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Herbicidal Activity of Hydrolyzed Corn Gluten Meal on Three Grass Species under Controlled Environments

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Abstract. US Patent No. 5,030,268 discloses that corn gluten meal, the protein fraction of corn (*Zea mays* L.) grain, can be used as a natural preemergence herbicide. However, corn gluten meal is insoluble in water, and this characteristic renders it difficult to apply as a herbicide. To seek a water-soluble material with more potent herbicidal activity, the phytotoxicity of various samples derived from corn gluten meal and other related crop materials were evaluated by using three different grass species under controlled environments. Greenhouse and growth chamber bioassays showed that the sample of enzymatically hydrolyzed corn gluten meal was more herbicidally active than the corn gluten itself and was highly water soluble. Gluten hydrolysate prepared with bacterial source proteinase had the greatest inhibitory activity to the root growth of germinating seeds. This water-soluble material derived from corn gluten meal had a growth-regulating effect on the root system and can be used as a natural herbicide.

The use of herbicides for weed control has been practiced to some extent since 1927. Synthetic and organic pesticides have become a major component of agricultural, forestry, and turfgrass management systems (Balogh and Anderson 1992). Although chemicals with increased efficacy and safety have been developed in recent years, the agrochemical industry faces continuing and growing criticism over the toxicity and residue problems of pesticides (Huppatz 1990). Because many pesticides are toxic and all are biologically active by design, there is a concern regarding their effects on human health and environmental quality. Environmental and human health problems associated with pesticides have been documented (Balogh and Anderson 1992).

The judicious application of herbicides has become an integral part of agriculture (Pimentel 1986). Naturally occurring bioactive compounds, particularly those shown to be nontoxic, would be useful in integrated weed-control programs. The growing awareness of the environmental and public health consequences of agricultural chemical use has stimulated interest in the search for new, environmentally safe herbicides (Lydon and Duke 1987). The use of natural pesticides may help to change the traditional approaches to agricultural science and open the way to the development of sustainable systems (Lovett 1991).

The objective of this study was to search for herbicidally active and water-soluble materials derived from corn gluten meal. US Patent No. 5,030,268 discloses that corn gluten meal, which is a byproduct of corn from the wet-milling process, can be used as a natural preemergence herbicide (Christians 1991). Corn gluten meal can inhibit the establishment of germinating weeds by preventing root formation of germinating plants (Christians 1993), but corn gluten meal is quite insoluble in water and this characteristic renders it difficult to apply as a herbicide. If water-soluble, corn gluten meal–related materials could be developed and used as an alternative to conventional herbicides, they would be environmentally and economically desirable. They could be used as a substitute for synthetic chemical herbicides or as a supplement to synthetic herbicides to reduce the concentration of these pesticides in the environment. The phytotoxicity of various samples derived from corn gluten meal and other related crop materials were evaluated by using three different grass species grown in the greenhouse and growth chamber.

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Table 1. List of samples derived from corn gluten meal and other crop materials.

Sample	Description
1	corn gluten meal
2	corn gluten meal treated with fungal proteinase
3	corn gluten meal treated with bacterial proteinase
4	corn gluten filtrate
5	alcohol-soluble fraction of corn gluten filtrate
6	alcohol-insoluble fraction of corn gluten filtrate
7	corn gluten filtrate treated with cation resin
8	corn gluten meal mixed with corn hulls and germs
9	corn gluten meal without corn hulls and germs
10	corn gluten meal treated with bacterial proteinase
11	corn gluten hydrolysate, total ion exchanged
12	soluble corn steep liquor solids
13	insoluble corn steep liquor solids
14	wheat gluten
15	wheat gluten hydrolysate
16	corn gluten meal treated with bacterial proteinase
17	soybean meal

Materials and Methods

Seventeen different samples derived from corn gluten meal or other grain materials (Table 1) were obtained from Grain Processing Corporation (Muscatine, IA, USA). A number was assigned to each sample according to the order of arrival. Samples were tested in greenhouses and growth chambers for their inhibitory effects on test plants.

Study 1

Seven corn gluten meal–related samples with designated numbers 1–7 were used to study the effects on germinating seeds under controlled environmental conditions (Table 1).

Smooth crabgrass (*Digitaria ischaemum* Schreb.) was the test species in the greenhouse bioassays. Plastic pots with a surface area of 58 cm^2 and a depth of 5 cm were filled with a Nicolett (fine-loamy mixed mesic Aquic Hapludolls) soil that had a pH of 7.5. Seeds of crabgrass weighing 0.11 g were spread evenly on top of the soil. Preweighed, dry samples of the test materials were applied uniformly on the soil surface at rates of 0, 0.86, 1.72, 3.44, and 6.88 g/dm^2, except sample 6 that did not have enough material for the 3.44 and 6.88 g/dm^2 rates. Three replicates of the treatments and a completely randomized experimental design were used. All pots were placed randomly on a mist bench where constant moisture was available for 6 days. After germination, the pots were moved to a greenhouse bench and watered every 2 to 3 days by using a fine-mist nozzle. The greenhouse temperature was maintained within a range of 18 to 32°C.

Data were collected on the number of live grass shoots at 18 days after seeding (DAS). Fresh clipping weights were taken at 35 DAS. These two measurements from each pot were averaged for each treatment.

Smooth crabgrass and creeping bentgrass (*Agrostis palustris* Huds.) were used for the Petri dish bioassays. The seven test samples were applied at rates of 0, 0.118, 0.236, 0.355, and 0.473 g/dm^2 to two layers of seed-germination blotter paper (Packaging Converters, Hudson, WI, USA) measuring 42.25 cm^2. Before the treatment, the blotter paper was placed in a 9-cm-diameter Petri dish (Fisher Scientific Co., Pittsburgh, PA, USA) and moistened with 7 mL of deionized distilled water (D.D. H_2O). Eighteen smooth crabgrass seeds were placed on the blotter paper. The dishes were covered with a lid and sealed with Parafilm®. They were incubated in a growth chamber at a day/night temperature of 25 ± 0.5°C. A 16-h photoperiod was used, and radiation levels were maintained at 70 $\mu mol/s/m^2$ with fluorescent lamps. After 21 days, the germination rate, expressed as a percentage, was derived by dividing the number of germinated seeds by 18, the number of seeds used in each plate, and multiplying that number by 100.

Study 2

In the second study, the effects of six corn gluten meal–derived samples numbered 8–13 were bioassayed by using creeping bentgrass and perennial ryegrass (*Lolium perenne* L.; Table 1). Plastic pots filled with the same type of soil as in study 1 were used for the bioassays. Creeping bentgrass was seeded on the 64-cm^2 surface area soil at a rate of 0.16 g/dm^2. Sample materials were applied in three replicates at rates of 0, 0.78, 1.56, 3.13, 4.69, and 6.25 g/dm^2 for all samples except for sample 13, which had only enough material for three levels of application, 0.78, 1.56, and 3.13 g/dm^2. All pots were randomly placed in the greenhouse after 6 days on the mist bench. The environments and irrigation method were maintained with the same conditions as in study 1. Data were taken at 28 DAS on fresh clipping weight of live plants.

Perennial ryegrass was seeded at 1.56 g/dm^2. The same seven rates used in the creeping bentgrass bioassay as described for samples 8–11 were used. Samples 12 and 13 were not tested because of insufficient material. The study was conducted as described for study 1. Data were collected as fresh clipping weight at 27 DAS.

Perennial ryegrass seeds were also used to test the bioactivity of these six samples, 8–13, in a Petri dish bioassay. A volume of 6 mL of D.D. H_2O was applied to two layers of blotter paper measuring 36 cm^2. The test materials were applied at 0, 0.028, 0.056, 0.139, 0.222, and 0.278 g/dm^2 to the surface of the blotter paper in the Petri dishes. The treatments had three replicates, and the control (0 g/dm^2) had six. Sixteen perennial ryegrass seeds were placed on the treated blotter paper. All 36 dishes were randomly placed in a growth chamber. Irradiance was from fluorescent light maintained at 70 $\mu mol/s/m^2$. A day/night temperature of 25/15°C and a 16-h photoperiod were maintained. The number of normal seedlings, which were defined as having roots longer than 0.5 cm, were counted at 14 DAS.

Study 3

In the third study, perennial ryegrass was used to investigate the effects of samples 14–17 on seedling growth in the greenhouse (Table 1). The same type of soil as described in study 1 was used. Perennial ryegrass was seeded at 0.78 g/dm^2 on 64-cm^2 plastic pots. The four test samples were evaluated at the rates of 0, 0.78, 1.56, 3.13, 4.69, and 6.25 g/dm^2 in three replicates. Pots were kept in the greenhouse and watered regularly in the same manner

Table 2. Effect of materials derived from corn gluten meal on the establishment[a] of crabgrass grown on soil in the greenhouse.[b]

	Amount of sample material			
Sample	0.86 g/dm^2	1.72 g/dm^2	3.44 g/dm^2	6.88 g/dm^2
1	51 (2.58)	31 (1.11)	5 (0.01)	0 (0)
2	49 (2.69)	47 (1.78)	3 (0.28)	0 (0)
3	23 (1.48)	4 (0.42)	2 (0.00)	0 (0)
4	52 (2.73)	43 (1.36)	32 (1.33)	5 (0.19)
5	55 (3.30)	37 (3.73)	8 (0.87)	9 (0.69)
6	100 (3.95)	61 (2.49)	—	—
7	47 (1.21)	25 (1.56)	10 (0.88)	6 (0.78)
MSD[c]	9 (0.77)	9 (0.53)	4 (0.20)	1 (0.10)

[a] Effect of each treatment was expressed as the number of plants; fresh clipping weight in gram per pot in parentheses.
[b] Number is the mean of three replicates for each of four treatment levels of samples. The control had 95 ± 12 plants, and fresh clipping weight was 1.24 ± 0.54 g.
[c] MSD is the mean of standard deviations of the values in each column. The LSD (0.05) were 21 and 1.28 g for number of plants and fresh clipping weight, respectively.

Table 3. Effect of materials derived from corn gluten meal on germination[a] of test grasses[b] bioassayed in the growth chamber.[c]

	% Germination			
Sample	0.118 g/dm^2	0.236 g/dm^2	0.355 g/dm^2	0.473 g/dm^2
1	89 (83)	78 (78)	56 (72)	56 (61)
2	83 (89)	72 (61)	61 (61)	28 (28)
3	6 (61)	6 (11)	0 (0)	0 (0)
4	61 (17)	39 (56)	6 (28)	0 (0)
5	50 (22)	11 (44)	0 (0)	0 (0)
6	72 (61)	56 (22)	22 (17)	11 (6)
7	39 (61)	11 (6)	0 (0)	0 (0)
MSD[d]	8 (7)	4 (4)	3 (3)	2 (2)

[a] A germinated seed was defined as a seed with a protruding radicle or shoot.
[b] Crabgrass and creeping bentgrass were tested; the germination of creeping bentgrass is in parentheses.
[c] Number is the mean of three replicates for each of four treatment levels of samples. Control germination was 67 ± 5% for crabgrass and 61 ± 9% for creeping bentgrass.
[d] MSD is the mean of standard deviations of values in each column. The LSD (0.05) were 12 and 15% for crabgrass and creeping bentgrass, respectively.

as described in study 1. Data were collected on fresh clipping weight of each treatment at 28 DAS.

Samples of 14–17 (Table 1) were tested in Petri dish bioassays at rates of 0, 0.028, 0.056, 0.139, 0.222, and 0.278 g/dm^2. The dry samples were applied to the top of two layers of 36-cm^2 blotter paper that were presoaked with 6 mL of D.D. H_2O. Sixteen perennial ryegrass seeds were placed on the blotter paper, and the dishes were sealed with Parafilm®. All sealed dishes, including four controls, were incubated for 15 days in the growth chamber under the same conditions as in the previous evaluation. Data were controlled on the number of normal seedlings with root growth greater than 0.5 cm at 15 DAS.

Statistical Analysis

An analysis of variance (ANOVA) was conducted on number of plants, fresh clipping weight, and germination variables for each study with the StatView computer software program (Abacus Concepts, Berkeley, CA, USA). Means were separated by the projected least-significant difference (LSD) by using Fisher's test (Snedecor and Cochran 1989).

Results and Discussion

Study 1

Compared with the number of plants in the control, all seven samples, except the alcohol-insoluble fraction of gluten filtrate (sample 6), reduced crabgrass establishment at the four test rates (Table 2). Corn gluten meal, sample 1, was less herbicidally active than the bacterial proteinase hydrolyzed gluten, sample 3, at the two lowest rates. The bacterial proteinase hydrolysate that reduced the establishment of crabgrass seedlings by 76, 96, 98, and 100% at the rates of 0.86, 1.72, 3.44, and 6.88 g/dm^2, respectively, was the most active of the test samples. Separate tests of the proteinase showed no effects of this material on seed germination (data not shown).

On the basis of the fresh clipping weight data at 35 DAS, all seven samples had a stimulatory effect on crabgrass growth at the rate of 0.86 g/dm^2 (Table 2). The stimulation was likely due to a nitrogen (N) response (Christians 1993). At rates of 3.44 and 6.88 g/dm^2, several treatments reduced fresh clipping weight, with the bacterial proteinase hydrolyzed gluten sample (3) being the most effective.

In Petri dish tests, the bacterial proteinase hydrolyzed gluten sample (sample 3) and the gluten filtrate treated with a cation resin (sample 7) inhibited the germination of crabgrass at all test rates and of creeping bentgrass at rates higher than 0.118 g/dm^2 (Table 3). The fungal proteinase hydrolysate (sample 2) inhibited germination only at the rate of 0.473 g/dm^2. Corn gluten meal (sample 1) was less active than the bacterial proteinase hydrolyzed gluten, sample 3, for the inhibition of both species.

The definition of germination in the Petri dish study included any seed with a protruding radicle or shoot. This may have been misleading because many of the seeds counted as germinated seed had greatly reduced root growth. Therefore, in the later

Table 4. Effect of materials derived from corn gluten meal on the fresh clipping weight of creeping bentgrass and perennial ryegrass[a] grown on soil in the greenhouse.[b]

	Fresh clipping weight (g)				
Sample	0.78 g/dm^2	1.56 g/dm^2	3.13 g/dm^2	4.69 g/dm^2	6.25 g/dm^2
8	1.48 (0.66)	1.15 (0.73)	0.51 (0.68)	0.07 (0.63)	0 (0.76)
9	1.86 (1.01)	1.21 (0.81)	0.16 (1.07)	0 (1.56)	0 (1.05)
10	2.24 (0.81)	1.02 (1.23)	0.32 (1.08)	0 (0.76)	0 (0)
11	1.49 (1.08)	1.64 (1.25)	1.19 (1.15)	0.46 (1.82)	0.54 (1.13)
12	1.94	1.38	1.03	2.51	1.98
13	2.25	1.66	0.42	—	—
MSD[c]	0.31 (0.08)	0.17 (0.09)	0.04 (0.08)	0.08 (0.08)	0.05 (0.06)

[a] Fresh clipping weight of perennial ryegrass is in parentheses. Sample 13 was not evaluated at the two highest rates because of the lack of material for testing.
[b] Number is the mean of three replicates for each level of samples. The control had 0.85 ± 0.13 g for creeping bentgrass and 0.78 ± 0.07 g for perennial ryegrass.
[c] MSD is the mean of standard deviations of values in each column. The LSD (0.05) were 0.39 and 0.23 g for creeping bentgrass and perennial ryegrass, respectively.

studies, germination was redefined to include any radicle measuring 0.5 cm or longer.

Study 2

Plant response in the greenhouse study was based on the fresh clipping weight of creeping bentgrass plants at 28 DAS (Table 4). All samples stimulated growth at rates of 0.78 and 1.56 g/dm^2. The inhibition effect was seen at rates of 3.13 g/dm^2 and higher. The bacterial proteinase hydrolysate of corn gluten solution (sample 10), which was equivalent to sample 3 in study 1, was the most effective inhibitor in this study. The corn steep liquor samples 12 and 13, were ineffective and stimulated growth of creeping bentgrass at most rates.

Perennial ryegrass was also used as a bioassay species because of its rapid germination. It generally required greater amounts of the samples to reduce plant establishment of the ryegrass than of the bentgrass (Table 4). The bacterial proteinase hydrolysate sample (sample 10) started showing inhibition at rates higher than 3.13 g/dm^2 on creeping bentgrass and 4.69 g/dm^2 on perennial ryegrass. It completely inhibited establishment of ryegrass at the rate of 6.25 g/dm^2 and was the most effective of the materials tested.

Germinated seeds in the Petri dish study were defined as those with a radicle longer than 0.5 cm extruding from the seed coat. The gluten hydrolysate sample (sample 10) was the most active among all six test samples in this study (Table 5). It inhibited germination at all test rates and stopped germination at rates of 0.056 g/dm^2 and higher. The total-ion-exchange gluten hydrolysate sample (sample 11) had slightly less activity than the gluten hydrolysate (sample 10). Two gluten meal samples (8 and 9) were less active than gluten hydrolysate. The soluble corn steep liquor sample (sample 12) had no inhibitory effect at all test rates, whereas the insoluble corn steep liquor (sample 13) inhibited germination at rates higher than 0.139 g/dm^2.

Table 5. Effect of materials derived from corn gluten meal on germination[a] of perennial ryegrass bioassayed in the growth chamber.[b]

	% Germination				
Sample	0.028 g/dm^2	0.056 g/dm^2	0.139 g/dm^2	0.222 g/dm^2	0.278 g/dm^2
8	81	100	38	0	0
9	100	100	94	50	4
10	12	0	0	0	0
11	62	12	0	0	0
12	75	88	100	94	88
13	100	94	12	0	0
MSD[c]	6	6	4	2	1

[a] A germinated seed was defined as a seed with a radicle longer than 0.5 cm.
[b] Number is the mean of three replicates. The control germination of perennial ryegrass was 94 ± 5% (n = 6).
[c] MSD is the mean of standard deviations of values in each column. The LSD (0.05) was 12%.

Study 3

In study 3, samples derived from corn gluten, wheat gluten, and soybean were bioassayed by using perennial ryegrass, which had more rapid germination and served as a more conservative species than the

Table 6. Effect of grain-derived materials on fresh clipping weight of perennial ryegrass tested on soil in the greenhouse.[a]

	Fresh clipping weight (g)				
Sample	0.78 g/dm^2	1.56 g/dm^2	3.13 g/dm^2	4.69 g/dm^2	6.25 g/dm^2
14	2.20	1.99	2.10	2.08	1.87
15	2.39	1.99	2.25	2.01	1.81
16	3.16	2.45	1.04	0	0
17	3.87	2.80	1.76	0	0
MSD[b]	0.45	0.34	0.25	0.13	0.10

[a] Number is the mean of three replicates for each level of samples. The control had 1.78 ± 0.44 g (n = 4).
[b] MSD is the mean of standard deviations of values in each column. The LSD (0.05) was 0.86 g.

Table 7. Effect of grain-derived materials on germination[a] of perennial ryegrass bioassayed in the growth chamber.[b]

	% Germination				
Sample	0.028 g/dm^2	0.056 g/dm^2	0.139 g/dm^2	0.222 g/dm^2	0.278 g/dm^2
14	100	100	100	88	44
15	94	100	62	19	6
16	94	12	0	0	0
17	69	62	19	0	0
MSD[c]	7	6	4	2	1

[a] A germinated seed was defined as a seed with a radicle longer than 0.5 cm.
[b] Number is the mean of three replicates for each level of samples. The control germination of perennial ryegrass was 96 ± 6% (n = 4).
[c] MSD is the mean of standard deviations of values in each column. The LSD (0.05) was 12%.

other two test grass species. All test samples had a greater fresh clipping weight than the control at 28 DAS at the rates of 0.78 and 1.56 g/dm^2 (Table 6). Corn gluten hydrolysate (sample 16) and soybean meal (sample 17) started to show inhibition of plant growth at the rate of 3.13 g/dm^2 and completely stopped plant establishment at higher levels of treatment. Wheat gluten (sample 14) and its hydrolysate (sample 15) showed no inhibition.

In the Petri dish study, the soybean meal and corn gluten hydrolysate showed inhibition at all test rates, and corn gluten hydrolysate completely inhibited radicle emergence at rates of 0.139 g/dm^2 and higher (Table 7). However, the wheat gluten showed no inhibition at the rate of 0.139 g/dm^2, and its hydrolysate had less than 40% reduction in germination of perennial ryegrass. The wheat gluten hydrolysate had more inhibitory activity than wheat gluten itself.

Conclusion

It was demonstrated in all three studies that the corn gluten hydrolysate was a more effective inhibitor than corn gluten meal. The corn gluten hydrolysate is a proteinase-hydrolyzed product of corn gluten meal. It is formed by a process of treating the corn gluten slurry with amylases and then treating it with one or more proteinases, followed by the removal of the water (Christians et al. 1993). The proteinase used to hydrolyze corn gluten meal did not contribute to the inhibition of the germination of perennial ryegrass (data not shown).

On the basis of the results from four studies, both in the greenhouse and growth chamber, the corn gluten hydrolysate prepared with bacterial proteinase contained the most potent inhibitory activity for three test grass species. In soil bioassays, treatments with rates above 4.69 g/dm^2 of the gluten hydrolysates resulted in complete inhibition of plant establishment. However, rates of 6.88 g/dm^2 were required for the corn gluten meal to achieve complete inhibition.

In the Petri dish bioassay, the corn gluten hydrolysate prevented germination of creeping bentgrass at application levels above 0.118 g/dm^2 and prevented germination of crabgrass at application levels above 0.236 g/dm^2, as seen in study 1 (sample 3). The same material was tested on perennial ryegrass in study 2 (sample 10), and it completely inhibited root emergence of perennial ryegrass seed at 0.056 g/dm^2. The Petri dish bioassay required less of the sample than the soil bioassay to inhibit germination. The Petri dish bioassay is a faster and more economical method to monitor inhibitory activity than soil bioassay. Root-length reduction of seedlings was the best measurement of herbicidal activity.

The bacterial proteinase gluten hydrolysate was the most highly bioactive water-soluble material. This made it suitable for the isolation and identification of the bioactive compounds. This material also has the potential of being used as a natural herbicide, and further work to evaluate its effectiveness in various cropping systems is planned.

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