Historic, archived document

Do not assume content reflects current
scientific knowledge, policies, or practices.

A99.1
F-7644
Csp. 3

A SEARCH FOR PHYTOTOXINS INFLUENCING GERMINATION AND EARLY GROWTH OF PONDEROSA PINE

**RICK G. KELSEY
MICHAEL G. HARRINGTON**

USDA Forest Service Research Paper INT-216
INTERMOUNTAIN FOREST AND RANGE EXPERIMENT STATION
FOREST SERVICE, U.S. DEPARTMENT OF AGRICULTURE

THE AUTHORS

RICK G. KELSEY, is currently a senior research associate with the Wood Chemistry Laboratory, Department of Chemistry, University of Montana at Missoula. He received his B.S. (1970) and Ph.D. (1974) in forestry from the University of Montana.

MICHAEL G. HARRINGTON is currently a research forester with the Rocky Mountain Forest and Range Experiment Station in Tempe, Arizona. The research reported in this paper was conducted in cooperation with the Intermountain Forest and Range Experiment Station's Northern Forest Fire Laboratory, Missoula, Montana. Mr. Harrington received his B.S. in forestry (1970) and his M.A. in botany (1977) from the University of Montana.

USDA Forest Service
Research Paper INT-216
February 1979

A SEARCH FOR PHYTOTOXINS INFLUENCING GERMINATION AND EARLY GROWTH OF PONDEROSA PINE

**Rick G. Kelsey
Michael G. Harrington**

INTERMOUNTAIN FOREST AND RANGE EXPERIMENT STATION
Forest Service
U.S. Department of Agriculture
Ogden, Utah 84401

ACKNOWLEDGMENTS

The authors thank Mr. William Fischer of the Northern Forest Fire Laboratory and Dr. Fred Shafizadeh, Director and Professor of the Wood Chemistry Laboratory, for their advice and technical and financial support throughout this study. They also thank the many scientists, faculty, and technicians at the Northern Forest Fire Laboratory and the University of Montana who provided facilities and expertise.

Special thanks are extended to Missoula County for use of greenhouse facilities and Montana Fish and Game Department for the field study site on the Blackfoot-Clearwater Game Range.

This report was prepared as a cooperative project of the Intermountain Forest and Range Experiment Station and the Wood Chemistry Laboratory, University of Montana, Missoula.

RESEARCH SUMMARY

A series of laboratory and field experiments were conducted to determine if mature ponderosa pines produce a substance (phytotoxin) that inhibits the germination and growth of seedlings directly under the tree crown.

Neither live nor dead materials collected from ponderosa pines produced either volatile or water-soluble phytotoxins that drastically inhibited germination of seeds or growth of seedlings.

Seeds overwintering beneath the canopy of mature pines, or planted in soils collected there, showed reduced germination. Exact cause of the reduction was not determined. If weak phytotoxins were responsible, they did not inhibit growth of seedlings that germinated.

CONTENTS

	Page
INTRODUCTION.	1
LITERATURE REVIEW.	2
EXPERIMENTAL STUDIES AND RESULTS	4
Seed Source and Preparation.	4
Tests for Volatile Inhibitors	4
Tests for Water Soluble Toxins	5
Toxicity of Plant Leachates on Soils.	10
Field Soil Bioassay.	13
Seedbed Effect on Germination.	15
ALLELOPATHY AND MYCORRHIZAE	19
SUMMARY.	19
CONCLUSIONS	20
PUBLICATIONS CITED	21

INTRODUCTION

Pinus ponderosa Laws. is found throughout the western United States from Canada to Mexico making it the most widely distributed pine species in North America (Curtis and Lynch 1965). Although it grows under a variety of environmental conditions and different forest types, ponderosa pine is most commonly associated with sites that are characterized by low soil moisture and high surface temperature at some time during the growing season (Pearson 1951, Foiles and Curtis 1973). Because of its drought resistance ponderosa pine can survive in areas that are too dry for other conifer species (Foiles and Curtis 1973). Only on the driest sites does ponderosa pine become the climax species.

Observations indicate that virgin ponderosa pine stands were uneven-aged stands composed of even-aged groups (Cooper 1960, Weaver 1943). The age class structure of the stand was maintained by a series of continually occurring natural events. A few trees or groups of trees would be killed or die from insect attack, disease, or old age. Fires, which are known to have been frequent in virgin pine (Weaver 1959), would consume the remains of these trees, often leaving an opening and good seedbed for young ponderosa pine seedlings. Because of the optimum conditions, a large number of seedlings would likely become established on this seedbed if a seed source was available. Subsequent fires would remove the smaller trees from the dense areas of reproduction until the most vigorous and rapidly growing saplings were no longer susceptible. The result was a group of even-aged trees that continued their growth until the cycle was repeated (Weaver 1943). Seedlings occasionally became established beneath the larger mature and overmature trees, but they seldom survived the fires that burned the deep layers of litter and debris. The elimination of reproduction within the pine stand left an open forest floor, usually covered with nutritious vegetation and providing a park-like appearance to the forest as described by early travelers (Cooper 1960, Biswell 1973).

Seedlings that grew beneath mature trees were very susceptible to fire because of stunted growth and poor vigor. Cooper (1960) reported that even seedlings that grew within an open canopy of mature ponderosa pine grew very slowly and were stunted and unhealthy. The poor growth was attributed to competition for soil moisture rather than light. The effect of light should not be overlooked, however, because light shading of pine seedlings will reduce growth compared to those in full sunlight, and heavy shade can cause death (Pearson 1936, 1940). McDonald (1976) discovered that seedling height growth was significantly reduced by the presence of mature seed trees 20 feet or closer, and the greater the seed tree density the poorer the height growth. Also, removal of the seed trees did not immediately eliminate the inhibitory effect. Moisture depletion by the seed trees was considered the major limiting factor, but it did not explain continued inhibition after the seed trees were removed. Possible explanations were nutrient depletion and toxic substances produced by decaying seed-tree roots.

The following report describes the results from five experiments (a test for volatile inhibitors, a test for water soluble toxins, the toxicity of plant leachates on soils, a field soil bioassay, and the effect of seedbeds on germination) that were conducted to determine if phytotoxins are released from mature ponderosa pine trees or the decomposition of its litter, and if these phytotoxins drastically reduced the germination or growth of ponderosa pine seeds and seedlings.

LITERATURE REVIEW

In recent years, natural plant chemicals have been shown to influence the ecological relationships of plant-plant, plant-animal, and plant-insect interactions (Sondheimer and Simeone 1970, Rice 1974). Plant-plant chemical interaction, or allelopathy, is defined by Rice (1974) as "any direct or indirect harmful effect by one plant (including micro-organisms) on another through the production of chemical compounds that escape into the environment." This definition provides for three factors that must be present in order for allelopathy to be functioning in a plant community: (1) a plant must produce chemicals, (2) these chemicals must get out of the plant and into the environment, and (3) once in the environment, they must have a harmful effect on the plants in the area either directly or indirectly. If any one of these three requirements is absent, then allelopathy is not functioning. It is also important to emphasize that allelopathy is independent of competition. Allelopathy involves the placement of natural chemicals into the environment, whereas competition involves the rivalry for environmental factors such as light, water, nutrients, etc. (Rice 1974).

The mechanisms for releasing phytotoxins into the environment are quite variable and dependent upon environmental characteristics. The mechanisms include the leaching of the aerial portions of the plant by rain or fog drip, exudation from the roots, residue left or produced during the decay of plant material, and volatilization (Whittaker 1970, Rice 1974).

Whittaker (1970) considers allelopathy, or the use of a chemical defense by plants, as a universal phenomenon; however, he states that the actual significance of allelopathy is difficult to judge for three reasons. First, the influence of chemistry on a community is not always conspicuous to the observer of community relationships; second, there are different degrees of chemical interaction; and third, species may have an evolved tolerance to the chemical defense of others.

Allelopathy or chemical defense in plants is usually thought of as a mechanism by which one species is reduced or eliminated by another species in a community. Occasionally, the chemicals released are not species specific and they are toxic to the producer species as well. An excellent example occurs in the old-field succession in central Oklahoma and southeast Kansas.

Old-field succession in Oklahoma is characterized by a 2- to 3-year pioneer weed stage, 9- to 13-year annual grass stage, a perennial bunch grass stage of 13 to 30+ years, and finally true prairie, which has never been reached in old-field succession. The initial pioneer weed stage is short because these plants have been shown to eliminate themselves through the production of phytotoxins. These compounds have little effect on the species of the annual grass stage, which then became abundant (Rice 1974). A similar phenomenon has been observed in the replant problem of fruit trees including cherries, grapes, apples, peaches, apricots and plums. When seedlings of these species are replanted in orchards of the same species, they frequently exhibit retarded shoot and root growth or occasionally even die. Young trees that produce fruit provide lower yields than trees of comparable age planted in nonorchard sites. Detailed studies with peaches and apples indicate that compounds produced by the trees, particularly in the roots, are broken down or rearranged by micro-organisms to toxic compounds that retard seedling growth (Rice 1974).

Allelopathic interactions have been well documented in a variety of deciduous tree species. *Eucalyptus globulus* Labill. and *E. camaldulensis* Dehn. influence herbaceous vegetation through chemicals that escape into the environment (del Moral and Muller 1969, 1970). Sycamore, *Platanus Occidentalis* L. (Al-Naib and Rice 1971), and hackberry, *Celtis laevigata* Willd. (Lodhi and Rice 1971, Lodhi 1975) are characterized by sparse vegetation beneath their canopies. Experiments with decaying leaves, leaf leachates, and soils collected beneath the trees lead to the conclusion that phytotoxins were a significant factor. Several oak species including *Quercus stellata* Wang., *Q. marilandica* Muench. (McPherson and Thompson 1972) and *Q. falcata* var. *pagodaefolia* Ell. (DeBell 1971) produce compounds that inhibit the growth in understory plants.

Another tree species, *Grevillea robusta* Cunn., in the subtropical rain forest of Australia cannot regenerate in pure stands (Webb and others 1967). Experiments were conducted to show that a water-transferable factor in the rhizosphere from the older trees was responsible for this phenomenon. The commercial production of these trees may not be possible in pure stands, and a polyculture will be required unless special care and treatment is used to remove toxins from the soil.

Since ponderosa pine is climax only on the driest of sites, the production of an autotoxic chemical could provide survival value to the species. When moisture is limiting, adequate growth and vigor can only be maintained at lower tree densities. Therefore, to insure lower densities, chemicals from the mature trees could, perhaps, reduce the germination and growth of seedlings, which could then be eliminated by drought or frequent fires. This would prevent overcrowding, stagnation, and competition between members of the same species. Hall (1976) stated that he suspected "... a selective inhibitory substance in ponderosa pine litter that is destroyed with periodic underburning. Without fire, this substance is free to build up in the soil and reduce pine growth." If this is the case then fire played a dual role in the ecology of ponderosa pine, it eliminated unhealthy seedlings from within mature pine stands, and it prepared seedbeds for pine seeds in open areas by destroying phytotoxins, releasing nutrients and reducing competition from other species.

There is some experimental evidence that suggests ponderosa pine produces phytotoxins. Jameson (1961) reported that water extracts of ponderosa pine needles inhibited wheat radicle growth by 86 percent. Needle extracts also inhibited the radicle growth of two native grass species, *Sitanion hystrix* (Nutt.) J. G. Smith and *Bouteloua gracilis* (H.B.K.) Lag., and *Pinus ponderosa* (Jameson 1968). Del Moral and Cates (1971) observed inhibition of barley by ponderosa pine volatiles and litter water extracts, although field analysis suggested only weak allelopathic influence with associated native species. Eckert (1975) has revealed results which indicate that ponderosa pine needles produce chemicals that adversely affect some associated understory species, creating obvious ecotones near ponderosa pine trees.

EXPERIMENTAL STUDIES AND RESULTS

Seed Source and Preparation

The seeds used for experiments during the summer of 1974 were obtained from the U.S. Department of Agriculture Forest Service Nursery at Coeur d'Alene, Idaho. These seeds had been collected from the Seeley Lake Ranger District at an elevation of 4,000 feet $\pm$ 500 feet.

During the fall of 1974, approximately 1,000 pine cones were clipped from large ponderosa pine trees on the Blackfoot-Clearwater Game Range, which is adjacent to the Seeley Lake Ranger District, and the location of our field study site where field germination and growth experiments were conducted. The field results will be reported in a separate paper. Approximately 10 pounds of clean seeds were collected. All experiments conducted from the fall of 1974 through 1975 used the seeds from this source.

The seeds in all experiments were soaked in warm water for 24 hours and then placed in 3 percent Chlorox solution for 15 minutes to kill seedcoat pathogens. After soaking in Chlorox, the seeds were stratified at 1° to 5°C for a minimum of 2 weeks. Just prior to use, the stratified seeds were again soaked for 15 minutes in the 3 percent Chlorox solution.

Tests for Volatile Inhibitors

Materials and Methods

To test for the presence of volatile toxins in pine tissues, green needles, surface litter (dried pine needles), decomposing duff (decaying pine needles), roots (1 to 4 cm in diameter), and bark, were collected at the field study site, placed in plastic bags and returned to the laboratory.

The technique used to test for toxic volatile compounds was similar to that described by Muller, and others (1964). Sterilized cellulose sponges were brought to maximum water holding capacity by soaking them in distilled water and allowing them to drain. The wet sponges were placed in sterile 100 x 80 mm petri dishes, and each sponge was covered with 7.0 cm filter paper soaked with distilled water. Fifteen pine seeds were then placed on top of the filter paper.

Two grams of each fresh plant material, described above, were broken up by hand and placed into separate 5 x 2 cm plastic vials. One plastic vial containing plant material (treatment) or no plant material (control) was placed into each petri dish adjacent to the sponge seedbed, permitting only gaseous contact between plant material and seeds. The petri dish was covered and placed randomly into a dark conditioning chamber set at 25°C $\pm$ 1°. Five replications were set up for each of the five plant materials and the control.

Germination was recorded daily during which time the seeds were exposed to approximately 1 hour of light. After germination was completed, the radicle lengths were measured from root tip to seed coat or from root tip to the start of the stem in

Table 1.--*Effect of volatile compounds from five ponderosa pine plant materials on the germination and radicle growth of ponderosa pine seedlings.*

Treatment	Germination		Growth rate ²	
	Experiment	Experiment	Experiment	Experiment
	A	B	A	B
	-----Percent-----		-----cm/day-----	
Control	¹ 40.0a	57.3a	0.74a	0.86a
Root	40.0a	70.0a	.77a	.85a
Litter	33.3a	52.0a	.84a	.93a
Green needle	32.0a	65.3a	.80a	.85a
Duff	29.3a	40.0a	.95a	.90a
Bark	28.0a	61.3a	.79a	.84a

¹All numbers followed by different letters are significantly different at the 5 percent level or greater.

²Growth rate was obtained by dividing average seedling length per replication by average seedling age per replication.

larger seedlings. Since seedling age certainly influences seedling size, growth rates were computed to eliminate the effect of age differences and allow concentration on treatment differences.

The entire experiment was repeated using fresh plant material collected on the same date but stored in plastic bags in a cold room. The two experiments were identical except for the length of the germination periods. In the first experiment A, the germination period was 14 days and in the second experiment B, only 10 days. The germination period was longer in experiment A because of slow germination rates.

For statistical analysis, one-way analysis of variance tests were used. Following these, the Newman-Keuls test for multiple comparisons was utilized to compare treatment means (Hamilton 1965). The homogeneity of the variances was analyzed in all experiments prior to the analysis of variance.

Results

The results of experiments A and B are provided in table 1. It is apparent that there were no volatile compounds in the green needles, roots, bark, surface litter, and decomposing duff of ponderosa pine that inhibited the germination or radicle growth of pine seedlings.

Tests for Water Soluble Toxins

Materials and Methods

One month prior to the start of this experiment, buckets lined with plastic bags and covered with a double layer of cheesecloth secured by rubber bands were placed in openings and beneath the crowns of large ponderosa pine trees at the game range. The "pure" rain water was collected in the openings while that collected beneath the crown canopy was considered at natural crown leachate or throughfall and a potential source of phytotoxins. In addition, the rain water that ran down the bark (stemflow) was

collected by placing metal troughs around the base of several trees. The rain water, throughfall, and stemflow collected was stored in plastic containers in a cold room at 3° to 4°C until used.

Several days prior to the start of this experiment, fresh plant material including green needles, litter, decomposing duff, bark, and roots were collected. These samples were returned to the laboratory, placed in an oven set at 40°C, and allowed to dry for 48 hours. All the dried samples except the roots were run through a grinder. The roots were cut into small pieces by hand. The grinder was adjusted so that it would break and crush the dried plant material into smaller pieces but not reduce it to a powder. Each sample was then extracted by placing 225 grams of plant material into 2,025 ml of distilled water to give a 10 percent solution (w/v). The solutions were placed on a shaker for 30 minutes and left overnight, 16 to 20 hours. The plant material was then removed by filtering through paper. Five percent solutions were made for each sample by diluting (1:1) with distilled water. In addition, two of Hoagland's solutions were prepared, a standard Hoagland's (1x) and 5x concentrated Hoagland's, to check potential osmotic effects on germination. A total of 16 different test solutions was analyzed in this experiment: 10 plant extracts, 3 field rain samples (throughfall, stemflow, rain water), 2 concentrations of Hoagland's solution, and 1 distilled water control.

The pH was measured for all the test solutions, and the osmotic potential was determined with a Fiske Osmometer for the 10 percent plant extracts, both Hoagland's solutions, and all the rain samples from the field.

Sterilized cellulose sponges were soaked to water holding capacity in each of the test solutions, and excess solution was allowed to drain off. Each moist sponge was placed into the bottom of a 9.0 cm plastic sterile petri dish and covered with a 7.0 cm piece of filter paper soaked in the corresponding solution. Fifteen stratified and sterilized ponderosa pine seeds were placed on the filter paper. Each petri dish was covered and placed randomly into a dark conditioning chamber set at 25°C ± 1°. Ten replications were set up for each treatment.

This entire experiment was conducted twice, the first time (experiment A) was in the summer of 1974 and the second time (experiment B) in the summer of 1975. The germination time for experiment A was 9 days and in experiment B, 11 days. During the summer of 1974, the stemflow was collected from two different rainstorms. The first storm was a drizzling rain, and the second was a very short cloudburst. The drizzling rain produced a light brown stemflow, and the cloudburst produced a very dark brown solution. Because of the color differences, these two samples were kept separate and each tested independently. The stem flow for 1975 was a combination of samples from several storms.

In order to better understand the effect that pH might have on germination, a small germination experiment was conducted using three pH solutions, 3.0, 5.0, and 7.0. Twenty-five stratified ponderosa pine seeds were placed on top of a filter paper and sponge previously soaked in the test solutions. Six replicates were made for each solution, and all dishes were randomly placed into a conditioning chamber. The germination was checked daily for 9 days. A one-way analysis of variance followed by the Newman-Keuls test was used for statistical analysis.

Results and Discussion

A variety of comparisons can be made between the different solutions tested in this experiment, but the most meaningful for evaluating the presence of a phytotoxin are the comparisons between the plant extracts and the distilled water and "pure" rain controls. In addition, the effect of osmotic potential and pH of the test solutions (table 2) should also be evaluated to ensure that they have not produced the experimental responses observed.

Table 2.--*The pH and osmotic potential of test solutions obtained by artificial extraction and natural leaching of ponderosa pine plant materials*

Solution	Experiment A		Solution	pH
	pH	Osmotic Potential (Atms.)		
Stemflow-2	2.9	-0.3	Bark 10%	3.5
Stemflow-1	3.2	-0.2	Stemflow	3.6
Bark 10%	3.2	-0.4	Green Needle 10%	3.8
Bark 5%	3.5	¹ --	Green Needle 5%	3.9
Green Needle 5%	4.0	--	Bark 5%	3.9
Green Needle 10%	4.0	-2.3	Litter 10%	4.1
Litter 5%	4.0	--	Duff 10%	4.3
Litter 10%	4.1	--	Duff 5%	4.4
Roots 5%	4.1	--	Litter 5%	4.4
Throughfall	4.4	< -0.2	Throughfall	4.7
Roots 10%	4.5	-0.7	Roots 10%	4.9
5x Hoagland	4.5	-3.2	Roots 5%	5.0
Hoagland	4.8	-0.7	Rain	5.9
Duff 10%	5.4	--	Control-Distilled H ₂ O	7.1
Duff 5%	5.9	--	5x Hoagland	7.1
Rain	6.1	<-0.2	Hoagland	7.3
Control-Distilled H ₂ O	6.9	<-0.2		

¹The osmotic potentials were not measured for the 5 percent solutions. Accurate osmotic potentials could not be obtained for the litter and duff 10 percent solutions because of suspended particulates.

In experiment A, green needle 5 percent was the only test solution with a significantly lower germination than the distilled water control (table 3). The germination of pine seeds in rainwater was not significantly different from any of the plant extracts. In experiment B, the germination of the control was significantly greater than green needle 10 percent, whereas the rain was greater than duff 5 and 10 percent, roots 5 and 10 percent, green needle 5 and 10 percent, bark 10 percent, and stemflow.

In both experiments A and B, there was a tendency for those solutions with a low pH to also have a low germination, whereas those solutions with a high pH had a high germination. In a side experiment testing the effects of solution pH only, germination was unaffected by pH values, 3.0, 5.0, and 7.0. Seed germination values varied insignificantly from a low of 80.7 percent in the pH 3.0 solution to a high of 84.0 percent in the pH 7.0 solution. However, the pH of the solutions in experiments A and B could have changed the toxicity of the solutions if the toxic compounds were sensitive to acid or base.

It has been well documented that ponderosa pine seeds exhibit the greatest germination when under some water stress. Germination is usually the highest in solutions with an osmotic potential near -3 atmospheres (Larson and Schubert 1969, Djavanshir and Reid 1975, Rietveld 1975). The lowest osmotic potential recorded in experiments A and B was -3.2 atmospheres for the 5x Hoagland's solutions (table 2); consequently, osmotic potential was not responsible for poor germination in these experiments.

Table 3.--Effect of water soluble compounds from ponderosa pine plant materials on germination of ponderosa pine seeds (percent)

Experiment	Germination treatment																
	H	D-5	C	D-10	TF	5xH	L-5	Rain	L-10	R-10	SF-2	B-10	SF-1	R-5	B-5	GN-10	GN-5
A	42.0	39.3	32.7	30.0	29.3	28.7	26.0	25.3	24.7	24.0	22.0	21.3	20.0	20.0	19.3	19.3	15.3
B	Rain	H	5xH	L-10	B-5	TF	C	L-5	B-10	D-5	SF	GN-5	R-5	D-10	R-10	GN-10	
	79.3	74.0	68.0	66.7	66.7	66.7	64.7	64.0	62.7	62.0	54.0	52.7	51.3	50.7	49.3	38.7	

Abbreviations: H = lx Hoaglands; 5xH = 5x Hoaglands; D = Duff, 5 or 10 percent concentration; C = Distilled water control; TF = Throughfall; L = Litter; Rain = Natural rain water; R = Root; SF = Stemflow, 1 and 2 represents collections from two different rainstorms; B = Bark; GN = Green needle. .
Numbers not underscored by the same line are significantly different at the 5 percent level.

Table 4.--The effects of water soluble compounds from ponderosa pine plant materials on the radicle growth of ponderosa pine seedlings (cm/day)

Experiment	Growth treatment																
	L-5	TF	SF-1	B-5	Rain	B-10	H	C	D-5	SF-2	D-10	L-10	R-5	R-10	GN-5	5xH	GN-10
A	1.00	0.99	0.94	0.92	0.92	0.91	0.86	0.85	0.85	0.81	0.81	0.73	0.70	0.66	0.45	0.45	0.44
B	0.70	0.70	0.68	0.66	0.65	0.65	0.64	0.62	0.62	0.62	0.61	0.52	0.50	0.50	0.28	0.15	

Abbreviations: same as Table 3.

Abbreviations: same as table 3.
Numbers not underscored by the same line are significantly different at the 5 percent level.

Numbers not underscored by the same line are significantly different at the 5 percent level. Growth rates were obtained by dividing average seedling length per replication by average seedling age per replication.

Examining growth rates for experiment A, table 4 shows that both the distilled water control and the rain were significantly greater than the green needle 5 and 10 percent, and the 5x Hoagland's solution. In addition, the rain was greater than the roots 5 and 10 percent. In experiment B, both the distilled water and the rain were significantly greater than roots 5 and 10 percent, duff 10 percent, and green needle 5 and 10 percent.

The pH of the test solutions had very little influence on the radicle growth of pine seedlings. However, the radicle growth rates of seedlings in solutions with low osmotic potential were significantly lower than for seedlings in solutions of high osmotic potential, suggesting that osmotic potential was important. The growth of ponderosa pine radicles is very sensitive to osmotic potential and generally decreases quite rapidly as the osmotic potential decreases (Larson and Schubert 1969, Djavanshir and Reid 1975, Rietveld 1975). Therefore, some of the reduction in radicle growth associated with 5x Hoagland's (-3.2 atms) and green needle 10 percent (-2.3 atms) can be attributed to their low osmotic potentials.

The green needle extracts both 5 and 10 percent might have contained a phytotoxin, because they significantly reduced germination and radicle growth rates. However, the significance of the green needle results was reduced by the occurrence of fungal and bacterial growth in both experiments A and B. It was the only solution tested that became significantly contaminated. Daily visual observations suggested that the bacteria and fungi did not become important until the later half of each experiment and probably had less influence on germination than on radicle growth. From these visual observations, it was clear that the green needle extracts did reduce radicle growth of the pine seedlings, but the final results were a combination of extract and fungal effects. In addition, the osmotic potential of the green needle extracts was low enough to be a contributing factor to the reduced radicle growth, but not low enough to have reduced germination. It can be concluded that the germination results for the green needle extracts are reliable, but the results for the radicle growth are questionable. If the green needles of ponderosa pine do contain a phytotoxin, then the toxin could be washed from the needles during a rain storm. Therefore, the activity of throughfall could help in evaluating the presence of a green needle toxin. There was no significant difference in germination or growth between throughfall and pure rain treatments in either experiment A or B. This, however, does not eliminate the presence of a very weak toxin in the throughfall that accumulates with time on the forest floor.

There are three other plant extracts, roots, duff, and bark that could also contain a phytotoxin. Both the 5 and 10 percent solutions of roots significantly reduced radicle growth compared to the rain in experiments A and B and to the distilled water control in experiment B. The root extracts had less effect on germination, with a significantly lower germination than rain in experiment B. This suggests the presence of a growth inhibitor within the roots of ponderosa pine. The severity of inhibition is certainly not great as seen in table 3. In the field, pine seedlings germinate on or near the soil surface well above the roots of pine trees; it is very unlikely that this weak toxin would influence seed germination and initial root growth; however, it might influence the later root development.

The germination of seeds in duff 5 and 10 percent solution was significantly lower than rain only in experiment B. Duff 10 percent also reduced radicle growth rates significantly below the control and rain in experiment B; there was no significant difference in experiment A. If there is a toxin present in duff extracts, it is very weak because only the 10 percent solution demonstrated much activity.

The bark 10 percent and its natural counterpart, stemflow, reduced germination compared to rain in experiment B. Neither solution interfered with the radicle growth.

To summarize, the results of this experiment indicated that the water extract of green needles, roots, duff, and bark of ponderosa pine might contain growth inhibitors. Closer observation, however, indicates that the inhibition of radicle growth by the green needle extracts may have been the combined effects of inhibitor, osmotic potential, and fungal activity. The reduced germination caused by the green needles was most likely the work of the inhibitor alone. Throughfall, which is a natural leachate of pine needles, had no effect on the germination or radicle growth of the pine seeds. Therefore these results do not support the presence of a strong inhibitor, but they do not completely eliminate the presence of a weak toxin that may occur in low concentrations in the leachate and accumulates on or in the soil. The water extract of roots reduced germination only slightly, but consistently reduced radicle growth rates. Although the root extracts were inhibitory, they did not severely damage the growing ability of the pine radicles. The duff extracts were marginally toxic because the 5 and 10 percent solutions were inhibitory to germination in experiment B; also the 10 percent solution reduced radicle growth in experiment B. Germination reduction by bark and stemflow was minimal.

It can be concluded from this experiment that the green needles and roots of ponderosa pine are the most likely tissues containing phytotoxins that interfere with the germination and growth of ponderosa pine seedlings. Bark and duff may contain a weak toxin, but it is probably not significant unless it accumulates in the soil.

Toxicity of Plant Leachates on Soils

Materials and Methods

This experiment was conducted twice, experiment A in the summer of 1974 and experiment B in the summer of 1975. There were some technical differences in the setup of each, so the two experimental designs will be discussed separately.

In experiment A, large samples of green needles, bark, litter, and decomposing duff were collected at the field site and returned to the laboratory. Each plant material was separately placed on a plastic-lined trough and sprayed with a fine mist of distilled water on a ratio of 1 part plant material to 4 parts of water. The leachate was collected and filtered through paper, approximately one-third was stored in a cold room at 1° to 4°C, and the remaining two-thirds was frozen until needed.

Soil was collected in large openings away from pine tree influence at the game range. These soils were passed through a 2.0 mm sieve, mixed thoroughly, and then placed into 4-inch plastic pots. Ten replicates were set up for each leachate and a distilled water control. Fifteen stratified and sterilized ponderosa pine seeds were added to each pot. The ten treatment replicates were each watered with 75 ml of their corresponding leachate or distilled water in the case of the control. The treatments were then randomly placed in the greenhouse. The pots were watered when needed with 25 ml of the corresponding solution. Germination was checked every day for 15 days, then all seedlings were allowed to grow an additional 10 days at which time all but the largest three seedlings were removed. The remaining three seedlings were allowed an additional 1 month growing period at which time the seedlings were harvested, oven-dried at 70°C for 48 hours, and weighed.

In experiment B the same four plant materials were collected and prepared as in experiment A. In addition to the distilled water control, a Hoagland's solution was included in the list of test solutions. This treatment was added as a nutrient-enriched control for comparison with pine leachates that might have contained higher quantities of nutrients than the distilled water.

It had been observed in experiment A that the roots of the 2-month-old seedlings were coiled around the bottom of the 4-inch pots. In order to eliminate physical

barriers for root growth in experiment B, the sieved soil was placed in 30 by 30 by 4 cm root boxes in the greenhouse. Thirty stratified and sterilized ponderosa pine seeds were planted in each box. Three replicates were set up for each of the four leachates, the Hoagland's solution, and the distilled water control. Each root box was initially watered with 200 ml of the appropriate test solution followed by 100 ml every other day.

The seedlings were allowed to germinate and grow for 35 days, then all but the largest 10 seedlings were removed from each root box. Germination was checked daily until the thinning. The remaining 10 seedlings were allowed to grow an additional 29 days at which time the soil was washed away from the roots and the seedlings collected. The root lengths, stem lengths, number of laterals over 1.0 cm, and biomass of the roots and crowns were measured for each treatment.

The one-way analysis of variance and Newman-Keuls tests were performed for statistical analysis.

Results and Discussion

In both experiments A and B, there was no significant difference in germination between any of the plant leachates and the distilled water control (table 5). However, the seeds watered with the Hoagland's solution in experiment B had a significantly lower germination than the seeds watered with the four plant solutions and the control. These data show that under these conditions, none of the leachates contained phytotoxins that inhibited the germination of pine seeds. The reason for low germination in the Hoagland's treatment is uncertain.

In experiment A there was no significant difference in total seedling weight and shoot weight between the treatments. There was, however, a significant difference in root weights for duff and bark compared to the control. The reduced root weights of the duff treatment resulted in a shoot/root ratio that was significantly larger than all other treatments and the control.

In experiment B there was no significant difference in total seedling weight, root and shoot weight, or shoot/root ratios between the plant leachate treatments and the distilled water control. The Hoagland's solution was significantly lower in root weight than the other solutions, which again resulted in a very large shoot/root ratio.

In experiment B the entire seedlings were extracted from the root boxes, the roots and crown lengths were measured, and the number of lateral roots greater than 1.0 cm were counted. The taproots of seedlings grown in the Hoagland's solution were equal in length with the taproots from seedlings grown in the other solutions (table 5), but the Hoagland's seedlings had a reduced number of lateral roots. A reduction in the number of laterals resulted in reduced root weights for the Hoagland's treatment but is probably not representative of a disadvantaged seedling under these growing conditions. A similar situation could have existed in experiment A reducing the root weights of seedlings watered with duff and bark leachates. Neither bark nor duff leachates reduced root growth in experiment B.

Although this experiment produced no evidence of a germination inhibitor, the experimental design did not eliminate all mechanisms of inhibition associated with leachates. Plant compounds may have to accumulate in the soils over a period of time to reach active concentrations. In this experiment, the soils were not watered with plant leachate until the seeds were sown, and the germination was quite rapid. Therefore, there may not have been sufficient time for the inhibitor to accumulate to the point where it could have affected germination. It is also possible that compounds in the leachates are broken down or rearranged to produce the inhibitor and there may not have been sufficient time for the breakdown product to reach inhibitory concentrations. Therefore, this experiment did not disprove these two mechanisms.

Table 5.--The effect of artificial leachates from ponderosa pine on seed germination and seedling growth of ponderosa pine in soil.

Parameter	Artificial leachate											
	Litter			Green needle			Bark			Duff		
	A	B		A	B		A	B		A	B	
Germination (percent)	66.0a	76.7a		64.0a	67.9a		63.3a	71.1a		62.0a	68.9a	
Total seedling weight (g)	0.0863a	0.0891a		0.0850a	0.0755a		0.0805a	0.0890a		0.0806a	0.0771a	
Shoot weight (g)	0.0490a	0.0486a		0.0457a	0.0425a		0.0473a	0.0493a		0.0511a	0.0430a	
Root weight (g)	0.0373	0.0405a		0.0393	0.0330a		0.0332	0.0397a		0.0295c	0.0341a	
	a,b,c			a,b			b,c					
Shoot/root by weight	1.36b	1.24b		1.19b	1.31b		1.46b	1.24b		1.80a	1.30b	
Stem length (cm)		6.28a			5.78a			6.61a			5.89a	
Taproot length (cm)		24.8a			24.6a			26.0a			24.7a	
Average no. lateral roots longer than 1.0 cm		18.8a			13.8a			19.6a			15.9a	
											17.8a	

¹ Numbers within an experiment followed by different letters are significantly different at the 5 percent level or greater. The results of experiments A and B were not compared statistically.

Seedling growth was not affected by green needle and litter leachates. There was an indication that duff and bark leachates may, under certain conditions, slightly reduce root weights, but the results were not reproducible. Seedlings grown in Hoagland's solution also exhibited reduced root weights.

Field Soil Bioassay

Materials and Methods

This experiment was conducted twice, experiment A in the summer of 1974 and experiment B in the summer of 1975. Because of differences in the experimental design, each will be discussed separately.

In experiment A, the upper 5.0 cm of soil was collected from five areas in the open and five areas under pine canopies. Each of the 10 different soils was sifted twice through a 2.0 mm sieve to remove large pieces of organic matter and then placed into 10 greenhouse pots (4-inch size). Fifteen stratified and sterilized ponderosa pine seeds were planted into each pot, and the pots were randomly placed in the greenhouse. All soils were given equal volumes of tap water at periodic intervals as needed. The germination was recorded for 15 days, and then all but the largest three seedlings were removed from each pot. The remaining three seedlings were harvested after an additional 16-day growing period. The biomass of both roots and shoots was measured after oven-drying at 70°C for 48 hours.

Experiment B was very similar to A in that soils were collected from five open areas and from five areas under pine canopies. However, in experiment B, the upper 15 cm of the soil was lifted from the ground with minimal disturbance and placed directly into 6-inch greenhouse pots. Three replications were taken from each of the 10 different collection sites. Each pot was saturated with tap water and planted with 30 stratified and sterilized ponderosa pine seeds and randomly placed in the greenhouse. All pots were given equal volumes of water at intervals as needed. Germination and initial growth was observed for 21 days when all but the largest seven seedlings were removed from each pot. These seedlings were grown an additional 56 days, at which time they were harvested, and the root and shoot weighed after drying 70°C for 48 hours.

A series of subsamples from the two soil groups were taken for nutrient analysis. The concentration of eight cations was measured by atomic absorption. The various treatment subsamples could not be paired statistically, so a nested experiment analysis of variance was used and followed by the Newman-Keuls test.

Results and Discussion

Although the experimental design was different between A and B, the results were essentially the same. In both A and B, the germination of pine seeds in soils collected from the openings was significantly greater than the germination of pine seeds in soils collected beneath a pine canopy (table 6). Although germination of the canopy treatment was significantly lower than the opening treatment, it was certainly not a drastic difference. Since both treatments received equal amounts of water and sunlight and were, therefore, influenced by similar temperatures, soil factors, would have to account for germination differences.

It was observed that the canopy soil contained larger amounts of organic matter than the opening soils, even in experiment A where the soils were sieved twice. Soil surfaces in which the seeds were planted, dried quite rapidly after watering. It is possible that the higher amounts of organic matter in the canopy soils created greater moisture tensions that reduced germination.

Table 6.--*The effect of soil collected in the opening and beneath a ponderosa pine canopy on the germination and growth of ponderosa pine seedlings*

Parameter	Germination and Growth Experiments					
	1	2	3	4	5	Mean ¹
Germination (Percent)	EXPERIMENT A					
Opening Sites	76.0	64.0	66.7	70.7	65.3	68.5a
Canopy Sites	66.0	55.3	58.0	60.0	58.7	59.7b
	EXPERIMENT B					
Opening Sites	83.8	64.4	81.1	63.3	73.3	73.1c
Canopy Sites	40.0	57.8	55.6	53.3	74.4	56.2d
Total Seedling weight (g)	EXPERIMENT A					
Opening Sites	0.1486	0.1190	0.1105	0.1408	0.1439	0.1326a
Canopy Sites	0.1282	0.1353	0.1217	0.1265	0.1215	0.1264a
	EXPERIMENT B					
Opening Sites	0.1253	0.0883	0.1076	0.1202	0.1177	0.1111b
Canopy Sites	0.1414	0.1532	0.1010	0.0749	0.1578	0.1257b
Shoot weight (g)	EXPERIMENT A					
Opening Sites	0.1077	0.0906	0.0851	0.1037	0.1069	0.0988a
Canopy Sites	0.0949	0.0975	0.0912	0.0965	0.0923	0.0945a
	EXPERIMENT B					
Opening Sites	0.0708	0.0488	0.0603	0.0647	0.0689	0.0626b
Canopy Sites	0.0807	0.0835	0.0554	0.0424	0.0857	0.0695b
Root weight (g)	EXPERIMENT A					
Opening Sites	0.0419	0.0283	0.0255	0.0371	0.0370	0.0339a
Canopy Sites	0.0333	0.0378	0.0294	0.0310	0.0292	0.0321a
	EXPERIMENT B					
Opening Sites	0.0545	0.0395	0.0473	0.0555	0.0488	0.0487b
Canopy Sites	0.0608	0.0698	0.0456	0.0325	0.0720	0.0561b

¹Means in the same experiment followed by different letters are significantly different at the 5 percent level.

It is also possible that weak phytotoxins could have been responsible. The extract experiment revealed potential phytotoxins in green needles, bark, duff, and roots, and the soil leachates experiments failed to eliminate the possibility of weak toxins or breakdown products accumulating on the soil. Therefore, weak toxins in the canopy soil could have reduced the germination.

Another explanation is an interaction or the combined effects of moisture tension and a weak phytotoxin working synergistically to reduce germination in the canopy soils. The two factors working simultaneously may be more inhibitory than either factor separately.

The factors influencing germination apparently had no effect on the growth and development of young pine seedlings, since there was no significant difference in total seedling weights, shoot weights, or root weights between treatments in either experiment A or B. There were greater concentrations of nutrients measured in the canopy soils, but the differences were apparently not great enough to affect seedling growth.

Seedbed Effect on Germination

Materials and Methods

The possibility that the germination and growth of ponderosa pine seeds and seedlings are influenced by the seedbed in which the seeds overwintered was investigated by a field and a laboratory experiment.

In the field seedbed experiment, seven different seedbeds were prepared at the edge of a small opening at the game range field site on November 20, 1974. Six of the seedbeds were set up in the opening and one under the canopy of several trees bordering the opening. The seedbed beneath the canopy was prepared by scraping away the litter and duff to expose the surface soil. This treatment shall be referred to as the canopy topsoil (canopy). The vegetation and litter were scraped away from six small areas in the opening, and each was covered with one of the following materials: topsoil collected from under a ponderosa pine canopy, referred to as canopy topsoil (opening); topsoil from a large opening; subsoil from a large opening; litter; decomposing duff; and decomposing duff with litter on top. A large number of unstratified ponderosa pine seeds were then placed on each of the seven seedbeds. The seeds on the soil seedbeds were not treated further, while those in plant materials were covered with the corresponding plant material. In the duff and litter combined treatment, the pine seeds were placed on top of decomposing duff, and the litter was then placed over the top of the seeds. Small hardware cloth enclosures were placed over each seedbed to ban rodents.

On May 9, 1975, the seeds were collected and taken to the laboratory where the germination tests were conducted. Sterilized cellulose sponges were soaked in distilled water, drained, and placed in the bottom of 9 cm plastic petri dishes. Each sponge was covered with a wet 7.0 cm piece of filter paper. Twenty seeds from each treatment were placed in respective petri dishes. Four replications were set up for each treatment. The petri dishes were covered and randomly placed in a growth chamber set at 25° to 27°C. The germination was checked daily for 1 week.

For the laboratory experiment, litter, duff, and topsoil from under a ponderosa pine canopy were collected along with topsoil from a nearby opening on November 20, 1974. These materials were returned to the laboratory to be used as overwintering seedbeds for ponderosa pine seeds. The litter and duff samples were soaked separately for two hours in distilled water and then transferred to 100 by 80 mm petri dishes. A third seedbed was set up using a combination of duff covered with litter. The opening and canopy soils were placed into petri dishes and brought to saturation with distilled water. The control was silica (sand) brought to saturation. Eight replications were

Figure 1.--Artificial overwintering treatments for laboratory seedbed experiment.

*After stratification, subsamples were placed directly into the germination chamber.

**After stratification, subsamples were opened, germinants were counted, and the remaining seeds were placed into chamber.

made for each seedbed material. One half (four) of the replicates for each seedbed were sterilized by autoclaving for 30 minutes. The remaining four replicates were left unsterilized. Two of the autoclaved and two of the unautoclaved replicates received ponderosa pine seeds that had been treated with a powder fungicide. The remaining two autoclaved and unautoclaved replicates received untreated pine seeds. The seeds were allocated by weight rather than by numbers. This design resulted in four degrees of sterilization in each seedbed; i.e., duff autoclaved and seeds coats sterilized, duff autoclaved and seeds coats not sterilized, duff not autoclaved and seeds coats sterilized, and duff not autoclaved and seeds coats not sterilized, with duplicate samples for each (fig. 1). The covered petri dishes were then randomly placed into a dark coldroom at 2° to 3°C for 5 months.

On April 22, 1975, the petri dishes were removed from the coldroom. It was observed that some germination had already taken place within the dishes, so one dish from each duplicated sterilization treatment for each seedbed was opened and the germination percentage determined by counting all the seeds. This, however, was only a partial germination, so in order to determine complete germination, two 15-seed subsamples were taken from the ungerminated seeds. The seeds of each subsample were placed into petri dishes containing a moist sponge covered with filter paper; the petri dishes were covered and placed into a dark growth chamber at 24° to 26°C. The germination was recorded daily for 7 days.

The other half of the artificial overwintering petri dishes were placed directly from the coldroom into the dark growth chamber set at 24° to 26°C. After a 7-day period the dishes were removed and the percentage germination determined by counting all the seeds.

The statistical analysis for the field seedbed experiment included the one-way analysis of variance and the Newman-Keuls test for multiple comparisons. Statistics were not needed in the laboratory seedbed test because of similarity of results.

Results and Discussion

In the field seedbed experiment, germination was fairly high in most treatments, ranging from 62.5 percent to 91.3 percent. Seeds overwintering on the opening topsoil, the canopy topsoil (opening) and the duff seedbeds had significantly better germination than the seeds on the opening subsoil, litter, litter and duff, and canopy topsoil (canopy). The germination of the seeds from the canopy topsoil (canopy) was significantly lower than for seeds on all other seedbeds as shown in the following tabulation:

<i>Seedbed</i>	<i>Percent</i> ¹
Opening topsoil	91.3 a
Canopy topsoil (open)	91.3 a
Duff	88.8 a
Litter and duff	80.0 b
Litter	78.8 b
Opening subsoil	77.5 b
Canopy topsoil (canopy)	62.5 c

Periodic observations in late winter and early spring revealed that soils under the pine canopies accumulated less snow than in the openings, and the canopy soils were the first to lose their snow cover in the spring. The viability of the seeds from the canopy could be reduced by exposure to cold and dry conditions after the snow melts, causing lower germination. A phytotoxin could have also been responsible for the reduced germination in the canopy topsoil (canopy) seedbed. If so, the movement of the canopy topsoil to the opening eliminated its effect, possibly due to increased moisture. Also, both moisture stress and phytotoxins may have been acting simultaneously.

The poorest germination of seeds overwintering in the open occurred from beds of litter, litter and duff, and opening subsoil. It is very unlikely that opening subsoil contains phytotoxins; physical soil properties may have been responsible. The germination of seeds from litter, and litter and duff seedbeds was reduced, but it was not a drastic reduction. Phytotoxins may have been responsible, although opening subsoil seeds were not influenced by phytotoxins and their germination was reduced. Thus other environmental factors such as those in the subsoil seedbeds could have reduced germination in the duff, and litter and duff seedbeds as well.

This experiment demonstrates that pine seeds overwintering on a topsoil seedbed in the open, away from the influence of the pine canopy, have the greatest potential for germination at the end of winter.

In the laboratory seedbed experiment, the results of the duplicate samples--one assayed after transferring the petri dish to the growth chamber and the other assayed by first counting the number of seeds that germinated during the experiment and transferring subsamples of the ungerminated population to the growth chamber--were very similar, so they were averaged. In the design of this experiment, fungal growth was not expected to occur in seedbeds that had been autoclaved and the seedcoats treated with fungicide; however, these samples did become contaminated. Seedbeds composed of

¹Numbers followed by different letters are significantly different at the 5 percent level.

Table 7.--*The germination of ponderosa pine seeds stratified in the laboratory on a variety of seedbeds with different fungal control measures.*

Treatment ¹		Seedbed						
Seedbed	Seed	Duff	Litter	Litter and duff	Opening topsoil	Canopy topsoil	Silica	Average
-----Percent-----								
--	--	80.2	84.3	87.8	88.3	88.4	81.4	85.1 2±9.2
A	--	87.1	88.7	88.7	83.5	87.5	84.8	86.7 ±4.8
--	F	90.2	91.1	87.2	82.5	84.8	90.5	87.7 ±7.8
A	F	87.0	77.4	81.8	82.4	83.2	76.6	81.4 ±8.6
Average		86.1 ±7.3	85.4 ±10.4	86.4 ±5.4	84.2 ±4.8	86.0 ±4.2	83.3 ±10.1	

¹A = Autoclaved, F = Fungicide

² ± Standard Deviation

plant material were more heavily infected than the topsoil seedbeds, and the silica could not support a fungus because of insufficient nutrients. The combined autoclaved-fungicide seedbeds were probably contaminated by fungal spores introduced with the seeds. In general, all seedbeds that were autoclaved had a heavier fungal growth than those that were not. This may have been due to the elimination of competing bacterial and fungal populations, the breakdown of a fungicidal compound in the seedbed during autoclaving, or the release of nutrients by autoclaving.

The seedcoat fungicide was quite effective in eliminating seedcoat contamination when the seedbeds were not autoclaved. The fungicide was probably less effective in the autoclaved seedbeds because of the larger fungus populations in these dishes.

Table 7 shows that within any one seedbed there was essentially no difference in germination between any of the treatment groups. Consequently, the results for the treatments within each seedbed were averaged and compared. When this was done, it became obvious that there was no difference in germination due to the seedbed on which the seeds were stratified.

Similar results were obtained by comparing the germination of seeds from different seedbeds that had received the same treatment. Again, there were no differences, so the results for the six seedbeds were averaged within each treatment and then compared. No differences were observed in germination between treatments.

Unlike the field experiment, no seedbed in the laboratory hindered the germination potential of the ponderosa pine seeds. The absence of fungus on the seedbed (silica seedbed) or the absence of fungus on the seedcoats (seedcoat fungicide treatments) did not improve the germination, or conversely, the presence of fungus did not significantly reduce germination.

In the laboratory seedbed experiments, all of the seedbed materials were very moist, with uniform temperatures. In the field seedbed experiments, the opening seedbed received more rain and snow than the canopy seedbed. The opening seeds all had greater germination, although there was some difference between opening treatments. Together these two experiments suggest that the seedbed on which pine seeds overwinter is not greatly significant providing there is adequate moisture.

ALLELOPATHY AND MYCORRHIZAE

It is well known that most forest trees require mycorrhizal associations for good growth and survival. Although trees can survive without mycorrhizae, under natural conditions they usually become stunted and may die (Meyer 1973). Therefore, chemicals from natural foliage leachates, the decomposition of litter, or chemicals from micro-organisms produced during litter decomposition that inhibit the growth and development of mycorrhizae could indirectly reduce the growth and development of conifer seedlings.

Handley (1963) describes the suppression of tree growth when planted on ancient *Calluna* heathlands in Europe. He attributed the inhibition to antibiotics (phytotoxins) produced by the *Calluna* roots or its associated mycorrhizae that inhibited the growth and development of mycorrhizal associates on the sensitive tree species. Without sufficient mycorrhizae tree growth was checked. Brown and Mikola (1974) conducted experiments that indicated that lichens, particularly *Cladonia alpestris*, produce toxic chemicals that adversely affect the mycorrhizae of pine and spruce seedlings in Finland and consequently reduce their growth.

The effect of pine leachates and extracts were not tested for toxicity to associated ectomycorrhizae in these experiments, but the mycorrhizae were examined on pine seedlings grown under a variety of conditions at the field study site. The field results will be presented in a separate publication.

Future allelopathic studies of tree species with ectomycorrhizal associates should include investigation into both the direct suppression of seedlings by phytotoxins, as well as the indirect suppression of seedlings through the inhibition of mycorrhizae.

SUMMARY

Volatile compounds present in ponderosa pine tissues (green needles, surface litter, decomposing duff, roots, and bark) did not inhibit the germination of pine seeds and the growth of seedling radicles. In the tests for water-soluble inhibitors, the germination of ponderosa pine seeds was inhibited by the 10 percent bark solution, the 5 and 10 percent duff solution, the natural stemflow, the 5 and 10 percent green needle solution, and the 5 and 10 percent root solution compared to the rainwater controls. Throughfall,

a natural leachate of green needles, did not inhibit seed germination. However, the two artificial green needle solutions were probably more concentrated than the throughfall, hence the increased inhibition. The natural stemflow may have been more concentrated than either of the prepared bark solutions because it was slightly inhibitory, whereas the prepared solutions were not.

The radicle growth rates of seedlings exposed to pure rainwater were significantly greater than growth rates in the 5 and 10 percent root solutions, the 5 and 10 percent green needle solutions, the 10 percent duff, and the 10 percent litter solutions. Again both green needle solutions were inhibitory, but throughfall was not. Some of the inhibitory effects of the green needle solution could be attributed to the osmotic potential of the solution and fungal growth. Recognizing that the solutions used in this experiment were highly concentrated, the green needle and root extracts were the two most toxic solutions, and the bark and duff extracts were marginally active.

In the soil leachate experiments, no significant differences in germination were observed between the distilled water control and any of the leachate treatments. In one replication of this experiment, duff and bark leachates reduced root weights compared to controls; however, in the second replication, neither of these solutions were inhibitory. In most cases, both shoot and root weights of seedlings watered with plant leachates were similar to the distilled water controls.

In the field soil bioassay, pine seed germination was significantly lower in the canopy soils than in the opening soils in duplicate experiments. This may have been caused by differences in soil structure due to larger amounts of organic matter in the canopy soils creating water potential differences. It is also possible that a phytotoxin was responsible. Although leachate experiments failed to find strong germination inhibitors in pine tissues, it is possible that weak phytotoxins accumulated on canopy soils or were produced by microbial activity. An interaction between a weak phytotoxin and moisture tensions may have also reduced the germination. Shoot, root, and total seedling weights were very similar regardless of treatment.

Two separate experiments were performed to test the overwintering seedbed effect on germination. In the first experiment, the seeds overwintered in the field on various seedbeds with and without a canopy cover. Seeds away from the canopy influence showed the greatest germination. This may have been caused by increased moisture and the better stratification of seeds under a deep layer of snow in the openings, a weak phytotoxin under the canopy, or both. Seeds stratified on litter, and litter and duff seedbeds had the poorest germination for those seedbeds located away from the canopy influence.

The second seedbed experiment was set up in the laboratory where the seeds were stratified on a variety of seedbed materials in a coldroom. Germination percentages were very similar regardless of the seedbed. Fungal contamination did not reduce seed viability.

CONCLUSIONS

No highly toxic compounds in the live or dead tissues of ponderosa pine, volatile or water soluble, drastically reduced the germination or early growth of ponderosa pine seedlings.

Germination was reduced if the seeds overwintered beneath the canopy of mature pine trees, or if the seeds were germinated in soil collected from beneath the canopy of mature trees. The exact cause of this reduction was not determined although it could be explained by weak phytotoxins, the moisture conditions of the seeds, or a combination of the two. If phytotoxins were responsible, they did not affect the growth of the seedlings that did germinate.

PUBLICATIONS CITED

- Al-Naib, F. A., and E. L. Rice
1971. Allelopathic effects of *Platanus occidentalis*. Bull. Torrey Bot. Club 98:75-82.
- Biswell, H.
1973. Fire ecology in ponderosa pine-grassland. Proc. Annu. Tall Timbers Fire Ecol. Conf. 12:69-96.
- Brown, R. T., and P. Mikola
1974. The influence of fruticose soil lichens upon the mycorrhizae and seedling growth of forest trees. Acta For. Fenn. 141:1-22.
- Cooper, F.
1960. Changes in vegetation, structure, and growth of southwest pine forests since white settlement. Ecol. Monogr. 30:129-164.
- Curtis, J. D., and D. W. Lynch.
1965. Ponderosa pine. In Silvics of forest trees of the United States. USDA For. Serv. Agric. Handb. 271, p. 217-431.
- DeBell, D. S.
1971. Phytotoxic effects of cherrybark oak. For. Sci. 17:180-185.
- del Moral, R., and R. Cates.
1971. Allelopathic potential of the dominant vegetation of western Washington. Ecology. 52:1030-1037.
- del Moral, R., and C. H. Muller.
1969. Fog drip: a mechanism of toxin transport from *Eucalyptus globulus*. Bull. Torrey Bot. Club 96:467-475.
- del Moral, R., and C. H. Muller.
1970. The allelopathic effects of *Eucalyptus camaldulensis*. Am. Midl. Nat. 83:254-282.
- Djavanshir, K., and C. P. P. Reid.
1975. Effect of moisture stress on germination and radicle development of *Pinus eldarica* Medw. and *Pinus ponderosa* Laws. Can. J. For. Res. 5:80-83.
- Eckert, J. R.
1975. Roles of fire and allelopathy in ponderosa pine forest of the Colorado Front Range. J. Colorado-Wyoming Acad. Sci. 7:31.
- Foiles, M. W., and J. D. Curtis.
1973. Regeneration of ponderosa pine in the northern Rocky Mountains-Intermountain Region. USDA For. Serv. Res. Pap. INT-145, 44 p. Intermt. For. and Range Exp. Stn., Ogden, Utah.
- Hall, F. C.
1976. Fire and resource dynamics. Symp., Fire Manage. Executive, Pajaro, Calif., Jan. 19-23, 1976.
- Hamilton, M. A.
1965. Multiple comparison procedures. USDA For. Serv. Res. Note RM-44, 12 p. Rocky Mt. For. and Range Exp. Stn., Fort Collins, Colo.

- Handley, W. R. C.
1963. Mycorrhizal associations and calluna heathland afforestation. For. Comm. Bull. 36, 70 p.
- Jameson, D. A.
1961. Growth inhibitors in native plants of northern Arizona. USDA For. Serv. Res. Note 61, 2 p. Rocky Mt. For. and Range Exp. Stn., Fort Collins, Colo.
- Jameson, D. A.
1968. Species interaction of growth inhibitors in the native plants of northern Arizona. USDA For. Serv. Res. Note RM-113, 2 p. Rocky Mt. For. and Range Exp. Stn., Fort Collins, Colo.
- Larson, M. M., and G. H. Schubert.
1969. Root competition between ponderosa pine seedlings and grass. USDA For. Serv. Res. Pap. RM-54, 12 p. Rocky Mt. For. and Range Exp. Stn., Fort Collins, Colo.
- Lodhi, M. A. K.
1975. Soil-plant phytotoxicity and its possible significance in patterning of herbaceous vegetation in a bottomland forest. Am. J. Bot. 62:618-622.
- Lodhi, M. A. K., and E. L. Rice
1971. Allelopathic effects of *Celtis laevigata*. Bull. Torrey Bot. Club 94:83-89.
- McDonald, P. M.
1976. Inhibiting effect of ponderosa pine seed trees on seedling growth. J. For. 74:220-224.
- McPherson, J. K., and G. L. Thompson.
1972. Competitive and allelopathic suppression of understory by Oklahoma oak forests. Bull. Torrey Bot. Club 99:293-300.
- Meyer, F. H.
1973. Distribution of ectomycorrhizae in native and man-made forests. In Ectomycorrhizae, p. 79-105, Academic Press, New York.
- Muller, C. H., W. H. Muller, and B. L. Haines.
1964. Volatile growth inhibitors produced by aromatic shrubs. Science 143:471-473.
- Pearson, G. A.
1936. Some observations on the reaction of pine seedlings to shade. Ecology 17:271-276.
- Pearson, G. A.
1940. Shade effects in ponderosa pine. J. For. 38:778-780.
- Pearson, G. A.
1951. A comparison of the climate in four ponderosa pine regions. J. For. 49:256-258.
- Rietveld, W. J.
1975. Phytotoxic grass residues reduce germination and initial root growth of ponderosa pine. USDA For. Serv. Res. Pap. RM-153, 15 p. Rocky Mt. For. and Range Exp. Stn., Fort Collins, Colo.
- Rice, E. L.
1974. Allelopathy. 353 p. Academic Press, New York.
- Sondheimer, E., and J. B. Simeone.
1970. Chemical ecology. 336 p. Academic Press, New York.
- Weaver, H.
1943. Fire as an ecological and silvicultural factor in the ponderosa pine region of the Pacific slope. J. For. 41:7-14.
- Weaver, H.
1959. Ecological changes in the ponderosa pine forests of the Warm Springs Indian Reservation in Oregon. J. For. 57:15-20.
- Webb, L. J., J. G. Tracy, and K. P. Haydock.
1967. A factor toxic to seedlings of the same species associated with living roots of the nongregarious subtropical rain forest tree *Grevillea robusta*. J. Appl. Ecol. 4:13-25.
- Whittaker, R. H.
1970. The biochemical ecology of higher plants. In Chem. Ecol., p. 43-70, Academic Press, New York.

Kelsey, Rick G., and Michael G. Harrington.

1979. A search for phytotoxins influencing germination and early growth of ponderosa pine. USDA For. Serv. Res. Pap. INT-216, 28 p. Intermt. For. and Range Exp. Stn., Ogden, Utah 84401.

Live and dead tissues of ponderosa pine were examined for volatile and water-extractable phytotoxins that inhibit the germination and early growth of ponderosa pine seedlings. No highly toxic substances were discovered. Germination of ponderosa pine seeds was reduced if the seeds were sown on soils collected from beneath a pine canopy.

KEYWORDS: Pinus ponderosa Laws., germination, allelopathy, phytotoxins, chemical ecology.

Kelsey, Rick G., and Michael G. Harrington.

1979. A search for phytotoxins influencing germination and early growth of ponderosa pine. USDA For. Serv. Res. Pap. INT-216, 28 p. Intermt. For. and Range Exp. Stn., Ogden, Utah 84401.

Live and dead tissues of ponderosa pine were examined for volatile and water-extractable phytotoxins that inhibit the germination and early growth of ponderosa pine seedlings. No highly toxic substances were discovered. Germination of ponderosa pine seeds was reduced if the seeds were sown on soils collected from beneath a pine canopy.

KEYWORDS: Pinus ponderosa Laws., germination, allelopathy, phytotoxins, chemical ecology.

Headquarters for the Intermountain Forest and Range Experiment Station are in Ogden, Utah. Field programs and research work units are maintained in:

Billings, Montana
Boise, Idaho
Bozeman, Montana (in cooperation with Montana State University)
Logan, Utah (in cooperation with Utah State University)
Missoula, Montana (in cooperation with University of Montana)
Moscow, Idaho (in cooperation with the University of Idaho)
Provo, Utah (in cooperation with Brigham Young University)
Reno, Nevada (in cooperation with the University of Nevada)

U.S. DEPT. OF AGRICULTURE
FOREST SERVICE
LIBRARY
WASHINGTON, D.C.

APR 20 '79

PROPERTY OF FOREST SERVICE
CURRENT SERIAL RECORDS