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SOME FACTORS LEADING TO DEATH OF SMOOTH BROMEGRASS (BROMUS INERMIS LEYYS.) ON ORGANIC SOIL IN MICHIGAN

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Kristen E. Myhr

1964

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By

Kristen E. Myhr

AN ABSTRACT

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Crop Science

1964

Approved Milo B. Tesar

ABSTRACT

SOME FACTORS LEADING TO DEATH OF SMOOTH BROMEGRASS (*BROMUS INERMIS* LEYYS.) ON ORGANIC SOIL IN MICHIGAN

by Kristen E. Myhr

Experiments were conducted in the field and under greenhouse conditions to determine why smooth bromegrass dies on organic soil, and the effect of fungicide treatments, irrigation, and different levels of fertility and temperature on the time of death.

Irrigation depressed yields of bromegrass and orchardgrass approximately 16%. Irrigated plots of Lincoln bromegrass treated with Dexon fungicide (a p-Dimethylaminobenzenediazo sodium sulfonate) yielded significantly more in the third harvest and exhibited a better stand late in the fall of the first harvest year than untreated plots.

Fusarium spp. and *Pythium* spp. were isolated from decayed bromegrass roots in muck soil in the field. *Fusarium poae* was significantly associated with killing of roots and reduction of top growth of Lincoln bromegrass under controlled conditions. *Pythium* spp. may have contributed to such death.

Dexon fungicide, which is highly specific against *Pythium* spp. but relatively uneffective for control of *Fusarium* spp. was not very effective in controlling root rot under greenhouse conditions. In unfertilized cultures

it depressed the yield significantly, but in fertilized soil the fungicide increased yields, although not significantly.

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INTRODUCTION

With proper management, smooth bromegrass (*Bromus inermis* Leyss.) may last indefinitely on mineral soil in Michigan. On organic soil, however, the stands start to die out after one year and are almost gone after four years. On muck soil the stand seems to be hurt during the summer time rather than during the winter. Careful examinations have revealed that roots and rhizomes often are heavily infected with micro-organisms.

Two ecotypes of *Bromus inermis* are recognized according to origin. The northern type is indigenous to Siberia and is now grown for seed mainly in Canada. The southern type was introduced from Hungary and is now grown for seed in the Midwest. The southern type is usually recommended for the Corn Belt area. It is more vigorous, earlier maturing, higher yielding, less competitive to the legume, and has fewer leaf diseases than the northern type. On organic soil, however, varieties of the southern type are first to be killed.

Difficulties in getting stands of bromegrass established have been reported from Canada and several states in the Midwest. The seedlings are frequently killed by a root rot complex which usually is referred to as "damping off." The most active organisms in this complex have been *Pythium* spp., *Helminthosporium* spp. and *Fusarium* spp. In Michigan, "damping off" seldom causes poor establishment and has not affected the establishment of bromegrass on the Michigan State University Muck Farm.

The experiments reported herein were designed to determine the cause of death of bromegrass after its establishment on muck soil, especially as it might be affected by 1) soil-borne fungi causing diseases, and 2) the interaction of water, temperature and fertilizer on diseases.

LITERATURE REVIEW

The present subject concerning death of established stands of smooth bromegrass on organic soil has not been reported in the literature. The problem is identified, however, by Tesar and Shepherd (10) who evaluated forage species on organic soil in Michigan. Hay fields of eight grasses and four legumes in pure stand were compared for a four year period. Grimm alfalfa, Ladino clover, and Empire birdsfoot trefoil were unproductive after the first harvest year and were severely invaded by grasses and weeds in the second harvest year. Big trefoil did not survive the first winter. Failure in survival of legume species indicates that the forage species on muck soil necessarily have to be grasses. Reed canarygrass (4.28 tons) and tall fescue (3.89 tons) gave the greatest yields, but the authors did not recommend these species because they have not given good animal performance. Orchardgrass (3.43 tons) had resistance to invasion by weeds and other grasses. Canadian (2.80 tons) and Lincoln (2.42 tons) bromegrass, representing the northern and southern ecotypes, respectively, were lower in yield but with excellent animal performance were preferable to other grass species in the Mid-West if more permanent. These results indicate that Lincoln produced a high yield the first harvest year (4.41 tons), but dropped gradually to (0.06 tons) in the fourth year. Canadian produced a more constant yield - 3.06 tons in the first and 1.74 tons in the four year - but even this yield is not considered satisfactory.

Vanterpool (12) who studied *Pythium* root rot of grasses in Saskatchewan, Canada, classifies four types or phases of *Pythium* damage to grasses:

1. Pre-emergence killing of the seedlings in which both roots and shoots are attacked;

2. Damping off or early seedling killing due to the rotting of the roots and the bases of the stems;

3. Lesioning of the larger roots and an invasion of many of the fine laterals in the late seedling stage while the plants are becoming established; and

4. Lesioning of the new roots of perennial grasses during subsequent growth periods.

According to Vanterpool, pathogenic species of *Pythium* are most readily isolated from diseased roots of young plants up to the third week of June. Virulent species are not easily obtained in culture from the older roots of plants two or more years old. On the basis of several series of greenhouse experiments, Vanterpool concluded that the factors limiting yield were more effective in dry soil than in moist soil and that *Pythium* root rot was more severe at high than at low temperatures. He suggested fall planting of grasses as a means of obtaining better stands, and the use of phosphate fertilizers applied to naturally infested field soil as a means of increasing yields.

Hawk and Welch (6), Andrews (1), and Buchholtz (2), working in Iowa, Minnesota and South Dakota, respectively, all indicate the difficulties in obtaining a good stand when grasses are planted in the spring. Poor establishment was associated with several indigenous fungi.
Hawk and Welch tested the resistance of several bromegrass varieties to Pythium graminicola. Their results indicate that the southern ecotype was more tolerant of injury than the northern type. Injury was less pronounced at lower temperatures. Andrews isolated Helminthosporium sativum, Fusarium spp., Pythium graminicola, and Rhizoctonia solani from lesioned roots of grasses. The first three mentioned were pathogenic to seedlings of bromegrass.

Nielsen, Dickson and Smith (8) report that varieties and seed treatment are factors in seedling establishment in Wisconsin. Bromegrass treated with the commercial fungicides Captan, Ceresan, and Arasan had better stands than not treated. The southern ecotype exhibited slightly greater tolerance to Helminthosporium sativum, Fusarium roseum, and Pythium graminicola than the northern type of bromegrass.

Thomason and Dickson (11) isolated root rot organisms from roots and crown tissue of bromegrass in four locations in Wisconsin and one location in South Dakota. Of 2,788 isolations 40.7% proved to be Fusarium spp., 32.7% Pythium spp. and 16.7% Helminthosporium spp. In a factorial experiment, seedlings of Lincoln and Canadian bromegrass were infected with the isolated organisms and allowed to grow at temperatures of 16, 20, 24, and 28° C. Canadian bromegrass showed better stands in the presence of *Pythium graminicola* and *Helminthosporium sativum* than Lincoln. The best stands were obtained at 16 and 20° C.

Kaufmann, Drolsom, and Nielsen (7) have reported differences in reaction to infection when seedlings of strains and experimental synthetics were inoculated with *Pythium graminicola* and *Helminthosporium sativum*. The variety Saratoga and four synthetics appeared significantly more tolerant than other strains.

Drolsom and Nielsen (3) have continued their efforts to develop new varieties of smooth bromegrass resistant to root rot diseases detrimental to seedling establishment.

EXPERIMENT I

Field

Effect of fungicide and irrigation on established stands

The objective of this experiment was to determine the production and persistency of Lincoln and Canadian bromegrass (*Bromus inermis* Leyss.) and commercial orchardgrass (*Dactylis glomerata* L.) in the first harvest year when treated with fungicide under irrigated and non-irrigated conditions. The research hypothesis was that the fungicide would by reducing pathogenic soil fungi such as *Pythium* spp. improve the production and longevity of bromegrass on a muck soil.

Materials and Methods

The experiment was conducted on a well-drained Houghton muck soil on the Michigan State University Muck Farm. Lincoln bromegrass, Canadian bromegrass and commercial orchardgrass were each planted in pure stand on August 18, 1963. The seed was drilled one inch deep, in contact with 500 pounds per acre of 12-12-12 fertilizer in 7-inch rows in drill-width plots. Both bromegrass varieties survived the winter without injury and had a 100% stand on May 2, 1964. Orchardgrass had a 50% stand due to considerable winter damage. The stand recovered after several weeks and by harvest time it had reached optimum density.

Figure 1. Orchardgrass, in the center, showed considerable winter damage while bromegrass, left and right, exhibited an excellent stand on May 2, 1964.

During the first harvest year no additional fertilizers were applied because the stand was very vigorous and earlier experiments had shown the soil released sufficient nitrogen for a satisfactory hay crop.

Dexon* (p-Dimethylaminobenzenediazo sodium sulfonate), a non-mercurial fungicide compound of possible protective value to germinating and growing plants from phycomycetons fungi, was applied to the stand on April 22-23, 1964, in the four following ways:

1. Check, no fungicide
2. 50 lbs. active Dexon per acre worked 4 inches deep into the soil between the rows with a tine rake.
3. 50 lbs. active Dexon per acre on the soil surface.
4. 100 lbs. active Dexon per acre - twice the recommended rate - on the soil surface.

One half of each replication was irrigated according to the following schedule:

<u>Second cutting:</u>

June	16	1"
"	18	2"
"	25	1"
"	30	1"
July	2	2"
Total		7"

<u>Third cutting:</u>

July	29	2"
Aug.	6	1"
"	7	1"
"	18	1"
"	19	1"
Sept.	3	1"
Total		7"

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*obtained from Chemagro Corporation, Kansas City, Missouri

The experiment was designed as a split-plot, with 4 replications of 3 species, 4 fungicide treatments, and 2 water levels, giving a total of 96 subplots, each 8 x 18 feet in size. A strip 3 feet wide and 15 feet long was mowed from each plot for yield. Moisture samples were obtained from one replication. Yields are reported as tons per acre of hay containing 12% moisture.

Results

Bromegrass lodged heavily in the two first harvests. In the second cutting Lincoln had 50% lodging and Canadian had 30% lodging. Orchardgrass was not lodged. The experiment was free from weeds and volunteer grasses. The yield data are presented in Table 1.

Table 1. Tons of hay per acre for various treatments at each harvest on irrigated (I) and not irrigated (NI) muck soil.

Dexon fungicide	Harvest date	Lincoln I	Lincoln NI	Canadian I	Canadian NI	Orchard I	Orchard NI	Average I	Average NI
1. None, check	June 1	1.27	1.31	1.55	1.38	1.76	2.02	1.53	1.57
	July 23	1.30	1.32	1.00	1.45	1.67	1.75	1.32	1.50
	Sept. 25	0.51	0.85	0.52	0.71	0.85	1.04	0.62	0.86
	Total	3.08	3.48	3.07	3.54	4.28	4.81	3.47	3.93
2. 50 lbs/ acre mixed into 4" depth	June 1	1.42	1.27	1.51	1.54	1.76	1.58	1.56	1.46
	July 23	1.29	1.29	1.17	1.38	1.49	2.01	1.32	1.56
	Sept. 25	0.52	0.83	0.62	0.64	1.02	1.01	0.72	0.83
	Total	3.23	3.39	3.30	3.56	4.27	4.60	3.60	3.85
3. 50 lbs/ acre broad-cast on surface	June 1	1.40	1.53	1.30	1.34	1.87	1.85	1.52	1.57
	July 23	1.34	1.38	1.11	1.43	1.51	1.89	1.32	1.56
	Sept. 25	0.62	0.83	0.40	0.63	1.14	0.97	0.72	0.81
	Total	3.36	3.74	2.81	3.40	4.52	4.71	3.56	3.94
4. 100 lbs/ acre broad-cast on surface	June 1	1.36	1.24	1.37	1.65	1.70	1.66	1.47	1.51
	July 23	1.03	1.23	1.21	1.33	1.40	1.88	1.21	1.48
	Sept. 25	0.85	0.85	0.64	0.73	0.83	1.09	0.77	0.89
	Total	3.24	3.32	3.22	3.71	3.93	4.63	3.45	3.88

5% LSD for 1st harvest = 0.33
2nd harvest = 0.24
3rd harvest = 0.22
total yield = 0.45

Irrigation has depressed total yield for all combinations of fungicide treatments and species. The effects of irrigation are shown in Table 2. No water was added prior to the first harvest and the yields were similar at the first cutting. In the next two harvests irrigation depressed yields approximately 16%.

Table 2. The average effect of irrigation in tons of hay per acre of Lincoln bromegrass, Canadian bromegrass, and orchardgrass on a muck soil.

Cutting	Cutting date	Irrigated	Not irrigated
First	June 1	1.52	1.53
Second	July 23	1.29	1.53
Third	Sept. 25	0.71	0.85
	Total	3.52	3.91

Table 3 shows total yield on irrigated plots for the last two harvests in percent of those not irrigated. On irrigated soil, the heaviest amount of fungicide resulted in greater yields of both bromegrasses.

Table 3. Yield on irrigated plots in percent of those not irrigated for the two last harvests.

Dexon fungicide	Lincoln bromegrass	Canadian bromegrass	Orchard-grass
1. Check, no fungicide	83	70	90
2. 50 lbs./acre mixed in	81	88	83
3. 50 lbs./acre on surface	89	73	92
4. 100 lbs./acre on surface	90	90	75

The beneficial effects of the fungicide were not significant until the third harvest. The interaction fungicide x species x irrigation was significant in the second and third harvest. Under irrigated conditions; of primary significance was that the yield of Lincoln bromegrass in the third harvest was significantly higher when the soil was treated with the 100-pound rate of fungicide, than when not treated or treated with the 50-pound rate, table 1. The significance of the 3rd-order interaction in the second harvest was mainly due to Orchardgrass. On irrigated land the heaviest amount of Dexon significantly depressed its yield.

The yields of Lincoln bromegrass showed positive, but non-significant effects of the fungicide in the first harvest. Because no irrigation was applied before the first cutting, the data for irrigated and not irrigated plots were pooled. Treatment 3 - recommended fungicide rate broadcast - produced 0.18 tons more per acre than the untreated check, but in the first harvest the other treatments were similar to untreated plots. Twice the commended rate of the fungicide resulted in reduced yields in the second harvest under irrigated conditions.

Three persons, estimating independently, determined that the stand of irrigated Lincoln bromegrass was significantly better on fungicide treated plots than on untreated plots at the end of the first harvest year, table 4.

Table 4. Percent stand on untreated and fungicide treated plots of 4 replications Lincoln bromegrass on irrigated land on November 4, 1964, at the end of the first harvest year.

Treatment	Estimator 1	Estimator 2	Estimator 3	Average
Untreated	68	63	63	65
Dexon fungicide	76	69	73	73

5% LSD = 6

The effect of fungicide treatments on Canadian bromegrass was somewhat different than on Lincoln bromegrass. Treatment 3 - recommended fungicide rate broadcast - had lower production in both the first and third cutting compared with the untreated check, but the differences were not significant.

EXPERIMENT II

Greenhouse

Effect of fungicide, fertilizer, and temperature on established grass

A comparison was made of the performance of several grass species grown in untreated and fungicide treated muck soil under greenhouse conditions. It was assumed that it would be possible to get as many cuttings in the greenhouse in nine months as in two harvest years in the field. Assuming that bromegrass is weakened to a certain degree after defoliation, and since many plants died during the second growing season in Tesar and Shepherd's experiment (10), there was reason to expect thinning and killing during the latter part of the experimental period. The hypothesis was that bromegrass grown in fungicide treated soil would yield more and live longer than in untreated soil.

Materials and Methods

The following variables were included in the experiment:

2 Fungicides - untreated and treated soil

2 Fertilizers - none and heavy

4 Species - Lincoln bromegrass, Canadian bromegrass, orchardgrass, and reed canarygrass

2 Night temperatures - 70°F and 80°F

4 Replications

The soil used in the experiment was obtained by compositing soil samples collected around the periphery of the field experiment. Only the upper 4 inch layer was used. After screening, 1850 grams of soil was mixed with fungicide and fertilizer, according to the design, and put into a 6-inch gallon can.

Fifty pounds of active Dexon fungicide per acre, and 2000 lbs. per acre of a 5-20-20 fertilizer was added to one half of the pots.

Fifty seeds of each of Lincoln bromegrass, Canadian bromegrass, commercial orchardgrass, and reed canarygrass (<u>Phalaris</u> <u>arundinacea</u> L.) were planted in separate pots one inch below the soil surface on December 23, 1963. After reseeding reed canarygrass on January 9, because of poor germination, and a few transplantations of bromegrass on January 17, the stand reached adequate density in all cultures. The first shoots from rhizomes on bromegrass appeared on February 11.

One half of the experiment was placed in a section of the greenhouse where the night temperature was approximately 80°F., the other half at a night temperature of approximately 70°F. Day temperature varied and during the first three harvests day temperature averaged about 10 degrees higher in the high temperature house. As the summer season approached the day temperature in the two houses was similar.

Water was added weekly to bring each container up to a weight of 2400 grams to adjust for uneven consumption. When necessary water was supplied daily.

Yields were obtained by cutting to a height of 3 cm. on the following days in the low temperature house:

February 29	July 1
March 30	August 12
May 18	September 30

The experiment was terminated after the first four harvests in the high temperature house. Yields are expressed in grams dry matter per culture. After each cutting all cultures were fertilized with a water solution of nitrogen at a rate of 100 lbs. N per acre.

The pots were grouped together in replications at all times, but the position of a particular pot, as well as the replication in which it occurred was systematically changed to avoid border effects in the experiment.

Statistical analysis of the data was carried out separately for each temperature section according to a factorial model. For the calculations of F-values and for interpretation of the data, all treatments were considered as fixed constants.

Results

The stand was healthy and vigorous during the three first cuttings in all pots, with the exception of the fungicide treated reed canarygrass. During the month of June, the plants began to suffer from unfavorable conditions. The bromegrass lost leaf turgor, luster and green color. Orchardgrass and reed canarygrass exhibited the same symptoms, although to a lesser extent. Close examinations did not reveal wilting symptoms were associated with the treatments. Because the experiment was inspected daily, deficient water supply was not a possible cause. A more reasonable explanation is that the extreme high temperatures which occurred in the greenhouse during hot sunny summer days were unfavorable for these cool season grasses. After the fourth and fifth cuttings, the plants showed the same wilting symptoms when they reached a height of 4-5 inches, and production was reduced.

The data for the low and high temperature section are presented separately in table 5 and table 6, respectively. Because the data do not correspond exactly, and there were two more harvestings on the low temperature material, the two parts were not pooled.

Table 5. Grams dry matter per pot for various treatments in a greenhouse maintained at a 70°F. night temperature.

Treatment	Harvest date	Lincoln bromegrass	Canadian bromegrass	Orchard grass	Reed canarygrass
		NOT FERTILIZED			
No fungicide	Feb 29	6.24	7.55	5.66	5.83
	Mar 30	5.08	5.65	6.80	8.15
	May 18	11.15	10.86	13.28	16.75
	July 1	6.71	5.60	6.76	9.28
	Aug 12	2.54	2.26	3.35	3.48
	Sept 30	1.90	0.93	2.30	2.26
	Total	33.62	32.85	38.15	45.75
Fungicide	Feb 29	5.53	5.96	5.41	0.81
	Mar 30	4.94	4.74	6.51	3.98
	May 18	10.62	10.60	12.47	19.09
	July 1	5.18	5.62	5.51	15.14
	Aug 12	2.27	2.44	2.51	5.51
	Sept 30	1.93	1.29	2.24	3.05
	Total	30.47	30.65	34.65	47.58
		FERTILIZED			
No fungicide	Feb 29	7.81	9.94	6.42	6.13
	Mar 30	8.40	8.61	9.41	9.85
	May 18	15.72	14.55	17.01	18.83
	July 1	12.91	13.03	12.64	15.83
	Aug 12	6.71	5.99	6.04	6.12
	Sept 30	2.93	2.28	4.30	3.43
	Total	54.48	54.40	55.82	60.19
Fungicide	Feb 29	6.73	8.78	7.06	1.89
	Mar 30	8.18	8.86	9.36	5.68
	May 18	17.63	16.82	18.52	20.93
	July 1	14.36	14.56	13.28	20.22
	Aug 12	5.97	5.74	3.48	9.08
	Sept.30	2.33	2.07	3.89	4.82
	Total	55.20	56.83	55.59	62.62

5% LSD for 1st harvest = 0.92 4th harvest = 1.38
2nd harvest = 0.74 5th harvest = 0.96
3rd harvest = 1.34 6th harvest = 0.68

total yields = 2.84

Table 6. Grams dry matter per pot for various treatments in a greenhouse maintained at 80°F. night temperature.

Treatment	Harvest date	Lincoln bromegrass	Canadian bromegrass	Orchard grass	Reed canary
		NOT FERTILIZED			
No fungicide	Feb 29	6.22	7.62	6.35	7.09
	Mar 30	7.13	7.00	6.49	8.13
	May 18	8.62	6.65	8.63	12.62
	July 1	5.23	4.19	5.60	7.33
	Total	27.20	25.46	27.07	35.17
Fungicide	Feb 29	5.77	6.29	6.35	1.84
	Mar 30	6.84	6.95	7.82	7.69
	May 18	9.58	6.89	10.32	16.36
	July 1	4.73	3.24	5.02	9.56
	Total	26.92	23.37	29.51	35.45
		FERTILIZED			
No fungicide	Feb 29	7.03	9.23	6.50	6.13
	Mar 30	9.51	9.95	8.38	8.32
	May 18	13.39	10.25	12.04	16.31
	July 1	12.41	10.12	10.21	13.69
	Total	42.34	39.55	37.13	44.45
Fungicide	Feb 29	6.02	7.58	6.35	3.52
	Mar 30	9.14	10.78	10.46	10.27
	May 18	14.19	9.54	13.31	19.23
	July 1	12.57	8.27	9.64	13.87
	Total	41.92	36.17	39.76	46.89

5% LSD for 1st harvest = 0.73; 2nd harvest = 1.14; 3rd harvest = 2.04; 4th harvest = 1.50; total yield = 3.70

The fungicide treatment caused a significant or near significant reduction in yield in the Lincoln bromegrass in the first cutting and sometimes in the second cutting at both fertility and temperature levels. In the third and fourth cuttings under low night temperature, the fungicide significantly improved the yield of Lincoln in fertilized soil. For the two last cuttings on August 12 and September 30, the plants were not vigorous; the yields were low and slightly depressed in fungicide treated fertilized soil.

Canadian bromegrass reacted similarly to Lincoln in the response to fungicide, except that it has been harmed more by the fungicide under high temperature.

Total yield of orchardgrass was significantly depressed by the fungicide in unfertilized cultures at low temperatures. At high temperatures the orchardgrass has given greater yields in fungicide treated cultures.

Reed canarygrass reacted differently than the other two species. It gave a very small yield in the first and second cutting following fungicide treatment in the low temperature house. Also in the high temperature a similar depression is recorded at the first cutting. In subsequent harvests the yield was significantly increased, but differences in total yield were not significant.

The effect of fertilizer was highly significant for all cuttings. The fungicide x fertilizer interaction for total yield at low temperature is shown in table 7.

Table 7. Yield in grams per pot for combinations of fungicide and fertilizer for four grasses grown at night temperature of 70°F.

Treatment	Fungicide	No fungicide
Fertilizer	57.54	56.16
No fertilizer	35.80	37.56
Average	46.67	46.86

5% LSD = 1.42

The interaction indicates that the fungicide significantly depressed the yield in non-fertilized cultures. In those that were fertilized the fungicide tended to induce increased production.

In table 8 yields of different species in non-fertilized pots are expressed in percent of the production of those fertilized, for the two temperature levels separately. Data for the fifth and sixth cutting are not included in the table because of very small yields.

Table 8. Yield of the first four harvests of non-fertilized grass as percent of fertilized grass.

Species	Night temperature		
	Low	High	Average
Lincoln bromegrass	58	64	61
Canadian bromegrass	57	64	61
Orchardgrass	65	73	69
Reed canarygrass	75	77	76

Percentagewise, orchardgrass and reed canarygrass performed better on unfertilized soil than the bromegrass varieties.

After the last cutting the roots were washed, examined, dried and weighed. Rotted roots were found in both untreated and fungicide treated cultures. Visual estimation of the roots did not reveal any significant differences. The heaviest root-weights in Lincoln bromegrass were found in untreated soil, but since the data were variable as indicated by a large error-term, there was no significant difference in the effect of fungicide in root weights.

EXPERIMENT III

Greenhouse

Evaluation of fungicide treatments

This experiment was set up to determine the effectiveness of Dexon, the soil fungicide used in the two prior experiments, and propylene oxide in comparison to heat sterilization as methods of killing soil pathogens theorized as causing death of bromegrass on muck soil.

Materials and Methods

The following variables were used in greenhouse cultures at a night temperature of 70°F., 6 fungicide treatments:

a. Check, no fungicide

b. Dexon, 50 lbs. active ingredient per acre on top of soil

c. Dexon, same rate as b, mixed into the upper 2" of soil

d. Dexon, same rate as b, mixed into the entire soil volume

e. Autoclaving for 1 hour, soil in pots; and

f. Propylene oxide gaseous sterilization

3 species - Lincoln bromegrass, Canadian bromegrass and orchardgrass

4 replications

Propylene oxide sterilization is described by Hansen and Snyder (5). Compared with heat sterilization this method causes little change in the physical-chemical nature of the soil.

The experiment was conducted in the same manner as the other greenhouse experiment. It was designed so that the fertilized Lincoln bromegrass, Canadian bromegrass, and orchardgrass of experiment II in the low temperature house were included. The two experiments had two treatments in common: 1) fertilized check, and 2) fertilized cultures with Dexon mixed into the entire soil volume.

Results

Good stands were obtained and maintained until after the third cutting in early summer at which time the grasses began to wilt and die slowly in the same way as described for experiment II. The data are presented in table 9.

Autoclaving was the only treatment which increased the yield of bromegrasses in the first and occasionally in the second harvest. Propylene oxide decreased the yield of the bromegrasses during the first two cuttings. Dexon fungicide applied on the surface or in the upper two inches had a similar harmful effect on all three species in the first two harvests.

In the last two cuttings the two bromegrass varieties but not orchardgrass yielded more when treated with propylene oxide than when not treated. Yields of bromegrass in Dexon treated soil and in autoclaved soil were similar to yields of non-treated bromegrass.

Table 9. Yield in grams per pot of Lincoln bromegrass, Canadian bromegrass, and Orchardgrass when grown in untreated, fungicide treated, and sterilized soil.

Treatment	Harvest date	Lincoln bromegrass	Canadian bromegrass	Orchard grass	Average
Check, untreated	Feb 29	7.81	9.43	6.42	8.05
	Mar 30	8.40	8.61	9.41	8.80
	May 18	15.72	14.55	17.01	15.76
	July 1	12.91	13.63	12.64	12.86
	Aug 12	6.71	5.99	6.04	6.27
	Sept 30	2.93	2.28	4.30	3.17
	Total	54.48	54.40	55.82	54.91
Dexon on top	Feb 29	4.65	4.34	4.13	4.37
	Mar 30	5.77	5.55	7.60	6.31
	May 18	13.34	15.36	17.74	15.48
	July 1	10.87	14.26	12.42	12.52
	Aug 12	6.13	8.35	5.95	6.82
	Sept 30	3.44	3.59	3.96	3.67
	Total	44.20	51.55	51.80	49.17
Dexon in top 2 inches	Feb 29	5.23	7.13	5.32	5.89
	Mar 30	5.53	7.71	7.86	7.03
	May 18	13.24	15.83	17.21	15.42
	July 1	9.48	13.53	10.70	11.24
	Aug 12	6.35	6.66	5.01	6.00
	Sept 30	3.48	2.74	3.32	3.17
	Total	43.31	53.60	49.42	48.75
Dexon mixed in	Feb 29	6.73	8.78	7.06	7.52
	Mar 30	8.18	8.86	9.36	8.80
	May 18	17.63	16.82	18.52	17.65
	July 1	14.36	14.56	13.28	14.06
	Aug 18	5.97	5.74	3.48	5.06
	Sept 30	2.33	2.07	3.89	2.76
	Total	55.20	56.83	55.59	55.85

Table continues on next page

Table 9. continued

Treatment	Harvest	Lincoln	Canadian	Orchard	Average
Autoclaved soil	Feb 29	10.52	13.11	9.33	10.99
	Mar 30	9.44	8.84	9.28	18.19
	May 18	16.39	14.94	18.20	16.51
	July 1	12.15	12.12	12.68	12.32
	Aug 18	6.03	6.21	6.76	6.33
	Sept 30	3.85	3.52	4.58	3.99
	Total	58.38	58.74	60.83	68.33
Propylene oxide	Feb 29	5.08	6.91	5.47	5.82
	Mar 30	6.06	6.85	8.75	7.22
	May 18	14.97	17.80	18.59	17.12
	July 1	13.25	15.52	12.35	13.71
	Aug 18	7.06	8.55	6.27	7.29
	Sept 30	4.17	4.20	4.38	4.25
	Total	50.59	59.83	55.81	55.41

5% LSD for 1st harvest = 1.74
2nd harvest = 1.56
3rd harvest = 1.94
4th harvest = 2.08
5th harvest = 1.70
6th harvest = 1.42
total yield = 4.30

Lincoln bromegrass appeared to be more sensitive to both Dexon fungicide and propylene oxide than Canadian bromegrass.

After the last harvest the roots were washed, examined, dried and weighed, but because of a large experimental error no differences between treatments were obtained. The roots were partially decayed in all pots.

EXPERIMENT IV

Greenhouse

Pathogenic effect of fungi isolated from infected bromegrass roots

The objective of this study was to determine, under controlled conditions, how bromegrass is affected by the fungi isolated from decaying roots of bromegrass on muck soil.

Materials and Methods, General

Roots of Lincoln bromegrass were brought in from the Muck Farm on June 18, July 9, and August 18. The roots were carefully washed in running water for 3 hours. Partially lesioned sections of young white roots were then selected and surface sterilized in a 1% chlorazene solution for 2 minutes. A section of root 5 mm long was obtained from the border zone between healthy and decayed tissue, and placed on sterile water agar in a petri dish for three days at room temperature.

Approximately 40 such transplants were made on each date. In most of the dishes fungus mycelia grew out radially from the root sections and spread over the agar. Some small bits of mycelial growth from the periphery of various colonies were transferred to potatodextrose agar in test tubes for further experimentation.

The major interest in this investigation was to inoculate established bromegrass with isolates of the more pathogenic fungi. In order to screen out the less harmful organisms, however, seedling tests were conducted first. The assumption made was that pathogenic fungi would harm a young seedling rapidly and more severely than an established plant.

Seedling tests to screen "harmless" fungi

One hundred and twenty cc of vermiculite and 50 cc of a standard potato juice (200 gm potato extract in a liter of water) was put into 1 pint, widemouth Mason jars, covered with a half petri dish and autoclaved. Mycelial cultures were then transferred aseptically from the test tubes into the vermiculite and allowed to establish for a few days before bromegrass seeds, surface-sterilized with chlorazene were planted at a rate of 20 per jar. Seedlings were counted after 14 days. To make comparative evaluations of damage caused by some known pathogenic species of Pythium, pure cultures of a few identified isolations were obtained from the Department of Botany and Plant Pathology.

The number of seedlings developed in the presence of the most harmful isolates is shown in table 10. The data for the different inoculums are given as percentage of the check (non-inoculated).

Table 10. Bromegrass seedlings developed in sterile and inoculated vermiculite medium 14 days after planting. Stands given as percent of check.

Inoculum	Test June 18 Lincoln bromegrass	Test July 9 Lincoln bromegrass	Test July 9 Canadian bromegrass	Test Aug 18 Lincoln bromegrass
Check, sterile medium	100	100	100	100
Pythium ultimum	55	72	85	
Pythium irregulare	71	47	80	
Culture 18	31	6	20	
Fusarium, culture L3				28
Fusarium, culture L8				23
Fusarium, culture L23				23
Fusarium, culture L32				50
Fusarium, culture L33				32

In addition to the 8 inoculums recorded in table 10, 43 others were tested. Number of developed seedlings in those ranged from 60 to 100 percent of check.

Fusarium spp. and to a lesser extent, *Pythium* spp. reduced the number of seedlings significantly. Canadian bromegrass was more resistant than Lincoln bromegrass to the fungi tested July 9. Two (L8 and L23)of the *Fusarium* isolations made on August 18 were identified as *Fusarium poae* (Pk.) Wr., Gilman (4). Three others recorded in table 10

(L3, L32, and L33) were probably the same species with similar red-brown mycelia. Culture 18 which was isolated on June 18 and tested twice was difficult to purify and identify because it was an inextricably mixed combination of a Pythium species and a Fusarium species with similar growth habits. Its effect on bromegrass in these tests is illustrated in Fig. 2.

Seeds, seedlings, and the length of the longest root were examined when the experiments were terminated. The lower numbers of seedlings in the inoculated jars were due to pre-emergence killing of the seedlings. Both root primordium and coleoptile had been attacked and severed by the fungus. The average root length in the checks in table 10, was 20mm. Inoculated seedlings had much shorter roots ranging from 2 to 5 mm.

Test of the fungal isolates on established plants

Materials and Methods

On June 17, surface sterilized seeds of Lincoln bromegrass were planted in 4-inch circular peat pots which previously had been filled with muck soil, embedded in flats and autoclaved. When these plants had passed the seedling stage and had been cut once, the cultures were taken out of the flats, cleaned carefully outside, transferred to 6-inch gallon cans, and embedded in vermiculite. This vermiculite had previously been moistened with potato juice, autoclaved, and inoculated with some of the fungus isolates which had previously proved most detrimental to bromegrass-seedlings, table 10.

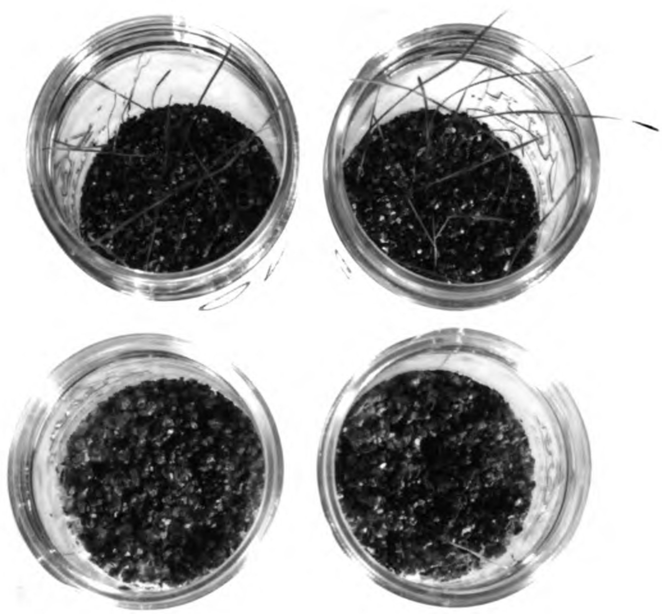

Figure 2. Relative survival of Lincoln (left) and Canadian bromegrass (right) inoculated with fungus culture 18 isolated June 18 from decayed roots in the field (lower row) compared with check (upper row).

On August 2, eight bromegrass cultures were inoculated with each of the following treatments separately:

1. Check. No inoculum
2. *Pythium irregulare*
3. Culture 18
4. Culture T50

On September 29 four cultures of each of the following treatments were added:

5. Check. No inoculum
6. *Fusarium*, culture L3
7. *Fusarium*, culture L8
8. *Fusarium*, culture L33

Culture T50 was included in this experiment as a form of control treatment because it had produced a heavy mycelium without, however, visibly harming the small bromegrass plants in the seedling test.

When the experiment was terminated on October 29, the tops were severed from the roots, dried and weighed. Root weights had to be determined indirectly because the roots had infiltrated the vermiculite and could not be separated by washing, figure 3. The roots were cut close to the wall outside the peat pot, and then the roots and vermiculite together were dried and weighed. The dry weight of vermiculite added initially was subtracted to get dry root weight.

Figure 3. Method of growing bromegrass to determine susceptibility of established plants to fungi.
From left to right:
1. The gallon pots in which the peat pots were embedded
2. Peat pots with vermiculite
3. Roots and vermiculite after separation from peat pot
4. Peat pots after roots and vermiculite were removed
5. Peat pots after the tops were removed

Results

Relative dry weights of tops and roots on October 29 are reported in table 11.

Table 11. Relative top and root weights for Lincoln bromegrass on October 29 after being established June 17 in sterile muck soil and inoculated with fungus on August 2 and September 29.

Inoculum			Top weight	Root weight
	Check		100	100
1)	Group 1:	<u>Pythium irregulare</u>	85	66
		Culture 18	83	68
		Culture T50	86	69
		5% LSD	N.S.	N.S.
2)	Group 2:	<u>Fusarium</u>, culture L3	72	57
		<u>Fusarium</u>, culture L8	74	66
		<u>Fusarium</u>, culture L33	65	64
		5% LSD	19	25

1) 88 days experimental period (August 2 - October 29)
2) 30 days experimental period (September 29 - October 29)

The <u>Fusarium</u> isolations in Group 2 significantly reduced both top-growth and root weights, compared with the check. The fungi in Group 1, did not reduce top or root growth significantly because of extreme variability.

The data strongly suggest, however, that an actual reduction in top growth and especially root growth, resulted when Lincoln bromegrass was inoculated with these fungi.

DISCUSSION

The yields of both bromegrass and orchardgrass in the field were approximately 16% lower when irrigated during a hot and dry season. That result would ordinarily not be expected. Whether this depression was due to an increase in or an activation of the parasites in the soil or to harmful side effects (such as oxygen deficiency) of the irrigation itself was not determined. Before the third cutting Lincoln bromegrass lacked vigor and luster, and the production was obviously depressed on irrigated land. The dry matter percent was higher on irrigated plots - Lincoln bromegrass had 22.1% dry matter on the irrigated plots and 16.8% on non-irrigated plots in the third harvest. The higher dry matter percent on the wetter plots suggests that the parasites in the soil may have severed the roots and in that way limited the water supply for the plants during the last three weeks before cutting when no irrigation was provided.

All the roots sampled from the field for isolation of pathogenic organisms on August 18 were obtained from irrigated plots of Lincoln bromegrass. Of 35 isolations 5 proved to be pathogenic *Fusarium* spp. Two were later identified as *Fusarium poae.* The other three tested, and an additional three which were discarded because of contaminations were probably all of the same species. These data indicate that *Fusarium* spp. are very frequent parasites on bromegrass roots in wet soil. Inoculations of bromegrass plants in the greenhouse showed *Fusarium poae* to be very harmful to Lincoln bromegrass.

Dexon fungicide was selected for use because it was believed that *Pythium* spp. were a major cause of the root rot. If *Fusarium* spp. had been initially considered the major fungus causing root death, another fungicide would preferably have been selected. Dexon is highly specific against *Pythium* spp. but is relatively uneffective for control of *Fusarium* spp. It significantly improved the stand of Lincoln bromegrass, however, for the third cutting on the irrigated plots in the field.

In experiment IIand III conducted in the greenhouse only small differences between fungicide treatments and the check were obtained. Dexon fungicide and propylene oxide gaseous sterilization were detrimental to yield until after the second cutting. Plant nutrients not utilized in these first two periods may have contributed to later yield increases. Heat sterilization gave increased production for the first two harvests, a response which may have been due to released nitrogen, as mentioned by Vanterpool (12). After the experiments were terminated rotted roots were found in all cultures, regardless of treatment, indicating that none of the fungicide treatments used gave completely protection. Recontamination with spores and propagules of the pathogenic fungi could later produce root infection in the sterilized soil. Such recontaminations may come from dust, splashing water or handling. Once begun it can be more severe than the original infestation present before sterilization.

Testing for pathogenic effects of fungi on roots of established plants by the method described in experiment IV has several drawbacks.

The major objection is that the vermiculite cannot be washed away from the roots when the experiment is terminated. Therefore a fixed weight of vermiculite must be put into the pots initially so the root weights finally can be determined by subtraction. In experiment IV dry vermiculite weighed 200 grams per pot while the root averaged only 20 gram. Under such conditions great care must be exercised if results are to be obtained.

This investigation has not finally solved the problem why bromegrass is killed on organic soil. The results obtained, however, substantiate the hypothesis that fungi reduce the growth and longevity of Lincoln bromegrass. The data indicate that, especially under wet conditions, *Fusarium poae* may well be one of the principal fungi causing root death and dying of Lincoln bromegrass as early as the latter part of the first harvest year. Plant feeding nematodes were not recovered from either soil or from decaying bromegrass roots in samples from the Muck Farm, examined by the Michigan State University Nematode Laboratory, and may therefore be excluded as a possible cause of root death of bromegrass on organic soil.

Further elucidation of the conditions favoring disease and possibly synergistic relationships with other soil fungi should be attempted. *Fusarium poae* has been considered a weak parasite, Sprague (9), but may prove to be more adapted to parasitism in a highly organic environment, such as in muck soil, than on mineral soils. It is intriguing that the disease appears to be of much less consequence on mineral soils where most of the experience with *Fusarium poae* has been gained.

Results cited by Tesar and Shepherd (10) show that the dying of bromegrass is not readily detected by yield differences until in the second harvest year. Results in the present experiment indicate root death and decreased stands may be evidenced as early as the end of the first harvest year.

SUMMARY

Investigations were conducted in the field and under controlled conditions to determine why smooth bromegrass dies on organic soil during the growing season, and its reaction to fungicide treatments, and different levels of water supply, fertility, and temperature.

1. Isolations of fungi from decayed bromegrass roots in the field show that *Fusarium* spp. are frequent and apparently harmful parasites in muck soil.

2. *Fusarium poae* was particularly identified with killing of Lincoln bromegrass roots in the greenhouse. *Pythium* spp. appeared to be associated with similar injury.

3. Dying of Lincoln bromegrass in the field was detected in the third cutting near the end of the first harvest year.

4. Dexon fungicide selected for its specific action against *Pythium* spp. was not as helpful as desired in reducing the dying of roots later attributed to *Fusarium poae*. Dexon improved Lincoln bromegrass yields in third cutting and stands after the end of the first year in the field.

5. Dexon fungicide increased yields of bromegrass in fertilized, but not in unfertilized cultures in the greenhouse.

6. Yields were reduced approximately 16% as a result of relatively high irrigation on a muck soil.

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