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). The plots are maintained with good fertilizer, weed control, irrigation and 2 cm mowing practices.

Our basic laboratory evaluation for allelopathy is the Lemna minor L. (duckweed) bioassay. The Lemna bioassay measures allelopathic effects of extracts of plant tissues against the growth rate of duckweed fronds. Extracts from shoots are applied to duckweed cell plates at three concentrations. Loretta, Derby Supreme, Envy and Brightstar inhibited duckweed at certain concentrations. Stimulation of duckweed occurred from most other concentrations and cultivars. Root extracts from Gator, Derby, Saturn, SR 4200, Brightstar and Assure were tested at three concentrations. Full strength extracts from Gator, Saturn and Brightstar stimulated duckweed, but no effects were found from other concentrations. Interesting and promising results so far.

We have developed a ryegrass extract-agar-crabgrass seed bioassay. Tissue extracts are added to agar in the cell plates then crabgrass seeds are placed on the agar and seedling germination and development are measured. Problems with procedures to improve crabgrass germination and surface sterilize the seed to prevent fungal contamination have been largely overcome. No data yet, but this will be very important bioassay.

Determination of Acremonium endophyte content of stem samples from field plots showed actual infection levels different from those expected. So monitoring endophytes is important.

One half of each field plot was overseeded to crabgrass in March. Crabgrass stands ranged from 16 to 23% of plot cover by late June, but the correlation between perennial ryegrass density in May and crabgrass cover was not statistically significant.

The original field plots were touch-up overseeded with small amounts of seed from the original seed lots in mid October. Bermudagrass "fairway" plots were overseeded with new seed lots of the 12 cultivars on October 25, 1994. Half of all plots will be overseeded to crabgrass early next spring and evaluated for crabgrass suppression.

ANNUAL REPORT FOR USGA GREEN SECTION RESEARCH GRANT PROJECT AT THE UNIVERSITY OF ARKANSAS - October 30, 1994

"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.

In anticipation of USGA grant funding, 12 perennial ryegrasses were selected for evaluation of allelopathic crabgrass suppression. 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.

The good news of the USGA grant was received on January 14, 1994. That same day I applied to NTEP for the National Perennial Ryegrass Test. This was granted and seeded in September. A strip in each plot will be overseeded to crabgrass next spring so each of the 99 cultivars can be evaluated for crabgrass suppression. The bad news of the grant being cut in half to $10,000 per year resulted in revising the objectives downward. Also, the U of A attorney's wanted some details of the contract changed. The various processes resulted in funding not being available to me until July 1, 1994. Nevertheless, the research work commenced in the spring as planned.

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. (Recent discussions with Dr. West indicate the need for this more extensive endophyte monitoring.)

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

The 12 ryegrasses were hand seeded after vertical grooving at 55 g/2.25 sq m (1.5 x 1.5 m plots) (5 lb/1000 sq ft) with six replications on October 28 & 29, 1993. Ryegrasses germinated and grew slowly. Mowed as needed at 2.5 inch height. Applied 1 lb N/1000 sqft from 25-4-20 (50% SCU) on December 9 and February 17. After spiking, crabgrass was overseeded in the west half of each plot on March 12 at 0.5 kg/100 sq m (1.1 lb/M) rate. On March 18 benefin (Balan 2.5G) was applied to east half of each plot. 2,4-D, meccoprop, and dicamba (Trimec) was applied at 1 lb 2,4-D/A on March 18. Began mowing at 2 cm height. Irrigated well during summer, but tree root competition caused damage to rep 1 plots. A trench to cut roots is planned for this winter. On August 2, 9, and 16 MSMA was applied to control crabgrass. On September 6 and October 28, 24-8-16 (40% slow release) was applied at 1 lb N/M. After spiking the very limited supply of the original seed lots (stored in freezer) were overseeded at 10 g/plot on crabgrass half on reps 2-6 and 15 g on whole plot for rep 1 on October 11 (to avoid confounding Acremonium infection levels with new seed lots). (Dr. Shearman's idea of stand maturity will be followed by overseeding crabgrass on opposite side of plots next year.) On October 25 the overseeding into bermudagrass "fairway" turf was done. The process was to vertical groove, lightly rake off debris, hand seed at 60 g/plot, cross rake, mow at 2 cm with mulching mower and irrigate. Regular 2 cm mowing will continue as needed. The overseeded plots will be fertilized in November, December and February. Half of each plot will be overseeded to crabgrass after spiking in March, 1995.

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 no 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 is a serious problem, but we believe present procedures will allow a 7 to 10 day germination/growth period before fungi overrun the cells.

The petri dish bioassay method was to cut a 5 cm diameter plug from each ryegrass field plots, cut grass off at crowns, cut to a one (1) cm surface soil thickness, place in a petri dish, overseed with 50 crabgrass seeds, water as needed, and keep covered on the greenhouse bench.

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.

RESULTS AND DISCUSSION

Laboratory Bioassays

The Lemna bioassay of the 12 cultivars was conducted during the late spring of 1994 (Table 1). Derby Supreme, Envy, Loretta, and Brightstar inhibited duckweed. Derby Supreme showed a pattern of inhibition, non-significant stimulation, and significant stimulation as the extract concentration was lowered over full, half, and 1/4 strength. Envy and Loretta showed significant inhibition at full and half concentration and non-significant stimulation at 1/4 concentration. Brightstar showed significant inhibition at full extract concentration and non-significant stimulation at half and 1/4 concentration. Manhattan II (E) and SR 4200 showed non-significant inhibition at full strength concentration. All other concentrations and cultivars showed significant stimulation, ranging from about 50 to 150% increases in duckweed growth.

The Lemna bioassay was applied to root extracts from six cultivars (Table 2). Full strength extracts from Brightstar, Gator and Saturn significantly stimulated duckweed. All other concentrations and cultivars gave non-significant levels of stimulation of duckweed. Dr. Skulman's general experience with the Lemna bioassay with allelopathy in rice accessions is that root tissue is less allelopathic than stem tissue.

Four cultivars, Loretta, Envy, Derby Supreme and Brightstar, showed significant allelopathic inhibition of duckweed. This is an exciting, promising result. But the unanticipated result was that most cultivars and concentrations of extract stimulated duckweed.

Discussions with Dr. Briggs Skulman who adapted the Lemna bioassay here for testing allelopathy in rice, tall fescue, and now perennial ryegrass cultivars and thus is very experienced with this bioassay assures me that unexpected and sometimes murky and inconsistent results are not uncommon in allelopathy research.

Dr. Skulman wrote the following comments in early summer, "Since there are no known control (non-allelopathic) ryegrass cultivars to serve as reference or controls, we had to first establish what the maximum concentrations for extracted plant material were appropriate for the bioassay. From that concentration we always included diluted solutions as a way to assess the potency of the particular cultivar being examined. By straightforward assumption then those cultivars that exhibit phytotoxicity at the most dilute concentrations are potentially the most allelopathic. In the case of ryegrass there are far more of the cultivars showing some form of phytostimulation rather than toxicity. The question to ask ourselves is whether this stimulation is significant from a crabgrass control viewpoint. Could poorly timed growth be deleterious to crabgrass? Or could this stimulatory effect be similar to some form of plant hormonal response and hence be exploitable for weed control? On the other hand there is data showing some phytotoxic

effects by 4 of the cultivar shoot extracts. Envy and Loretta seem to exhibit the strongest phytotoxic effects (e.g. significant effect at 1/2 concentration). Another twist to this data is seen in those cultivars that exhibit phytotoxic effects at high concentrations and appear to become somewhat stimulatory at diluted concentrations (eg Derby Supreme and Brightstar). Are we seeing a loss of toxicity through dilution of substance that at lower concentrations becomes a stimulant? If yes, then maybe all the cultivars that show stimulation just don't produce enough allelochemical(s) to become effective phytotoxicants. At this point it may be well worthwhile to test whether extracts from selected ryegrass cultivars will stimulate/injure crabgrass seed germination and crabgrass plants grown in ryegrass extract/nutrient culture solutions."

Therefore, Dr. Skulman and our lab technicians have worked out a ryegrass extract-agar-crabgrass seed bioassay. Much preliminary work was required. The procedure is outlined in the laboratory methods section. This bioassay will become our most direct way of determining allelopathy against crabgrass.

Petri Dish Bioassay

The petri dish-surface soil bioassay failed because of inadequate control of moisture levels (and perhaps high temperature 'scald' under the covers) in the greenhouse tests. In addition to the reason discussed above, the ryegrass extract-agar-crabgrass seed bioassay was developed as one response to this failure. But the petri dish test is important from the standpoint of determining whether the allelochemical(s) are present in the soil under ryegrass plants. Next spring I will try again with improved techniques, such as, teasing the surface cm of soil from around roots, mixing imbibed light treated seed, placing soil over filter papers in petri dishes, adding water, sealing lid with Parafilm and placing in a growth chamber or seed incubator.

Acremonium Infection Monitoring

Acremonium endophytes infection level has been determined for three replications of the 12 cultivars (Table 3). So far the results show much less endophyte infection in Assure, Manhattan II and Omega II than expected. Gator and Loretta have more infection than expected. Also, the variation of infection among replications is quite large for several cultivars. The expected endophyte infection level was supplied by the seed suppliers. Obviously something changed infection levels between their tests and ours. My original selection of the 12 ryegrass cultivars included the goal of having two cultivars in each range from zero to 95% Acremonium infection. These results will be important to deciding on which six cultivars to continue using in the bioassays. These results also led to my decision to do "touch-up" overseeding of plots of the original 12 ryegrasses with the very small amounts of original seed lot available rather than "contaminate" the plots by

overseeding with the new seed lots. These results underscore the need for thorough monitoring of actual endophyte infection levels.

Field Plot Data

The turfscore quality and density ratings and percent crabgrass versus ryegrass cover ratings are presented in Table 4. Crabgrass cover in late June varied from 16 to 23 %. Contrary to expectations, there was not a significant correlation between May ryegrass density and crabgrass cover.

GENERAL DISCUSSION

We are well started on this research into allelopathy vs. competition vs. endophyte affects on crabgrass suppression by these 12 perennial ryegrass cultivars. Good progress has been made toward meeting objectives.

The spring Lemna bioassay leaf-stem tissue from the 12 ryegrass cultivars was accomplished (obj. 1). In addition, this bioassay was conducted with root tissue from six cultivars. However, the summer bioassay with leaf-stem tissue was not conducted. (A young man who was working on this project for his M.S. thesis research quit in late July; worse yet he managed to dangle me along about this until late August. I now have three part-time workers in the lab working about 44 hours per week on the Lemna bioassay and the ryegrass extract-agar-crabgrass seed bioassay. Working with a succession of part-time workers is difficult. I am progressing toward appointing a graduate student to work with this project.) Work is well along on a fall leaf-stem tissue Lemna bioassay on the 12 cultivars. The development of new ryegrass extract-agar-crabgrass bioassay is an important step. Overall we are exceeding our objective 1 goals.

Crabgrass was overseeded into plots of the 12 ryegrass cultivars in March 1994. Although the results were disappointing, objective 2 was accomplished.

New seed lots of the original 12 ryegrass cultivars were overseeded into bermudagrass 'fairway' turf on October 25, 1994. Sufficient quantities of seed were obtained to repeat the overseeding in 1995. Thus objective 3 is being accomplished and exceeded.

The petri dish bioassay was attempted (obj. 4), but my methods were poor and that bioassay failed. Improved methods will be developed next winter and spring.

Although the immunoassay procedure was not completely developed and verified until late summer, we have determined Acremonium infection in three replications of the 12 ryegrasses this fall and are progressing toward testing the remaining samples. We will also determine Acremonium infection in the plants from the new seed lots used in the fall overseeding. This seed is being stored in a freezer to preserve endophyte infection. Thus objective 5 is being accomplished.

The larger overall objective of selecting six of the original 12 ryegrasses for continued allelopathic testing has not been accomplished yet. In so far as possible we will choose cultivars that fit into the desired Acremonium infection ranges and have shown allelopathy to crabgrass and/or duckweed. When the Acremonium infection determinations and the fall runs of the Lemna and the ryegrass extract-agar-crabgrass seed bioassays are completed, we will confer and make these hard choices.

FINANCIAL ACCOUNTING

As of October 25, 1994, expenses posted to the USGA grant account were $373.60 for payroll and $63.74 for materials (repair of Mataway for overseeding) for a total of $437.34.

Obviously, this does not accurately reflect the payroll and other costs of the work accomplished to date. So far this fiscal year a disproportionate portion of payroll costs have been charged to the tall fescue allelopathy grant account. These payroll costs will balance out over the course of the year. Furthermore, no transfers of funds to cover material costs for laboratory bioassays and Acremonium determinations have been made yet. Please don't get the notion that we can accomplish so much, even here at the U of A, for so little money.

FINAL COMMENTS

Loretta, Derby Supreme, Envy, and Brightstar perennial ryegrass cultivars have shown allelopathic inhibition of duckweed. This is an exciting and promising result. As I commented to my turfgrass field day audience on October 10, we are a long, long way from being able to recommend specific ryegrass cultivars to control crabgrass. Nevertheless, we have a well-rounded research approach and team for accessing crabgrass suppression by (up to 99) perennial ryegrass cultivars. Eventually, proper selection of ryegrass cultivars may become a more important part of IPM programs for turfgrass culture. We appreciate greatly the support of the USGA Green Section Research grant in pursuing these research goals.

Table 1

Ryegrass Shoot Phytotoxicity to Lemna minor (at 10g/30ml initial extraction) Spring 1994					
Cultivar	Extract Concentration	Final # Fronds	Control # Fronds	% of Control	SD
Gator (2)	Full (10g/30ml)	22.7	15.2	149.3	S
Gator (2)	1/2 (5g/30ml)	29	15.2	190.8	S
Gator (2)	1/4 (2.5g/30ml)	24.2	15.2	159.2	S
Omega II (6)	Full (10g/30ml)	22.2	15.2	146.1	S
Omega II (6)	1/2 (5g/30ml)	27.7	15.2	182.2	S
Omega II (6)	1/4 (2.5g/30ml)	25.5	15.2	167.8	S
Derby Supreme (4)	Full (10g/30ml)	5	13.7	36.5	Y
Derby Supreme (4)	1/2 (5g/30ml)	18.8	13.7	137.2	N
Derby Supreme (4)	1/4 (2.5g/30ml)	20.2	13.7	147.4	S
Envy (5)	Full (10g/30ml)	-3	13.7	-21.9	Y
Envy (5)	1/2 (5g/30ml)	7.8	13.7	56.9	Y
Envy (5)	1/4 (2.5g/30ml)	17.3	13.7	126.3	N
Loretta (1)	Full (10g/30ml)	-3	14.3	-21.0	Y
Loretta (1)	1/2 (5g/30ml)	-2.8	14.3	-19.6	Y
Loretta (1)	1/4 (2.5g/30ml)	15.3	14.3	107.0	N
Manhattan (7)	Full (10g/30ml)	13.2	14.3	92.3	N
Manhattan (7)	1/2 (5g/30ml)	26	14.3	181.8	S
Manhattan (7)	1/4 (2.5g/30ml)	21.7	14.3	151.7	S
Saturn (8)	Full (10g/30ml)	16.8	10	168.0	S
Saturn (8)	1/2 (5g/30ml)	23	10	230.0	S
Saturn (8)	1/4 (2.5g/30ml)	20.3	10	203.0	S
SR 420 (9)	Full (10g/30ml)	9.5	10	95.0	N
SR 420 (9)	1/2 (5g/30ml)	25.3	10	253.0	S
SR 420 (9)	1/4 (2.5g/30ml)	20.2	10	202.0	S
Derby (3)	Full (10g/30ml)	19.3	11.7	165.0	S
Derby (3)	1/2 (5g/30ml)	26.3	11.7	224.8	S
Derby (3)	1/4 (2.5g/30ml)	22.3	11.7	190.6	S
Brightstar (10)	Full (10g/30ml)	-3	11.7	-25.6	Y
Brightstar (10)	1/2 (5g/30ml)	12.3	11.7	105.1	N
Brightstar (10)	1/4 (2.5g/30ml)	16.8	11.7	143.6	N
Assure (11)	Full (10g/30ml)	18.8	10.5	179.0	S
Assure (11)	1/2 (5g/30ml)	25.2	10.5	240.0	S
Assure (11)	1/4 (2.5g/30ml)	20.2	10.5	192.4	S
Yorktown III (12)	Full (10g/30ml)	17.3	10.5	164.8	S
Yorktown III (12)	1/2 (5g/30ml)	21.7	10.5	206.7	S
Yorktown III (12)	1/4 (2.5g/30ml)	23	10.5	219.0	S
SD = significant difference at one standard deviation					
Y = significant pytotoxic effect compared to the control					
N = No significant phytotoxic effect compared to the control					
S = Significant phytostimulation effect when compared to the control					

Table 2

Ryegrass Root Phytotoxicity to Lemna minor (at 10g/30ml initial extraction) Spring 1994					
Cultivar	Extract Concentration	Final # Fronds	Control # Fronds	% of Control	SD
Gator (2)	Full (10g/30ml)	16.2	10.2	158.8	S
Gator (2)	1/2 (5g/30ml)	14	10.2	137.3	N
Gator (2)	1/4 (2.5g/30ml)	11.7	10.2	114.7	N
Saturn (8)	Full (10g/30ml)	15	10.3	145.6	S
Saturn (8)	1/2 (5g/30ml)	14.7	10.3	142.7	N
Saturn (8)	1/4 (2.5g/30ml)	12.2	10.3	118.4	N
SR 420 (9)	Full (10g/30ml)	15.2	10.3	147.6	N
SR 420 (9)	1/2 (5g/30ml)	14	10.3	135.9	N
SR 420 (9)	1/4 (2.5g/30ml)	13.8	10.3	134.0	N
Derby (3)	Full (10g/30ml)	15.7	10.2	153.9	N
Derby (3)	1/2 (5g/30ml)	13.8	10.2	135.3	N
Derby (3)	1/4 (2.5g/30ml)	11	10.2	107.8	N
Brightstar (10)	Full (10g/30ml)	13.3	8.5	156.5	S
Brightstar (10)	1/2 (5g/30ml)	9.8	8.5	115.3	N
Brightstar (10)	1/4 (2.5g/30ml)	11.2	8.5	131.8	N
Assure (11)	Full (10g/30ml)	9.8	8.5	115.3	N
Assure (11)	1/2 (5g/30ml)	9.8	8.5	115.3	N
Assure (11)	1/4 (2.5g/30ml)	9.7	8.5	114.1	N
SD = significant difference at one standard deviation					
Y = significant pytotoxic effect compared to the control					
N = No significant phytotoxic effect compared to the control					
S = Significant phytostimulation effect when compared to the control					

IMMUNOASSAY DATA Table 3

Dr. John King's ryegrass field study

Date of report: 10-20-94

CULTIVAR	PERCENT INFECTION						MEAN	EXPECTED
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6		
Assure		0	0	0			0	94-96
Brightstar		95	95	95			95	90
Derby		20	10.5	0			10.2	5-10
Derby Supreme		30	20	50			33.3	40-45
Envy		25	100	90			71.7	40
Gator		35	25	0			20	0
Loretta		5	10	15			10	0
Manhattan II		0	25	0			8.3	50-98
Omega II		0	55	5			20	76
Saturn		0	15	60			25	80
SR4200		90	100	100			96.7	80-85
Yorktown III		67	80	90			79	97

Table 4

1994 Ryegrass Evaluation

Code	Variety Name	Turf Score Mean	April	May	July	September	Density Score Mean	May	July	Crabgrass Percent Cover	Ryegrass Percent Cover
1	Loretta	4.83c	5.00a	5.16c	4.83bc	4.33cd	5.50bc	6.00ab	5.00cd	19.16a	77.50a
2	Gatir	5.25bc	5.33a	6.00b	5.00bc	4.66bcd	5.58abc	6.00ab	5.16bcd	19.16a	55.00a
3	Derby	4.87c	5.33a	5.66bc	4.33c	4.16d	4.50d	5.16bc	3.83e	22.50a	85.83a
4	Derby Supreme	5.08bc	5.33a	5.50bc	4.66c	4.83bcd	5.25bcd	5.50abc	5.00cd	21.66a	76.67a
5	Envy	5.20bc	5.00a	5.83bc	5.33bc	4.66bcd	5.33bc	5.66abc	5.00cd	23.33a	70.00a
6	Omega II	5.08bc	4.83a	5.33bc	4.66c	5.50ab	4.83cd	4.83c	4.83cde	21.66a	69.17a
7	Mahattan II	4.83c	4.50a	5.33bc	4.83bc	4.66bcd	5.33bc	5.50abc	5.16bcd	23.33a	69.17a
8	Saturn	5.45b	5.33a	5.66bc	5.33bc	5.50ab	4.83cd	5.16bc	4.50de	18.33a	41.67a
9	SR 4200	5.45b	5.16a	6.00b	5.33bc	5.33abc	5.75ab	5.83ab	5.66abc	16.66a	91.50a
10	Brightstar	6.29a	5.66a	7.00a	6.50a	6.00a	6.33a	6.33a	6.33a	17.50a	63.33a
11	Assure	5.54b	5.16a	5.16c	5.83ab	6.00a	5.83ab	5.50abc	6.16ab	20.00a	75.83a
12	Yorktown III	5.45b	5.50a	5.50bc	5.16bc	5.66ab	5.58abc	6.00ab	5.16bcd	22.50a	46.67a