery in Ottawa County, Michigan was the source of all plants in this study. Trees were grown on a dark, loamy sand with 4.6% organic matter. Soil was drained by means of open ditches. Five fertilizer treatments were randomly assigned to each of five blocks. Nitrogen in the form of urea was applied in row bands in 1988 on May 17 and July 27 and in 1989 on May 11 and June 9. Three rows spaced 50 cm apart with trees spaced 45 cm on center were assigned one of the following fertilizer applications: (1) 100 lb N per acre in spring, (2) 200 lb N per acre in spring, (3) 300 lb N per acre in spring, (4) 100 lb N per acre applied in spring and 100 lb N per acre applied in the summer, (5) no fertilizer.

In the fall of 1988, four 30-38 cm trees were hand-dug and potted from each fertilizer treatment in each block during the last week of August, September, October and November. In 1989, 38-45 cm trees were harvested during the last week of the month as in 1988. One hundred trees were transported each month, immediately after harvest, to the Michigan State University, Horticulture Teaching and Research Center in East Lansing, Michigan. Trees were placed in quonset style houses in a randomized block design similar to the blocking system used in the field. Trees were stored over winter in unheated quonset houses which were covered with 4-mil white polyethylene on August 31, 1988 and November 9, 1989. Polyethylene

covers were removed on May 10, 1989 and May 23, 1990. Harvested trees were periodically observed for the development of symptoms. On the first day of every month, plants were evaluated on a visual scale of 1-5 where 1=no symptoms of disease, 2=less than one-third of the plant surface exhibiting symptoms of needle loss or discoloration, 3=more than one-third, but less than two-thirds of the plant surface showing signs of disease, 4=more than two-thirds of the plant surface showing needle loss, needle discoloration, extensive cankering, and death of shoots, and 5=entire plant dead. Visual evaluations recorded during December, January and February were deleted from the linear regression analysis since the condition of the plants did not change during this period. The relationship between rating and evaluation date was analyzed using a linear regression analysis for data recorded in September, October, November, March, April, May and June. Comparisons of regression coefficients were made using a T-test.

Foliar samples were collected from field plants each month just before plants were harvested. In 1988, three samples of the most current growth were collected from each of twenty trees in each treatment to form one composite sample. Composite samples were collected from each of five blocks. In 1989, samples were collected from each fertilizer treatment, but samples from blocks were combined into one composite sample for each treatment with no replications. Needles were clipped from the stems and samples were submitted to the Soil Testing Laboratory at Michigan State University. The nitrogen content was analyzed using the semi-micro

Kjeldahl procedure.

The new growth of the terminal leaders of each harvested tree and field trees was measured the summer following harvest. Growth of plants harvested in 1988 was measured in July 1989 and growth of plants harvested in 1989 was measured in July 1990. Growth of undisturbed field plants was measured in July 1988 and 1989. Visual ratings and growth of terminal leaders after harvest were analyzed as a blocked 2-way factorial experiment with four harvest dates, five fertilizer rates, and five blocks.

RESULTS AND DISCUSSION

Plants harvested during the last week in August began losing needles within fifteen days after harvest. Older, interior needles from the lower one-third of the plant and needles from the tips of lower branches dropped simultaneously. Needles dropped from areas where extensive cankers had developed. At the time of harvest, only infrequent, very small cankers were evident on a few plants. Cankers rapidly developed on the trunk and stems of the trees four to six weeks after harvest. The entire inner bark area of most trunks was completely discolored by the spring following harvest. On younger stems, cankers were frequently found centered around nodes or found at the tips of the last season's growth. The only readily visible symptom other than needle drop was the development of necrotic areas that were revealed only when the outer bark was scraped away. In late fall, when temperatures dropped below freezing, needle drop ceased, but when temperatures warmed again in the spring, trees began dropping needles. Trees also began to seep a resinous exudate. The first watering of trees in the spring raised the humidity inside the overwintering house. This provided an environment for the release of conidia from pycnidia on the infected trunk and older-stem tissue. In the field, nonharvested trees were free from needle drop and extensive cankers. Pycnidia were found only once on field plants and then were observed on a partially decomposed twig that appeared to have been dead for quite some time. Many harvested plants were dead by the time of bud break. On some plants, dead needles remained on branches that had died by spring. Of

those that survived, growth was stunted and expanding shoots curled downward, turned brown and died.

The overall progression of needle drop was similar in 1988 and 1989 (Figure 2.1 and 2.2). At the time of fall harvest, plants were symptomless, were in good form, and had good color. Plants harvested in August or September began dropping green needles several weeks after harvest. By November temperatures had dropped to freezing and needle drop ceased. Plants that were harvested at the end of October and November showed no needle drop until March. In March, plants that were harvested during early fall (August and September) started dropping needles once again and plants harvested during late fall (October and November) dropped needles from the lower part of the plant. Potted Colorado blue spruce are normally shipped to retailers during March and April. Nursery growers do not sell plants that rate higher than two on the arbitrary scale used in this study. In March, 1988 and 1989, trees harvested in October and November had developed very few symptoms, most trees were rated as symptomless. Trees harvested in August or September had an average rating of near two by March. Most of the trees harvested in early fall, and all of the trees harvested in late fall, would be scored as healthy and would be shipped. By April, a few more fall harvested trees would have declined and would be graded out before shipment. Although many of these trees would appear healthy at the time of the sale and shipment, they would probably decline in retail outlets and landscapes. Credits claims from customers receiving infected Colorado blue spruce have confirmed this.

Figure 2.1. The monthly visual rating of Colorado blue spruce infected with *Phomopsis occulta* and harvested in the fall of 1988 where 1 = no symptoms of disease, 2 = less than one-third of the plant surface exhibiting symptoms of needle loss or discoloration, 3 = more than one-third, but less than two-thirds of the plant surface showing signs of disease, 4 = more than two-thirds of the plant surface showing needle loss, needle discoloration, extensive cankering, and death of shoots, and 5 = entire plant dead. Observations were recorded during the first few days of each month. Points represent the monthly rating of trees averaged over all fertilizer treatments.

HARVEST FALL 1988

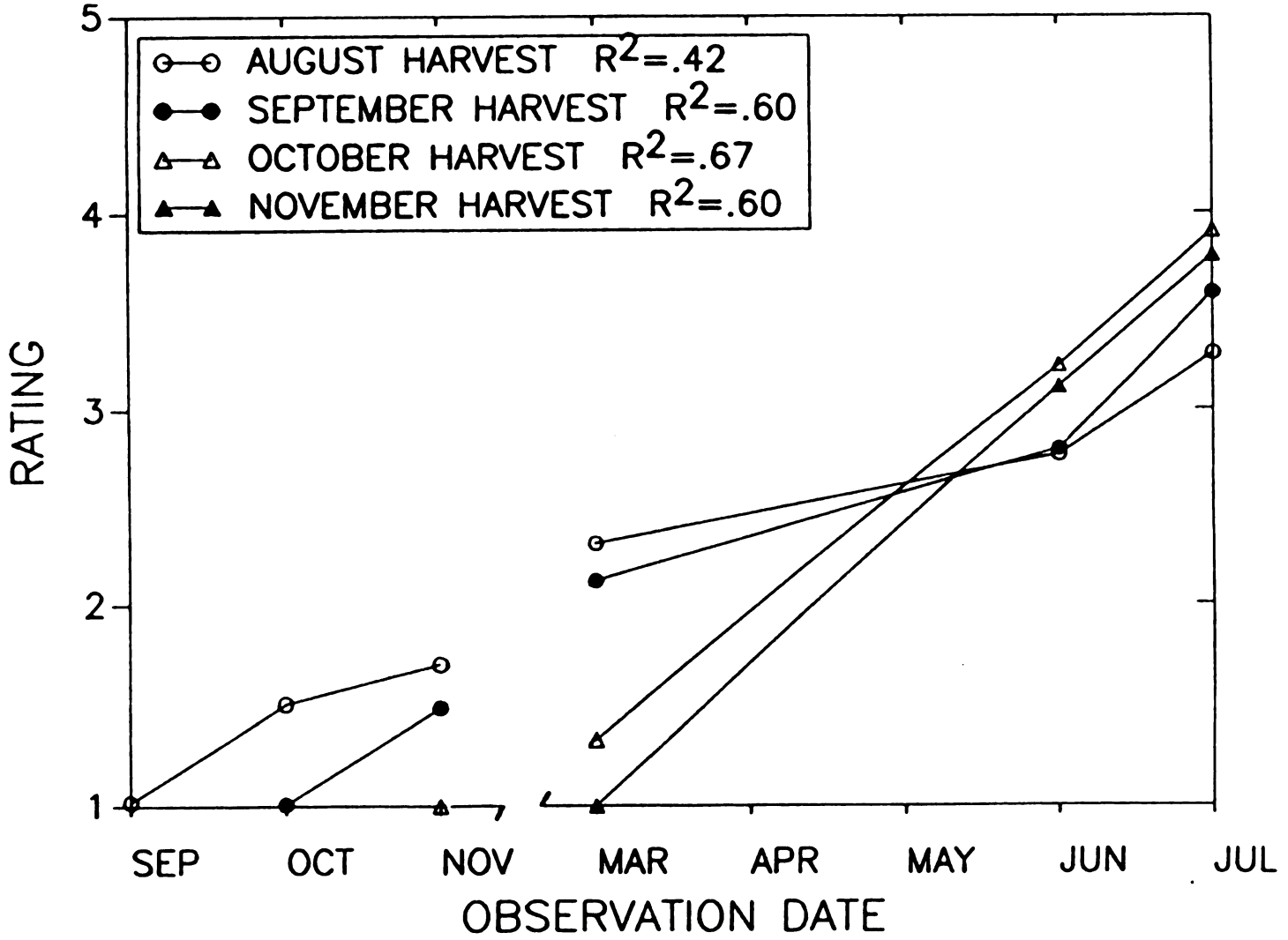
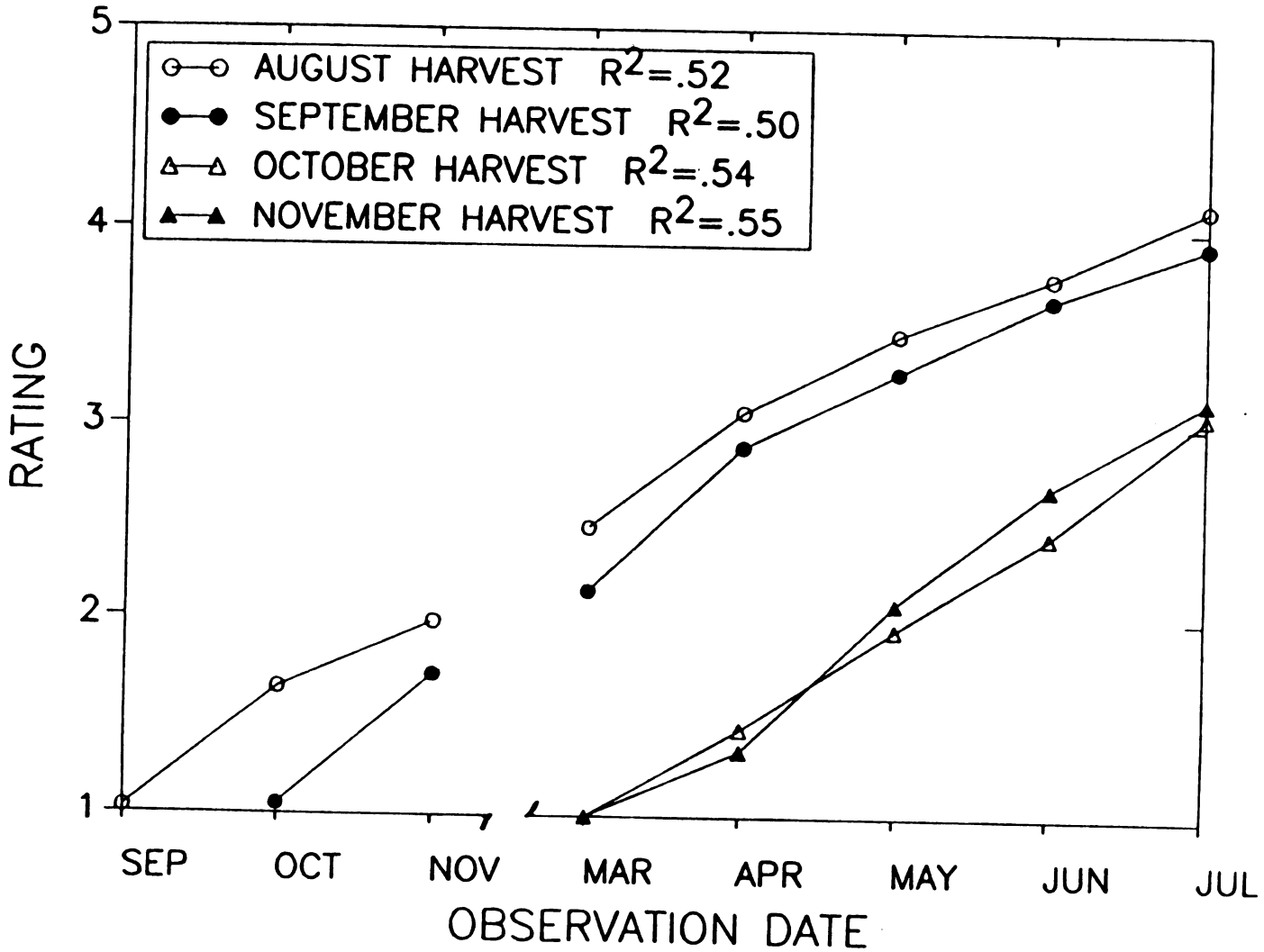


Figure 2.2. The monthly visual rating of Colorado blue spruce infected with *Phomopsis occulta* and harvested in the fall of 1989 where 1 = no symptoms of disease, 2 = less than one-third of the plant surface exhibiting symptoms of needle loss or discoloration, 3 = more than one-third, but less than two-thirds of the plant surface showing signs of disease, 4 = more than two-thirds of the plant surface showing needle loss, needle discoloration, extensive cankering, and death of shoots, and 5 = entire plant dead. Observations were recorded during the first few days of each month. Points represent the monthly rating of trees averaged over all fertilizer treatments.

HARVEST FALL 1989



The linear correlation between the visual rating and time of evaluation ranged between .42 and .67 for the harvest dates. It appears as though plants would continue to decline even after the final observation date in July if they remained in the pots. Decline rates of plants harvested during different months in 1989 were not significantly different from each other. Trees harvested in October and November of 1988 declined more rapidly than trees harvested in August or September of 1988.

The result of final evaluation of plants in July differed between 1988 and 1989. Trees harvested in August, 1988 were significantly less symptomatic than trees harvested in October or November of the same year (Figure 2.1). In 1989, the reverse occurred (Figure 2.2). Trees harvested in August, 1989 were significantly more symptomatic than trees harvested in October or November. In 1988, the October harvest received the worst rating (3.9) and the August harvest received the best rating (3.3). In 1989, the October harvest was rated the best harvest date (3.1) and the August harvest the worst (4.1). These results can be partially explained by the very different weather conditions between the two years. May and June rainfall levels in 1988 totaled only 2.11 cm (Table 2.1). During the same period in 1989, trees received 19.3 cm of rain. The 1988 drought in this area was the driest time recorded since 1948 and precipitation remained below average until September. However, the total 1988 rainfall during September, October, and November, was well above average. Precipitation levels in 1989 were much closer to the ten-year average recorded on the nursery site. In 1988, plants harvested in August were drought hardened. It is known

Table 2.1. Average monthly rain levels (cm) at the nursery in Ottawa County, Michigan.

Month	1988	1989	1980-1989
May	0.89	14.86	9.40
June	1.22	4.44	6.07
July	5.97	3.81	6.86
August	6.22	14.60	9.35
September	13.84	4.83	10.64
October	11.81	3.43	6.96
November	14.22	9.02	8.51

that plants experiencing water stress prior to a drought, suffer less injury than plants not previously stressed (10). Because plants harvested at the end of August in 1988 had been exposed to a drought for four months, they may have been better able to tolerate the water stress induced by harvesting. Trees harvested in August or September of 1989 were more symptomatic than later harvest dates because of warmer air and soil temperatures, and possibly lower root regeneration potential during this period. Lower temperatures of October and November would reduce transpirational water loss, and would be less conducive to growth of the pathogen. Colorado blue spruce root regeneration potential may also be higher during the later fall months.

Regardless of harvest date, the most severely infected plants had minimal or no root regeneration in the pots by the spring following fall harvest. Plants that do not regenerate roots following transplanting are more likely to remain water stressed (24). Generally, plants that were less severely affected had good root regeneration as evidenced by the long white roots extending to the bottom of pots. The question arises whether the most serious cases of infection are a result of (1) the plant's inherent inability to rapidly regenerate roots which causes the plant to remain stressed for a longer period during which the weak pathogen is relatively more aggressive or (2) the extensive cankering of stem tissue which interferes with the process of root regeneration.

Symptoms observed in this study were similar to those reported on spruce in Wisconsin (12). Symptoms of shoot blight in Wisconsin nurseries

appeared in June and July. From the results of artificial inoculations Sanderson and Worf concluded that spring infections on spruce may not develop until later in the season as conditions become warm and humid. They saw greater symptom development when controlled environments were warm and humid. Since new growth is most susceptible, infection of Colorado blue spruce by *Phomopsis occulta* probably occurs from spring bud break until new growth hardens in mid-summer (Chapter one). However, symptoms may not occur in the field until temperatures warm or until the plants are environmentally stressed. The trees in this study had latent infections that were disturbed by harvesting in the fall of 1988 and 1989. During the 1988 drought, many field plants were stressed and died by August. There was no field-plant mortality in 1989.

Fertilization of infected Colorado blue spruce made no difference in the visual rating of 1988 fall harvested plants (Table 2.2). There was also little difference in the 1988 fall foliar nitrogen levels of fertilized plants. Levels were comparable to a previous report of foliar N levels in which 568 samples from commercial nursery evergreen stock averaged 2.14% N (18). In 1989, plants that were not fertilized contained only 1.75% N while those plants that received 100 lb N/acre in the spring and another 100 lb N/acre in the summer contained 2.35% N in needles. Both of of these levels fall within the limits of the sufficient range of foliar N for evergreens (19). The 1989 harvested trees with higher foliar N levels were significantly less symptomatic than 1989 harvested trees that had not been fertilized in two years. If the higher rates of N-fertilization contributed to a more vigorous

Table 2.2. The effect of nitrogen fertilization on the growth, appearance and foliar nitrogen content of field-grown, fall-harvested, Colorado blue spruce naturally infected with *Phomopsis occulta*.

	Fertilizer level (lb N/acre)				
Measurement	**None**	**100**	**200**	**300**	**200 split application**
Growth after one season (cm)					
Field plants					
1989	11.4	10.8	10.1	11.4	11.0
1990	23.1	22.3	22.8	25.1	21.2
Harvested plants (all dates)					
1989 (1988 harvest)	3.7	3.5	3.7	3.7	3.2
1990 (1989 harvest)	3.3	3.0	3.8	4.0	4.9
Visual Rating (1-5)					
Field plants					
1989	-	2.4	-	-	-
1990	-	1.1	-	-	-
Harvested plants (all dates)					
1989 (1988 harvest)	3.5	3.7	3.6	3.6	3.8
1990 (1989 harvest)	4.0	3.8	3.5	3.4*	3.2*
Needle N (% dry weight, avg all dates)					
1988	2.08	2.21	2.23	2.27	2.25
1989	1.75	2.09	2.15	2.22	2.35

* Significantly different from the no fertilizer treatment on the same line, according to Tukey's HSD at 5% level.

tree which was less susceptible to development of infections, it was not evident from the growth of terminal leaders the spring following harvest. No difference between fertilizer treatments was observed when comparing the length of terminal leaders of trees that were growing in the field or of fall harvested trees. The only outstanding difference was the amount of growth of harvested trees compared to non-harvested trees. The growth of the terminal leader of 30-38 cm field grown trees was about three times greater than trees of the same size that were harvested the preceding fall. This difference was even larger between 38-45 cm harvested and control trees. Also, 30-38 cm trees harvested during the year of the drought (1988) grew only half as much as 38-45cm trees harvested during a normal year (1989).

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