

# Phytotoxic Grass Residues Reduce Germination and Initial Root Growth of Ponderosa Pine

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### Abstract

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Extracts of green foliage of Arizona fescue and mountain muhly significantly reduced germination of ponderosa pine seeds, and retarded speed of elongation and mean radicle length. Three possible routes of release of the inhibitor were investigated: (1) leaching from live foliage, (2) root exudation, and (3) overwinter leaching from dead residues. The principal route remains uncertain. The ecological implications of the inhibitory substance are discussed.

**Keywords:** *Muhlenbergia montana* (Nutt.) Hitchc., *Festuca arizonica* Vasey, *Pinus ponderosa* Laws., allelopathy, phytotoxic substances, plant competition, chemical ecology.

COVER: An example of the ponderosa pine-bunchgrass community of northern Arizona. Clearcut logging in the early 1920's allowed the subclimax bunchgrasses, *Festuca arizonica* and *Muhlenbergia montana*, to capture and dominate the site in nearly pure stands for over 50 years.

## **Phytotoxic Grass Residues Reduce Germination and Initial Root Growth of Ponderosa Pine**

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# Phytotoxic Grass Residues Reduce Germination and Initial Root Growth of Ponderosa Pine

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## INTRODUCTION

The widespread pine-bunchgrass community of northern Arizona consists of ponderosa pine (*Pinus ponderosa*), Arizona fescue (*Festuca arizonica*), and mountain muhly (*Muhlenbergia montana*) as principal species. Typically, the ponderosa pine forest supports a grass understory of varying density which extends through parklike landscapes. Best development of the community occurs on better soils where the pine overstory is less than 20 square feet basal area per acre, with frequent larger openings.

In parklike stands and openings created by fire or logging, the bunchgrasses promptly capture the site, and develop into dense, exclusive communities seemingly impenetrable by other species. Numerous examples are found near Flagstaff where the ponderosa pine climax has not returned in 50 to 100 years after fire or timber harvesting (Schubert 1974). Seeds from surrounding trees repeatedly fall in these openings, but few seedlings emerge. Pine seedlings transplanted directly into the grasses typically fail to survive or grow poorly. Even where dense grass cover is mechanically removed, survival and growth of planted trees may be depressed.

In addition to competition for available water, nutrients, and growing space, chemical interactions (allelopathy) may play an important role in plant competition. Jameson (1961, 1968) tested for the presence of growth inhibitors in Arizona fescue and other northern Arizona native species, and found that an aqueous extract of fescue foliage inhibited radicle elongation of squirreltail (*Sitanion hystrix*), blue grama (*Bouteloua gracilis*), and ponderosa pine. Elongation of pine radicles in fescue extract was only 40 percent of that in water. In a small pilot test, ponderosa pine seed overwintered under Arizona fescue litter were found to have reduced germination the following summer (USDA-FS 1957). Seeds subjected to fescue leachings had only 63 percent germination, compared to 90 percent for uncovered seed.

This Paper reports research to determine (1) if various grass residues contain substances phytotoxic to ponderosa pine, and (2) their route of release.

## CHEMICAL ECOLOGY: CHEMICAL INTERACTIONS AMONG SPECIES

Many interactions between plants can be attributed to such specific factors as competition for light, water, nutrients, and growing space; susceptibility or immunity to insects and diseases; and the differential effects of other environmental stresses. However, it has become increasingly apparent that there are some interactions involving chemical substances released from one plant which negatively influence another plant. The study of these interactions is the subject of the new and rapidly expanding field of chemical ecology (Sondheimer and Simeone 1970). Many terms have been applied to the toxic chemicals and chemical effects of one species on another, but the term allelopathy (meaning mutual harm) is most commonly used. Whittaker (1970, p. 44) defined allelopathic substances as "chemicals that are released from higher plants (directly or by way of decay processes) that inhibit the germination, growth, or occurrence of other plants."

Evidence regarding the occurrence and ecological roles of allelopathy is voluminous, and has been reviewed repeatedly and comprehensively.<sup>2</sup> Allelopathic effects have been reported for agricultural and wild species of most growth forms and habitats.

The routes by which allelopathic substances reach the soil from aboveground plants include adsorption of volatile materials on soil particles, rain washing of soluble materials from living plant surfaces, leaching of materials from plant litter (or seedcoats) on the soil surface, and release of toxic compounds during microbial decomposition of litter. Routes of release within the soil include root exudation, leaching from living and dead roots, and decomposition of dead roots and incorporated residues (Whittaker 1971).

Liberation of phytotoxic substances from grasses and grasslike plants capable of inhibiting germination

<sup>2</sup>Published reviews include those by Bonner (1950), Börner (1960), Evenari (1949), Garb (1961), McCalla and Haskins (1964), Muller (1966), National Academy of Sciences (1971), Rice (1974), Sondheimer and Simeone (1970), Whittaker and Feeny (1971), and Woods (1960).

and/or growth of another species appears to be a widespread phenomenon.<sup>3</sup> The nature of the allelopathies, route of release, and mode of action in most cases is species specific, that is, the allelopathic mechanism has evolved to fulfill a particular ecological role. Barley, for example, inhibits all other plants around it. *Hordeum spontaneum* grows in the wild state in almost pure stands (Went 1970), and the cultivated species *Hordeum sativum* successfully excludes weeds from fields by excretion of inhibitors from its roots (Overland 1966). Quackgrass (*Agropyron repens*), a widespread weed, inhibits growth of cultivated plants by release of inhibitors from decaying roots which interfere with mineral nutrition (Grümmer 1961). The tillage practice of stubble mulching, which places residues of oat, wheat, corn, or sorghum on the soil surface, has resulted in reduced growth of subsequent crops. Liberation of phenolic acids to the soil during microbial decomposition of the residues was found to be responsible. Bacterial metabolism of these compounds in the soil resulted in the formation of other toxic substances (Guenzi and McCalla 1966).

Allelopathic effects also may be a significant influence in the sequence and timing of succession of plant communities. A dominant species may, by allelopathic suppression, speed its invasion of a community and delay its replacement by other species (Whittaker 1970, 1971). Studies on old-field succession in Oklahoma (Rice 1971, 1972; Wilson and Rice 1968) showed that sunflower (*Helianthus annuus*), one of the dominants of the first stage of succession, produces substances inhibitory to most other species of the stage. Threeawn (*Aristida oligantha*), an annual grass, quickly invades because it is tolerant of the toxic substances as well as low soil nitrogen levels. Threeawn maintains dominance for several years by allelopathic suppression of nitrogen-fixing bacteria and blue-green algae, thereby keeping soil nitrogen at levels too low to support species from later stages of succession. Eventually, broomsedge (*Andropogon virginicus*), which is also tolerant of low-fertility soils, replaces threeawn by means of chemical inhibition and competition, and for many years delays replacement by its successor, little bluestem (*Andropogon scoparius*).

<sup>3</sup>The occurrence of toxic substances has been reported in western wheatgrass (Jameson 1961), squirreltail (Jameson 1961, 1968), cheatgrass (Jameson 1961), Johnsongrass (Hoveland 1964), Bermudagrass (Hoveland 1964), blue grama (Jameson 1968), Sudangrass (Patrick, Toussoun, and Snyder 1963), quackgrass (Grümmer 1961), timothy (Patrick and Koch 1958), rye (Holm 1969, Patrick and Koch 1958; Patrick, Toussoun, and Snyder 1963), false flax (Grümmer 1960), brome (Myers and Anderson 1942), as well as oat, wheat, and barley (Guenzi, McCalla, and Norstadt 1967; Holm 1969; Patrick, Toussoun, and Snyder 1963).

Of 20 species involved in the old-field successions, 16 species had some and 10 considerable inhibitory activity against nitrogen-fixing and nitrifying bacteria (Abdul-Wahab and Rice 1967; Rice 1964, 1965; Rice and Parenti 1967).

There is considerable potential for chronic and indirect allelopathic effects. Relative allelopathic effects vary with the levels of phytotoxic substances, the kind of soil and other environmental conditions, and the sensitivity of different plant species. It is reasonable to postulate that the observed cases of allelopathy stand out from a background of more widespread, less conspicuous effects on plant growth and plant distribution. The combined influence of allelopathy and competition could explain why certain species quickly capture a site following disturbance, and persist for so long in almost pure stands before the climax vegetation is ultimately restored.

## EXPERIMENTAL STUDIES AND RESULTS

### Germination of Seeds and Growth of Seedlings in Aqueous Extracts of Grass Residues

A laboratory experiment was conducted to determine the phytotoxic effects of various residues of fescue and muhly on seed germination and primary root elongation of ponderosa pine.

### Materials and Methods

Samples of fresh green foliage (including seed-stalks), new litter (standing dead grass litter), old litter (matted dead litter accumulated in the center of the clump), and roots (live roots clipped from growing grass plants) of fescue and muhly were collected in mid-September on the Fort Valley Experimental Forest near Flagstaff, Arizona. Excess soil was shaken from the grass roots, but they were left unwashed to avoid losing any excreted phytotoxins. The residues were air-dried for 26 days, then ground in a mill to pass a 20-mesh screen. Powdered residues were refrigerated in sealed metal cans until use.

Each extract was prepared by homogenizing 20 g of powdered residue with 200 ml of sterile distilled water in a blender at low speed for 15 minutes. The extracts were centrifuged (5,600 rpm for 10 minutes) and filtered, using suction to remove suspended matter. The control "extract" consisted of sterile distilled water homogenized for 15 minutes. The pH and osmotic potential (by cryoscopy, Harris and Gortner 1914) of the freshly prepared extracts were measured.

Extracts used in the germination tests were not sterilized since heat might affect stability of inhibitory substances in the extracts, and the chemical sterilants sodium benzoate and sodium hypochlorite were found

to inhibit pine seed germination. To reduce fungal contamination, seeds were surface-sterilized by soaking in 30 percent hydrogen peroxide for 15 minutes and rinsed with sterile distilled water, and all glassware and glass filter paper were sterilized by autoclaving. The interior of the growth chamber was washed with sterilizing chemicals. Five ml of unsterilized extract was added to each petri dish only once; sterile distilled water was added when additional water was needed. Control dishes were wetted with 5 ml of sterile distilled water.

The experiment was conducted in a controlled environment chamber held at a constant temperature of 75°F and a photoperiod of 16 hours of combined fluorescent and incandescent lighting daily. The incandescent lights were turned off 15 minutes after the fluorescent lights to expose the seeds to light in both the red and far-red regions at the end of each day, as in the natural condition. Relative humidity was maintained above 80 percent. The arrangement of petri dishes, four per treatment combination, in the growth chamber was completely randomized; locations were shifted daily to distribute the effects of microclimatic variation.

Seeds were considered germinated when the radicle length equaled the length of the seed. The number of germinated seeds was recorded daily for 25 days. Radicle length (in millimeters) from the seedcoat or cotyledons to radicle tip, of the first 10 seeds to germinate in each petri dish, was measured daily until the seedlings began to decline due to exhaustion of the endosperm and growth factors contained in the extracts. Fungal contamination was not serious until the last days of the experiment, but 90 percent of the germination had occurred by that time.

Percentage germination, speed of germination (Maguire 1962), time for 50 percent germination, speed of elongation (adapted from Maguire 1962), and mean radicle length before decline were calculated and tested for significant differences by factorial analysis of variance. Speed of germination and elongation take both the promptness and completeness of the respective processes into account.

## Results and Discussion

Extracts prepared from live foliage of fescue (FF) and muhly (MF) by far produced the most dominant effects (fig. 1). With few exceptions, FF and MF extracts significantly ( $\alpha = 0.01$ ) reduced percentage germination and speed of germination, increased time for 50 percent germination, and reduced speed of elongation and mean radicle length. FNL similarly inhibited seed germination, speed of elongation, and mean radicle length, but to a lesser degree than extracts from live foliage. The magnitude of these

effects is apparent in figure 1 in which speed of germination of seeds and speed of elongation of radicles in the extracts are expressed as percentages of the control values. A few inconsistent, less significant effects appear in the data: MR extract inhibited mean radicle length and FOL increased percentage germination and speed of germination. These results could be reverse responses to low concentration of a growth substance or due to secondary metabolites in the extracts. However, the variance for mean radicle length was high, and these results may simply be chance events.

Retarded pine germinants tended to have a stocky appearance with a shortened, stubby radicle and short hypocotyl. Other symptoms were an enlarged root collar and dark brown sheath covering both the root collar and radicle tip. In the most toxic extracts the radicle tip eventually died.

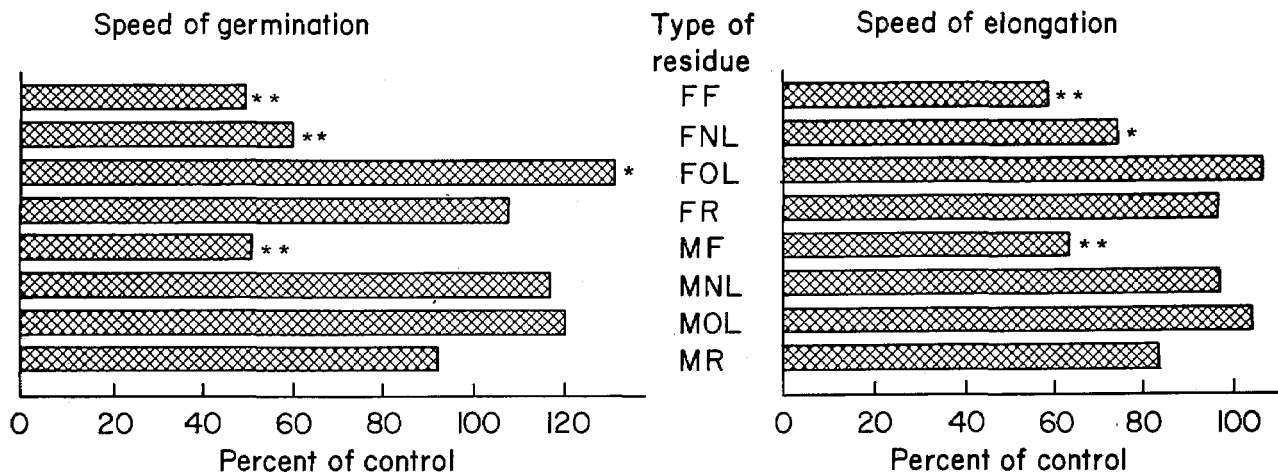
The pH and osmotic potential of aqueous extracts of grass residues were as follows:

Source of extract	pH	Osmotic potential (atm)
FF	5.70	-2.60
FNL	4.58	-0.50
FOL	5.80	-0.30
FR	6.14	-0.60
MF	5.67	-1.60
MNL	4.87	-0.60
MOL	5.59	-0.30
MR	5.80	-0.60
Control	5.49	0.00

The pH of the control "extract" was slightly acidic due to the incorporation of carbon dioxide by the blender. Although no tests were run, pH values are not considered to be low enough to significantly affect seed germination or initial growth. Moreover, the pH values of the extracts that induced the strongest responses (FF, FNL, MF) are similar to the control.

Extract osmotic potential decreased with increasing age and decomposition of residues. Extracts that significantly inhibited germination and initial development also had the lowest osmotic potentials, especially FF and MF. Larson and Schubert (1969a) reported that moderately low osmotic potentials (down to -3 bars) benefit germination of ponderosa pine seeds, but the reason for this response, as well as the effects on speed of germination, and speed of elongation of pine, are unknown.

The separate effects of extract osmotic potential were tested in a followup experiment in which pine seeds were germinated in solutions of polyethylene glycol (PEG, molecular weight 20,000) with osmotic potentials of -1, -2, -4, and -8 atmospheres. Percentage germination, speed of germination, and



\*Differs significantly from control at 5% level.  
 \*\*Differs significantly from control at 1% level.

Figure 1.—Germination and growth responses of ponderosa pine to extracts prepared from grass residues.

speed of elongation were measured under the same experimental conditions described previously. The PEG solutions and distilled water control were changed daily to minimize the increase in concentration of the solutions by seed imbibition of water. Percentage germination of pine seeds (fig. 2A) is enhanced by low levels of osmotic potential, beyond which germination drops off gradually. This pattern is in general agreement with the results of Larson and Schubert (1969a). Speed of germination of pine seeds (fig. 2B) followed a trend similar to percentage germination within the range tested, but to a lesser

degree. Speed of elongation of pine radicles (fig. 2C) was found to be quite sensitive to osmotic potential at all potentials tested below -1 atmosphere. Overall, in the range 0 to -3 atmospheres, which includes the osmotic potential of the extracts, osmotic potential should not interfere significantly with percentage or speed of germination of pine seeds. Speed of elongation of pine is sensitive to the osmotic potential of the wetting solution.

Thus, extract osmotic potential was a principal confounding factor which inconsistently accounted for part of the observed responses to the extracts.

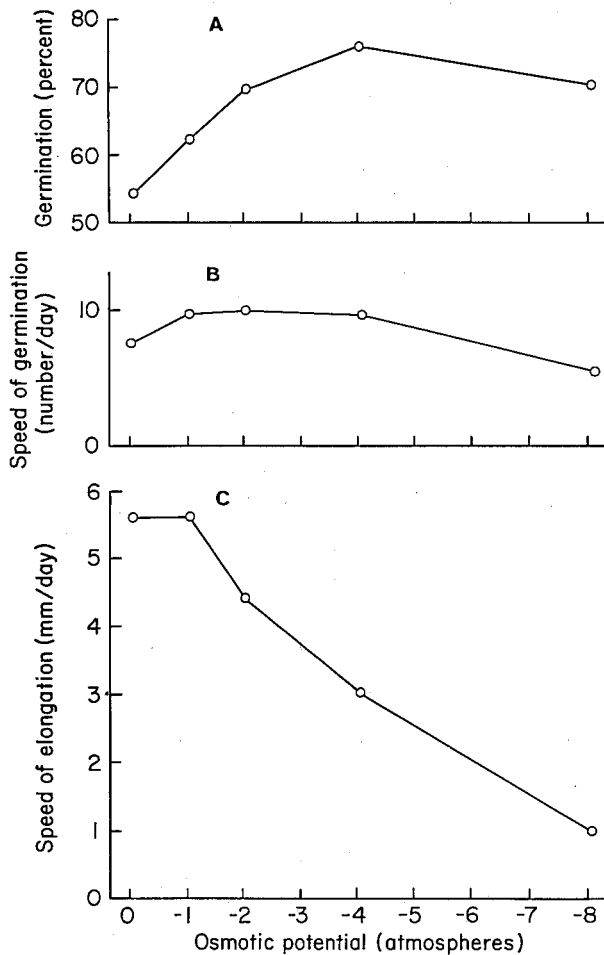


Figure 2.—Effects of different osmotic potentials imposed by solutions of polyethylene glycol (PEG) on: **A**, percentage germination; **B**, speed of germination; and **C**, speed of elongation of ponderosa pine.

When extract osmotic potential is taken into account, the inhibitory effects of FF, FNL, and MF extracts on percentage germination and speed of germination (figs. 3A and 3B) would be greater, rather than lesser, compared to the control values. Speed of elongation of pine (fig. 3C) radicles would likely not be significantly different from the respective controls when the separate effects of extract osmotic potential are taken into account. If the controls consisted of PEG solutions with osmotic potentials equivalent to the extracts, the actual depression of germination of pine seeds by FF extract, for example, would be 61.4 percent (fig. 3A) of the control compared to 44.2 percent calculated from the tabulation in figure 1. Speed of germination of pine seeds was depressed 50 percent of the control value in figure 1, and 64.3 percent if extract osmotic potential is taken into account (fig. 3B). However,

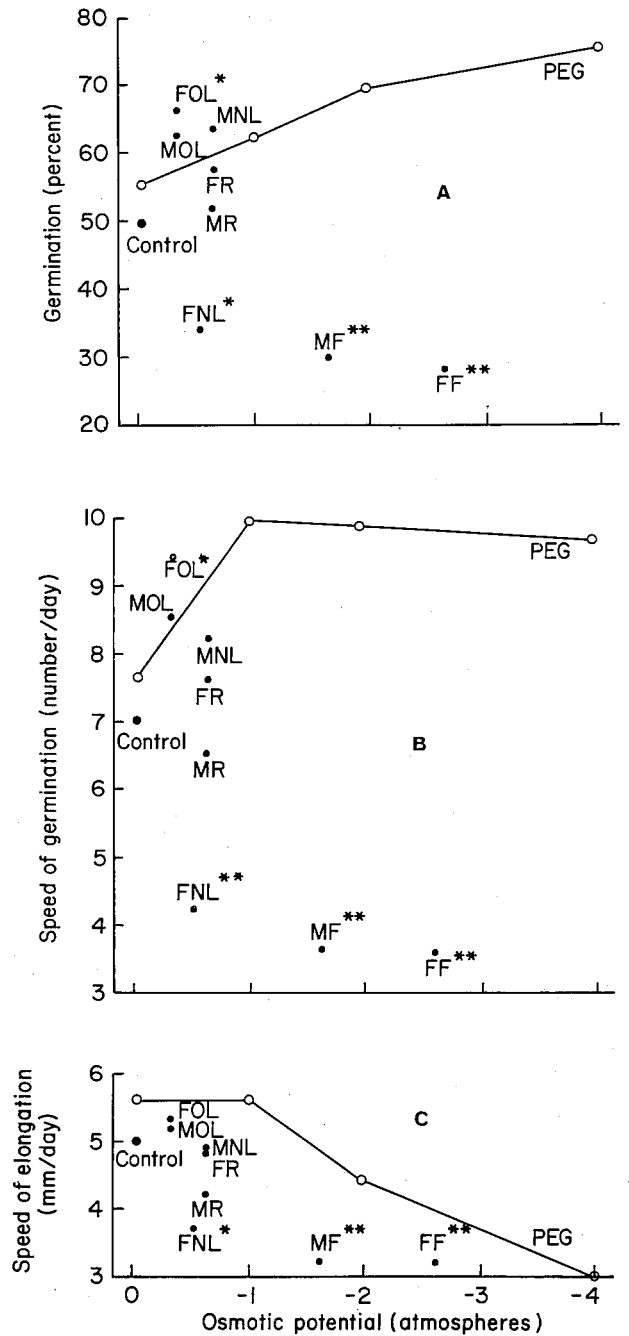


Figure 3.—Comparisons of: **A**, percentage germination; **B**, speed of germination; and **C**, speed of elongation of ponderosa pine in polyethylene glycol (PEG) solutions and grass residue extracts of different osmotic potentials. One asterisk indicates the difference between the extract and control values is significant at the 0.05 probability level; two asterisks indicate significance at the 0.01 probability level.

for speed of elongation of pine radicles, the actual depression is 16.5 percent (fig. 3C) compared to 36 percent calculated from the tabulation in figure 1. Thus, elongation of pine radicles appears to be much more sensitive to the osmotic potential of the wetting solution than is seed germination.

The extract experiment also revealed that pine seeds germinate much better under conditions of limited water than under wet conditions. Apparently the improved aeration and small negative water potential afforded by minimum wetting stimulated germination. This is illustrated also by the ability of ponderosa pine seeds to withstand air-drying during germination (Larson and Davault 1975).

The low percentage germination of 49.3 for the pine control treatment (fig. 1) is attributed to overwetting (5 ml water per 9 cm petri dish). Also, fungal contamination may have interfered. To test these possibilities, a small experiment was performed to test the effects of sterilized FF and MF extracts, 3 ml per petri dish, on germination of surface-sterilized pine seeds. All glassware was similarly sterilized by autoclaving. The test was run in a small germinator without light at 75°F with high humidity. Germination in the control treatment was much higher, 84.8 percent, than in the original experiment (fig. 4), but the differences in germination between the extracts and the control persisted:

Source of extract	Germination	
	(Percent)	(No./day)
FF	62.3	11.9
MF	60.8	11.8
Control	84.8	17.9

As a consequence of higher percentage germination, speed of germination was correspondingly higher than in the original experiment. However, the treatment differences are consistent.

Another followup experiment was run to determine the effects of extract added after germination. Fescue foliage extract, 1 or 2 ml per 7 cm petri dish, was added to pine germinants with radicles ½ or 1 inch long. Response was evaluated during a 3-day incubation period. The results were similar regardless of initial radicle length: 2 ml of extract per dish retarded radicle elongation by 30 percent. Thus, it appears that the inhibitory substance retards both germination and initial growth processes, rather than the latter being a consequence of the effects upon germination.

#### Growth of Various Species Associations in Sand Culture

The sand culture experiment was one of three experiments conducted to investigate possible routes

of release of the inhibitory substance. Paired combinations of pine, fescue, and muhly were grown in intimate contact to (1) determine if inhibitory substances are excreted by grass roots, and (2) measure their relative toxicity to an associated pine or grass plant.

#### Materials and Methods

Pine, fescue, and muhly seeds were germinated in petri dishes. Pairs of plants in all possible combinations of the three species were then planted in 4½-inch-diameter by 10-inch deep metal pots. The pots were filled with an equal weight of number 60 silica sand and a 1-inch layer of Perlite on the surface to reduce evaporation. Group A consisted of 36 pots of plants with simultaneous planting times; Group B contained 36 pots of plants with age differing by 3 months.

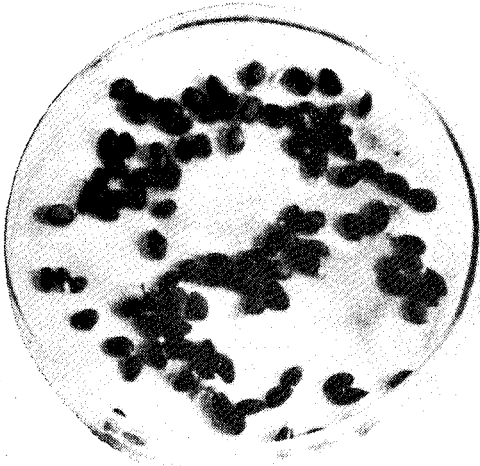
The experiment was conducted in a controlled environment chamber programed for two periods of plant growth separated by a 10-week cool period. The warm environment consisted of a thermoperiod of 75°F during the day and 60°F during the night, and photoperiod of 16 hours of combined fluorescent and incandescent lighting. The transition period of temperature changes coincided with light changes, and a 15-minute period of twilight was provided at day end as described previously. After 4 months under the warm environment, the growth chamber was set for a cool environment of 10 weeks' duration, consisting of a thermoperiod of 50°F during the day (8 hours) and 35°F at night. Following the cool period, the warm environment was resumed for an additional 3 months.

A watering system based on the relationship between matric potential and water content was developed which regulated soil moisture potential within the range of -0.3 atmosphere (field capacity) and -3.0 atmospheres by a corresponding range in pot weight. The plants were watered with one-half strength Hoagland solution at 2- to 4-day intervals during the entire experiment to maintain water and nutrients at adequate levels. Leaching of the pots at monthly intervals was necessary to remove accumulated salts. Each pot was leached with 2,000 ml of water. Since phytotoxins excreted from roots could be lost in the water leached from the pots, the first 250 ml of leachate from each pot in Group A was collected and tested for toxicity to yellow sweetclover (*Melilotus officinalis*) seed germination.<sup>4</sup> A 25 ml

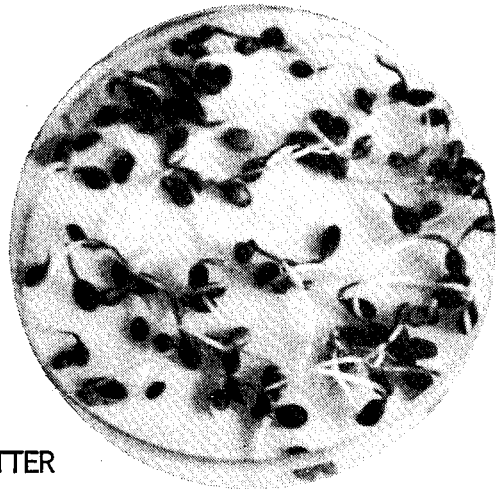
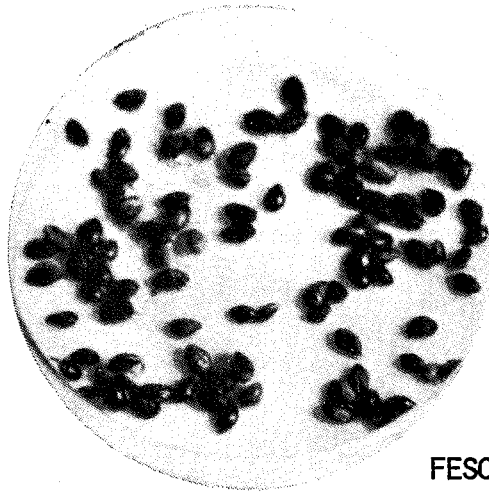
<sup>4</sup>Germination and initial growth of yellow sweetclover were found to be extremely sensitive to the phytotoxins produced by Arizona fescue and mountain muhly, and therefore provided a quick bioassay for the presence of the inhibitor(s).

STERILIZED EXTRACT

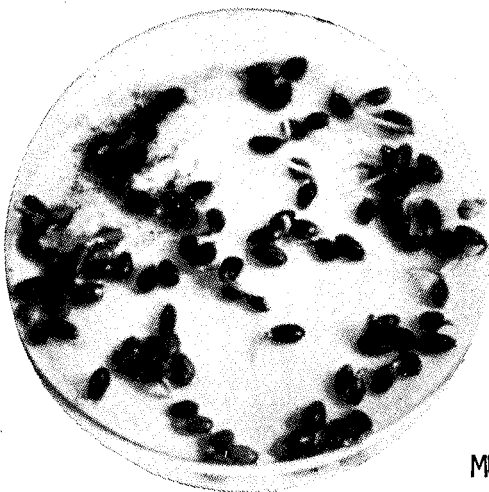
CONTROL



FESCUE FOLIAGE



FESCUE NEW LITTER



MUHLY FOLIAGE

Figure 4.—Germination of surface-sterilized pine seeds in sterilized extracts compared with the control.

aliquot of the leachate from each pot was evaporated to dryness, rewetted with 3 ml of distilled water and added to 100 clover seeds in a petri dish; distilled water only was used as a control. The osmotic potential of the leachates was not determined.

Foliage height (distance from cotyledons to shoot tip) of pine, and total height and basal diameter of the two grasses, were measured monthly for the 9-month duration of the experiment. At the end of the experiment, dry weight of the top and roots of individual plants was determined. These data and calculated top/root weight ratios and plant volumes (grasses) were analyzed for significant differences by analysis of variance.

## Results and Discussion

Though the experiment was designed to test for toxic root excretions in the absence of competition for light, water, and nutrients, strong plant competi-

tion developed as early as 3 months into the experiment and intensified as the experiment progressed. Because of competition, it was very difficult to establish the second species in the later planting, especially where large fescue and muhly plants were present. Toward the end of the experiment, the pots were distinctly crowded by large grass plants (fig. 5), and most pine seedlings planted 3 months after the grass were suppressed or dead. This awesome demonstration of the competitive power of fescue and muhly is in accord with the results of Larson and Schubert (1969b), especially with regard to planting pines in an established grass stand. Seedlings that died were assigned a value of zero for their corresponding data.

The data presented in table 1 for Group A show mainly the effects of plant competition, or lack of it, on various growth parameters. Fescue plants made superior growth in terms of top and root dry weight and top volume when associated with pine compared to another fescue or muhly plant. The lack of competition from the associated pine is undoubtedly

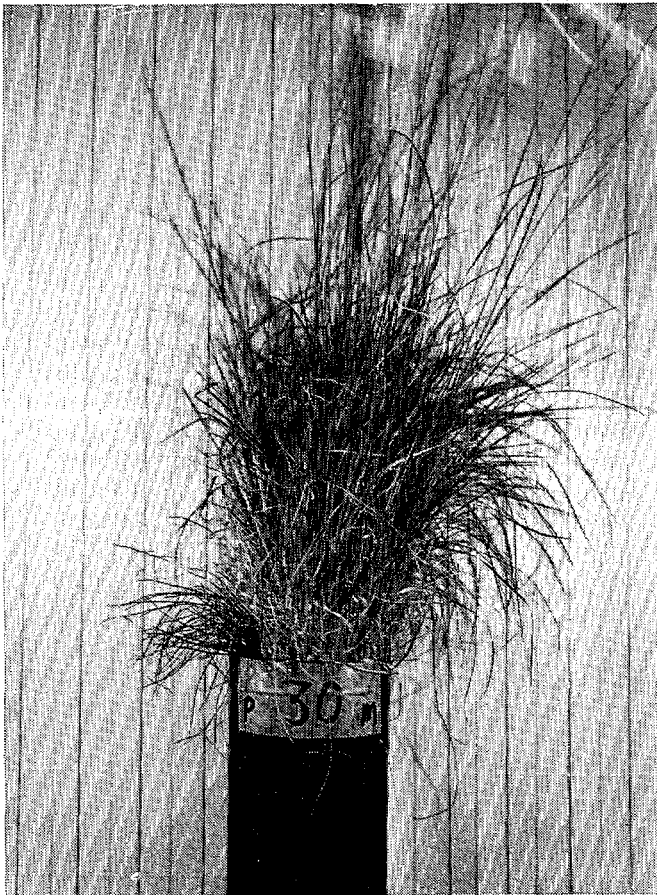


Figure 5.—The pine-muhly species combination, planted simultaneously (Group A), at age 9 months. Competition for growing space occurred as early as 3 months into the experiment.

Table 1.--Comparative growth of ponderosa pine, Arizona fescue, and mountain muhly

Group	Species evaluated	Species combination	Mean total height	Mean top dry weight	Mean root dry weight	Mean top/root weight ratio	Pine mean foliage height	Grass mean top volume
			<i>cm</i>	<i>g</i>	<i>g</i>		<i>cm</i>	<i>cm<sup>3</sup></i>
GROUP A <sup>1</sup>	Ponderosa pine	Pine/Pine	20.0	0.75	0.26	3.96	17.5	
		Pine/Fescue	16.8	.49	.14	3.79	14.3	
		Pine/Muhly	16.7	.55	.24	3.27	14.2	
	Arizona fescue	Fescue/Fescue	67.8	8.82	7.07	1.52		259.5
		Fescue/Pine	64.0	13.60*	11.09*	1.56		376.9*
		Fescue/Muhly	61.2	7.79	4.18	1.98		216.2
	Mountain muhly	Muhly/Muhly	57.0**	18.38	7.31	4.43		275.1
		Muhly/Pine	69.3	35.47	9.93	3.95		585.0*
		Muhly/Fescue	68.2	32.73	7.14	5.25		460.5
GROUP B <sup>2</sup>	Ponderosa pine	Pine/Pine	19.5 a	1.34 a	0.60 a	2.26	17.0 a	
		Pine/Fescue	19.6 a	1.55 a	.68 a	2.30	17.1 aj	
		Pine/Muhly	24.2 a	1.80 a	.94 a	1.99	21.7 ai	
		Pine/Pine	7.2 be	.12 b	.06 b	3.13 e	5.3 be	
		Fescue/Pine	4.2 bf	.02 b	.01 b	1.75 f	2.9 bf	
		Muhly/Pine	.0 bf	.00 b	.00 b	.00 f	.0 bf	
	Arizona fescue	Fescue/Pine	67.8 a	18.86 a	21.65 a	.98 a		447.5 a
		Fescue/Fescue	63.0 a	20.35 a	25.63 a	.89 a		526.5 a
		Fescue/Muhly	68.2 a	20.78 a	18.70 a	1.46 a		503.9 a
		Pine/Fescue	37.7 beg	.39 b	.66 b	.63 b		3.4 b
		Fescue/Fescue	.0 bf	.00 b	.00 b	.00 b		.0 b
		Muhly/Fescue	.0 bfh	.00 b	.00 b	.00 b		.0 b
	Mountain muhly	Muhly/Pine	71.4 a	40.42 a	11.42 a	3.67 a		695.5 a
		Muhly/Fescue	73.9 a	43.80 a	13.66 a	3.52 a		713.5 a
		Muhly/Muhly	75.6 a	42.71 a	13.66 a	3.91 a		709.2 a
		Pine/Muhly	23.4 beg	.80 b	.35 b	2.00 bg		8.4 b
		Fescue/Muhly	.0 bfh	.00 b	.00 b	.00 bh		.0 b
		Muhly/Muhly	.0 bf	.00 b	.00 b	.00 b		.0 b

\* Differs significantly from other two members of group at 5 percent level.

\*\* Differs significantly from other two members of group at 2.5 percent level.

<sup>1</sup>Three species planted simultaneously in various combinations; plants grown for 9 months in controlled environment.

<sup>2</sup>Three species planted at different times in various combinations; second species planted 3 months after first. Species left of / planted early; species right of / planted 3 months later.

Comparison

Interpretation

- a,a,a vs. b,b,b Collectively, early-planted species combinations (a,a,a) differ significantly from late-planted species combinations (b,b,b).
- e vs. f,f Pine or grass species planted late with an existing pine (e) differs significantly from the same species planted late with an existing grass plant, considered collectively (f,f).
- g vs. h Fescue or muhly planted late with an existing pine (g) differs significantly from fescue or muhly planted late with the alternate grass species (h).
- i vs. j Pine planted early with muhly (i) differs significantly from pine planted early with fescue (j).

related to the difference in growth. Muhly plants were significantly shorter when associated with another muhly and had only half as much top volume after 9 months compared to muhly plants associated with pines.

Data for Group B (table 1) and interpretation of significant specific comparisons show that the responses generally, are the same as in Group A. In all cases, except mean top/root weight ratio of pine, plants of the species evaluated established early (a,a,a) collectively differed significantly from plants established 3 months later (b,b,b). This is an expected difference due to the difference in age of the plants. The top/root weight ratio of the grasses apparently changes with age, as indicated by the difference between early- (a,a,a) and late-planted (b,b,b) seedlings. Pine or grass seedlings established late with an existing pine seedling (e) demonstrated superior height growth compared to seedlings planted late with the two grasses considered collectively (f,f).

Considering the grasses independently, fescue or muhly seedlings planted late with an existing pine (g) grew taller than when planted late with the other grass species (h). Although the significant (e) vs (f,f) and (g) vs (h) comparisons might be considered meaningless because of almost complete mortality of seedlings planted late with an existing grass plant, they are important in that they depict the intense vegetative competition expressed by an established grass plant. Finally, pines planted early with muhly (i) had significantly greater foliage height than when planted early with fescue (j). A similar difference developed in mean total height of pines, but is not significant due to high variation. In all cases, the observed effects can be ascribed to the lack of competition because of the presence of a pine seedling, or conversely, due to intense competition from an existing grass plant. Muhly appears to be slightly less competitive toward the pines than fescue.

There were no significant differences in germination of yellow sweetclover seeds incubated either in leachate from pots representing the species combinations in Group A or distilled water, indicating no detectable amount of inhibitory substance arising from grass roots was leached from the pots.

#### **Seed Germination in Leachates From Grass Residues**

The results of the extract experiment indicated that a phytotoxic substance is contained in the residues of grass plants, and is particularly concentrated in live foliage. In this experiment, leaching from live foliage was explored as a possible route of release of the inhibitor.

## **Materials and Methods**

Samples of fescue and muhly live grass blades and seedstalks were collected in mid-September on the Fort Valley Experimental Forest. Fescue stalks had begun to dry when collected, whereas muhly stalks were still green; blades of both species were green. In the laboratory, the samples were separated into seed-stalks and blades. A 50 g sample (fresh weight) of each of the four residue types was soaked in 100 ml of distilled water in a large beaker for either 5 minutes or 12 hours. Each was agitated for 5 minutes to wet all of the material. At the end of the soaking periods the eight leachates were drained off and refrigerated.

Surface-sterilized pine seeds were used to test the leachates for phytotoxicity. Ten ml of leachate were transferred to each of three 9 cm petri dishes, evaporated to dryness, autoclaved, and cooled. In a separate test, it was found that autoclaving grass residue extracts did not alter their growth-inhibiting potential. Three ml of sterile distilled water and 100 unstratified surface-sterilized seeds were added to each petri dish. Germination tests were conducted in darkness in a small Mangelsdorf germinator with temperature set at 75°F and high humidity. Other procedures and germination criteria were the same as described previously. The number of germinated seeds was recorded daily for 11 days. From these data, percentage germination and speed of germination were calculated and tested for significant differences by analysis of variance.

## **Results and Discussion**

None of the leachates affected percentage germination or speed of germination of pine seed. Seeds germinating in the 12-hour leachates exhibited the same appearance symptoms as in the extract experiment. The dark brown sheaths at the root collar and radicle tip may simply be the result of staining by the extract or leachate.

#### **Overwintering of Pine Seeds Under Various Plant Residues**

Newly fallen pine seeds normally lie on the ground in the presence of grass and pine residues until the following July, 8 months or more, before environmental conditions are right for germination. In this experiment, samples of ponderosa pine seed were overwintered under various plant residues in different stages of decomposition to determine if continuous exposure to leachates of plant residues over the natural period would adversely affect seed viability.

## Materials and Methods

Fescue, muhly, and pine residues were collected on the Fort Valley Experimental Forest in mid-November. The grasses had entered dormancy by that time, so the residues collected were FNL, FOL, FR, MNL, MOL, MR (all described in the extract experiment), plus freshly fallen pine needles (PNL) and partially decomposed pine needles (POL). All of the residues were chopped into smaller pieces so they would form a dense mat over the seeds.

The experiment was conducted at the Fort Valley Experimental Nursery, which is surrounded by a rodentproof fence (fig. 6). The area utilized had been kept free of vegetation, including conifers, for several years. The soil surface was leveled and covered with approximately 2 inches of washed sand to eliminate interference from substances in the soil. Thirty-six metal retaining rings 12 inches in diameter and 2 inches deep were arranged in a grid pattern on the sand with 2 feet between rings. The rings were randomly assigned to treatment.

Approximately 225 ponderosa pine seeds, measured by weight, were evenly distributed on top of the sand in the retaining rings and covered with the predetermined type of residue to the top of the ring. The control treatment consisted of rings filled with washed sand. Each ring was covered with a square of 1/2-inch hardware cloth for further protection.

On June 5 the seeds were recovered from the individual retaining rings. They were not washed before the germination test, but were shaken on a

Figure 6.—Pine seeds were overwintered under various residues of pine, fescue, and muhly. The seeds were recovered and germinated the following summer.

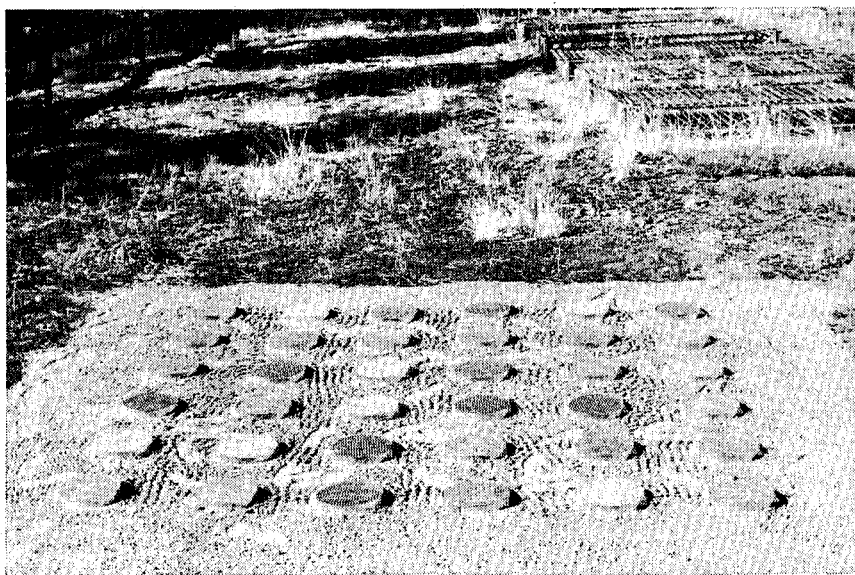
screen to remove extraneous materials. One hundred seeds from each replication were germinated in a 9 cm petri dish under the same conditions as the extract experiment. The seeds were wetted with a minimum amount of sterile distilled water. Germination was recorded daily; seeds were considered germinated when radicle length equaled the length of the seed. Locations of dishes in the growth chamber were randomized after each germination count. After 18 days the ungerminated seeds were cut to determine soundness. Percentage germination data, based on sound seeds, were tested for significant differences by analysis of variance.

## Results and Discussion

The winter of 1969-70 was an "open winter" with only 9.74 inches of precipitation during the period November 20 to June 5; 7.14 inches of this total fell during March. Consequently, during most of the winter there was only minimal moisture available for leaching.

There were no significant differences in percentage germination of pine seeds overwintered under the different plant residues:

Type of residue	Percentage germination
FNL	89.0
FOL	85.4
FR	85.7
MNL	88.5
MOL	89.1
MR	79.7
PNL	90.5
POL	88.4
Control	92.6



The germination in each case was quite high, indicating no general loss of viability due to the treatments.

These results do not agree with findings from an informal study conducted in the winter of 1956-57 (USDA-FS 1957). Pine seeds overwintered under Arizona fescue litter yielded 63 percent germination the following summer, compared to 85 percent for pine needle litter and 87 percent for no cover. Leaching conditions were somewhat better during the period November to June 1956-57, with 12.27 inches of precipitation, of which 6.17 inches fell during January. Furthermore, seeds were placed directly on the surface of a clay soil, which potentially would allow some accumulation of leached substances. Such accumulation would be unlikely in the sand layer used in the present experiment.

## DISCUSSION OF ECOLOGICAL SIGNIFICANCE

The route of release of the inhibitory substance remains uncertain, in spite of several efforts to identify a pathway. Release of the inhibitor underground as a root excretion can probably be ruled out, judging from the lack of any evidence pointing to chemical effects in the sand culture experiment, and since an extraction of fescue and muhly roots was not inhibitory in the extract experiment. The inhibitory substance is apparently present in aboveground grass residues and most concentrated in live, green foliage.

The question reduces to: how is the inhibitor released to the external environment, and how does it enter the receiver species? Since little microbial decomposition occurs in standing dead residues, the principal mechanisms of breakdown would be biochemical degradation during senescence, and normal weathering processes. Any inhibitory substance in the standing residues at the time senescence begins would subsequently (1) undergo biochemical degradation, (2) oxidize as a result of weathering processes, or (3) remain intact for some indefinite period. The principal processes for removal of breakdown products or an intact inhibitor would be translocation, volatilization, or leaching.

Overwinter leaching does not appear to be the route of release, unless there is accumulation in the soil up to and during the time of germination and growth of the receiver species. Considering the high water solubility and probable subjectivity to microbial decomposition, it is unlikely that the inhibitor could persist, even in clay soils, until the following July. Leaching should not be ruled out, but it appears that leaching at or near the time of seed germination and initial growth of the receiver species is more likely to be effective. Leachates prepared from mid-September live foliage and new litter of

fescue and muhly were not toxic to germinating pine seeds, however. To fully understand the role of leaching as a route of release, research is needed on the presence, toxicity, and leachability of the inhibitor in live and dead grass residues during the growth period of the receiver species.

A potential mechanism of release of the inhibitor which was not studied is the formation of phytotoxic substances during microbial decomposition of dead residues on or in the soil. The liberation of substances toxic to living plants during decomposition of plant residues appears to be a widespread phenomenon (McCalla and Haskins 1964, Patrick 1971, Patrick and Koch 1958, Patrick, Toussoun, and Koch 1964). Soil toxicity due to decomposition of organic constituents is most frequently associated with heavy, poorly aerated, or waterlogged soils. However, localized pockets of anaerobiosis are widespread in the soil and may prevail for varying periods of time following rainfall (Patrick 1971). Conditions that lead to the formation of high concentrations of phytotoxic decomposition products may, therefore, be more common than is generally realized, and not necessarily confined to waterlogged soils. Additional research is needed on microbial decomposition of dead residues on or in the soil as a possible mechanism of release of the inhibitor.

There is evidence to suggest that the inhibitor must be present at a time of physiological activity (germination and initial development) of the receiver species in order to be effective. Extracts prepared from FF, FNL, and MF residues significantly depressed germination of ponderosa pine seeds in the extract experiment (fig. 1), whereas similar residues collected 2 months later had no effect on germination of pine seeds in the overwintering experiment. The newer grass residues employed in the overwintering experiment must have contained a considerable amount of the inhibitor,<sup>5</sup> but overwinter exposure of dormant pine seeds to the inhibitory substance leached from the residues did not have any effect on germination in distilled water the following summer.

The results of the followup experiment in which a postgermination addition of extract from fescue foliage retarded radicle elongation of pine seedlings further supports this suggestion. This point could be clarified by (1) a study on low-temperature soaking of seeds in fescue foliage extracts and subsequent germination (after rinsing) in distilled water at a higher temperature, and (2) a study to determine if the effects of the inhibitor can be reversed.

<sup>5</sup>FNL in the extract experiment had been dead nearly 12 months, whereas the residue designated FNL in the overwinter leaching experiment had been dead only 2 months.

An important question that should be considered is the relation between the amounts of the residues used to prepare the extracts in the experiments and the amounts that occur in nature in an arbitrary area of influence around a single seed. The amount of herbage production depends upon the extent of tree cover. Arnold (1956) estimated that herbage production would vary from 1,000 pounds dry weight per acre per year in forest openings to 50 pounds per acre per year under dense forest canopies. In these openings, assuming no grass utilization, the potential addition of residues to the soil each year is 1,000 pounds dry weight per acre. If 1 square inch is conservatively estimated to be the area of influence around a single pine seed, then an average of 0.072 g of grass residue would be introduced into this area each year. In the extract experiment, 5 ml of extract obtained from 0.67 g of dry residue (extract yield from 20 g of residue and 200 ml of water was 150 ml) was sufficient to produce the reported effects in 100 seeds, which is equivalent to 0.0067 g of residue per seed. The followup experiment utilizing sterilized extracts found that 0.004 g of residue per seed was inhibitory. Dividing the yearly production of 0.072 g of residue per 1 square inch by the calculated experimental rate per seed shows that 10.8 times as much residue is added to the area of influence per year than was necessary to induce the observed effects in the extract experiment.

These calculations serve only to illustrate that the amount of the residues used to prepare the extracts is conservative compared to the amounts that occur in nature. The primary difference between the two cases is the concentration of inhibitor around a seed at any one time. In the extract experiment, the inhibitory substance contained in the residues was almost completely released into solution at one time, whereas in nature the phytotoxins are undoubtedly released more slowly. Under natural conditions, even though greater amounts of residues may occur, the concentration of inhibitor at a particular time may not approach that of the extract experiment. The net influence of plant sensitivity to the inhibitor and the effect of interacting environmental variables that affect the concentration of inhibitor present would determine the extent of allelopathy. At this time we can only state that there is potential for allelopathy in the ponderosa pine — bunchgrass community.

Allelopathic effects would be conditioned by a host of factors, including the route and rate of release of the inhibitor, timing of release with growth processes of the receiving species, persistence of the inhibitor in the soil, distribution of seeds and residues, and weather conditions. The outcome of interactions among these environmental factors could vary within broad limits, ranging from striking examples of allelopathy to fluctuations or chronic expressions, or no effect at all. Furthermore, it is entirely possible

that the existing severe competition for water, nutrients, and growing space (Larson and Schubert 1969b) is the near-total mechanism.

If allelopathy occurs in the ponderosa pine — bunchgrass community of northern Arizona, what is its ecological role? The grasses are superior competitors for available water and nutrients (Larson and Schubert 1969b), and the sand culture experiment indicated they also compete successfully for growing space. These characteristics alone would seem to enable the grasses to dominate the site. But they do not explain the observed phenomenon where bunchgrasses have persisted on a site for 100 years or more (Schubert 1974), encompassing numerous seed crops and moist periods that are suitable for the establishment of pines. But no new pines emerge unless the grass cover is disturbed. The combined effects of an allelopathic mechanism and plant competition is a more feasible explanation for the long-term exclusion of new pine seedlings and other species from the community. Therefore, allelopathy may prolong the bunchgrass successional stage and forestall the ponderosa pine climax.

## CONCLUSIONS

A growth-inhibitory substance is produced predominantly in live foliage, and to a lesser extent in dead residues, of Arizona fescue and mountain muhly in the northern Arizona ponderosa pine — bunchgrass community. The inhibitor is capable of substantially reducing total germination and retarding germination rate and initial radicle development of ponderosa pine. The osmotic potential of the extracts confounds to some extent their phytotoxic effects.

The route of release of the inhibitor remains uncertain, although three possible routes were studied. Neither winter-long leaching of dead residues nor root excretion appears to be the principal route of release. Since dormant seeds and seedlings are insensitive to the inhibitor, it was suggested that leaching from live grass tissues and release of substances by microbial decomposition of dead residues during the growing season are potentially significant routes of release. Further research is recommended on these pathways.

Further research is also needed to clarify the ecological significance of the inhibitor. On the basis of the concentrations found toxic in laboratory experiments, there is considerable potential for toxic concentrations to occur under natural conditions. Actual inhibitory effects would be conditioned by a number of environmental variables, including those which (1) affect the rates of leaching and residue decomposition, and (2) influence the persistence or rate of degradation of the free inhibitory substance in

the soil. Coincidence of toxic levels of the inhibitor with initial growth processes appears to be necessary.

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