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CULTURAL AND CHEMICAL CONTROL OF *PHOMOPSIS* SHOOT BLIGHT OF COLORADO BLUE SPRUCE (*PICEA PUNGENS GLAUCA*)

presented by

Susan Marie Gruber

has been accepted towards fulfillment
of the requirements for

M.S. degree in **Horticulture**

N C Peterson
Major professor

Date 2-24-92

CULTURAL AND CHEMICAL CONTROL OF *PHOMOPSIS* SHOOT BLIGHT OF COLORADO BLUE SPRUCE (*PICEA PUNGENS GLAUCA*).

By

Susan Marie Gruber

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

1992

ABSTRACT

CULTURAL AND CHEMICAL CONTROL OF *PHOMOPSIS* SHOOT BLIGHT OF COLORADO BLUE SPRUCE (*PICEA PUNGENS GLAUCA*).

By

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Phomopsis occulta Trav., causal agent of shoot blight of Colorado blue spruce (*Picea pungens glauca*), was isolated from six spruce species in Michigan nurseries. Cultural and chemical methods for control of *Phomopsis* on Colorado blue spruce were tested. Fall root pruning prior to spring digging increased postharvest symptom severity, while spring root pruning before fall harvest was beneficial. Improving postharvest root regeneration did not alleviate symptom severity on naturally infected field grown plants. Overwintering spruce inside a polyhouse reduced disease severity compared to those held outdoors. *In vitro* and greenhouse screening of fungicides showed several compounds have potential as chemical controls. Spruce shoots inoculated two weeks after budbreak were more susceptible to tip blight than those at earlier or later stages of development. Postharvest applications of benomyl were effective in reducing disease severity.

for Anne

ACKNOWLEDGMENTS

My major professor, Dr. Curt Peterson, has been supportive and encouraging throughout this project, and I thank him for all his time and tolerance. The members of my guidance committee - Drs. Muralee Nair, Dave Roberts, and Bob Schutzki, have each given generously of their time, advice, and resources. Their interest in my research and professional goals has been very helpful, and is greatly appreciated.

Zelenka's Evergreen Nursery generously supported this project, and provided plant materials, supplies, and the time and help of staff members. Thanks to all of you; this research is also the fruit of your labors.

I can't list all of my fellow grad students who have shared their knowledge and good humor with me over the last two years, but thank-you especially - Bill, Jim, Sue, Diana, Shawn, Anu and Mark. Vicki Buell and Tom Fink spent many hours on this research, and I thank them for their hard work. Thanks also to the staff of the HTRC, John, Lou, Gary, and Bill. You guys are the best!

My husband Karl has worked and waited for the last two years, and I thank him for his love and friendship, and for reminding me how good it would feel when this is finished. Finally, I thank our parents for their love and all they have done over many years to make this possible.

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INTRODUCTION

Members of the genus *Picea* (spruce) are widely used in landscapes and as Christmas trees. In recent years the production of spruce, particularly Colorado blue spruce (*Picea pungens glauca*) in Michigan has been affected by shoot blights and stem cankers caused by the imperfect fungus *Phomopsis occulta* (Trav.) (12).

P. occulta is not a newly described, or newly introduced organism. reports of this fungus and it's association with conifers were first described by Funkel who isolated it from cone scales of spruce (*Picea excelsea*) in Germany in 1875 (6). Other reports of *Phomopsis occulta* infestations were published in the late 1920's in conjunction with research on *P. juniperovora*. The pathogenicity of *P. occulta* on spruce was suggested, but was not investigated (21,22).

G.G.Hahn reported the isolation of *P. occulta* from 14 coniferous genera including *Picea*, and found it widely distributed in the United States and Europe (8). Hahn considered this fungus to be only slightly parasitic, although he held the position that shoot blight agents *Phomopsis thujae* and *Phomopsis criptomeriae* were actually strains of *P. occulta*. He also stated that other physiologic strains of the fungus may indeed be plant pathogens. *P. occulta* has been categorized in references as a saprophyte or secondary pathogen, on the basis of Hahn's later work which showed the organism failed to parasitize red cedar (9,11,18). There are many references to *Phomopsis occulta* and other *Phomopsis sp.* as endopyhtes of conifer needles and non pathenogenic members of the microflora found on cones, twigs, and bark (5,7,10,17). *Phomopsis* species have also been reported to cause cankers and tip blights of Douglas-Fir (10).

In the late seventies and early eighties, growers in the state of Wisconsin experienced losses in the production of young spruce due to a shoot blight which caused 'severe damage' to Colorado blue spruce liners, costing one grower an estimated $25,000 per year (1). Researchers from The University of Wisconsin isolated *P. occulta* from infected trees in these growing areas, and demonstrated that it was a causal agent of tip blight on blue spruce seedlings (16).

Michigan growers also experienced losses of field grown blue spruce during the early eighties. Lower branches of the trees became defoliated following harvest, or in the field during periods of stress, such as the 1987 and 1988 growing seasons, when the state experienced a flood followed by a severe drought. Severe shoot blight symptoms were also found on larger sizes of blue and white spruce in Christmas tree production. Isolation of fungi from samples submitted to the Michigan State University Plant and Pest Diagnostic Lab consistently yielded cultures of *Phomopsis occulta*. Research done by Igoe confirmed that this fungus was a causal agent of the postharvest disorder of Colorado blue spruce (12).

Inoculation trials have also provided information about the susceptibility of spruce species to *Phomopsis*. Sanderson and Worf inoculated Siberian

(*P. obovata*), Black Hills (*P. glauca* 'Densata'), Norway (*P.abies*), and Colorado blue spruce, and found that all four species were infected, but Colorado blue spruce was the most susceptible (16).

A pattern of symptomless infection and disease development following the incidence of stress is common among members of the genus *Phomopsis* (13,14,15,20). The correlation between root injury and severity of *P. occulta* infection on Colorado blue spruce was demonstrated in studies by Igoe, who provided a review of the genus, and the role of stress in disease development (12).

Management strategies for the suppression of *P. occulta* in spruce production were sought by the nursery industry to reduce losses which have exceeded the economic tolerance of many Michigan growers. The fungus has been successfully controlled by one Wisconsin nursery by applying benomyl fungicide (23), but this single management tool is not a reliable long term method of disease control. Considering the great potential for fungicide resistance to develop under such conditions (2,3,4,19), a combination of cultural and well designed chemical controls will provide the most successful control of *P. occulta*.

This research was undertaken to develop cultural and chemical strategies for long term disease management of *P. occulta* on Colorado blue spruce. Cultural practices designed to reduce harvest and postharvest stress were examined for their effect on the development of latent infections. Fungicidal compounds were tested, and experiments were carried out to help determine the most effective timing of fungicide applications.

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CHAPTER I

THE EFFECT OF CULTURAL PRACTICES ON THE SEVERITY OF *PHOMOPSIS OCCULTA* (TRAV.) CANKER DEVELOPMENT IN COLORADO BLUE SPRUCE (*PICEA PUNGENS GLAUCA* ENGLM.).

ABSTRACT

Cultural management techniques were examined for their effectiveness in reducing the severity of postharvest *Phomopsis* disease symptoms on Colorado blue spruce (*Picea pungens glauca*). Root pruning ten year old (30-36") blue spruce by cutting roots with a landscape spade in October prior to April harvest resulted in more severe symptom expression following harvest. In contrast, root pruning in April prior to October harvest significantly reduced symptom severity. Postharvest root regeneration of field grown blue spruce was significantly improved by treating root systems with 1000 ppm IBA (indole-3-butyric acid), or by potting plants in a wood-chip container medium instead of native field soil. Improved root regeneration had no effect on the severity of subsequent canker development. Overwintering fall field-potted blue spruce inside a polyhouse reduced both mean symptom severity and number of dead plants compared to those kept outdoors.

INTRODUCTION

Long term solutions to disease problems are most successful if integrated pest management (IPM) techniques are employed. Although IPM is more widely applied to insect pests, a combination of chemical and cultural tools should be applied to manage plant pathogens in order to minimize the use of chemical agents. This is important for environmental and economic reasons, but also in helping to delay or prevent the occurrence of resistance to chemical controls (5). Cultural control of plant vigor can be effective in reducing damage caused by plant pathogens, because stress plays an important role in plant susceptibility to infection and disease development. (23,24).

A relationship between stress and the development of shoot blights and stem cankers has been reported for many species of *Phomopsis* and their woody hosts (14). The severity of *P. occulta* shoot blight of Colorado spruce as related to plant stress was first observed by White in 1925 who found the fungus causing tip blight on trees in overcrowded and flooded beds (29). Hahn, who studied in detail the pathogenicity of *P. occulta* observed that it acted as a pathogen only on wounded or stressed conifers (11). A survey of the disease in Michigan nurseries have correlated well with these findings. Igoe demonstrated this effect by root wounding artificially inoculated *Picea pungens* seedlings. Root pruned and intact plants had the same number of infections, but the amount of canker development and number of dead terminal shoots was significantly greater in the seedlings with injured root systems. Igoe's research also showed that time of harvest (spring vs. fall), and levels of nitrogen fertilization programs had little effect on the severity of *P. occulta* symptoms on naturally infected, field grown plants (14).

While water stress from drought or flooding has been observed by growers to cause outbreaks of *P. occulta* in the field, the stress of harvest is usually most severe, and is also potentially more manageable by growers. Harvest stress in spruce is mainly the result of two factors: 1) large reduction of the total root mass at the time of digging, and 2) slow regeneration of the root system after harvest.

Root mass reduction at harvest is generally controlled in nurseries by root pruning trees one or more seasons prior to digging. Many studies have demonstrated increased survival of spruce seedlings due to root pruning and root wrenching prior to harvest (12,17,22). Few reports have been published on the effect of root pruning larger conifers. In one study, Watson (28) improved the harvested root mass of landscape sized Colorado spruce by pruning the root system 4 years prior to harvest.

Improving root regeneration following potting, balling, or transplanting has also been examined by many researchers. Much of this work has focused on environmental conditions including light, temperature, and media fertility and physical properties. Media aeration and bulk density influence the amount of root regeneration in conifers (19). Spruce seedlings have been shown to perform poorly in compacted, low oxygen media, and in soils with high bulk density (8).

Applications of IBA (indole-3-butyric acid, a synthetic auxin) to roots of harvested trees is another potential method of improving the postharvest plant status. Carter and Tripepi observed a significant increase in root number, weight, and length when 1000 ppm IBA was applied to fall harvested (3+2) Colorado blue spruce seedlings. While they concluded such treatment was not economically justified, the economic value of reduced symptom severity on plants with *P. occulta* could justify the expense of IBA treatment if losses from the disease were significantly reduced (4).

Another source of difficulty in maintaining plant vigor is the handling and storage of potted plants during the winter. Harvesting spruce in fall for early spring sales is a common practice in Michigan nurseries. Winter storage of these plants outdoors can cause stress from root injury, desiccation and freezing. Indoor storage in quonset style polyhouses may help to alleviate these problems, but may simultaneously create an environment more favorable for fungal growth and canker development. Higher temperatures and relative humidity occur inside the structures, particularly during periods of warm weather in late fall and early spring. Such conditions have been shown to favor the development of *Phomopsis* blight of Colorado spruce (20).

MATERIALS AND METHODS

Root Pruning. Plants used in this experiment were 24-36", naturally infected Colorado blue spruce randomly selected from a uniform field at a large commercial nursery in Ottawa County, Michigan. Previously these trees had been root pruned between rows only, with a tractor and U-blade late in September or early October 1988, 1989, and 1990. A landscape spade was used to cut roots starting just under the edge of the first whorl of branches (approximately 30 cm from the trunk), and cutting at an angle of about 60°. Roots were cut around the whole circumference of the trees to the depth of the spade (35cm). Twenty trees were treated on October 26, 1990, and harvested on April 3, 1991, along with 20 untreated controls from the same farm. Disease symptom severity was visually rated on the 15th of June, July, and August using a scale of 1 to 5, where 1 = symptomless, 2 = less than-one third of the plant showing needle loss, 3 = one-third to two-thirds of the branches affected, 4 = more than two-thirds of the plant showing needle loss, cankering, and death of shoots, and 5 = dead, as described by Igoe (14).

The root pruning procedure was repeated on April 24, 1991. Ten root pruned trees and controls were harvested on October 24, 1991. The trees were moved to a heated greenhouse (24°C/75°F day, 18°C/65°F night) on November 7, 1991 to accelerate symptom development. Symptoms were visually rated on December 1 and 20, 1991 and January 13, 1992, (5, 8, and 11 weeks after harvest).

Root Regeneration. Two hundred 15-18" Colorado blue spruce (*Picea pungens glauca*) seedlings were harvested on October 26, 1990 from a uniform field in a large commercial nursery in Ottawa County, Michigan. None of the harvested plants showed any symptom of *Phomopsis* infection, but were growing in an area known to contain high levels of *P.occulta* inoculum. The trees were bare rooted and placed in groups of 8 - 10 in 30 gallon plastic bags with wet burlap wrapped around the roots to prevent desiccation during transport to East Lansing. Immediately upon arrival the trees were heeled into moist peat moss in a 1.5°C (35°F) cooler and held overnight.

The following day plants were removed from the peat beds, and the root systems were washed with running water. All root systems were trimmed to a uniform size for planting in a Classic 1600™ pot. Lateral roots were cut to 12 cm from the central axis, and the vertical length of the root mass was standardized at 20 cm. A small number of white roots were found on 12 trees, and were removed during this process.

The experiment was set up as a 3X2 factorial, with 30 replications. The roots were treated with a ten second dip in one of the following solutions: 1000 ppm IBA (indole-3 butyric acid, Sigma Chemical Corporation) in a 10% EtOH solution in water, 10% EtOH, or water. Immediately following treatment, the spruce were potted in either soil from the growing area, or in a container medium. Properties of these two potting media are summarized in Table 1.1.

Table 1.1 Properties of media used in the root regeneration study.

MEDIA PROPERTY +x	FIELD SOIL	CONTAINER MIX
	Granby fine sandy loam*	40% hardwood chips 40% rice hulls 10% peat moss 10% field soil
pH	6.5	7.0
soluble salts (mmhos)	.75	.58
nitrate (ppm)	32	3
phosphorus (ppm)	0.5	5.8
potassium (ppm)	25	93
calcium(ppm)	86	57
magnesium (ppm)	20	22
sodium (ppm)	10	7
chloride (ppm)	9	11
zinc (ppm)	18	24
manganese (ppm)	11	31
copper (ppm)	1	1
iron (ppm)	83	95
organic matter %	4.6 (digestion)	50.8 (ignition)
% solids	76.94	37.03
% air space	5.41	29.87
% water space	17.65	33.10

* Classification from: Soil Survey of Ottawa County, U.S. Soil Conservation Service, USDA, Washington D.C.

\+ Media chemical properties determined by the Soil Testing Laboratory, Michigan State University.

x Physical properties of media calculated using containers identical to those used in the experiment.

The potted plants were placed in randomized complete blocks in an open quonset style polyhouse at the Horticulture Teaching and Research Center at Michigan State University. Poly cover (white 4ml, 18% shade) was placed on the house on November 8, 1990, and removed on April 2, 1991.

Root and shoot fresh and dry weights were measured from 20 trees at the initiation of the experiment. Shoot weights for this experiment were taken from 3 branches collected from the top whorl of the tree. Measurements of root regeneration were made on December 17, 1990 and May 22, 1991. Ten plants per treatment were measured at each sampling. In December, the number of white roots over 1 mm were counted, and fresh and dry weights of the shoots were measured. In May, white roots were separated from the rest of the root system for analysis. Data on white (new) and brown (old) root fresh and dry weights, and shoot fresh and dry weights were collected.

Symptom severity was measured on the plants when root data was collected. A complete set of treatments was held for evaluation of disease severity in June, July, and August 1991. Symptoms were visually rated during the summer on a scale of 1 (no disease symptoms) to 5 (dead), as previously described.

Overwintering. Eighty 12-25" Colorado blue spruce were harvested from a commercial nursery in Ottawa County, MI on October 11, 1990. None of these trees had *P. occulta* disease symptoms. Forty plants had been treated in the field with benomyl applied by nursery personnel as Benlate 50wp 2.2kg/378*l* of water

(1lb/100 gal) once a month in June, July, and August of 1990. Forty trees received a single application in June. Twenty plants from each group were overwintered inside the polyhouse. The remaining 20 were overwintered just outside the house, on a gravel surface identical to the inside. The plants were arranged in a randomized complete block design with four blocks of five replications in each location. All containers were moved as close together as possible for overwintering when the house was covered with poly. The trees were spaced out in spring when the plastic was removed. Disease symptoms were visually rated during the summer on a scale of 1 (no disease symptoms) to 5 (dead), as previously described.

RESULTS AND DISCUSSION

Root Pruning. Trees root pruned in October prior to field potting in April were significantly worse than control plants. In the field prior to harvest, and for three weeks following potting, there was no evidence of canker development or needle cast on any of the plants. Following bud break and shoot expansion, differences between treatments became quite obvious. As illustrated in Figure 1.1, the average root pruned plant was not suitable for sale by June, and the quality continued to decline throughout the summer. The untreated controls developed much milder symptoms early in the season, and never became as severely diseased as the root pruned plants.

Trees which were root pruned in April prior to October field potting were also symptomless at harvest. Needle loss on the lower limbs of trees in both treatments began after one week in the greenhouse. Eight weeks after harvest new growth was beginning, and defoliation had become severe on many trees. By eleven weeks, the difference between treatments was pronounced. In contrast to the fall experiment, symptoms were significantly less severe on the root pruned plants in this group (Figure 1.2).

There is a need for more research into the preharvest conditioning of landscape sized spruce. There is limited information available in the liturature on the periodicity of root growth in Colorado blue spruce, or the effects of root pruning and other cultural practices on root development.

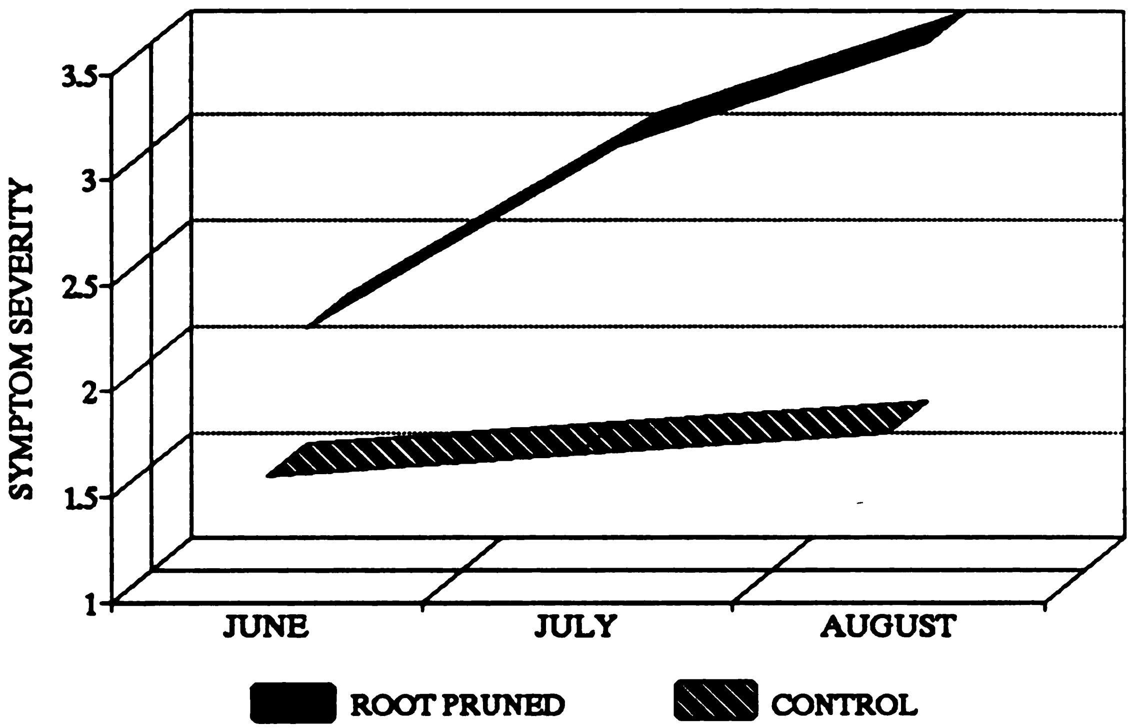

Figure 1.1. *Root pruning I:* June, July, and August evaluations of *Phomopsis* symptom severity on naturally infected, field-grown Colorado blue spruce root pruned October 26, 1990 and harvested April 3, 1991.

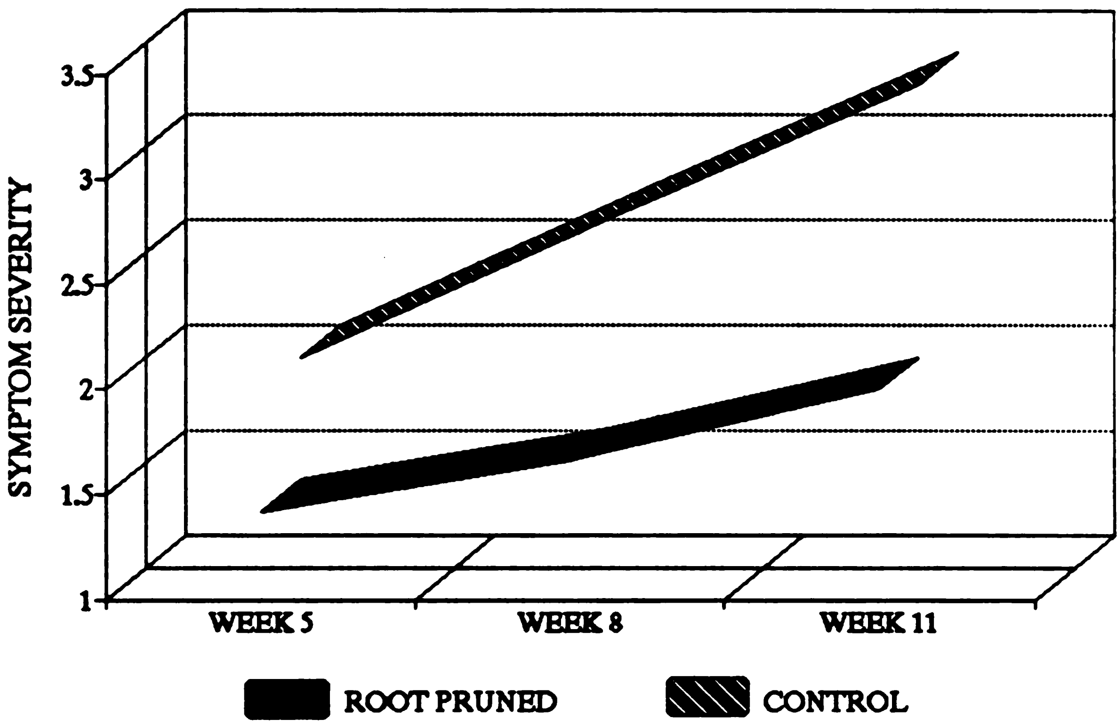

Figure 1.2 *Root pruning II*: *Phomopsis* symptom severity on naturally infected, field-grown Colorado blue spruce root pruned April 24, 1991 and harvested October 24, 1991. Trees were placed in a heated greenhouse November 7, 1991, and evaluated 5, 8, and 11 weeks after harvest.

Root growth for white (*P. glauca*) and Sitka spruce (*P. stichensis)* generally occurs in two peaks during the growing season. The first peak occurs from early spring until shoot expansion begins, the second begins after shoot growth in midsummer (7,9,15). Root growth capacity of Colorado blue spruce has been shown to increase from August to October (21). Root regeneration in conifers depends mainly on current photosynthate, and not on starch reserves (18,27). Trees root pruned in October and harvested the following April did not have the benefit of the growing season to regenerate roots. The improved quality of spring treated plants may be due to increased density of roots in the harvested root system compared to the fall root pruned plants. Root pruning in early September would also allow more time for the regeneration of roots than late October treatment.

Many growers root prune in the fall because spring root pruning can reduce subsequent top growth (3,13), and because the spring season is very busy. However, shoot growth on the root pruned plants in these experiments was comparable to controls. July root pruning has been shown to increase long term survival of spruce seedings (6,17). Also, spring transplanting of young Colorado blue spruce results in no loss of quality (26). If further research shows that disease severity were reduced by spring root pruning, this practice may deserve further consideration where *P. occulta* is causing postharvest losses. Reduction in top growth one or two seasons prior to harvest would be helpful if top pruning, and the resulting number of wounds were reduced. Needle and twig injuries provide an entry point for *P. occulta* mycelia to colonize tissue and cause cankers (14).

If the number of fibrous roots inside the root ball is increased, the trees should experience less stress, and be more resistant to canker development after harvest (2,23,25). Large increases in the amount of roots inside the root ball have been reported for landscape sized Colorado blue spruce which were root pruned only once, several years before harvest (28). Multiple root prunings before harvest may actually be detrimental (10). More testing of the frequency and timing of root pruning landscape sized spruce is needed, since spring root pruning shows some potential to improve the performance of spruce infected with *P. occulta*.

Root Regeneration. The first evaluation of postharvest root regeneration was made six weeks following potting on December 17, 1990. At that time, there were very small white tips of newly formed roots visible on most plants. The white roots were formed primarily at the tips of uncut fibrous roots. The cut ends of the large primary roots of several plants had small amounts of callus formation, but the secondary roots were the site of most new root development.

Very few roots had white tips over 5mm when the data were collected. The size and quantity of root regrowth during this period may have been greater than indicated by this data. A drop in the media temperatures occurred during the two weeks previous to the evaluation, causing rapid suberization of the new roots, and making them impossible to distinguish from the existing root system.

Differences between the treatment means are large, but are not statistically significant due to high variation within treatments (Figure 1.3). The variation between blocks was not significant for any variable in this experiment.

There was very little symptom development before the first sampling, and all treatments were similarly affected (Table 1.2). Shoot moisture content was similar for all treatments, and comparable to the samples measured at the beginning of the experiment (Table 1.3).

Evaluation of treatments was repeated on May 22 1991. Most of the spruce had begun the spring growth flush, indicating the end of the first peak period of root growth. New roots were numerous on most trees, and originated primarily from fibrous secondary roots as indicated by the December observations. The cut ends of large primary roots occasionally gave rise to new roots, but these were usually short and contributed little to the mass of new roots. Trees harvested for this study were seedlings, and therefore varied in size and mass of the root system. In order to reduce the impact of their variation on the results, data presented in Figure 1.4 are fresh weight of new roots as a percentage of the entire root system. IBA treatment and media type significantly affected the amount of root regeneration. There was no significant interaction between factors.

A significant improvement in root system regeneration was achieved through the use of a root dip in a solution containing 1000 ppm IBA, confirming results obtained by Carter and Tripepi on younger blue spruce (4).

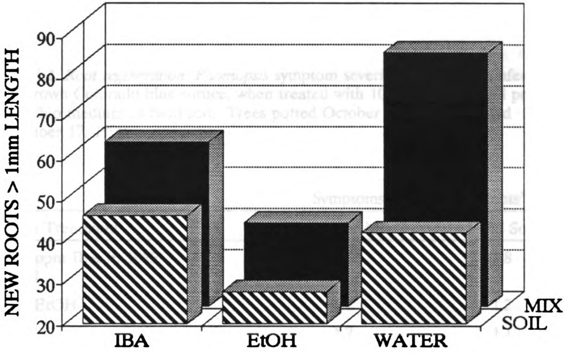

Figure 1.3 *Root regeneration:* Number of white roots > one millimeter in length on field-grown Colorado blue spruce, when treated with 1000 ppm IBA and potted in wood chip medium or field soil. Trees potted October 27, and evaluated December 17, 1990.

Table 1.2. *Root regeneration*: *Phomopsis* symptom severity on naturally infected, field-grown Colorado blue spruce, when treated with 1000 ppm IBA and potted in wood chip medium or field soil. Trees potted October 27, and evaluated December 17, 1990.

	Symptoms of Sampled Plants[x]	
Auxin Treatment	Container Mix	Field Soil
1000 ppm IBA in 10% EtOH	1.5	1.8
10% EtOH	1.2	1.2
water	1.7	1.1

Source of Variation	
Media	NS
Auxin	NS
Media X Auxin	NS

[x] Symptom severity rated on a scale of 1 (no symptoms) to 5 (dead); trees rating higher than 2 would be considered unsalable.
NS = Not significant at P = 0.05, according to F test.

Table 1.3. *Root regeneration:* Shoot moisture content of naturally infected, field-grown Colorado blue spruce, when treated with 1000 ppm IBA and potted in wood chip medium or field soil. Trees potted October 27, and evaluated December 17, 1990.

	Shoot Moisture Content (% of fresh weight)	
Auxin Treatment	Container Mix	Field Soil
1000 ppm IBA in 10% EtOH	58.04	56.63
10% EtOH	56.19	57.90
water	55.37	57.16
	Pre-test samples[x]	54.84

Source of Variation	
Media	NS
Auxin	NS
Media X Auxin	NS

[x] Measurements from twenty plants taken at the beginning of the experiment.
NS = Not significant at P = 0.05, according to F test.

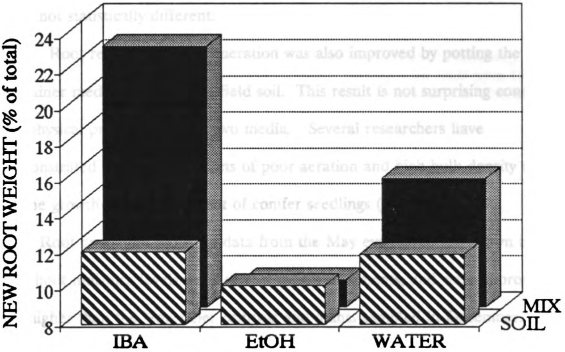

Figure 1.4 *Root regeneration:* Fresh weight of new roots (as a percentage of root system fresh weight) on field-grown Colorado blue spruce, when treated with 1000 ppm IBA and potted in wood chip medium or field soil. Trees potted October 27, and evaluated May 22 1991.

In their work, 1000 ppm IBA in 100% ethanol solution reduced root regeneration by over 50% compared to IBA in 20% ethanol. Reduction in new root fresh weight was also observed in this study, but ethanol and water controls were not statistically different.

Root regrowth and regeneration was also improved by potting the trees in container medium rather than field soil. This result is not surprising considering the physical properties of the two media. Several researchers have demonstrated the harmful effects of poor aeration and high bulk density media on the growth and development of conifer seedlings (8,16,19).

Root and shoot moisture data from the May evaluation are shown in Table 1.4. Shoot moisture levels were similar for all treatments, and were approximately 15% higher than the November controls and plants sampled in December. This difference between fall and spring sampled plants is consistent with the stage of growth during the evaluations and with normal seasonal variations in plant water content. It is doubtful that this difference occurred as a consequence of the new root growth. Treatment differences would be expected if there were any correlation with the amount of new root growth. There were also no significant differences between treatments for root moisture content.

The goal of this study was to determine if the severity of *Phomopsis* symptom development could by reduced by improving the postharvest root regeneration of blue spruce. Unfortunately, the expression of *Phomopsis* shoot blight symptoms was not significantly affected by the amount of root regeneration.

Table 1.4. *Root regeneration:* Root and shoot moisture content of naturally infected, field-grown Colorado blue spruce, when treated with 1000 ppm IBA and potted in wood chip medium or field soil. Trees potted October 27, 1990 and evaluated May 22, 1991.

	Moisture Content (% of fresh weight)			
	New Roots		Shoots	
Auxin Treatment	Mix	Soil	Mix	Soil
1000 ppm IBA in 10% EtOH	92.24	91.57	71.80	61.50
10% EtOH	91.15	89.98	63.63	70.26
water	92.10	91.43	65.17	71.04

Source of Variation	Roots	Shoots
Media	NS	NS
Auxin	NS	NS
Media X Auxin	NS	NS

NS = Not significant at P = 0.05, according to F test.

The evaluation of plants sampled in May, and of a third complete set of treatments observed through the summer showed no meaningful reduction in symptom severity in any of the treatments (Table 1.5 and Table 1.6).

The visible shoot blight symptoms did not appear serious on these plants until late May. By this time the new shoots were expanded, temperatures were higher, and the trees were transpiring rapidly. *Phomopsis* cankers can expand under the bark of woody hosts without causing blight symptoms until the host is under stress. The lesions did not develop enough during the cool months of early spring to interfere with root regeneration, but became injurious following bud break. Cultural control of this disease would be more successful if efforts address reducing stress during harvest, and preventing the growth of incipient infections.

Overwintering Study. The winter storage conditions of these spruce proved to be an important factor in their performance during the following growing season. The difference in treatments is due mainly to the increased number of severely or fatally affected trees. Table 1.7 shows the number of trees in each group which were unsalable (living), and the number which were dead by August. Spruce which were overwintered outdoors developed symptoms earlier than the other group, and continued to decline through the season (Figure 1.5). Wind desiccation and freezing injury to the root systems of these plants may have resulted in water stress, which is known to make conditions more favorable for canker development (25).

Table 1.5. *Root regeneration: Phomopsis* Symptom severity on naturally infected, field-grown Colorado blue spruce, when treated with 1000 ppm IBA and potted in wood chip medium or field soil. Trees potted October 27, and evaluated May 22, 1991.

	Symptoms of Sampled Plants[x]	
Auxin Treatment	Container Mix	Field Soil
1000 ppm IBA in 10% EtOH	1.8	1.7
10% EtOH	1.9	1.6
water	1.4	1.7

Source of Variation	
Media	NS
Auxin	NS
Media x Auxin	NS

[x] Symptom severity rated on a scale of 1 (no symptoms) to 5 (dead); trees rating higher than 2 would be considered unsalable.
NS = Not significant at P = 0.05, according to F test.

Table 1.6. *Root regeneration*: Symptom severity on naturally infected, field-grown Colorado blue spruce, when treated with 1000 ppm IBA and potted in wood chip medium or field soil. Trees potted October 27, and evaluated August 15, 1991.

	Symptom Severity[x]	
Auxin Treatment	Container Mix	Field Soil
1000 ppm IBA in 10% EtOH	1.9	2.0
10% EtOH	1.8	2.3
water	1.3	1.5

Source of Variation	
Media	NS
Auxin	NS
Media X Auxin	NS

[x] Symptom severity rated on a scale of 1 (no symptoms) to 5 (dead); trees rating higher than 2 would be considered unsalable.
NS = Not significant at P = 0.05, according to F test.

Table 1.7. *The effects of overwintering and benomyl applications*: *Phomopsis* symptom severity on naturally infected, field-grown Colorado blue spruce when treated with benomyl once in June, or monthly in June, July, and August 1990, harvested October 11, 1990, and overwintered outdoors or inside a poly covered quonset house. Plants evaluated August 15, 1990.

Treatment	Symptom Severity[x]	Unsalable (living)	Dead
Inside / One Spray	1.6	1	1
Inside / Three Sprays	1.7	1	0
Outside / One Spray	2.65	1	6
Outside / Three Sprays	2.05	2	2

Source of Variation	(Tmt Means)
Overwintering Method	**
Benomyl Applications	NS
Overwintering x Benomyl	NS

[x] Symptom severity rated on a scale of 1 (no symptoms) to 5 (dead); trees rating higher than 2 would be considered unsalable.
NS = Not significant at P = 0.05, according to F test.
** = Significant at P= 0.01, according to F test.

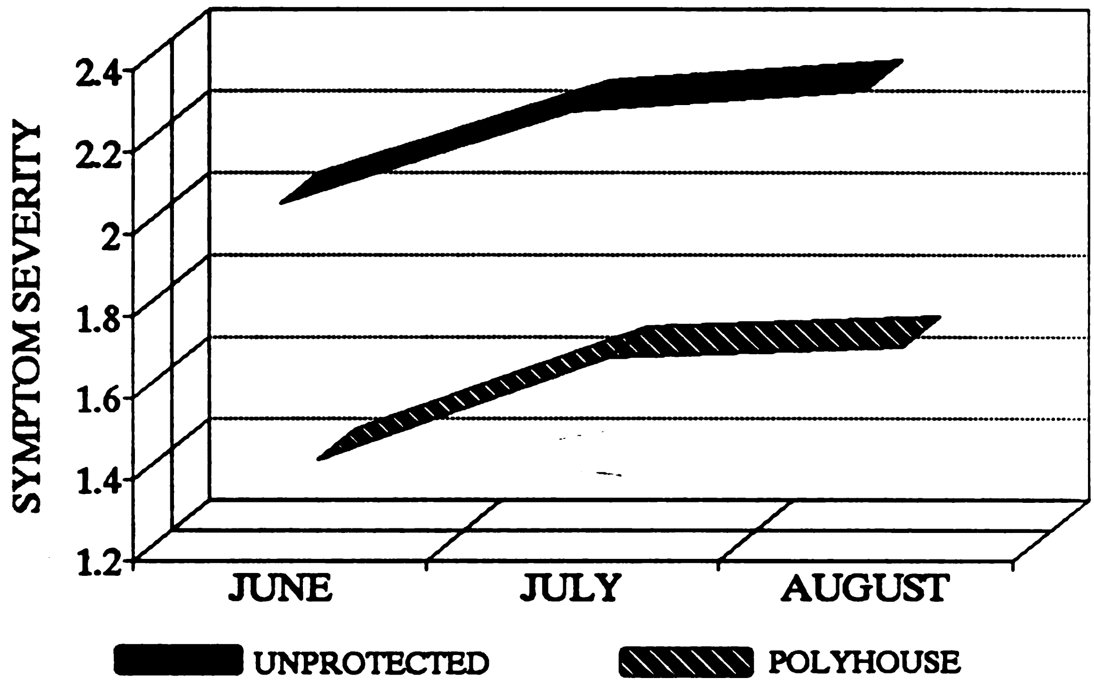

Figure 1.5. *Overwintering: Phomopsis* symptom severity evaluations for June, July, and August on naturally infected, field-grown Colorado blue spruce harvested October 11, 1990, and overwintered outdoors or inside a poly-covered quonset house. Plants evaluated August 15, 1990. (Data averaged over fungicide treatments).

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CHAPTER II

INCIDENCE, HOST RANGE, AND CHEMICAL MANAGEMENT OF *PHOMOPSIS* CANKER OF COLORADO BLUE SPRUCE (*PICEA PUNGENS GLAUCA*) IN MICHIGAN.

ABSTRACT

Phomopsis occulta was isolated from spruce tissue collected in several counties across southern lower Michigan. Five species of spruce were found to be hosts, including Colorado blue (*Picea pungens glauca*), white (*P.glauca*), Black Hills (*P.g.'Densata*), Norway (*P. abies*), and Engelmann spruce (*P. engelmanii*). Inoculation showed that Spartan spruce (*P. pungens* x *P. glauca*) is also susceptible. Colorado blue spruce seedlings at six stages of shoot development were inoculated with conidia of *P. occulta*. Shoots which expanded for two weeks following budbreak were more susceptible to shoot blight than dormant plants, as well as those inoculated after 4, 6, or 8 weeks of shoot expansion. *In vitro* screening of eight fungicides in amended potato dextrose agar (PDA) showed that *P. occulta* was completely inhibited by 1 ppm benomyl, 10 ppm myclobutanil and propiconazole, 100 ppm triadimefon, trifiorine, mancozeb, and 1000 ppm bordeaux. Fenarimol supressed growth, but provided incomplete control at 1000 ppm. Benomyl was applied to field grown plants before and after fall field potting. Sprays in July and August prior to digging had no significant effect on disease development. Sprays applied on the first day after harvest, or three times during shoot expansion in the spring following harvest reduced symptom severity.

INTRODUCTION

Shoot blights and stem cankers caused by *Phomopsis occulta* result in serious production and postharvest losses of spruce (*Picea sp.*) grown in Michigan landscape and Christmas tree nurseries. Colorado blue spruce (*P. pungens glauca*) has been the most severely affected species in recent years. Postharvest canker development results in substantial losses and increased production costs which have caused some growers to reduce the number of blue spruce entering the production cycle. Long term suppression of *P. occulta* will be achieved by implementing a program which combines several appropriate fungicides and proper timing to use the least amount of pesticide for adequate control. This strategy, combined with effective cultural control measures, should be adopted in order to avoid fungicide resistance (5,19,22).

Chemical control of *Phomopsis occulta* was examined by Igoe (10) who reported the *in vitro* activity of four fungicides against *Phomopsis occulta*. Her research showed that ipriodine and chlorothalonil were very inhibitory to mycelial growth and benomyl arrested growth of the fungus at 1ppm. However, the fungicides failed to reduce symptoms on naturally infected, field grown plants which were sprayed once a month in June, July, and August prior to fall digging.

The failure of those treatments was probobly due to the presence of advanced cankers in the trees before treatment. The effectiveness of benomyl in the field has been reported by a grower in Wisconsin, who has successfully managed *Phomopsis* on their spruce crop by applying 2.2kg of benomyl/358*l* of water (1lb/100gal) three times during budbreak and shoot expansion at approximate 3 week intervals to protect the plants from infection (1). However, an effective long-term program to control *P. occulta* should not rely on a single fungicide. Many fungicides have been tested against other species of *Phomopsis* which cause blights and cankers on woody hosts which may have potential for controlling *P. occulta*. Benomyl is the primary compound used in the control of *Phomopsis* diseases of juniper, blueberry, arizona cypress and russian olive (2,11,13,18,20). Piperazine controls stem cankers on grape and blueberry, but failed to reduce the severity of juniper tip blight (14). *Phomopsis* fruit rot of grape can be controlled by two applications of mancozeb (15).

Fungicide applications should be timed to protect the crop during periods of maximum susceptibility. These conditions occur when inoculum level is high and the host plant is at a stage of growth which is vulnerable to infection. For most species of *Phomopsis*, the optimum conditions for infection and colonization include warm, humid weather and a host with tender new growth (4,8,9,12). The mycelia of *P. occulta* grows most rapidly at around 25°C (77°F) (10), which is close to the peak temperature for growth and conidia germination in many *Phomopsis* species (8,16,15). Sanderson and Worf found the highest rates of infection of Colorado blue spruce when seedlings were incubated at warm temperatures (28 °C/ 82 °F) and high relative humidity (17), though Incipient infections may also form during less ideal conditions (10,17).

The experiments described in this chapter were designed to investigate an effective fungicide program for the suppression of *Phomopsis occulta* in Colorado blue spruce production by determining the most susceptible stage of shoot growth for infection. An attempt was also made to understand the relative activity of several fungicides, and the effectiveness of pre and postharvest benomyl spray applications.

MATERIALS AND METHODS

Host Range and Distribution in Michigan. During May and June of 1990, samples of symptomatic spruce tissue were collected from nurseries in eastern, central, and western lower Michigan. Samples were washed with running water, soaked in 10% bleach for 5 minutes, then rinsed repeatedly in sterile water. The outer bark was removed and small sections were taken from canker margins and placed on potato dextrose agar (PDA) plates. The cultures were incubated for 5-7 days before evaluation.

Susceptibility of Four Spruce Species to *P. occulta*. Seedling trees of Norway spruce (*Picea abies*), Colorado blue spruce (*Picea pungens glauca*), white spruce (*Picea glauca*), and Spartan spruce (*Picea pungens* x *Picea glauca*) were purchased as 6-8" A-G (accelerated growth) plugs from Van's Pines, West Olive, MI. All the trees were grown in a peat based media (Baccto, Michigan Peat Company) under accelerated optimal growth conditions (3). The trees were at approximately the same stage of shoot expansion when inoculated (three weeks after budbreak). Sixteen plants of each species were inoculated. Eight trees of each species were root pruned by removing the pot and cutting the root ball in half, severing approximately 60% of the roots.

Inoculum was prepared from sporulating cultures of *P. occulta* by placing 3 ml of sterile deionized water onto the plates and scraping the surface gently with a sterile glass rod. The resulting suspension was filtered through a sterile triple layered cheesecloth and the concentration of α conidia determined with the aid of a hemacytometer. The suspension was further diluted with sterile water to a final concentration of approximately 1.21×10^7 α conidia/ml.

The inoculum was sprayed onto the seedlings with a spray bottle until droplets formed on the stems and leaves (about 30 ml per plant). The control trees were sprayed similarly with sterile deionized water. All plants were placed on a greenhouse mist bench and covered with a 4ml poly tent. Mist was kept on for 30 second intervals every 8 minutes for 72 hours. The mist heads were shielded to prevent water splash from inoculated plants to controls. Temperature in the chamber was 30°± 5°C (86°± 8°F), with 88% relative humidity. Disease symptoms were visually rated 6 weeks after inoculation. The ratings are based on a scale of 1 to 5, where 1 = no symptoms, and 5= dead.

Plant Growth and Pathogen Susceptibility. Colorado blue spruce seedlings used in this experiment were purchased as 6-8" A-G (accelerated growth) plugs from Van's Pines Nursery in West Olive, MI. They were potted Classic 600 nursery containers using a peat based media (Baccto, Michigan Peat Company). The seedlings were grown in a greenhouse under accelerated optimal growth conditions (3) for 4 months. The photoperiod was then reduced to 8 hours to allow budset. On March 10 1991, 90 plants were moved to a 4.4°C (40°F) vernalization cooler with an 8 hour photoperiod. Fifteen trees were moved from the cooler to the greenhouse every 2 weeks beginning on July 15 1991. After two weeks in the greenhouse the spruce were just beginning to break bud.

Bud development within each group was quite uniform, and each successive set responded in the same way. When the last set was brought to the greenhouse on September 23 1991, 10 trees from each group were sprayed with a conidial suspension of *P. occulta*. Six groups of seedlings were treated. The stages of shoot development were: dormant, breaking bud, and 2, 4, 6, and 8 weeks of shoot growth after budbreak. By eight weeks of expansion, lateral buds appeared to be fully formed with bud scales present.

A suspension containing $7.1X10^6$ α (alpha) conidia/ml was prepared from sporulating cultures of *P. occulta* grown on PDA. The conidia were harvested by placing 3 ml of sterile deionized water onto the plates and scraping the surface with a glass rod. The suspension was filtered through several layers of sterile cheesecloth, and the concentration of spores was determined with the aid of a hemacytometer. The solution was sprayed onto the seedlings with a spray bottle to the point of saturation. Control plants were sprayed similarly with sterile deionized water. After inoculation, all plants were placed on a greenhouse mist bench and covered with a 4ml poly tent. Mist was applied automatically for 30 second intervals every 2.5 minutes. The mist heads were shielded to prevent water splash from inoculated plants to controls. Temperature in the chamber was $30^\circ \pm 5^\circ C$ ($86^\circ \pm 8^\circ F$). Relative humidity was maintained at approximately 92%. After 72 hours the mist was turned off, and poly cover removed from the bench. Disease severity was determined four weeks later by counting the number of infected branches per tree.

***In Vitro* Fungicide Screening.** Fungicide amended agar was used to test the relative effectiveness of several fungicides *in vitro*. This 'poison agar' technique was used to evaluate the eight fungicides listed in Table 2.1. Potato dextrose agar (PDA) was combined with each compound to produce standard 100X15 mm petri plates containing 20 ml each of agar amended with 1, 10, 100, or 1000 ppm of fungicide. Ten plates were prepared for each treatment, including 10 unamended PDA controls.

Plugs of *P. occulta* were removed from 20 day old cultures using a 10 mm cork borer. The top half of each disk was transferred to the agar, where it was placed inverted in the center of the plate. Plates were placed in plastic bags in randomized complete blocks, and incubated in the dark at 25°C. Radial growth of each colony was measured after 10 days of incubation. The experiment was repeated once using 5 replicate plates per treatment.

Field Test of Benomyl. An experiment was conducted to test the effectiveness of benomyl applications on 15-18" Colorado blue spruce at various intervals before and after harvest. Benomyl was applied as Benlate 50 WP at 2.2kg/358*l* of water (1lb/100 gal) for all treatments. Preharvest sprays were applied by trained nursery personnel using a tractor sprayer. Postharvest treatments were applied with a hand sprayer equipped with a flat fan nozzle. The benomyl was sprayed to the point that droplets formed on the foliage. The experiment was set up as a 2X2X2 factorial. Plants were arranged in the holding area in randomized complete blocks.

Table 2.1. *In vitro fungicide screening*: Properties of the tested fungicides.

TRADE NAME	PRODUCER	COMMON NAME	CHEMICAL NAME	FORM
Banner	Ciba-Geigy	propiconazole	1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl] methyl]-H-1,2,4-triazole	1.6 EC
Bayleton	Mobay	triadimefon	1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-R-butanone	50 WP
Benlate	du Pont	benomyl	[methyl 1-(butyl carbamoyl)-2-benzamidazole carbamate]	50 WP
Bordow	Dow	bordeaux	copper sulfate and calcium hydroxide	12.75
Funginex	FMC	triforine	(n,n'-[1,4-piperazinediyl-bis-(2,2,2-trichloro-ethylidene)]-bis-formamide)	1.6 EC
Manzate 200	du Pont	mancozeb	manganese- ethylenebisdithiocarbamate	200 DF
Nova	Rohm & Haas	myclobutanil	σ-n-butyl-σ-(4-chlorophenyl) -1,4-1,2,4 triazole-1-propanitrile	40 W
Rubigan	Dow	fenarimol	σ-(2-chlorophenyl)-σ-(4-chlorophenyl)-5-pyrimidine methanol	1.2 EC

The three intervals of spray applications examined were: 1) field applications once per month during the weeks of June 21, July 22, and August 23 1990, prior to harvest, 2) a single application on October 19 (the day after field potting), and 3) three applications during shoot expansion the spring after harvest: April 29, May 20 and June 10, 1991. The check for factor one was a single application of benomyl on June 21, 1990. Factors 2 and 3 included unsprayed controls.

Disease symptom severity was evaluated on the 15th of the month in June, July, and August 1991, using a scale of 1 to 5, where 1 = symptomless, 2 = less than-one third of the plant showing needle loss, 3 = one-third to two-thirds of the branches affected, 4 = more than two-thirds of the plant showing needle loss, cankering, and death of shoots, and 5 = dead, as described by Igoe (10).

RESULTS AND DISCUSSION

Host Range and Distribution in Michigan. Inspection of landscape and Christmas tree nurseries across southern lower Michigan provided ample evidence that *P. occulta* is present and causing production losses. Cultures of *P. occulta* were isolated from tissue samples collected in Allegan, Ingham, Mason, Montcalm, Ottawa, and Saginaw Counties. In small plant culture, either in seed and liner beds or greenhouse plug production, it was very difficult to find evidence of *Phomopsis* infection. Seedlings in these production areas are intensively managed. Cultural programs usually include the use of the best growing conditions such as well drained sites, sterilized soil, supplemental irrigation, constant monitoring and rouging, weed control, and frequent applications of fungicides compared to normal field culture. However, *P. occulta* was isolated from one nursery bed where Colorado blue spruce liners (2+3) were planted over an old driveway, and were crowded in the bed. These stressed trees displayed curled and browned new shoots and needle loss, while plants in all other areas of the nursery expressed no injury.

Symptoms of *Phomopsis* infection were found consistently on trees recently transplanted (in 1990) to growing fields. Inspections of growing fields during 1990-1991 showed only an occasional flagging branch in older fields. Very small brown and purple cankers could be found under the bark on lower branches on established trees in fields where plants showed no obvious signs of blight.

Landscape sized (12-15" to 5-6') field potted and balled and burlapped spruce develop symptoms in holding areas of production nurseries, wholesale yards, and garden centers. Lower and older branches are most severely affected. These branches shed needles, and new shoots expand then wilt and die. One Michigan nursery operation suffered a postharvest loss as high as 65% of fall harvested stock which was kept in the nursery for evaluation after winter storage. Isolations from symptomatic plants yielded *Phomopsis occulta* from Colorado blue spruce, white (*P.glauca*), Black Hills (*P. glauca 'Densata'*) and Norway spruce (*P. abies*). *Phomopsis* was also found on symptomatic Engelmann (*P. engelmanii*) and Colorado blue X Engelmann hybrid spruce growing on a marginal site at Michigan State University. Other spruce species may also be hosts to this fungus. Due to the difficulty in distinguishing *Phomopsis* from other canker and needle cast diseases of spruce, it is strongly recommended that samples are submitted to a diagnostic service for positive identification before control measures are initiated.

Susceptibility of Four Spruce Species to *P. occulta*. Colorado blue spruce (*Picea pungens glauca*) was the most susceptible species of spruce tested in this study (Figure 2.1). The symptoms of *Phomopsis* were worsened somewhat by damaging the root system of blue spruce prior to inoculation, but the symptoms were severe to fatal without the added stress of root wounding. White spruce (*Picea glauca*) and Norway spruce (*Picea abies*) both showed some evidence of *P. occulta* infection, but showed a limited symptom development in plants with intact root systems. Mean values for plants with disturbed root systems were not

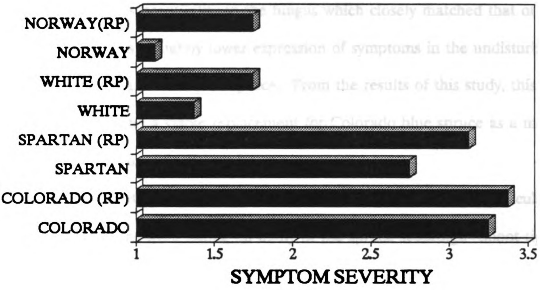

Figure 2.1. Severity of *Phomopsis* shoot blight symptoms on seedlings of Norway, Colorado, white, and Spartan spruce, evaluated six weeks after inoculation. (RP) = root system reduced by approximately 60% before inoculation.

significantly different for either species. The root pruning treatment did visibly affect several white and Norway spruce, which showed moderate *Phomopsis* symptoms, while others in the group remained symptomless.

Spartan spruce, a hybrid of blue by white spruce (*Picea pungens x P. glauca* 'Hyblue') showed susceptibility to the fungus which closely matched that of blue spruce. There was a slightly lower expression of symptoms in the undisturbed Spartan spruce than in the blue spruce. From the results of this study, this hybrid does not seem to be a viable replacement for Colorado blue spruce as a means of avoiding losses caused by *P. occulta.*

Plant Growth and Pathogen Susceptibility. Four weeks after inoculation tip blight symptoms were visible on many of the spruce seedlings. Shoot tips were curled, brown and beginning to drop needles. Seedlings from each set were symptomatic in various degrees, with the exception of those which were fully dormant when inoculated. The set which had been developing longest (eight weeks of shoot expansion) also appeared to be more resistant than groups with younger shoots, although the difference was not statistically significant. In each group there were individuals which were unaffected at four weeks after inoculation, resulting in a high degree of variation within treatments. The overall variation between treatments was still highly significant ($P > F = .0016$). The most severe symptoms appeared on plants which had undergone 2 weeks of shoot growth prior to inoculation (Figure 2.2). These seedlings were significantly more susceptible to colonization and canker development than plants in the other 5

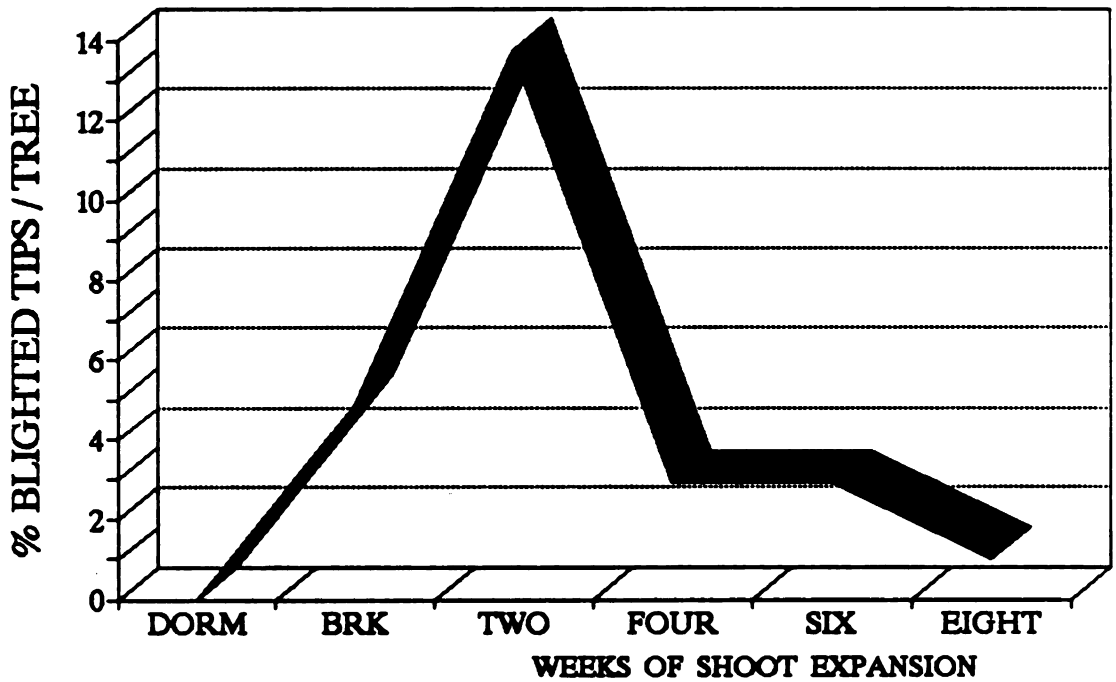

Figure 2.2 *Plant growth and pathogen susceptibility*: Percent of shoot tips blighted on Colorado blue spruce seedlings at six stages of shoot development. Plants evaluated 4 weeks after inoculation with conidia of *Phomopsis occulta*. Stages of shoot growth: DORM= dormant, BRK = budbreak, 2, 4, 6 and 8 weeks of shoot growth after budbreak.

groups. This does not prove that spruce are immune to infection by *P. occulta* during dormancy or after the shoots have hardened off. The *Phomopsis* fungi are known to form incipient infections without producing symptoms until conditions are favorable. However, the tissue which is the most vulnerable to canker development would likely .provide favorable conditions for an increased percentage of the incident conidia to successfully penetrate the host tissue under field conditions.

Fungicide sprays should be timed to protect the new growth from conidia penetration, and to suppress the development of existing infection sites when the plants are most susceptible. A series of applications of protectant fungicides should commence immediately at the first indication of bud break, and continue until the new shoots are fully developed, 8-10 weeks after budbreak. Though *P. occulta* conidia may be present later in the season, the number and severity of resulting infections should not result in unacceptable aesthetic or physiological damage. If the levels of incipient infections are reduced, and the trees are under no severe stress, they will perform well following harvest.

***In Vitro* Fungicide Screening.** Data from both replications of this study were pooled for analysis and presentation in the following discussion.

All of the compounds tested in this experiment were effective in inhibiting *P. occulta* mycelial growth at high concentrations (Figure 2.3). The colonies were inhibited completely at 1000 ppm a.i. of all the compounds except fenarimol, where colonies grew only about 7mm in radius in 10 days.

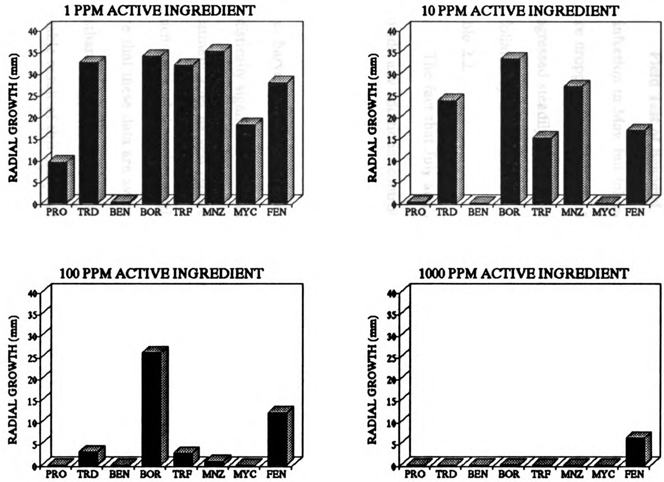

Figure 2.3. *In vitro fungicide screening*: Radial growth of *Phomopsis* colonies on fungicide amended agar after 10 days of incubation at 25°C. 10 mm mycelial plug transferred to plate from 21 day-old cultures. Data represent 15 replications. PRO = propiconazole, TRD = tridiamefon, BEN = benomyl, BOR = bordeaux, TRF = trifiorine, MAN = mancozeb, MYC = myclobutanil, FEN = fenarimol.

Propiconazole, benomyl, and myclobutanil were clearly the most effective at very low levels (1 ppm). Data obtained by Igoe for ipriodine and chlorothalonil are in agreement with the results found in this study for piperazine (10).

Field Test of Benomyl. The trees in this experiment showed few symptoms of infection in May, but following budbreak new shoots began to wilt, and needles were dropping by mid June. The symptom development for most treatments progressed steadily throughout the season. Cankered branches were completely defoliated by the August evaluation. The August evaluation data are presented in Table 2.2.

The fact that July and August applications of benomyl in the field did not provide any additional control was also observed in another study (Chapter 1). The June spray may have been beneficial since shoots are more susceptible to infection when succulent foliage is present. Benomyl applications on the first day after harvest and three times during shoot expansion after harvest were statistically significant factors in reducing disease severity. One fungicide treatment after harvest reduced average symptom severity from as well as trees which received three benomyl sprays the spring after harvest. It is important to note that these data are averaged over all other treatments. It is doubtful that postharvest applications alone will provide satisfactory control of *P. occulta*. The notable result of this study is the gradient in disease severity which decreases with additional applications of fungicide. Continuous spraying with fungicide helped to prevent the advance of the fungus. Trees in the treatment which received all seven sprays remained symptomless until the July evaluation, then showed only the slightest disease expression during the following months. However, it is unlikely such applications would be cost effective or practical in nursery production and subsequent handling. Retarding the growth of existing cankers is an inefficient strategy. Chemical control should be applied to protect the trees from infection. This approach will provide better control with less pesticide input and labor.

The long term suppression of *P. occulta* with chemical control agents will be most successful if anti-resistance strategies are adopted at the initiation of the control program. Minimizing the total use of fungicides and particularly the use of any one agent reduces the selection pressure on the pathogen, delaying or preventing the buildup of resistant strains (6,22). Methods for management of resistance to benzamidole fungicides include cultural practices, resistant genotypes, and the use of a variety of fungicides (7). Lacking effective cultural control methods or resistant blue spruce, the primary management tool available in the case of *Phomopsis* is the use of several different fungicides. Companion fungicides can be used in alternation, mixtures, or combinations of mixtures and alternations. The fungicides should be chosen from two or more classes of chemicals with different modes of action, in order to avoid cross-resistance (21)

Table 2.2. *Field test of benomyl*: *Phomopsis* symptom severity on naturally infected, field-grown Colorado blue spruce when treated with benomyl in the field and after harvest. Trees harvested October 18, 1990, and evaluated August 15, 1991.

	Symptom Severity[x]			
TIMING	Sprayed 1st Day Postharvest		Unsprayed 1st Day Postharvest	
	3 Applications During Shoot Expansion√			
Field Applications	YES	NO	YES	NO
June Only	1.2	1.3	1.4	2.1
June, July, and August	1.0	1.2	1.2	1.8

Source of Variation	
Field	NS
1st Day Postharvest (PH)	**
Field x PH	NS
Shoot Expansion√ (SE)	**
Field x SE	NS
PH x SE	NS
Field x PH x SE	NS

[x] Symptom severity rated on a scale of 1 (no symptoms) to 5 (dead); trees rating higher than two would be considered unsalable.

√ Benomyl applied at budbreak, then three weeks, and seven weeks later.

NS, ** = nonsignificant at P = 0.05, and significant at P = 0.01 respectively, according to the F test.

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