Biological Activity of Extracts from *Zea mays* L. and *Pinus densiflora* L.

Young K. Joo, Soul Chun[1], Nick E. Christians[2]

Dept. of Biological Resources and Tech., Yonsei University, Wonju, 222-710, Korea

[1]Dept. of Applied Biological Chemistry, University of Tokyo, Yayoi 1-1-1, Tokyo, 113, Japan

[2]Dept. of Horticulture, Iowa State University, Ames, IA 50011-1100, USA.

옥수수(*Zea mays* L.)와 소나무(*Pinus densiflora* L.) 추출물의 생물학적 활성

주영규 · Soul Chun[1] · Nick E. Christians[2]

연세대학교 환경자원학과, [1]동경대학교 생물화학과, [2]아이오와주립대학교 원예학과

ABSTRACT

Environmental concerns arising from synthetic herbicides in plant management systems have led to an interest in plant-derived compounds as natural herbicides. Inhibitory effects of compounds extracted with 50% methanol from corn (*Zea mays* L.) and pine (*Pinus densiflora* L.) were evaluated on large crabgrass (*Digitaria sanguinalis* (L.) Scop.), annual bluegrass (*Poa annua* L.), radish (*Raphanus sativus* L.), and perennial ryegrass (*Lolium perenne* L.) The aqueous extracts inhibited seed germination and had postemergence activity on the four species. The stability of biological activity of corn grain, stover, and root extracts was not affected by heating to 135℃ or freezing/thawing treatments when applied at levels above 0.25 kg m^{-2} based on dry weights of powders before extraction. Heating reduced the activity of pine litter and bark extracts at all levels except the highest application level but had little effect on pine needle extracts.

Key words: natural herbicide, turfgrass, *Zea mays*, *Pinus densiflora*

INTRODUCTION

Synthetic pesticides are a major component of agricultural, forestry, and turfgrass management systems (Balogh and Anderson, 1992). The growing concern over environmental and public health consequences of agricultural chemical use has stimulated interest in the search for new, environmentally safe herbicides (Lydon and Duke, 1987). Naturally-occurring bioactive herbicidal compounds, particularly those shown to be nontoxic, would be useful in integrated weed-control programs. The use of natural pesticides may help to change the traditional approaches to agricultural science and development of sustainable systems (Lovett, 1991).

Potential sources of natural herbicides are extracts from corn or pine. It has been found that corn gluten meal contains biologically active compounds, and gluten meal is used as a natural preemergence control of weeds in turf (Christians, 1993; Liu and Christians, 1994a, 1994b, 1996a, 1996b). It also has been reported that *Pinus* spp. have allelochemical effects on understory plants (Rice, 1995).

The objectives of this study were to investigate preemergence and postemergence herbicidal activity of extracts of corn and pine on four plant species. The stability of the compounds following heating and freezing was also evaluated.

Materials and Methods

Bioactivity experiments

Mature corn grain, stover, and root parts were harvested for petri dish and pot test. Pine needles in the green leaf stage, bark, and pine litter were also used. The materials were dried at 60℃ for 72 h and ground to a powder with a particle size<1 mm. The separate powders of corn and pine were dissolved for 24 h in 50% methanol and filtered through Whatman™ No.3 (0.2-μm) filter paper. The methanol mixture solution was vacuum evaporated at room temperature leaving a concentrated aqueous extract of each of the plant materials.

In the first test, bioassays were conducted using the concentrated extracts on large crabgrass, annual bluegrass, radish, and perennial ryegrass. For this test, the grain, stover, and root components were combined in equal parts to form the corn extract, and the needle, bark, and litter extracts were combined in equal parts to form the pine extract. The extracts were applied at 0 (control), 0.5, and 1.0 kg m^{-2} based on dry weights of the powders before extraction. One hundred seeds of each species were placed on one layer of Whatman™ No.3 filter paper in 55-mm diameter petri dishes. The treatments were applied in distilled water, and the petri dishes were covered with a lid and parafilm and placed in a controlled environmental chamber at 27℃ with a 12-h photo period. Germination rates were evaluated in each petri dish after 10 d.

In the second assay, seedlings of perennial ryegrass at the one- and two-leaf stage of development were placed on the filter paper. The one-leaf stage was defined as a selected seedling with one leaf shorter than 20 mm, and the two-leaf stage was defined as a selected plant with at least two leaves that were 20 mm or more in length. Separate aqueous extracts of grain, stover, and root from corn and needles, bark, and litter from pine were applied to the filter paper at 0 (control), 0.5, and 1.0 kg m^{-2} levels based on dry weights of the powder before extraction. Data on the elongation of leaves and roots were collected after 15 d and expressed as a percentage inhibition of growth compared to the control [100×(Length of control-length of treated plant) / length of control].

Temperature stability

The separate aqueous corn extracts from corn grain, stover, root, and pine extracts from needles, bark, and litter were tested for temperature stability. The extracts were prepared in the same way as described in the bioactivity experiments. The extracts from each part of corn and pine were autoclaved at 135℃ for 20 min, at 140 Kpa pressure. The extracts were also subjected to freezing and thawing at -10 and 25℃ three times over a 15-h period. One hundred seeds of perennial ryegrass were placed on one layer of Whatman™ No.3 filter paper in 55-mm diameter petri dishes. The extracts were applied at 0 (control), 0.25, 0.5, and 1.0 kg m^{-2} based on dry weights of the powder before extraction. Germination rates were evaluated in each petri dish after 10 d under the same controlled environmental conditions as the bioactivity experiments.

Pot experiments

Plastic pots with a 28.3-cm^2 surface area were filled with clay loam soil, and 50 perennial ryegrass seeds were placed on the surface and covered with 2 mm soil. Treatments included the ground powder of corn grain, stover, root, and pine needles that had not been extracted and the extracts from each of these materials at 0 (control) and 0.5 kg m^{-2} based on dry weights of the powder before extraction. In this experiment, distilled water was used for the extraction in place of the 50% methanol.

The pots were covered with polyethylene and cultured for 20 d in a controlled environmental chamber at 27℃ with a 12-h photo period. Germination rates and dry weight yield were evaluated in each pot after 20 d.

Statistical analysis

All experiments were conducted as randomized complete blocks with three replications. Data were analyzed by using the analysis of variance (ANOVA) procedure of SAS (SAS Institute, 1990).

Results and Discussion

Bioactivity experiments

The corn extract reduced germination of the large crabgrass, annual bluegrass, radish, and perennial ryegrass by 100, 97, 86, and 100%, respectively, at the 0.5 kg m^{-2} level

Table 1. Effect of corn and pine aqueous extracts on the germination of four species.

Material	Level[z] (kg m^{-2})	Species			
		Large crabgrass	Annual bluegrass	Radish	Rerennial ryegrass
		% Germination			
Control	0	51	96	24	93
Corn	0.5	0	3	16	0
	1.0	0	0	3	0
Pine	0.5	24	10	8	3
	1.0	6	0	3	0
$LSD_{0.05}$		3	3	3	3

ANOVA

Source of variation	df	Pr>F
Replication	2	
Species	*3*	*0.0001*
Material	1	0.0043
Level	2	0.0001
Species×Material	3	0.0003
Species×Level	6	0.2358
Material×Level	2	0.0027
Species×Material×Level	*6*	*0.0202*

[z]Based on dry weights of the powders before extraction.

(Table 1). The 1.0 kg m^{-2} level reduced germination of the large crabgrass, annual bluegrass, and perennial ryegrass by 100%, whereas radish germination was reduced by 97% at that treatment level.

The pine extract was less effective on every monocotyledonous species except radish which is a dicotyledon. Only annual bluegrass and perennial ryegrass germination was reduced by 100% at the 1.0 kg m^{-2} level of the pine extract. The 0.5 kg m^{-2} level reduced germination of all species, although none was reduced by 100% at that treatment level.

The corn grain, stover, and root extracts reduced the shoot growth of perennial ryegrass in the one-leaf stage by an average of 91% (Table 2). The corn extracts were less effective on the perennial ryegrass shoots in the two-leaf stage than those in the one-leaf stage. Root length of the perennial ryegrass was reduced an average of 95% by the three corn components at the one-leaf stage and by 94% at the two-leaf stage.

The pine leaf extract reduced the shoot growth of perennial ryegrass in the one- and two-leaf stage by 96 and 93% and reduced root growth by 97 and 98% (Table 2). The pine litter and bark extracts were very effective at reducing shoot growth of plants in the one-leaf stage but were less effective on plants in the two-leaf stage. The extracts of these two materials were equally effective at reducing root elongation of plants in the

Table 2. Effect of corn and pine extracts[z] on the shoot and root length of perennial ryegrass seedlings at the one- and two-leaf stage of development.

Material	Part extracted	% Growth inhibition[y]			
		Shoot length		Root length	
		One-leaf[x]	Two-leaf[w]	One-leaf	Two-leaf
Corn	Grain	93	68	95	94
	Stover	91	51	96	94
	Root	89	45	95	94
	$LSD_{0.05}$	NS	20.1	NS	NS
Pine	Green leaf	96	93	97	98
	Litter	93	27	99	90
	Bark	93	49	97	89
	$LSD_{0.05}$	NS	25	NS	4

[z]Extract rate applied of 0.5 kg m^{-2} based on dry weights of the powders before extraction.
[y][(Length of control-length of treated plant) / length of control] × 100.
[x]Defined as perennial ryegrass seedling with one leaf shorter than 20 mm.
[w]Defined as perennial ryegrass seedling with two leaves longer than 20 mm.

one- and two-leaf stages.

The bioactivity study indicates that there are biologically active substances in both corn and pine that can be extracted with methanol. These materials were effective in inhibiting the germination of both dicotyledonous and monocotyledonous species.

Temperature stability

There was a significant temperature-by-rate interaction for the effect of corn and pine

Table 3. Analysis of variance for the effects of corn and pine extracts treated with high and low temperature on the germination of perennial ryegrass.

Material	Source of variation	df	Pr>F
Corn	Replication	2	
	Temperature	2	0.0529
	Part extracted	2	0.0921
	Temp. × Part	4	0.1348
	Rate	3	0.0001
	Temp. × Rate	6	0.0002
	Part × Rate	6	0.1842
	Temp. × Part × Rate	12	0.1065
Pine	Replication	2	
	Temperature	2	0.1342
	Part extracted	2	0.0064
	Temp. × Part	4	0.2628
	Rate	3	0.0001
	Temp. × Rate	6	0.0043
	Part × Rate	6	0.0002
	Temp. × Part × Rate	12	0.0155

extracts on the germination of perennial ryegrass (Table 3). The germination of the perennial ryegrass treated with corn grain extracts at the 0.1 kg m^{-2} level increased from 6 to 16% when the extracts were heated to 135℃ and from 6 to 60% when the extract was subjected to freezing and thawing treatments (Table 4). At higher treatment levels, heating did not reduce biological activity. There was no effect of freezing and thawing above the 0.25 kg m^{-2} level. The effectiveness of corn stover extract was reduced by freezing and thawing at the 0.1 kg m^{-2} level only and was unaffected by heating.

The effectiveness of the pine needle extract was reduced by heating and cold temperature treatments at the 0.1 kg m^{-2} level only. The activity of pine litter and bark extracts were reduced by heating at all levels except 1.0 kg m^{-2}. The effectiveness of the pine litter and bark extract was reduced at the 0.1 kg m^{-2} level by the high temperature treatments.

Table 4. Effect of corn and pine extracts treated with high and low temperatures on the germination of perennial ryegrass[z].

Part extracted	Level[y] (kg m^{-2})	Treatment		
		No temp. treatment	Heating[x]	Freezing/thawing[w]
		------------------	%Germination	------------------
Corn grain	0.10	6	16	60
	0.25	0	0	19
	0.50	0	0	0
	1.00	0	0	0
Corn stover	0.10	0	0	41
	0.25	0	0	0
	0.50	0	0	0
	1.00	0	0	0
Corn root	0.10	0	1	9
	0.25	0	0	0
	0.50	0	0	0
	1.00	0	0	0
Pine needle	0.10	0	7	5
	0.25	0	0	0
	0.50	0	0	0
	1.00	0	0	0
Pine litter	0.10	8	16	4
	0.25	3	12	3
	0.50	0	9	1
	1.00	0	0	0
Pine bark	0.10	12	52	4
	0.25	13	11	3
	0.50	0	12	0
	1.00	0	0	0
$LDS_{0.05}$		5	5	4

[z]The germination rate of the untreated control was 95±2%.
[y]Based on dry weights of the powders before extraction.
[x]Means the sample autoclaved at 135℃ for 20 min.
[w]Means the sample treated three times at -10/25℃, 15 h alternatively.

The inhibitory substances in both corn and pine are effective in reducing both shoot and root formation of perennial ryegrass. All materials were generally more effective on the inhibition of roots than shoots. The inhibition of shoot growth reduced as the plant matured. The extract of pine needles was more effective than litter and bark on the inhibition of growth of perennial ryegrass in the two-leaf stage, indicating that either different compounds are present in the needle extract or the compounds are present in higher concentrations in the needles.

Pot experiments

The powder treatment and the aqueous extracts from corn grain and pine needle had an inhibitory effect on germination and dry yield of perennial ryegrass (Table 5). Germination rate was reduced 87% by the corn grain aqueous extract but was reduced only 20% by the corn grain powder. Similar effects of the corn grain extract and powder were observed on dry weight production which was reduced by 89 and 20%, respectively. The extracts of corn stover and corn root and the ground powders of these two materials were less effective at inhibiting both germination and tissue growth. The ground powder of the stover was less effective than the extract in reducing tissue growth, and the powdered root was less effective at reducing germination than was the root extract. There were no differences between the pine needle extract and the ground powder of the pine needles on either germination or tissue growth.

The results of these tests indicate that there may be different inhibitory substances found in the corn grain than are present in corn stover and root. The substances in grain are evidently water soluble and can be easily extracted in aqueous solution. Liu

Table 5. Effect of corn and pine extracts on the germination and dry weight of perennial ryegrass[z] in the growth chamber pot test. Application rate of both powder and extracts was based on the weight of 0.5 kg m^{-2} of the powders before aqueous extraction.

Part extracted	Treatment	Germination inhibition	Yield inhibition
		% Inhibition	
Corn grain	Extract	87	89
	Powder	20	20
Corn stover	Extract	46	50
	Powder	42	27
Corn root	Extract	52	41
	Powder	34	23
Pine needle	Extract	83	82
	Powder	75	70
$LSD_{0.05}$		13	20

[z]The germination of perennial ryegrass in the control was 77±6%(n=50). The dry weight yield of perennial ryegrass in the control was 74±6 mg(pot surface area; 28.3 cm^2).

and Christians (1994b and 1996b) have previously reported that the protein fraction of corn grain contains several dipeptides and at least one pentapeptide capable of inhibiting plant germination. The pine needles contain an inhibitory substance equivalent in activity to the corn grain extract. The nature of the inhibitory substance in the pine is unknown; however, similar inhibitory activity has been reported in pine forest understory by Kil (1989) and Kil et al.(1991). Future works will be conducted to isolate and identify the biologically active compounds in these materials and tests will be conducted to determine if they have potential for use as natural herbicides.

Literature Cited

Balogh, J.C., and J.L. Anderson. 1992. Environmental impacts of turfgrass pesticides, p. 221-354. In: J.C. Balogh and W.J. Walker (eds.). Golf course management and construction-environmental issues. Lewis Publ., Chelsea.

Christians, N.E. 1993. The use of corn gluten meal as a natural preemergence control in turf, p. 284-290. In: R.N. Carrow, N.E. Christians, and R.C. Shearman (eds.). Turfgrass Soc. Res. J. 7. Intertec Publishing Corp., Overland Park, KS.

Kil, B.S. 1989. Allelopathic effects of five pine species in Korea, p. 81-99. In: C.H.Chou and G.R.Waller (eds.). Phytochemical ecology: Allelochemicals, mycotoxins and insect pheromones and allomones. Inst. of Bot., Academia Sinica Monogr. Ser. No. 9, Taipei, ROC.

Kil, B.S., D.Y. Kim, Y.S. Kim, and S.Y. Lee. 1991. Phytotoxic effects of naturally occurring chemicals from *Pinus koraiensis* on experimental species. Korean J. Ecol. 14:149-157.

Liu, D.L. and N.E. Christians, 1994a. Herbicidal activity of hydrolyzed corn gluten meal on three grass species in controlled environments. J. Plant Growth Reg. 12:221-226.

Liu, D.L. and N.E. Christians. 1994b. Isolation and identification of root-inhibiting compounds from corn gluten hydrolysate. J. Plant Growth Reg. 13:227-230.

Liu, D.L. and N.E. Christians. 1996a. Bioactivity of a pentapeptide isolated from corn gluten hydrolysate on *Lolium perenne*. J. Plant Growth Reg. 15:13-18.

Liu, D.L. and N.E. Christians. 1996b. Inhibitory activity of corn gluten hydrolysate on monocotyledonous and dicotyledonous species. HortSci. 32:243-245.

Lovett, J.V. 1991. Changing perceptions of allelopathy and biological control. Biol. Agri. Hort. 8:89-100.

Lydon, J. and S.O. Duke. 1987. Progress toward natural herbicides from plants. Herbs Species Medical Plants Dig. 5:1-4.

Rice E.L. 1995. Allelopathy in forestry, p. 353-355. In: Biological control of weeds and plant diseases. Univ. of Oklahoma Press, Norman.

SAS Institute. 1990. SAS/STAT user's guide. Vol. 2, 4th ed. SAS Institute, Cary, NC.