Phytotoxic Effects of Bunchgrass Residues on Germination and Initial Root Growth of Yellow Sweetclover

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Highlight: The subclimax bunchgrasses Arizona fescue and mountain muhly promptly invade disturbances in the climax ponderosa pine forest and develop into dense, persistent, impenetrable communities. Yellow sweetclover and several weed species invade disturbances in the bunchgrass community, flourish briefly, then decline as the bunchgrasses recover the site. Extracts prepared from green foliage and straw of fescue and muhly significantly reduced sweetclover seed germination and retarded speed of elongation and mean radicle length. Leachates from live grass foliage significantly inhibited sweetclover seed germination, suggesting that leaching may be a route of release of the inhibitor.

The ponderosa pine-bunchgrass community of northern Arizona occupies openings created by fire or logging in once dense stands of ponderosa pine (*Pinus ponderosa*). The subclimax bunchgrasses Arizona fescue (*Festuca arizonica*) and mountain muhly (*Muhlenbergia montana*) promptly take over the site following disturbance and develop into dense, exclusive communities, seemingly impenetrable by other species. In many instances, the ponderosa pine climax has not returned in 50 to 100 years, despite repeated seedfall from surrounding stands and favorable moisture conditions (Schubert 1974).

Yellow sweetclover (*Melilotus officinalis*) is a biennial herb commonly found along roadsides and drainages and in fields and parks in northern Arizona. It is capable of producing high yields of forage, especially when seeded, but accounts for only a small fraction of the total forage resource because of its short duration. When present in the ponderosa pine-bunchgrass community, it is an invader on areas where the bunchgrass cover has been disturbed. Sweetclover may form dense, fairly pure stands where the grasses are recently and completely removed (Fig. 1); but as the grasses recover the site, the clover and other

Fig. 1. *An experimental plot which had been plowed in the spring developed a heavy cover of yellow sweetclover by midsummer (right), whereas none occurred in the intact bunchgrass stand. Clover seeds were apparently distributed over the entire area, but only on the plowed plot did they germinate and survive.*

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forbs decline in density and height and disappear within 2 to 3 years.

In addition to the intense competition displayed by the bunchgrasses for available water, nutrients, and growing space (Larson and Schubert 1969), there is evidence that chemical interactions may play an important role in determining plant associations. 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. The author recently reported that aqueous extracts of fescue and muhly foliage reduced percentage germination and speed of germination of ponderosa pine seeds by 60% or more. Radicle elongation was similarly retarded but was found to be sensitive to the osmotic potential of the extracts. The route of release of the inhibitor was not uncovered (Rietveld 1975).

This paper reports the phytotoxic effects of bunchgrass residues on germination and initial growth of yellow sweetclover, and a study to explore leaching as a possible route of release of the inhibitor.

Seed Germination and Radicle Elongation 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 yellow sweetclover.

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, Ariz. Excess soil was shaken from the grass roots, but they were left unwashed to avoid losing any excreted phytotoxins. The plant materials were airdried for 26 days, then ground in a mill to pass a 20-mesh screen.

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 (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 germination of clover seeds. To reduce fungal contamination, seeds were surface-sterilized by soaking in 30% hydrogen peroxide for 15 minutes and rinsed with sterile distilled water[1]; all glassware and glass filter paper were sterilized by autoclaving. The interior of the growth chamber was washed with sterilizing chemicals. Five milliliters 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.

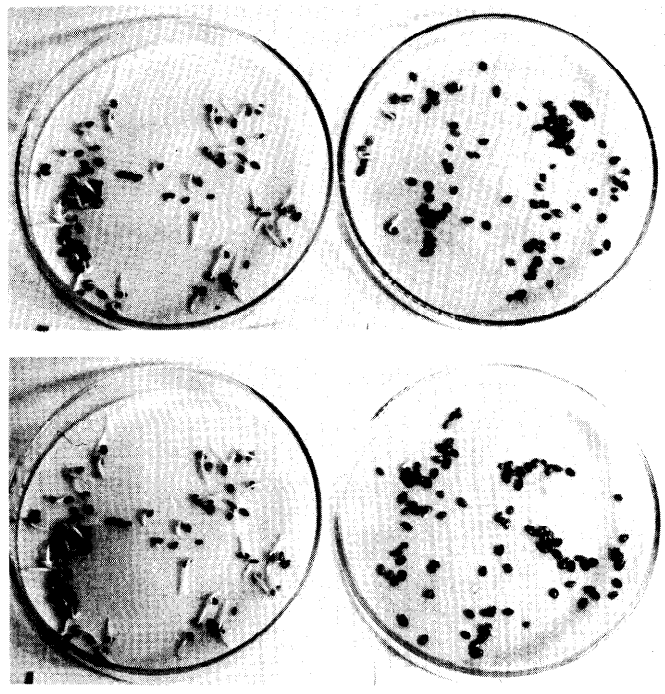


Fig. 2. *Germination of surface-sterilized yellow sweetclover seeds in extracts of fescue foliage (top, right) and muhly foliage (bottom, right) compared with the control (left).*

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%. 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.

[1]Hydrogen peroxide may stimulate germination through chemical scarification of seed coats, but may also increase sensitivity to phytotoxins. However, all seeds used in the study were pretreated uniformly.

Table 1. Germination and growth responses of yellow sweetclover to extracts prepared from grass residues.

Source of extract	Mean percentage germination (%)	Mean speed of germination (no./day)	Mean time for 50% germination (days)	Mean radicle length (mm)	Mean speed of elongation (mm/day)
Fescue foliage (FF)	32.1**	4.7**	6.5**	4.5**	1.1**
Fescue new litter (FNL)	68.8	13.8**	4.9**	11.0	4.0
Fescue old litter (FOL)	70.3	25.6	3.0	13.4	5.1
Fescue roots (FR)	66.9	26.6	1.9	15.3*	5.2
Muhly foliage (MF)	26.0**	6.3**	3.0	1.3**	0.7**
Muhly new litter (MNL)	73.6	23.5	2.6	15.0*	3.6
Muhly old litter (MOL)	69.4	29.0	2.0	15.8*	6.1
Muhly roots (MR)	71.8	26.7	2.2	12.3	4.0
Control	68.0	29.7	2.6	10.7	5.1

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

Percentage germination, speed of germination (Maguire 1962), time for 50% germination, speed of elongation (adapted from Maguire 1962), and mean radicle length before decline were calculated and tested for significant differences by analysis of variance and least significant difference (LSD). Speed of germination and elongation take both the promptness and completeness of the respective processes into account, and are weighted to emphasize early germination and growth.

Results and Discussion

Fescue foliage and muhly foliage extracts induced highly significant ($\alpha = 0.01$) reductions in amount and rate of germination and radicle elongation of clover seeds (Table 1, Fig. 2). Speed of germination and speed of elongation were retarded to rates less than one-fourth of the control. Time for 50% germination was correspondingly increased. Fescue new litter retarded speed of germination to approximately one-half that of the control, and as a result significantly increased the time required for 50% germination. The magnitude of these effects is apparent in Figures 3A and 3B, in which speed of germination and speed of elongation of clover seeds and radicles, respectively, are expressed as percentages of the control values. Three extracts (fescue roots, muhly new litter, and muhly old litter) stimulated radicle elongation significantly ($\alpha = 0.05$). No definitive explanation is offered for these root growth responses, but they could be caused by metabolites in the extracts.

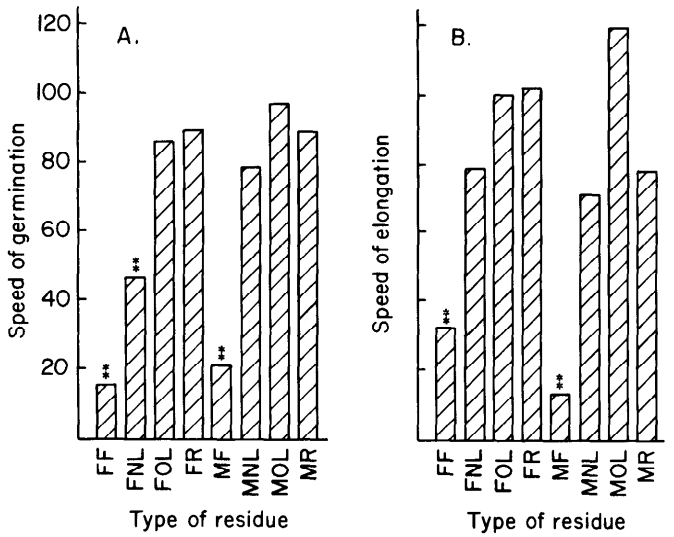


Fig. 3. *Speed of germination (A) of clover seeds and speed of elongation (B) of radicles expressed as percentages of the respective control values. Asterisks designating significance correspond to Table 1. (FF = fescue foliage, FNL = fescue new litter, FOL = fescue old litter, FR = fescue roots, MF = muhly foliage, MNL = muhly new litter, MOL = muhly old litter, MR = muhly roots.)*

Retarded seedlings tended to be stocky, with a thickened, stubby radicle and short hypocotyl. In the most toxic extracts, the radicle tip eventually died.

The control "extract" was slightly acidic (pH 5.5, Table 2) due to the incorporation of carbon dioxide by the blender. Although no tests were run as to the effects of pH on seed germination and seedling growth, measured pH values were not considered to be low enough to affect these processes. The extracts which induced the strongest responses (fescue foliage, fescue new litter, muhly foliage) had pH values similar to the control.

Osmotic potential of the extracts (Table 2) decreased with increasing age and decomposition of residues. Fescue foliage and muhly foliage extracts, which significantly inhibited germination and radicle elongation, also had the lowest osmotic potentials, −2.6 and −1.6 atmospheres respectively. Since the separate effects of osmotic potentials (equivalent to water potential here) were unknown, a follow-up experiment was conducted in which clover seeds were germinated in solutions of polyethylene glycol 20,000 (Sharma 1973) with osmotic potentials of −1, −2, −4, and −8 atmospheres. The polyethylene glycol solutions and distilled water control were changed daily to minimize the increase in concentration of the solutions as seed imbibed water.

Table 2. pH and osmotic potential of aqueous extracts of grass residues prepared by homogenizing in a blender. Deionized water was used as a standard with zero osmotic potential.

Source of extract	pH	Osmotic potential (atmospheres)
Fescue foliage	5.7	−2.6
Fescue new litter	4.6	−0.5
Fescue old litter	5.8	−0.3
Fescue roots	6.1	−0.6
Muhly foliage	5.7	−1.6
Muhly new litter	4.9	−0.6
Muhly old litter	5.6	−0.3
Muhly roots	5.8	−0.6
Control	5.5	0.0

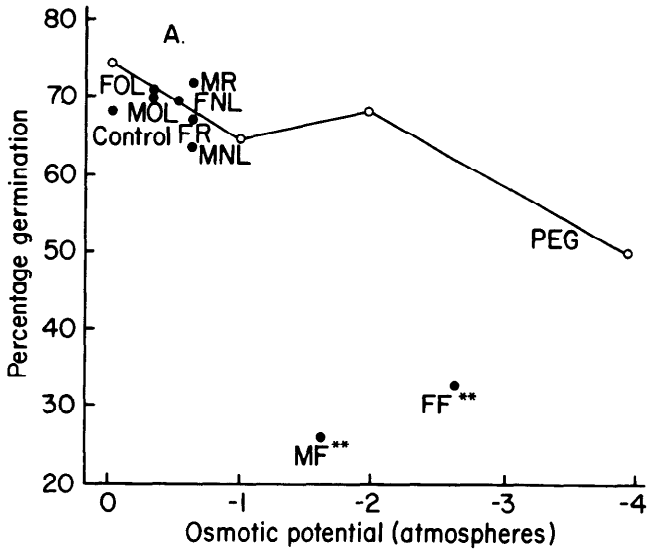
In species or processes sensitive to water stress, polyethylene glycol solutions with stresses (osmotic potentials) equivalent to the extracts would be a better measure of the control response. Percentage germination, speed of germination, and speed of elongation of clover in polyethylene glycol solutions are compared with the responses in grass residue extracts in Figure 4. Response data for the extracts are from Table 1 and osmotic potential data are from Table 2. Percentage germination (Fig. 4A) was stimulated by osmotic potentials above −1 atmosphere, and declined at a moderate rate below −2 atmospheres. Although osmotic potential accounts for part of the significant response to fescue foliage and muhly foliage extracts, the difference in response from polyethylene glycol solutions of equivalent osmotic potential remains substantial. The magnitude of the observed extract effects on speed of germination and speed of elongation (Figs. 4B and 4C) are diminished considerably by the separate effect of osmotic potential, but the differences from polyethylene glycol remain significant. Thus, osmotic potential of extracts was a principal confounding factor which accounted for part of the observed responses to the extracts. In the range 0 to −3 atmospheres, which includes the osmotic potentials of the extracts, osmotic potential should not interfere with percentage germination, but may account for a substantial portion of the observed speed of germination and speed of elongation responses.

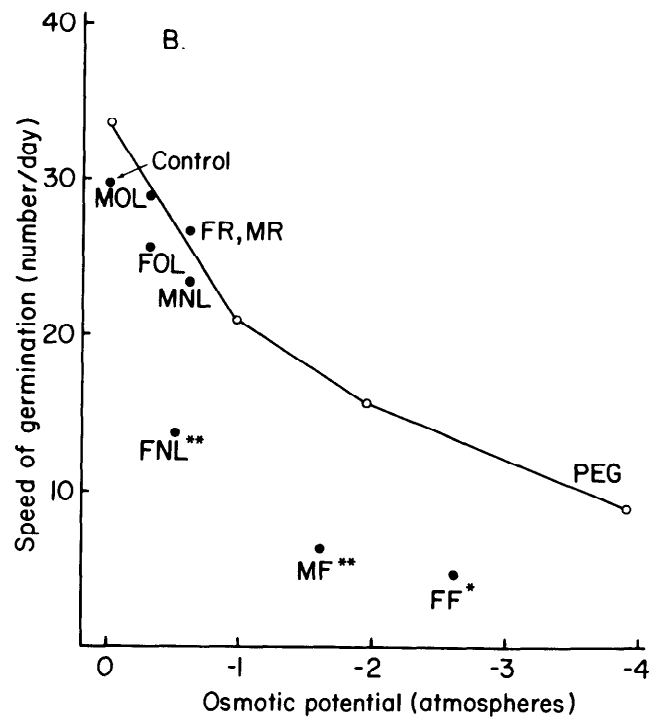
Seed Germination in Leachates from Grass Residues

The results of the extract experiment indicated that a phytotoxic substance is contained in residues of Arizona fescue and mountain muhly, and that it is particularly concentrated in live foliage. The following experiment explored leaching as a possible route of release of the inhibitor.

Materials and Methods

Samples of fescue and muhly live foliage were collected in mid-September on the Fort Valley Experimental Forest. In the laboratory,





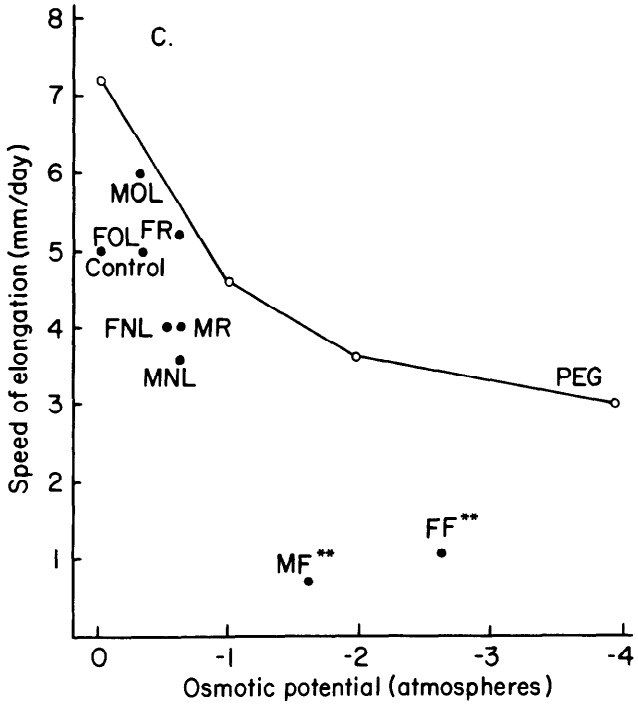


Fig. 4. *Comparison of osmotic potential effects of grass residue extracts and polyethylene glycol (PEG) solutions on (A) percentage germination, (B) speed of germination, and (C) speed of elongation of yellow sweetclover. One asterisk indicates the difference between the extract and PEG is significant at the 5% probability level; two asterisks indicate significance at the 1% level. (FF = fescue foliage, FNL = fescue new litter, FOL = fescue old litter, FR = fescue roots, MF = muhly foliage, MNI. = muhly new litter, MOL = muhly old litter, MR = muhly roots.)*

the samples were separated into basal leaves and culms (seed stalks, including the inflorescence). Fescue culms had begun to dry when collected, whereas muhly culms were still green; basal leaves of both species were green. A 50-g sample (fresh weight) of each of the four plant materials was soaked in 100 ml of distilled water in a large beaker for 12 hours. Each was agitated for 5 minutes to wet all of the material. At the end of the soaking period the four leachates were drained off and refrigerated.

Surface-sterilized clover seeds were used to test the four leachates for phytotoxicity. Ten milliliters 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 reduce their growth-inhibiting activity.) Three milliliters of sterile distilled water and 100 unstratified seeds were added to each petri dish. A control with no leachate was also run. Germination tests were conducted in darkness in a germinator with temperature set at 75°F and high humidity. Other procedures and germination criteria were the same as described previously. Germination was recorded daily for 11 days.

Results and Discussion

All of the leachates, except from muhly culms, markedly reduced percentage germination and retarded speed of germination (Table 3). The magnitude of the observed responses and appearance symptoms of retarded seedlings were similar to those in the extract experiment.

Unfortunately, the osmotic potentials of the leachates were not determined, since they undoubtedly would account for a portion of the observed responses. Osmotic potentials within the range 0 to −3 atmospheres were found to have only a small depressing effect on percentage germination, but speed of germination was quite sensitive (Figs. 4A and 4B). Since it is unlikely that the osmotic potentials of the leachates were lower than any of the extracts, the results can be qualified in terms of the osmotic potentials measured in the extract experiment. If the control values in Table 3 are adjusted downward for the separate effect of osmotic potentials equivalent to fescue foliage and muhly foliage extracts (Figs. 4A and 4B), the percentage germi-

Table 3. Effects of leachates of Arizona fescue and mountain muhly live foliage on percentage germination and speed of germination of yellow sweetclover seeds.

Source of leachate	Mean percentage[1] germination	Mean speed of germination (no./day)
Fescue, basal leaves	20.8**	2.1**
Fescue, culms	19.8**	2.0**
Muhly, basal leaves	20.6**	2.0**
Muhly, culms	43.8	11.3**
Control	52.0	18.6

1 arc sin $\sqrt{p}$ transformation.
*Differs significantly from control at 5% probability level.
**Differs significantly from control at 1% probability level.

nation response would be little changed, but speed of germination would be diminished by two-thirds. The depression in percentage germination would remain highly significant (α = 0.01), but speed of germination responses would be lowered to the 5% level of significance. However, because of the sensitivity of speed of germination of clover seed to extract or leachate osmotic potential, the validity of the qualified responses is uncertain. Thus leaching may be a route of release of the inhibitory substance; the toxic substance contained in grass foliage may be slowly leached into the ground, where it inhibits the growth of species susceptible to it. However, more research is needed on the presence, toxicity, and leachability of the inhibitor in live and dead grass residues during the germination and growth period of the receiver species. Other possible routes of release, such as microbial decomposition of dead residues on the soil, should be investigated.

Discussion of Ecological Significance

The results of the extract experiment showed that substances contained in live foliage of fescue and muhly are highly toxic to germination and initial root development of yellow sweetclover in the laboratory. Osmotic potential of the extracts or leachates accounts for a large portion of the observed responses. The actual degree of inhibition is about the same as for ponderosa pine, but ponderosa pine is less sensitive to plant water stress (Rietveld 1975).

Under natural conditions, the occurrence of allelopathic effects would be conditioned by an assortment 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—especially the diluting and leaching effects of precipitation. The outcome of interactions among these environmental factors could vary within broad limits.

It is interesting to note that yellow sweetclover has been reported to have inhibitory effects on certain grass species. Extracts prepared from sweetclover roots inhibited both germination and seedling growth of crested wheatgrass, Russian wildrye, intermediate wheatgrass, bromegrass, and timothy (Lawrence and Kilcher 1962). White sweetclover (*Melilotus albus*) contains a large amount of coumarin and related compounds, as do most sweetclovers, which inhibit seed germination of several associated species (Knapp and Furthmann 1954). Later studies have shown that the coumarin is converted to melilotic acid, which is apparently the responsible phytotoxin (Kosuge and Conn 1961).

While sweetclovers may release toxic substances which inhibit the development of associated species in a cultivated forage crop, this effect may be of little advantage in determining species distribution of wild sweetclovers, since other factors play a much more important role. Since wild yellow sweetclover frequently occurs in nearly pure stands, chemical interactions may take place with other herbaceous species, although they have not yet been documented. Because sweetclover is very sensitive to water stress, at least during germination and initial growth, there seems to be little need for an allelopathic mechanism to exclude it from the bunchgrass community. The bunchgrasses are acknowledged to be superior competitors for available water and nutrients (Larson and Schubert 1969), and for growing space (Rietveld 1975). Moreover, sweetclover is biennial, which presents no threat to the bunchgrass community should it invade during a moist year. Sweetclover would be ousted from the community when growth factors again become limiting. The fact that sweetclover usually occupies roadsides, drainage banks, and disturbed sites also supports the contention that it cannot endure vegetative competition.

Do yellow sweetclover and the bunchgrasses engage in reciprocal chemical warfare, i.e. allelopathy? If so, the toxins produced by sweetclover are of little avail. Sweetclover is distinctly on the losing side, since it is easily overcome by the defense mechanisms of the bunchgrasses. Yellow sweetclover appears to be one of the weaker herbaceous aggressors against the bunchgrass community. The strong defense mechanisms of the bunchgrasses have apparently evolved to meet stronger challengers. One of those challengers is most likely ponderosa pine, the climax species. It seems reasonable to postulate that the combination of intense competition and production of phytotoxins benefit the bunchgrasses most by forestalling the ponderosa pine climax, thus prolonging their subclimax successional stage.

Conclusions

A growth-inhibitory substance is present 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 yellow sweetclover.

Percentage germination of sweetclover seeds is less sensitive to the osmotic potential of the extracts than are speed of germination and speed of radicle elongation, which are retarded by even small negative osmotic potentials.

The inhibitory substance appears to be leachable from live grass foliage collected in mid-September. Leaching could be a route of release, but further research is needed on the leachability and toxicity of the substance during the germination period of the receiver species.

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