

**UNIVERSITY OF ARKANSAS, FAYETTEVILLE**

**Allelopathy vs. Acremonium Endophytes vs. Competition Affect on Crabgrass Suppression by 12 Perennial Ryegrasses - Dr. John W. King.**

Twelve (12) perennial ryegrasses which range from moderate to high stand density and zero to 95% endophyte infection were selected and six replications of field plots were planted in late October, 1993. The cultivars and their expected percent endophyte infection are Loretta (0), Gator (0), Derby (5-10), Derby Supreme (40-45), Envy (40), Omega II (76), Manhattan II (50-90), Saturn (80), SR 4200 (80-85), Brightstar (90), Assure (95), and Yorktown III (97). Determination of Acremonium endophyte content showed actual infection levels different from those expected in the original and later seedlots. New seedlots were obtained for fairway overseeding trials for 1994, 1995 and 1996. All plots are maintained with good fertilizer, weed control, irrigation and 2 cm mowing practices. One half of each original field plot was overseeded to crabgrass in spring of 1994, 1995 and 1996. Bermudagrass "fairway" plots were overseeded with new seedlots of the 12 cultivars in the fall of 1994 and 1995. Half of each plot was overseeded to crabgrass in each spring and evaluated for crabgrass suppression. No differences in crabgrass stand could be attributed to any of the 12 cultivars. A range of crabgrass stands occurred when it was overseeded into a strip in each plot of the 99 cultivars of my 1994 NTEP Perennial Ryegrass Test.

Our basic laboratory evaluation for allelopathy is the Lemna minor L. (duckweed) bioassay. It measures allelopathic effects of extracts of plant tissues against the growth rate of duckweed fronds. All cultivars have affected duckweed growth, but inconsistently. Development of a ryegrass extract-agar-crabgrass seed bioassay was attempted, but problems with fungal contamination and poor seed germination persisted. Bioassays using soil from under each cultivar or mixing dried powdered leaf-stem tissue of each cultivar into soil in petri dishes showed inhibition of crabgrass seed germination and growth, but inconsistent results per cultivar over the tests. All objectives, except determining Acremonium endophyte effect on allelopathy, have been met or exceeded.

We are conducting a well-rounded research approach to allelopathy in perennial ryegrasses, but inconsistencies of results over bioassays are very disappointing. Perhaps eventually, selection of ryegrass cultivars for crabgrass inhibition may become an important part of our IPM programs, but I think plant breeders will have to select for allelopathic effect before this comes to pass on the basis of allelopathy. We appreciate greatly the support of the \$30,000 USGA Green Section Research grant for this evaluation.

**ANNUAL REPORT FOR USGA GREEN SECTION RESEARCH GRANT PROJECT  
AT THE UNIVERSITY OF ARKANSAS**

October 30, 1996

**"Allelopathy vs. Acremonium Endophytes vs. Competition Affect on Crabgrass  
Suppression by 12 Perennial Ryegrasses"**

Prepared by Dr. John W. King, Principal Investigator, in collaboration with Dr. Terry L. Lavy and his associate Dr. Briggs Skulman of the Soil Residue Laboratory and Dr. Charles P. West of the Forage Physiology Laboratory.

Twelve perennial ryegrasses were selected for evaluation of allelopathic crabgrass suppression with funding from \$20,000 per year USGA proposed grant; the actual grant was \$10,000 per year over three years. The 12 cultivars are Loretta, Gator, Derby, Derby Supreme, Envy, Omega II, Manhattan II, Saturn, SR 4200, Brightstar, Assure, and Yorktown III. These were selected to cover a range of density and Acremonium endophyte infection. These perennial ryegrasses were planted in October, 1993, and investigations into allelopathy began in the spring of 1994. To augment these investigations the 1994 NTEP National Perennial Ryegrass Test was planted in the fall of 1994.

**REVISED OBJECTIVES: 1/31/94**

1. Conduct Lemna bioassays for the allelopathic effects from leaf-stem tissue extracts from the 12 field grown cultivars in spring of 1994, then conduct Lemna tests for 6 selected cultivars in the summer of 1994 and spring and summer of 1995 and with root tissue in the spring of 1995.
2. Conduct crabgrass seedling bioassays by overseeding crabgrass into the existing field plots of the 12 PR cultivars in the spring of 1994, 1995, and 1996.
3. Evaluate crabgrass suppression in practical bermudagrass culture by overseeding the selected 6 cultivars into a common bermudagrass "fairway" area in the fall of 1994 and 1995 and overseeding with crabgrass in the following late winters.
4. Conduct crabgrass seedling bioassays by overseeding crabgrass into petri dishes containing the surface 1 cm of soil from a 5 cm diameter plug. Do this in the spring of 1994 with the 12 cultivars and then with the selected 6 cultivars in the summer of 1994 and the spring and summer of 1995 and 1996.
5. Determine Acremonium endophyte content of field grown plant stems of the 12 cultivars in the spring of 1994 and from these plots in the spring of 1995 and 1995. Determine Acremonium endophyte content of plants grown from the seed lots of the 12 cultivars used for overseeding the original plots and "fairway" overseeded plots.

6. Determine Acremonium endophyte contribution to allelopathy in the cultivar showing the strongest allelopathic effects associated with endophyte in previous bioassays. Do this by growing plants from E- and E+ seed in pots in the greenhouse in 1995-96 and conducting Lemna and petri dish bioassays.

7. Report research results at ASA convention in the fall of 1995 and 1996.

8. Submit research article(s) for publication in the fall of 1996.

## **CULTURE OF FIELD PLOTS**

As explained in the 1995 annual report seeds stored in a deep freeze which was unplugged died and fall overseeding of the overseeding of the 12 cultivars into bermudagrass "fairway" plots failed. These plots were sprayed with Roundup and Finale on March 1 to control winter weeds and the limited stand of perennial ryegrass. On March 4 the plots were vertical grooved twice, overseeded with the new perennial ryegrass seedlots, raked carefully and irrigation initiated. The fairly warm weather of late February was punctuated by 8 degree F temperature on March 8 and still very cold with 15 degrees on March 9th. Concern developed as seedlings were not found until March 25. Thereafter good stands grew in.

On April 12-15 crabgrass was overseeded (after three passes with the spiker) into the west half of the plots of both the perennial ryegrass allelopathy and overseeding test plots and a strip in the NTEP perennial ryegrass studies. The other half of the plots was treated with Balan. Scott's 25-3-10 fertilizer was applied at one half pound of N per 1000 sq ft on June 6. Plots were mown at 3/4 inch height. Data were collected in July.

## **LABORATORY METHODS**

The Lemna bioassay involves collecting tissue samples from the field plots. These are stored in a freezer. Samples are weighed (10 g), chopped, ground with water, coarsely filtered, centrifuged, filtered 3 times and full strength or diluted extracts are placed in 24 celled plates (6 reps per cultivar or concentration) with duckweed fronds and nutrient media. These plates are kept in a growth chamber for a week. Then the number of fronds are counted and inhibition (or stimulation) is calculated. A corollary process keeps multiplying the duckweeds so plenty of uniform 3-frond duckweeds are available for transfer to cell plates. The final filtration and transfer of fronds especially are done aseptically. Fungal contamination in the cell plates is a problem sometimes.

A ryegrass extract-agar-crabgrass seed bioassay has been developed but only preliminary data has been collected yet. The extracts are prepared as outlined above, then added to liquid agar in the cell plates. Then scarified and surface sterilized crabgrass seeds are placed on the solidified agar. Germinated crabgrass plants are counted and root and shoot

lengths are rated. Fungal contamination and poor crabgrass seed germination rate are still serious problems.

The petri dish bioassay method was changed from earlier attempts. Five cm diameter plugs from each ryegrass field plot were cut to a one (1) cm surface soil thickness. Thirty seven gms of each moist soil sample was placed in a petri dish, overseeded with about 50 crabgrass seeds, watered with 8 ml, taped closed and kept in a growth chamber for one week. Then germination counts and visual rating of crabgrass seedling yellowing was done. The six replications in field plots corresponded with the 6 reps in the petri dishes. The soil used in the check dishes was similar except that perennial ryegrass had not been grown in it recently and it was pasteurized.

Another type of petri dish bioassay examined the effect of rates of dried powdered leaf-stem tissue on crabgrass seed germination. Forty gm of the pasteurized soil as above was thoroughly mixed with 0, 500, 1000 or 1500 mg placed in a petri dish and watered with 14,16,18 or 20 ml to achieve a "moist, but not, wet" appearance. The dishes were taped closed, placed in the growth chamber and data were collected one week later.

Acremonium endophyte infection is determined by a sero-immunoassay that Dr. Charles P. West and his associate Melody Marlatt have developed in their Forage Physiology Laboratory. Fundamentally, the technique involves using an antibody developed by rabbits fed Acremonium infected tall fescue. The antibody has been proven to react against the Acremonium species in both tall fescue and perennial ryegrasses. Basically, a grass stem is cut off near the crown and the juice from a lower 1-2 mm section is squeezed onto a paper appropriately treated with the antibody solution. A red stain develops if that stem is infected with Acremonium. Thus this is a qualitative test -- Acremonium is or is not present. We collect 20+ stems randomly from each plot, test 20 stems for Acremonium, and calculate the percent infection. This method is much faster than microtome slicing/staining and examination under a microscope.

Our attempts to determine if Acremonium endophyte affects allelopathy have not been completed yet. The endophyte in seed will die off over time or in response high temperatures (about 50 C) and high humidity treatment in an incubator over a few days time. The difference between killing the endophytes and the seed is small. We haven't gotten it quite right yet.

## RESULTS AND DISCUSSION

Results and discussion will be organized around the :

Revised Objectives: 1/31/94

1. Conduct Lemna bioassays for the allelopathic effects from leaf-stem tissue extracts from the 12 field grown cultivars in spring of 1994, then conduct Lemna tests for 6 selected

cultivars in the summer of 1994 and spring and summer of 1995 and with root tissue in the spring of 1995.

All-in-all efforts devoted to this objective exceed original intentions greatly. Lemna bioassays have been accomplished for the spring and fall of 1994 and the late spring of 1995 at full, half and quarter extract concentration. The spring 1995 bioassays were directly over 6 reps of field plots-a huge amount of work. See previous reports. The late spring 1996 field plots sample have been collected. Loretta, Manhattan II, and Yorktown III were assayed and varying amounts of allelopathy were found; the remaining cultivars are still in process. All 12 cultivars are still being assayed. Only part of the cultivars were tested on the basis of root extracts. Great inconsistencies exist in allelopathy against Lemna by cultivars and extract dilution.

2. Conduct crabgrass seedling bioassays by overseeding crabgrass into the existing field plots of the 12 PR cultivars in the spring of 1994, 1995, and 1996.

This has been done all three years, but no statistically significant evidence exists showing differences in crabgrass suppression among the 12 PR cultivars.

The 1994 NTEP PR plots were damaged severely last winter so this test has very little validity this year. Tissue samples of APM and TopHat which suppressed crabgrass most last year and DVS NA 9402 and Linn which were least suppressive need to be tested in the Lemna bioassay.

The work of this objective has been exceeded, however disappointing the results.

3. Evaluate crabgrass suppression in practical bermudagrass culture by overseeding the selected 6 cultivars into a common bermudagrass "fairway" area in the fall of 1994 and 1995 and overseeding with crabgrass in the following late winters.

This was done with 12 cultivars both years. In 1995 no differences in crabgrass suppression among cultivars were found. As the result of a plug being pulled on a deep freeze and loss of seed viability, the 1995 overseeding with PR was delayed to early March of 1996. No differences in crabgrass suppression occurred this year either. Since the 12 cultivars were overseeded both years this objective was exceeded.

4. Conduct crabgrass seedling bioassays by overseeding crabgrass into petri dishes containing the surface 1 cm of soil from a 5 cm diameter plug. Do this in the spring of 1994 with the 12 cultivars and then with the selected 6 cultivars in the summer of 1994 and the spring and summer of 1995 and 1996.

The first attempt to do this failed. Since then a great deal of effort has been devoted to developing a crabgrass/agar bioassay, but many problems with poor crabgrass seed germination and surface sterilization of seed have limited these results severely.

This year crabgrass seeds were planted (in petri dishes) in soil from near the top of plugs collected from each of the 12 cultivars. Crabgrass germination was inhibited, compared to the control soil, by soils from under all 12 cultivars. The germination for the control was 65%. The germination for Derby Supreme, Gator, and Assure was 47, 46, and 44, respectively, and not significantly different from the control. The remaining cultivars gave significantly lower germination percentages as follows: Manhattan II 42, Brightstar 40, Loretta 40, Derby 39, Envy 39, Yorktown III 39, SR 4200 36, Saturn 35 and Omega II 35%.

In another 1996 test 0, 500, 1000 or 1500 gm dried, powdered stem-leaf tissue of each of the 12 cultivars was mixed into 40 gm of a pasteurized soil in a petri dish and overseeded with crabgrass. As shown in Table 1, the degree of inhibition of germination tended to increase with increasing rates of debris and varied among cultivars. There was a tendency (data not shown) for the crabgrass seedlings to be yellower in the treated dishes than in controls.

The inconsistencies continue in these petri dish soil and soil/debris versus crabgrass germination bioassays. For example, in the soil test soil from under Derby Supreme, Gator and Assure did not affect crabgrass germination, but debris from these cultivars gave mixed effects on germination. Furthermore, debris from Derby and Omega II did not affect germination.

5. Determine Acremonium endophyte content of field grown plant stems of the 12 cultivars in the spring of 1994 and from these plots in the spring of 1995 and 1996. Determine Acremonium endophyte content of plants grown from the seed lots of the 12 cultivars used for overseeding the original plots and "fairway" overseeded plots.

This has been done in the original plots in 1994 and 1995, but not in 1996.

6. Determine Acremonium endophyte contribution to allelopathy in the cultivar showing the strongest allelopathic effects associated with endophyte in previous bioassays. Do this by growing plants from E- and E+ seed in pots in the greenhouse in 1995-96 and conducting Lemna and petri dish bioassays.

The loss of the seed stored in the unplugged deep freeze set this back. We have ordered a humistat instrument to facilitate the process of high temperature and humidity killing of endophyte in the seed. We have worked on the methods of killing endophytes while retaining seed viability. This is an important part of the project which is not done yet.

7. Report research results at ASA convention in the fall of 1995 and 1996.

I have not submitted ASA papers. Can I publish "negative" results?

8. Submit research article(s) for publication in the fall of 1996.

The whole project needs to be finished up and thoroughly statistically analyzed and organize results into just a few large tables to determine if any results are publishable.

### **GENERAL DISCUSSION**

Our research into allelopathy vs. competition vs. endophyte affects on crabgrass suppression by these 12 perennial ryegrass cultivars is nearing completion. All objectives, except determining endophyte effect on allelopathy, have been met or exceeded by the fact of still testing 12 cultivars. Allelopathy has been shown to exist in the Lemna bioassays and the various crabgrass seedling bioassays. The lack of consistent levels of allelopathy from the 12 cultivars over various assays and the seasons is a serious problem. No significant differences were found in field plots for crabgrass inhibition by any of the 12 selected cultivars, but ranges of differences were found among the 99 cultivars in our NTEP perennial ryegrass test. If or when plant breeders start selecting for allelopathy against crabgrass the methods used and developed in this research project should be helpful.

### **FINANCIAL ACCOUNTING**

As of October 30, 1996, expenses posted to the USGA grant account were \$17,618.90 for payroll, \$609.60 for materials and supplies, \$591.68 for other direct costs, \$5,000 for lab analysis, \$1026.04 for Acremonium determination supplies, and \$753.68 for travel for a total of \$25,599.90. We are somewhat under budget right now since the project finishes in few months.

### **FINAL COMMENTS**

Most of 12 perennial ryegrass cultivars have shown allelopathic inhibition of duckweed and crabgrass in one bioassay or another. This is exciting and promising in many ways, but the inconsistencies of results over bioassays is very disappointing. Thus we are a long, long way from being able to recommend specific ryegrass cultivar(s) to control crabgrass. Nevertheless, we have conducted a well-rounded research approach for accessing crabgrass suppression by (up to 99) perennial ryegrass cultivars. Perhaps, proper selection of ryegrass cultivars for crabgrass inhibition may become a more important part of IPM programs for turfgrass culture, but I think plant breeders will have to become greatly involved before this comes to pass on the basis of allelopathy. We appreciate greatly the support of the USGA Green Section Research grant in pursuing this research.

Table 1. Crabgrass germination suppression by powdered tissue mixed into soil in petri dishes.

Cultivar	Tissue	% Germ.	Sign.	Cultivar	Tissue	% Germ	Sign
Loretta	0 g	51	-	Gator	0	58	-
	500	42	N		500	46	Y
	1000	43	N		1000	26	Y
	1500	41	Y		1500	24	Y
Derby	0	42	-	Derby Supreme	0	58	-
	500	40	N		500	44	Y
	1000	33	N		1000	49	N
	1500	34	N		1500	30	Y
Envy	0	34	-	Omega II	0	29	-
	500	18	Y		500	35	N
	1000	13	Y		1000	21	N
	1500	15	Y		1500	24	N
Manhattan II	0	28	-	Saturn	0	60	-
	500	15	Y		500	51	N
	1000	9	Y		1000	32	Y
	1500	9	Y		1500	31	Y
SR 4200	0	48	-	Brightstar	0	46	-
	500	44	N		500	42	N
	1000	35	N		1000	27	N
	1500	29	Y		1500	17	Y
Assure	0	33	-	Yorktown III	0	38	-
	500	32	N		500	19	Y
	1000	34	N		1000	16	Y
	1500	15	Y		1500	6	Y

Sign = significant difference at one standard deviation

Y = Significant phytotoxic effect compared to control

N = No significant phytotoxic effect compared to control